February 2022

QIAgility User Manual

For use with the QIAgility and the QIAgility Setup Manager v1.4







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Introduction

1 Introduction

Thank you for choosing the QIAgility[®]. We are confident it will become an integral part of your laboratory.

Before using the QIAgility, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

1.1 About this user manual

This user manual describes the operation of the QIAgility instrument with the QIAgility Setup Manager Software. It provides information about the QIAgility in the following sections:

- 1. Introduction
- 2. <u>Safety information</u>
- 3. General description
- 4. Installation procedures
- 5. Getting started
- 6. **Operating procedures**
- 7. <u>QIAgility Setup Manager Software</u>
- 8. <u>Maintenance procedures</u>
- 9. <u>Troubleshooting</u>
- 10. <u>Glossary</u>
- 11. Appendices

The user manual can be accessed by simply pressing the context-sensitive help functionality (**F1** key) provided by the QIAgility Setup Manager Software. Alternatively, the user manual can be accessed, read and printed as ***.pdf** file.

The functionality of the QIAgility Setup Manager Software and its available plug-ins are described in detail in the QIAgility Setup Manager Software section (section 7).

The user manual includes the following appendices:

- <u>Appendix A</u>:
 - o Technical data
 - o Environmental conditions
 - o Waste Electrical and Electronic Equipment
 - o FCC declaration
 - o Declaration of conformity

- <u>Appendix B: Liability clause</u>
- Appendix C: French (FR) translation of Safety Information
- Appendix D: German (DE) translation of Safety Information
- Appendix E:
 - o License terms
 - Copyright information

Check <u>www.giagen.com/goto/QIAgility</u> for a newer version of the user manual.

1.2 General information

1.2.1 Technical assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Services Departments are staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of QIAGEN products. If you have any questions or experience any difficulties regarding the QIAgility or QIAGEN products in general, do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please visit our Technical Support Center at **www.support.giagen.com**.

1.2.2 Policy statement

It is the policy of QIAGEN to improve products as new techniques and components become available. QIAGEN reserves the right to change specifications at any time.

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual. Please contact QIAGEN Technical Services.

1.3 Intended use of the QIAgility

The QIAgility is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

The QIAgility, if used in combination with QIAGEN kits indicated for use with the QIAgility instrument, is intended for the applications described in the respective QIAGEN kit handbooks.

If the QIAgility instrument is used with kits other than QIAGEN kits, it is the user's responsibility to validate the performance of such product combination for any particular application.

The QIAgility instrument is intended for use by professional users trained in molecular biological techniques and the operation of the QIAgility instrument.

1.4 Requirements for QIAgility users

This table covers the general level of competence and training necessary for transportation, installation, use, maintenance and servicing of the QIAgility.

Task	Personnel	Training and experience
Delivery	No special requirements	No special requirements
Installation	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Routine use (running experiments)	Laboratory technicians or equivalent	Appropriately trained and experienced personnel familiar with use of computers and automation in general
Servicing and annual preventive maintenance	QIAGEN Field Service Specialists only	Regularly trained, certified and authorized by QIAGEN

Safety information

2 Safety information

Before using the QIAgility, it is essential that you read this user manual carefully and pay particular attention to the safety information. The instructions and safety information in the user manual must be followed to ensure safe operation of the instrument and to maintain the instrument in a safe condition.

Note: Translations of the Safety Information in French and German are available in <u>Appendix C</u> and <u>Appendix D</u>.

The following types of safety information appear throughout this manual.

WARNING	The term WARNING is used to inform you about situations that could result in personal injury to you or other persons.
	Details about these circumstances are given in a box like this one.

CAUTION	The term CAUTION is used to inform you about situations that could result in damage to the instrument or other equipment.
	Details about these circumstances are given in a box like this one.

The advice given in this manual is intended to supplement, not supersede, the normal safety requirements prevailing in the user's country.

2.1 Proper use

Risk of personal injury and material damage [W1] Improper use of the QIAgility may cause personal injuries or damage to the instrument. The QIAgility must only be operated by qualified personnel who have been appropriately trained.
Servicing of the QIAgility must only be performed by QIAGEN Field Service Specialists.

	Risk of overheating [W2] To ensure proper ventilation, maintain a minimum clearance of 10 cm at the sides and rear of the QIAgility. Slits and openings that ensure the ventilation of the QIAgility must not be covered.
--	--

WARNING/	Risk of personal injury and material damage [W3]
CAUTION	The QIAgility is too heavy to be lifted by one person. To avoid personal injury
	or damage to the instrument, do not lift the instrument alone.

	Risk of personal injury and material damage [W4] Do not attempt to move the QIAgility during operation.
--	---

	Explosive atmosphere [W5] The QIAgility is not designed for use in an explosive atmosphere.
--	---

CAUTION Damage to the instrument [C1] Direct sunlight may bleach parts of the instrument and cause damage to plastic parts. The QIAgility must be located out of direct sunlight.	
---	--

CAUTION	Damage to the instrument [C2]
	Avoid spilling water or chemicals onto the QIAgility. Damage caused by water or chemical spillage will void your warranty.

	Risk of personal injury [W7] The robotic arm moves during position calibration while the instrument lid is raised. Never click any buttons while parts of your body are within the instrument workspace.
--	--

In case of emergency, switch off the QIAgility using the power switch at the rear of the instrument and unplug the power cord from the power outlet.

2.2 Electrical safety

Disconnect the line power cord from the power outlet before servicing.

	 Electrical hazard [W8] Any interruption of the protective conductor (earth/ground lead) inside or outside the instrument or disconnection of the protective conductor terminal is likely to make the instrument dangerous. Intentional interruption is prohibited. Lethal voltages inside the instrument When the instrument is connected to line power, terminals may be live, and opening covers or removing parts is likely to expose live parts.
--	--

	UV irradiation hazard [W9] An electronic interlock ensures that the instrument lid must be closed for operation of the UV lamp. Under no circumstances should this interlock be disabled.
--	---

To ensure satisfactory and safe operation of the QIAgility:

- Connect the line power cord to a line power outlet that has a protective conductor (earth/ground).
- Do not adjust or replace internal parts of the instrument.

- Do not operate the instrument with any covers or parts removed.
- If liquid has spilled inside the instrument, switch off the instrument, disconnect it from the power outlet and contact QIAGEN Technical Services.

If the instrument becomes electrically unsafe, prevent other personnel from operating it, and contact QIAGEN Technical Services. The instrument may be electrically unsafe when:

- The line power cord appears to be damaged.
- It has been stored for a prolonged period of time in conditions which are outside of the "Storage Conditions" outlined in <u>Appendix A</u>.
- It has been subjected to severe transport stresses.

2.3 Biological safety

Specimens and reagents containing materials from humans should be treated as potentially infectious. Use safe laboratory procedures as outlined in publications such as Biosafety in Microbiological and Biomedical Laboratories, HHS (www.cdc.gov/od/ohs/biosfty/biosfty/biosfty.htm).

2.3.1 Samples

Samples may contain infectious agents. You should be aware of the health hazard presented by such agents, and should use, store and dispose of such samples according to the required safety regulations.

Samples containing infectious agents [W10] Some chemicals used with this instrument may contain infectious agents. Handle such samples with the greatest of care and in accordance with the required safety regulations. Always wear safety glasses, 2 pairs of gloves and a lab coat.
The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of infectious agents as defined in the applicable Safety Data Sheets (SDSs) or OSHA, [*] ACGIH [†] or COSHH [‡] documents.
Venting for fumes and disposal of wastes must be in accordance with all national, state and local health and safety regulations and laws.

* OSHA: Occupational Safety and Health Administration (United States of America).

- [†] ACGIH: American Conference of Government Industrial Hygienists (United States of America).
- [‡] COSHH: Control of Substances Hazardous to Health (United Kingdom).

2.4 Chemicals

Hazardous chemicals [W11] Some chemicals used with this instrument may be hazardous or may become hazardous after completion of the protocol run.				
Always wear safety glasses, gloves and a lab coat.				
The responsible body (e.g., laboratory manager) must take the necessary precautions to ensure that the surrounding workplace is safe and that the instrument operators are not exposed to hazardous levels of toxic substances (chemical or biological) as defined in the applicable Safety Data Sheets (SDSs) or OSHA, [*] ACGIH [†] or COSHH [‡] documents.				
Venting for fumes and disposal of wastes must be in accordance with all national, state and local health and safety regulations and laws.				

* OSHA: Occupational Safety and Health Administration (United States of America).

[†] ACGIH: American Conference of Government Industrial Hygienists (United States of America).

[‡] COSHH: Control of Substances Hazardous to Health (United Kingdom).

2.4.1 Toxic fumes

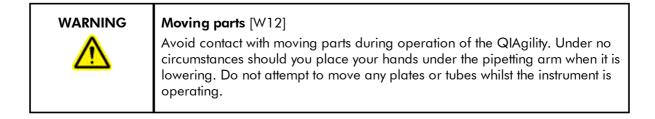
If working with volatile solvents or toxic substances, you must provide an efficient laboratory ventilation system to remove vapors that may be produced.

2.5 Waste disposal

Used plasticware and containers may contain certain hazardous chemicals, or contagious/biohazardous materials. Such wastes must be collected and disposed of properly according to local safety regulations.

For information about how to dispose of the QIAgility, see Appendix A.

2.6 Mechanical hazards



WARNING Moving parts [W13] To avoid contact with moving parts during operation of the QIAgility, the instrument must be operated with the lid closed. If the in-built lid sensor is not functioning correctly, contact QIAGEN Teal Services.	
--	--

CAUTION Damage to the instrument [C3] Do not apply any manual force to the Y-arm (i.e. the pipetting head). We strongly recommend restarting the system if the Y-arm was accidentally manually moved.

To ensure satisfactory and safe operation of the QIAgility, follow the guidelines below:

- Only use recommended tips and tip racks.
- Keep the instrument lid closed whenever possible.
- When calibrating with the lid open, always stand clear of the instrument when making adjustments. The lid interlock is disabled during calibration. Therefore, do not click with the mouse or press any of the keys on the keyboard when looking closely at calibrations. Wait until the robotic arm has completed its movements before moving any plates or tubes.
- Do not place any items onto the robotic arm.

During run, click on the **Pause** button to pause the instrument before removing plates or tubes from the worktable. Wait until the robotic arm has completed the current movement. This may take up to 10 seconds.

2.7 Maintenance safety

Perform the maintenance as described in the <u>Maintenance procedures</u>. QIAGEN charges for repairs that are required due to incorrect maintenance.

Risk of electric shock [W15] Do not open any panels on the QIAgility.			
Risk of personal injury and material damage Only perform maintenance that is specifically described in this user manual.			

CAUTION	Damage to the instrument [C4]				
	Do not use solvents or reagents containing acids, alkalis, or abrasives to clean the QIAgility.				

	Damage to the QIAgility lid [C5] Do not use alcohol, solvents, reagents containing acids, alkalis, or abrasives to clean the instrument lid. Alcohol will damage the lid. To clean the lid, use distilled water only.
--	---

CAUTION	Damage to the QIAgility side panels [C6]				
	Never clean the instrument side panels with alcohol or alcohol-based solutions.				

Relocating the QIAgility [C7] The most important component of the packaging is the wooden support that immobilizes the Y-arm. This support must be used when the QIAgility is moved.
noved.

2.8 Symbols on the QIAgility

Symbol	Location	Description
	On the back of the instrument, and on the front edge of the worktable	Warning symbol
	Near the tip ejector outlet, and on the front edge of the worktable	Biohazard symbol
	On the front edge of the worktable	Squeezing symbol
CE	Type plate on the back of the instrument	CE mark for European Conformity
	Type plate on the back of the instrument	CSA listing mark for Canada and the USA
	Type plate on the back of the instrument	Legal manufacturer
X	Type plate on the back of the instrument	Waste Electrical and Electronic Equipment (WEEE)
F©	Type plate on the back of the instrument	FCC mark of the United States Federal Communications Commission
\bigcirc	Type plate on the back of the instrument	RCM (former C-Tick) for Australia (supplier identification N17965)
2 5	Type plate on the back of the instrument	RoHS mark for China (the restriction of the use of certain hazardous substances in electrical and electronic equipment)
li	Type plate on the back of the instrument	Consult instructions for use

General description

3 General description

The QIAgility is a compact benchtop instrument that enables rapid, high-precision PCR setup. For added flexibility and ease of use, the QIAgility has been designed to support almost all tube and plate formats, as well as the Rotor-Disc[®] for the Rotor-Gene[®] Q instruments. The QIAgility is operated via a computer with an user-friendly software. Further information on the QIAgility Setup Manager Software are available <u>here</u>.

The QIAgility instrument provides:

- Automated PCR setup in almost all formats
- Convenient, easy-to-use software
- Standardized results and increased productivity
- Elimination of manual pipetting steps
- Seamless integration with QIAGEN sample technologies

3.1 QIAgility instrument



7

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- 1 Lid
- 2 Feet
- 3 Tip ejector
- 4 HEPA filter
- 5 UV lamp

3.1.1 Lid

CAUTION	Damage to the QIAgility lid [C5]					
	Do not use alcohol, solvents, reagents containing acids, alkalis, or abrasives to clean the instrument lid. Alcohol will damage the lid. To clean the lid, use distilled water only.					

Tip rack holder and tips

Tip rack holder and tips

Master mix block and reagent block

The lid of the instrument provides both an isolated enclosure and a safety mechanism. The instrument lid incorporates a magnetic sensor to determine whether or not the lid is closed. Under no circumstances should this sensor be bypassed.

The instrument lid must be closed for a run to proceed. Opening the instrument lid while the instrument is performing a run causes the run to pause.

The instrument lid may remain open during calibration. Do not interfere with robotic arm movement during these calibrations and keep all body parts out of reach of the robotic arm while it is moving.

Important: Care should be taken when opening the lid during a run, because it takes up to 10 seconds for the instrument to complete the current movement and for the pause to take effect. Under no circumstances should the sensor be bypassed.

3.1.2 Feet

The feet of the instrument have a non-slip base to ensure that the QIAgility remains securely positioned on a laboratory bench. Do not attempt to slide the instrument on a bench. Always lift the instrument to avoid damage to the base of the feet.

3.1.3 Tip ejector



Tips used on the worktable are discarded through the tip ejector located on the left side of the QIAgility. The tip ejector is held in place by a locating block, allowing easy placement and removal for cleaning.

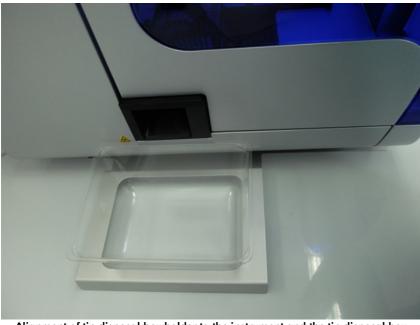
Note: Details on removing and replacing the tip ejector are described in section <u>Cleaning the tip ejector</u>.

Tip ejector

3.1.4 Tip disposal box

Discarded tips are collected in a tip disposal box, located at the side of the QIAgility. To ensure correct positioning under the tip ejector, the tip disposal box is held in place by the tip disposal box holder. The tip disposal box has a capacity of approximately 350 tips.

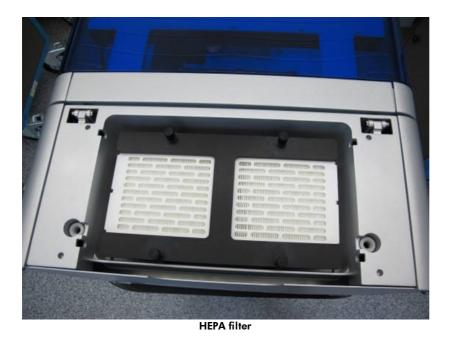
Lock the tip disposal box holder with the instrument's feet on the left side of the instrument and then place the tip disposal box into the tip disposal box holder.



Alignment of tip disposal box holder to the instrument and the tip disposal box.

3.1.5 HEPA filter

The HEPA filter system provides positive clean air pressure on the worktable beneath the lid. The HEPA filter system consists of two HEPA filter cartridges.



3.1.6 UV lamp

A UV lamp in the instrument lid provides efficient worktable decontamination and helps to prevent crosscontamination.

Note: Before using the UV lamp, the tip ejector must be placed into the instrument.

For increased safety, an electronic interlock ensures that the UV lamp operates only when the instrument lid is closed. Under no circumstances should the interlock be disabled.

During UV irradiation, the robotic arm moves. All items must be removed from the instrument to ensure the worktable is fully exposed.

The UV lamp does not irradiate certain areas, including the pipettor head, due to construction constraints.

To operate the UV lamp, the UV lamp bulb needs to be connected to the UV plug. For more details, see section <u>Using UV lamp</u>.

3.1.7 Pipettor head and robotic arm

Liquid-handling tasks are performed by the pipettor head, which provides a motor-driven, backlashcompensated pipetting mechanism. Tips attach to the pipettor piston of the pipettor head.

The robotic arm controls the movement of the pipettor head in the X-, Y- and Z-directions.

The Y-axis arm of the robotic arm provides support for the pipettor head. Under no circumstances should any force be applied to the Y-arm. This may misalign the arm and cause pipetting errors.

Note: To prevent accidental damage, the wooden Y-arm support should always be fixed or locked before the QIAgility is moved.

3.1.8 Worktable

The worktable is the instrument surface upon which items, such as adapters, labware and tips are placed. The QIAgility Setup Manager Software automatically calculates the worktable layout before each run.

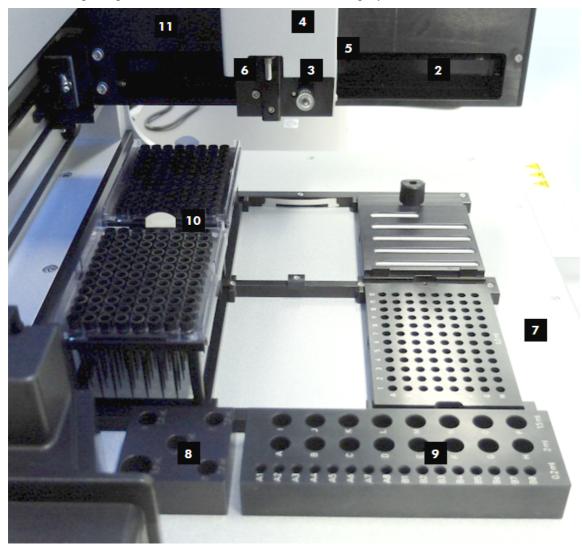
3.1.9 Accessory panel

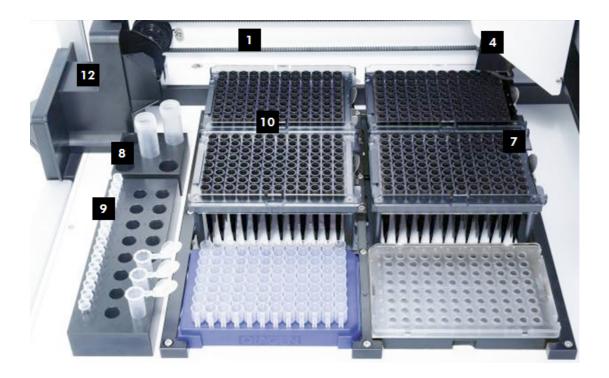
All instrument electronics are housed in the rear of the instrument. The accessory panel, located at the rear of the QIAgility, provides ports for connection to the instrument.

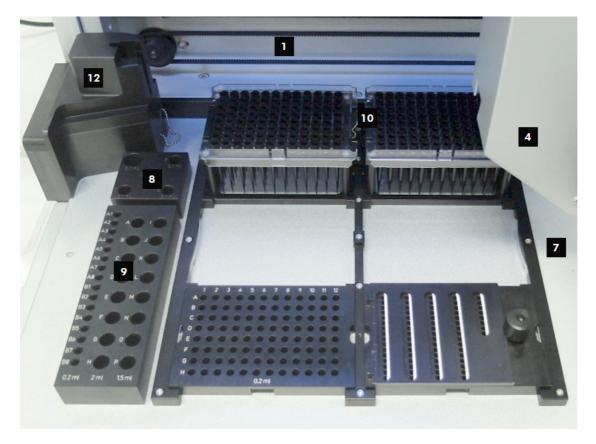
Ac	coessory 3	Accessory 2	Accessory 1	S	enal U	JSB	ACCESSORY POWER RATINGS REFER INPUT	
		əə)e	-				1017
-	3	2	1	4	5		6	7 8
QIAgility accessory panel								
1	Accessory	/ 1		5	USB socket			
2	Accessory 2			6	Accessory power outlet			
3	3 Accessory 3		7	Main power input socket				
4	4 Serial RS-232 socket			8	Power switch			

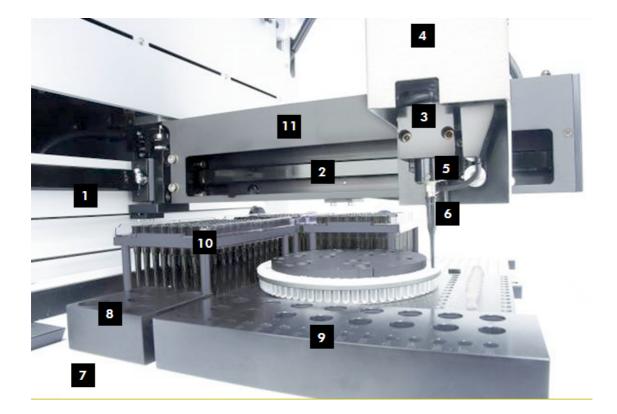
3.1.10 Internal features of the QIAgility

The following images show the internal features of the QIAgility.









1 X-axis

Y-axis

Z-axis

Pipettor head cover

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- 7 Worktable
- 8 Master Mix Block
 - 9 Reagent Block
- 10 Tip Rack Holders and tips
- 5 Pipettor head 11 Y-arm
- 6 Pipetting hub 12 Tip ejector

Part	Description
X-axis	The X-axis directs the left/right movement of the pipettor head.
Y-axis	The Y-axis directs the forwards/backwards movement of the pipettor head.
Z-axis	The Z-axis directs the upwards/downwards movement of the pipettor head.
Tip ejector	The tip ejector enables tip removal from the pipettor head.
Pipettor head cover and pipettor head	The pipettor head is a single channel, able to pipette 1–200 μ l and is located under the protective pipettor head cover. The pipettor head consists of a motor-driven backlash-compensated pipetting mechanism.
Y-arm	The Y-arm supports the pipettor head. Under no circumstances should the user apply force to the Y-arm. This may misalign the Y-arm and cause pipetting errors.
Worktable	The worktable has 6 SBS positions (127.76 mm x 85.48 mm) in which adapters and labware can be placed. In addition, the worktable supports both Master Mix Blocks and Reagent Blocks.

3.2 Liquid-level tracking

The QIAgility can track the liquid level using liquid-level sensing or liquid-level estimate. The QIAgility Setup Manager Software ensures optimal use of the feature during runs.

Liquid-level sensing

For maximum accuracy and precision, the QIAgility is equipped with a liquid-level sensor. This feature enables the instrument to automatically detect when the pipet tip makes contact with the liquid as the tip descends. When aspirating, the tip is placed below the liquid surface, at the minimum insertion depth required to collect the aspirate volume. When dispensing, the tip is placed at the liquid surface and is retracted as liquid is ejected. This minimizes contact between the tip and the liquid, reducing liquid carryover.

The liquid-level sensor relies on conductive tips to detect the change in capacitance when the tip contacts the liquid inside a tube – the smaller the volume of liquid inside the tube, the lower the change in capacitance. For correct functioning of the liquid-level sensor, it is essential that the labware (plates, tubes, adapters) located on the instrument worktable has been correctly selected in the software. See section <u>Managing labware</u> for details about how to do this.

If a liquid is nonconductive (e.g., pure water, oil), the level sensor will not detect any change in capacitance.

Liquid-level estimate

This feature enables the instrument to estimate the liquid level, using information from the run configuration. The software tracks the movement of liquid between wells and estimates the liquid level during all liquid transfers. This information is used to determine the optimal tip placement. Levels of accuracy and precision comparable to those obtained with liquid-level sensing are obtained. The pipet tip moves to a calculated position so that, after aspiration, the pipet tip remains below the liquid level.

3.3 Computer

The QIAgility is operated via a computer.

A laptop with the correct specifications for operation of the QIAgility and QIAgility Setup Manager Software is supplied as part of the QIAgility system. However, if a different computer is used to operate the QIAgility or to run the QIAgility Setup Manager Software, then a computer hosting system with the following specifications is recommended.

Computer specifications:

- Windows[®] 7 Professional (32- or 64-bit) with Service Pack 1 or higher; Windows 10 (64-bit) with minimum required version 1607
- At least 1 GHz or faster CPU
- At least 1 GB RAM for 32-bit OS, or 2 GB RAM for 64-bit OS
- At least 16 GB free hard drive capacity for 32-bit OS, or 20 GB free hard drive capacity for 64-bit OS
- At least 1360 x 768 screen resolution
- Pointer device (mouse or similar)

To view reports generated in PDF format, a PDF reader must be installed on the computer. Adobe[®] Reader[®] can be downloaded at <u>www.adobe.com</u>.

Additional configuration steps:

- Turn off all automatic processes or services such as indexing or similar, which could produce a high workload.
- Ensure that energy saving options, hibernation and the screen saver are deactivated during the operating times of the QIAgility.
- For Windows 10 only if UEFI Boot is used, the Secure Boot option must be disabled. Otherwise, the Windows Device Driver Installation of the QIAgility device driver will fail during installation of the QIAgility Setup Manager Software.
- Ensure that the Federal Information Processing Standards (FIPS) policy is disabled.

Note: In case of any concern regarding the Windows configuration, please contact your local IT support.

Note: If you close the laptop lid during a run, the run will be interrupted and stopped.

If connecting the computer to a network, be sure to consider the following:

- Ensure that update processes are scheduled to download and install only outside the operating times of the QIAgility.
- Turn off auto-restart after an installation of Windows security patches to prevent automatic computer restart during the operating times of the QIAgility.

Note: In order to complete a security update installation, perform a manual restart later.

If virus protection software is required, ensure that the following tasks are scheduled to run outside the operating times of the QIAgility:

- Scans (also ensure that files that are created by the QIAgility Setup Manager Software are not scanned during creation or on access)
- All kind of updates

Note: Some virus protection software might interfere with QIAgility Setup Manager Software and cause the software to run very slowly.

Note: The QIAgility Setup Manager Software is tested by QIAGEN using the original image supplied with your laptop without connecting the laptop to the internet. Updates on your operating system might lead to unexpected behavior of the QIAgility Setup Manager Software. In such cases, please contact your local IT support.

Note: The minimal screen resolution can be used in combination with small fonts. However, medium size fonts can be used in combination with an improved resolution (e.g., the medium font size (125%) setting in Windows 7 requires a minimum screen resolution of 1680 x 1050). Larger font sizes are not supported.

Installation procedures

4 Installation procedures

This section provides instructions on unpacking and installing the QIAgility.

Note: For your own safety and to prevent damage to the instrument, observe the safety instructions.

4.1 System delivery

The following items are delivered:

- QIAgility instrument
- Laptop
- QIAgility Setup Manager Software and instructions for use
- Unpacking Instructions (printed version)
- Packing List (printed version)
- Volume Calibration Report (printed version)
- QIAgility Calibration Kit
- Accessories

Note: We recommend checking the packing list before installation. If anything is missing, contact QIAGEN Technical Services.

Adapters

The QIAgility is supplied with the following adapters:

- 2 x Adapter, 96 x 0.2 ml PCR Tubes
- 1 x Adapter, Flip-Cap 32 x 1.5 ml Tapered Base
- 1 x Adapter, 72 x 0.1 ml Strip Tubes
- 1 x Master Mix Block, 5 x 5 ml
- 1 x Master Mix Block 1 x 5 ml, 4 x 2 ml
- 1 x Master Mix Block, 1 x 5 ml, 4 x 1.5 ml
- 1 x Reagent Block, 16 x 0.2 ml / 2 ml Flat / 1.5 ml Tapered Tubes
- 4 x Tip Rack Holder for 96-Tip Rack

The adapters are intended to hold tubes, plates and tips.

Tubes and plates

Several types of tubes and plates can be used with the QIAgility. A set of labware is already preinstalled in the software and can be found in the **Configuration** environment. The following tubes are supplied with the instrument:

- 5 ml free-standing tubes
- 200 µl PCR tubes

Note: Supplied tubes are not guaranteed to be sterile.

Important: Tubes supplied by different manufacturers may have slightly different dimensions, despite a similar appearance. Such differences can significantly affect the calibration settings, in particular the height calibration (Z-axis calibration). We recommend that once a particular tube type is chosen, to always purchase that consumable from the same manufacturer to avoid frequent recalibration.

Conductive Filtered Tips

 $200 \,\mu$ l or $50 \,\mu$ l Conductive Filtered Tips can be used on QIAgility. Supplied tips are carbon impregnated. See section <u>Insert tips to tip rack holder</u> for details about how to insert tips into a Tip Rack Holder.

Important: Do not use tips from other suppliers.

4.2 Requirements

QIAgility is a high-precision liquid-handling device and has specific requirements regarding the installation site. These requirements are explained in the following chapter.

4.2.1 Installation site

Choose the position for the QIAgility carefully. A change in position necessitates a recalibration. Place the QIAgility in its final position.

The requirements for the QIAgility location and position are given below:

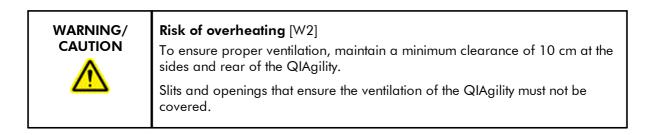
- The QIAgility must be located out of direct sunlight, away from heat sources and away from sources of vibration and electrical interference. Refer to <u>Appendix A</u> for the operating conditions (temperature and humidity).
- The site of installation should be free of excessive drafts, moisture and dust, and should not be subject to large temperature fluctuations.
- Use a level workbench that is dry, clean, vibration-proof and large enough to accommodate the QIAgility, computer and tip disposal box. Refer to <u>Appendix A</u> for the weight and dimensions of the QIAgility.
- The QIAgility must be placed within approximately 1.5 m of a properly grounded (earthed) AC power outlet. The power line to the instrument should be voltage regulated and surge protected.
- Ensure that the switch and the main power input socket at the rear of the instrument are easily reachable. Maintain sufficient clearance at the rear to unplug the power line from the main power input socket.
- Ensure that the instrument lid can fully open upwards without obstruction.

- The QIAgility must not be installed near generators of static electricity (e.g., ionizers, large-screen televisions).
- The QIAgility should be installed in a laboratory environment that is free of excessive airborne dust.

Note: We recommend that the QIAgility does not share a wall socket with other laboratory equipment, and that the QIAgility is plugged directly into its own wall socket.

Important: The wall socket shall be easily accessible.

Important: Position the QIAgility in a way that allows easy access to the power supply cable at the rear. Meeting these location and position requirements will allow you to immediately disconnect the instrument from the power supply should a need or emergency arise.



Damage to the instrument [C1] Direct sunlight may bleach parts of the instrument and cause damage to plastic parts.
The QIAgility must be located out of direct sunlight.

4.2.2 Power requirements

The QIAgility operates at these power conditions: 100–240 V, 250 VA, 50–60 Hz, 5 A fuse.

Make sure the voltage rating of the QIAgility and laptop are compatible with the AC voltage available at the installation site. Main supply voltage fluctuations are not to exceed \pm 10% of nominal supply voltages.

4.2.3 Grounding requirements

To protect operating personnel, and for correct functioning of the liquid-level sensor, ensure that the QIAgility is correctly grounded (earthed). The instrument is equipped with a 3-conductor AC power cord that, when connected to an appropriate AC power outlet, grounds (earths) the instrument. To preserve this protection feature, do not operate the instrument from an AC power outlet that has no ground (earth) connection.

4.3 Unpacking and installation

4.3.1 Unpacking the QIAgility

Important: For unpacking of the QIAgility, refer the unpacking instructions delivered with the instrument. The unpacking instructions can also be downloaded at <u>www.qiagen.com/goto/QIAgility</u>.

Before unpacking the QIAgility, move the package to the site of installation and check whether the package is damaged. In case of damage, contact the transporter of the package.



Risk of personal injury and material damage [W3]

The QIAgility is too heavy to be lifted by one person. To avoid personal injury or damage to the instrument, do not lift the instrument alone.

- 1. Remove the lid from the QIAgility shipping container.
- 2. Check that the QIAgility is not damaged and that there are no loose parts. If anything is damaged, contact QIAGEN Technical Services.
- 3. Read the packing list to check that you have received all items (see <u>System delivery</u>). If anything is missing, contact QIAGEN Technical Services.
- 4. Keep the package in case you need to transport or ship the QIAgility in the future.
- Carefully place the QIAgility onto the workbench where it will be used. For site requirements, refer to section <u>Installation site</u>. When lifting the QIAgility, slide your fingers under both sides of the instrument and keep your back straight.
- 6. <u>Remove transport lock</u> from pipetting arm.

4.3.1.1 Releasing transport locks

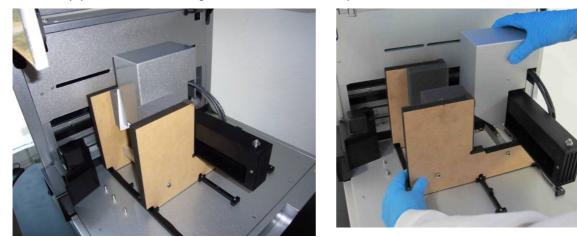
CAUTION Relocating the QIAgility [C7] The most important component of the packaging is the wooden support that immobilizes the Y-arm. This support must be used when the QIAgility is moved.

To release the transport locks:

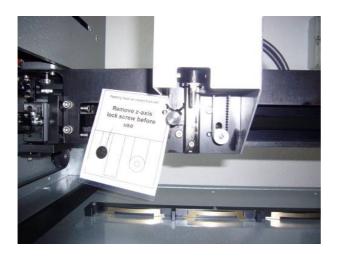
1. Unscrew the two screws and remove the horizontal bar.



2. Move the pipettor head to the right and remove the transport lock from the worktable.



3. Unscrew the Z-axis locking screw. When this screw is removed, the pipettor head will drop.



- 4. Store the transport lock and the Z-axis screw with the rest of the packaging.
- 4.3.2 Installation of AC power cord

Connect the QIAgility to the power outlet as follows:

- 1. Ensure that the power switch of the QIAgility is set to the "Off" position.
- 2. Plug the power cord into the main power input socket.
- 3. Plug the power cord into a grounded power outlet.

Important: The wall socket should be easily accessible.

Important: The QIAgility Setup Manager Software must be installed on the computer before the QIAgility is switched on (see section <u>Software installation</u>).

4.3.3 Connecting the computer to the QIAgility

Before attempting to connect the QIAgility to the laptop, ensure the following conditions have been met:

- The QIAgility has been properly set up and is in its final location (see section Unpacking the QIAgility).
- The laptop is in its final location near the instrument.

Note: Before connecting the QIAgility to the computer for the first time, ensure that the QIAgility Setup Manager Software has been installed (see section <u>Software installation</u>).

Note: Connect the computer to the QIAgility via the USB interface.

To connect via the USB interface:

Connect the USB cable between the USB port on the instrument and the USB port on the computer.



USB cable attached to the accessory panel.

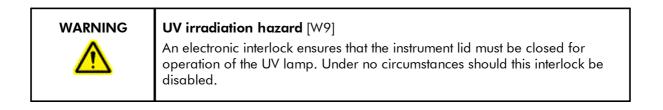
Note: The QIAgility Setup Manager Software recognizes connection to the QIAgility as a USB device even when the instrument is switched off.

4.3.4 UV lamp

The UV plug is integrated within the lid. To connect the UV lamp, proceed as follows:

- 1. Open the lid.
- Connect the delivered UV lamp bulb into the UV plug and turn it by 90°. 2.

Note: Hold the UV lamp bulb at both ends when fixing it into the UV plug. Always wear safety gloves.



Software installation 4.4

The QIAgility Setup Manager Software must be installed before the QIAgility is connected to the laptop for the first time. The software will generally be installed on the laptop by a QIAGEN Field Service Specialist on the day of instrument installation, if the QIAgility was purchased with an installation and startup training. A user with Windows administrator privileges is required to install the QIAgility Setup Manager Software, but such privileges are required for software installation only. If the QIAgility was purchased without an installation and startup training, follow the steps below to install the software:

For computer system requirements, see section Computer.

1. Set up the computer as described in the computer installation instructions provided with the computer.

- 2. Before installing the QIAgility Setup Manager Software, shut down all other software and make sure that the QIAgility is not connected to the computer.
- If a previous version of the software is already installed on the computer, refer to <u>Updating QIAgility</u> <u>Setup Manager Software</u>. Otherwise, follow the instructions in <u>Installing QIAgility Setup Manager</u> <u>Software</u>.

Note: We recommend having no other software installed on the laptop that controls the QIAgility other than the QIAgility Setup Manager Software. In particular, the legacy QIAgility Software must not be installed.

Note: Default settings are recommended where appropriate for all installation steps.

Note: The QIAgility Setup Manager Software version 1.2 is the first version supporting Windows 10 (64-bit).

4.4.1 Installing QIAgility Setup Manager Software

Things to do before starting:

- 1. Ensure that the computer meets the minimum requirements (see <u>Computer</u>).
- Use a Windows account with administrator privileges.
 Note: Windows administrator privileges are required for software installation only.
- 3. Close any programs running on the computer and make sure that the QIAgility is not connected to the computer (i.e., unplug the USB cable from the computer).

To install the QIAgility Setup Manager Software from a Removable Media:

4. Insert the QIAgility Setup Manager Software Removable Media into the computer. The setup wizard automatically opens the QIAgility Setup Manager Software setup window. Proceed to step 7.

Note: Automatic setup is disabled on laptops provided by QIAGEN.

Note: If the setup does not start automatically, double-click the **setup.exe** file in the Removable Media drive folder. Proceed to step 7.

Note: Depending on the Windows configuration, there may be a pop-up message asking if you want to allow the program to install. Allow the program to install.

To install the software from a web download:

QIAgility Setup Manager Software is available for download at <u>www.qiagen.com/goto/QIAgility</u>. Before installing, read the information provided on the web page.

4. Download the installation package .zip file.

Note: If the computer running the QIAgility Setup Manager Software is not connected to a network, download the installation package from another computer to a USB stick. Copy the installation package to the QIAgility Setup Manager Software computer.

- 5. Extract the installation package .zip file.
- 6. Double-click on the **setup.exe** file. Proceed to step 7.

Note: Depending on the Windows configuration, there may be a pop-up message asking you if you want to allow the program to install. Allow the program to install.

- 7. The appropriate version of the Microsoft .NET Framework will be installed during setup if it is not already present. In this case, another dialog opens. Click **Yes** to install the Microsoft .NET Framework. This may take some time. If you click **No**, the QIAgility Setup Manager Software will not be able to operate.
- 8. Click **Next** to start the installation.
- Follow the instructions that appear in the setup wizard.
 Note: Default settings are recommended where appropriate for all installation steps.
- 10. Before the installation is completed, the appropriate version of the device driver will be installed if it is not already present. In this case, the Device Driver Installation Wizard will open. Click **Next** to proceed. Depending on the Windows configuration, a Windows security confirmation message may be displayed to indicate that QIAGEN GmbH is attempting to install drivers. Click **Install** to install the QIAgility driver. After driver installation, click **Finish** in the Device Driver Installation Wizard.

Note: If you do not install the driver software, the QIAgility Setup Manager Software will not be able to operate the QIAgility.

- 11. After installation, click **Exit Setup** in the Removable Media menu, or **Finish** in the wizard.
- 12. Connect the QIAgility to the computer.
- 13. The QIAgility Setup Manager Software can now be started.

Note: If the computer is connected to a network, network policy settings may prevent you from completing this procedure. For more information, contact your local IT support.

Note: In case other messages are displayed, refer to the troubleshooting section System setup.

4.4.2 Install plug-ins

The QIAgility Setup Manager Software setup automatically installs all currently available plug-ins. To view the list of available plug-ins, click **Help** in the menu bar of the QIAgility Setup Manager Software, and then **About**. In the dialog that is shown click the tab **Loaded plug-ins**. Click **Close** to close the dialog. For further details about plug-ins, refer to <u>Plug-in concept</u>.

New plug-in versions will be made available over time. Please check the QIAgility website at **www.giagen.com/goto/QIAgility** for additional information.

Update the QIAgility Setup Manager Software to upgrade plug-ins to a newer version. Follow the instructions in <u>Updating QIAgility Setup Manager Software</u>.

After the QIAgility Setup Manager Software has been upgraded, the new versions of plug-in wizards will be available in the **Home** environment, having replaced the previous versions.

4.4.3 Uninstalling QIAgility Setup Manager Software

Important: During uninstallation of the QIAgility Setup Manager Software, all experiments, templates, reports, saved output files, etc. that have been saved in any subfolder of **C:** **Users\Public\QIAGEN\QIAgility** will be removed.

We recommend moving all user files to another location outside C:\Users\Public\QIAGEN\QIAgility before uninstalling the software.

Note: It is not possible to uninstall an individual plug-in.

To automatically uninstall the QIAgility Setup Manager Software together with all its plug-ins, follow these steps:

- 1. In the Windows Start menu, select: All Programs / QIAGEN / QIAgility / Uninstall QIAgility Setup Manager Software
- 2. Follow the instructions of the installer to uninstall the software.

To manually uninstall the QIAgility Setup Manager Software or any plug-ins or wizards, follow these steps:

- 1. Open the **Control Panel** by clicking on the **Windows Start** menu and selecting **Control Panel**.
- 2. Depending on your layout settings, either click **Programs and Features** or **Programs** / **Uninstall Programs**.
- Select QIAgility Setup Manager Software to be uninstalled and click Uninstall/Change.
 Note: Depending on the Windows configuration, there may be a pop-up message asking if you want to allow the program to install. Allow the program to install.
- 4. Follow the instructions of the installer to uninstall.

4.4.4 Updating QIAgility Setup Manager Software

If a previous version of the QIAgility Setup Manager Software is already installed, it can be updated using the software package that is available at <u>www.giagen.com/goto/QIAgility</u>. Before updating, read the information provided on the web page. Experiments and user settings will be preserved. Customized labware will be deleted and must be installed again after the software is updated.

Things to do before starting:

- 1. Ensure that the computer meets the minimum requirements (see <u>Computer</u>).
- Use a Windows account with administrator privileges.
 Note: Windows administrator privileges are required for software installation only.
- 3. Close any programs running on the computer and make sure that the QIAgility is not connected to the computer (i.e., unplug the USB cable from the computer).

To install the software from a web download:

QIAgility Setup Manager Software is available for download at <u>www.qiagen.com/goto/QIAgility</u>. Before installing, read the information provided on the web page.

4. Download the installation package .zip file.

Note: If the computer running the QIAgility Setup Manager Software is not connected to a network, download the installation package from another computer to a USB stick. Copy the installation package to the QIAgility Setup Manager Software computer.

- 5. Extract the installation package .zip file.
- 6. Double-click on the **setup.exe** file. Proceed to step 7.

Note: Depending on the Windows configuration, there may be a pop-up message asking you if you want to allow the program to install. Allow the program to install.

- 7. The appropriate version of the Microsoft .NET Framework will be installed during setup if it is not already present. In this case, another dialog opens. Click **Yes** to install the Microsoft .NET Framework. This may take some time. If you click **No**, the QIAgility Setup Manager Software will not be able to operate.
- 8. Click **Next** to start the installation.
- 9. If you are updating from QIAgility Setup Manager Software version 1.0 or 1.1, a message will appear to inform you that a wrong SiLabs USB driver version has been detected that will be replaced during installation by the new driver version. This new driver version is required to operate the QIAgility Setup Manager Software. Click **OK** to proceed with the installation.
- 10. Follow the instructions that appear in the setup wizard.

All new software components will be installed. Existing plug-ins will be updated and newly available plug-ins will be added automatically. User profiles, labware settings and calibrations created before the update will be available after the update. Experiments, reports and sample files will also be available.

Note: Default settings are recommended where appropriate for all installation steps.

Note: A message will appear to inform you that some files that need to be updated by the setup are still in use by the QIAgility Core Engine Service. The QIAgility Core Engine Service is a service of the QIAgility Setup Manager Software that always runs on the computer. Select the option **Close the applications and attempt to restart them.** The setup will stop and restart the QIAgility Core Engine Service during installation. If you select the option **Do not close applications. A reboot will be required**, the installation will continue, but it will only be completed after the computer is rebooted.

Note: If you are updating from version 1.0, a message may appear to inform you that during uninstallation of the QIAgility Setup Manager Software, all experiments, templates, reports, saved output files, etc. that have been saved in any subfolders of **C:\Users\Public\QIAGEN\QIAgility** will be removed. Click **OK** to acknowledge the message. Despite the message, these items will still be available after the setup.

11. If the message appeared at the beginning of installation to inform you that the SiLabs USB driver will be replaced by a new driver version, the Device Driver Installation Wizard will open. Click **Next** to proceed. A security confirmation message is shown that QIAGEN GmbH drivers attempt to be installed. Click Install to install the driver. After driver installation click Finish in the Device driver Installation Wizard.

Note: If you do not install the new driver version, the QIAgility Setup Manager Software might not be able to operate the QIAgility properly.

12. After installation, click **Finish** in the wizard.

Note: If you selected the option **Do not close applications. A reboot will be required.** during installation, you will need to restart the computer before the QIAgility Setup Manager Software is ready to operate.

- 13. Connect the QIAgility to the computer.
- 14. The QIAgility Setup Manager Software can now be started.
- 15. Use one of the previously created user IDs for login.

Note: If the computer is connected to a network, network policy settings may prevent you from completing this procedure. For more information, contact your local IT support.

Note: In case of other messages displayed, refer to the troubleshooting section System setup.

Note: Experiments from a previous plug-in version can be opened with a newer plug-in version.

4.4.5 Upgrading from legacy QIAgility Software

Before upgrading from old versions of the QIAgility Software (version 4.X) to the QIAgility Setup Manager Software, you must first uninstall the legacy QIAgility Software. It is not possible to have both the legacy version of the QIAgility Software and the QIAgility Setup Manager Software installed on the same computer. Data migration of protocols from old to new software is not possible and not provided.

After the successful uninstallation, install the QIAgility Setup Manager Software as described in section Installing QIAgility Setup Manager Software.

Getting started

5 Getting started

This chapter provides information about basic configuration that needs to be completed before the QIAgility can be used for runs.

Some of the tasks need to be repeated occasionally, such as instrument calibration after servicing the QIAgility, or labware managament and labware calibration when changing labware or supplier.

5.1 Start the software

The QIAgility Setup Manager Software can always be started without having the instrument connected or switched on, for example, to prepare experiments for the next day. However, in this case, certain areas of the software that require the instrument to be switched on will not be available, such as worktable and run step for experiments, calibration or system checks.

After starting the computer on which the QIAgility Setup Manager Software will run, wait two minutes before opening the application.

You can switch on the QIAgility later after the software has already started. The QIAgility automatically connects to the QIAgility Setup Manager Software. This may take around 5 to 10 seconds.

To start the QIAgility:

1. Double-click on the **SetupM** desktop icon, or open the **Windows Start menu** and select: **All Programs / QIAGEN / QIAgility / QIAgility Setup Manager Software**.



- The software starts and displays the login dialog.
 Note: Authentication is always required.
- If you are starting the software for the first time after installation, log in by entering the user name admin into the User ID field and entering the password admin into the Password field. Click OK. Otherwise, log in with your user ID and password.
- 4. The software opens in the **Home** environment.
- 5. If you are starting the software for the first time, continue with <u>Customizing settings</u>.

Note: The QIAgility will not initialize when the software is started; however, it will initialize when someone attempts to use the instrument.

Note: If you go to the **Customer** settings for the first time, a "**VER**" will show on the icon because the instrument is not fully calibrated. Before you can start a run, <u>instrument calibration</u> needs to be performed or completed.

The QIAgility User Manual (this publication) can be accessed at any time from within the software by pressing the **F1** key, or by clicking the help button ² located above the main toolbar.

5.2 Customizing settings

The **Global Settings** tab of the **Configuration** screen enables users to define general settings such as default directories, e-mail server details (for e-mail notifications), printing and password options, and more.

File Edit View Help	~1	•
QIAGEN Home	Experiment Service Configuration	Instrument
Settings Labware	Manager Calibration User Manager	
Gene Expression User Settings	Maintenance E-mail notifications	
Pathogen Detection User Settings Generic PCR User Settings Generic PCR Liquid Classes Global Settings	UV lamp cleaning duration 15 minudes Security Password policy Deactivate user account Never Never Never Never Arter failed login attempts Support package Standard export path C.VUsers/Public/QLAGENIOLAgility/Support Barcode printing Barcode type Barcode type Barcode type Barcode type Barcode type No printer Select.	
	Save Cancel	
	July 22, 2019 admir	n admin 🛛 🔿 🖡

Maintenance options

Enter the time duration for the UV lamp cleaning procedure in the **Maintenance** section of the window. The UV lamp cleaning procedure can be initiated from the **Cleaning Procedure** tab of the **Service** environment. QIAGEN recommends a duration of 15 minutes.

Maintenance

UV lamp cleaning duration 15 minutes	

Security options

Specify the security and authentication settings in the **Security** section of the window.

Security

Password policy Standard policy (8-40 arbitrary characters Password expiration (in days)	Deactivate user account Never After failed login attempts
--	--

Option Password policy	Description There are two different options for the password policy:
	 Standard policy (8–40 arbitrary characters): select this option if authentication is required.
	 No policy (0–40 characters): select this option if no authentication is required. This policy allows you to use short passwords or even to use an empty password.
Deactivate user account	Specify whether the user account should be deactivated after failed login attempts:
	• Never: select this option if an account should never be deactivated.
	 After failed login: select this option if a user account should be deactivated after a certain number of failed login attempts. Enter the number in the field below.
Password expiration (in days)	Specify the number of days after which user passwords expire. Users will be forced to change their passwords after the set amount of time.

Support package

Set the standard export path for the support package by clicking the **Browse** button. This path will be used as the initial path when a support package is created from the **Support package** tab of the **Service** environment.

Support package

Standard export path	
C:\Users\Public\QIAGEN\QIAgility\Support	Browse

Barcode printing

Use this option if you intend to print a barcode for output labware. Select the barcode type that fits your needs. Select the barcode printer by clicking **Select**. A dialog offers a list of printers that are available from the computer, i.e., all printers which have been configured in Windows. Select **No printer** if you do not want to use this option. Barcode printing is then not available.

Barcode printing

Barcode type	Barcode printer	
Code 128 🔹	No printer	Select

E-mail notifications

If you would like to be informed via e-mail about certain instrument events, such as errors or finish of a run, activate the **Enable notifications** option. The actual notification options can be found in the **User Settings** of the individual plug-ins.

The general network settings for the mail server must to be entered in order for the e-mail notification to function. In case you do not know these details, contact your network administrator.

E-mail notifications

Enable notifications	
SMTP server address	Port 25
SMTP user	2.0
SMTP password	Send test notification
Sender Address	
Protocol	
None	

Option	Description
SMTP server address	Specify the address of the SMTP server.
SMTP user	Specify the user for authentication on the SMTP server.
SMTP password	Enter the users password for authentication on the SMTP server.
Sender address	Specify the address of the sender of notifications.
	Note: Some SMTP servers require that the sender address is related to the SMTP user and password.
Protocol	Specify whether the SMTP server requires encryption (SSL or TLS) or not.

Use the **Send test notification** button to test if an e-mail is sent to the e-mail address that is configured for the currently logged in QIAgility Setup Manager Software user.

5.2.1 User settings for Gene Expression experiments

The **Configuration** environment provides settings for the Gene Expression Plug-in. These settings are specific for a user.

Report / Export

Option Display report after	Description Check this option if the report shall open automatically after it has been created.
saving	Note: The report will always be saved first and then opened.
	Note: To view reports, a PDF reader must be installed on the computer.
Report type	The Gene Expression Plug-in provides a Basic report and an Advanced report. For details, refer to section <u>Report</u> .
Report format	Select the paper format: A4 or US Letter.
Report path	Select Save to origin experiment directory if you want to save the report side by side with the experiment file.
	Select Save to standard directory if you want to save all reports together in a defined directory. See below for directory configuration.
	Note: This selection serves as a starting point for the file dialog that opens when you create a report.
Sample output file path	Select Save to origin experiment directory if you want to save the output file side by side with the experiment file.
	Select Save to standard directory if you want to save all output files together in a defined directory. See below for directory configuration.
	Note: This selection serves as a starting point for the file dialog that opens, when you save the output file.

Standard directories

To change a standard directory, press the **Browse** button in the appropriate row.

Subject	Description
Experiment	The selected directory serves as a starting point for the file dialog when you save an experiment.
	Note: When opening an experiment from Home environment with the Open other experiment button, for the first time after software start the starting point is C: \.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Experiments.
Template	The selected directory serves as a starting point for the file dialog when creating an experiment from a template from Home environment with the Create from other template button.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Templates.
Reports	The selected directory serves as a starting point for the file dialog that opens when you create a report, if the Save to standard directory option has been selected for the report path (see above).
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Reports.
Sample input files	The selected directory serves as a starting point for the file dialog that opens when you load a sample input file or an output file in the Labware Selection step.
	Also, when you scan the input or output labware barcode, the software will search in this directory for a file to load.
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.
Sample output files	The selected directory serves as a starting point for the file dialog that opens when you save an output file after the run.
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.

Notification

Two kinds of notifications can be configured: a sound notification or a notification via e-mail, for various kinds of events.

Notification

Send notification on:	e-Mail	Sound	
Errors		✓	Error
Warnings			Warning V
User interaction required		✓	Information 🛛 🔻
Run completed		✓	Information 🛛 🔻

A sound will be played when the run is complete, or during the run to indicate errors and situations that require user interaction (e.g., refill tips).

Note: E-mail notification options are only enabled if the following have been performed by the software administrator in the **Global Settings** (see chapter <u>Customizing settings</u>):

- The e-mail notifications have been enabled and configured.
- An e-mail address for the user account has been configured.

Option	Description
E-mail notification	To enable e-mail notification for a certain event, check the corresponding checkbox in the E-mail column.
Sound notification	To enable sound notification for a certain event, check the corresponding checkbox in the Sound column. If the checkbox is checked, a drop-down menu becomes available, allowing you to select different sounds.

HEPA filter

Option	Description
Switch on HEPA filter during run	Select this option if you want to use the HEPA filter for runs. The HEPA filter will be used for every run as long as this option is selected.
	Note: Consider that all options are user specific. If another user also wants to use the HEPA filter during runs, that use also needs to select this option.
Switch off after run	Enabled only if the option Switch on HEPA filter during run has been selected.
	Select Immediately to switch off the HEPA just after the last run action of the QIAgility.
	Select When the lid is opened for the first time if you want to continue the HEPA filter until you open the lid for the first time after the run.

5.2.2 User settings for Pathogen Detection experiments

The **Configuration** environment provides settings for the Pathogen Detection Plug-in. These settings are specific for a user.

Report / Export

Option	Description			
Display report after	Check this option if the report shall open automatically after it has been created.			
saving	Note: The report will always be saved first and then opened.			
	Note: To view reports, a PDF reader must be installed on the computer.			
Report type	The Pathogen Detection Plug-in provides a Basic report and an Advanced report. For details, refer to section <u>Report</u> .			
Report format	Select the paper format: A4 or US Letter.			
Report path	Select Save to origin experiment directory if you want to save the report side by side with the experiment file.			
	Select Save to standard directory if you want to save all reports together in a defined directory. See below for directory configuration.			
	Note: This selection serves as a starting point for the file dialog that opens when you create a report.			
Sample output file path	Select Save to origin experiment directory if you want to save the output file side by side with the experiment file.			
	Select Save to standard directory if you want to save all output files together in a defined directory. See below for directory configuration.			
	Note: This selection serves as a starting point for the file dialog that opens, when you save the output file.			

Standard directories

To change a standard directory, press the **Browse** button in the appropriate row.

Subject	Description				
Experiments	The selected directory serves as a starting point for the file dialog when you save an experiment.				
	Note: When opening an experiment from Home environment with the Open other experiment button, for the first time after software start the starting point is C:\.				
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Experiments.				
Templates	The selected directory serves as a starting point for the file dialog when creating an experiment from a template from Home environment with the Create from other template button.				
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Templates.				
Reports	The selected directory serves as a starting point for the file dialog that opens when you create a report, if the Save to standard directory option has been selected for the report path (see above).				
	For experiments created with this plug-in.				
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Reports.				
Sample input files	The selected directory serves as a starting point for the file dialog that opens when you load a sample input file or an output file in the Labware Selection step.				
	Also, when you scan the input or output labware barcode, the software will search in this directory for a file to load.				
	For experiments created with this plug-in.				
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.				
Sample output files	The selected directory serves as a starting point for the file dialog that opens when you save an output file after the run.				
	For experiments created with this plug-in.				
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.				

Notification

Two kinds of notifications can be configured: a sound notification or a notification via e-mail, for various kinds of events.

Notification

Send notification on:	e-Mail	Sound	
Errors		✓	Error
Warnings			Warning V
User interaction required		✓	Information 🛛 🔻
Run completed		✓	Information 🛛 🔻

A sound will be played when the run is complete, or during the run to indicate errors and situations that require user interaction (e.g., refill tips).

Note: E-mail notification options are only enabled if the following have been performed by the software administrator in the **Global Settings** (see chapter <u>Customizing settings</u>):

- The e-mail notifications have been enabled and configured.
- An e-mail address for the user account has been configured.

Option	Description
E-mail notification	To enable e-mail notification for a certain event, check the corresponding checkbox in the E-mail column.
Sound notification	To enable sound notification for a certain event, check the corresponding checkbox in the Sound column. If the checkbox is checked, a drop-down menu becomes available, allowing you to select different sounds.

HEPA filter

Option	Description
Switch on HEPA filter during run	Select this option if you want to use the HEPA filter for runs. The HEPA filter will be used for every run as long as this option is selected.
	Note: Consider that all options are user specific. If another user also wants to use the HEPA filter during runs, that user also needs to select this option.
Switch off after run	Enabled only if the option Switch on HEPA filter during run has been selected.
	Select Immediately to switch off the HEPA just after the last run action of the QIAgility.
	Select When the lid is opened for the first time if you want to continue the HEPA filter until you open the lid for the first time after the run.

5.2.3 User settings for Sample Pooling experiments

The **Configuration** environment provides settings for the Sample Pooling Plug-in. These settings are specific for a user.

Report / Export

Option	Description				
Display report after saving	Check this option if the report shall open automatically after it has been created.				
	Note: The report will always be saved first and then opened. Note: To view reports, a PDF reader must be installed on the computer.				
De la cut fe une et					
Report format	Select the paper format: A4 or US Letter.				
Report path	Select Save to origin experiment directory if you want to save the report side by side with the experiment file.				
	Select Save to standard directory if you want to save all reports together in a defined directory. See below for directory configuration.				
	Note: This selection serves as a starting point for the file dialog that opens when you create a report.				
Sample output file path	Select Save to origin experiment directory if you want to save the output file side by side with the experiment file.				
	Select Save to standard directory if you want to save all output files together in a defined directory. See below for directory configuration.				
	Note: This selection serves as a starting point for the file dialog that opens, when you save the output file.				
Select xslt file	If you need the output file in another format other than the QDEF format, an .xslt file can be selected that defines a transformation from the QDEF format to another format. If an .xslt file has been selected, every time you save an output file from the Run screen (see <u>Run completion</u>), the transformation will be started automatically based on the created QDEF file using the selected .xslt file. As a result, two output files will be created: one in QDEF format, and one in the format defined by the .xslt file. The file created by the .xslt will have the same file name as the QDEF file, but with an extension .csv . It will be saved side by side with the QDEF file.				
	As an example, the Sample Pooling Plug-in provides one .xslt file that transforms the output file into a .csv file:				
	C:\Program Files\QIAGEN\QIAgility\QIAgilityData\KitConfiguration\PSP\PSP_SampleInformati onFile.xslt				
	Note: If any other file format needs to be generated, please contact an Application Service Specialist.				

Default pipetting order

To define the default pipetting order used for sample input batches (first line) and pool batches (second line).

Default pipetting order			
Type of Labware	Rectangular	Not rectangular	
Sample Batches	Row by Row 🛛	Ascending	
Pool Batches	Row by Row 🛛	Ascending	

For regular rectangular labware, e.g., 96-well plates, you can select between **Row by Row** and **Column by Column** order. For all other labware, select between **Ascending** and **Descending** pipetting order. For sample input batches, the selected pipetting order will be used for all sample input batches. For pool batches, you can select the pipetting order that is most often used. During an experiment setup, define the pipetting order for the pool batch individually in the <u>Sample-pool mapping dialog</u>.

Standard directories

To change a standard directory, press the **Browse** button in the appropriate row.

Subject	Description
Experiments	The selected directory serves as a starting point for the file dialog when you save an experiment.
	Note: When opening an experiment from Home environment with the Open other experiment button, for the first time after software start the starting point is C:\.
Templates	The selected directory serves as a starting point for the file dialog when creating an experiment from a template from Home environment with the Create from other template button.
Reports	The selected directory serves as a starting point for the file dialog that opens when you create a report, if the Save to standard directory option has been selected for the report path (see above).
	For experiments created with this plug-in.
Sample batch input files	The selected directory serves as a starting point for the file dialog that opens when you load a sample input file in the Labware Selection step.
	Also, when you scan the input labware barcode, the software will search in this directory for a file to load.
	For experiments created with this plug-in.
Pool batch output files	The selected directory serves as a starting point for the file dialog that opens when you save a batch file after the run.
	For experiments created with this plug-in.
Unused samples	The selected directory serves as a starting point for the file dialog that opens when you save an unused samples file after the run.
	For experiments created with this plug-in.

Notification

Two kinds of notifications can be configured: a sound notification or a notification via e-mail, for various kinds of events.

Notification

Send notification on:	e-Mail	Sound	
Errors		✓	Error
Warnings			Warning
User interaction required		✓	Information
Run completed		✓	Information

A sound will be played when the run is complete, or during the run to indicate errors and situations that require user interaction (e.g., refill tips).

Note: E-mail notification options are only enabled if the following have been performed by the software administrator in the **Global Settings** (see chapter <u>Customizing settings</u>):

- The e-mail notifications have been enabled and configured.
- An e-mail address for the user account has been configured.

Option	Description
E-mail notification	To enable e-mail notification for a certain event, check the corresponding checkbox in the E-mail column.
Sound notification	To enable sound notification for a certain event, check the corresponding checkbox in the Sound column. If the checkbox is checked, a drop-down menu becomes available, allowing you to select different sounds.

HEPA filter

Option	Description
Switch on HEPA filter during run	Select this option if you want to use the HEPA filter for runs. The HEPA filter will be used for every run as long as this option is selected.
	Note: Consider that all options are user specific. If another user also wants to use the HEPA filter during runs, that user also needs to select this option.
Switch off after run	Enabled only if the option Switch on HEPA filter during run has been selected.
	Select Immediately to switch off the HEPA just after the last run action of the QIAgility.
	Select When the lid is opened for the first time if you want to continue the HEPA filter until you open the lid for the first time after the run.

5.2.4 User settings for Generic PCR experiments

The **Configuration** environment provides settings for the Generic PCR Plug-in. These settings are specific for a user.

Report / Export

Option	Description
Display report after	Check this option if the report should open automatically after creation.
saving	Note: The report will always be saved first and then opened.
	Note: To view reports, a PDF reader must be installed on the computer.
Report type	The Generic PCR Plug-in provides a Basic report and an Advanced report. For details, refer to section <u>Report</u> .
Report format	Select the paper format: A4 or US Letter.
Report path	Select Save to origin experiment directory if you want to save the report side by side with the experiment file.
	Select Save to standard directory if you want to save all reports together in a defined directory. See below for directory configuration.
	Note: This selection serves as a starting point for the file dialog that opens when you create a report.
Sample output file path	Select Save to origin experiment directory if you want to save the output file side by side with the experiment file.
	Select Save to standard directory if you want to save all output files together in a defined directory. See below for directory configuration.
	Note: This selection serves as a starting point for the file dialog that opens, when you save the output file.
Select output file converter directory	The selected directory contains the files that define conversion of the sample output file from the QDEF format to another format that is required, e.g. by a PCR cycler software.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\OutputConverters.
Normalization	

Option	Description
Normalization target	Select one of Sample mass or Sample concentration . Depending on the selection, normalization calculation will be based on mass or concentration. When normalizing to a specific concentration the sample will be diluted to the chosen target value and afterwards transferred to the output labware.
	Changing the selection will take effect for new experiments, only.
	Note: The default normalization target is Sample mass.

Sample validation

Option	Description
Enable sample loading validation	Select this option if you want to validate loading of sample tubes using a hand- held scanner during worktable loading. If checked, the Validate sample loading button will be available in the Worktable step.

Standard directories

To change a standard directory, press the **Browse** button in the appropriate row.

Subject	Description
Experiments	The selected directory serves as a starting point for the file dialog when you save an experiment.
	Note: When opening an experiment from Home environment with the Open other experiment button, for the first time after software start, the starting point is C:\.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Experiments.
Templates	The selected directory serves as a starting point for the file dialog when creating an experiment from a template from Home environment with the Create from other template button.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Templates.
Reports	The selected directory serves as a starting point for the file dialog that opens when you create a report, if the Save to standard directory option has been selected for the report path (see above).
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\Reports.
Sample input files	The selected directory serves as a starting point for the file dialog that opens when you load a sample input file or an output file in the Labware Selection step.
	Also, when you scan the input or output labware barcode, the software will search in this directory for a file to load.
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.
Sample output files	The selected directory serves as a starting point for the file dialog that opens when you save an output file or a dilution file after the run.
	For experiments created with this plug-in.
	Initially, the path is set to C:\Users\Public\QIAGEN\QIAgility\SampleFiles.

Notification

Two kinds of notifications can be configured: a sound notification or a notification via e-mail, for various kinds of events.

Notification

Send notification on:	e-Mail	Sound	
Errors		✓	Error
Warnings			Warning
User interaction required		✓	Information 🔹
Run completed		✓	Information 🛛

A sound will be played when the run is complete, or during the run to indicate errors and situations that require user interaction (e.g., refill tips).

Note: E-mail notification options are only enabled if the following have been performed by the software administrator in the **Global Settings** (see chapter <u>Customizing settings</u>):

- The e-mail notifications have been enabled and configured.
- An e-mail address for the user account has been configured.

Option	Description
E-mail notification	To enable e-mail notification for a certain event, check the corresponding checkbox in the E-mail column.
Sound notification	To enable sound notification for a certain event, check the corresponding checkbox in the Sound column. If the checkbox is checked, a drop-down menu becomes available, allowing you to select different sounds.

HEPA filter

Option	Description
Switch on HEPA filter during run	Select this option if you want to use the HEPA filter for runs. The HEPA filter will be used for every run, as long as this option is selected.
	Note: Consider that all options are user specific. If another user also wants to use the HEPA filter during runs, that user also needs to select this option.
Switch off after run	Enabled only if the option Switch on HEPA filter during run has been selected.
	Select Immediately to switch off the HEPA just after the last run action of the QIAgility.
	Select When the lid is opened for the first time if you want to continue the HEPA filter until you open the lid for the first time after the run.

5.2.5 Generic PCR Liquid Classes

Liquid classes define the pipetting and tip handling for respective tip transfers. Liquid classes are defined by QIAGEN.

You can view information about the liquid classes in the **Configuration** environment, in the **Generic PCR Liquid Classes** tab, or you can refer to the table below.

Name	Version	Description
Aqueous liquid to reaction (master) mic	1.0.0	Example: MgCl ₂ solution - use for all reagents with low viscosity (main solvent water) added to a final reaction (master) mix
Control or Std. to output or for dilution	1.0.0	Example: Positive/Negative control or Standards - use for all assay controls or standards (incl. stock) transferred to final assay well or for creation of a standard dilution series.
		Note: In case NTC is used from the water provided in the kit, without using a separate input tube for NTC and the water, the liquid class "Water to reaction (master) mix" is recommended
Diluent for dilution	1.0.0	Example: Diluent - use for all water-based diluents for preparation of standard series or for normalization of

		samples/templates, or any kind of dilution
Diluent to dilution	1.0.0	Example: Diluent - use for all water-based diluents which are pipetted to the dilution plate during normalization. This liquid class is specifically predefined within the advanced pipetting paramater of the Normalization sceen, when performing normalization requiring dilution labware
Enzyme to reaction (master) mix	1.0.0	Example: DNA polymerase - use for all pure enzyme reagents (main solvent glycerol (or similar)) added to the reaction (master) mix
Mix of reaction (master) mix (final)	1.0.0	Example: Reaction mix created by QIAgility - use to optimize mixing of the final reaction (master) mix.
		This liquid class is automatically selected within the advanced pipetting parameter of the Mixes screen.
		In case the data reveal a non- homogenous final reaction (master) mix, the mixing repetitions can be adjusted (e.g. might be required for reaction (master) mixes requiring preparation in a 5 ml tube).
Primer-Probe or IC to reaction (master) mix	1.0.0	Example: Primer/Probe or internal control - use for all primer/probes or internal controls with low viscosity (main solvent water) added to the final reaction (master) mix.
Reaction (master) mix (final) to output	1.0.0	Example: Final reaction (master) mix - use for distribution of final reaction (master) mix to final assay wells.
		This liquid class is automatically selected in the Mixes screen and has been developed for reaction

		(master) mixes within a standard viscosity range.
Sample for dilution	1.0.0	Example: Sample/Template - use for transfer of samples/templates to dilution plate during normalization.
		This liquid class is automatically selected within the advance pipetting parameter of the Normalization screen, if normalization requires dilution labware.
		In case data reveal a non- homogenous mixing of the sample/templates and dilution buffer, the mixing repetitions and/or maximum volume for the dilution well can be adjusted.
Sample to output	1.0.0	Example: Sample/Template - use for transfer of samples/templates to the final assay well.
		This liquid class is specifically predefined in the Sample/Labware screen and has been developed for water-based samples/templates.
		In case data reveal a non- homogenous mixing of sample/templates and final reaction (master) mix, the mixing repetitions can be adjusted within the custom pipetting parameters.
Sample to output include premix	1.0.0	Example : Sample/Template – use for transfer of samples/templates to final assay well.
		This liquid class is specifically predefined in Sample/Labware screen and has been developed for water-based samples/templates.
		In case data reveal a non- homogenous mixing of

		sample/templates and final reaction (master) mix, the mixing repetitions (pre and post) can be adjusted within the custom pipetting parameters.
Viscous liquid to reaction (master) mix	1.0.0	Example: 10x buffer - use for all viscous reagents containing glycerol or similar components added to the final reaction (master) mix.
Water to reaction (master) mix	1.0.0	Example: Nuclease-free water - use to add water to the final reaction (master) mix.
		Note: This liquid class is recommended to be used for NTC, if NTC shall be served from the same water tube as used for the final reaction (master) mix preparation.
		In case of any dilutions (normalization or standard series), it is recommended to define a separate dilution liquid and assign the respective "Diluent to dilution" liquid class.

5.3 Managing users

When starting the QIAgility Setup Manager Software for the first time, we recommend that you <u>change the</u> <u>password</u> from the default **admin**, and create user profiles to suit your needs.

Users assigned the administrator role are able to add new user profiles or activate, deactivate and modify existing user profiles. User profiles can be deleted, if necessary.

Manage users in the User Manager tab of the Configuration environment.

- 1. Click **Configuration** in the main toolbar.
- 2. Click the User Manager tab.

3. The User Manager screen is displayed.

	QIAgility Setup Manag							– 🗆 🗙
	Edit View Help 🜌							6
QI	CACCEN Home Experiment Service Configuration							
-		are manager	Calibration User					
	egistered users			F	1 4 M	C 11-2	Deler	0-41
				First Name	Last Name	E-Mail	Roles	Actions
	admin		dc-b839-9409e04a5bd2	admin	admin	admin@example.com	Administrator	
	janes	ddb144b2-06b6-447	78-919f-e4762d8d0a99	Jane	Doe	jane.doe@example.com	Operator	
~	johnsmith	32c2c281-4c05-449	9d-a059-a5c65cc7a21a	John	Smith	john.smith@example.com	Operator	

Registered users table in the User Manager

modi

Creating a user

Note: To configure the QIAgility, a user profile with administrator role is required. To operate the QIAgility, the user role operator is sufficient. The initially provided user **admin** has the user role administrator and is sufficient to configure and operate the QIAgility. For details refer to the <u>user roles concept</u>.

Note: If an audit trail is required, create individual user profiles with user role operator for each person working with the QIAgility. Additionally, ensure that the standard password policy has been configured in the **Global Settings**. We recommend to create two user profiles with role administrator.

1. Click the **New user** button on the bottom right.

2. The **Add user** dialog is shown:

Add user	
First name John Last name Smith E-mail	Roles Administrator Operator
User ID John1 Password	
Confirm password	
	OK Cancel

3. Enter the first name, last name, e-mail and an user ID in the corresponding fields. **Note:** E-mail is mandatory for administrators, but optional for operators.

Note: The following restrictions apply to the User ID and name fields:

Field	Restriction				
User ID	Must be unique for each user				
	May not be empty				
	May not contain more than 40 characters				
	Numerical and non-numerical characters are allowed				
	Must contain at least one non-numerical character				
	Spaces are not allowed (in general, white spaces are not allowed)				
	Once the user profile has been created, the field can no longer be changed				
First/Last	May not contain more than 50 characters				
name	Numerical and non-numerical characters are allowed				
	May not be empty				

- 4. Enter a password in the **Password** field, and enter it again in the **Confirm password** field. **Note:** As long as the entered password does not meet the configured <u>password policy</u>, the field will remain yellow and a hint will be shown in a tooltip. The **Confirm password field** will remain yellow if it differs from the **Password** field.
- The Activate user check box (Activate user) is checked by default. This means that the created user profile may be used for the next login.
 Note: To prepare a user profile for later use, create a deactivated user profile by removing the check from this check box. To activate it later, follow the steps below for activating/deactivating a user.
- Activate the check box in the **Roles** table that should be assigned to the user. Note: At least one role must be assigned.
- 7. Click **OK.**
- 8. The Add user dialog closes and the new user is added to the Registered users table.

Modifying a user

The data, password and roles of a user can be modified using the **Edit user** icon in the **Actions** row of the **Registered users** table.

Re	egistered users							
	User ID 🔺	UUID	First Name	Last Name	E-Mail	Roles	Actions	
~	admin	7a5881b4-a74d-4edc-b839-9409e04a5bd2	admin	admin	admin@example.com	Administrator		
~	janes	ddb144b2-06b6-4478-919f-e4762d8d0a99	Jane	Doe	jane.doe@example.com	Operator	· 🖉 🗙	
~	johnsmith	32c2c281-4c05-449d-a059-a5c65cc7a21a	John	Smith	john.smith@example.com	Operator	0 ×	

- 1. Open the **User Management** screen by clicking **Configuration** in the main toolbar and then clicking the **User Manager** tab.
- 2. Click the **Edit user** icon (2) in the **Actions** row for the user you want to change.

3. The Edit User dialog is opened:

C Edit User	
First name admin Last name admin E-mail admin@example.com User ID admin	Roles Administrator Operator
Password Confirm password Activate user	OK Cancel

4. Modify the user data as needed.

Note: The User ID can no longer be modified.

Note: The password fields show 8 bullets and not the actual password. If you click in the password field, both fields will become empty and you will need to enter and confirm a new password that meets the configured password policy.

In case you clicked in the password field and do not want to change the password, click **Cancel**.

- 5. Click **OK**.
- 6. The changes to the user's data are saved.

Note: If you change your own user account, you will need to log out and log back in again for the changes to take effect.

Deleting a user

This will permanently delete a user. As an alternative to the permanent deletion, you can temporarily deactivate a user.

1. Open the **User Management** screen by clicking **Configuration** in the main toolbar and then clicking the **User Manager** tab.

2. Click the **Delete user** icon in the **Actions** row for the user you want to delete.



3. The following confirmation dialog is opened:

Confirm:	ation	
Are you sure you w profile permanently		er 🔺
	Ok	▼ Cancel

4. Click **OK** to permanently delete the user.

Activating/deactivating a user

A user profile can temporarily be deactivated and be activated later.

- 1. Open the **User Management** screen by clicking **Configuration** in the main toolbar and then clicking the **User Manager** tab.
- 2. Click the **Edit user** icon (2) in the **Actions** row for the user you want to change.
- 3. The **Edit User** dialog is opened.
- 4. Use the **Activate user** check box (**Activate user**) to activate or deactivate a user:

To activate a user:	Add a check to the check box	~
To deactivate a user:	Remove the check from the check box	

- 5. Click OK.
- 6. The user will be activated or deactivated according to your changes.

There is an option on the **User Manager** screen to show only activated user profiles. Add or remove the check from the check box to activate or deactivate the option, respectively:

Show only activated user profiles

Note: If you deactivate a user profile while this option is activated, the user profile will not be listed in the **Registered users** table. To show the deactivated user, you must first remove the check from this box to deactivate this option.

5.3.1 Changing password

User passwords may be changed by following the steps below. Each user may change his or her own password.

- 1. From the File menu select Change password.
- 2. The Change password dialogs opens.

Ø	Change	passw	ord.		
Old password	1				
••••					
New passwor	d				
•••••	•				
Confirm new	password				
•••••	•				
			ОК	Cance	2
				Cance	51

Change password dialog

- 3. Enter the current password into the **Old password** field.
- 4. Enter a new password into the **New password** field, and enter it again in the **Confirm new password** field.

Note: As long as the entered password does not meet the configured <u>password policy</u>, the **New password** field will remain yellow and a hint will be shown in a tooltip. The **Confirm new password** field will remain yellow if the contents differ from the **New Password** field.

Note: The **No policy** option allows users to set and save an empty password. To set an empty password, leave the **New password** and **Confirm new password** fields empty. However, if the **New password** field remains yellow, this indicates that the **Standard policy** is configured and an empty password is not allowed.

5. Click **OK** to save the new password.

Use the new password for the next login.

If an empty password has been set, leave the **Password** field empty during login.

5.4 Managing labware

The QIAgility Setup Manager Software contains a labware library that includes all labware supported by the QIAgility.

During run setup, the software automatically plans the worktable for the run. As a basis for this plan, you must first indicate which labware (e.g., adapters, plates or tubes) is available in your laboratory. This information is managed in the **Labware Manager**.

Access the Labware Manager by clicking the Labware Manager tab in the Configuration environment.

QIAgility		
<u>F</u> ile Edit View Help		0
CIAGEN Home Experiment Service Configuration		Instrument
Settings Labware Manager Calibration User N	Manager	
Adapter > Filter options	Select adapter	
Plates Category All	Select available adapter and press the "Add" button. 24 x 5 ml Fiat-Base Tubes Catalog number Catalog number	
Tubes Name	32 x 1.5 ml Tipic-Cap Tapered-Base Tubes 33 x 1.5 ml Tapered-Base Tubes 33 x 1.8 ml screwtop flat baseCryo Name	
Part number	33 x 2 ml Flat-Base Tubes 36 x 0.2 ml PCR Tubes 36 x 0.5 ml PCR Tubes 364-Well Plate	
Socket All V Reset filter Apply filter	48 x 0.5 ml Tubes 96-Well Plate Light Cycler 480, 384-Well Plate Light Cycler 480, 96-Well Plate Master Mix Block, 1 x 5 ml, 4 x 1.5 ml Tubes Master Mix Block, 1 x 5 ml, 4 x 2 ml Tubes	A v
	Add Adapter name Currently available	
	32 x 1.5 ml Flip-Cap Tapered-Base Tubes X Master Mix Block, 5 x 5 ml Tubes X Reegent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Taper X	
	4 Back Save	Cancel
	June 16, 2015 ad	min admin 🛛 🔿 🗍

This screen contains a step marker with three steps (**Adapter**, **Plates**, **Tubes**) on the left. The software distinguishes between the different labware types, because they must be treated differently during worktable planning:

• Adapter: An adapter is placed on the worktable in order to hold other labware such as tubes or plates.

- Plates: For example, 96-well plates, 48-well plates or Rotor-Discs for the Rotor-Gene Q. Some plates can be placed directly on a worktable position, while others require an adapter. Plates usually occupy an entire worktable position, while a Rotor-Disc occupies 2 positions.
- Tubes: Single tubes or tube strips. Tubes always need an adapter to be placed on the worktable.

The Filter options area in the middle is used to search for a specific labware.

The **Select adapter**, **Select plates**, or **Select tubes** area on the right shows the search results together with a picture and further details.

Use the **Add** button to confirm and add the selected labware to the **Available in laboratory** list. The **Next** and **Back** buttons in the button bar allow you to advance or move backwards one screen.

Defining a specific labware as available

- 1. Click **Configuration** to change to the **Configuration** screen.
- 2. Click the Labware Manager tab.
- 3. The Labware Manager screen will be displayed, and the Adapter step will be active.
- 4. Use the options in the **Filter options** area to filter for a specific adapter. The available filter options are:

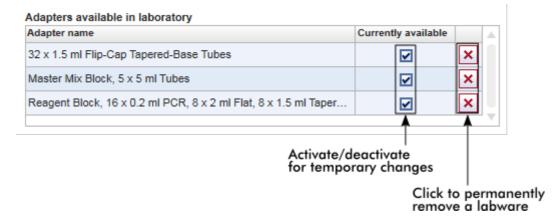
Option	Description
Category	Labware category, such as standard 96-well, Rotor-Gene Q, etc.
Name	Labware name
Part number	Catalog number of the labware
Socket (available in Adapter step only)	Labware suitable for a specific position on the worktable
Manufacturer (not available in Adapter step)	List of labware manufacturers
Tube/Well size (not available in Adapter step)	Tube/well size range from < 0.5 ml to > 2 ml

- 5. Click Apply filter to commit your filter query.
- 6. All adapters matching your query are listed in the Select adapter area.
- 7. Click on the desired adapter and then click Add to add it to your available in laboratory list.
- 8. The selected adapter is added to the Adapters available in laboratory list at the bottom.
- 9. Click **Save** to save the list of available adapters.
- Repeat steps 4–9 for the **Plates** and the **Tubes** steps.
 Either click on the desired step in the step marker or click **Next** in the button bar.

Important: Tubes or plates supplied by different manufacturers may have slightly different dimensions, despite a similar appearance. Such differences can significantly affect the calibration settings, in particular the height calibration (Z-axis calibration). Therefore, it is important to select the correct plates and tubes. We recommend that once a particular tube type is chosen, to always purchase that consumable from the same manufacturer to avoid frequent recalibration.

Removing labware permanently or temporarily

Use the interactive buttons in the **Adapters available in laboratory** table to temporarily or permanently remove a labware. Use the temporary deactivation if a labware is out of stock but will be available later. Remove it permanently if you will not be using this labware in the future. If you permanently remove a labware and then change your mind, you can add the labware again.



To **temporarily** remove a labware

To **permanently** remove a labware

- 1. Deactivate the check box in the **Currently available** column.
- 2. The icon changes from 🗹 to 🔲.
- 3. The selected labware will be deactivated and will no longer be available in the **Experiment** environment.

To reactivate the labware, simply activate the check box again.

- 1. Click the **Remove labware** icon (\Join) in the column of the labware you want to remove.
- 2. A confirmation dialog will be shown (the text may vary depending whether you are removing an adapter, plate or tube):

Confirmation
Removing the availability of adapter may affect the availability of its corresponding plates and tubes. Do you want to continue ? (2090010)
OK Cancel

3. Click **OK** to permanently remove the selected labware.

Before the labware can be used, instrument calibration is required.

5.5 Instrument calibration

QIAgility is a high-tech precision instrument, which is able to perform movements in the micrometer range. An instrument calibration must be performed before the QIAgility can be used, to ensure absolute precision positioning and pipetting. No runs can be started if the required calibrations have not been performed.

Calibration includes:

• Volume calibration

The actual volume calibration is performed at the manufacturer's site and only requires that the user enters the measured calibration data.

Volume calibration defines the number of units that the P-axis motor must move to accurately deliver a given volume of liquid.

• X-Y and Z-axis calibration

The X-Y and Z-axis calibration must be performed at the user's site and is required for the tip ejector, the tip racks and all labware used on the QIAgility.

X- and Y- axis calibration defines the distinct position of a labware on the worktable to ensure proper operation of the instrument. Z-axis calibration defines the worktable height of each labware to maximize precision of liquid transfers and to ensure optimal conditions for liquid-level detection. Before using any items on the worktable, X-Y and Z-axis calibration must be performed for each labware to be used on each worktable position.

The lid interlock is disabled during calibration. The robotic arm will move during calibration when the lid is raised.

	Risk of personal injury [W7] The robotic arm moves during position calibration while the instrument lid is raised. Never click any buttons while parts of your body are within the instrument workspace.
--	--

WARNING Moving parts [W12] Avoid contact with moving parts during operating. Avoid contact with moving parts during operating.	under the pipetting arm when it is
--	------------------------------------

Important: Clicking on **Perform calibration** or a positioning button will immediately move the robotic arm in the selected direction for a specific movement step.

To ensure your personal safety:

- Always stand clear of the instrument when starting calibration or making adjustments.
- Do not click any of the software buttons with the mouse or press any of the keys on the keyboard while parts of your body are within the instrument worktable or when looking closely at calibration positions.
- Wait until the robotic arm has completed its movements before moving any plates or tubes.

The calibration tasks are bundled in the **Calibration** tab of the **Configuration** environment. All calibration steps are built in a wizard-style format, that allows you to move forward or backward one screen using the **Next** or **Back** button in the button bar, respectively.

📃 QIAgility Set	up Manager								
ile Edit View	Help 🦛 🦟	۱							
QIAGEN	Home	Experiment	XT Service	Con	Ç[©] figuration				
Settings	Labware Ma	anager	Calibration	n	User I	Manager			
Volume	>	Volume Calibration							
Tip Ejector		Enter the calibration values into the table below. Refer to the calibration report shipped with your QIAgility.							
Tip Rack		Pipettor hea 123456-0	d serial num	ber					
		Targ	jet volume		Value	e (P-Axis units))		
Labware				1 µl		51.44			
				2 µl		75.64			
				5 µl		152.81			
				10 µl		287.22			

The calibration comprises the following steps:

Calibration step

Volume

Tip Ejector

Tip Rack

Labware



Enter the volume calibration data that was shipped with the instrument in the calibration report. No further interaction is required. See <u>Calibrate volumes</u>.

Perform, and if necessary, correct and test the calibration of the tip ejector. Manual adjustment of XY-axis calibration is possible if necessary. Z-Axis calibration is performed automatically without user interaction. See <u>Setup tip ejector</u>.

Perform and manually correct the XY-axis calibration of the tip rack. See <u>Calibrate tip racks</u>.

Perform X-Y and Z-axis calibration for all labware that will be used on the QIAgility. See <u>Calibrate labware</u>.

Select the labware, the corresponding adapter(s) and/or cooling block and the worktable position for which the labware should be calibrated, and add to a calibration work list.

Based on the work list, the labware calibration wizard will guide you through all XY-axis calibrations, followed by the Z-axis calibrations for each selected labware.

Manually correct the XY-axis calibration for each XY-axis calibration step.

It is strongly recommended to perform the Z-axis calibration automatically whenever possible. However, the software also provides the possibility to perform Z-axis calibration manually.

Note: QIAGEN recommends that you follow the steps from top to bottom starting with the **Volume** calibration and finishing with the **Labware** calibration step. For the first calibration, this order is required, and the next step will only be available after the previous step has been completed.

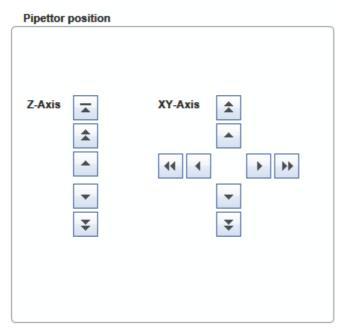
Note: If the QIAgility is moved, we recommend recalibration, starting from step **Tip Ejector**. Click on the **Tip Ejector** step to start there.

Note: After servicing the pipettor head, recalibration is required, starting from step Volume.

Note: Calibration data can be saved and restored for backup purposes using the **Export calibration data** and **Import** buttons at the bottom of the screen. For details, see <u>Saving and restoring calibration data</u>.

5.5.1 Calibrate positioning buttons

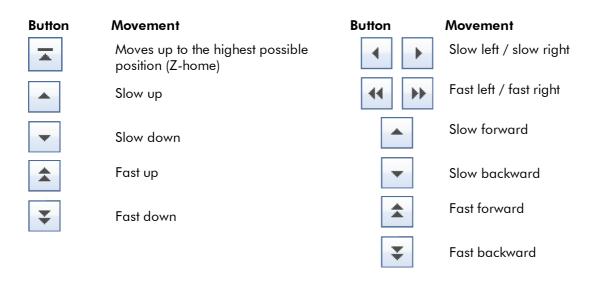
For manual corrections to calibration of positions, the software provides several positioning buttons. The positioning buttons are arranged in two groups, one for the Z-axis and one for the XY-axis.



The following table shows the movement steps for the positioning buttons:

Z-axis

XY-axis



5.5.2 Calibrate volumes

Volume calibration defines the number of units that the P-axis motor must move to accurately deliver a given volume of liquid.

The QIAgility is shipped with a calibration report. The data in this calibration report have been measured and adjusted by the manufacturer individually for each individual pipettor head to ensure accuracy of volumes. The data in the calibration report must be entered in the software before the QIAgility can be used.

QIAGEN	Home	Experiment	XT Service	Config	ф uration		
Settings	Labware M	anager Z	Calibration		User N	/lanager	
Volume	>	Volume Cali	bration				
Tip Ejector			ibration values tion report ship				
Tip Rack		Pipettor hea 9403-0	d serial numb	er			
		Targ	jet volume		Value	(P-Axis units)	
Labware				1 µl		51.4	
				2 µl		75.6	
				5 µl		152.8	
				10 µl		287.2	
			:	20 µl		556.1	
			:	50 µl		1371.3	
			1	00 µl		2689.7	
			1	50 µl		4012.6	
			2	00 µl		5319.3	
				Cance		Save value	5

Follow these steps to enter the volume calibration data:

1. Click on **Configuration** to change to the **Configuration** screen.

2. Click on the **Calibration** tab.

Note: If the instrument is not connected, there will be a warning sign on the **Calibration** tab. When you click it, a message will be displayed that the instrument needs to be connected first. The volume step will be displayed without the pipettor head serial number and with the column **Value (P-axis units)** empty. No calibration functionality will be available, because all calibration information is related to an individual pipettor head. In this case, connect the instrument and relaunch the software.

- 3. The **Volume** step is displayed by default.
- 4. Enter the pipettor head serial number from the calibration report into the **Pipettor head serial number** field. Ensure that the calibration report shows the same serial number.
- 5. For the first target volume entry in the table $(1 \ \mu I)$ enter the corresponding P-axis value from the calibration report to the **Value** column.

Note: If no volume calibration data has been saved yet for this pipettor head, the cells of the **Value** column are empty. They are displayed in yellow to indicate that a value is mandatory.

- 6. Repeat the previous step for all volumes from 2 μ l to 200 μ l .
- 7. Click **Save values** to save the data or click **Cancel** to cancel the volume data input and revert any values to their previous values.

Click the Next button to continue with Tip Ejector calibration.

Note: The **Next** button is available only if volume calibration data for the current pipettor head have been saved.

5.5.3 Setup tip ejector

The **Tip Ejector** step allows the instrument to determine the location of the tip ejector. The location of the tip ejector must be calibrated to ensure proper operation of the instrument. This must be done carefully to avoid damaging the instrument.

File Edit View Help Im main 00000 α° QIAGEN Home Experiment Service Configuration Calibration Settings Labware Manager User Manager Tip Ejector Calibration Volume Place the tip ejector. Empty the worktable. Press "Perform calibration" to start the automatic calibration **Tip Ejector** > process. Correct the xy-position if necessary. Press "Save position". Then press "Next". **Tip Rack** Pipettor position Labware Perform calibration . Z-Axis XY-Axis * -. ₽ ► Ŧ Ŧ Ensure your personal safety! After you click a button, the robotic arm will move while the instrument lid is open. Test tip ejection Cancel Save position

The tip ejector calibration is available only if volume calibration has already been performed.

The lid interlock is disabled during calibration. The robotic arm will move during calibration when the lid is raised.

	Risk of personal injury [W7] The robotic arm moves during position calibration while the instrument lid is raised. Never click any buttons while parts of your body are within the instrument workspace.
--	--

WARNING Moving parts [W12] Avoid contact with moving parts during operation of the QIAgility. Under no circumstances should you place your hands under the pipetting arm when it is lowering. Do not attempt to move any plates or tubes whilst the instrument is operating.

Important: Clicking on **Perform calibration** or a positioning button will immediately move the robotic arm in the selected direction for a specific movement step.

To ensure your personal safety:

- Always stand clear of the instrument when starting calibration or making adjustments.
- Do not click any of the software buttons with the mouse or press any of the keys on the keyboard while parts of your body are within the instrument worktable or when looking closely at calibration positions.
- Wait until the robotic arm has completed its movements before moving any plates or tubes.

Follow these steps to calibrate the tip ejector:

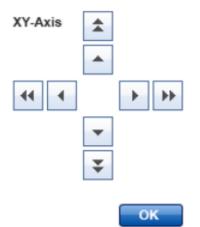
1. Install the tip ejector. For details, refer to <u>Tip ejector</u>.

Ensure that the tip ejector is securely mounted to the worktable. During the process, the body of the pipettor head is touched against the tip ejector.

- 2. Install the tip disposal box. For details, refer to Tip disposal box.
- 3. Ensure that the pipettor head is not holding any tips.
- 4. Empty the worktable.
- 5. Keep the lid open during the calibration procedure.
- 6. Click **Perform calibration** to start the automatic calibration process. The head starts to move and automatically locates the tip location.
- 7. The message dialog opens asking whether the pipettor hub is above the ejector slot. Carefully visually check the positioning on the instrument (from both the front and the side).

8. Click **Yes** if the positioning is correct. In this case, the XY-axis calibration of the tip ejector will be automatically saved, and the Z-axis calibration will start immediately.

Or click **No** to manually adjust the XY positioning. In this case, the manual XY-axis positioning buttons will be enabled. Use the buttons to position the pipettor head directly centered above the tip ejector. To confirm and save the XY-axis calibration, click the **OK** button on the bottom right of the positioning button. The Z-axis calibration will start immediately.



For details about the buttons, refer to Calibration positioning buttons.

- 9. The Z-axis calibration starts automatically. The pipettor head moves up and down and stops when the calibration is finished. No user interaction is needed for the Z-axis calibration. The **Save Position** button will be enabled after the Z-axis calibration has finished.
- 10. Click Save Position to save the tip ejector calibration. The calibration information is saved.
- 11. We recommend testing whether the calibration was successful by clicking **Test tip ejection** (see procedure below). Afterwards, continue with the next calibration step (Tip rack) either by clicking **Next** in the button bar or **Tip Rack** in the step marker pane.

Testing the tip ejector calibration

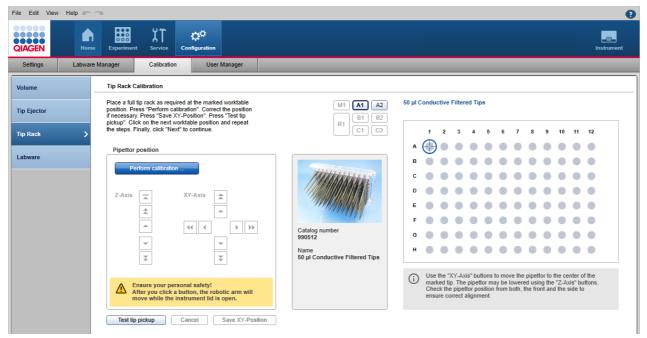
The following procedure starts a test to determine whether the calibration of the tip ejector was successful. During the test, the pipettor head will move.

- 1. Click the **Test tip ejection** button to start the test.
- 2. A confirmation dialog opens. When you click **OK**, the pipettor head starts to move immediately to the right to its home position.
- 3. Another dialog opens requesting you to place a tip onto the pipettor head. Manually place a tip onto the pipettor head and click **OK**. The pipettor head starts to move immediately.
- 4. The pipettor head moves to the tip ejector and ejects the tip. If the tip is not correctly ejected, there might be an error with the calibration. Repeat the tip ejector calibration as described above.

5.5.4 Calibrate tip racks

The tip rack calibration is essential to ensure a reliable pickup of tips.

Tip rack calibration is available only if the volume and tip ejector calibrations have already been performed.



Proceed as follows:

- 1. Carefully place a full tip rack of 50 μ l tips in worktable position A1. It is important to follow the instructions in <u>Insert tips to tip rack holder</u> for details about how to place tips.
- 2. Carefully place a full tip rack of 200 μ l tips in worktable position A2.
- 3. If not activated, click A1 from the worktable selector:



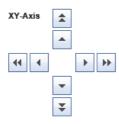
- 4. Keep the lid open during the calibration procedure.
- 5. Click **Perform calibration** to start the calibration. The pipettor head starts to move and positions itself above position A1 of the rack in worktable position A1.

6. Carefully inspect whether the pipettor head is positioned over the center of the tip in position A1. Look

from the front and from the side. In case you have difficulties seeing the position, use the buttons of the Z-axis position control to lift and lower the pipettor head.

Note: If you still have difficulties seeing the position, you can click **Cancel** just below the pipettor position control. Remove the tip from A1 and any tips around it. Then click **Perform calibration** and calibrate into the A1 position of the tip rack, instead. Replace the tips after step 9.

7. Adjust the XY-positioning manually. Use the buttons of the XY-axis control to position the pipettor head directly centered just above the calibration position. For details about the buttons, refer to <u>Calibration positioning buttons</u>.



- 8. Click Save XY-Position to save the calibration data for tips at worktable position A1.
- 9. A message pops up indicating that the pipettor will move to the home position. Confirm and stand clear of the instrument while the pipettor is moving.
- 10. To test if the calibration was successful, click **Test tip pickup** (see below).
- 11. Click A2 from the worktable selector and repeat steps 4 to 9 for the tip rack at worktable position A2.

Note: Tip rack calibration on A1 and A2 is the minimum requirement. We recommend that you also calibrate 50 μ l tip racks on positions B1 and B2 in the later <u>Labware calibration</u> step. This is because the software will plan additional tip racks on these positions, if possible, to support completely using previously started tip racks and minimizing tip reloading during the run.

Testing the tip rack calibration

The following procedure starts a test to determine whether the calibration of the tip rack was successful. During the test, the pipettor head moves to the tip rack, picks up a tip and drops it at the tip ejector.

- 1. If not activated, click the worktable position you want to test: A1 or A2.
- Click the Test tip pickup button to start the test.
 Note: The button is active only if a calibration has been already performed on that worktable position.
- 3. The pipettor head moves to the tip ejector, ejects the attached tip and picks up the next available tip from the tip rack at the selected worktable position.
- 4. Confirm the message with **OK**. If the tip pickup failed, repeat the tip rack calibration for the selected worktable position. If the instrument picked up the tip successfully, continue with the next calibration.

5.5.5 Calibrate labware

All labware and adapters must be calibrated before use, to ensure that the pipettor knows their exact positions on the worktable. In addition, every labware, adapter and worktable position combination must be calibrated, because the same labware with different adapters at different positions has different coordinates. For example, the QIAGEN Elution Microtubes RS labware used in both positions B1 and B2 must be calibrated twice: once in position B1 and a second time in position B2. Also, different adapters of the same type may show calibration variances. Therefore, be sure to always calibrate the adapter that will be used.

Labware calibration is performed in the software using a convenient wizard, which guides users through all necessary steps for the set of labware to be calibrated.

The first step of labware calibration is to select the set of labware (or labware/adapter combinations), and the worktable positions on which the labware will be calibrated. This information is collected in the work list. See <u>Select labware to calibrate</u>. The calibration wizard will then guide the user through the following calibration steps.

The second step is the preparation of the worktable, i.e., placing required tips. See <u>Prepare worktable for</u> <u>labware calibration</u>.

In several steps, all labware and labware/adapter combinations will be XY-axis calibrated on the worktable positions as defined in the work list, one step for each labware or labware/adapter combination in a specific worktable position. The user will correct the XY-positioning manually and save the calibration data for each step, before continuing with the next step. See <u>Calibrate X- and Y-axis</u>.

Afterwards, in several steps, all labware and labware/adapter combinations will be Z-axis calibrated, one step for each labware and labware/adapter combination in the work list. For most labware, the software provides an automatic Z-axis calibration function, which should be used whenever possible. However, it is also possible to decide on each step individually to calibrate the Z-axis manually. Calibration data needs to be saved for each step, before continuing with the next step. See <u>Calibrate Z-axis</u>.

Note: Make sure to calibrate each individual adapter that is used with the QIAgility, even if you have already calibrated an adapter of the same type. When performing experiments, only use the exact adapter that was calibrated.

5.5.5.1 Select labware to calibrate

The first step of labware calibration is to select a set of labware along with the adapters and worktable positions on which the labware will be calibrated in the work list. Initially, the work list is empty.

The labware calibration step will only be available if all previous calibration steps have been performed.

All labware that has already been calibrated for the currently connected instrument will be listed in the **Calibration history** table. Initially, the table is empty.

Note: The calibration history will also be empty after the pipettor head has been changed, because calibration data are individual for an instrument and a pipettor head.

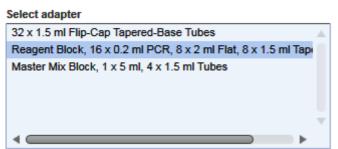
le Edit View Help 🜌								
IAGEN H	me Experiment Service Ca	Configuration						nstrumen
Settings Labw	are Manager Calibration	User Manager						
lume	Labware Calibration		1					
Ejector	Select the labware to be calibra Finally, press "Start calibration"		Calibration hi	story	Adapter	Worktable pos.	Cooling block	
	Select labware		1.5 ml tapered	d tube (QIAGEN)	Master Mix Block, 1 x 5 ml, 4 x 1.5 m	M1		Ī
Rack	QIAGEN S-Block 96 Well	De	1.5 ml tapered	d screw cap tube (QIAG	Master Mix Block, 1 x 5 ml, 4 x 1.5 m	M1		
bware S	QIAGEN Elution Microtubes RS QIAGEN 96 Well Microplate RB			se tube (QIAGEN)	Master Mix Block, 1 x 5 ml, 4 x 1.5 m	M1		
	200 µl PCR tube (QIAGEN) 1.5 ml tapered tube (QIAGEN	D	1.5 ml tapered	d tube (QIAGEN)	Reagent Block, 16 x 0.2 ml PCR, 8 x	R1		
	1.5 ml tapered screw cap tube		1.5 ml tapered	d screw cap tube (QIAG	Reagent Block, 16 x 0.2 ml PCR, 8 x	R1		•
	2.0 ml colf standing corow car Select adapter	n tuba (OLACENI)			·			
	32 x 1.5 ml Flip-Cap Tapered-	-Base Tubes	Work list Labware		Adapter	Worktable pos.	Cooling block	
	Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tap			ibe (QIAGEN)	Reagent Block, 16 x 0.2 ml PCR, 8 x	R1		×
	Master Mix Block, 1 x 5 ml, 4	x 1.5 ml Tubes	1.5 ml tapered	d tube (QIAGEN)	32 x 1.5 ml Flip-Cap Tapered-Base T	B2		×
			1.5 ml tapered	d screw cap tube (QIAG	Master Mix Block, 1 x 5 ml, 4 x 1.5 m	M1		×
	•							
		ect accessory Cooling block						
	M1 A1 A2 0	Cooling block						
	R1 C1 C2	Add to work list					Start calibrati	on
			-					
			Back	Next 🕨	Export c	alibration data	Import	

Follow these steps to define the list of labware that should be calibrated:

1. Select the desired labware from the **Select labware** list. Only one item can be selected.

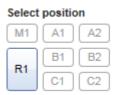
Select labware	
QIAGEN S-Block 96 Well	
QIAGEN Elution Microtubes RS	
QIAGEN 96 Well Microplate RB	
QIAGEN 96 Well Microplate FB	
200 µI PCR tube (QIAGEN)	U
1.5 ml tapered tube (QIAGEN)	
5.0 ml flat-base tube (QIAGEN)	•

2. If the selected labware can be used with adapters, select the desired adapter from the **Select adapter** list. Only one item can be selected.



Select accessory Cooling block

3. Select the worktable position in which the selected labware will be used. Only one position can be selected.



4. **Optional step:** if you intend to use a cooling block for the labware on the selected worktable position, select the **Cooling block** option.

Note: The cooling block check box will only be available if the currently selected labware or labware/adapter combination can be used with a cooling block.

5. Click **Add to work list** to save the selected combination of labware, adapter (optional), position and cooling block (optional) to the work list. The combination is added to the the **Work list** table:

w	or	k	I	i	e	t
	~		•	•	-	•

Labware	Adapter	Worktable pos.	Cooling block	
QIAGEN 96 Well Microplate FB	-	B1	\checkmark	×
1.5 ml tapered tube (QIAGEN)	Reagent Block, 16 x 0.2 ml PCR, 8 x	R1		×

The work list above shows for example that the first labware "QIAGEN 96 Well Microplate FB" has no alternative adapters to be selected and is calibrated with a cooling block. The last entry is calibrated with a specific adapter on R1 without cooling block.

- 6. Repeat steps 1–5 for all labware, adapter and worktable position combinations to be calibrated together in a session.
- 7. Finally, click **Start calibration** to start the calibration wizard. Based on the work list, the wizard creates the necessary XY-axis and Z-axis calibrations.

After all calibration steps have been performed, all items from the work list will appear in the calibration history table and the work list will be empty.

Note: If the calibration wizard is canceled, the work list will still contain the items that have not been completely calibrated.

Note: A labware item is correctly calibrated only if both XY- and Z-calibration have been performed. When creating the work list, take into account the time required for a complete calibration. Sometimes it is better to create a smaller work list and to calibrate the labware in subsets.

It is not necessary to calibrate every labware on every worktable position. As a starting point, use the following recommendations as a guide (for details, refer to the kit handbooks):

- Calibrate labware that will contain the samples (input) on worktable position C1. In case you intend to use Rotor-Gene Q adapters for output, calibrate the input labware on worktable position C2 instead.
- Calibrate labware that is intended for output on worktable position C2. However, Rotor-Gene Q adapters must be calibrated on B1/C1.
- In addition to the tip rack calibration, calibrate 50 μ l tips and 200 μ l tips on B1, B2, C1 and C2. The software will plan additional tip racks on these positions, if possible, to support completely using previously started tip racks and minimizing tip reloading during the run. If you plan to do sample pooling experiments, you will need to additionally calibrate 50 μ l tips on A2 and 200 μ l tips on A1.

Note: It is not possible to combine tip racks and other labware in one work list.

• Calibrate the labware provided with the QIAgility and the tubes provided with the QIAgility Calibration Kit on worktable positions M1 and R1, because this labware is used for QIAGEN Kits.

5.5.5.2 Prepare worktable for labware calibration

The worktable must be prepared before the labware calibration can be started. The calibration requires 50 μ l and 200 μ l tips. The required number of each type of tips will be displayed in the **Required** column of the **Worktable preparation** dialog. Use the tip availability buttons at the bottom right of the dialog to define the available tip positions.

Worktable preparation													
Place the required tip rack on the marked worktable position. Adjust tip availability at the right. Click "Loaded". Click on the next worktable position and repeat the steps. Finally, click "Next" to continue.		R1	A1 A2 B1 B2	50 µl Co		e Filtere 2 3		5 (5 7	8	9 10	11	12
			C1 C2	А	•	• •	•	• •		•		•	•
A1 - tip rack	-			в	•		•	• •		•		•	•
Tip type	Required	Available	Loaded	с	•	• •	•	•		•		•	•
Tip50	6	96		D									
				_									-
				E	•			•		•			•
				F	•		•	•		•		•	•
				G	•	• •	•	• •		•		•	•
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				i	Define tip acknowle	p availa edge by	bility by clicking	selecting the '+' o	i tip pos r '-' butte	itions an ons.	d		
				(j)	acknowle	edge by	clicking	the '+' o	r'-' butte	ons.			

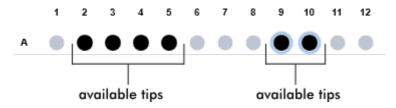
Preparing the worktable

1. Place the required tip rack containing a sufficient number of tips in worktable position A1.

The dialog displays the required number of 50 μ l and 200 μ l tips in the **Required** column of the table. Initially, the buttons A1 and A2 are displayed in yellow to indicate that the number of available tips, initially set to zero, is insufficient and that the loaded state has not yet been confirmed. 2. A visualization of the tip rack is displayed on the right. Use the tip availability buttons below the rack graphic to set the availability of the rack.

Option	Required action
To set all tips to available	Click on:
	Set all tips 🕂 To available
To set all tips to unavailable	Click on:
	Set all tips
	 To unavailable
To set selected tips to available	Select the desired tip positions. Click on the letter representing a row to select the whole row, the column number to select the whole column or individual tip positions to select single positions. Then click on:
	Set selected tips 🕂 To available
To set the selected tips to unavailable	Click on:
	Set selected tips
	 To unavailable

Available tip positions are displayed in dark color (A2–5, A9–10) and unavailable positions are displayed in gray (A1, A6–8, A11–12). Selected tips are surrounded by a light blue circle (A9–10):



Note: Ensure that the visualization on the screen is identical to the actual tip loading of the tip rack on the worktable.

3. Click the **Loaded** check box in the last column of the tip rack table on the left to confirm that you have loaded the tip rack onto the worktable. The color changes from yellow to blue:

A1 - tip rack			
Tip type	Required	Available	Loaded
Tip50	6	26	

4. Click **A2** in the worktable position:

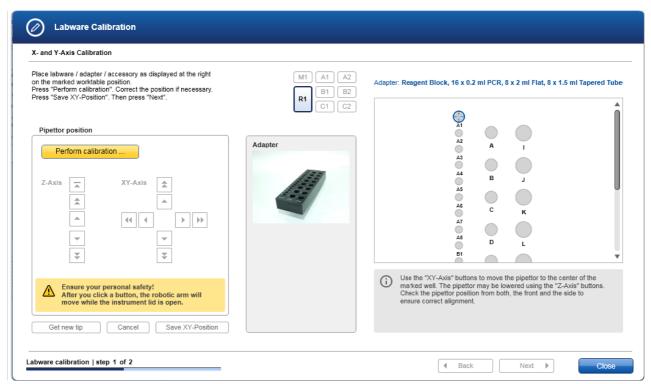
M1	A1	A2
R1	B1	B2
	C1	C2

- 5. Place the required tip rack into worktable position A2.
- 6. Repeat steps 2–3 for the tip rack at position A2.
- 7. Once you have placed a sufficient number of available tips into both positions A1 and A2, and have confirmed the **Loaded** state for both tip racks, the **Next** button will be activated.
- 8. Click **Next** to continue to the next step, the XY-axis calibration of the labware.

5.5.5.3 Calibrate X- and Y-axis

Each individual XY-axis calibration step is provided in a separate screen.

The calibration <u>positioning buttons</u> are displayed on the left side of the window, along with advice and notifications regarding the calibration. An image of the labware or labware/adapter combination to be calibrated in this step is displayed in the center. The name of the labware or names of the labware/adapter combination is displayed on the right side. The graphic on the right shows a schematic of the labware with the position used for the calibration marked with crosshairs. A progress bar is displayed at the bottom left indicating the number of calibration steps remaining for the actual work list.



XY-Axis calibration of the reagent block adapter. Position A1 will be used for calibration.

To perform XY-axis calibration for one labware item, follow the steps below:

1. Place the labware, adapter or accessory listed at the top right onto the worktable position indicated in the worktable indicator:

M1	A1	A2
R1	B1	B2
	C1	C2

- 2. Click Perform calibration to start the XY-axis calibration.
- 3. The pipettor head starts moving and positions itself above the dedicated calibration position (marked with crosshairs in the schematic).

Note: If a tip rack is calibrated, any attached tip will be ejected from the pipettor head before it moves to the calibration position.

- 4. **Optional step:** If no tip is attached to the pipettor head, click **Get new tip**. The pipettor head picks up the next 200 μ l tip and returns to the dedicated calibration position.
- 5. Carefully inspect whether the pipettor head is positioned in the center of the marked position. Look

from the front and from the side. In case you have difficulties seeing the positioning, use the buttons of the Z-axis position control to lift and lower the pipettor head. Adjust the XY-positioning manually. Use the buttons of the XY-axis control to position the pipettor head directly centered above the calibration position. For details about the buttons, refer to <u>Calibration positioning buttons</u>.



- 6. Click **Save XY-position** to save the XY-calibration position. A message will be displayed, and the pipettor head will move to the home position, while keeping an attached tip. Alternatively, click **Cancel** and repeat from step 2 on.
- 7. Click **Next** to move forward to the next calibration step.

Note: The Next button will only be activated after the Save XY-position button has been clicked.

Note: If the work list contains remaining XY-calibrations for any labware, the next step will be the XY-calibration for the next labware item. The first Z-calibration step will only be displayed once all XY-calibration steps for the work list have been performed.

You can return to the previous calibration step by clicking the **Back** button. From the first calibration step, this will lead back to the initial screen of the labware calibration (work list).

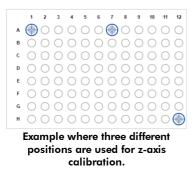
You can stop the labware calibration by clicking the **Close** button on the bottom right. In this case, none of the labware items from the work list are completely calibrated, because all Z-calibrations must still be

performed. The labware calibration wizard will be closed and the initial labware calibration screen will be shown. All labware items will remain in the work list, allowing you to start calibration again at a later time.

5.5.5.4 Calibrate Z-axis

Each individual Z-axis calibration step is provided in a separate screen. The first Z-calibration step will only be displayed after all of the XY-calibration steps for the work list have been performed.

The screen is very similar to the XY-calibration screen. The graphic on the right shows a schematic of the labware with the positions used for the calibration marked with crosshairs.



To perform Z-axis calibration for one labware item, follow the steps below:

1. Place the labware, adapter or accessory listed at the top right onto the worktable position indicated in the worktable indicator.

For tubes, ensure that tubes are placed in all positions marked with crosshairs in the schematic. Use the scroll bars of the schematic to scroll down, in case some labware positions are not visible.

M1	A1	A2
R1	B1	B2
	C1	C2

2. You can either perform an automatic calibration by leaving the check box next to **Automatic** activated or make a manual calibration by deactivating the option. We recommend using the automatic calibration option whenever possible.

Pipettor position



- 3. Click the **Perform calibration** ... button. The pipettor head ejects the tip, picks up a new 50 μ l tip and moves to the calibration position.
- 4. The pipettor head automatically moves several times down to the bottom of the labware using several tips. The pipettor head repeats this procedure for all calibration positions marked with crosshairs.
- 5. Click **Save Z-position** to save the Z-axis calibration data.

6. Click **Next** to move forward to the next Z-calibration step, or click **Close** if this was the last calibration step. To determine if this was the last calibration step, look at the progress bar in the lower left of the window.

Note: The **Next** button will only be activated after clicking the **Save Z-position** button, if there is a following Z-calibration step. The **Next** button is not activated after the last step.

You can return to the previous calibration step by clicking the **Back** button. From the first calibration step, this will lead back to the initial screen of the labware calibration (work list).

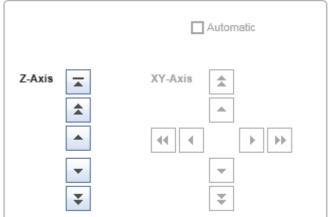
You can stop the labware calibration by clicking the **Close** button at the bottom right. Only the labware items from the work list for which both the XY- and Z-calibrations have been performed are completely calibrated. The labware calibration wizard will be closed and the initial labware calibration screen will be shown. All labware items which have not been completely calibrated will remain in the work list, allowing you to start calibration again at a later time.

Manual calibration

Note: We recommend using the automatic calibration option whenever possible.

- 1. Click the **Perform calibration** ... button. The pipettor head ejects the tip and picks up a new 50 μ l tip.
- 2. The pipettor head moves to the calibration position. There is only one calibration position for the manual calibration procedure. The Z-axis positioning buttons become activated.
- 3. Click **Get new tip**. The pipettor head picks up the next 50 μ l tip and returns to the calibration position.
- 4. Use the Z-axis positioning buttons to carefully lower the tip so that it is close to the base of the tube or well (approximately 0.5 mm above tube or well base). The distance may be checked by carefully lifting the well from its support. In case the tip is damaged, click **Get new tip** and repeat to ensure a good calibration result.





5. Click **Save Z-Position** to save the Z-axis calibration data. Alternatively, click **Cancel** and repeat from step 3 on.

6. Click **Next** to move forward to the next Z-calibration step, or click **Close** if this was the last calibration step. To determine if this was the last calibration step, look at the progress bar in the lower left of the window.

Note: The **Next** button will only be activated after clicking the **Save Z-position** button, if there is a following Z-calibration step. The **Next** button is not activated after the last step.

5.5.6 Saving and importing calibration data

The QIAgility Setup Manager Software includes a function to save and restore the calibration data. Use this function if for any reason another computer will be used to control the QIAgility, or if the QIAgility Setup Manager Software must be reinstalled.

Calibration data files have the file extension *.xcd.

Saving calibration data

- 1. Click the **Export calibration data** button in the button bar.
- 2. The file dialog is shown.
- 3. Select the target directory and enter a file name.
- 4. **Optional step:** Select the desired information to be saved by activating the corresponding check boxes:

Options

Volume calibration Instrument calibration (Tip Ejector, Tip Rack) Labware calibration

Option Volume calibration	Description Include volume calibration data.
Instrument calibration	Include general instrument calibration data (e.g., for tip ejector, tip rack)
	Note: Instrument calibration data can only be saved together with volume calibration data.
Labware calibration	Include labware calibration data.
	Note: Labware calibration data can only be saved together with volume and general instrument calibration data.

Note: If you intend to change the laptop that controls the QIAgility, or if the QIAgility Setup Manager Software needs to be reinstalled, check all options.

5. Click **OK** to confirm and close the file dialog.

6. The calibration data will be saved under the provided file name with the file extension .xcd.

Importing calibration data

To import calibration data that was previously saved on another laptop for the currently connected QIAgility, proceed as follows.

Note: The import function will only be available if volume calibration has already been performed. This ensures that only calibration data that match the current instrument and pipettor head are imported.

- 1. Click on **Import** in the button bar.
- 2. The file dialog is shown.
- 3. Navigate to the directory containing the calibration data file and select it.
- 4. From the options area of the file dialog, select the calibration data you want to import by activating the corresponding check boxes.

Options	
Volume calibration	
Instrument calibration	
(Tip Ejector, Tip Rack)	
Labware calibration	

Option	Description
Volume calibration	Include volume calibration data
Instrument calibration	Include general instrument calibration data (e.g., for tip ejector, tip rack)
	Note : Instrument calibration data can only be imported together with volume calibration data.
Labware calibration	Include labware calibration data.
	Note : Labware calibration data can only be imported together with volume and general instrument calibration data.
	Important : If you have already calibrated labware using this laptop before importing, use this option cautiously. Importing the labware calibration data may result in a situation in which data for labware items that are not part of the file will be kept, and calibration data for labware items that are part of the file will be replaced.

Note: If you changed the laptop that controls the QIAgility, or if the QIAgility Setup Manager Software has been reinstalled, check all options.

Click Open to import the selected calibration data set from the selected calibration file.
 Note: If the selected calibration data file to be imported does not correspond to the current instrument and pipettor head, the import will be declined.

Note: During import, the selected calibration data will be read from the file, and will replace the current data as appropriate.

6. The selected calibration data will be imported and will be used for subsequent runs.

Operating procedures

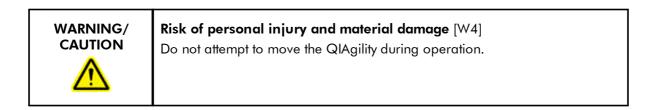
6 Operating procedures

This section describes how to operate the QIAgility.

Before proceeding, we recommend that you familiarize yourself with the features of the QIAgility by referring to the section <u>General description</u>.

The QIAgility lid must remain closed during operation of the instrument. Only open the lid when the instrument is not operating or when instructed to do so by the software.

Note: Opening the lid during operation of the QIAgility will cause the system to pause. The instrument will complete the current movement, which may take up to 10 seconds.



	Moving parts [W12] Avoid contact with moving parts during operation of the QIAgility. Under no circumstances should you place your hands under the pipetting arm when it is lowering. Do not attempt to move any plates or tubes whilst the instrument is operating.
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	Moving parts [W13] To avoid contact with moving parts during operation of the QIAgility, the instrument must be operated with the lid closed. If the in-built lid sensor is not functioning correctly, contact QIAGEN Technical Services.
--	---

6.1 Starting a run

The QIAgility Setup Manager Software provides two plug-ins that have been developed to directly facilitate specific PCR applications. Each plug-in supports several QIAGEN Kits. A third plug-in has been solely designed to meet your sample pooling requirements for convenient setup of your PCR experiments. A fourth plug-in is available for a kit-independent, easy and convenient, qPCR setup, which includes a sample normalization function. The fifth plug-in allows you to set up PCR experiments that incorporate serial dilution. For details on different experimental setup possibilities, refer to <u>Working with experiments</u>.

Before starting a run, read the QIAgility User Manual and the kit handbook for the kit that will be used.

To start a new run, proceed as follows:

- Start the QIAgility Setup Manager Software (see <u>Start the software</u>) if it is not yet operating.
 Note: For the first steps, the instrument does not need to be switched on.
- 2. If the **Home** screen is not visible, click on the **Home** icon from the main tool bar.
- 3. Create a new experiment using one of the following alternatives:
 - a) Create a new empty experiment by clicking an entry in the **Create Experiment** list. For details, refer to <u>Create an empty experiment</u>.
 - b) Use a template to create an experiment by clicking on it from the **Favorite Templates** list. If the desired template is not listed here, click on the **Create from other template** button at the bottom to open the file dialog. For details, refer to <u>Create an experiment using a template</u>.
 - c) Use a saved experiment as the starting point for the new experiment. In the **Recent Experiments** list, position the mouse pointer over the name of the desired experiment and select the **Use as template for new experiment** option from the context menu icon that appears. For details, refer to <u>Create an experiment from an existing experiment</u>.

The new experiment will be created and displayed in the **Experiment** environment.

Step by step, enter all necessary parameters and sample data. Follow the instructions given by the software. Click **Next** to go to the next step. Before entering the **Worktable** step, proceed with step 5.
 Note: For details about the steps and parameters, press the **Help** button to access the help page for the current step.

Note: The number and kind of steps and parameters may vary, depending on the plug-in to be used. For details, refer to <u>Working with experiments</u>.

Note: Some steps may only become available after the previous one has been completed and after all required data have been entered. Required data are indicated with a yellow field. For details, refer to <u>General software usage</u>.

- 5. Empty the tip disposal box.
- 6. Before entering the **Worktable** step, ensure that the instrument is connected to the software. If the instrument is not yet connected, switch it on now.

Note: The instrument needs to be switched on and connected to the software before entering the **Worktable** step.

- 7. Close the lid.
- 8. Click **Next** to enter the **Worktable** step. The software automatically plans the worktable and checks the instrument calibration status. If the instrument has not been used before, it will initialize.
- 9. Prepare the reagents and samples.
- 10. Open the instrument lid.
- Load the worktable. Follow the instructions of the software as it guides you through all worktable positions. For each item to be loaded, add a check to the Loaded check box to confirm.
 Note: In order to confirm all Loaded check boxes in one step, e.g., for an input plate, click the Confirm all button below the table.

Follow the instructions in <u>Loading tips</u> and <u>Insert tips to tip rack holder</u> for details about how to place tips.

Follow the instructions in <u>Loading adapters and labware</u> for details about how to place adapters and labware.

Follow the instructions in Loading reagents and samples to ensure run accuracy.

Note: For details, press the F1 key or the Help button to open the help page.

Note: If you want to load the worktable in a different order from that proposed by the software, click the worktable position from the worktable selector. The software jumps to the corresponding step. Load the position and click **Next** or another yellow worktable position to load. Blue worktable position buttons will indicate when all worktable positions have been loaded.

Note: A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started. Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a <u>labware calibration worklist</u>. Ensure that you have the empty labware at hand for the calibration. For tubes from the kit, refer to the QIAgility Calibration Kit that was shipped together with the QIAgility. The first <u>XY-calibration</u> screen is displayed. Follow the loading instructions on the screen, and perform all XY- and <u>Z-calibration steps</u>. When the calibration steps have been completed, the worktable screen will be displayed again. Load (or re-load) all calibrated positions that are displayed in the table.

- 12. Close the instrument lid.
- 13. If the experiment has not yet been saved, save it now by clicking the **Save** button in the button bar. The file dialog opens. Select a folder and enter a name.

Note: If an experiment has been saved before, the software will always automatically save the latest changes to the experiment when the run is started.

- 14. Click **Start run** to start the run.
- 15. The run will start and the progress will be displayed in the **Run** step.

Note: The instrument follows a specified order when pipetting liquids. First, the master mix is pipetted, followed by the standards and controls, and then the samples.

6.1.1 Loading tips

The software tracks the tip usage during operation. The interactive graphic on the right side of the screen shows the current tip availability according to that tracking. However, the software cannot detect if tip racks have been reloaded or removed in the mean time.

An example below demonstrates the confirmed loading of a tip rack with 50 μ l tips on worktable position B2. The tip rack has been filled with 40 tips. A total of 136 tips are available for the run because the loading of a full tip rack with 50 μ l tips has already been confirmed for another worktable position.

B2 - Tip rack

Tip type	Required	Available	Loaded	
50 µl Conductive tip (QIAGEN)	105	136	V	

Tip loading instruction.

Required tip type

The required tip type to be loaded to the worktable position is displayed in the **Tip type** column of the table. Ensure that the tip rack you load matches the tip type specified here.

Note: To ensure optimal tip pickup, it is important to follow the instructions in the section <u>Insert tips to tip</u> rack holder.

Required number of tips

The required number of tips for the run is displayed in the **Required** column.

Note: The software always displays the total number of required tips of that type for the run.

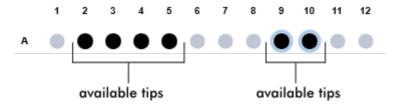
Note: If according to the tip tracking, the number of currently available tips of a particular tip type is not sufficient for the run, or if more than 96 tips of the same tip type will be required, the software will plan an additional worktable position for tips of that tip type, if possible. However, it is possible not to make use of this additional tip rack for a run (see **Confirm loading** below).

Tip availability

The software tracks the tip usage during operation. The interactive graphic on the right side of the screen shows the current tip availability according to that tracking. However, the software cannot detect if tip racks have been reloaded or removed in the mean time. For the run, the software relies on the information displayed in the graphic. Therefore, it is important to set the same tips available in the graphic as the tips that are placed on the tip rack on the worktable.

50 µl Cor	nducti	ve Fil	tered	Tips									
	1	2	3	4	5	6	7	8	9	10	11	12	
Α								•	•	ullet	ullet	•	
в								•	•	ullet	ullet	•	
с								•	•	•	ullet	•	
D								•	•	•	ullet	•	
E								•	•	ullet	ullet	•	
F								•	•	ullet	ullet	•	
G								•	•	ullet	ullet	•	
н								•	•	ullet	ullet	•	
Set all tip:	s 🕂	To av	ailable	;		Set a	all sele	ected	tips .	+ То	availa	able	
	-		availa							_		ailable	
		l	Intera	ctive r	ack to	defin	ne tip	availe	ability				
Use the ti	ip av	ailab	ility b	outtor	ns be	low t	he gi	raph	ic to	set th	ie av	ailabilit	y of the rack.
Option									-	uire		ion	
To set all	tips	to av	ailab	le						k on:			
									Set	all tips	+	To availa	ble
To set all	tips	to un	avail	able					Clic	k on:			
									Set	all tip:	s		
											—	To unava	ailable
To set se	lected	d tips	to a	vaila	ble								positions. Click on the letter select the whole row, the column
									num	nber 1	o sel	ect the	whole column or individual tip
										itions n clic			gle positions.
									Set	select	ed tips	s 🛨 To	available
To set the	e sele	ected	tips t	oun	avail	able			Clic	k on:			
									Set	select	ed tips	\$	
												- To	unavailable

Available tip positions are displayed in dark color (A2–5, A9–10) and unavailable positions are displayed in grey (A1, A6–8, A11–12). Selected tips are surrounded by a light blue circle (A9–10):



Available number of tips

According to the settings selected in the tip rack graphic on the right, the software will display the number of available tips of that tip type in the column **Available**.

Note: The software will display the total number of available tips of that type, summarizing the available tips of that tip type from all worktable positions.

Note: You can start the run, even if it is not possible to load enough tips. The software will pause the run at a certain time and ask you to reload more tips.

Confirm loading

Confirm that:

- you have loaded a tip rack
- of the correct tip type, and
- you have correctly set the tip availability in the graphic on the right

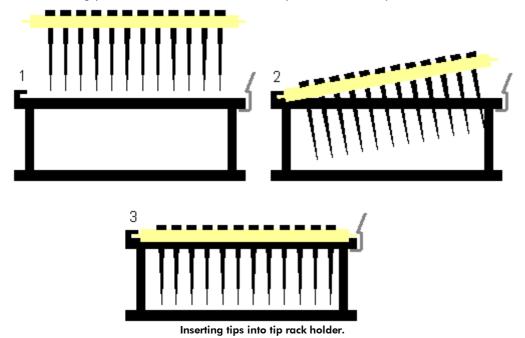
To confirm, either check the box next to **Loaded** in the table, or click on **Confirm all**.

Note: In order to indicate that a planned tip rack on a worktable position will not be loaded for the run (e.g., because there are already enough tips of that tip type on other worktable positions), set the tip availability for all tips of that worktable position to **Unavailable** and do not check the **Loaded** check box.

6.1.2 Insert tips to tip rack holder

To ensure optimal tip pickup, insert the tips as described below.

The following picture outlines how to insert a tip rack into the Tip Rack Holder.



Note: Sometimes, the tip rack holder may not fit perfectly into the worktable position. In this case, check that the tip rack holder is shifted towards the upper left corner of the worktable position and is aligned with the corner, to ensure calibration and run accuracy.

Note: Sometimes, the tip rack may not fit perfectly into the tip rack holder. In this case, check that the tip rack itself is shifted towards the upper left corner of the tip rack holder, to ensure calibration and run accuracy.

6.1.3 Loading adapters and labware

To ensure calibration and run accuracy, it is important that all adapters and labware are loaded correctly onto the worktable.

Adapters

Sometimes, an adapter may not fit perfectly into the worktable position. In this case, check that the adapter is shifted towards the upper left corner of the worktable position and is aligned with the corner.

Plates

Sometimes, plates that can be loaded directly onto the worktable may not fit perfectly into the worktable position. In this case, check that the plate is shifted towards the upper left corner of the worktable position and is aligned with the corner.

If the plate is loaded into an adapter, the plate may not fit perfectly into the adapter. In this case, check that the plate itself is shifted towards the upper left corner of the adapter.

Tubes

Check that the tubes fit correctly into the adapter. Sometimes, labels on the outside of tubes may prevent the tubes from sitting deep in the adapter. In this case, remove the label from the outside of the tube, or manually push the tube so that it sits deep in the adapter.

6.1.4 Loading reagents and samples

To ensure run accuracy, it is important that all reagents and samples loaded onto the worktable are free from air bubbles, including in the bottoms of the tubes. If necessary, briefly centrifuge the tubes to remove any bubbles.

For gene expression and pathogen experiments:

Use original QIAGEN kit reagents in their original tubes to ensure correct liquid level detection. The volumes displayed in the software are the minimum required volumes, so consider adding extra to avoid liquid level errors (i.e., "Insufficient liquid") during the run.

For viscous liquids (e.g., Master Mix), it is particularly important that you use the original kit reagent tubes, because they already contain extra liquid. If you transfer only the minimum required volume to another tube, the liquid level detection may detect insufficient liquid and display an error message.

For generic PCR. generic PCR with normalization, PCR with serial dilution and other workflow experiments:

For generic PCR runs, original QIAGEN kit reagents or self-developed reagents can be used in any available tubes.

The volumes displayed in the software are the minimum required volumes, already taking extra volume into consideration, to avoid liquid level errors (i.e., "Insufficient liquid") during the run. Keep in mind, especially for viscous liquids (e.g., Master Mix), that original tubes already contain extra liquid by production. It is also possible to define an additional volume for the input liquid.

6.2 Monitoring a run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table.

The instrument icon in the main toolbar as well as the progress indicator on the run step show the overall run progress.

Run progress

Progress
· · · · · · · · · · · · · · · · · · ·
U V
Approximate end time
17:59

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

6.3 Pause/cancel a run

A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Pausing and resuming a run

Every pause of a run will be recorded and listed in the Exceptions section of the report.

To pause a run

1. Click the **Pause** button.

2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished!

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	Pause
Overall progress		¥
Start time 13:05	Paused at 13:21	Resume

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

Stopping a run

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished!

6.4 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

Depending on the plug-in, several options may be available on the right side of the **Run** step screen. For details, refer to the specific chapters on <u>gene expression</u>, <u>pathogen detection</u>, <u>sample pooling</u>, <u>generic PCR</u>, <u>generic PCR with normalization</u>, and <u>PCR with sample dilution</u> experiments or click **Help** from the run screen.

Run info	
Operator	
John Smith	
Comment	
	ļ
Output labware	
Print barcode	Save output file
	Create report

Options of the Gene Expression plug-in

As an example, the Generic PCR Plug-in offers the following options:

Option	Description
Comment	You can enter a comment about the run in the Comment field. It will be part of the report.
Print barcode	You can print a barcode for the output labware; this requires that a printer is selected in the Global Settings tab of the Configuration environment.
Save output file	An output file can be saved for the cycler; this depends on the cycler configuration.
Create report	A report can be created for documentation purposes.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in <u>Cleaning the QIAgility</u>.

6.5 Finishing work and shutting down

For instructions on how to clean the QIAgility, refer to section Cleaning the QIAgility.

To shutdown the software and the instrument:

- 1. Close the lid if the instrument has been used. This allows the pipettor head to move to the home position.
- 2. Click in the top right corner of the screen, or select **Exit** from the **File** menu to shut down the software.

Note: The software cannot be closed if a run is still active. In this case, the software will display an error message.

- 3. The software shows a confirmation message if there are any unsaved changes in experiments. If you are unsure, click **Cancel** and save the changes first. Otherwise, click **OK** to confirm that the software should discard all changes and shut down.
- 4. If the QIAgility has been used, the instrument discards the tip, completes its homing routine, and then the pipettor head lowers to a known safe location.
- 5. If the QIAgility has been used, the software displays a message for a few seconds when it is safe to switch the instrument off. Then the software closes.
- 6. If the instrument is on, switch it off now.
- 7. As the instrument is switched off, a small click occurs as the pipettor head drops 1–2 mm further. All the instrument axes are disengaged and can be moved freely.

Note: We recommend that you close the software at the end of the working day.

Note: Normally, you should not switch off the instrument without closing the software. If the pipettor head holds a tip it will not be discarded. As the instrument is switched off, the pipettor head will drop approximately 70 mm. However, if it is necessary to switch off the instrument without the software, this will not damage the instrument but may result in a tip being picked up.

QIAgility Setup Manager Software

7 QIAgility Setup Manager Software

QIAgility Setup Manager Software is an easy-to-use application with a modern user interface to assist you in your workflow. It has been specially developed for use with the QIAgility instrument and uses a modern task-focused GUI-design to facilitate the interaction with the software.

QIAgility Setup Manager Software is optimized for the use of QIAGEN kits. It provides a plug-in concept whereby each plug-in has its own experiment setup, specialized to the needs of the kind of experiments and supporting a specific set of kits.

Find more information on the plug-in of your choice in the following sections:

- Working with Gene Expression experiments
- <u>Working with Pathogen Detection experiments</u>
- <u>Working with Sample Pooling experiments</u>
- <u>Working with Generic PCR experiments</u>
- <u>Working with Generic PCR with normalization experiments</u>
- Working with PCR with serial dilution experiments
- <u>Working with Other Workflow experiments</u>
 - Working with Normalization Only experiments
 - Preparing Reaction Mix experiments
 - Preparing and Distributing Reaction Mix experiments
 - Preparing Standard experiments

For detailed information about which kits are supported, refer to www.giagen.com/goto/QIAgility.

7.1 Basic concepts and general software usage

The following chapters will give you an insight into the underlying concepts and the general software usage.

7.1.1 Concepts

The following concepts are essential to understand how QIAgility Setup Manager Software works and get the most out of your workflow:

- User roles
- Experiments and templates
- Plug-in concept
- Protection of files

7.1.1.1 User roles concept

User roles are an essential concept in the QIAgility Setup Manager Software. A user role defines the rights and permissions of a user. At least one user role must be assigned to every user. There are two user roles in the QIAgility Setup Manager Software:

- Operator
- Administrator

The user roles define the rights and permissions a user can have in the QIAgility Setup Manager Software. The following table describes the permissions required to enter the tabs for specific environments:

Envi	Home Experiment Configuration						Instrument
Ensitonment Role			User Manager	Labware Manager	Calibration	Settings	
Operator	~	~	×	~	√ (except Volume)	(except Global Settings	~
Administrator	~	\checkmark	\checkmark	\checkmark	\checkmark	✓	\checkmark

Envi	Service							
Environment Role	Cleaning Procedure	Maintenance	System Check	Support Package	Audit Trail			
Operator	\checkmark	\checkmark	✓	\checkmark	×			
		(except Replace HEPA Filter)						
Administrator	\checkmark	\checkmark	✓	✓	\checkmark			

The user role is assigned when a new user is created.

Note: Multiple user roles can be assigned to a user. A specific user can have both operator and administrator user roles. However, in this case, use the user role administrator.

Note: For the initial instrument setup, it is convenient to work with a user who has the administrator user role assigned.

7.1.1.2 Experiments and templates concept

To facilitate the process of defining experiments, the QIAgility Setup Manager Software provides the option to use templates.

The general workflow is to select a plug-in from the **Home** environment, define the experiment in the <u>Experiment environment</u> and finally <u>start the run</u>. It is not necessary to finish the definition of an experiment in one step. A save function is available at any time, allowing you to save your progress and continue later. To facilitate the experiment definition, you can use <u>templates</u> or reuse previously run experiments.

• Save an experiment as a template for later reuse.

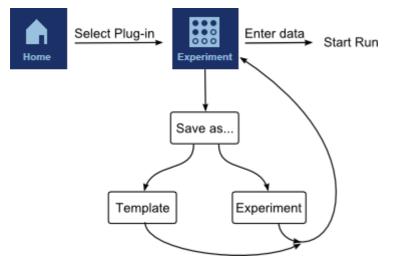
Templates are previously saved experiments that inherit the data of the experiment. The software allows you to save the experiment as template at different steps. For more details, refer to <u>Create a template</u>. <u>Experiments created using a template</u> will automatically be populated with data from the selected template. Use this function to facilitate frequently performed runs with the same experiment setup.

• Save an experiment as a quick-order template for later reuse.

This option is available for experiments created with the Generic PCR Plug-in, only. Use this function to facilitate runs on a regular basis with exactly the same experiment setup. Quick-order templates are previously saved experiments that inherit the complete data of the experiment, including assignment and layout of the output labware. For more details, refer to <u>Create a template</u>. Experiments created using a quick-order template will automatically use the complete run setup data from the selected template. The settings are not visible and cannot be modified for the current run setup. Instead, experiment setup is reduced to sample definition and loading the worktable. Always keep the original experiment for your reference. Sample names need to be defined for the same input positions as in the original experiment, however, not all of these input positions need to be used in the run. In this case, worktable calculation will create different positioning and volume of reagents.

• Use a previously saved experiment to create a new experiment. Use this function if you want to repeat the same experiment again. For more details, refer to <u>Create an experiment from an existing experiment</u>. If you often create an experiment based on the same experiment, consider creating a template and to adding it to the favorite templates list.

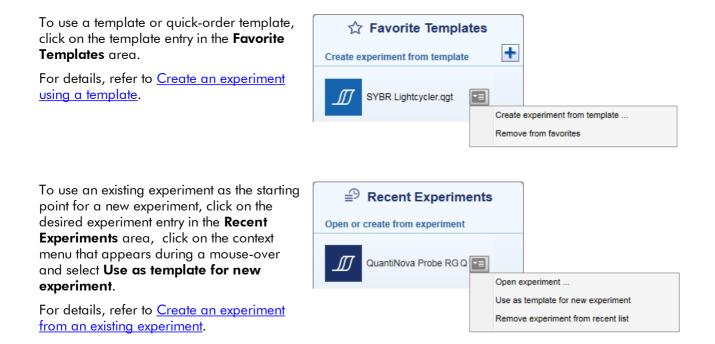
The following graphic explains these functions:



Experiments and templates have different file extensions, which are also plug-in specific. For example, the file extension for gene expression experiments is ***.qsmgx**, and for templates is ***.qsmgt** (**Q**|Agility **S**etup **M**anager **G**ene Expression Experiment and Template, respectively). Generally, the file extension for an experiment ends with **x**, and for a template with **t**. The file extension for a quick order template ends with **qt**.

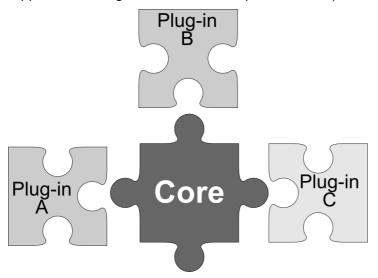
Templates and experiments can be selected from the **Home** environment, to set up a new experiment based on the experiment data in the selected template.

🔜 QIAgility Setup Manager				– 🗆 X
File Edit View Help 🖛 🦡				0
QIAGEN	Experiment Service Configuration			Instrument
ſ	Create Experiment	☆ Favorite Templates	≣ ⁹ Recent Experiments	
	Create experiment by wizard	Create experiment from template	Open or create from experiment	
	Gene-Expression	C:\Users\Public\QIAGEN\QIAglilty\Templates	PCR -experiment.qsmrx	
	Pathogen Detection		PCR and normalization expe	



7.1.1.3 Plug-in concept

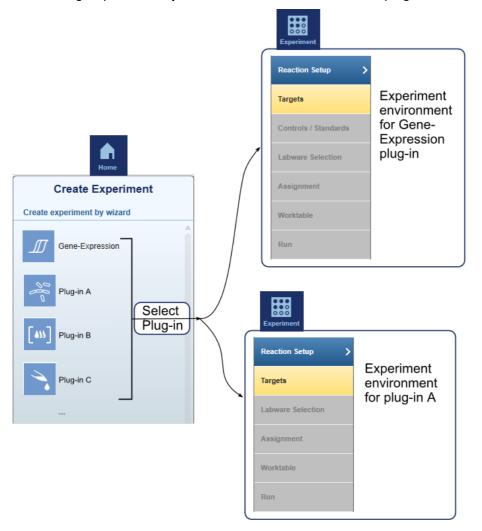
Plug-ins provide users with more specialized ways to set up experiments (e.g., more or less restrictions, support in handling controls and other special features).



A plug-in comes with its own experiment setup, report and settings (e.g., notification settings).

All available plug-ins are listed in the **Create Experiment** area of the **Home** environment. When you click on one of the plug-ins to create a new experiment, the **Experiment** environment will be opened and the steps of the step marker will be created according to the instructions of the plug-in. The steps, step names, number of steps and their order are all plug-in dependent. Refer to following sub-chapters of <u>Working with</u> <u>experiments</u> for detailed information about the different experiment setups.

The following graphic illustrates the relationship between selecting a plug-in in the **Home** environment and the resulting steps in the **Experiment** environment for different plug-ins:



7.1.1.4 Protection of files

QIAgility Setup Manager Software guarantees the protection of records. The system ensures that no user can modify or delete any record.

Files, configuration files and experiments are protected using checksum, which enables detection of inappropriate modifications. A checksum is a numerical value assigned to a file by the QlAgility Setup Manager Software, and is based on the content of the file. The file is invalid if the checksum does not match the file's content or is missing.

In the event that a file is modified outside the QIAgility Setup Manager Software, the file can no longer be used. If the modified file is a configuration file, it is possible that the software can no longer be used. If the modified file is an experiment, the experiment can no longer be loaded.

Note: We strongly recommend not altering the software files outside the software environment.

7.1.2 General software usage

The following section describes the GUI (graphic user interface) and the user interaction provided in the QIAgility Setup Manager Software. Fundamental topics such as entering data and working with tables are explained:

- Use of color
- Displaying errors and warnings
- Entering data
- Working with tables
- <u>Selecting samples</u>

7.1.2.1 Controlling the mouse

The following terms for controlling the mouse are used in this manual.

Left-click	Click with the left mouse button.
Click	Click with the left mouse button.
Right-click	Click with the right mouse button.

7.1.2.2 Use of color

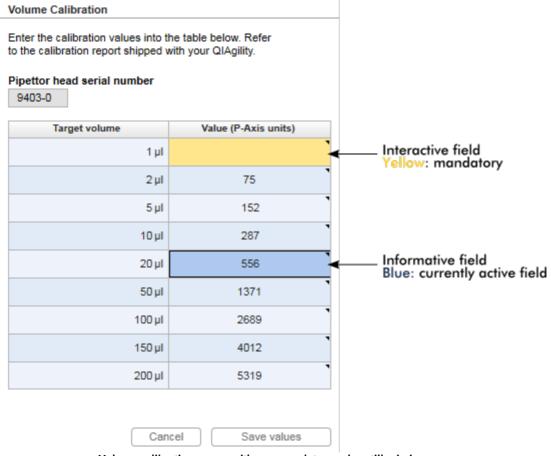
For an optimal user interaction, the QIAgility Setup Manager Software uses a dedicated color concept for presenting information. The coloring assists you in your workflow.

The following table provides an overview of the different colors used in the software and their meanings:

Color	Description
Light blue	The field is interactive and clickable.
Dark blue	The field is selected or focused.
Gray	The field is read-only and cannot be activated.
Yellow	The field requires input.

The following examples give an explanation of the color concept.

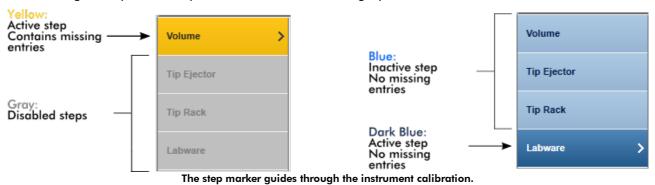
Example 1



Volume calibration screen with one mandatory value still missing.

Example 2

During experiment setup and instrument calibration, a step marker guides you through the necessary steps. The coloring concept of the step buttons is illustrated in the graphic below:



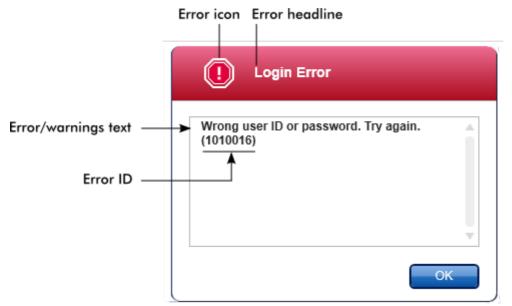
The active **Volume** step still requires missing values. Because the **Volume** calibration is required before any other calibration, the following steps are still disabled and inactive. If the **Volume** step contains all mandatory entries, it changes to blue color, and the next step **Tip Ejector** becomes available. It will remain yellow as long as the required entries or tasks are missing.

7.1.2.3 Displaying errors and warnings

Errors and warnings are essential information that indicates a problem or an erroneous situation. QIAgility Setup Manager Software differentiates between 4 different problem levels:

Priority	Name	lcon	Description of the functionality	User action
1	System error		A combination of unacceptable incidents	User interaction required
2	Validation error	Δ	An error that occurs due to a missing or invalid user input	User interaction required
3	Warning	Δ	Situation could be optimized by further input	User interaction possible, but not mandatory
4	Information	(j)	A message containing additional information about the current situation	User interaction not possible

The general structure of an error message is described below. When communicating with QIAGEN Technical Services, provide the error ID, which is unique and unambiguously characterizes the error situation. If you receive a message that contains a **Help** button, click on it to get more information.



7.1.2.4 Entering data

Shortcuts

The following hot keys are available in QIAgility Setup Manager Software:

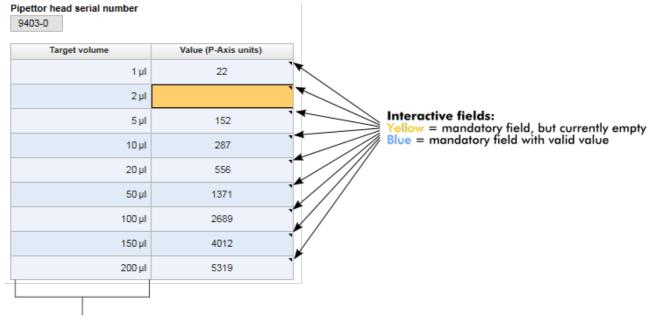
- F1 to open the help file
- Copy and paste operations (CTRL + C and CTRL + V)
- Navigation (tab key, cursor keys)

While entering data, the following keyboard shortcuts can be used:

- Escape to cancel the input
- Return to commit an input

Identifying interactive fields in tables

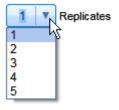
In <u>tables</u>, all interactive fields into which data can be entered are marked with a black triangle symbol (\mathbb{N}) in their upper right corner. Left-click in the field with the mouse, and then enter data.



The following example is taken from the volume calibration step in the Configuration environment:

Non-interactive fields: Preset values, cannot be modified No ▼-icon available

Editable menu: adding new data to an editable menu

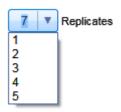


If a menu is editable, its text becomes selected if you click on it. The value list can be expanded by clicking on it.

If the value you need is missing, simply start typing the value as long as the text is selected. If the value list is empty, simply start typing into the (light-blue) menu field.



In this example, the value 7 has been entered.



The entered data (in this example, 7) can be used as value. However, it is not added to the list of values.

Date picker: entering date in date fields

Dates can either be entered manually into date fields using the keyboard or by using an interactive date picker in the form of a pop-up calendar (). A calendar icon is positioned to the right of every date field.

Kit expiry date Kit expiry date **==** May, 2015 Su Tu We Мо Th Fr Sa Today : 5/18/2015

Change to the previous or next month by clicking the arrow icons (and). Hovering over the year label displays additional control arrows, which can be used to quickly jump to the next (up arrow) or previous year (down arrow):



Clicking on the calendar icon opens the pop-up calendar.

Step-by-step procedure to enter a date using the date picker

- 1. Click on the calendar icon () next to the date field. The calendar pop-up is shown.
- 2. Continue using the following scheme:

То	Do this		
Change the year	Hover the mouse pointer over the year.		
	Date is displayed in blue. Additional control arrows are shown.		
	Click the up arrow to change to the next year.		
	Click the down arrow to change to the previous year.		
Change the month/day	Click the left arrow to change to the previous month.		
	Click the right arrow to change to the next month.		
	Click the date of the desired day.		

3. The date picker disappears and the date field is populated with the selected date.

7.1.2.5 Working with tables

Tables are an essential element of the QIAgility Setup Manager Software. They are widely used to display and enter data. The interactive icon (****) marks cells in which you can enter data.

Example: Table to enter sample names

Inpu	ut				Graphic	List	
Pos.	Sample ID		Conc. [ng/µl]	Description			
1	Sample 1	٦	•	1			
2	Sample 2	٦	٦				
3	Sample 3	٦	٦				
4	Sample 4	٦	•				
5	Sample 5	٦	•				
6	Sample 6	٦	•				
7	Sample 7	٦	•				
8	Sample 8	٦	•				
9	Sample 9	٦	•				
10	Sample 10	٦	`				
11	Sample 11	٦	`				

Entering data:

- To enter data into one cell, left-click in the cell and then enter the data.
- To move to the cell below, press the **Enter** key.
- To move to the right cell, press the **Tab** key.

This table has a context menu that is opened when you right-click on a cell:

Сору	Ctrl+C
Paste	Ctrl+V
Auto-generate sample names	

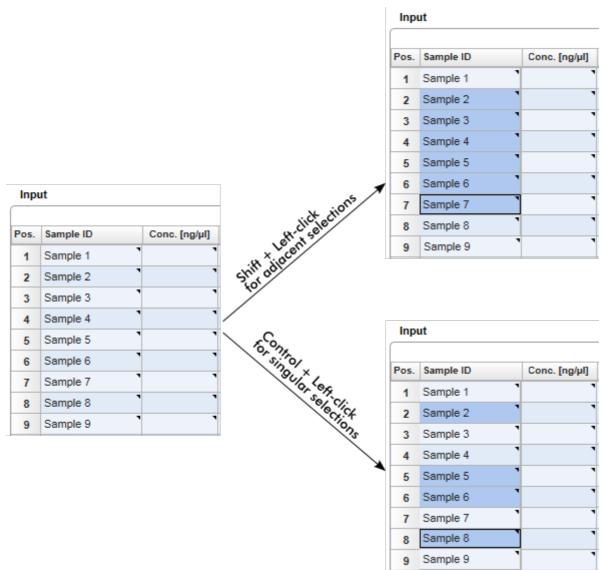
Option	Description
Сору	Copy the content of the selected cell(s) to the clipboard
Paste	Paste the clipboard content to active table cell(s). If the clipboard contains data from multiple cells, the table cells will be consecutively filled with the pasted data.
Auto-generate sample names	Sample names are generated automatically for the selected cells by taking the text in the selected cell and adding an incrementing number to the text. Example: If a cell contains "Sample," then the sample IDs Sample 1, Sample 2, etc. are generated.

7.1.2.6 Selecting samples

You can use left-clicking in combination with the **Shift** and the **Control** keys to make selections in a table or in a sample graphic:

Note: For selecting of samples, click the Pos. column and not the Sample ID column.

Shift + Left-click	To select adjacent table cells, first click on the starting cell for the selection, hold down the Shift key, move the cursor to the last cell in the selection, left-click, and release the Shift key.
Control + Left-click	To select singular cells, hold down the Control key while left-clicking on the desired cells. The selected cells will be added to the selection.
Left-click and drag	To select a rectangular area of positions.
	For rectangular sample graphics only.



The following illustrates the selection in the sample names table:

7.1.3 General elements

QIAgility Setup Manager Software uses a modern task-focused GUI design to facilitate the user's interaction with the application. The following general user interface elements are described in this section:

<u>Menu</u>

<u>Main toolbar</u>

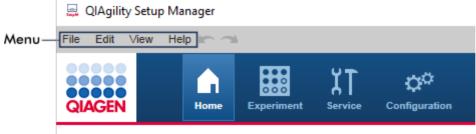
Button bar

<u>Status bar</u>

7.1.3.1 Menu

The main menu has four menu entries:

- File
- Edit
- View
- Help



Main menu

File menu

File	Edit View Help			
	Create new experiment	-	Gene-Expression	
	Create new experiment from template		Pathogen Detection	
	Open experiment		Sample Pooling	
	Save		Generic PCR setup	
	Save as		Generic PCR setup with Normalization	
	Login	PCR Setup with Serial Dilution		
	Logout		Other workflows	
	Change password	nt	by wizard	Create
	Service login			
	Exit	Exp	ression	C:\Use

Option	Description
Create new experiment	Create a new experiment without any data. The default experiment name is set to Experiment_MM_DD_YYYY.
Create new experiment from template	Create a new experiment by selecting a previously saved template file. The data of the new experiment will be populated with all available experiment data from the template file; sample information will not be included.
Open experiment	Open an experiment.
Save	Save the current experiment. If you have not changed the default name (Experiment_MM_DD_YYYY), the file selection dialog is displayed in which you can enter the new file name along with an optional comment.
Save as	Save the current experiment under a new file name.
Login	Log in to the application. This option is only available after software start or logout.
Logout	Log out of the application. The login screen will be shown.
Change password	Change your current password. The Change password dialog is shown in which you will enter your current password and the new password.
Service login	This login is used by QIAGEN service personnel only.
Exit	Close the application.

Edit menu

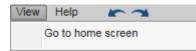


Option	Description
Undo	Undo the last operation.
Redo	Redo the last operation.

Note: As an alternative to using the Edit menu, you can use the undo and redo commands by clicking on the following icons in the menu:

File	Edit	View	Help	-	~
				 Undo	¶ Redo

View menu

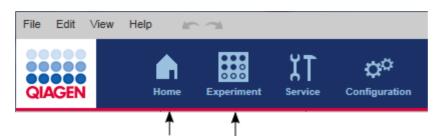


Option

Description

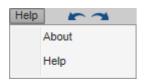
Go to home screen

Change to the home screen.



Note: As an alternative to using the View menu to change to the Home screen, you can also use the Home and Experiment buttons from the main toolbar to switch between these two environments.

Help menu

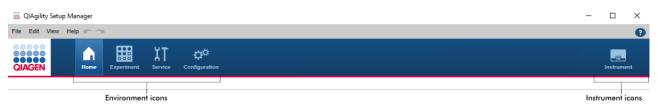


Option	Description
About	Opens the About dialog with the copyright notice and version number (at the top right) of the software.
Help	Opens the software help file.

7.1.3.2 Main toolbar

The main toolbar contains two areas:

- Environment icons
- Instrument icons



Environment icons

You can switch to another environment by clicking the appropriate button from the main toolbar. The icon of the currently active environment is highlighted.

Environment	Description
Home	Used to jump to the home screen. From here, you can create or open experiments. (<u>Home</u>)
Experiment	Used to set up experiments. (<u>Experiments</u>)
XT Service	Bundles tasks for maintenance, system check and support of QIAgility. (<u>Service</u>)
Configuration	Bundles the tasks' custom settings, user and labware management, and calibration. (<u>Configuration</u>)
Instrument icons	

The instrument icon is placed on the right of the toolbar. Use it to inspect the connection, running and maintenance status of the instrument.

If you click the instrument icon, the software switches to:

- The run screen of the running experiment, if a run is in progress
- The maintenance schedule, if no run is in progress

Symbol	Description	Action						
Instrument	Instrument connected.	A run can be started.						
۱ ۰ ۰	Instrument not connected.	Prior to starting a run, switch on the instrument.						
Instument		Note: It can take some time until the software connects to the instrument. For details, refer to <u>Start the software</u> .						
		Note: The icon cannot be clicked.						
Instrument	Instrument running. The progress bar below the icon shows the overall run progress.	If you are in the process of setting up the next experiment, you can click the icon to switch to the run step of the running experiment.						
		To return to the setup, click the experiment name of the experiment you worked on.						
	Run has just finished. The progress bar shows run completion.	The same as for running instrument.						
Instrument	An error occurred during the run.	Click the icon to switch to the run step of the current experiment to show the error message.						
VER	The instrument is not fully calibrated.	Before you can start a run, <u>instrument</u> <u>calibration</u> needs to be performed or completed.						
Definition	A service task is due. After the reminder, the symbol persists until the service task has been completed successfully.	If no run is in progress, click the icon to switch to the <u>maintenance schedule</u> for details.						

7.1.3.3 Button bar

The button bar is placed at the bottom of the screen. It contains buttons specific for the selected screen of an environment. Generally, the buttons for saving, exporting and importing can be found here.

📃 QIAgility Setup Mar	nager														-		×
File Edit View Help																	0
QIAGEN	Home Experiment	Service C	CC onfiguration													Instrume	nt
Settings L	abware Manager	Calibration	User Manager														
Volume	> Volume Calib	ration															
Tip Ejector			o the table below. Refe d with your QIAgility.	r													
Tip Rack	Pipettor head 123456-0	l serial number															
Labware	Targe	et volume	Value (P-Axis	units)													
Labware		1															
		2															
		5	ul 152.81														
		10	ul 287.22														
		20	ul 556.13														
		50	ul 1371.31														
		100	ul 2689.65														
		150	ul 4012.85														
		200	ul 5319.34		1												
		C	ancel Save	values													
					A Bac	ck)	No	d 🕨	_		 - Far	ort calibra	tion data		Import		
						on	Ne			_	Ext	ion calibra	mori uata		Impon		
												Au	gust 19, 20	019	admin ad	lmin	⇒₽

7.1.3.4 Status bar

The status bar is always visible and gives an overview of the session status.

	May 27, 2015	admin admin	→阗
<u></u>	Date	User name	Log out button

Button	Description
Date	Show the current date.
User name	Shows first and last name of the user who is currently logged in.
Log out button	Logs out the current user. If there are unsaved data, a warning will appear.

7.1.3.5 File dialog

The file dialog is used in various situations to open or save experiments or templates, to import sample information, or to save a report or an output file after a run.

File Path UNC path	
Look in Experiments	T
Path C:\Users\Public\QIAGEN\QIAgility\Experiments	
Name	▲ Last modified
Rotor-Gene SYBR Green PCR Demo Kit (80).qsmgx	7/26/2019 2:03:14 PM
ile name	File type
ile name	File type Gene-Expression (*.qsmgx)
ile name	

Path indicates the current path. The initial path shown when the file dialog opens can be configured in the user settings for the plug-in.

Note: When the file dialog opens for the first time after clicking the **Open other experiment** or **Create** from other template button from the **Home** screen, the initial path will be **C**:.

The list in the middle of the dialog shows the sub-folders of the current path and all files with the extension selected in the **File type** menu.

Name 🔺	Last modified	
Examples	10/24/2016 3:51:07 PM	
Experiments	12/6/2016 1:33:43 PM	
Log	10/24/2016 3:54:27 PM	U
QIAgilityData	11/30/2016 5:27:16 PM	
Reports	11/2/2016 5:57:17 PM	
SampleFiles	10/31/2016 10:51:37 AM	▼

List of sub folders.

To open a file:

Navigate to the folder where the file is stored. 1.

Use the **button** to navigate to the parent folder.

The **Look in** menu provides the option to select another drive by clicking the drive letter, or to select one of the parent folders by clicking it. The list shows all drive letters that are configured in the Windows file explorer.

Look in	Experiments v	
	C: Users Public QIAGEN QIAGEN G: F: G: J: P: S: U:	

To navigate to a sub folder, either left-click the sub folder name in the list and then click **Open**, or double-click the name.

Name 🔺	Last modified	
Examples	10/24/2016 3:51:07 PM	
Experiments	12/6/2016 1:33:43 PM	
Log	10/24/2016 3:54:27 PM	U
QIAgilityData	11/30/2016 5:27:16 PM	
Reports	11/2/2016 5:57:17 PM	
SampleFiles	10/31/2016 10:51:37 AM	▼

The **Path** changes to the sub folder and the list shows the content of the selected sub folder.

2. Ensure that the correct file type is selected. The **File type** menu lists all file extensions that are available for the current situation. The various file extensions are described further below.

•

Experiment types

If you change the selected file type, the list of files will be updated to show all files of the newly selected file type.

3. To open the file, either left-click the file name in the list and then click **Open**, or double-click the file name to open the file.

To save a file:

- 1. Navigate to the folder where you want to store the file. Use the navigation options described above.
- 2. Ensure that the correct file type is selected.
- 3. Enter the file name into the **File name** field.
- 4. Click **OK** to save the file.

File extension	Description
*.qsmgx	Gene expression experiment
*.qsmgt	Gene expression template
*.qsmpx	Pathogen detection experiment
*.qsmpt	Pathogen detection template
*.qsmpoolix	Sample pooling experiment
*.qsmpoolit	Sample pooling template
*.qsmrt	Generic PCR experiment
	Generic PRC with normalization experiment
	PCR with serial dilution experiment
	Other workflows
*.qsmrt	Template
*.qsmrqt	Quick order
*.xml, *.csv, *.txt	For sample input files
	Note: Find examples in the folder: C:\Users\Public\QIAGEN\QIAgility\Examples
	Note: For *.csv and *.txt files it is important to adhere to the syntax. In general, the file represents a comma separated table (CSV), where each line in the file represents a row in the table and a comma (,) represents the cell border to the next column. Like in a table, it is possible to leave the content of a table cell empty, but you cannot omit the commas in a line.
*.xml	For saving an output file
*.smp	For saving an output file using the XSLT converter for Rotor-Gene
*.txt	For saving an output file
*.pdf	Note: The file type of output files depends on the selected converter. For reports

The QIAgility Setup Manager Software provides the option to use **UNC** syntax to open files from and save files to network shares that are not directly known in the Windows file explorer.

Open Experiment	
File Path UNC path	
Enter UNC path	
Enter the path to the network share in UNC syntax, e.g. \\path\to\network\share. Then press "Test UNC path" to check if the entered path is valid and can be accessed.	Test UNC path
File name	File type *.qsmgx
Short description	
	Open Cancel

- 1. In the file dialog, click **UNC path**.
- 2. Enter the path to the network share using the UNC syntax (e.g., \\path\to\network\share).
- 3. Press the **Test UNC path** button to test whether the entered path is valid and can be accessed.
- 4. Enter the file name without the file extension into the **File name** field.
- 5. Select the file extension from the **File type** menu.
- 6. Optionally, enter a short description of the file.
- 7. Click **Open** or **OK** to open or save the file, respectively.

If you encounter issues accessing the network share, contact your network administrator and ensure that the path has been entered correctly and is accessible.

7.1.3.6 Import data dialog

The **Import data** dialog is used in various workflows of the Generic PCR Plug-in to import sample information from a **.csv** file. The dialog is available from the **Samples/Labware** screen by clicking **Import data from CSV file**.

- The dialog for the input labware can be opened from the Labware identifier menu at the top.
 Note: If you are using more than one labware, you have to choose first the correct input plate from the Labware identifier menu.
- Click Select file. The file dialog opens and shows all files with file type .csv that are available in the Sample Input Files folder, as specified in the <u>QG_PGP_user_settings</u>. Select the desired file and click Open. For details about the file dialog refer to <u>File dialog</u>.
- 3. The software loads the file into the dialog. The **Raw data** field shows the sample data as present in the file. In general, the data in the file are expected to represent a comma separated table, where each line in the file represents a row in the table, corresponding to a sample position, and a column delimiter represents the cell border to the next column. The **Preview** table below shows how the data would be imported into the samples table of the software.

Mart Data
Labware IdentifierInput_1_20180503_141101
Import from file C:\Users\Public\QIAGEN\QIAgility\SampleFiles\Samples Example.csv Select file
Raw data Sample ID;Concentration 1;0.001 2;0.01 3;0.04 4;0.1
Import format profile none
Preview Conc. [ng/µl] A1 1 0.001
C1 3 0.04 ▼ ✓ Advanced import format parameter Column delimiter Cell delimiter Start import from row
Semicolon (;) V None I Import data row contains header information Read every V row Import data sequentially to selected labware Keep original well position Sequence Start at position Import positions ColumnByColu A1 V V Import sample ID Import conc. values Import sample description
Sample ID Concentration Save current parameters as profile OK Cancel

Import data dialog

- 4. If the data in the **Preview** table are displayed correctly, proceed to step 9.
- 5. **Optional:** If the parameters to import from certain file types have been saved previously (see step 7), select them from the **Import format profile** menu. The **Preview** table changes according to the parameters in the selected profile. If the data are displayed correctly, proceed to step 9.

- 6. Tick the box next to **Advanced import format parameters**. Additional options and parameters become visible.
- 7. Change the parameters according to your needs. The **Preview** area changes according to the currently selected parameters.

Option	Description
Column delimiter	Click the menu below to select the character that delimits the columns in a row, i.e., this character is repeated between the different properties of the sample position in the Raw data field.
	If you change the selection, a previous column selection in options Import sample ID , Import conc. values and Import sample description , below, will be cleared.
Cell delimiter	Click the menu below to select a cell delimiter. Such a character could be used to surround the characters of a (e.g., description) property. This might be necessary, if the property value includes the column delimiter character. If you are unsure, start by selecting None .
	Example: In this example, the column delimiter is , (comma), and the file contains a description text of a sample like this – Could be difficult, but give it a try. With cell delimiter None , the "," inside the description text would lead to an incomplete import of the description value Could be difficult . To solve the incomplete import of the description text, use a cell delimiter in the following way: in the file, surround the description of that sample with quotation marks, e.g.," Could be difficult, but give it a try. " Then for import, select this cell quotation mark delimiter ("). The software will recognize that all characters between the quotation marks will belong to the same cell and will import the description text correctly – Could be difficult, but give it a try.
	If you change the selection, a previously made column selection in options Import sample ID , Import conc. values and Import sample description , below, will be cleared.
Start import from row	Count the lines in the Raw data field until the line where the actual sample data start (including a possible header row describing the columns). If the data starts in the very first line, enter 1.
First data row contains header information	Tick the box if the line with the number entered in the Start import from row field contains the column header. If ticked, the headers are available for

	selection in options Import sample ID , Import conc. values and Import sample description , below.
Read every row	The software expects exactly one row in the .csv file per sample/replicate .
	If the sample file contains exactly one row per sample/replicate, do not tick the box. During import the software will read every data row from the file.
	Tick the box, if the sample file contains several rows for each sample/replicate, e.g., based on target. In this case specify which rows shall be read.
	Initially, the box is not ticked.
	Note: If the selections made lead to the wrong rows per sample, enter the number of the first row of the file to be read into the field Start import from row and clear the box next to First data row contains header information , even if the file contains column header information.
Import data sequentially to selected labware	Select the option, if you want to import the sample data from the file subsequently to the positions in the labware. As a consequence, positions would be filled up, if the sample file does not contain information for all subsequent positions.
	Specify Sequence and Start at position.
	If selected, the options Sequence and Start at position become active and the option Keep original well position becomes unselected.
Sequence	Only active, if the option Import data sequentially to selected labware has been selected and if the selected labware supports this option.
	Select one of Column by column or Row by row.
Start at position	Only active, if the option Import data sequentially to selected labware has been selected.
	Click the menu below to select the position on the labware for which the import shall start. The menu contains all positions of the currently selected labware in the order specified by option Sequence . The first labware position is preselected. You can select another position, if, for example, you want to leave the first row or column empty.
	Example: If you selected Column by column and Start at position A2, the first column on the labware will be empty, the data of the sample described in the

	first data row (after a header, if present) of the file will be imported into position A2, the data from the sample in the next row of the file into position B2, and so on.
Keep original well position	Select this option, if you would like to keep the sample positions.
	Specify the file column that contains the sample positions in the option Import positions .
	If selected, the option Import positions becomes active and the option Import data sequentially to selected labware becomes unselected.
Import positions	Only active if the option Keep original well position has been selected.
	From the menu below, select the column that contains the sample positions.
	If the option First data row contains header information has been selected, the menu contains the column headers. Select the appropriate column. Otherwise, the menu contains Column 1 , Column 2 . In this case, count the columns in the Raw data field starting at the left and select the appropriate column.
Import sample ID	Tick the box, if you want to import sample IDs (sample names). In the menu below, select the file column which contains the sample IDs.
	If the option First data row contains header information has been selected, the menu contains the column headers. If the menu does not contain columns as expected, check the selection of the Column delimiter. Otherwise, the menu contains Column 1, Column 2. In this case count the columns in the Raw data field starting at the left.
	Select the appropriate column. Check if the correct column has been selected in the Preview table.
	If data in the Preview table are not as expected, check the parameters above and correct if needed. The column selection is cleared, if the column or the cell delimiter have been changed.
Import conc. values	Tick the box, if you want to import concentration values. In the menu below, select the file column that contains the concentration values.
	If the option First data row contains header information has been selected, the menu contains the column headers. If the menu does not contain columns

	as expected, check the selection of the Column delimiter . Otherwise, the menu contains Column 1 , Column 2 . In this case count the columns in the Raw data field starting at the left.
	Select the appropriate column. Check if the correct column has been selected in the Preview table.
	If data in the Preview table are not as expected, check the parameters above and correct if needed. The column selection is cleared, if the column or the cell delimiter have been changed.
	Note: If the concentration value for a sample is given as N/A in the file, the sample will be imported, but its concentration value will be empty.
Import sample description	Tick the box, if you want to import sample descriptions. In the menu below, select the file column that contains the sample descriptions.
	If the option First data row contains header information has been selected, the menu contains the column headers. If the menu does not contain columns as expected, check the selection of the Column delimiter. Otherwise, the menu contains Column 1, Column2. In this case, count the columns in the Raw data field starting at the left.
	Select the appropriate column. Check if the correct column has been selected in the Preview table.
	If data in the Preview table are not as expected, check the parameters above and correct if needed. The column selection is cleared, if the column or the cell delimiter have been changed.

- 8. Repeat steps 5-7 until the data are correctly displayed in the **Preview** area.
- 9. **Optional:** Save the currently selected parameters for reuse in an import profile by clicking **Save** current parameters as profile, if you would like to load files with the same format for future experiments (in this case go to step 3).
- 10. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 2–9.
- 11. Click **OK** to import the file and to close the dialog.

7.1.3.7 Using the labware browser

The **Labware Browser** is used to select the input and output labware for a run, if you decide not to use the labware proposed by the protocol. It provides access to the labware that is supported by the QIAgility Setup Manager Software. Filter options can be used to restrict the search to labware that has been indicated as **Available in the laboratory** in the **Labware Manager**.

You can access the **Labware Browser** from the **Labware Selection** step of an experiment, by selecting **Choose other labware** from the **Input labware type** or **Output labware type** drop-down menu.

Shows available labware only QIAGEN S-Block 96 Well Name QIAGEN S-Block 96 Well Name QIAGEN S-Block 96 Well Advanced options Dead Volume 40 µl Dead Volume 40 µl 1	Iter options ategory Standard 96 Well	Labware Select the item that most closely matches the labw QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS	Catalog number	
Name of labware Dead Volume Part number 1 Part number 1 Manufacturer All All V Labware adapter Select the adapter to be used. Catalog number Catalog number			Name	
Part number A 000000000000000000000000000000000000	-			
Manufacturer All Image: Constraint of the state of th	Part number			A 000000000000000000000000000000000000
All The / Well size All The Apply filter Reset filter Apply filter Apply filter	Vanufacturer			
All All All All All All Catalog number Catalog number			T	
Reset filter Apply filter				
			Catalog number	
			Name	

Labware Browser

The **Filter options** area on the left is used to search for a specific labware.

The **Labware** and **Labware adapter** area in the middle shows the search results together with a picture and further details.

To select input or output labware for a run:

1. In the filter options, select the labware category from the **Category** drop-down menu. Find a description of all filter options below.

2. Select the option **Shows available labware only**.

Note: For the run, only labware that has been indicated as **Available in the laboratory** in the **Labware Manager** can be used.

- 3. Use **Apply filter** to show all available labware of the selected category.
- 4. In the middle of the screen, select the desired labware from the list at the top. The software displays a picture and further details about the selected labware on the right.

If the desired labware is not listed, either select another available labware, or uncheck the **Shows available labware only** option to search it among all supported labware. You can use the **Advanced** filter options to restrict the search by other criteria.

Note: Before you can use the labware for a run, it must be indicated as **Available in the laboratory** in the **Labware Manager**. If the labware has never been used before on the QIAgility, it must also be calibrated.

5. If the selected labware requires an adapter, the software will list all adapters that can be used with the selected labware in the **Labware adapter** area below. Select the adapter. The software displays a picture and further details about the selected adapter on the right.

Note: If the option **Shows available labware only** has been checked, the software lists only adapters that have been indicated as **Available in the laboratory** in the **Labware Manager**.

If none of the matching adapters has been indicated as **Available in the laboratory** in the **Labware Manager**, the list will be empty and the **OK** button will remain disabled. Either choose another available labware or indicate one of the adapters as **Available in the laboratory** in the **Labware Manager**.

6. Click **OK** to confirm the selection and to close the **Labware Browser**.

Note: The **OK** button will only be enabled if a labware has been selected. In addition, if the labware requires an adapter (see the details area of the selected labware), the **OK** button will become enables after you have selected an adapter from the **Labware adapter** area.

Important: Tubes or plates supplied by different manufacturers may have slightly different dimensions, despite a similar appearance. Such differences can significantly affect the calibration settings, in particular the height calibration (Z-axis calibration). Therefore, it is important to select the correct plates and tubes.

Filter options

Option Category	Description Labware category, such as standard 96-well, Rotor-Gene Q, etc.
Shows available labware only	If checked, the search will be restricted to labware that has been indicated as Available in the laboratory in the Labware Manager .
	Note: The option applies also to the Labware adapter area.
	Uncheck the option if a desired labware is supported in general, but does not appear in the list if the option is checked.
Advanced options	Check to enable the advanced filter options.
	Enter or select details only for those advanced options for that you want to restrict the search. Leave all other options empty/unselected.
Name of labware	Labware name
Part number	Catalog number of the labware
Manufacturer	List of labware manufacturers
Tube/well size	Tube/well size range from < 0.5 ml to > 2 ml
Reset filter	If you click the button, the software clears all filter options to the initial state.
Apply filter	If you click the button, the software lists all labware that matches the search criteria.

7.1.3.8 Editing sample names

If several input labware will be used in a run, sample names can be defined in one dialog for all input labware.

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.

Select an input labware identifier to enter, edit or view the samples provided on that labware Labware identifier Input1_20161205_101957 Pos. Sample ID A1 A2 A3 A4 A4 A5 A6 A7 A8 B1 B2 B3 B4 B6 B7 B8 B8 C1 C2 C3 C4 Conc. [ng/µ] Description T C4 Pos. Sample ID Conc. [ng/µ] Description A1 Conc. [ng/µ] Description A2 Conc. [ng/µ] Description A3 Conc. [ng/µ] Description A4 Conc. [ng/µ] Description A4 Conc. [ng/µ] Description A4 A5 Conc. [ng/µ] Description A4 Conc. [ng/µ] Description A5 Conc. [ng/µ] Conc. [ng/µ] <		Sample editor		
Input1_20161205_101957 Imput1_20161205_101957 Pos. Sample ID Conc. [ng/µI] Description A1 Conc. [ng/µI] Description A2 Conc. [ng/µI] Description A3 Conc. [ng/µI] Description A4 Conc. [ng/µI] Description A5 Conc. [ng/µI] Description A4 Conc. [ng/µI] Description A5 Conc. [ng/µI] Description A6 Conc. [ng/µI] Description A7 Conc. [ng/µI] Description A8 Conc. [ng/µI] Conc. [ng/µI] B1 Conc. [ng/µI] Conc. [ng/µI] B2 Conc. [ng/µI] Conc. [ng/µI] B4 Conc. [ng/µI] Conc. [ng/µI] B5 Conc. [Sele	ct an input labware identifier t	o enter, edit or vi	ew the samples provided on that labware
Pos. Sample ID Conc. [ng/µ] Description A1 Conc. Description A2 Conc. Conc. A3 Conc. Conc. A4 Conc. Conc. A4 Conc. Conc. A5 Conc. Conc. A6 Conc. Conc. A6 Conc. Conc. A7 Conc. Conc. B1 Conc. Conc. B2 Conc. Conc. B3 Conc. Conc. B4 Conc. Conc. B5 Conc. Conc. B6 Conc. Conc. B7 Conc. Conc. B8 Conc. Conc. B7 Conc. Conc. C1 Conc. Conc. C2 Conc. Conc.	Laby	ware identifier		
A1A1A2A3A3A3A4A3A5A3A6A3A7A3A8A3B1A3B2A3B3A3B4A3B4A3B5A3B6A3B7A3B8A3B9A3B1A3B3A3B4B4B5B4B6B4B7B4B8B4B9B4 </td <td>In</td> <td>out1_20161205_101957</td> <td></td> <td>▼</td>	In	out1_20161205_101957		▼
A2A3A3A4A4A6A7A5A6A7A6A7A8B1A8A8B2A8A8B3A9A9B4A9A9B5A9A9B6A9A9B7A9A9B8A9A9B7A9A9B8A9A9B9A9B9A9<	Pos.	Sample ID	Conc. [ng/µl] De	scription
A3A3A3A4A6IA5IIA6IIA7IIB1IIB2IIB3IIB4IIB5IIB6IIB7IIB8IIB7IIB8IIB9IIB1IIB2IIB3IIB4IIB5IIB6IIB7IIB8IIB9II	A1		1	· · · · · · · · · · · · · · · · · · ·
A3 A3 A4 A3 A5 A3 A6 A3 A7 A3 B3 A3 B4 A3 B3 A3 B4 A3 B5 A3 B6 A3 B7 A3 B8 A3 B7 A3 B8 A3 B9 A4 B1 A4 B2 A4 B4 A4	A2	`	1	
A4A6A6A5A6A7A6A7A8B1A7A8B2A8A8B3A8A8B4A8A8B5A8A8B6A8A8B7A8A8B8A9A9B7A9A9B8A9A9B8A9A9B8A9A9B8A9A9B9A9B9A9 <t< td=""><td>A3</td><td>1</td><td>1</td><td></td></t<>	A3	1	1	
A5 A6 A6 A7 A7 A8 B4 A8 B3 A8 B4 A8 B4 A8 B5 A8 B6 A8 B7 A8 B8 A8 B1 A8 B2 A8 B3 A8 B4 A8 B5 A8 B6 A8 B7 A8 B8 A8 B9 A8 B1 A8 B2 A9 B3 A9 B4 A9 B5 A9 B6 A9 B7 A9 B8 A9 B9 A9 B1 A9 B2 A9 B3 A9 B4 A9 B5 A9 B6 A9 B7 A9 B8 A9 B9 A9 B1 A9 B2 A9 B3 A9 B4 A9 B5 A9 B6	A4	1	•	
A6A7A8A8A8A8A8B1A8A8B2A8A8B3A8A8B4A8A8B5A8A8B6A8A8B7A8A8B8A8A8B8A8A8C1A8A8C2A8A8B8A9B8A9B8A9B9A9 <td>A5</td> <td>1</td> <td>1</td> <td></td>	A5	1	1	
A7A8A8A8B1CB2CB3CB4CB5CB6CB7CB8CB8CB9C<	A6	1	•	
A8 A8 B1 A8 B2 A8 B3 A8 B4 A8 B5 A8 B6 A8 B7 A8 B8 A8 B8 A8 B8 A8 B8 A8 B8 A8 B9 A8 B9 A8 B9 A8 B9 A8 B9 A8 B9 A9 B9	A7			
B1 B2 B2 C B3 C B4 C B5 C B6 C B7 C B8 C B1 C B2 C	A8	`	•	
B2 B3 B3 C B4 C B5 C B6 C B7 C B8 C B1 C C1 C C2 C C3 C	B1	•	•	
B3 B6 B4 C B5 C B6 C B7 C B8 C B7 C C1 C C2 C C3 C	B2	٦	•	
B4 B6 B5 Image: Constraint of the second of	B3	٦	•	
B5 B6 B6 Image: Constraint of the second of	B4	٦	•	
B6 B7 C1 C1 C1 C2 C1	B5	٦	•	
B7 B8 C1 C1 C2 C1 C3 C1	B6	٦	•	
B8 Image: Constraint of the second of the	B7	٦	•	
C1 C2 C3 C3 C3 C4	B8	1	٦	
C2 C3	C1	•	٦	
C3	C2		•	
C4	C3	•	۲	
	C4		1	
OK				OK

Note: If you are using more than one input plate, at least one sample must be defined on each plate.

- 3. Enter each sample ID and optionally, enter the concentration and sample description.
 - Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column **Sample ID**. Press and hold the **SHIFT** key and left-click to select multiple adjacent cells, or press and hold the **CTRL** key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on **Auto-generate sample names**. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on **Auto-generate sample** names.

Сору	Ctrl+C
Paste	Ctrl+V
Auto-generate sample names	

Note: For more details how to enter information into a table refer to the section Entering data.

Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

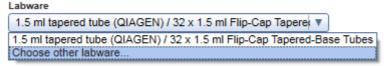
7.1.3.9 Add input labware dialog

If several input labware will be used in a run, it can be defined in the Add new input labware dialog.

To define a new input labware:

1. Click on the Labware type drop-down menu.

2. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.



3. Select your input labware in the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

er options legory tandard 96 Well	Labware Select the item that most closely matches the labw QIAGEN 96 Well Microplate RB	are to be used	Catalog number	
Shows available labware only	QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well		19585 Name QIAGEN S-Block 96 Well	
Advanced options			Dead Volume 40 µl	Association
art number				1 6 1 A 00000000000000000000000000000000000
fanufacturer All		Ţ		н 000000000000000000000000000000000000
ube / Well size	Labware adapter			
	Select the adapter to be used.			
		1	Catalog number	
Reset filter Apply filter			Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

4. **Optional:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

6. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- If you want the software to automatically generate sample names for all positions on the labware, check the option Auto-generate sample IDs for all samples.
 Note: This option will not be available if the dialog was opened using the Load sample input from file or the Scan barcode button.
- 9. Click **OK** to close the dialog.

7.1.3.10 Add output labware dialog

If several output labware will be used in a run, it can be defined in the **Add new output labware** dialog.

To define new output labware:

- 1. Click on the Labware type drop-down menu.
- 2. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

Labware type	
Select output labware type	•
Select output labware type	
QIAGEN S-Block 96 Well	
Eppendorf twin.tec PCR Plate 96 semi-skirted / 96	5-Well Plate
Choose other labware	

3. Select your output labware. If not listed, click **Choose other labware** to open the **Labware browser**.

Iter options	Labware		
ategory Standard 96 Well T Shows available labware only	Select the item that most closely matches the la QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name QIAGEN S-Block 96 Well	
Advanced options Name of labware		Dead Volume 40 µl	ALCOLUDIDA
Part number			1 6 1 A 0000000000 00000000000 D 00000000000 0000000000
Manufacturer V			н 000000000000000000000000000000000000
Tube / Well size	Labware adapter		
Ali	Select the adapter to be used.		
		Catalog number	
Reset filter Apply filter		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

4. **Optional:** If you would like to use the cooling block for the output labware, check the option Use cooling block under the Output labware type drop-down menu.

Labware type
Eppendorf twin.tec PCR Plate 96 semi-skirted / 96 🔻
Use cooling block Output labware is pre-filled with reagents

Note: Whenever you select another labware type, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

5. **Optional:** If you would like to indicate that the output labware has been pre-filled, tick the box next to **Output labware is pre-filled with reagents**.

Labware type		
Eppendorf twin.tec F	PCR Plate 96 semi-skirted / 96 🔻	
Vse cooling block	Output labware is pre-filled with	reagents

6. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report.

Note: Whenever you select another labware type, this identifier will be automatically generated again by the software.

7. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

8. **Optional:** If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to **Output converter** to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, **None** is the only entry in the list.

Output Converter

Select
Select
ABI7500_QuantiplexHyres.xslt
ABI7500_SDS_v1_4.xslt
ABI7500_SDS_v2.3_HID_v1.2.xslt
CE Loading (AL).xslt
ConvertToCsv.xslt
RGQ-SW_QREX.xslt

Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

9. Click **OK** to close the dialog.

7.1.3.11 Scan labware barcode dialog

To define samples, the QIAgility Setup Manager Software provides the option to load the sample information based on the barcode on the labware. Click the **Scan labware barcode** button to open the **Scan labware barcode** dialog.

Scan labware barcode
Labware barcode
OK Cancel
Scan labware barcode dialog used for Gene Expression and Pathogen Detection experiments.
Scan labware barcode
Import a batch from file using Batch ID. Scan the batch's barcode or manually enter the Batch ID.
Enter Batch ID
OK Cancel

Scan labware barcode dialog used for Sample Pooling experiments.

- Scan the labware barcode. Alternatively, you can enter the labware ID manually.
 Note: The software supports 1-D and 2-D barcodes. 2-D barcodes are supported if they encode a string that is used as labware ID.
- 2. Click **OK** to confirm.
- 3. For input labware, the software will automatically search for a sample information file in the **Sample input files** directory (defined in the user settings). The software loads the sample information from the sample file.

Note: The software will first search for an ***.xml** file in QDEF format, which defines the samples for the labware. It is not required that the *****.xml file has the labware barcode as its file name. The software looks into the file and compares the labware barcode with the plate ID described in the file. If no file in QDEF format is present, the software will then search for a ***.csv** file which has the labware barcode as its file name.

4. For output labware, the software automatically searches for a QDEF format file (*.xml) describing the output labware in the Sample output files directory (defined in the user settings). Based on the information from the loaded file, the software will automatically select the Output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

7.1.3.12 Fill empty output positions dialog

The **Fill empty position of output labware** dialog is used in various workflows of the Generic PCR Plug-in to define output positions which are not used for PCR setup, but will be filled, e.g., with a buffer. The dialog is available from the **Assignment** screen if empty positions of the output labware have been selected by clicking **Fill selected**.

Note: This functionality should only be used after all other positions have been assigned, so that any sample or standard/control assignments are not overwritten.

- 1. Click the **Liquid** menu to select the liquid that shall be used to fill the selected positions. The menu lists all liquids of type Diluent/Water. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 2. Enter the volume in μ l into the **Volume** field.
- 3. Click **OK** to confirm and to close the dialog.
- 4. The positions on the **Output** panel will be marked with the symbol for Buffer. During the run, the QIAgility will fill the selected positions with the defined amount of the liquid.

Fill empty position of output plate	
Liquid Select	Volume µl
Select Dilution buffer	
	OK Cancel

Dialog to select the liquid to fill output positions during run.

To clear a filled position select the position on the **Output** panel and then click **Remove assignment**.

7.1.3.13 Advanced sample pipetting parameters dialog

The **Define advanced pipetting parameters** dialog is used in various workflows of the Generic PCR Plugin. The dialog is available from the **Samples/Labware** screen, panel **Sample Pipetting**, by ticking the box **Use custom pipetting parameters** and then clicking **Edit parameters**.

Define adv	anced pipetting parameters
Prepare extra volume Extra volume 4 µl and	Extra volume per assignment
Mix before aspiration	Mix after dispense sample to output well Repetitions
	OK Cancel

Advanced sample pipetting parameters dialog

 If you tick the box next to Prepare extra volume, parameters become visible to define extra volume for samples. Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute Extra volume, the Extra volume per assignment shall cover variations depending on sample assignments later in the Assignment step (e.g., volume loss per tip) and is added per replicate.

However, if you face any liquid level detection issue increase **Extra volume per assignment** if issues are for higher number of assignments of a sample only. Increase absolute **Extra volume** if issues are not related to the number of assignments of a sample.

- 2. If you tick the box next to **Mix before aspiration**, you can specify the mixing repetitions in the input labware before the sample is aspirated for sample transfer. The box is enabled only if the selected liquid class for sample transfer includes mixing before aspiration.
- 3. If you tick the box next to **Mix after dispense sample to output well** you can specify the mixing repetitions in the output labware after the sample has been dispensed. The box is enabled only if the selected liquid class for sample transfer includes mixing after dispense.
- 4. Click **OK** to confirm your selections and to close the dialog.

7.1.4 Environments

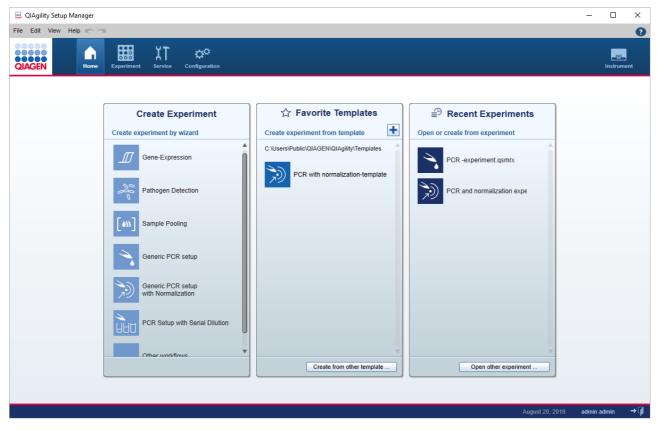
QIAgility Setup Manager Software contains 4 different environments.

You can switch to another environment by clicking the appropriate button from the main toolbar. The icon of the currently active environment is highlighted.

Environment	Description
Home	Used to jump to the home screen. From here, you can create or open experiments. (<u>Home</u>)
Experiment	Used to set up experiments. (<u>Experiments</u>)
XT Service	Bundles tasks for maintenance, system check and support of QIAgility. (<u>Service</u>)
Configuration	Bundles the tasks' custom settings, user and labware management, and calibration. (<u>Configuration</u>)

7.1.4.1 Home

The **Home** environment bundles all tasks for creating and opening of experiments and for working with templates.



The Home environment is divided in three areas:

Creates an experiment using the wizard. Each installed plug-in Create Experiment comes with its own wizard. The new experiment is empty and does not contain any data. Most data fields are empty, except those that Create experiment by wizard are prefilled by the wizard. Use this to start an experiment from scratch. For details, refer to Create an empty experiment. Creates an experiment using a previously saved template. All data A Favorite Templates fields in the new experiment are populated with existing data from + Create experiment from template the template. For details, refer to Create an experiment using a template. All favorite templates are listed in tabular form below the headline. For information regarding how to add a template to the list, refer to Manage favorite templates. Click the button Create from other template to look for a template that is not in the list using the file dialog. Opens a previously saved experiment. Use this to modify or adapt a Recent Experiments saved experiment or alternatively, use it to set up a new experiment that inherits the data from an old experiment. For details, refer to Open or create from experiment View recent experiments or Create an experiment from an existing experiment, respectively.

All recently saved experiments are listed in tabular form below the headline.

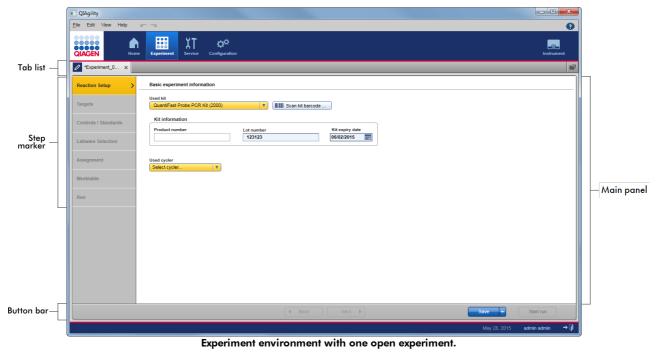
Click the button **Open other experiment** to look for an experiment that is not in the list using the <u>file dialog</u>.

7.1.4.2 Experiments

The **Experiment** environment is the environment in which all user interaction with experiments takes place. This is where experiments are set up and run and reports are created. In order to interact with an experiment, the experiment must be opened. If a new experiment is created, it is open by default.

All changes to an experiment are performed in the computer's memory. In order to make them permanent, it is necessary to save the experiment. Before a run is started, the corresponding experiment must be saved.

The maximum number of experiments that can be opened at one time is 8. Attempting to open more than 8 experiments will result in a warning message.



The screen of the **Experiment** environment has four main areas:

Area Description

Experiment

The experiment tab control is displayed at the top of the **Experiment** environment. It shows tab list all currently open experiments and offers basic experiment management.

> A separate tab is displayed for each open experiment. If an experiment name is too long to be displayed within the available space, the displayed name will be truncated, and ellipses (...) will be shown at the end of the name. In order to display the full name, simply let the mouse pointer remain over the experiment name for a few seconds. A tooltip will display the full name of the experiment.

> When you make modifications in any step, an asterisk symbol (*) will be displayed in front of the experiment name. This is a visual reminder for you that there are modifications that have not yet been saved. When you save the experiment, the asterisk symbol disappears.

> Clicking on the cross to the right of an experiment name will close the experiment. If any modifications have not been saved, then you will be asked whether the experiment should really be closed. Closing the experiment without saving the modifications will result in loss of the modifications.

The following icon appears on the far right of the tab list: 🔳. This is a shortcut to open an experiment; clicking this displays a file selection dialog that allows you to open an experiment.

Step markers Step markers allow you to navigate between different aspects of one experiment. The step markers control the content of the main panel; each step marker is associated with a certain content of the main panel. To switch to another step, click on the step marker step or use the **Back** and **Next** buttons in the button bar.

> **Note:** It is not required to follow the steps in order, but it is recommended. You can jump any active step at any time.

- All experiment-specific information is shown and selected in the main panel. The content Main panel depends on the selected step marker.
- Button bar The button bar is displayed at the bottom of the **Experiment** environment. It contains buttons for experiment management functions that are not related to a specific step marker, including Back, Next, Save and Start run.

The **Start run** button is only enabled if all requirements for a new run are met (e.g., all required values have been defined during setup) and if the experiment has not already been run. For details regarding a run, refer to Starting a run.

Selecting the **Save** button will save the current experiment. If the experiment has not yet been named, a dialog will be shown, prompting you to enter a name for the experiment. If the experiment already has a name (for example, if an existing experiment was opened), the existing experiment will be overwritten.

The Save button also has a sub-function: Save experiment as. Clicking on the small arrow to the right of the button will display a menu with the additional choice. The Save experiment/template as file dialog allows you to enter a new experiment name and save the experiment with the new name. Use this option to also create a template from the current experiment by selecting the file extension for a template in the file dialog.

7.1.4.3 Service

The **Service** environment bundles all tasks needed to check and maintain the QIAgility along with tools to facilitate the communication with QIAGEN Technical Services for technical assistance.

	QIAgility							
Ē	<u>F</u> ile Edit View He	elp 🦾 🕋						
	QIAGEN		eriment	XT Service	Configuration			
	Cleaning Procedure	Maintenance	System 0	Check	Support Package	Audit Trail		
							1	

Service environment is organized in five tabls

This environment is organized in five different tabs. You can switch between these tabs by clicking on the respective button in the tab bar.

Tab Cleaning Procedure	Description Offers to start the UV lamp as the last step of the <u>cleaning procedure</u> . The UV		
Cleaning Proceable	radiation cleans possible biological contaminations on the worktable.		
Maintenance	<u>Maintenance scheduler</u> keeps track of service tasks. Additionally, HEPA filter <u>replacement</u> can be managed here.		
System check	Provides several <u>self-diagnostic tools</u> :		
	 Checking the connection between computer and instrument and within the instrument itself 		
	Cycling the pipettor head		
	Checking for movement reliability		
	 Checking the reliability of the liquid-level sensor 		
Support Package	Create a <u>support package</u> to assist your communication with QIAGEN Technical Services, in case you have an issue.		
Audit Trail	Allows you to view the system <u>audit trail</u> . This tab is only available for users with administrator user role.		

7.1.4.4 Configuration

The **Configuration** environment bundles all tasks to customize the instrument for your needs. You can set application settings, select the labware available in your laboratory, perform the instrument calibration and manage users.

QIAgility			
<u>F</u> ile Edit View	Help 🖛 🦟		
QIAGEN	Home Experiment	t Service Cor	¢¢ Ifiguration
Settings	Labware Manager	Calibration	User Manager
•			

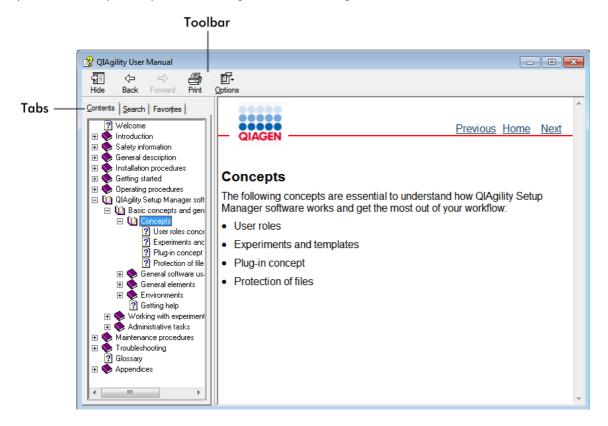
Configuration environment is organized in four tabs

This environment is organized in four different tabs. You can switch between these tabs by clicking on the respective button in the tab bar.

Tab	Description
Settings	Specify <u>general settings</u> including directories for experiments and report customization, set the password policy and e-mail notifications, etc.
Labware Manager	Select the labware (adapter, plates, tubes) available in your laboratory.
Calibration	Perform an <u>instrument calibration</u> , which is essential for accurate and reliable results.
User Manager	Manage users:
	 Create and modify users and their roles
	 Activate and deactivate users

7.1.5 Getting help

QIAgility Setup Manager Software comes with a detailed help system. The help is provided as both a user manual in PDF format and also as a context-sensitive help system. To access the context-sensitive help, press the **F1** key while you are viewing screens or dialogs.



The help file contains two functional areas:

- Toolbar
- Tabs

The toolbar contains the following buttons:

	0			
Name	lcon	Description		
Hide or Show	मांde	Hides the left-hand side navigation tab. To display the navigation tab again, click Show . This button appears instead of Hide .		
Back	< ⊖ Back	Returns to the previous s	creen.	
Forward	⇒ Forward	Returns to the screen dis button.	played before using the Back	
Print	e	Provides two options:		
	Print	• Print the selected topic.		
		• Print the selected head	ling and all subtopics.	
		Select one option and co to go back.	onfirm with OK or select Cancel	
Options	Options	Opens the Options mer	nu with the following entries:	
		Hide Tabs		
		Back		
		Forward		
		Home		
		Stop		
		Refresh		
		Internet Options		
		Print		

Search Highlight Off

The navigation tab contains the following tabs:

Name	Description
Contents	In the Contents tab the help content can be browsed by topics.
Search	Specific help topics can be found by entering search terms.
Favorites	Shortcuts to individual help topics can be added and managed.

7.2 Working with experiments

The <u>Home</u> environment bundles all tasks for creating and opening experiments and for working with templates. It offers the following functions:

- Creating a new, empty experiment from the Create Experiment section
- Creating a new experiment from a template from the Favorite Template section
- Creating a new experiment from a previously run experiment from the Recent Experiments section

The general workflow is to select a plug-in from the **Home** environment, define the experiment in the <u>Experiment environment</u> and finally <u>start the run</u>. It is not necessary to finish the definition of an experiment in one step. A save function is available at any time, allowing you to save your progress and continue later. To facilitate the experiment definition, you can use <u>templates</u> or reuse previously run experiments.

Refer to the following sections about available experiment setups:

- <u>Working with gene expression experiments</u>
- <u>Working with experiments for pathogen detection</u>
- <u>Working with sample pooling experiments</u>
- <u>Generic PCR experiments</u>
- <u>Generic PCR setup with normalization experiments</u>
- PCR setup with serial dilution experiments

The QIAgility Setup Manager Software provides the following other workflows:

- Normalization only experiments
- <u>Prepare reaction mix experiments</u>
- <u>Prepare and distribute reaction mix experiments</u>
- <u>Prepare standards experiments</u>

7.2.1 Managing experiments and templates

Experiments and templates are managed in the **Home** environment. The following section describes how to work with them.

For details regarding how to create a template and about the template concept, refer to <u>Experiments and</u> <u>templates</u>.

7.2.1.1 Create a template

An experiment can be saved as a **template** for future reuse. Use this function if you plan to enter the same values for experiment setup for several experiments (e.g., for routinely performed experiments or a series of runs). Templates do not inherit the assignment and worktable information from the experiment.

Additionally, the Generic PCR Plug-in offers the option to save a **quick order template**. Quick order templates inherit the sample input positions, assignment and layout on the output labware.

Prepare an experiment as the basis for the template or the quick order template:

- 1. <u>Create a new, empty experiment</u>. Alternatively, <u>create an experiment from an existing experiment</u>. For a quick order template, <u>open the original experiment</u>.
- 2. For a template, enter all values that should be available for reuse in the template.

Note: If you created the experiment from an existing experiment, remove all values that should not be reused. When using the template, it is easier to recognize that some values are still missing than to recognize that some values should be changed.

For a quick order template, define the experiment including sample input positions and assignment, i.e., all wizard steps before the worktable step.

Note: When defining a quick order template, do not use lot numbers/expiry date in the liquid definitions of the original experiment. Instead, enter lot numbers, if needed, into the comment field on the run screen.

Note: When using a quick order template, fewer samples than defined in the original experiment can be run. However, in this case worktable calculation will create different positioning and volumes of reagents.

3. For a quick order template, save the experiment (as experiment) for later reference, because when using the quick order template, the definitions made will not be visible anymore. Select, e.g. the directory that has been defined as default directory for templates.

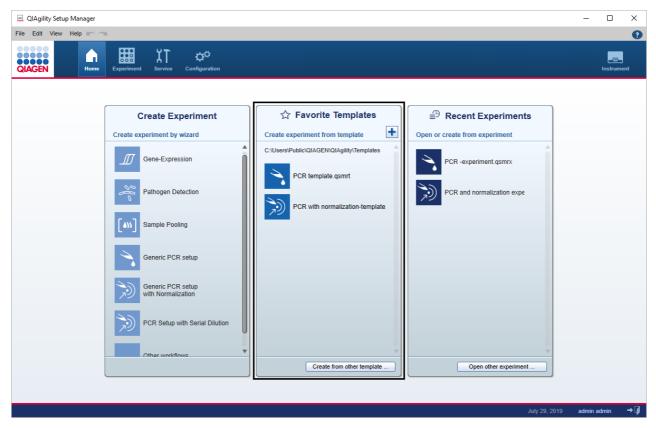
To save an open experiment as a template or quick order template:

- 1. Click on the small arrow to the right of the **Save** button in the buttof the on bar. From the displayed menu, select **Save experiment as**.
- 2. The Save experiment/template as file dialog opens.
- 3. Select the directory where the template should be saved, e.g. the directory that has been defined as default directory for templates in the settings of the plug-in. Initially, the default directory for experiments is shown.
- 4. Enter a name for the template in the **File name** field.
- 5. From **File type**, for a template select the file extension ending with a **t** (e.g., *.qsmg**t** for a template for use with the Gene Expression Plug-in). To save a quick order template for use with the Generic PCR Plug-in, select the file extension ending with a **qt**.
- 6. Click Save.

Add the created template to the <u>favorite templates</u> section.

7.2.1.2 Manage favorite templates

The software offers the possibility to manage favorite templates in the **Favorite Templates** list of the **Home** environment. For information about how to create a template, refer to <u>Create a template</u>.



A maximum of 11 templates can be displayed. When this limit is reached, the oldest template will be removed from the list. The template name will be truncated if the available space is exceeded, and ellipses (...) will be shown at the end of the template name. To display the full name, move the mouse pointer over the experiment name and after a few seconds, a tooltip will be displayed showing the full name.

Templates can be added or removed from this list, and <u>new experiments can be created</u> based on these templates. In addition, this section offers the option to create experiments based on templates that are not contained in the list of favorite templates.

Adding a folder with templates

- 1. Click on the Home icon in the main toolbar to change to the Home environment.
- 2. The **Favorite Templates** list in the middle will display all favorite templates.

3. Click on the plus icon in the list header:



4. Select the folder where your templates are located. The file extension of a template depends on the plug-in that it's compatible with. See the table below for more information about file extensions. Click **OK**.

Plug-in	File Extension
Gene-Expression	*.qsmgt
Pathogen Detection	*.qsmpt
Sample Pooling	*.qsmpoolit
Generic PCR	*.qsmrt
Generic PCR Quick Order	*.qsmrqt

٢	Select folder
Look in Path	Templates C: Users Public QIAGEN QIAGIN QIAgility Templates D:
Folder name	
L	OK Cancel

Folder selection

٢	Select folder
Look in	Templates
Path	C:\Users\Public\QIAGEN\QIAgility\Templates
Name	Last modified
Folder nam	ne
~	OK Cancel

Selected folder

Note: The **Select folder** dialog does not show the contents of the selected folder. To view the contents of the folder, use your preferred file manager application.

5. Templates from the selected folder will be added to the Favorite Templates list.

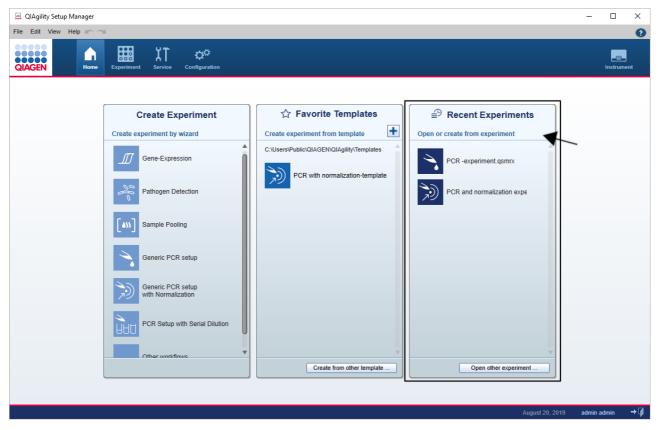
Removing a template

- 1. Click on the Home icon in the main toolbar to change to the Home environment.
- 2. The **Favorite Templates** list in the middle displays all favorite templates.
- 3. Move the mouse pointer over the template name.
- 4. The context menu icon appears (¹¹).
- 5. Click on the icon.
- 6. The context menu is shown.
- 7. Click on the **Remove from favorites** command.
- 8. The selected template is removed from the **Favorite Templates** list.

Note: Removing a template using the steps above will not physically delete or modify the template file. It will only be removed from the **Favorite Templates** list.

7.2.1.3 View recent experiments

When an experiment is saved or run, it is automatically added to the **Recent Experiments** table in the **Home** environment:



The most recent experiment appears at the top of the list. A maximum of 11 experiments can be displayed. When this limit is reached, the oldest experiment will be removed from the list.

The experiment name will be truncated if the available space is exceeded, and ellipses will be shown at the end of the experiment name. To display the full name, move the mouse pointer over the experiment name, and after a few seconds, a tooltip will appear with the full name.

Opening an existing experiment

- 1. Click on the **Home** icon in the main toolbar to change to the **Home** environment.
- 2. The **Recent Experiments** table on the right displays all recent experiments.

- Click on the experiment name you want to open. Alternatively, position the mouse pointer over the experiment name. Click on the context menu icon that appears (), and select Open experiment. Optional step: If the experiment is not listed because it has been pushed off the list by newer experiments, click on the Open other experiment button at the bottom and select the experiment from the file dialog.
- 4. The experiment is opened in the Experiment environment

Note: Alternatively, you can use the **Open experiment** command from the **File** menu to open an experiment.

II Q	IAgility
File	Edit View Help 🖛 🦡
	Create New Experiment
	Create New Experiment from Template
	Open experiment
	Save 13
	Save as
	Login
	Logout
	Change Password
	Service login
	Exit

Removing an experiment from the list of recent experiments

Follow these instructions to remove an experiment from the Recent Experiments list:

- 1. Click on the Home icon in the main toolbar to change to the Home environment.
- 2. The **Recent Experiments** table on the right displays all recent experiments.
- 3. Move the mouse pointer over the experiment name you want to remove.
- 4. The context menu icon (🛄) is displayed.
- 5. Click on the context menu icon to open the context menu.
- 6. Select the **Remove experiment from recent list** command.
- The selected experiment is removed from the recent experiments list.
 Note: The experiment will still be available via the Open other experiment button.

7.2.2 Creating an experiment

You can create an experiment either by

- Creating a <u>new</u>, blank experiment without any data, or
- Creating an experiment with data inherited from a previously saved <u>experiment</u> or <u>template</u>.

7.2.2.1 Create an empty experiment

There are two ways to create an empty experiment:

• Using the Home environment

In the **Home** environment, all plug-ins are listed in the **Create Experiment** list. Move the mouse pointer over one of the plug-in entries and click on it. The new experiment is created and displayed in the **Experiment** environment.

💂 QIAgility Setup Manager		
File Edit View Help	~i	
QIAGEN	Experiment Service Configuration	
	Create Experiment Create experiment by wizard Gene-Expression Pathogen Detection	Favorite Templates Create experiment from template C:\Users\Public\QIAGEN\QIAgility\Templates Decreate experiment from templates

• Using the File menu

To create a new experiment using the main menu, select **File** from the main menu and move the mouse pointer over the **Create New Experiment** command. A submenu containing all available plug-ins is shown. Click on the desired plug-in to create a new, blank experiment.

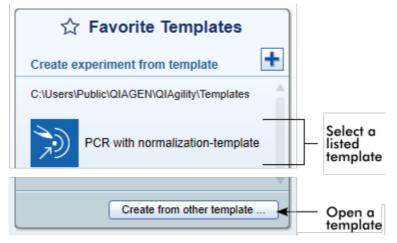
LogH.	🔜 QIAgility Setup Manager				
File	Edit View Help 🖛 🐋				
	Create new experiment		Gene-Expression		
	Create new experiment from template		Pathogen Detection		
	Open experiment		Sample Pooling		
	Save		Generic PCR setup		
	Save as		Generic PCR setup with Normalization		
	Login		PCR Setup with Serial Dilut	tion	1
	Logout		Other workflows		
	Change password	nt hu	universe		Create
	Service login	IT Dy	/ wizard		Create
	Exit	Expre	ession		C:\Users

7.2.2.2 Create an experiment using a template

There are two ways to create an experiment based on a template:

• Using the **Home** environment

In the **Home** environment, favorite templates are listed in the **Favorite Templates** list. You can either click on one of the listed templates, or if the desired template is not listed, click on the **Create from other template** button at the bottom to open the file dialog that allows you to select a folder that contains the template. In both cases, a new experiment will be created containing all data from the selected template.



For details on how to add a template to the favorite list, refer to Manage favorite templates.

• Using the File menu

To create a new template-based experiment using the main menu, select **File** from the main menu and click on the **Create New Experiment from Template** command. The file open dialog is opened, allowing you to select a template. When you confirm the file dialog, a new experiment will be created containing all data from the template.

🔳 Q	IAgility
File	Edit View Help 🖛 🐋
	Create New Experiment
	Create New Experiment from Template
	Open experiment
1	Save
	Save as
	Login
	Logout
	Change Password
	Service login
	Exit

For details of the template concept, refer to Experiments and templates.

7.2.2.3 Create an experiment from an existing experiment

There are two ways to use an existing experiment as a starting point for creating a new experiment:

- Like a template: inherit the data of the experiment, but not the sample information. Use this option if you want to provide new sample name only. If you often create an experiment based on the same experiment, consider creating a template and add it to the favorite templates list.
- Make a copy: inherit the data of the experiment, including the sample information. Use this option if you want to rerun the same experiment again with the same sample names and the samples placed in the same positions on the input labware.

To use the existing experiment like a template, follow this procedure:

1. Change to the **Home** environment.

2. Move the mouse pointer to the desired experiment in the **Recent Experiments** list.

Note: In case the desired experiment is not listed here, click on the **Open other experiment** button at the bottom to open it once using the file dialog that appears. Now the experiment will be listed in the **Recent Experiments** list. Close it and start again with step 1.

- 3. Hold the mouse pointer over the desired experiment.
- 4. The context menu icon (💴) appears.
- 5. Click on it.
- 6. The context menu is opened.

Open or create from experiment	
Multiplex GPCR-like 5 Var2.	
Multiplex GPCR-like 5 Var1.	Open experiment
Multiplex GPCR-like 5.qsmg:	Use as template for new experiment

- 7. Click on Use as template for new experiment.
- 8. A new experiment will be created based on the data from the selected experiment (excluding the sample information), and will be displayed in the **Experiment** environment.

To use the existing experiment as a copy, including the sample information, follow this procedure:

- 1. Change to the **Home** environment.
- 2. Click on the desired experiment in the **Recent Experiments** list to open it.
- 3. Note: In case the desired experiment is not listed here, click on the **Open other experiment** button at the bottom to open it once using the file dialog that appears.
- 4. The existing experiment will be displayed in the **Experiment** environment.
- 5. Click on the small arrow to the right of the **Save** button in the button bar. From the displayed menu, select **Save experiment as**.
- 6. The Save experiment/template as file dialog opens.
- 7. Select the directory where the new experiment should be saved.
- 8. Enter a name for the new experiment in the **File name** field.

- 9. From **File type**, select the file extension ending with a **x** (e.g., *.qsmg**x** for an experiment for use with the Gene Expression Plug-in).
- 10. Click Save.
- 11. A new experiment will be created based on the data from the existing experiment (including the sample information), and will be displayed in the **Experiment** environment. The existing experiment will close automatically.

7.2.3 Working with gene expression experiments

The Gene Expression Plug-in enables easy and convenient qPCR setup of your gene expression experiments using dedicated lines of QIAGEN kits including QuantiTect, QuantiFast, QuantiNova and Rotor-Gene.

New gene expression experiments are defined in the **Experiment** environment of the software. When a new experiment is created, only the **Reaction Setup** step will initially be enabled. All other steps will become available once the required information has been entered in the **Reaction Setup** step. The coloring of the **Targets** step will change to yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 7 steps that guide you through the run setup process:

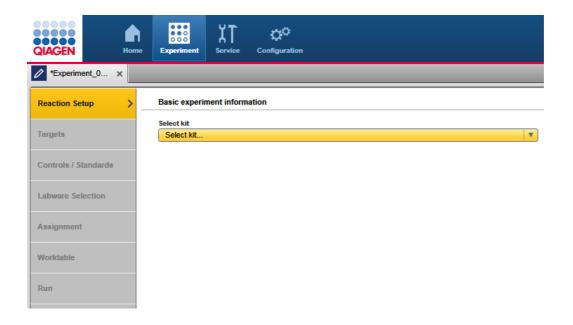
- 1. Reaction setup
- 2. <u>Targets</u>
- 3. Controls/standards
- 4. Labware selection
- 5. Assignment
- 6. <u>Worktable</u>
- 7. <u>Run</u>

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. The file extension for gene expression experiments is ***.qsmgx**, and for templates ***.qsmgt** (**Q**|Agility **S**etup **M**anager **G**ene Expression Experiment, and **T**emplate, respectively). The **Start run** button will be disabled as long as there are incomplete or invalid steps or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.3.1 Reaction Setup step

The **Reaction Setup** step is used to make basic selections, such as the kit to be used, the preferred cycler and the volume definitions. When you create a new experiment, the **Reaction Setup** step will be the only active step, and within this step, only the **Used kit** menu will be active. The other controls will initially remain inactive, but will become visible once you have selected the kit to be used in the reactions.



1. Select the kit to be used from the **Select kit** drop-down menu.

5	elect Kit				
	Rotor-Gene Multiplex PCR Kit (400)	•	\odot	For fast multiplex real-time PCR, two-step RT-PCR, and one-step RT-PCR	
			\odot	using sequence-specific probes on Rotor-Gene cyclers	

Once selected, a kit description is displayed next to the selection menu. When you are using a kit for the first time, we recommend reading it carefully. It contains important information regarding the experiment setup.

2. The Kit information and Used cycler fields are displayed.

Kit information			
Product number	Lot number	Kit expiry date	
204774			

3. Optionally, enter the **Lot number** and the **Kit expiry date** in the corresponding fields for documentation purposes.

Note: You can use the date picker (E) to open an interactive calendar for entering the dates.

- 4. Select the cycler to be used from the Select cycler and output format drop-down menu.
- 5. If the selected cycler requires a passive reference, the **Passive reference** option will be enabled. Select this option to use passive reference.

Note: If the selected kit contains a passive reference and the passive reference option is selected, the software will automatically add the passive reference to the additional reagents list (see <u>Target step</u>) along with the recommended volume for your setup.

6. The **Volume definitions** area is displayed. The fields may vary depending on your specific kit and cycler selection.

Volume definitions		
Sample input volume 2	Samples are covered with top-elute	Use option "Use ready-made reaction mix" if a prepared reaction mix is used that already contains the kit master mix and all target-specific components (such as primers and probes). Additional reagents and/or water may be included by the instrument.

Note: The default values that appear in the volume fields are the recommended values from the kit handbook for your setup.

7. Enter the volume data in the corresponding fields:

Field	Description
Sample input volume	Enter sample input volume. This is the volume of sample, controls and standards that will be added to the reaction mix.
Total reaction volume	Enter the total reaction volume. This is the volume of the final reaction mix including reaction mix and sample, control or standard.

- 8. If your samples are covered with TopElute Fluid, indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
- 9. Check the box next to **Use ready-made reaction mix** to indicate whether you have already prepared the reaction mix containing the kit master mix and all target components. Enter the reaction mix volume per reaction. Later in the worktable step, you will be asked to place this prepared mix onto the worktable.
- 10. Click **Next** to proceed.

7.2.3.2 Targets step

The **Targets** step is used to <u>define primer-probe mixes or single components</u> for the targets. All defined targets and primer-probe mixes are listed in the **Targets** tables. At least one target needs to be defined.

Optionally, <u>additional reagents can be defined</u> to be added to the reaction mix. Defined reagents are listed in the **Additional reagents** table. The QIAgility will add these reagents to the assay points during run, if you select the option **Use ready-made primer-probe mixes** in the <u>Reaction Setup step</u>.

Click **Next** to proceed.

ction Setup	Define targets and reagents					
gets >	Targets Target name	Content	Target type	Reporter	Actions	_
cts /	GAPDH	GAPDH rev (0.5 µM); GAPDH for (0.5 µM); GAPDH Probe (0.2 µM);	Reference	HEX		1
ols / Standards	GPCR-like 5	5 rev (0.5 µM); 5 for (0.5 µM); 5 Probe (0.2 µM);	Test	JOE		
					New targe	et
nment table					New targe	et
		Additional reagents			New targe	et.
		Additional reagents Name		Content	New targe Actions	et
					Actions	
		Name				
		Name			Actions	
		Name			Actions	
		Name			Actions	

Targets step

7.2.3.2.1 Defining targets and mixes

You can choose whether the QIAgility should use single components (primers and probes) or ready-made primer-probe mixes.

To define:

- a new primer-probe mix for one or several targets
- or single components for one target

proceed as follows:

- 1. Click on **New target** below the **Targets** table.
- 2. The Add target dialog will be displayed. Refer to Add target dialog for details.
- 3. New rows for the defined primer-probe mixes or single components for the targets will be added to the **Targets** table.



The buttons in the Actions column
Button Action

But	Button			
0				

Description

To edit a target

The **Edit target** dialog will open, displaying the values that have been entered in the **Add target** dialog.

of the **Targets** table have the following functions:

Ŀ	To duplicate a target	The Duplicate target dialog will open, displaying the values that have been entered for the original target. The target names will initially display a copy of the target.
×	To delete a target	Deletes the row in the Targets table.

7.2.3.2.2 Add target dialog

The **Add target** dialog contains two major options at the top:

Use ready-made primer-probe mix	Select this option if you intend to load a ready-made primer-probe mix in one single tube for the run.
primer-probe mix	You can define several target names. There is also the option to define the components for documentation purposes.
Use single	Select this option if you intend to load primers and probes in individual tubes for the run.
components	Define one target name and the components (primers and probes) related to this target name.



O Use ready-made primer-probe mix

Use single components

		Target name		Target type	Reporter
GAPDH Refer V HEX					
omp	onents				
ube	Name	Conc. in assay reaction [µM]	Stock concentration [µM]	Volume per reacti	on [µl]
1	GAPDH rev	0.5	20		0.5 🗙
2	GAPDH for	0.5	20		0.5 🗙
3	GAPDH Probe	0.2	4		1 🗙
				Add primer	Add probe
				(

Dialog to load primers and probes in individual tubes for the run.

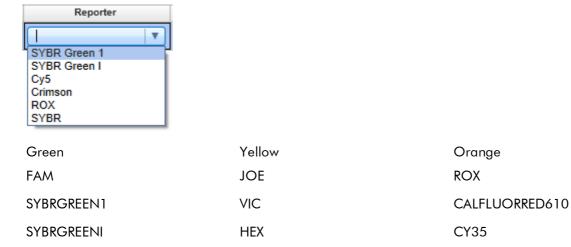
To define single components for one target

- 1. Select the option **Use single components**.
- 2. Enter a target name. The **Target name** cell will be colored yellow as a reminder that this entry is required.
- 3. Click on the menu in the **Target type** column and select a target type:

	Target type
	Test 🔻
٦	Test
	Reference
	IC

Target type	Description
Test	Use this target type for your gene of interest or the targeted region.
Reference	Use this target type for reference targets, such as housekeeping genes or other endogenous controls.
IC	Use this target type for internal controls (IC). Internal controls are either added during sample extraction or before PCR setup.
	If the kit contains an IC, define a corresponding IC target. Later, when <u>assigning</u> <u>samples and targets</u> to the output labware, either select that your samples already contain the IC or that the QIAgility shall add an IC to the reaction.
	If you use an own IC, you can also define an IC target. If you want to use the IC for selected samples only, the samples on the input labware must already contain the IC when loading them onto the worktable. Alternatively, if the IC shall be used with all the samples in the run and the samples do not yet contain the IC, you can define the IC as an additional reagent. The IC will then be added by the QIAgility to all reaction mixes.

4. **Optional step:** Click on the menu in the **Reporter column** and select an appropriate reporter dye for the target:



FLUORESCEIN	TET	TEXASRED
ALEXAFLUOR488	MAX	ALEXAFLUOR568
	CALFLUOR	
	GOLD540	
	YAKIMAYELLOW	
Red	Blue	Crimson
CY5	MARINABLUE	QUASAR705
QUASAR670	EDANS	ALEXAFLUOR680
ALEXAFLUOR633	BOTHELLBLUE	LIGHTCYCLERRED705
LIGHTCYCLERRED640	ALEXAFLUOR350	
	AMCAX	

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section <u>Entering data</u>.

- 5. In the **Components** table, define the primers and the probe (steps 6–10).
- 6. Click on Add primer to add a primer or click on Add probe to add a probe.
- 7. A row for the primer or probe will be added to the **Components** table and the **Name** cell will be colored yellow.

Note: A **Delete** icon () will be displayed in the last column of each component row. Click on it if you would like to delete a component.

- 8. Enter the name of the primer or probe. The QIAgility Setup Manager Software will prompt you at a later step to place a tube with the name of the primer or probe onto the worktable.
- 9. Enter the **Volume per reaction** of the primer or probe. Alternatively, you can specify the concentration in the assay reaction along with the stock concentration, and the software will automatically calculate the **Volume per reaction**.
- 10. To define all components, repeat steps 6-10.
- 11. If the single components of the target have been defined, click **OK**. The dialog will close.
- 12. The target will be listed in the **Targets** table.

Note: The software automatically assigns a color to each defined target name. If you would like to select a different color, you can click on the colored rectangle in the first column to open the **Colors** dialog:



	ready-made primer-probe mix single components	
arge	t definition (list the individual targets of the mix here) Target name	Target type Reporter
G	APDH	Refere V HEX V
G	PCR-like 5	Test V JOE V X
	e per reaction μ μι [0 - 20 μ]	Add target
4		Add target
4 Comp	μι [0 - 20 μl] conents (optional, for documentation only)	
4 Comp	μι [0 - 20 μι] onents (optional, for documentation only) Name	Conc. in assay reaction [µM]
4 Comp	μ μl [0 - 20 μl] onents (optional, for documentation only) Name GAPDH rev	Conc. in assay reaction [µM]
4 Comp Tube	μ μ [0 - 20 μ] onents (optional, for documentation only) Name GAPDH rev GAPDH for	Conc. in assay reaction [µM] 0.5 × 0.5 ×

Dialog to load a ready-made primer-probe mix in one single tube for the run.

To define a ready-made primer-probe mix

- 1. Select the option Use ready-made primer-probe mix.
- 2. Enter a target name. The **Target name** cell will be colored yellow as a reminder that this entry is required.

Note: To name a target mix, you can either choose one name that indicates the target mix name, or you can define several different target names. In the software, these targets will always be handled together, because they are part of the same mix. The QIAgility Setup Manager Software will prompt you at a later step to place a tube with the name of the target mix onto the worktable. If you defined several different target names, the software will display a list of the targets for that tube.

3. Click on the menu in the **Target type** column and select a target type.

	Target typ	e
	Test	•
1	Test Reference	
	IC	

Target type Test	Description Use this target type for your gene of interest or the targeted region.
Reference	Use this target type for reference targets, such as housekeeping genes or other endogenous controls.
IC	Use this target type for internal controls (IC). Internal controls are either added during sample extraction or before PCR setup.
	If the kit contains an IC, define a corresponding IC target. Later, when <u>assigning</u> <u>samples and targets</u> to the output labware, either select that your samples already contain the IC or that the QIAgility shall add an IC to the reaction.
	If you use an own IC, you can also define an IC target. If you want to use the IC for selected samples only, the samples on the input labware must already contain the IC when loading them onto the worktable. Alternatively, if the IC shall be used with all the samples in the run and the samples do not yet contain the IC, you can define the IC as an additional reagent. The IC will then be added by the QIAgility to all reaction mixes.

4. **Optional step:** Click on the menu in the **Reporter column** and select an appropriate reporter dye for the target:

Reporter		
SYBR Green 1 SYBR Green I		
Cy5 Crimson ROX SYBR		
Green	Yellow	Orange
FAM	JOE	ROX
SYBRGREEN1	VIC	CALFLUORRED610
SYBRGREENI	HEX	CY35
FLUORESCEIN	TET	TEXASRED
ALEXAFLUOR488	MAX	ALEXAFLUOR568
	CALFLUOR	

	GOLD540	
	YAKIMAYELLOW	
Red	Blue	Crimson
CY5	MARINABLUE	QUASAR705
QUASAR670	EDANS	ALEXAFLUOR680
ALEXAFLUOR633	BOTHELLBLUE	LIGHTCYCLERRED705
LIGHTCYCLERRED640	ALEXAFLUOR350	
	AMCAX	

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section <u>Entering data</u>.

- Optional step: To add further target names, click on Add target and repeat the steps 2–5.
 Note: After you have created at least two targets, a Delete icon (X) will be displayed in the last column of each target row. Click on it if you would like to delete a target.
- 6. Enter the volume per reaction of the primer-probe mix in the Volume per reaction field.
- In the Components table, you can define the primers and the probe (steps 8–12).
 Note: Steps 8–12 are optional.
- 8. Click on Add primer to add a primer or click on Add probe to add a probe.
- 9. A row for the primer or probe will be added to the **Components** table and the **Name** cell will be colored yellow.

Note: A **Delete** icon () will be displayed in the last column of each component row. Click on it if you would like to delete a component.

- 10. Enter the name of the primer or probe.
- 11. Enter the Concentration in the assay reaction in the corresponding cell.
- 12. Optional step: To add further primers or probes, repeat steps 8-11.
- 13. If the target or primer-probe mix has been defined, click **OK**. The dialog will close.
- 14. The primer-probe mix will be listed in the **Targets** table. The targets defined in the dialog share the same **Content** cell.

7.2.3.2.3 Defining additional reagents

Additional reagents can be managed (added, duplicated or deleted) using the **Additional reagents** table at the bottom. The table lists the reagent name and the volume per reaction.

Adding an additional reagent

- 1. To add a new reagent click on New additional reagent.
- 2. The **Add additional reagent** dialog will open. For details, refer to section <u>Add additional reagent</u> <u>dialog</u>.
- 3. The reagent will be displayed as an additional row in the **Additional reagents** table.



The buttons in the **Actions** column

Button	Action	Description
0	To edit a reagent	The Edit additional reagent dialog will open, displaying the values that have been entered in the Add additional reagent dialog.
L	To duplicate a reagent	The Duplicate additional reagent dialog will open, displaying the values that have been entered for the original reagent. The reagent names will initially display a copy of the reagent.
×	To delete a reagent	Deletes the row in the Additional reagents table.

7.2.3.2.4 Add additional reagent dialog

Add additional reagent	
Reagent name	
Mg2+	
Concentration in assay reaction Stock concentration	Volume per reaction 1 μl [0 - 20 μl]
	OK Cancel

- 1. Enter the reagent name.
- 2. Enter the **Volume per reaction**. Alternatively, you can specify the concentration in the assay reaction along with the stock concentration, and the software will automatically calculate the **Volume per reaction**.
- 3. Click OK.
- 4. The reagent will be displayed as an additional row in the Additional reagents table.

Note: The QIAgility will add the additional reagent to the assay points during run.

7.2.3.3 Controls/Standards step

This optional step is used to define controls and standard series. If you do not intend to use controls or standards for the run, proceed by clicking **Next**.

The screen contains two tables: **Controls** and **Standard series**. Both tables have an **Actions** column with interactive buttons to manage the controls and standard series.

Adding a control or standard series

- 1. Click on Add control or Add standard series.
- 2. The **Add control** or **Add standard series** dialog will be opened. For details, refer to <u>Add control</u> <u>dialog</u> or <u>Add standard series dialog</u>, respectively.
- 3. The control will be added to the **Controls** table, and the standard series will be added to the **Standard series** table.
- 4. Click **Next** to proceed.

ction Setup	Define cor	trois and standards						
	Controls Sequence	N	ame	Target	Target type	Control type	Replicates	Actions
jets				PDH	Reference	NTC	Replicates	
ntrols / Standards >	1-4	NTC GAPDH + GPCR-like 5	G	CR-like 5	Test	NTC	4	0 🖪 🗙
signment	Output laby	vare order						Add control
orktable	Output laby Standard s Sequence		Concentration	Target	Target type	Туре	Replicates	Add control



The buttons in the **Actions** column of the **Controls** table and the **Standard series** table have the following functions:

Button	Action	Description
D	To edit a control or standard series	The Edit control or Edit standard series dialog will open, displaying the values that have been entered in the Add control or Add standard series dialog.
Ľ	To duplicate a control or standard series	The Duplicate control or Duplicate standard series dialog will open, displaying the values that have been entered for the original control or standard series. The control or standard series names will initially display as a copy of the control or standard series.
×	To delete a control or standard series	Deletes the row in the Controls or Standard series table.

Changing the order of the output labware

You can change the order of the controls and standard series using the up and down arrows below each table. Use this option to change the order of several controls within the controls section or the order of several standard series within the standard series section. The order of the output plate will be based on the order indicated in these tables.

Output labware order

Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.3.3.1 Add control dialog

Ģ	Ø	Add control				
Cont	Control name					
NTC GAPDH + GPCR-like 5 4 T Replicates						
		Target	Target type	Reporter	Control type	
✓		GAPDH	Reference	HEX	NTC	
		GPCR-like 5	Test	JOE	NTC	
				ок	Cancel	

NTC for a multiplex assay

- 1. Enter a control name in the **Control name** field.
- 2. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:

1	•
1	
2	
3	
4	
5	

Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

3. The software lists all defined targets and target mixes in the table below. Select at least one of the targets by activating the check box in the first column of the desired target(s).

Note: Selecting more than one target per control will result in a multiplex assay, including all selected targets in one reaction mix.

4. Select the control type using the drop-down menu in the **Control type** column:

	Control	type	
	Select	•	
4	Select		
	POS		
	NTC		

Option Description

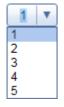
POS A positive control will be added to the assay points.

- NTC Water from the kit will be added to the assay points.
- 5. Click **OK** to confirm and close the **Add control** dialog.
- 6. The control will be added to the **Controls** table.

7.2.3.3.2 Add standard series dialog

Standar	d Series 5				3	Replicate	s
Target							
GPCR-	like 5			•			
C-target							
				•			
				_			
Use re	adv-made standard series						
	ady-made standard series				Con	centration unit	
Use re Dilution s	-	Stock			_	centration unit pies/µl	•
Dilution s	-	Stock		on rat	CO		V
Dilution s	teps	Stock)	on rat	CO	pies/µl	V
Dilution s 3 V	Name of standard	Stock) Dilutio	on rat	CO io	pies/µl Concentration	•

- Dialog for standard series
- 1. Enter a name for the series in the **Standard series name** field.
- 2. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

- 3. Select a target from the **Target** drop-down menu.
- 4. Optionally, you can select one IC target from the **IC target** drop-down menu.
- 5. Check the option **Use ready-made standard series**, if you intend to load the ready-made dilutions individually for the run.

Uncheck the option if you plan to load one tube containing the standard stock and would like for the QIAgility to automatically create the dilutions for the standard series during the run.

6. Select the number of dilution steps from the **Dilution steps** menu. **Dilution steps**



The number of rows in the table at the bottom is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering</u> <u>data</u>.

Note: In order to use the actual stock as the first standard, define the first standard to have the same concentration as the stock (dilution ratio will be 1:1 in this case). If the option **Use ready-made standard series** has not been checked, the QIAgility will directly use the stock as template for the assay points of this standard.

 If the option Use ready-made standard series has not been checked, enter the stock concentration in the Stock field and select the concentration unit using the Concentration unit drop-down menu. Concentration unit

	▼
%	
IU/ml	
IU/µl	
copies/reaction	
copies/ml	
copies/µl	
µg/µl	
ng/reaction	
ng/µl	
pg/µl	
%mut	
μM	

For each dilution step, enter either a dilution ratio or a concentration. The other value will be calculated automatically by the software. Optionally, enter a meaningful name into the Name of standard cell. This is especially important if you plan to define more than one standard series.
 Note: The dilution ratio refers to the stock concentration.

Note: Concentration values lower than 0.01 will be displayed in scientific format, e.g., 1.00E-03 represents 0.001.

Note: This step is optional if the option Use ready-made standard series has been checked.

- 9. Click **OK** to confirm and close the **Add standard series** dialog.
- 10. The standard series will be added to the **Standard series** table.

7.2.3.4 Labware Selection step

Use the **Labware Selection** screen to select the labware for the input side and the output side and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side.

QIAGEN Horr			instrument
✓ *Experiment_0 ×			10 A A A A A A A A A A A A A A A A A A A
Reaction Setup	Define input labware, samples, and output labware		
	Input labware	Output labv	vare
Targets	Use sample IDs or existing sample input file	Options	
Controls / Standards	 Enter sample IDs Load existing sample file 	-	output labware
Labware Selection >	Load sample file)		put labware file
Assignment	Input labware type QIAGEN S-Block 96 Well Use cooling block	Output labw	are type lotor-Disc 100 / Rotor-Gene Q, Rotor-Disc 100 Ea 🔻 🗌 Use cooling block
Worktable	Define samples		
Run			
	Labware Selection	tep	I

To define the input labware:

- 1. Check the option Use sample IDs or existing sample input file.
- 2. Select either **Enter sample IDs** to define sample IDs manually using the **Sample editor**, and proceed to step 3.

Alternatively, select Load existing sample file to use a sample input file, and proceed to step 11.

To select the input labware type:

...

- 3. Click on the **Input labware type** drop-down menu.
- 4. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

Input labware type	
1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Taperei 🔻 🔲 🗌 Us	e cooling block
QIAGEN S-Block 96 Well	
1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered-Base Tube	s
Choose other labware	

5. Select your input labware in the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Iter options	Labware		
ategory Standard 96 Well T Shows available labware only	Select the item that most closely matches the lat QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name QIAGEN S-Block 96	
Advanced options Name of labware		Dead Volume 40 µl	1
Part number Manufacturer			A
Tube / Well size	Labware adapter Select the adapter to be used.		
Reset filter Apply filter		Catalog number	
		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then also select the adapter below. Click **OK** to close the **Labware browser**.

The selected labware will be displayed and added to the Input labware types drop-down menu

Note: For more details about the Labware browser, refer to the section Using the labware browser.

6. **Optional step:** If you want to use the cooling block for the input labware, check the option **Use** cooling block next to the **Input labware type**.

To define samples manually:

7. Click **Define samples**.

°05.	Sample ID	Conc. [ng/µl]	Description
A1			•
A2			
A3			
Α4			
A5			·
A6			
Α7			
A8			
A9			
A10			
A11			
A12			
B1			
B2			
B3			
B4			
B5			
B6			
B7			
B8			
В9			
B10			
B11			
B12			

8. The **Sample editor** will be opened, and the **Sample ID** fields will be colored yellow to indicate that this information is required.

9. Enter each sample ID and optionally, enter the concentration and sample description.

Note: Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells. Press the **SHIFT** key and left-click to select multiple adjacent cells or press the **CTRL** key and left-click to select multiple non-adjacent cells. Then right-click to open the context menu and click on **Auto-generate sample names**.

Сору	Ctrl+C
Paste	Ctrl+V
Auto-generate sample names	

Note: For more details about the sample editor, refer to the section Editing sample names.

10. Click OK to close the Sample editor. Proceed to step 16.

To define samples by using a sample input file:

11. The Load sample file and Scan labware barcode buttons are enabled, if the option Load existing sample file had been checked.

Use sample IDs or existing sample input file

O Enter sample IDs

Load existing sample file

Load sample file ... Scan labware barcode ...

- 12. Click the Load sample file button and select the desired sample file. For details, refer to File dialog.
- Alternatively, if you would like to load the sample information based on the barcode on the labware, click the Scan labware barcode button. The Scan labware barcode dialog opens. Scan the barcode and confirm by clicking OK. For details, refer to <u>Scan labware barcode dialog</u>.
- 14. The software loads the sample information from the sample file and displays the sample editor. Optionally, edit the sample information. Then click **OK** to confirm.
- If the sample information file also contains the labware type, it will automatically be selected in the Input labware type drop-down menu.
 Note: This is only possible for files in QDEF format.

To define the output labware:

16. You can either **Use an empty output labware** or **Use an existing output labware**. The option **Use empty output labware** is initially selected and can be used for most runs.

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case select the option **Use an existing output labware** and proceed to step 19.

17. Select the output labware from the **Output labware type** menu on the right. It includes all labware suitable for the selected cycler. A fitting adapter is automatically added if marked as available in the labware manager. Proceed to step 22.

Note: If the selection is yellow, you are required to mark the labware or a fitting adapter as available in the **Labware manager**.

Note: If the labware to be used is not listed, select **Choose other labware** to open the **Labware** browser.

18. **Optional step:** If you want to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

19. The Load output labware file and Scan labware barcode buttons are enabled, if the Use existing output labware option had been selected.

Options

O Use empty output labware

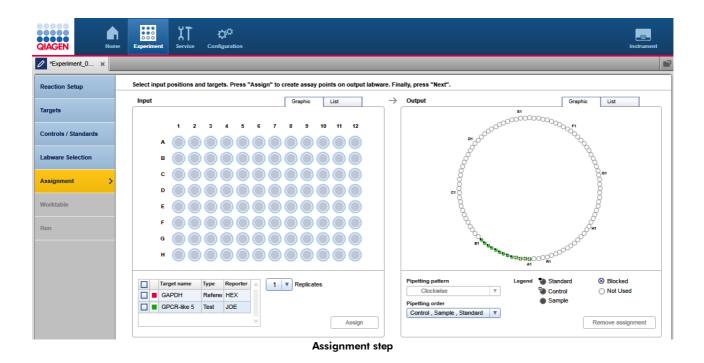
Use existing output labware (partially filled labware from a previous run)

Load output labware file ... Scan labware barcode ...

- 20. Click the **Load output labware file** button and select the desired labware file. For details, refer to <u>File</u> <u>dialog</u>.
- 21. Alternatively, click the **Scan labware barcode** button. In the **Scan labware barcode** dialog, scan the barcode and confirm by clicking **OK**. For details, refer to <u>Scan labware barcode dialog</u>. Using the information from the loaded file, the software will automatically select the **Output labware type**. In addition, the positions that were used with this labware for a previous run will be blocked.
- 22. Click **Next** to proceed.

7.2.3.5 Assignment step

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of sample-target combinations to the output labware. Single and multiplex assay points can easily be generated for selected samples.



Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware. The meaning of the different symbols is described below:

•	Name Sample	Description Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been selected once for assay setup on output labware.
	Selected sample	Currently selected sample.
•		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
•		Note: It is possible to use the same sample multiple times to create assay points on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. In the **List** tab, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this sequence is used when assigning selected samples to the output labware.

At the bottom of the panel is a table showing all targets defined in the **Targets** step, along with a **Replicates** menu.

In order to assign a sample to an assay point, select the sample position(s) and then the target(s) you would like to assign to the selected sample(s). Then set the number of replicates. When you click the **Assign** button, the selected samples and targets will be assigned to assay points created on the output labware. Each combination of targets assigned to the output labware will result in the setup of a target reaction mix including the target-specific components.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. When entering the screen, the software automatically places all standards and controls from the **Controls/Standard** step. The creation of assay points containing samples depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at the input side. The assay points are colored with the targets. Standards and controls are indicated by an additional small symbol. If you hover with the mouse over one target color of an output position, the software displays the sample ID, the target and the target type for that position.

Output			Graphic	L	ist 🔓		
Pos.	Sample ID		Target			Conc. [µM]	
A1	NTC GAPDH + GPCR-I		GAPDH , Reference GPCR-like 5 , Test	;			0
A2	NTC GAPDH + GPCR-I		GAPDH , Reference GPCR-like 5 , Test	•			
A3	NTC GAPDH + GPCR-I		GAPDH , Reference GPCR-like 5 , Test	•			
A4	NTC GAPDH + GPCR-I		GAPDH , Reference GPCR-like 5 , Test	•			
A5	1		GAPDH , Reference GPCR-like 5 , Test	•	_		
A 6	1	-	GAPDH , Reference GPCR-like 5 , Test	•			
A7	1		GAPDH , Reference	;			
Pipetting pattern		Legend 🏾 🍓 Star	ndard	Blocked			
Clockwise 🛛		Cor		Not Used			
÷	ting order	-	San	nple			
Con	trol, Sample, Standard	*			Remov	ve assignmen	ıt

Use the List tab of the output panel to view the assay points that have been created.

The List tab of the Output panel: 4 replicates of the NTC control are placed at A1-A4. Both targets have been assigned to sample 1, with 3 replicates at A5-A7.

The two menus at the bottom of the output panel can be used to set the **Pipetting pattern** (e.g., row by row vs. column by column) and the **Pipetting order** (e.g., first standards, then controls, then samples). You can also define a new pipetting order.

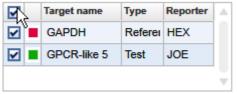
7.2.3.5.1 Assigning samples with targets to the output labware

Proceed as follows:

1. From the **Input** panel, select the sample position you want to assign a target.

Note: To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions, or drag the mouse to select adjacent samples in a rectangle. Selecting in a rectangle is only possible on rectangular plates.

2. From the targets table below, check the box next to the target you want to assign. You can check multiple targets at the same time in case you want to make multiple target assignments.



All targets selected.

Note: To select all targets, check the box in the table header.

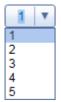
3. If an <u>IC target</u> has been selected, additional options will be displayed.

If the kit contains an IC, use the option **Samples contain IC** if the samples on the input labware already contain the IC. Use **Add IC to reaction** if the QIAgility shall add the IC to the assay points during the run.

The option Add IC to reaction is enabled only if the kit contains an IC.

If the kit does not contain an IC, the samples on the input labware must already contain the IC. The option **Samples contain IC** is the only available option. However, but only, if the IC shall be used for all assay points and the samples do not yet contain the IC, you can define the IC as an <u>additional</u> reagent. As every additional reagent, the IC will be added by the QIAgility to all reaction mixes.

4. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:

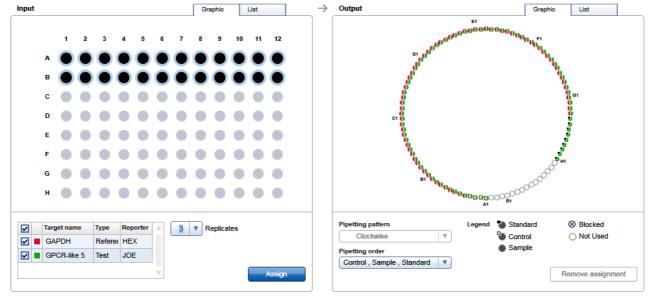


Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

- 5. Click Assign.
- 6. The software creates assay points on the **Output** panel on the right side of the screen. The selected target(s) are assigned to the selected samples. If you have selected more than one replicate, the sample positions are multiplied accordingly. The positioning of the created assay points on the output side follows the selections made for the **Pipetting pattern** and the **Pipetting order**.
- 7. Repeat the steps until all positions of the output side have been defined for the planned run.
- 8. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 9. Close the lid.
- 10. Click **Next**. If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable step**, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the **Worktable step**.

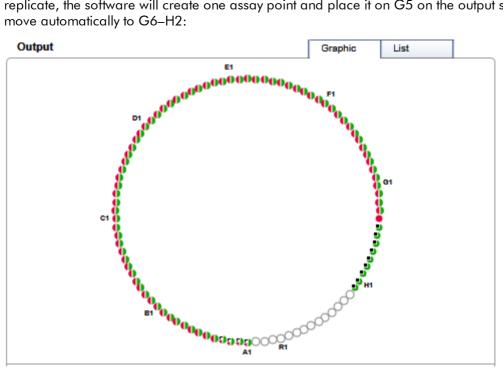
Example:



Select input positions and targets. Press "Assign" to create assay points on output labware. Finally, press "Next".

Both targets have been assigned to the samples of rows A and B, with 3 replicates. Controls have been placed automatically at A1-A4, standards at G5-H1.

In this example, the created assay points for rows A and B have been placed between controls and samples. If you would now select the sample on C1 on the input plate, assign only target GAPDH with one replicate, the software will create one assay point and place it on G5 on the output side. The standards will move automatically to G6–H2:



New assay point placed at G5 (red position).

7.2.3.5.2 Removing target assignment

To remove an assigned target from the output side,

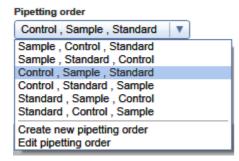
- 1. Select the desired sample position on the **Output** side. Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.
- 2. Click on **Remove assignment**. The assignment will be removed, and the sample position will be cleared from the output side.

Note: The software removes all replicates of the desired sample(s).

7.2.3.5.3 Creating a new pipetting order

Generally, the pipetting order defines the placement order of the components (samples, controls, standards) on the output side. It can also be used to define blocked positions on the output labware that shall not be used for the run.

The QIAgility Setup Manager Software comes with a set of predefined pipetting orders, which you find in the **Pipetting order** drop-down menu. At the end of the menu are two commands to **Create a new pipetting order** and to **Edit a pipetting order**. Use this function to define output layouts for reuse. Once defined, they are also listed in the menu, if the same output labware is used.



- 1. Click on the **Pipetting order** drop-down menu under the output side.
- 2. Click on Create new pipetting order.

3. The Create new pipetting order dialog is shown:

Pipetting	ordei	r nam	e)			
											Sav	'e	J			
output lai				(- II												
QIAGEI	N S-E	SIOCK	96 W	eli					•							
utput la	bware	9													Position	
	1	2	3	4	5	6	7	8	9	10	11	12	(С	Blocked	Ø
А	•	•	•	•	•	•	•	•	•	•	•	•	(С	Standards Only	1
в	•	•	•	•	•	•	•	•	•	•	•	•	(С	Controls Only	•
С	•	•	•	•	•	•	•	•	•	•	•	•	(С	Samples Only	С
D	•	•	•	•	•	•	•	•	•	•	•	•	(С	Any Kind	
E	•	•	•	•	•	•	•	•	•	•	•	•				
F	•	•	•	•	•	•	•	•	•	•	•	•	Pi	pet	ting pattern	
G	•	•	•	•	•	•	•	•	•	•	•	•			Line by Line	
н	•	•	•	•	•	•	•	•	•	•	•	•				

- 4. Enter a name for the pipetting order in the **Pipetting order name** field.
- 5. The **Output labware type** menu initially displays the labware that has been selected for the output labware before. This selection needs to be changed only, if you would like to create a pipetting order for another labware. In this case, select the desired labware from the list in the menu, or click on the **Choose other labware** command at the end of the list, in case the desired labware is not listed here. The selected labware will be used as output labware for the run.
- 6. Define the new pipetting order using the labware graphic. All positions are set to Any Kind by default:
 - a. Select the position in the graphic whose assignment you want to change.

Note: Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions, or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.

b. From the **Position** table at the right, click on the button you want to assign to the selected position:

Button	Description Blocked position
	Position for standards
•	Position for controls
0	Position for samples
	Any kind

- c. The selected position will be assigned to the selected position. The graphic symbol for the selected positions will be changed accordingly.
- 7. To change the pipetting pattern, select the appropriate command from the **Pipetting pattern** menu (e.g., **Row by Row** or **Column by Column** for rectangular plates).
- 8. Click **Save** to save the pipetting order to a file.
- 9. Click **OK** to close the dialog. The newly created pattern is now available in the **Pipetting order** menu in the **Output panel**.

7.2.3.6 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

M1	A1	A2		
	B1	B2		
R1	C1	C2		

- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded. The run cannot be started if any yellow positions remain.
- Worktable selector
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

The software displays the labware and required minimum volume of reagents to be loaded in a table located in the middle of the screen. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column.

Standard 1			
	200 µI PCR tube (QIAGEN)	0 µl	~
Mg2+	200 µI PCR tube (QIAGEN)	111 µl	✓
5 rev	200 µI PCR tube (QIAGEN)	110 µl	✓
5 for	200 µI PCR tube (QIAGEN)	110 µl	✓
5 Probe	200 µI PCR tube (QIAGEN)	110 µl	~
GAPDH rev	200 µI PCR tube (QIAGEN)	99 µl	✓
GAPDH for	200 µI PCR tube (QIAGEN)	99 µl	~
GAPDH Probe	200 µI PCR tube (QIAGEN)	99 µl	✓
Standard Series 5	200 µI PCR tube (QIAGEN)	21 µl	~
2x Rotor-Gene Multiplex PCR	2.0 ml self standing screw cap tube (969 µl	~
RNase-Free Water	2.0 ml self standing screw cap tube (285 µl	~
	5 rev 5 for 5 Probe GAPDH rev GAPDH for GAPDH Probe Standard Series 5 2x Rotor-Gene Multiplex PCR	5 rev200 µl PCR tube (QIAGEN)5 for200 µl PCR tube (QIAGEN)5 for200 µl PCR tube (QIAGEN)5 Probe200 µl PCR tube (QIAGEN)GAPDH rev200 µl PCR tube (QIAGEN)GAPDH for200 µl PCR tube (QIAGEN)GAPDH Probe200 µl PCR tube (QIAGEN)Standard Series 5200 µl PCR tube (QIAGEN)2x Rotor-Gene Multiplex PCR2.0 ml self standing screw cap tube (5 rev200 µl PCR tube (QIAGEN)110 µl5 for200 µl PCR tube (QIAGEN)110 µl5 Probe200 µl PCR tube (QIAGEN)110 µlGAPDH rev200 µl PCR tube (QIAGEN)99 µlGAPDH for200 µl PCR tube (QIAGEN)99 µlGAPDH Probe200 µl PCR tube (QIAGEN)99 µlStandard Series 5200 µl PCR tube (QIAGEN)21 µl2x Rotor-Gene Multiplex PCR2.0 ml self standing screw cap tube (969 µl

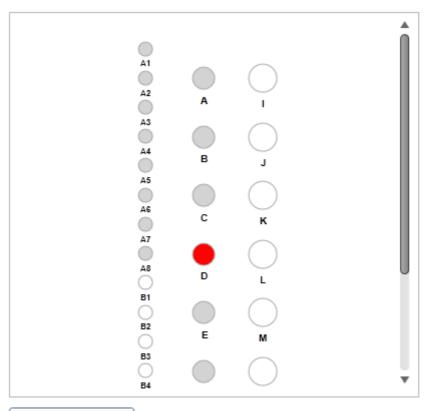
R1 - Reagent block

Table of tubes to be loaded to the worktable position R1.

It is possible to change the tube type for a reagent to be loaded.

Tube	
200 µI PCR tube (QIAGEN)	Ň
200 µI PCR tube (QIAGEN)	5
200 µl PCR tube (QIAGEN)	_

Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that are also marked as available in the laboratory in the **Labware Manager**. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type. For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. Tubes from QIAGEN kits are displayed with the same color as their tube caps.



Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

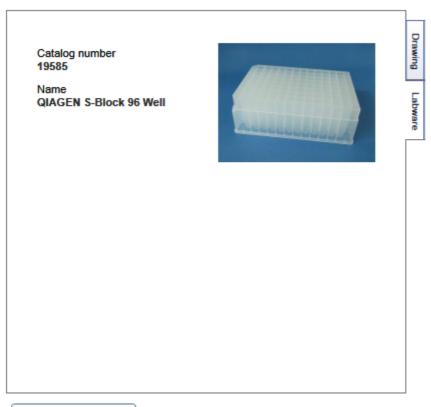
Calibrate labware ...

Schematic graphic for R1.

Click the Labware tab to get an image and details about the input and output labware.

Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

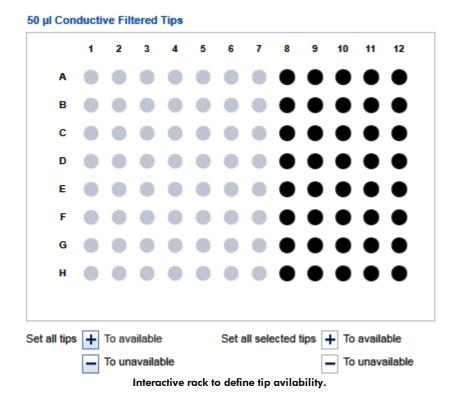
Labware: QIAGEN S-Block 96 Well



Calibrate labware ...

Image and details for input labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To available** or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate** button.

Preparing the worktable

The worktable preparation starts with the positions for tip racks:

- 1. Open the instrument lid.
- Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.
 Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u>
- Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started.

Click the **Calibrate labware** button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again.

4. Check that the interactive graphic on the right side of the screen shows the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to Loading tips.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 5. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 6. Click **Next** to continue with the next worktable position.
- 7. Repeat steps 2–6 for all tip rack positions.

For all other worktable positions:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

8. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps.

Note: Ensure that you have the empty labware available for the calibration. For tubes from the kit, refer to the QIAgility Calibration Kit that was shipped together with the QIAgility.

- 9. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 10. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed reagent is provided in the correct tube type. The volumes displayed are the minimum volume required, so consider adding extra to prevent liquid level detection errors. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
 - For samples: Ensure that samples are loaded using the correct labware type. Check that the samples are placed in the positions as displayed in the table.
- 11. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.

- 12. Click **Next** to continue with the next worktable position.
- 13. Repeat the steps 8–12 for all worktable positions.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 14. Close the lid.
- 15. Ensure that the tip disposal box is empty.
- 16. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

17. The <u>Run step</u> will be displayed.

7.2.3.7 Run step

The run progress can be <u>monitored</u> in the **Run** step. During the run, this is the only active step of the experiment. All other steps are read only. A run can be <u>paused or canceled</u> at any time. After the run, several <u>options</u> are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

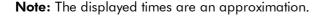
7.2.3.7.1 Monitoring a run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress

Task	Progress
Prepare Reaction Mix Base	
Prepare Standard Series	
Prepare Target Reaction Mix	
Distribute Liquids to Output Plate	
	v
Overall progress	
Start time	Approximate end time
17:40	17:59
Run prog	ress.



The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.3.7.2 Pause/cancel a run

A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	
Overall progress		
Start time 13:05	Paused at Resume	

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

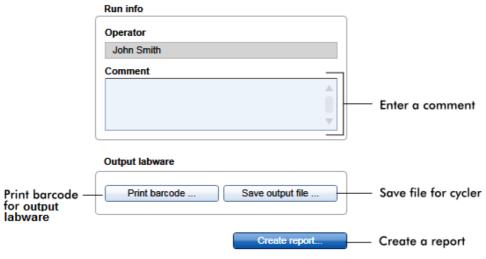
To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

Note: Pauses of a run are listed in the Exceptions/Pauses section of the Advanced report.

7.2.3.7.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.



After the run, several options are available in the right side of the **Run** step screen.

Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Print barcode	You can print a barcode for the output labware.
	Note: The option is available only if a printer has been selected in the Global Settings tab of the Configuration environment.
Save output file	An output file will be saved in xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>Customize user settings</u> to define a standard directory.
	The file may serve as sample input file for the Rotor-Gene Q operated with the Q-Rex software. The .xml file can also be used by a LIMS (Laboratory Information Management System). Find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QIAGEN\QIAgility\Examples.
	Note: Depending on the selected cycler, the software also creates a file in the format that is expected by the cycler. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension, and will also be saved in the specified directory. The input formats of the following cyclers are supported by the first release of the Gene Expression Plug-in: Rotor-Gene Q operated with the Rotor-Gene Software, version 2.3 or higher. Contact QIAGEN Technical Services if you need another file format.
	Note: Use this option also in the case that the sample input is limited and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Labware Selection step that you are using an existing output labware by selecting the specific option, and load this file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.

The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section <u>Cleaning the QIAgility</u>.

7.2.3.8 Report

The QIAgility Setup Manager Software offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results
- Exceptions
- Detailed run information

The **Advanced report** contains:

- General information
- Comment
- Kit information
- Reaction results
- Exceptions/Pauses
- Sample information
- Worktable information
- Detailed run information
- Advanced instrument information

Descriptions of the sections that are part of both report types are provided below.

General information

Some general information is provided at the beginning the report. This includes information such as the name of the kit, the experiment file and the output file (if any has been saved), the instrument serial number and the date, time and duration of the run.

Comment

The comment that has been entered on the **Run** screen.

Reaction results

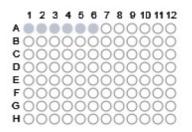
For regular rectangular output plates, the following table and an overview graphic are shown, visualizing each position on the output plate:

Symbol		Description
•	ok	The position was processed without any exceptions.
۲	warning	The position was processed with exceptions that allowed further processing (e.g., tip failure, liquid level detection error). These exceptions are listed in the Exceptions chapter below.
•	error	The position could not be processed as required due to an exception that did not allow further processing (i.e., the run was stopped due to an error). The exception is listed in the Exceptions chapter below.
0	empty	The position is empty. The position has not been used during the run.
	not used	This symbol is only used if an output file has been loaded as a starting point for the output plate (e.g., when using several runs to fill the output labware). The symbol represents a position that was not part of this run, although it is not empty.

Reaction results

Input plate ID: 90945_20150915_174454 Output plate ID: 90945_20150915_174455

ok
warning
error
empty



Note: If no input or output file has been loaded, the software automatically creates identifiers for the input and output labware.

A table listing the reaction result for each processed position on the output labware is also shown:

Column	Description
Output	The position on the output labware.
Identifier	Specifies the sample ID or the name of the particular standard or control.
Туре	One of Sample, Standard or Control.
Input	The corresponding position on the input labware. Valid for samples only. Empty for standards and controls.
Concentration	For standards, the concentration of the standard is listed. For samples, the final concentration is listed (only if the sample's concentration has been entered in the sample editor or loaded with a sample input file); otherwise, this is empty. Always empty for controls or NTC.
Flag	Shows the status of the position: either ok, warning or error, corresponding to the symbols of the graphic

Note: The table contains only those positions on the output labware that were subject to this run. Empty or unused positions are listed for reference below the table.

Exceptions

This section lists all events, if any, in a table:

Column Exception	Description The event that has been displayed during run.
Position	Lists the positions that were affected by the exception.
	First, the position is listed for which the exception occurred. After "Destination," all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.
	Example: "M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which might have impacted the output positions D1–D8 (row-wise).
	Note: If an output position is flagged OK in the reaction results table, even though it is listed here as possibly affected by an exception, then you can trust that the output position was completed successfully.
User response	A short form, which option (button) of the message dialog has been pressed.

Note: The **Basic** report does not list pauses. If you want to have also pauses listed, use the **Advanced** report, instead.

Detailed run information

This sections lists all tasks of the run as they were displayed on the **Run** step.

Descriptions of the sections that are only included in the **Advanced** report are below.

General information

Kit information is not listed here for the **Advanced** report; instead, this information is provided in more detail in a separate section.

Kit information

Kit name, lot number and expiration date. Comment provided with the kit.

Note: The Advanced report does not repeat this information in the General information section.

Reaction results table of the Advanced report

In addition to information provided in the **Basic** report, the **Reaction results** table of the **Advanced** report contains two additional columns:

Output	Identifier	Туре	Input	Actual content	Concentr ation	Final volume µl	Flag
A1	NTC GAPDH + GPCR-like 5	NTC		18 µl Mix1 [GAPDH,GPCR-like 5] + 2 µl RNase- Free Water		20	ok
G4	24	Sample	B12	18 μl Mix1 [GAPDH,GPCR-like 5] + 2 μl 24		20	ok
G5	Standard 1	Standard		18 μl Mix2 [GPCR- like 5] + 2 μl Standard 1	100 copies/µl	20	ok

Column	Description
Actual content	Lists the components. The software creates automatic names for each created reaction mix. The names of the targets used are given in brackets [].
	Examples:
	Output position A1 is the NTC for both targets and contains 18 μ l of the reaction mix Mix1 for GAPDH and GPCR-like 5, and 2 μ l RNase-free water.
	Output position G4 contains 18 μ l of the reaction mix Mix1 for GAPDH and GPCR-like 5, and 2 μ l of sample 24 which was on input position B12.
	Output position G5 is the first standard of the standard series and contains 18 μ l of the reaction mix Mix2 for target GPCR-like 5 and 2 μ l of the created first standard.
Final volume	Lists the final volume.

Exceptions/Pauses of the Advanced report

In contrast to **Basic** report, this section not only lists all events but also any pauses that may have occurred during the run in a table:

Column	Description
Exception	The event or pause that has been displayed during run.
Position/Duration	For an event:
	Lists the positions that were affected by the exception.
	First, the position is listed for which the exception occurred. After "Destination", all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.
	Example:
	"M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which might have impacted the output positions D1–D8 (row-wise).
	Note: If an output position is flagged OK in the reaction results table, even though it is listed here as potentially affected by an exception, then you can trust that the output position was completed successfully.
	For a pause: the duration of the pause.
User response	A short form, which option (button) of the message dialog has been pressed.

Sample information

Lists the sample information as it was entered in the sample editor or loaded by the sample input file, together with the identifier of the input labware.

Worktable information

Lists for each worktable position the liquids that have been placed there.

Detailed run information of the Advanced report

In addition to the run task list included in the **Basic** report, the assay setup information is also listed:

Assay Setup

Reaction mix [Target(s)]	Content
Mix1 [GAPDH,GPCR-like 5]	850 μl 2x Rotor-Gene Multiplex PCR Master Mix + 85 μl Mg+ + 76 μl RNase- Free Water + 76 μl GAPDH rev + 76 μl GAPDH for + 76 μl 5 rev + 76 μl 5 for + 76 μl GAPDH Probe + 76 μl 5 Probe
Mix2 [GPCR-like 5]	850 μl 2x Rotor-Gene Multiplex PCR Master Mix + 85 μl Mg+ + 36 μl RNase- Free Water + 9 μl 5 rev + 9 μl 5 for + 9 μl 5 Probe

This provides detailed information about each mix that was automatically created by the QIAgility.

Information about standard series is also provided if they were used in the run.

Advanced instrument information

This section lists not only the series numbers of the QIAgility and the pipettor head, but also information about HEPA filter usage, service due dates and volume calibration.

7.2.4 Working with experiments for pathogen detection

The Pathogen Detection Plug-in enables easy and convenient qPCR setup of your pathogen testing experiments using dedicated lines of QIAGEN kits including QuantiTect, QuantiFast and QuantiNova.

New experiments for pathogen detection are defined in the **Experiment** environment of the software. When a new experiment is created, only the **Reaction Setup** step will initially be enabled. All other steps will become available once the required information has been entered in the **Reaction Setup** step. The coloring of the **Targets** step will change to yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 8 steps that guide you through the run setup process:

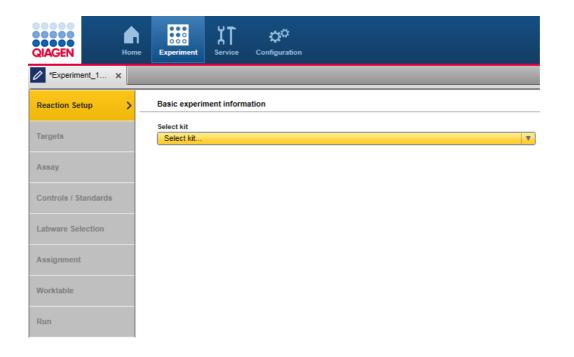
- 1. <u>Reaction setup</u>
- 2. Targets
- 3. Assay
- 4. Controls/standards
- 5. Labware selection
- 6. Assignment
- 7. <u>Worktable</u>
- 8. <u>Run</u>

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. The file extension for pathogen detection experiments is ***.qsmpx**, and for templates ***.qsmpt** (**Q**|Agility **S**etup **M**anager **P**athogen detection **Experiment**, and **T**emplate, respectively). The **Start run** button will be disabled as long as there are incomplete or invalid steps or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.4.1 Reaction Setup step

The **Reaction Setup** step is used to make basic selections, such as the kit to be used, the preferred cycler and the volume definitions. When you create a new experiment, the **Reaction Setup** step will be the only active step, and within this step, only the **Select kit** menu will be active. The other controls will initially remain inactive, but will become visible once you have selected the kit to be used in the reactions.



1. Select the kit to be used from the **Select kit** drop-down menu.

Select kit		
QuantiNova Pathogen + IC Kit (500)	G	For highly sensitive, ultrafast, real-time PCR and RT-PCR using sequence- specific probes

2. The **Kit information** and **Select cycler** fields are displayed.

Kit information

Product number	Lot number	Kit expiry date
204774		📰

3. Optionally, enter the **Lot number** and the **Kit expiry date** in the corresponding fields for documentation purposes.

Note: You can use the date picker () to open an interactive calendar for entering the dates.

- 4. Select the cycler to be used from the **Select cycler and output format** drop-down menu.
- 5. If the selected cycler requires a passive reference, the **Passive reference** option will be enabled. Select this option to use passive reference.

Note: If the selected kit contains one or more passive references, and the passive reference option is selected, the software will automatically add the passive reference according to the cycler you select to the additional reagents list (see <u>Target step</u>) along with the recommended volume for your setup.

6. The **Volume definitions** area is displayed. The fields may vary depending on your specific kit and cycler selection.

volume definitions			
Sample input volume 5 µl [1 - 20 µl]	Use ready-made assay mixes	i	Use option "Use ready-made assay mixes" if prepared assay mixes are used that already contain all components (such as kit master mix, primers, probes, PCR (C, and all additional reagents). Water may be added by the instrument.
Total reaction volume			
20 µl [10 - 40 µl]			

Note: The default values that appear in the volume fields are the recommended values from the kit handbook for your setup.

7. Enter the volume data in the corresponding fields:

Field	Description
Sample input volume	Enter sample input volume. This is the volume of sample, controls and standards that will be added to the assay mix.
Total reaction volume	Enter the total reaction volume. This is the volume of the final reaction mix including assay mix and sample, control or standard.

8. Check the box next to **Use ready-made assay mixes** to confirm that you have already prepared the assay mixes containing all the components. The assay mix must include the target components, the passive reference (if required by your cycler), and all other reagents. Enter the assay mix volume per reaction.

Later in the worktable step, you will be asked to place these already prepared mixes onto the worktable. During the run, the QIAgility will use the mixes directly for the assay points and add only water if the assay mix volume per reaction plus the sample input volume is less than than the total reaction volume you specified.

9. Click **Next** to proceed.

. . .

7.2.4.2 Targets step

The Targets step is used to define targets, internal controls and, optionally, additional reagents.

<u>Define primer-probe mixes or single components</u> for the targets. All defined targets and primer-probe mixes are listed in the **Targets** tables. At least one target needs to be defined.

<u>Define internal controls</u> (PCR or process controls) that will be used. All defined internal controls are listed in the **Internal controls** table.

If the selected kit contains an internal control (IC), the corresponding IC target and internal control(s) are already predefined.

Optionally, <u>additional reagents can be defined</u> to be added to all reaction mixes. Defined reagents are listed in the **Additional reagents** table. If the selected kit contains one or more passive references, and the passive reference option is selected, the software will automatically add the passive reference according to the cycler you selected to the additional reagents list with the recommended volume for your setup. If you selected the option **Use ready-made assay mixes**, the definitions in the **Additional reagents** table are for documentation purposes, only. Ensure that you include all listed reagents already in the ready-made assay mixes, because the QIAgility will not add them to the assay points during run.

Click Next to proceed.

AGEN Home		Çî ^{Çî} Figuration							Instrum
*Experiment_1 ×									
Reaction Setup	Define targets and reagents								
	Targets								
rgets >	Tar	get name			C	ontent	Target type	Reporter	Actions
	QuantiNova IC Probe Assay		QN IC F	orward Primer (0.8	µM); QN I	IC Reverse Primer (0.8 µM); QN IC Prob	IC	MAX	0 🖪 🗙
ssay	Pathogen Target 1		Forward	Primer 1 (0.8 µM);	Reverse f	Primer 1 (0.8 µM); Probe 1 (0.25 µM);	Test	FAM	
ntrols / Standards	Pathogen Target 2		Forward	Primer 2 (0.8 µM);	Reverse I	Primer 2 (0.8 µM); Probe 2 (0.25 µM);	Test	HEX	0 🗉 🗙
ssignment	Internal controls				Add	litional reagents			New target
	Name	Type	Content	Actions	Add	Name		Content	Actions
n	QN IC DNA	PCR control							
	QN IC RNA	PCR control	1 µl						
				Ţ					
				New IC				New ad	ditional reagent
			Tar	iets step					

7.2.4.2.1 Defining targets and mixes

You can choose whether the QIAgility should use single components (primers and probes) or ready-made primer-probe mixes.

To define:

- a new primer-probe mix for one or several targets
- or single components for one target

proceed as follows:

- 1. Click on **New target** below the **Targets** table.
- 2. The **Add target** dialog will be displayed. Refer to <u>Add target dialog</u> for details.
- 3. New rows for the defined primer-probe mixes or single components for the targets will be added to the **Targets** table.

Actions			
0	L	×	

The buttons in the **Actions** column

Button	Action	Description
0	To edit a target	The Edit target dialog will open, displaying the values that have been entered in the Add target dialog.
Ŀ	To duplicate a target	The Duplicate target dialog will open, displaying the values that have been entered for the original target. The target names will initially display a copy of the target.
×	To delete a target	Deletes the row in the Targets table.

Add target dialog 7.2.4.2.2

The **Add target** dialog contains two major options at the top:

Use ready-made primer-probe mix	Select this option if you intend to load a ready-made primer-probe mix in one single tube for the run.				
primer-probe mix	You can define several target names. There is also the option to define the components for documentation purposes.				
Use single components	Select this option if you intend to load primers and probes in individual tubes for the run.				
	Define one target name and the components (primers and probes) related to this target name.				

Note: If you selected the option **Use ready-made assay mixes** in the <u>Reaction Setup step</u>, the options are not displayed. Proceed with the steps **To define a ready-made primer-probe mix**, below.

Ø	Add target				
-	ready-made prime single component				
Targe	t definition				
		Target name		Target type	Reporter
Pa	athogen Target 1			Test 🔻	FAM 🔻
Tube	Name	Conc. in assay reaction [µM]	Stock concentration [µM]	Volume per reacti	
1	Forward Prim	0.8	16		1 🗙
	Reverse Prim	0.8	16		
2	Reveise Prim	0.0			· 🗠
3	Probe 1	0.25	5		1 🗙
					T
				Add primer	Add probe
				ОК	Cancel

Dialog to load primers and probes in individual tubes for the run.

To define single components for one target

- 1. Select the option **Use single components**.
- 2. Enter a target name. The **Target name** cell will be colored yellow as a reminder that this entry is required.
- 3. Click on the menu in the **Target type** column and select a target type:



Target type	Description
Test	Use this target type for your gene of interest or the targeted region.
Reference	Use this target type for reference targets, such as housekeeping genes or other endogenous controls.
IC	Use this target type for internal controls (IC). Internal controls are either added during sample extraction or before PCR setup.

4. **Optional step:** Click on the menu in the **Reporter column** and select an appropriate reporter dye for the target:

Reporter	
Ι	•
SYBR Green 1	
SYBR Green I	
Cy5	- 1
Crimson	- 1
ROX	- 1
SYBR	

Green	Yellow	Orange
FAM	JOE	ROX
SYBRGREEN1	VIC	CALFLUORRED610
SYBRGREENI	HEX	CY35
FLUORESCEIN	TET	TEXASRED
ALEXAFLUOR488	MAX	ALEXAFLUOR568
	CALFLUOR	
	GOLD540	
	YAKIMAYELLOW	
Red	Blue	Crimson
CY5	MARINABLUE	QUASAR705
QUASAR670	EDANS	ALEXAFLUOR680
ALEXAFLUOR633	BOTHELLBLUE	LIGHTCYCLERRED705
LIGHTCYCLERRED640	ALEXAFLUOR350	
	AMCAX	

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section <u>Entering data</u>.

- 5. In the **Components** table, define the primers and the probe (steps 6–10).
- 6. Click on Add primer to add a primer or click on Add probe to add a probe.
- 7. A row for the primer or probe will be added to the **Components** table and the **Name** cell will be colored yellow.

Note: A **Delete** icon () will be displayed in the last column of each component row. Click on it if you would like to delete a component.

8. Enter the name of the primer or probe.

The QIAgility Setup Manager Software will prompt you in later steps to place a tube with the name of the primer or probe onto the worktable.

9. Enter the **Volume per reaction** of the primer or probe.

Alternatively, you can specify the concentration in the assay reaction along with the stock concentration, and the software will automatically calculate the **Volume per reaction**.

- 10. To define all components, repeat steps 6–10.
- 11. If the single components of the target have been defined, click **OK**. The dialog will close.
- 12. The target will be listed in the **Targets** table.

Note: The software automatically assigns a color to each defined target name. If you would like to select a different color, you can click on the colored rectangle in the first column to open the **Colors** dialog:



Target definition (list the individual targets of the mix here) Target type Reports Target 1 Test FAM	r X
Painogen larget 1	
Λolume per reaction 1 μl [0 - 20 μl] Components (optional, for documentation only)	Add target
Tube Name Conc. in assay reaction [µ	-
	0.8 🗙
1 Reverse Primer 1	0.8 🗙
Probe 1	

Dialog to load a ready-made primer-probe mix in one single tube for the run.

To define a ready-made primer-probe mix

- 1. Select the option Use ready-made primer-probe mix.
- 2. Enter a target name. The **Target name** cell will be colored yellow as a reminder that this entry is required.

Note: To name a target mix, you can either choose one name that indicates the target mix name, or you can define several different target names. In the software, these targets will always be handled together, because they are part of the same target mix. The QIAgility Setup Manager Software will prompt you in a later step to place a tube with the name of the target mix onto the worktable. If you defined several different target names, the software will display a list of the targets for that tube.

3. Click on the menu in the **Target type** column and select a target type:

	Target type
	Test 🔹
ľ	Test Reference
	IC

Target type	Description
Test	Use this target type for your gene of interest or the targeted region.
Reference	Use this target type for reference targets, such as housekeeping genes or other endogenous controls.
IC	Use this target type for internal controls (IC). Internal controls are either added during sample extraction or before PCR setup.

4. **Optional step:** Click on the menu in the **Reporter column** and select an appropriate reporter dye for the target:

	Report	er
Γ		•
SYBR	Green 1	
SYBR	Green I	
Cy5		
Crimso	n	
ROX		
SYBR		

Green	Yellow	Orange
FAM	JOE	ROX
SYBRGREEN1	VIC	CALFLUORRED610
SYBRGREENI	HEX	CY35
FLUORESCEIN	TET	TEXASRED
ALEXAFLUOR488	MAX	ALEXAFLUOR568
	CALFLUOR	
	GOLD540	
	YAKIMAYELLOW	
Red	Blue	Crimson
CY5	MARINABLUE	QUASAR705
QUASAR670	EDANS	ALEXAFLUOR680
ALEXAFLUOR633	BOTHELLBLUE	LIGHTCYCLERRED705

LIGHTCYCLERRED640

ALEXAFLUOR350

AMCAX

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section <u>Entering data</u>.

- Optional step: To add further target names, click on Add target and repeat the steps 2–5.
 Note: After you have created at least two targets, a Delete icon (X) will be displayed in the last column of each target row. Click on it if you would like to delete a target.
- Enter the volume per reaction of the primer-probe mix in the Volume per reaction field.
 Note: If you selected the option Use ready-made assay mixes in the <u>Reaction Setup step</u>, this field is not available.
- In the Components table, you can define the primers and the probe (steps 8–12).
 Note: Steps 8–12 are optional.
- 8. Click on Add primer to add a primer or click on Add probe to add a probe.
- 9. A row for the primer or probe will be added to the **Components** table and the **Name** cell will be colored yellow.

Note: A **Delete** icon (\Join) will be displayed in the last column of each component row. Click on it if you would like to delete a component.

- 10. Enter the name of the primer or probe.
- 11. Enter the Concentration in the assay reaction in the corresponding cell.
- 12. Optional step: To add further primers or probes, repeat steps 8-11.

- 13. If the target or primer-probe mix has been defined, click **OK**. The dialog will close.
- 14. The primer-probe mix will be listed in the **Targets** table. The targets defined in the dialog share the same **Content** cell.

7.2.4.2.3 Defining internal controls

The QlAgility Setup Manager Software supports two kind of internal controls, the **PCR Internal Control** (PCR IC) and the **Process Internal Control** (Process IC).

• Use **PCR Internal Control** to control for successful amplification and to exclude, for example, the presence of PCR inhibitors. The PCR IC assumes that you include an internal control DNA/RNA as a reagent and that you define the corresponding internal control target.

On the <u>Assay step</u>, define an assay that combines the PCR IC with the internal control target and the actual test target.

On the <u>Assignment step</u>, assign the defined assay to the samples. The QIAgility Setup Manager Software will prompt you later in the <u>Worktable step</u> to load the tube with the internal control DNA/RNA. During the run, the QIAgility will add it directly to the reaction mix of that assay.

Note: The use of PCR ICs will result in an internal control signal detection in the no-template control (NTC) reactions.

• Use **Process IC** to control the whole workflow, from DNA/RNA purification to amplification. This option requires that you add the Process IC during sample lysis.

On the <u>Assay step</u>, define an assay that combines the Process IC with the Internal Control target and the actual test target.

On the Assignment step, assign the defined assay to all samples that contain the Process IC.

Note: No internal control signal will be detected in the NTC reactions, but will be detected in the sample reactions.

Note: Make sure that the corresponding target is defined in the Targets table.

To define a new internal control:

- 1. Click **New IC** below the **Internal controls** table.
- 2. The Add internal control dialog will be displayed. Refer to Add internal control dialog for details.
- 3. New rows for the defined internal controls will be added to the **Internal controls** table.

A	ction	s
0	L	×

The buttons in the **Actions** column

of the Internal controls table have the following functions:

Button	Action	Description
0	To edit an internal control	The Edit internal control dialog will open, displaying the values that have been entered in the Add internal control dialog.
L	To duplicate an internal control	The Duplicate internal control dialog will open, displaying the values that have been entered for the original internal control. The internal control name will initially display a copy.
×	To delete an internal control	Deletes the row in the Internal controls table.

Add internal control dialog 7.2.4.2.4

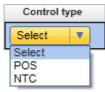
Volume per reaction 1 µl [0 - 25 µl]
OK Cancel

Dialog to define an internal control.

To define an internal control

Enter a name for the internal control. The IC name field will be colored yellow as a reminder that this 1. entry is required.

2. Click on the **Type** menu and select a type. The menu will be colored yellow as a reminder that this entry is required.



Target type	Description
PCR control	Use this internal control type to test for successful amplification.
	The QIAgility Setup Manager Software will prompt you in a later step to place a tube with the IC name onto the worktable. During the run, the QIAgility will add it to the reaction mix.
Process control	Use this internal control type to control the whole workflow, from DNA/RNA purification to amplification.
	You must have already added the Process IC during the sample lysis step.

For details about internal control types, refer to <u>Defining internal controls</u>.

- 3. If you selected **PCR control**, enter the **Volume per reaction** of the internal control.
- 4. Click **OK**. The dialog will close.
- 5. The internal control will be listed in the Internal controls tables.

7.2.4.2.5 Defining additional reagents

Additional reagents can be managed (added, duplicated or deleted) using the **Additional reagents** table at the bottom. The table lists the reagent name and the volume per reaction. During the run, the QIAgility will add all defined additional reagents to all assay mixes.

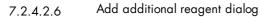
Adding an additional reagent

- 1. To add a new reagent click on New additional reagent.
- 2. The **Add additional reagent** dialog will open. For details, refer to section <u>Add additional reagent</u> <u>dialog</u>.
- 3. The reagent will be displayed as an additional row in the **Additional reagents** table.



The buttons in the **Actions** column

Button	Action	Description
0	To edit a reagent	The Edit additional reagent dialog will open, displaying the values that have been entered in the Add additional reagent dialog.
L	To duplicate a reagent	The Duplicate additional reagent dialog will open, displaying the values that have been entered for the original reagent. The reagent names will initially display a copy of the reagent.
×	To delete a reagent	Deletes the row in the Additional reagents table.



Add additional	reagent	
Reagent name Mg2+		
Concentration in assay reaction	Stock concentration	Volume per reaction 1 µl [0 - 20 µl]
		OK Cancel

Dialog to enter an additional reagent

1. Enter the reagent name.

The QIAgility Setup Manager Software will prompt you in a later step to place a tube with the reagent name onto the worktable.

- 2. Enter the **Volume per reaction**. Alternatively, you can specify the concentration in the assay reaction along with the stock concentration, and the software will automatically calculate the **Volume per reaction**.
- 3. Click **OK**.
- 4. The reagent will be displayed as an additional row in the **Additional reagents** table.

Note: If you selected the option **Use ready-made assay mixes**, the definitions of **Additional reagents** are for documentation purpose only. Ensure that you include all listed reagents already in the ready-made assay mixes, because the QIAgility will not add them to the assay points during run.

7.2.4.3 Assay step

The **Assay** step is used to <u>define one or more assays</u>, based on the defined targets and internal controls from the previous <u>Targets step</u>. All defined assays are listed in the **Assays** table. At least one assay must be defined.

Click Next to proceed.

QIAGEN	Home	0	riment Ser		Configuration							Instrume
*Experiment_1	×											
Reaction Setup		Defin	e assays									
Targets		Define an assay for each combination of targets and internal controls you want to use. Define at least one assay. If the internal control is planned to be added to the amplification reaction, select it from PCR controls, if the internal control was added during puffication procedure, select it from process controls. For more information use the Helo function.										
Assay	Assays											
Controls / Standards				Na	me		Target	Target type	PCR IC	Process IC	Vol/reaction	Actions
controlo / standarda					QuantiNova IC Probe Assay	IC						
Labware Selection		Assay	Assay A (IC +	ay A (IC + Target 1)		Pathogen Target 1	Test	QN IC DNA		4 µl		
	Assignment					QuantiNova IC Probe Assay	IC					
Assignment		Assay B (IC + Targe	Target		Pathogen Target 2	Test	QN IC DNA		4 µl	2 🖪 🗙		
Worktable							1			1		
Run												
												New assay
							٨٠٠٠	-				

Assay step

Note: If you selected the option Use ready-made assay mixes in the <u>Reaction Setup step</u>, the Volume per reaction cell will be empty.

7.2.4.3.1 Defining assays

An assay consists of one or more targets that were defined in the previous <u>Targets step</u>. An assay may also include one PCR IC and several Process ICs. If you want to use several combinations of targets and internal controls, several assays need to be defined: one for each combination of targets and internal controls, without taking into account the assignment of the samples. Samples can be defined and assigned in later steps (<u>Labware selection</u> and <u>Assignment</u> step).

Note: An assay allows simultaneous amplification of all defined targets within one reaction. However, for optimal performance of a multiplex assay, or if an internal control assay is used, special considerations need to be made with respect to primer and probe design and quality. Follow the guidelines provided in the kit handbook of the kit you are using for more information.

It is possible to define more assays than you actually need for one run. This can be helpful if you intend to prepare a general template for several test variants. For more information about templates and experiments, refer to the <u>Experiments and templates concept</u>.

To define a new assay, proceed as follows:

- 1. Click on **New assay** below the **Assays** table.
- 2. The Add assay dialog will be displayed. Refer to Add assay dialog for details.
- 3. New rows for the defined assays will be added to the **Assays** table.

Actions				
0	L,	×		

The buttons in the **Actions** column

Button	Action	Description
0	To edit an assay	The Edit assay dialog will open, displaying the values that have been entered in the Add assay dialog.
Ŀ	To duplicate an assay	The Duplicate assay dialog will open, displaying the values that have been entered for the original assay. The assay name will initially display a copy.
×	To delete an assay	Deletes the row in the Assays table.

7.2.4.3.2 Add assay dialog

Assay I	Add assay		
	A (IC + Target 1)]
argets	to be included in assa	y	
	Target	Target type	Use PCR control
	QuantiNova IC Probe	IC	IC_DNA 🔻
	Pathogen Target 1	Test	Use process controls
	Pathogen Target 2	Test	
			-
			OK Cancel

Dialog to combine targets and internal controls into assays

To define an assay:

1. Enter an assay name. The **Assay name** field will be colored yellow as a reminder that this entry is required.

If you selected the option **Use ready-made assay mixes** in the <u>Reaction Setup step</u>, the QIAgility Setup Manager Software will prompt you in a later step to place a tube with the assay name onto the worktable.

2. Select the targets that will be combined in the assay.

Targets to be included in assay Target Target type QuantiNova IC Probe IC Pathogen Target 1 Test Pathogen Target 2 Test

To select a target or target mix, check the box next to the target color(s). The checked targets or target mixes will become part of the assay. Later in the <u>Assignment step</u>, when assays are assigned to samples, these targets will be assigned together to the samples, because they are part of the assay.

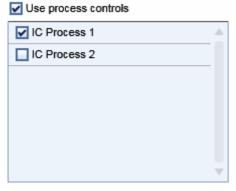
3. Optional step: If a PCR IC will be used in the assay, check the box next to Use PCR control.

Use PCR control	
Select PCR control	▼
IC_DNA	
IC_RNA	
Select PCR control	

Then select the particular PCR IC from the PCR ICs that were defined in the previous **Targets** step. Only one PCR IC can be used in a particular assay.

Note: If you selected the option **Use ready-made assay mixes** in the <u>Reaction Setup step</u>, you can use this option for documentation purposes. Ensure that you include the PCR IC already in the ready-made assay mix, because the QIAgility will not add it during run.

4. **Optional step:** If a Process IC will be used in the assay, check the box next to **Use Process control**.



Check the box next to the name of the Process IC that should be used in the assay. Several Process ICs can be used within one assay. Make sure that the Process IC was already added during sample lysis.

- 5. Click **OK**. The dialog will close.
- 6. The assay will be listed in the **Assays** table.

7.2.4.4 Controls/Standards step

This optional step is used to define controls and standard series. If you do not intend to use controls or standards for the run, proceed by clicking **Next**.

The screen contains two tables: **Controls** and **Standard series**. Both tables have an **Actions** column with interactive buttons to manage the controls and standard series.

Adding a control or standard series

- 1. Click on Add control or Add standard series.
- 2. The **Add control** or **Add standard series** dialog will be opened. For details, refer to <u>Add control</u> <u>dialog</u> or <u>Add standard series dialog</u>, respectively.
- 3. The control will be added to the **Controls** table, and the standard series will be added to the **Standard series** table.
- 4. Click **Next** to proceed.

QIAGEN Hor		Experimen	t Service	Configuration						Instrument	
*Experiment_1 ×											
Reaction Setup		Define con	itrols and stan	dards							
		Controls									
Targets		Sequence		Nam	•	Target	Target type	Control type	Replicates	Actions	
		1-4	NTC Assay A			QuantiNova IC Probe Assay	IC	NTC			
Assay	sav			NITO_Assuj A			Pathogen Target 1	Test	NTC	*	
		5-8				QuantiNova IC Probe Assay	IC	NTC	4		
Controls / Standards >		0-0	NTC_Assay B			Pathogen Target 2	Test	NTC			
Controls / Standards >						1					
Labware Selection		Output laby	ware order	\vee						Add control	
Assignment		Standard s									
		Sequence		Name	Concentration	Target	Target type	Туре	Replicates	Actions	
Worktable											
Run											
		Output laby	ware order	\vee					Add st	andard series	

Controls/Standards step

The buttons in the **Actions** column of the **Controls** table and the **Standard series** table have the following functions:

Button	Action	Description
0	To edit a control or standard series	The Edit control or Edit standard series dialog will open, displaying the values that have been entered in the Add control or Add standard series dialog.
Ľ	To duplicate a control or standard series	The Duplicate control or Duplicate standard series dialog will open, displaying the values that have been entered for the original control or standard series. The control or standard series names will initially display as a copy of the control or standard series.
×	To delete a control or standard series	Deletes the row in the Controls or Standard series table.

Changing the order of the output labware

You can change the order of the controls and standard series using the up and down arrows below each table. Use this option to change the order of several controls within the controls section or the order of several standard series within the standard series section. The order of the output plate will be based on the order indicated in these tables.

Output labware order

Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.4.4.1 Add control dialog

	Add control			
Co	ntrol name			
Ν	NTC_Assay A			4 Replicates
As	say			
	Assay A (IC + Target 1)		•	
	Target	Target type	Reporter	Control type
	QuantiNova IC Probe As	IC	MAX	NTC
	Pathogen Target 1	Test	FAM	NTC
			o	K Cancel

NTC for Assay A

- 1. Enter a control name in the **Control name** field.
- Select the number of replicates, using the **Replicates** drop-down menu next to the **Control name** field.
 Note: The replicates are listed in an editable menu. If the number of replicates you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering data</u>.
- 3. Select an assay from the Assay menu:



4. The software lists the defined targets and target mixes of the assay in the table below. Select the control type using the drop-down menu in the **Control type** column:

	Control type	
	Select 🗸	
4	Select	-
	POS	L .
	NTC	

Option Description

POS A positive control will be added to the assay points.

NTC No-template control. Water from the kit will be added to the assay points.

Note: The use of PCR ICs will result in an Internal Control signal detection in the NTC reactions.

- 5. Click **OK** to confirm and close the **Add control** dialog.
- 6. The control will be added to the **Controls** table.

7.2.4.4.2 Add standard series dialog

\oslash	Add standard series	5					
Standard s	series name						
Standard	d series A				4	 Replicate 	s
Assay							
Assa	y A (IC + Target 1)			•			
Diluent QuantiTe Dilution st	ect Nucleic Acid Dilution B	uffer Stock		• •	once	ntration unit	
5 🔻		100	0		copie	es/µl	V
Sequence	Name of standard		Dilutio	on ratio	C	oncentration	
1	Standard A1		1:10		1	100	
2	Standard A2	`	1:100		1	10	
3	Standard A3	•	1:1000)	1	1	
4	Standard A4	•	1 : 1000	0		0.1	
5	Standard A5	`	1 : 1000	00	1	0.01	
				C	ж	Cance	4

Dialog for standard series

- 1. Enter a name for the series in the **Standard series name** field.
- 2. Select the number of replicates, using the **Replicates** drop-down menu next to the **Standard series name** field.

Note: The replicates are listed in an editable menu. If the number of replicates you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering</u> <u>data</u>.

- 3. Select an assay from the **Assay** drop-down menu.
- 4. Check the option **Use ready-made standard series**, if you intend to load the ready-made dilutions individually for the run.

Uncheck the option if you plan to load one tube containing the standard stock and would like for the QIAgility to automatically create the dilutions for the standard series during the run.

- 5. **Optional:** if the option **Use ready-made standard series** has not been checked, select in the **Diluent** drop-down menu which diluent the QIAgility should use to create the dilutions.
- 6. Select the number of dilution steps from the **Dilution steps** menu.



The number of rows in the table at the bottom is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering</u> <u>data</u>.

Note: In order to use the actual stock as the first standard, define the first standard to have the same concentration as the stock (dilution ratio will be 1:1 in this case). If the option **Use ready-made standard series** has not been checked, the QIAgility will directly use the stock as template for the assay points of this standard.

 If the option Use ready-made standard series has not been checked, enter the stock concentration in the Stock field and select the concentration unit using the Concentration unit drop-down menu.
 Concentration unit

	V
%	
IU/ml	
IU/µl	
copies/reaction	
copies/ml	
copies/µl	
µg/µl	
ng/reaction	
ng/µl	
pg/µl	
%mut	
μM	

8. For each dilution step, enter either a dilution ratio or a concentration. The other value will be calculated automatically by the software. Optionally, enter a meaningful name into the **Name of standard** cell. This is especially important if you plan to define more than one standard series.

Note: The dilution ratio refers to the stock concentration.

Note: Concentration values lower than 0.01 will be displayed in scientific format, e.g., 1.00E-03 represents 0.001.

Note: This step is optional if the option Use ready-made standard series has been checked.

- 9. Click **OK** to confirm and close the **Add standard series** dialog.
- 10. The standard series will be added to the **Standard series** table.

7.2.4.5 Labware Selection step

Use the Labware Selection screen to select the labware for sample input and run output and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file. The QIAgility Setup Manager Software supports up to three input labwares.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side.

Proceed as follows:

1. Select the input labware and define the samples.

If the samples will be on a single plate or adapter, check the option **Samples will be provided on one single plate or adapter** on the left side of the screen and follow the steps in <u>Defining one input</u> <u>labware</u>.

If the samples will be on several plates or adapters, check the option **Samples will be provided on multiple plates or adapters (up to 3)** and follow the steps in <u>Defining up to 3 input labware</u>.

- 2. Define the output labware.
- 3. Click **Next** to proceed.

QIAGEN Home			
*Experiment_1 ×			
Reaction Setup	Define sample input and PCR output		_
Targets	Input labware	Output labware	
Assay	 Samples will be provided on one single plate or adapter Samples will be provided on multiple plates or adapters (up to 3) 		
Controls / Standards	Use a new input labware and enter sample names Load sample input from file	Use an empty output labware Load existing output from file	
Labware Selection >	Load sample input from file	Select file	
Assignment	Labware 1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Taperei ▼ Use cooling block Samples are covered with top-elute	Labware QIAGEN Rotor-Disc 72 / Rotor-Gene Q, Rotor-Disc 72 Adapl Use cooling block	
Worktable	Labware identifier Input1 20161205 100623 Print barcode	Labware identifier Output 2016/205 093306 Print barcode	
Run	Auto-generate Sample Ids for all samples Define samples		

Labware Selection step

7.2.4.5.1 Defining one input labware

If the option **Samples will be provided on one single plate or adapter** has been selected, define the input labware according to the following steps:

- Select Use a new input labware and enter sample names and proceed to step 2.
 Alternatively, select Load sample input from file to use a sample input file and proceed to step 11.
- 2. Click on the Labware drop-down menu on the left side of the screen.
- 3. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

Labware
1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Taperet 🔻
1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered-Base Tubes
Choose other labware

4. Select your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

options	Labware			
ory ndard 96 Well	Select the item that most closely matches the labw QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalo 19585 Name QIAG		1 6
t number hufacturer II V		v		A
e / Well size	Labware adapter			
	Select the adapter to be used.			
		Catalo	og number	
Reset filter Apply filter		Name		

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

5. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

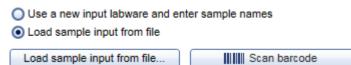
- If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
 Note: Whenever you select another labware, this option will become unchecked again.
- 7. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 9. Define the sample names. Check the option Auto-generate sample IDs for all samples if you want the software to automatically generate sample names for all positions on the labware. Alternatively, click the Define samples button to enter the sample names manually. It is also possible to edit the automatically generated sample names. For details about the sample editor refer to Editing sample names.
- 10. Proceed with step 2 in Labware Selection step.

To define samples by using a sample input file, follow these steps:

11. If the option **Load sample input from file** has been checked, the **Load sample input from file** and **Scan barcode** buttons will be enabled.



12. Click the **Load sample input from file** button and select the desired sample file. For details, refer to <u>File dialog</u>.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

The software will load the sample information from the sample file.

If the sample information file also contains the labware type, it will automatically be selected in the Labware drop-down menu. In this case, the software will automatically display the sample editor. Proceed to step 16.

Note: This is only possible for files in QDEF format.

Note: If the **Labware** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

14. If the sample information file does not contain the labware type, select the labware manually from the **Labware** drop-down menu. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Iter options	Labware		
ategory Standard 96 Well 🔹	Select the item that most closely matches the labwa QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name	
Advanced options		QIAGEN S-Block 96 Dead Volume 40 µl	
Part number			A 0000000000 0000000000 D 0000000000 D 00000000
Manufacturer T		•	0000000000 H 00000000000
Tube / Well size	Labware adapter		
	Select the adapter to be used.	Catalog number	
Reset filter Apply filter		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

15. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- 16. Optional step: Edit the sample information. If the sample editor does not open automatically, click Define samples to open it. Click OK to confirm the sample information and to close the sample editor. For details about the sample editor refer to Editing sample names.
- If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
 Note: Whenever you select another labware, this option will become unchecked again.
- 18. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 20. Proceed with step 2 in Labware Selection step.

7.2.4.5.2 Editing sample names

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.

	Sample editor		
Sele	ct an input labware identifier t	o enter, edit or	view the samples provided on that labware
Lab	ware identifier		
In	put1_20161205_101957		v
Pos.	Sample ID	Conc. [ng/µl]	Description
A1			in
A2			
A3			
A4			
A5			
A6			
A7			
A8			
B1			
B2			
B3			
B4			
B5	`		
B6			
B7			
B8			
C1			
C2	`		
C3			
C4	1		۲
			OK

Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.

Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column **Sample ID**. Press and hold the **SHIFT** key and left-click to select multiple adjacent cells, or press and hold the **CTRL** key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on **Auto-generate sample names**. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on **Auto-generate sample** names.

Сору	Ctrl+C
Paste	Ctrl+V
1 date	our v
Auto-generate sample names	

Note: For more details how to enter information into a table refer to the section Entering data.

Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

7.2.4.5.3 Defining up to three input labware

If the option **Samples will be provided on multiple plates or adapters (up to 3)** has been selected, you can define up to three input labware types.

For each input labware, you can decide whether to load the sample names from a file or to define the input labware manually. All defined input labware will be listed in the **Sample input labware** table.

1. Click the **Add new** button to define the labware manually. The **Add new input labware** dialog opens. Proceed with step 2.

Alternatively, click the **Load sample input from file** button and select the desired sample file. For details, refer to <u>File dialog</u>. Click **Open** to close the file dialog. The software will load the sample information from the selected sample file.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

The software will load the sample information from the sample file.

If the sample file does not contain the labware type, the **Add new input labware** dialog opens. In this case proceed with step 2. Otherwise, proceed with step 3.

- 2. Follow the steps in Add input labware dialog.
- 3. Repeat steps 1 and 2 until all input labware have been defined.
- 4. Define the sample names. The **Define samples** button will be colored yellow to indicate that this information is required.

Click the **Define samples** button to enter sample names manually. It is also possible to edit the automatically generated sample names. For details, refer to <u>Editing sample names</u>.

Note: This step is optional if the sample names were loaded from a file or automatically generated.

5. Proceed with step 2 in Labware Selection step.

The buttons in the right column of the **Sample input labware** table have the following functions:

Button	Action To edit an input labware	Description The Edit input labware dialog will open, displaying the values that have been entered in the Add input labware dialog.
e	To duplicate an input labware	The Duplicate input labware dialog will open, displaying the values that have been entered for the original input labware. The input labware identifier will initially display as a copy of the input labware.
×	To delete an input labware	Deletes the row in the Sample input labware table.

7.2.4.5.4 Add input labware type dialog

To define a new input labware:

- 1. Click on the **Labware type** drop-down menu.
- 2. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

Labware

1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapere
1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered-Base Tubes
Choose other labware

3. Select your input labware in the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

options	Labware		
jory Indard 96 Well Industry available labware only	Select the item that most closely matches the laboration of Well Microplate RB QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name QIAGEN S-Block 96 We	
dvanced options me of labware		Dead Volume 40 µl	1 6
rt number			A 000000000000000000000000000000000000
nufacturer		•	0000000000000000000000000000000000000
be / Well size	Labware adapter		
	Select the adapter to be used.		
		Catalog number	
Reset filter Apply filter		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

4. **Optional:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

- If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
 Note: Whenever you select another labware, this option will become unchecked again.
- 6. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 8. If you want the software to automatically generate sample names for all positions on the labware, check the option **Auto-generate sample IDs for all samples**.

Note: This option will not be available if the dialog was opened using the **Load sample input from file** or the **Scan barcode** button.

9. Click **OK** to close the dialog.

7.2.4.5.5 Defining output labware

To define the output labware:

1. You can either **Use an empty output labware** or **Load existing output from file**. The option **Use an empty output labware** will be selected by default and can be used for most runs.

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case select the option **Loading an existing output from file**, and proceed to step 6.

2. Select the output labware from the **Labware** menu on the right. This menu includes all labware suitable for the selected cycler. An appropriate adapter is automatically added if one has been marked as available in the labware manager.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

If the labware to be used is not listed, select Choose other labware to open the Labware browser.

r options	Labware		
andard 96 Well	Select the item that most closely matches the lat QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Vare to be used Catalog number 19585 QIAGEN S-Block 96 Well Dead Volume 40 µl	
All v tbe / Well size All v	Labware adapter Select the adapter to be used.		
Reset filter Apply filter		Catalog number Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Labware**.
- 4. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

Optional: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.

Alternatively, load a labware file for the output labware:

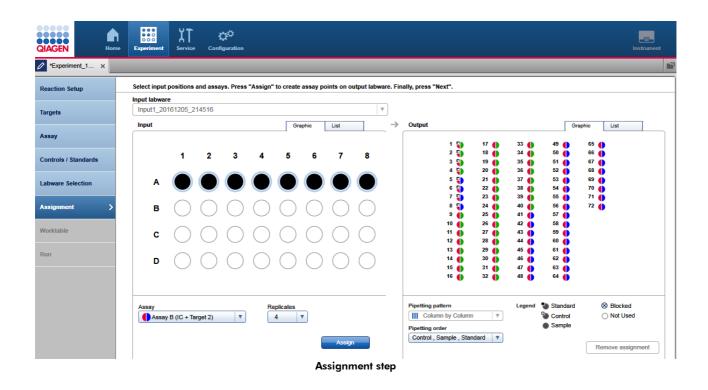
Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

6. The **Select file** and **Scan barcode** buttons will be enabled if the **Load existing output from file** option has been selected.



- 7. Click the Select file button and select the desired labware file. For details, refer to File dialog.
- 8. Alternatively, click the **Scan barcode** button. In the **Scan labware barcode** dialog, scan the barcode and confirm by clicking **OK**. For details, refer to <u>Scan labware barcode dialog</u>. Using the information from the loaded file, the software will automatically select the **Labware**. In addition, the positions that were used with this labware for a previous run will be blocked.
- 9. Click **Next** to proceed.
- 7.2.4.6 Assignment step

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of sample-assay combinations to the output labware.



Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware that have been selected in the **Input labware** menu. The meaning of the different symbols is described below:

	Name	Description
•	Sample	Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been selected once for assay setup on output labware.
	Selected sample	Currently selected sample.
•		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
•		Note: It is possible to use the same sample multiple times to create assay points on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. In the **List** tab, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this

sequence is used when assigning selected samples to the output labware.

At the bottom of the panel is a menu that lists all assays defined in the **Assays** step, along with a **Replicates** menu.

In order to assign a sample for assay setup, select the sample position(s) and then the assay you would like to assign to the selected sample(s). Then set the number of replicates. When you click the **Assign** button, the selected samples and the assay will be assigned to assay points created on the output labware. Each assay assigned to the output labware will result in the setup of an assay mix. Follow the procedure described in <u>Assigning samples with assays to the output plate</u>.

If the option **Samples will be provided on multiple plates or adapters** has been selected, you can switch between the different input labwares using the box above the **Graphic** and **List** tabs.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. When entering the screen, the software automatically places all standards and controls from the **Controls/Standard** step. The creation of assay points containing samples depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at the input side. The assay points are colored with the targets. Standards and controls are indicated by an additional small symbol. If you hover with the mouse cursor over one target color of an output position, the software displays the sample ID, the target and the target type for that position. Assay points containing samples can be removed. For details, refer to <u>Removing assay assignments</u>.

Outp	ut			Graphic		List	
Pos.	Sample ID		Assay			Conc. [µM]
1	NTC_Assay A		Assay A (IC + Tar	get 1), Control			
2	NTC_Assay A		Assay A (IC + Targ	get 1), Control			
3	NTC_Assay A		Assay A (IC + Targ	get 1), Control			
4	NTC_Assay A		Assay A (IC + Targ	get 1), Control			
5	NTC_Assay B		Assay B (IC + Tar	get 2), Contro	I		
6	NTC_Assay B		Assay B (IC + Tar	get 2), Contro	I		
7	NTC_Assay B		Assay B (IC + Tar	get 2), Contro	I		
8	NTC_Assay B		Assay B (IC + Tar	get 2), Contro	I		
9	Sample 1		Assay A (IC + Targ	get 1)			
10	Sample 1		Assay A (IC + Targ	get 1)			
11	Sample 1		Assay A (IC + Targ	get 1)			
12	Sample 1		Assay A (IC + Targ	get 1)			
Pipet	ting pattern		Legend 🏾 🐌 St	andard	8	Blocked	
	Column by Column	•	🕒 Co	ontrol	0	Not Used	
Pipet	ting order		🔵 Sa	ample			
Cor	ntrol, Sample, Standard	•		_			
					Remo	ve assignm	nent

Use the **List** tab of the output panel to view the assay points that have been created.

The List tab of the Output panel. Four replicates of the NTC controls for Assay A and B are placed at 1-8. Assay A has been assigned to sample 1, with four replicates at 9-12.

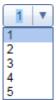
The two menus at the bottom of the output panel can be used to set the **Pipetting pattern** (e.g., row by row vs. column) and the **Pipetting order** (e.g., first standards, then controls and then samples). You can also define a <u>new pipetting order</u>.

7.2.4.6.1 Assigning samples with assays to the output labware

Proceed as follows:

1. From the **Input labware** menu on the left side of the screen, select an input labware that was defined in the previous **Labware selection** step. The software will display its defined samples below in the **Input** panel.

- From the Input panel, select the sample position for which you would like to assign an assay.
 Note: To select multiple samples, either press SHIFT and left-click with the mouse to select adjacent positions, CTRL and left-click with the mouse to select multiple, non-adjacent positions, or drag the mouse to select adjacent samples in a rectangle. Selecting in a rectangle is only possible on rectangular plates.
- 3. From the Assay menu below, select the assay you want to assign.
- 4. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

- 5. Click Assign.
- 6. The software creates assay points on the **Output** panel on the right side of the screen. The selected assay is assigned to the selected samples. If you have selected more than one replicate, the sample positions are multiplied accordingly. The positioning of the created assay points on the output side follows the selections made for the **Pipetting pattern** and the **Pipetting order**.
- 7. Repeat the steps until all positions of the output side have been defined for the planned run.
- 8. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 9. Close the lid.
- 10. Click Next. If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable step**, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the **Worktable step**.

Example:

Input Labware Input1_20161024_150731 Ŧ \rightarrow Input Graphic List Output Graphic List 1 🌒 17 🌔 33 🂧 49 65 0 66 0 67 0 68 0 69 0 70 0 71 0 72 0

 49

 50

 51

 52

 53

 54

 55

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 57

 58

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 60

 61

 62

 63

 64

 2 ŏ 18 🍈 34 🍯 2 3 7 8 1 4 5 6 35 **9** 36 **9** 3 🌔 19 🍈 20 🍈 4 ŏ 5 37 **1** 38 **1** 39 **1** 21 🍈 Α 22 🍈 7 ŏ 23 🍈 8 ŏ 24 🍈 40 40 0 41 0 42 0 43 0 44 0 45 0 46 0 47 0 48 0 В 9 **(**) 10 **(**) 11 **(**) 12 **(**) 25 🍈 26 🍈 27 **(**) 28 **(**) С 29 **(**) 30 **(**) 13 🍈 14 🍈 D 31 **()** 32 **()** 15 🍈 16 🍈 Pipetting pattern Legend Standard Blocked Assay Replicates Clockwise ₹ Control O Not Used Assay A (IC + Target 1) • 1 🔻 Sample Pipetting order Sample , Control , Standard Assign Remove assignment

Select input positions and assays. Press "Assign" to create assay points on output labware. Finally, press "Next".

Assay A (IC + Target 1) has been assigned to all samples, in replicates of 3. Controls have been placed automatically at 34–39. Standards were not defined.

In this example, the assay points created for all samples with the assay "A (IC + Target 1)" have been placed on the output side before the controls. If you now select the sample in position B1 of the input labware and assign assay "B (IC + Target 2)" with one replicate, the software will create one assay point and place it in position 34 on the output side. The controls will move automatically to positions 35–40:

tput			Gra	phic List	
1 🍈	17 🌗	33 🌗	49 🔾	65 🔿	
2 🌔	18 🍈	34 🌔	50	66 🔿	
3 🌗	19 🌓	35 🌓	51	67	
4 🍈	20 🌔	36 🌓	52	68 🔿	
5 🌗	21 🌔	37 🌓	53	69	
6 🍈	22 🍈	38 🌓	54 🔾	70 🔾	
7 🍈	23 🌔	39 🌓	55	71 🔿	
8 🌗	24	40 😜	56	72	
9 🍈	25 🌔	41 🔾	57		
10 🌗	26	42 〇	58		
11 🍈	27 🍈	43 🔾	59 🔾		
12 🍈	28 🌔	44 〇	60 🔿		
13 🌗	29 🌔	45 🔿	61		
14 🍈	30 🍈	46 🔾	62		
15 🍈	31 🍈	47)	63		
16 🍈	32 🍈	48)	64 🔾		

New assay point placed at position 34 (red/blue position).

7.2.4.6.2 Removing assay assignments

To remove an assigned assay from the output side, follow these steps:

- 1. Select the desired sample position on the **Output** side. Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.
- 2. Click **Remove assignment**. The assignment will be removed, and the sample position will be cleared from the output side.

Note: The software will remove all replicates of the desired sample(s).

7.2.4.6.3 Creating a new pipetting order

Generally, the pipetting order defines the placement order of the components (samples, controls, standards) on the output side. It can also be used to define blocked positions on the output labware that shall not be used for the run.

The QIAgility Setup Manager Software comes with a set of predefined pipetting orders, which you find in the **Pipetting order** drop-down menu. At the end of the menu are two commands to **Create a new pipetting order** and to **Edit a pipetting order**. Use this function to define output layouts for reuse. Once defined, they

are also listed in the menu, if the same output labware is used.

Pipetting order

Control, Sample, Standard	
Sample , Control , Standard Sample , Standard , Control	
Control, Sample, Standard	
Control, Standard, Sample	
Standard , Sample , Control	
Standard , Control , Sample	
Create new pipetting order Edit pipetting order	
Ear pipeting order	

- 1. Click on the **Pipetting order** drop-down menu under the output side.
- 2. Click on **Create new pipetting order**.
- 3. The Create new pipetting order dialog is shown:

Pipetting	orde	r nam	e								Sa	/e			
Dutput la	bwar	e type	•												
QIAGE	N S-E	Block	96 W	/ell					•						
Output la	bwar	е												Position	
	1	2	3	4	5	6	7	8	9	10	11	12	0	Blocked	8
А	•	•	•	•	•	•	•	•	•	•	•	•	0	Standards Only	
в	•	•	•	•	•	•	•	•	•	•	•	•	0	Controls Only	2
С	•	•	•	•	•	•	•	•	•	•	•	•	0	Samples Only	С
D	•	•	•	•	•	•	•	•	•	•	•	•	0	Any Kind	
E	•	•	•	•	•	•	•	•	•	•	•	•		1	
F	•	•	•	•	•	•	•	•	•	•	•	•	Pipet	ting pattern	
G	•	•	•	•	•	•	•	•	•	•	•	\bullet		Line by Line	
н	•	•	•	•	•	•	•	•	•	•	•	\bullet			

- 4. Enter a name for the pipetting order in the **Pipetting order name** field.
- 5. The **Output labware type** menu initially displays the labware that has been selected for the output labware before. This selection needs to be changed only, if you would like to create a pipetting order for another labware. In this case, select the desired labware from the list in the menu, or click on the

Choose other labware command at the end of the list, in case the desired labware is not listed here. The selected labware will be used as output labware for the run.

- 6. Define the new pipetting order using the labware graphic. All positions are set to **Any Kind** by default:
 - a. Select the position in the graphic whose assignment you want to change.

Note: Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions, or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.

b. From the **Position** table at the right, click on the button you want to assign to the selected position:

Button	Description
\otimes	Blocked position
•	Position for standards
•	Position for controls
0	Position for samples
	Any kind

- c. The selected position will be assigned to the selected position. The graphic symbol for the selected positions will be changed accordingly.
- 7. To change the pipetting pattern, select the appropriate command from the **Pipetting pattern** menu (e.g., **Row by Row** or **Column by Column** for rectangular plates).
- 8. Click **Save** to save the pipetting order to a file.
- 9. Click **OK** to close the dialog. The newly created pattern is now available in the **Pipetting order** menu in the **Output panel**.

7.2.4.7 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.



- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded. The run cannot be started if any yellow positions remain.

Worktable selector

- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

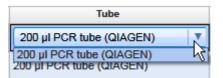
The software displays the labware and required minimum volume of reagents to be loaded in a table located in the middle of the screen. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column.

Pos.	Content	Tube	Req.vol.	Loaded
A	RNase-Free Water	2.0 ml self standing screw cap tube (535 µl	V
Т	QuantiNova IC Probe Assay	1.5 ml tapered screw cap tube (QIA	167 µl	~
J	Pathogen Target 1	1.5 ml tapered tube (QIAGEN)	46 µl	~
к	Pathogen Target 2	1.5 ml tapered tube (QIAGEN)	46 µl	✓
L	Reaction mix (Assay A (IC + Tar	1.5 ml tapered tube (QIAGEN)	0 µl	~
м	Reaction mix (Assay B (IC + Tar	1.5 ml tapered tube (QIAGEN)	0 µl	~

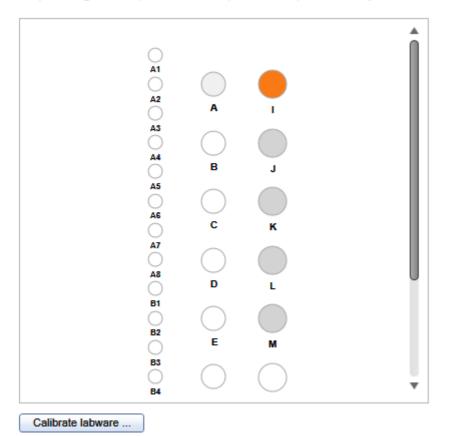
R1 - Reagent block

Table of tubes to be loaded to the worktable position R1.

It is possible to change the tube type for a reagent to be loaded.



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware Manager**. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type. For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. Tubes from QIAGEN kits are displayed with the same color as their tube caps.





Schematic graphic for R1.

Click the **Labware** tab to get an image and details about the input and output labware.

Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

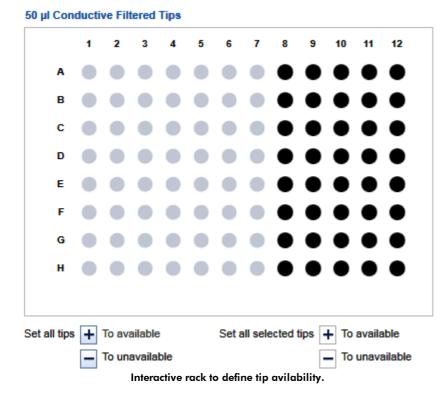
Labware: 1.5 ml tapered tube (QIAGEN) Adapter: 32 x 1.5 ml Flip-Cap Tapered-Base Tubes



Calibrate labware ...

Image and details for input labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To available** or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

The worktable preparation starts with the positions for tip racks:

- 1. Open the instrument lid.
- 2. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to Loading tips.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> <u>holder</u>.

 Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>. After the calibration steps have been completed, the worktable screen will be displayed again.

4. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to Loading tips.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 5. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm** all to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 6. Click **Next** to continue with the next worktable position.
- 7. Repeat steps 2–6 for all tip rack positions.

For all other worktable positions:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

8. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps.

Note: Ensure that you have the empty labware available for the calibration. For tubes from the kit, refer to the QIAgility Calibration Kit that was shipped together with the QIAgility.

- 9. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 10. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed reagent is provided in the correct tube type. The volumes displayed are the minimum volume required, so consider adding extra to prevent liquid level detection errors. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
 - For samples: Ensure that samples are loaded using the correct labware type. Check that the samples are placed in the positions as displayed in the table.

- 11. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 12. Click **Next** to continue with the next worktable position.
- 13. Repeat steps 8–12 for all worktable positions.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 14. Close the lid.
- 15. Ensure that the tip disposal box is empty.
- 16. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

17. The <u>Run step</u> will be displayed.

7.2.4.8 Run step

The run progress can be <u>monitored</u> in the **Run** step. During the run, this is the only available step of the experiment. A run can be <u>paused or canceled</u> at any time. After the run, several <u>options</u> are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.4.8.1 Monitoring a run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress

Task	Progress
Prepare Reaction Mix Assay	
Distribute assay mix to output labware	
Distribute controls to output labware	
Distribute samples to output labware	
	· · · · · · · · · · · · · · · · · · ·
Overall progress	
Start time	Approximate end time
10:17	10:4
Run progress.	

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.4.8.2 Pause/cancel a run

A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	
Overall progress		
Start time 13:05	Paused at Resume	

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

Note: Pauses of a run are listed in the Exceptions/Pauses section of the Advanced report.

7.2.4.8.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen.

Run info	
Operator	
John Smith	
Comment	
	$\overline{\mathbf{v}}$
Output labware	

Print barcode	Save output file
	Create report

Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Print barcode	You can print a barcode for the output labware.
	Note: The option is available only if a printer has been selected in the Global Settings tab of the Configuration environment.
Save output file	An output file will be saved in xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>Customize user settings</u> to define a standard directory.
	The file may serve as sample input file for the Rotor-Gene Q operated with the Q-Rex Software. The .xml file can also be used by a LIMS (Laboratory Information Management System). Find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QIAGEN\QIAgility\Examples.
	Note: Depending on the selected cycler, the software also creates a file in the format that is expected by the cycler. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension, and will also be saved in the specified directory. The input formats of the following cyclers are supported by the first release of the Pathogen Detection Plug-in: Rotor-Gene Q operated with the Rotor-Gene Software, version 2.3 or higher. Contact QIAGEN Technical Services if you need another file format.
	Note: Use this option also in case the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Labware Selection step that you are Loading an existing output from file by selecting that option, and load the file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.

The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section <u>Cleaning the QIAgility</u>.

7.2.4.9 Report

The QIAgility Setup Manager Software offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results
- Exceptions
- Detailed run information

The **Advanced report** contains:

- General information
- Comment
- Kit information
- Reaction results
- Exceptions/Pauses
- Sample information
- Worktable information
- Detailed run information
- Advanced instrument information

Descriptions of the sections that are part of both report types are provided below.

General information

Some general information is provided at the beginning the report. This includes information such as the name of the kit, the experiment file and the output file (if any has been saved), the instrument serial number and the date, time and duration of the run.

Comment

The comment that has been entered on the **Run** screen.

Reaction results

For regular rectangular output plates, an overview graphic is shown, visualizing each position on the output plate:

Symbol	ok	Description The position was processed without any exceptions.
0	warning	The position was processed with exceptions that allowed further processing (e.g., tip failure, liquid level detection error). These exceptions are listed in the Exceptions chapter below.
•	error	The position could not be processed as required due to an exception that did not allow further processing (i.e., the run was stopped due to an error). The exception is listed in the Exceptions chapter below.
0	empty	The position is empty. The position has not been used during the run.
	not used	This symbol is only used if an output file has been loaded as a starting point for the output plate (e.g., when using several runs to fill the output labware). The symbol represents a position that was not part of this run, although it is not empty.

Reaction results

Input plate ID:	Input1_20161205_104738	1 2 3 4 5 6 7 8 9 10 11 12
	Input2_20161205_104747	A 000000000000000000000000000000000000
Output plate ID:	Output_20161205_103805	C 000000000000000000000000000000000000
		GOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOOO

empty

For your reference, the labware identifiers for input and output labware will be listed even if the graphic is not shown. If multiple input labwares were used for the run, the labware identifiers will be listed in the same order in which they were defined on the **Labware selection** step.

A table listing the reaction result for each processed position on the output labware is shown:

Column Output	Description The position on the output labware.
Identifier	Specifies the sample ID or the name of the particular standard or control.
Туре	Sample, Standard or for controls, the Control type.
Input	The corresponding position on the input labware. Valid for samples only. Empty for standards and controls. If multiple input labwares were used for the run, the position is enhanced by a short input labware reference (e.g., A1/I1 refers to position A1 of the 1st Input labware, where "I" indicates Input, and "1" indicates the 1st input labware in the identifier list).
Concentration	For standards, the concentration of the standard is listed. For samples, the final concentration is listed (only if the sample's concentration has been entered in the sample editor or loaded with a sample input file); otherwise, this is empty. Always empty for controls or NTC.
Flag	Shows the status of the position: either ok, warning or error, corresponding to the symbols of the graphic

Note: The table contains only those positions on the output labware that were subject to this run. Empty or unused positions are listed for reference below the table.

Exceptions

This section lists all events, if any, in a table:

Column	Description		
Exception	The event that has been displayed during run.		
Position	Lists the positions that were affected by the exception.		
	First, the position is listed for which the exception occurred. After "Destination," all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.		
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.		
	Example: "M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which might have impacted the output positions D1–D8 (row-wise).		
	Note: If an output position is flagged OK in the reaction results table, even though it is listed here as potentially affected by an exception, then you can trust that the output position was completed successfully.		
	Example: If the "not enough liquid" liquid level detection error occurs in the assay mix tube while serving the last output position with an assay mix, all output positions that were designated to contain this assay will be listed here as potentially affected. However, most of these positions have already been served successfully and will be flagged OK in the reaction results table. You can trust an 'OK' flag in the reaction results table.		
User response	A short form, which option (button) of the message dialog has been pressed.		

Note: The **Basic** report does not list pauses. If you want to have also pauses listed, use the **Advanced** report, instead.

Detailed run information

This sections lists all tasks of the run as they were displayed on the **Run** step.

Descriptions of the sections that are only included in the **Advanced** report are below.

General information

Kit information is not listed here for the **Advanced** report; instead, this information is provided in more detail in a separate section.

Kit information

Kit name, lot number and expiration date. Comment provided with the kit.

Note: The Advanced report does not repeat this information in the General information section.

Reaction results table of the Advanced report

In addition to information provided in the **Basic** report, the **Reaction results** table of the **Advanced** report contains two additional columns:

Output	Identifier	Туре	Input	Actual content	Concentration	Final vol. µl	Flag
A1	Sample 1	Sample	A1/I1	15 μl Reaction mix (Assay A (IC + Target 1)) + 5 μl Sample 1		20	ok
B1	Sample 2	Sample	A2/I1	15 μl Reaction mix (Assay A (IC + Target 1)) + 5 μl Sample 2		20	ok
C1	Sample 3	Sample	A3/I1	15 μl Reaction mix (Assay A (IC + Target 1)) + 5 μl Sample 3		20	ok

Column	Description
Actual content	Lists the components. The software refers the assay name for each reaction mix created.
	Example:
	Output position A1 is the NTC for assay "A (IC + Target 1)" and contains 15 μ l of the reaction mix for assay "A (IC + Target 1)" and 5 μ l of RNase-free water.
Final volume	Lists the final volume.

Exceptions/Pauses of the Advanced report

In contrast to **Basic** report, this section not only lists all events but also any pauses that may have occurred during the run in a table:

Column	Description
Exception	The event or pause that has been displayed during run.
Position/Duration	For an event:
	Lists the positions that were affected by the exception.
	First, the position is listed for which the exception occurred. After "Destination", all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.
	Example:
	"M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which potentially impacted the output positions D1–D8 (row-wise).
	Note : If an output position is flagged OK in the reaction results table even though it is listed here as potentially affected by an exception, then you can trust that this output position has been completed successfully.
	For a pause: the duration of the pause.
User response	A short form, which option (button) of the message dialog has been pressed.

Sample information

Lists the sample information for each input labware as it was entered in the sample editor, loaded by the sample input file, or automatically generated by the software, together with the identifier of the input labware.

Worktable information

Lists for each worktable position the liquids that have been placed there.

Detailed run information of the Advanced report

In addition to the run task list included in the **Basic** report, the assay setup information is also listed:

Assay Setup

Reaction mix [Target(s)]	Content
Mix1 [GAPDH,GPCR-like 5]	850 μl 2x Rotor-Gene Multiplex PCR Master Mix + 85 μl Mg+ + 76 μl RNase- Free Water + 76 μl GAPDH rev + 76 μl GAPDH for + 76 μl 5 rev + 76 μl 5 for + 76 μl GAPDH Probe + 76 μl 5 Probe
Mix2 [GPCR-like 5]	850 μl 2x Rotor-Gene Multiplex PCR Master Mix + 85 μl Mg+ + 36 μl RNase- Free Water + 9 μl 5 rev + 9 μl 5 for + 9 μl 5 Probe

This provides detailed information about each mix that was automatically created by the QIAgility.

The selected cycler and information about standard series is also provided if they were used in the run.

Advanced instrument information

This section lists information about HEPA filter usage, service due dates and volume calibration.

Note: The series numbers of the QIAgility and the pipettor head are part of section **General information** at the beginning of the report.

7.2.5 Working with sample pooling experiments

The Sample Pooling Plug-in enables easy and convenient setup of your sample pooling experiments. The output from this experiment can be used to perform a manual or automated PCR setup using a plug-in of your choice.

New experiments for sample pooling are defined in the **Experiment** environment of the software. When a new experiment is created, only the **Sample Batches** step will initially be enabled. All other steps will become available once the required information has been entered in the **Sample Batches** step. The coloring of the **Pooling Setup** step will change to yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 4 steps that guide you through the run setup process:

- 1. <u>Sample batches</u>
- 2. Pooling setup
- 3. <u>Worktable</u>
- 4. <u>Run</u>

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. The file extension for sample pooling experiments is ***.qsmpoolix**, and for templates ***.qsmpoolit** (**Q**|Agility **S**etup **M**anager Sample **Pooli**ng **Ex**periment and **T**emplate, respectively). The **Start run** button will be disabled as long as there are incomplete or invalid steps or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

Sample Batches step 7.2.5.1

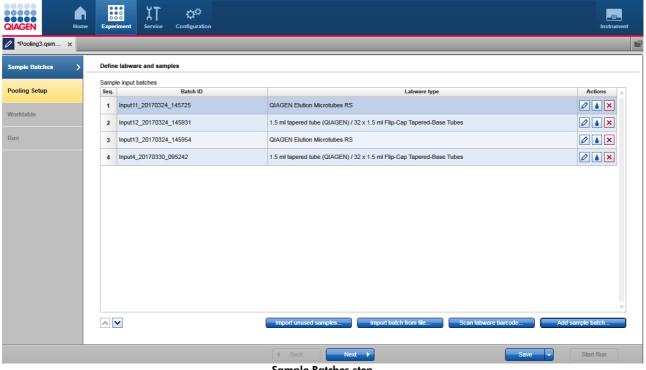
Use the **Sample Batches** screen to select the labware type for sample input and define the corresponding sample information. Several sample input batches can be defined for a pooling run. The QIAgility Setup Manager Software will plan reloading of input labware during the run, if appropriate.

A sample input batch is defined by a unique batch identifier, the labware type and sample names.

Tags can be assigned to samples to indicate which samples can be pooled together. Samples with different tags will not be pooled together. If samples need not be pooled together, e.g., samples originating from different sources, they can be assigned different tags. In other words, tags are used to group the samples before pooling. A pool will only contain samples with the same tag.

Tagging samples is independent of the pool size. The pool size will be defined under Pooling Setup step.

Note: The batch identifier (Batch ID) will be used in the report and in the sample output file.



Sample Batches step

For each sample input batch, you can decide whether to load the sample names from a file or to define them manually. All defined sample batches will be listed in the **Sample input batches** table.

Click the Add sample batch button to define the labware manually. The Add new sample batch 1. dialog opens. It offers the option to automatically generate sample names and pooling tag. Follow the steps in Add new sample batch dialog.

Alternatively, click the Import batch from file button or the Scan labware barcode button to import sample information from a file. Follow the steps in <u>Importing a sample batch from file</u>.

Alternatively, if you want to continue with unused samples from a previous run, click the **Import unused samples** button and select the desired sample file. The software will load the information from the file and open the **Import unused samples** dialog. Follow the steps in <u>Importing unused</u> <u>samples from previous runs</u>.

Note: In order to work with unused samples from a previous run, the 'unused samples' file should have been saved after that run. This file can then be used as a starting point for your current run.

If sample names have not been imported from a file or automatically generated, define them now. The
 Image: button will be colored yellow to indicate that the sample names still need to be defined.

Click the solution to enter sample names and pooling tags manually, or to change automatically generated sample names and pooling tags individually. For details, refer to <u>Editing samples and tags</u>.

Alternatively, click the 2 button if you want the sample names or a pooling tag to be generated automatically for all positions.

- Repeat steps 1 and 2 until all sample input batches have been defined.
 Note: If more sample input batches have been defined than can be placed on the worktable, the QIAgility Setup Manager Software prompts you later during the run to update the worktable with samples and tips.
- 4. Later, during pool planning and run, the software will use the order of sample input batches as defined on this screen. Select a table row and use the software buttons below the table to optimize the order of input batches as per your need. Finally, click **Next** to proceed to the <u>Pooling Setup step</u>.

The buttons in the right column of the Sample input batches table have the following functions:

Button	Action	Description
D	To edit a sample input batch	The Edit sample batch dialog will open, displaying the values that have been entered in the Add new sample batch dialog. It offers the option to automatically generate sample names and/or to assign a common pooling tag for all samples of the sample input batch.
		Note: If you select the option to automatically (re)generate sample names or pooling tag, existing sample names or pooling tags will be overwritten.
٨	To edit sample names and pooling tags	The Sample editor will open, displaying the sample names and pooling tags that have already been entered or automatically generated. Sample names and tags can be entered or changed individually.
×	To delete a sample input batch	Deletes the row in the Sample input batches table.

7.2.5.1.1 Add new sample batch dialog

The Add new sample batch dialog allows simple, manual setup of a sample batch.

Add new sample batch	
Batch ID	
nput17_20170207_135426	Print barcode
Labware type	
Select labware type	v
Options	
Samples require cooling	Samples with top elute
Auto-generate sample names	 Assign common tag
	Tag
	OK Cancel

Add new sample batch dialog

1. Optional:

If you would like to change the batch identifier that was automatically generated by the software, you can edit it in the **Batch ID** field.

Note: This identifier will be used in the report and in the sample output file.

2. Select the labware type of your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**. Initially, the drop-down list is colored yellow to indicate that this information is required.

Iter options	Labware		
ategory Standard 96 Well Shows available labware only	Select the item that most closely matches the labware QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name QIAGEN S-Block 96 Wel	
Advanced options Name of labware		Dead Volume 40 μl	1 6
Part number			A
All Tube / Well size	Labware adapter	Ŧ	H 00000000000
Reset filter Apply filter	Select the adapter to be used.	Catalog number	
reset liner		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

3. Optional:

If you would like to use the cooling block for the input labware, check the option **Samples require cooling**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will become disabled.

4. If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

- 5. If you would like the QIAgility Setup Manager Software to generate sample names automatically, check the option **Auto-generate sample names**. You can define a prefix with which every sample name will start. Sample names will be generated for all positions on the labware. It is possible to edit sample names individually or change sample names later under <u>Sample Batches step</u>, step 2.
- 6. If you would like to assign a common pooling tag to all samples automatically, check the option **Assign common tag**. Select a tag from the **Tag** drop-down menu.

Note: The **Tag** menu is an editable menu. If you want to use another tag or if the menu is still empty, just type the tag into the field. For details about editable menus, refer to <u>Entering data</u>.

It is possible to change tags later or assign tags individually to samples from the **Sample Batches** screen by clicking the button in the right column corresponding to the input batch.

Tags can be assigned to samples to indicate which samples can be pooled together. Samples with different tags will not be pooled together. If samples need not be pooled together, e.g., samples originating from different sources, they can be assigned different tags. In other words, tags are used to group the samples before pooling. A pool will only contain samples with the same tag.

- Optional: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 8. Click OK. The defined sample batch will be added to the Sample input batches table.

Continue with step 2 in Sample Batches step.

7.2.5.1.2 Importing a sample batch from file

To import a sample input batch from file:

1. a) Click the **Import batch from file** button and select the desired sample file. For details, refer to <u>File</u> <u>dialog</u>.

The software will load the sample information from the sample file. If the sample information file also contains the labware type, it will automatically be selected for the sample batch. In case of a ***.csv** or ***.txt** file, the file name will be used as identifier for the sample batch. In case of a ***.xml** file, the plate ID described in the file will be used as identifier for the sample batch.

b) Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan labware barcode** button. The **Scan labware barcode** dialog opens.

Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>. The software will load the sample information from the sample file.

2. If the sample file does not contain the labware type, the **Add new sample batch** dialog opens. Follow the steps in <u>Add input labware dialog</u>.

Continue with step 2 in <u>Sample Batches step</u>.

7.2.5.1.3 Editing samples and tags

	dit or view the samples provideo e input batch	Adapter:	
-	20170324_145725	Labware: QIAGEN Elution	Microtules BS
Pos			
A1	Sample name Input11_1	Tag	Tag
A1 A2	Input11_2	Blue	Blue
AZ A3	Input11_3	Blue	
A3	Input11_4	Blue	Assign tag to selected samples
A4	Input11_5	Blue	
A6	Input11_6	Blue	Sample prefix
A7	Input11_7	Blue	Input11_
A8	Input11_8	Blue	Auto-generate sample names
A9	Input11_9	Blue	
A10	Input11_10	Blue	
A11	Input11_11	Blue	
A12	Input11_12	Blue	
B1	Input11_13	Blue	
B2	Input11_14	Blue	
B 3	Input11_15	Blue	
B4	Input11_16	Blue	
B5	Input11_17	Blue	
B6	Input11_18	Blue	
B7	Input11_19	Blue	
B8	Input11_20	Blue	
B9	Input11_21	Blue	
B10	Input11_22	Blue	V OK Cancel

To define samples manually and to assign pooling tags:

- 1. In the **Sample Batches** step, click the **I** button in the right column of the **Sample input batches** table corresponding to the input batch. The **Sample editor** will be opened. The sample batch is indicated at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample names** for all samples, the **Sample ID** fields will be colored yellow to indicate that this information is required.
- 3. Enter each sample ID.

Note: Alternatively, you can automatically generate sample IDs. Select the relevant cells. Press the **SHIFT** key and left-click to select multiple adjacent cells, or press the **CTRL** key and left-click to select multiple non-adjacent cells. Optionally, define a prefix with which the generated sample names shall start in the **Sample prefix** field. Click the **Auto-generate sample names** button to generate sample names for the selected samples.

Note: For more details about the sample editor, refer to the section Working with tables.

4. Optionally, assign pooling tags to selected samples. Select the relevant sample cells. Press the SHIFT key and left-click to select multiple adjacent cells, or press the CTRL key and left-click to select multiple non-adjacent cells. Select a tag from the Tag drop-down menu. The Tag menu is an editable menu. If the Tag menu is still empty or if you want to use a tag that is not listed, just type the tag name into the field. For details about editable menus, refer to Entering data.

Note: Tags can be assigned to samples to indicate which samples can be pooled together. Samples with different tags will not be pooled together. If samples need not be pooled together, e.g., samples originating from different sources, they can be assigned different tags. In other words, tags are used to group the samples before pooling. A pool will only contain samples with the same tag.

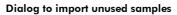
5. Click **OK** to close the **Sample editor**.

7.2.5.1.4 Importing unused samples from previous runs

Save the information about unused samples of any given run in a file by clicking the **Save unused samples** button on the **Run** screen. In order to work with the unused samples from an earlier run, simply load the 'unused samples' file and use it as a starting point. The software loads the information from the file and opens the **Import unused samples** dialog.

The upper part of the dialog shows the information about sample batches containing unused samples as loaded from the file. You can then select which of the sample batches shall be imported for use in your current run and they will appear in the lower part of the dialog.

Batches with unused	samples in myUnusedSamples.					Unused samples in In	put2_20170112_223232			
Batch ID	Labware type	Top elute	Count	Import		Sample ID		Tag	Pos.	
nput2_20170112_2	QIAGEN 96 Well Microplate RB		87			10		Blue	A10	
nput3_20170112_2	QIAGEN 96 Well Microplate RB		96			11		Blue	A11	Т
nput3_20170112_2	QIAGEN 96 Well Microplate RB		96		·U	12		Blue	A12	
nput4_20170112_2	QIAGEN 96 Well Microplate RB		96		•	13		Blue	B1	
nput5 20170112 2	QIAGEN 96 Well Microplate RB		96	V		14		Blue	B2	T
	QIAGEN 96 Well Microplate RB		96			15		Blue	B3	
nput6_20170112_2			96	Import	•	15		Blue	B3	
nput6_20170112_2 Reused batches as Rearrange sample:	they are		96				nput2_20170112_223232	Blue	B3	
 Reused batches as Rearrange samples 	they are	Top elute	(nput2_20170112_223232 From Batch ID	Blue Tag	B3	
nput6_20170112_2	they are s to optimize batch count		(Import		Imported samples in I		Tag		
nput6_20170112_2 Reused batches as Rearrange sample: mported batches latch ID nput2_20170112_2	they are s to optimize batch count	Top elute	Count	Import		Imported samples in I Sample ID	From Batch ID	Tag	Pos.	
nput6_20170112_2 Reused batches as Rearrange samplet mported batches Jatch ID nput2_20170112_2 nput3_20170112_2	they are a to optimize batch count Labware type QIAGEN 96 Well Microplate RB	Top elute	Count 87	Actions X X		Imported samples in I Sample ID 10	From Batch ID Input2_20170112_2	Tag Blue	Pos. A10	
nput6_20170112_2 Reused batches as Rearrange sample: mported batches Batch ID nput2_20170112_2 nput3_20170112_2	Labware type QIAGEN 96 Well Microplate RB QIAGEN 96 Well Microplate RB QIAGEN 96 Well Microplate RB	Top elute	Count 87 96 96	Actions X X X X		Imported samples in I Sample ID 10 11	From Batch ID Input2_20170112_2 Input2_20170112_2	Tag Blue Blue	Pos. A10 A11	
nput6_20170112_2 Reused batches as Rearrange sample: hported batches satch ID nput2_20170112_2 nput3_20170112_2 nput4_20170112_2	they are a to optimize batch count Labware type QIAGEN 96 Well Microplate RB QIAGEN 96 Well Microplate RB	Top elute	Count 87 96	Actions X X		Imported samples in I Sample ID 10 11 12	From Batch ID Input2_20170112_2 Input2_20170112_2 Input2_20170112_2	Tag Blue Blue Blue	Pos. A10 A11 A12	



1. Select the sample batches from the file that shall be used in this run. To do so, check the box in the column **Import** in the corresponding rows of the **Batches with unused samples** table at the top left.

To view the content of a sample batch before importing, click in the sample batch's row in the table **Batches with unused samples** at the left to see the list of its unused samples in a table at the right.

Hint: If you plan to use up all the unused samples in the next runs, you can mark to import all sample batches from the file at once, even though not all samples can be pooled in the immediate run. After the current run is finished, you can again export a file, this time containing fewer unused samples. You can repeat the import and save files step until all the initially unused samples have been pooled. This feature allows to define a large number of samples to be pooled in a single step, although several runs are needed to complete the pooling.

- 2. If the samples on a sample batch have been indicated in the previous run to be covered with TopElute Fluid, the corresponding check box in the **Batches with unused samples** table is checked. The QIAgility will pipet the samples accordingly.
- 3. Select one of the import options.

Use the option **Reuse batches as they are** if adapters shall be reused without rearranging sample tubes.

Use the option **Rearrange samples to optimize batch count** if you want to reduce the number of input batches for the run. If possible, the software will try to fill up adapters.

Note: The software will not rearrange samples that are placed on plates, i.e., plates will always be reused as they are independent of the selected option. For adapters with tubes, the software will fill up adapters only with samples that have the same tube type.

- Click the **Import** button. The software takes the sample input batches that have been marked for import from the **Batches with unused samples** table at the top left.
 Note: At least one of the **Import** check boxes needs to be checked in the **Batches with unused samples** table at the top left. Otherwise, the **Import** button is disabled.
- 5. The software lists the sample batches in the order they will be imported into the **Imported sample batches** table below the **Import** button. If the option **Rearrange samples to optimize batch count** has been enabled, the number of batches can be reduced.

To view the content of a sample batch after rearrangement, click in the sample batch's row in the table **Imported sample batches** at the left to see the list of its unused samples in the table at the right.

Note: If you click the **Import** button again, all previously listed batches in the **Imported sample batches** table will be replaced by this new action.

Click OK to import the sample batches as listed in the Imported sample batches table.
 Otherwise, click Cancel to discard the whole action to import sample batches from the selected file of unused samples.

Continue with step 2 in Sample Batches step.

7.2.5.2 Pooling Setup step

Use the **Pooling setup** screen to select the pooling protocol, the labware type for the output labware, and to define the sample pools itself.

QIAGEN	Home	Experiment	XT Service	Configuration							Instrument
Pooling3.qsm.	. ×										
Sample Batches	_			ooling options							
Pooling Setup	>	Pooling prot Nucleic ac			•						
		Pooling batch and options									
Worktable		Labware									
Run			Elution Microtu	bes RS		v S	ample pools require c	cooling			
		Batch ID PoolBat	h_170324_042	217	Print ba	code 🔽 A	uto-generate Pool IDs	5			
		Leave	samples unus	ed if last pool contai	ns fewer samp		lock positions on poo	Select patte	rn tern of blocked positions	▼	
		Sample poo									
		Sample	tag	Tagged samples 96	Pool size*	Volume/sample[µ]	Total volume[µl] 75	Total net volume[µl] 55	Last pool volume[µl]	Last pool net volume[µl]	Pools 20
		✓ bide		30	5			5	10		7
				32	5			30	20		7
		Green		96	5	5	25	5	5	0	20
						Back	Next	Empty pooling p	ositions: 42	View sample-pool	-mapping
						Datk	NCA				Burrrun
						Pooling Se	etup step				

5 1 1

Firstly, select the pooling protocol and define the pooling batch (output labware):

- 1. Select the protocol from the **Pooling protocol** drop-down list that fits best for your samples. Initially, the drop-down list is colored yellow to indicate that this information is required.
- Select the Labware for the pooling batch (i.e., the output labware of the run) from the drop-down list. If it is not listed, click Choose other labware to open the Labware browser. Initially, the drop-down list is colored yellow to indicate that this information is required.

Iter options	Labware		
ategory Standard 96 Well T Shows available labware only	Select the item that most closely matches the labw QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name QIAGEN S-Block 96 Well	
Advanced options Name of labware		Dead Volume 40 µl	1 6
Part number Manufacturer			A 000000000000000000000000000000000000
All Tube / Well size	Labware adapter	Ŧ	H 0000000000
All	Select the adapter to be used.		
Reset filter Apply filter		Catalog number Name	
	Select the adapter to be used.	Catalog number	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

3. Optional:

If the sample pools on the output labware require cooling, indicate this using the corresponding check box next to the **Labware**.

4. **Optional:**

If you would like to change the batch identifier that was automatically generated by the software for the pooling batch, you can edit it in the **Batch ID** field.

Note: This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

5. **Optional:**

Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been selected in the **Global Settings** tab of the **Configuration** environment.

6. Optional:

Check the box next to **Auto-generate Pool IDs**, if the software shall generate pool identifiers automatically. Pool IDs will be used in the report and in the sample output file to name the positions of

the pooling batch (on the output labware). Otherwise, pool IDs need to be defined later in the **Review** sample-pool-mapping dialog.

Note: The Auto-generate Pool IDs option is the most convenient way to define pool IDs.

7. Optional:

Select the option **Leave samples unused if last pool contains fewer samples**, if you want to create pools always with the complete pool size.

8. **Optional:**

If certain positions on the pooling batch shall not be used during the run, check the option **Block positions on pooling batch** and select a pattern from the drop-down list next to it. Select **Manage patterns** to <u>define a new pattern</u>.

The **Sample pools** table defines the sample pools along the tags that have been defined with the samples. The table lists all sample tags and the number of samples that have been tagged.

	Sample tag	Tagged samples	Pool size*	Volume/sample[µl]	Total volume[µl]	Total net volume[µl]	Last pool volume[µl]	Last pool net volume[µl]	Pools
2	Blue	96	5	15	75	55	15	0	20
2		32	5	5	25	5	10	0	7
~		32	5	10	50	30	20	0	7
2	Green	96	5	5	25	5	5	0	20

Sample pools table

For each row in the **Sample pools** table:

 Select the Tag if pools shall be created in this run by checking the box next to the sample tag name. If the tag has been selected, proceed with the next step to define the pools for it, otherwise proceed with step 12.

Note: At least one tag needs to be selected. The background of the boxes is yellow if no tag has been selected to indicate that a selection is required.

Note: If samples have been defined without an assigned tag, a row will be displayed with an empty sample tag name. Those samples will be treated as a separate tag group.

- 10. Enter the **Pool size** for the tag into the corresponding cell of the table, i.e., the number of samples that shall be combined together into one pool. The cell is initially colored yellow to indicate that this information is required.
- 11. Enter either the Volume per sample that shall be added to the pool or the Total volume of the resulting pool. If one value has been entered, the software will automatically calculate the other value. Both cells are initially colored yellow to indicate that the values are required.

Note: A minimum volume per sample is required, depending on the selected pooling protocol. The **Volume per sample** field is colored yellow, otherwise. Hover the mouse over the field to get a tool tip displayed with the minimum value. The **Total volume** field is colored yellow if the pool volume exceeds the labware capacity of the output labware.

The **Sample pools** table contains additional information about the pools in the other columns:

Table column	Description
Total net volume	If the pools shall serve as input for a further QIAgility run, this is the actual input volume that will be available in the next run. Depending on the dead volume of the chosen labware, the total net volume can be lower than the actual physical volume.
	Adjust the volumes if necessary to meet the required input volumes of the next run.
Last pool volume	The volume that will be available in the last pool. This volume can be lower than the total volume of a complete pool if the last pool will contain fewer samples. You can prevent the creation of incomplete last pools by selecting the option Leave samples unused if last pool contains fewer samples .
Last pool net volume	If the pools shall serve as input for a further QIAgility run, this is the actual input volume of the last pool that will be available in the next run. Depending on the dead volume of the chosen labware, the last pool net volume can be lower than the actual physical volume.
	Adjust the volumes if necessary to meet the required input volumes of the next run. Alternatively, you can prevent the creation of incomplete last pools by selecting the option Leave samples unused if last pool contains fewer samples .
Pools	The number of pools that will be created for the tag.

The number of **Empty pooling positions** on the output labware is displayed below the table.

12. Repeat steps 9–11 until all sample pools for the run have been defined.

The software plans pools according to the definitions in the **Sample pools** table. The following criteria are taken into consideration:

The order of the selected tags in the **Sample pools** table. Click a tag name in the table and use the \frown buttons below the table to optimize the order of tags as per your need.

The software uses the order of sample input batches as defined in the <u>Sample Batches</u> step.

Samples are taken from the sample input batches according to the default pipetting order for sample batches as defined in the user settings for sample pooling.

13. If the View sample-pool-mapping button is colored yellow, click it to define pool IDs in the upcoming dialog. This step is optional if the option Auto-generate Pool IDs has been selected. You can click the button at any time to see a list of the planned pooling batch (output labware), or to define/refine pool IDs individually. Refer to Sample-pool mapping dialog for details.

Note: Pool IDs must be defined in order to proceed to the next Worktable step.

14. If the required pool definition is completed, the **Next** button gets enabled.

_ ..

- 15. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 16. Close the lid.
- 17. Click Next.

Note: The software will show a message if more samples have been defined than the number of samples that will be pooled in this run, or if the number of defined sample pools exceeds the number of available positions on the output labware. Once the run is completed, you can save a file with unused samples to use it as a starting point for the next run.

If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable step**, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the **Worktable step**.

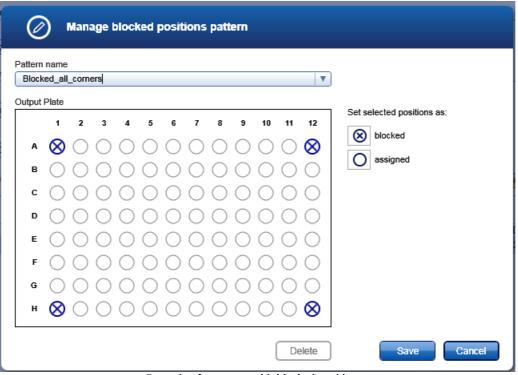
Proceed with the instructions described in <u>Worktable step</u>.

7.2.5.2.1 Defining a pattern for blocked positions

Use the **Manage blocked positions pattern** dialog to define blocked positions. The dialog shows a graphical representation of the selected output labware.

The dialog can be opened by selecting **Manage patterns** from the drop-down list next to the option **Block positions on pooling batch** on the **Pooling Setup** screen.

Note: The option **Block positions on pooling batch** needs to be selected in order to enable the dropdown list.



Example of a pattern with blocked positions.

To define a new pattern:

- Select New from the Pattern name drop-down list and enter a name for the pattern. This is an editable menu. For details, refer to <u>Entering data</u>. The pattern name will be displayed in the Pooling Setup step.
- 2. Select the position in the graphic whose assignment you want to change.

Note: Selecting multiple positions is possible by pressing **SHIFT** and left-clicking with the mouse to select adjacent positions, or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions. Use the mouse to select a rectangular area.

3. On the right, click on the button you want to assign to the selected position:

Button	Description
\otimes	Blocked position.
0	Position that can l

Position that can be used during the run.

- 4. The graphic symbol for the selected positions will be changed accordingly.
- 5. Click **Save** to finish the definition and to close the dialog. The newly created pattern is now selected in the <u>Pooling Setup step</u>.

Note: The pattern will be available in the drop-down list of blocked positions patterns in the **Pooling Setup** step whenever this output labware has been selected.

To refine a pattern:

- Select the pattern from the drop-down list in the **Pooling Setup** screen.
 Note: The list contains all patterns that have been defined for the currently selected output labware.
- 2. Select **Manage patterns** from the drop-down list in the **Pooling Setup** screen.
- 3. The Manage blocked positions pattern dialog opens. The pattern chosen in step 1 is selected in the Pattern name drop-down list.
- 4. Select the position in the graphic whose assignment you want to change.
- 5. On the right, click on the button you want to assign to the selected position.
- 6. The graphic symbol for the selected positions will be changed accordingly.
- 7. Click **Save** to finish the definition and close the dialog. Otherwise, click **Cancel** to leave the dialog without changing the selected pattern.

To delete a pattern:

- 1. Select Manage patterns from the drop-down list in the Pooling Setup screen.
- 2. The Manage blocked positions pattern dialog opens.
- 3. Select the **Pattern name** from the drop-down list which you want to delete.
- 4. Click the **Delete** button to delete the selected pattern. The pattern is deleted.
- 5. Click **Cancel** to leave the dialog.

7.2.5.2.2 Sample-pool mapping dialog

The **sample-pool mapping** dialog lists the sample pools on the pool batch (output labware) as currently planned for the run. For each output position, the list of samples to be pooled together is displayed along with the position on the sample input batch, the sample ID, the tag, and the input batch ID.

The definition of pool IDs is required in order to start the run. Pool IDs are displayed on the report. If pool IDs have not yet been generated or defined, the pool ID fields are colored yellow.

Column by	y Column		Auto-generate Pool IDs		
Output pos.	Pool ID	Input pos.	Sample ID	Tag	Input batch ID
		A1	Input11_1	Blue	Input11_20170324_145725
		A2	Input11_2	Blue	Input11_20170324_145725
A1	pool@A1-Blue	A3	Input11_3	Blue	Input11_20170324_145725
		A4	Input11_4	Blue	Input11_20170324_145725
		A5	Input11_5	Blue	Input11_20170324_145725
		A 6	Input11_6	Blue	Input11_20170324_145725
		A7	Input11_7	Blue	Input11_20170324_145725
B1	pool@B1-Blue	A8	Input11_8	Blue	Input11_20170324_145725
		A9	Input11_9	Blue	Input11_20170324_145725
		A10	Input11_10	Blue	Input11_20170324_145725

Review sample-pool mapping dialog with automatically generated pool IDs

1. Optional:

Select the pipetting order for the pool batch. Initially, the **Pool batch pipetting order** drop-down list displays the default pipetting order for pool batches as defined in the user settings.

Note: Each time the pipetting order is changed, pool IDs will be generated automatically by the software.

2. If not yet defined, define pool IDs.

Click the **Auto-generate pool IDs** button if the software shall create pool IDs automatically. The ID will contain the position on the pooling batch (output labware) and the tag.

Note: The Auto-generate Pool IDs option on the Pooling Setup screen is the most convenient way to define pool IDs.

Alternatively, enter the pool IDs into the **Pool ID** cells individually.

Note: Each time the pipetting order is changed, pool IDs will be generated automatically by the software.

Note: Each time the setup is changed on the **Pooling Setup** screen, pool IDs are removed. The software regenerates them automatically if the option **Auto-generate Pool IDs** is selected on the **Pooling Setup** screen.

3. Optional:

Review the list of pools.

4. Click **OK** to acknowledge the entered or generated pool IDs and leave the dialog. Otherwise, click **Cancel** to leave the dialog and to discard all changes.

Note: The OK button is enabled only if all pool IDs have been defined.

7.2.5.3 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status. If more sample input batches have been defined or more tips are required than can be placed on the worktable, the QIAgility Setup Manager Software will prompt you during the run to reload the worktable with sample batches and tips.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

M1 A1 A2	• B1 (black border): worktable position for which the loading details are displayed.
R1 B1 B2	 B1, B2, C1 (yellow): worktable positions that still need to be loaded. The run cannot be started if any yellow positions remain.
C1 C2 Worktable selector	• A1, A2, C2 (blue): worktable positions that have already been loaded.
	• M1, R1 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

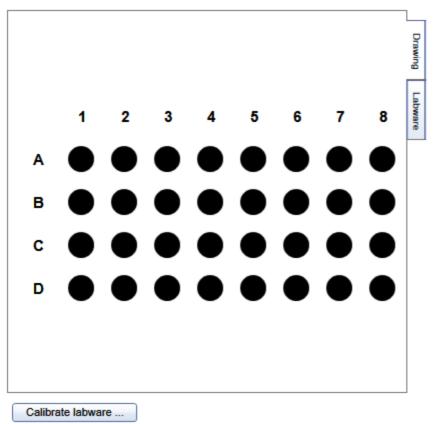
The software displays the labware and required minimum volume of sample to be loaded in a table located in the middle of the screen. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column.

B1 - I	nput	Batch id: Input12	20170324_14	593
Pos.	Sample ID	Required volume/sample	Loaded	
A1	Input12_1	10 µl	V	
A2	Input12_2	10 µl	V	
A 3	Input12_3	10 µl	V	
A4	Input12_4	10 µl	✓	U
A5	Input12_5	10 µl	V	
A 6	Input12_6	10 µl	V	
A7	Input12_7	10 µl	V	
A 8	Input12_8	10 µl	V	
B1	Input12_9	10 µl	V	
B2	Input12_10	10 µl	V	
B 3	Input12_11	10 µl	✓	Ŧ
			Confirm all	

Table of samples to be loaded to the worktable position B1.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. Click on the **Labware** tab to get an image and details about the required labware.

Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

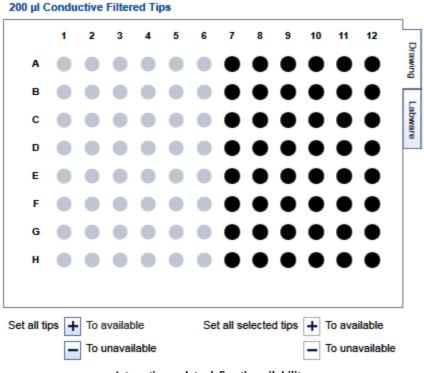


Adapter: 32 x 1.5 ml Flip-Cap Tapered-Base Tubes Labware: 1.5 ml tapered tube (QIAGEN)

Schematic graphic for B1.

If labware required for the run has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To** available or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to Loading tips.



Interactive rack to define tip avilability.

Preparing the worktable

- 1. Open the instrument lid.
- 2. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that a tip rack or labware has not yet been calibrated on the worktable position required for the run. It must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the Calibrate labware button. The software automatically prepares a labware calibration worklist containing all labware that is required for the current run and that has not yet been calibrated on the required worktable positions and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

Note: Ensure that you have the empty labware available for the calibration. Tip racks of both tip type will be required for labware calibration.

Note: Sample pooling runs use only one tip type. However, labware calibration requires both tip types. Ensure to correctly follow the tip loading instructions in the calibration wizard.

Note: The calibration worklist also considers all labware that will be reloaded later during the run that have not yet been calibrated on the planned worktable positions.

Note: If tip racks have not been calibrated for all required worktable positions, they will be added to the calibration worklist too.

After the calibration steps have been completed, the worktable screen will be displayed again.

Now, the worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips are sufficient. If not, load additional tips. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to Loading tips.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> <u>holder</u>.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 4. To confirm that the tip rack has been loaded, either check the box next to Loaded in the table or click on Confirm all to confirm. Refer to Loading tips for information about how to indicate that the tip rack should not be loaded for the run.
- 5. Click **Next** to continue with the next worktable position.
- 6. Repeat steps 3–6 for all tip rack positions.

For sample batches and pool batch:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

- 7. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 8. Perform the following steps for each row in the table:
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
 - For samples: Ensure that samples are loaded using the correct labware type. Check that the samples are placed in the positions as displayed in the table.
- 9. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 10. Click **Next** to continue with the next worktable position.
- 11. Repeat steps 8–12 for all worktable positions.

Note: If calibration has been performed between steps 3–12, ensure to load (or reload) all worktable positions as described in the steps.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 12. Close the lid.
- 13. Ensure that the tip disposal box is empty.
- 14. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

Note: If more sample input batches have been defined or more tips are required than can be placed on the worktable, a message is displayed to inform you that the QIAgility Setup Manager Software will prompt you during the run to update the worktable with samples and tips.

15. The <u>Run step</u> will be displayed.

7.2.5.4 Run step

The run progress can be <u>monitored</u> in the **Run** step. During the run, this is the only available step of the experiment. A run can be <u>paused or canceled</u> at any time. After the run, several <u>options</u> are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

The run can be paused in between, if more samples or tips are needed than was initially placed on the worktable.

7.2.5.4.1 Monitoring a run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups. The task list contains tasks to <u>update the worktable</u> with samples and tips during the run, if more sample input batches have been defined or tips are required than was initially placed on the worktable. The **Progress/Time** column lists the estimated time when the worktable update will be required.

The instrument icon in the main toolbar as well as the progress indicator on the run step show the overall run progress.

Run progress

	Task	Progress / Time
	Processing samples from batch Input11_20170324_145725	
	Processing samples from batch Input12_20170324_145931	
	Processing samples from batch Input4_20170330_095242	r
Э	Update worktable setup	15:36
	Processing samples from batch Input4_20170330_095242	r
	Processing samples from batch Input2_20170112_223232	r
	Processing samples from batch Input4_20170112_232228	r
Θ	Update worktable setup	15:52
	Processing samples from batch Input4_20170112_232228	r
	Processing samples from batch Input5_20170112_232234	r
	Processing samples from batch Input6_20170112_232240	· · · · · · · · · · · · · · · · · · ·

Start time	Approximate end tim	е
15:25	16:1	2
	Run progress.	

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be a part of the report. You may enter the comment during or after the run.

7.2.5.4.2 Reloading worktable during run

If samples or tips need to be reloaded during the run, the **Reload worktable** dialog is displayed. The instructions in the dialog are different for each experiment. The QIAgility Setup Manager Software optimizes these tasks to reduce the number of required reloads during the run.

	ns as specified below. Ensure that the poolir c Click on the "View sample batch details" I REMOVE Empty tip racks		Done
M1 A1 A2	REMOVE	LOAD	Done
R1 B1 B2	ID: Input12_20170324_145931	ID: Input4_20170112_232228	
C1 C2	View sample batch details	View sample batch details	
M1 A1 A2	REMOVE	LOAD	Done
R1 B1 B2	ID: Input11_20170324_145725	ID: Input2_20170112_223232	
C1 C2	View sample batch details	View sample batch details	
Ē	Empty the tip disposal box and close the lie	i.	Done

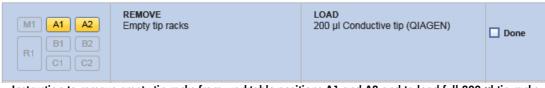
Relaod worktable dialog with instructions.

Each instruction is displayed in a row. The columns display from left to right: the affected worktable position(s), the item(s) that shall be removed and the item(s) that shall be loaded. Perform the actions described and check the "Done" boxes. Once all preparation steps are completed and confirmed, the Resume button will become active.

Follow the instructions listed in the dialog.

For **tip racks**:

1. Remove empty tip racks from the worktable positions highlighted on the left.



Instruction to remove empty tip racks from worktable positions A1 and A2 and to load full 200 μ l tip racks, instead.

Load full tip racks of the required tip type to the worktable positions.
 Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> <u>holder</u>.

Note: Full tip racks need to be loaded during worktable reloading.

To confirm that the tip racks have been loaded, check the box next to **Done**.
 The color of the worktable positions changes to blue to indicate that they have already been loaded.

For **sample input** batches:

4. Remove the sample input batch indicated in the second column from the highlighted worktable position.

M1 A1 A2 B1 B2	REMOVE ID: Input12_20170324_145931 View sample batch details	LOAD ID: Input4_20170112_232228 View sample batch details	Done
C1 C2			

Instruction to remove sample batch with ID Input12 20170324 145931 from worktable position B1.

You can click the View sample batch details button to get the sample list displayed.

Note: If no batch needs to be removed from the worktable position, the instruction displays "No unload needed."

5. Load the sample input batch indicated in the third column.

	M1 A1 A2 B1 B2 R1 C1 C2	REMOVE ID: Input12_20170324_145931 View sample batch details	LOAD ID: Input4_20170112_232228 View sample batch details	Done
--	-------------------------------	--	---	------

Instruction to load sample batch with ID Input4_20170112_232228 to worktable position B1.

Ensure that the samples are loaded using the correct labware type. Hover with the mouse over the batch ID to see a tool tip with labware information.

Check that the samples are placed in the correct positions. You can click the **View sample batch details** button to get the sample list displayed.

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and</u> <u>labware</u> and <u>Loading reagents and samples</u>.

Note: If no batch needs to be loaded, e.g., because only a finished sample batch can be removed, the instruction displays "No load needed."

- To confirm that the sample batches have been removed and loaded, check the box next to **Done**.
 Note: If the loading has been confirmed in the **View sample batch** dialog, the box is already checked.
- 7. Repeat steps 1–6 until all tips and sample batch instructions have been completed.
- 8. **Optional:**

To review the current pooling progress, click the **Review pooling progress** button. The <u>Review</u> <u>pooling progress</u> dialog opens. After closing the dialog, the **Reload worktable** dialog is displayed again.

To **continue** the run:

- 9. Ensure that the tip disposal box is empty.
- 10. Close the lid. To confirm, check the box next to **Done**.

Ē	Empty the tip disposal box and close the lid.	Done

Instruction to empty the tip box and to close the lid.

11. Click the **Resume** button to continue the run.

Note: If the completion of any instruction has not yet been confirmed, the run cannot be continued, and the **Resume** button will remain deactivated. The software will indicate the affected instruction with a yellow worktable position in the respective instruction row.

You can stop the run by clicking the **Stop** button. In this case, the run will be stopped. Pools may not have been completed. Click the **Review pooling progress** button to review the status of the pools before stopping the run. All samples that have not yet been added to a pool are treated as unused samples. After the run, a file of unused samples can be saved as a starting point for the next run.

7.2.5.4.3 View sample batch dialog

The **sample batch** dialog lists the samples of the sample batch. It lists information about unused samples for batches that shall be removed. For batches that shall be loaded, it provides the option to check the sample positions on the labware and to confirm sample loading.

Column	Description
Pos	The samples position on the sample batch.
Sample name	The name of the sample.
Tag	The tag that has been assigned to the sample during sample batch definition.

Req. Vol. [μl]	The required minimum amount of sample to be loaded.
Unused	Only for sample batches that shall be removed. Information if the sample has already been pooled or not.
	If the sample has already been pooled, the box is unchecked. If the sample has not been pooled, the box is checked.
Loaded	Only for sample batches that shall be loaded.
	Similar to the Loaded check boxes during worktable setup. Check the box to confirm the loading of the sample position individually. Alternatively, click the Confirm all button below the table to confirm loading of all samples with one click.

For sample batches that shall be loaded:

- 1. Check that the samples are placed in the positions as displayed in the table.
- 2. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 3. Click **OK** to close the dialog. The **Reload worktable** dialog is displayed again and the **Done** check box for this instruction is checked.

Alternatively, click the **Cancel** button to return to the **Reload worktable** dialog and confirm the loading there.

Note: If the loading of any position has not yet been confirmed, the OK button will remain deactivated.

	<u> </u>	batch input4_20170330_09		
Pos	Sample name	Tag	Req. Vol. [µl]	Loaded
A1	Input14_1		15	
A2	Input14_2		15	
A3	Input14_3		15	
A4	Input14_4		15	
A5	Input14_5		15	
A6	Input14_6		15	
A7	Input14_7		15	
A8	Input14_8		15	
B1	Input14_9		15	
B2	Input14_10		15	
B3	Input14_11		15	
B4	Input14_12		15	
B5	Input14_13		15	
B6	Input14_14		15	
B7	Input14_15		15	
B8	Input14_16		15	
C1	Input14_17		15	
C2	Input14_18		15	
C3	Input14_19		15	
C4	Input14_20		15	
C5	Input14_21		15	
C6	Input14_22		15	
C7	Input14_23		15	
C8	Input14_24		15	
D1	Input14_25		15	
D2	Input14_26		15	
D3	Input14_27		15	
D4	Input14_28		15	
			Confir	m all

Sample batch that shall be loaded.

For sample batches that shall be removed:

- 1. View the sample list.
- 2. Click **OK** to return to the **Reload worktable** dialog.

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View sample batch input12_20170324_145931

Pos	Sample name	Tag	Req. Vol. [µl]	Unused	
A1	Input12_1	pink	10		
A2	Input12_2	pink	10		
A3	Input12_3	pink	10		
A4	Input12_4	pink	10		
A5	Input12_5	pink	10		
A6	Input12_6	pink	10		
A7	Input12_7	pink	10		
A8	Input12_8	pink	10		
B1	Input12_9	pink	10		
B2	Input12_10	pink	10		
B3	Input12_11	pink	10		
B4	Input12_12	pink	10		
B5	Input12_13	pink	10		
B6	Input12_14	pink	10		
B7	Input12_15	pink	10		
B8	Input12_16	pink	10		
C1	Input12_17	pink	10		
C2	Input12_18	pink	10		
C3	Input12_19	pink	10		
C4	Input12_20	pink	10		
C5	Input12_21	pink	10		
C6	Input12_22	pink	10		
C7	Input12_23	pink	10		
C8	Input12_24	pink	10		
D1	Input12_25	pink	10		
D2	Input12_26	pink	10		
D3	Input12_27	pink	10		
D4	Input12_28	pink	10		

Sample batch that shall be removed from the worktable.

7.2.5.4.4 Reviewing pooling progress during run

While reloading the worktable, the current pooling progress can be reviewed. The table shows a row for each position on the pooling batch with the completion status and samples contained in the pool.

Outpu pos.	Pool ID	Complete	Input pos. pos.	Sample ID	Tag	Input batch ID	Result
			H7	Input11_91	Blue	Input11_20170324_145725	ок
			H8	Input11_92	Blue	Input11_20170324_145725	ок
C3	pool@C3-Blue	100%	Н9	Input11_93	Blue	Input11_20170324_145725	ок
			H10	Input11_94	Blue	Input11_20170324_145725	ок
			H11	Input11_95	Blue	Input11_20170324_145725	ок
D3 po			H12	Input11_96	Blue	Input11_20170324_145725	ок
			A10	10	Blue	Input2_20170112_223232	-
	pool@D3-Blue	20%	A11	11	Blue	Input2_20170112_223232	-
			A12	12	Blue	Input2_20170112_223232	-
			B1	13	Blue	Input2_20170112_223232	-
	pool@E3-Blue 0%		B2	14	Blue	Input2_20170112_223232	-
E3			B3	15	Blue	Input2_20170112_223232	-
		0%	B4	16	Blue	Input2_20170112_223232	-
			B5	17	Blue	Input2_20170112_223232	-
			B6	18	Blue	Input2_20170112_223232	-

Different stages of sample pooling (completed, started and not started).

Column	Description
Output pos.	The position on the pooling batch (output labware).
Pool ID	The name of the pool on that position on the pooling batch.
Complete	Completion status of the pool. Example: If a pool shall contain 5 samples and only one sample has been added so far, the completion status would be 20%.
Input pos.	The position of the sample on the original sample batch. Each sample that shall be added to the pool is displayed in a small row.
Sample ID	The name of the sample.
Tag	The tag that has been assigned to the sample during sample batch definition.

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Input batch ID	The identifier of the sample's input batch.
Result	OK , if the sample has been added to the pool, otherwise –

Click on the **OK** button to leave the dialog and to return to the <u>Reload worktable dialog</u>.

7.2.5.4.5 Pause/cancel a run

A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	
Overall progress	v	
Start time 13:05	Paused at Resume 13:21	

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click

OK to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.

3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

All samples that have not been added to a pool are treated as unused samples. After the run, a file of unused samples can be saved as a starting point for the next run.

Note: Pauses of a run are listed in the Exceptions/Pauses section of the report.

7.2.5.4.6 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen.

Run info	
Operator	
admin admin	
Comment	
	Ŧ

Pool batch

Print barcode	Save batch
Save unused samples	Create report

Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Print barcode	You can print a barcode for the pooling batch.
	Note: The option is available only if a printer has been selected in the Global Settings tab of the Configuration environment.
Save batch	An output file will be saved in xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the pool ID and the samples they originate from. The file may serve as sample input file for the next run of PCR setup using the QIAgility instrument operated with the QIAgility Setup Manager Software. The .xml file can also be used by LIMS (Laboratory Information Management System).
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>Customize user settings</u> to define a standard directory.
	Note: Depending on the <u>user settings</u> , the software also creates a file in another format that is expected by LIMS, for example. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension, and will also be saved in the specified directory. Contact QIAGEN Technical Services if you need another file format.
Save unused samples	A file will be saved with information about the samples that have not been pooled during this run.
	A file dialog opens to select directory and file name of the .csv file. Refer to <u>Customize user settings</u> to define a standard directory.
	Note: Use this option also in case the pool batch is limited, and one pooling run is not sufficient to completely pool the defined samples. In such a situation, you can perform more than one run to completely pool the defined samples. Save the unused sample file after the first run. To set up the second run properly, click in the Sample Batches step on the Import unused samples button and load the file from the first run as a starting point for the second run. If there are unused samples remaining even after the second run, save the unused samples file after this again as a starting point for the third run. Continue this step until all the samples that were initially defined in the Sample Batches step have been pooled.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.

The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.

3. Clean the worktable as described in section Cleaning the QIAgility.

7.2.5.5 Report

The report contains the following sections:

- General information
- Comment
- Pooling setup
- Pooling results
- Exceptions/Pauses
- Detailed run information
- Advanced instrument information

General information

Some general information is provided at the beginning the report. This includes information such as the name of the experiment file and the output file (if any has been saved), the instrument serial number and the date, time and duration of the run.

Comment

The comment that has been entered on the **Run** screen.

Pooling setup

Indicates the pooling protocol, pool batch ID and labware. Tags, number of samples with a certain tag, pool sizes, volumes per sample, volumes per pool and number of pools are also indicated.

Pooling results

For regular rectangular output plates, an overview graphic is shown, visualizing each position on the output plate:

Symbol		Description
•	ok	The position was processed without any exceptions.
۲	warning	The position was processed with exceptions that allowed further processing (e.g., tip failure, liquid level detection error). These exceptions are listed in the Exceptions chapter below.
•	error	The position could not be processed as required due to an exception that did not allow further processing (i.e., the run was stopped due to an error). The exception is listed in the Exceptions chapter below.
0	empty	The position is empty. The position has not been used during the run.
8	blocked	The position was marked as blocked in the Pooling Setup step and has not been used during the run.

A table listing the pooling result for each processed position on the output labware is shown:

Column	Description
Output Pos	The position on the output labware.
Pool ID	Specifies the pool ID.
Input Pos	The corresponding position on the sample input batch.
Sample ID	Specifies the sample ID.
Tag	The tag that has been assigned to the sample.
Input batch ID	The batch ID of the sample's input batch.
Result	Shows the status of the position: either ok, warning or error, corresponding to the symbols of the graphic.

Note: The table contains only those positions on the output labware that were subject to this run. Empty or unused positions are listed for reference below the table.

Exceptions/Pauses

This section lists all events but also any pauses that may have occurred during the run in a table:

Column	Description					
Exception	The event or pause that has been displayed during run.					
Position/Duration	For an event:					
	Lists the positions that were affected by the exception.					
	First, the position is listed for which the exception occurred. After "Destination," all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.					
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.					
	Example:					
	"B1 @ A1 Destination: output labware @ D1": The exception occurred on the worktable position B1 at position A1, which potentially impacted the output position D1.					
	Note: If an output position is flagged OK in the pooling results table even though it is listed here as potentially affected by an exception, then you can trust that this output position has been completed successfully.					
	For a pause: the duration of the pause.					
User response	A short form, which option (button) of the message dialog has been pressed.					

Detailed run information

This sections lists all tasks of the run as they were displayed on the **Run** step.

Advanced instrument information

This section lists information about HEPA filter usage, service due dates and volume calibration.

Note: The series numbers of the QIAgility and the pipettor head are part of section **General information** at the beginning of the report.

7.2.6 Generic PCR experiments

The Generic PCR Plug-in enables a kit-independent, easy and convenient, qPCR setup.

For pathogen testing and gene expression experiments using dedicated QIAGEN kits, refer to sections <u>Working with experiments for pathogen detection</u> and <u>Working with gene expression experiments</u>, respectively. For generic PCR setup experiments including sample normalization refer to section <u>Generic</u> <u>PCR setup with normalization experiments</u>. For PCR setup with serial dilution experiments, refer to the <u>PCR</u> <u>setup with serial dilution experiments</u> section. New experiments for generic PCR setup are created by clicking **Generic PCR setup** from the **Home** screen, and then defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and the **Mixes** step will be enabled initially. Subsequent steps will become available once the required information has been entered in the **Liquids** and **Mixes** step. The **Mixes** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 7 steps that guide you through the run setup process:

- 1. Liquids
- 2. <u>Mixes</u>
- 3. Controls/standards
- 4. Samples/Labware
- 5. <u>Assignment</u>
- 6. <u>Worktable</u>
- 7. <u>Run</u>

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is ***.qsmrx**, and for templates ***.qsmrt** (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is ***.qsmrqt** (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.6.1 Liquids step

The **Liquids** step is used to define all input liquids required for your PCR setup that are to be loaded onto the instrument's worktable, besides your samples. These are, for example:

- all kinds of reagents, that will , e.g. be part of reaction (master) mixes
- all kinds of controls and standards (stock)
- all kinds of diluents/water
- ready-made reaction mixes, i.e., final reaction (master) mixes that have already been prepared by the user and are ready to be used and distributed to the output labware by the QIAgility.

Detailed information for (final) reaction (master) mixes will be defined later in the **Mixes** step. Samples will be defined later in the **Samples/Labware** step.

The **Liquids** step also includes information about the kit that is used during the run.

PCR -experim ×							
uids >	Define input liquids						-
ies	Define required input liquids (reagent (master) mix, Standards (stock) and of Predefined liquid classes (LC) define	controls) for assay setup.	Kit information Kit Name	Lot number	Comment		
ntrols / Standards	task for liquid transfer (refer to LC tab screen). LC-naming: Liquid type + pipetting de For more information, press F1 key w	le in configuration estination/intended use	Product number	Kit expiry date			
nples / Labware	Name	Liquid type Liqu	uid class	Tube type		Actions	
ignment	Water	Diluent/Water Aqu	ueous liquid to reaction (master) mix	5.0 ml flat-base tube (QIAGEN)			
	Mastermix	Reagent Vis	cous liquid to reaction (master) mix	2.0 ml screw cap micro tube (Sarstedt)		2 🖪 🗙	
ktable	Primer Mix	Reagent Prin	mer-Probe or IC to reaction (master	2.0 ml screw cap micro tube (Sarstedt)		0 🖪 🗙	
	Standard 651	Standard Cor	ntrol or Std. to output or for dilution	2.0 ml screw cap micro tube (Sarstedt)		0 🖪 🗙	
	Standard 161	Standard Cor	ntrol or Std. to output or for dilution	2.0 ml screw cap micro tube (Sarstedt)		2 🖪 🗙	
	Diluent	Diluent/Water Dilu	uent for dilution	5.0 ml flat-base tube (QIAGEN)		🖉 🖪 🗙	
	-						
	∧ ∨					New liquid	

Liquids step

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The Add liquid dialog opens.
- 3. Define the liquid as described in <u>Add liquid dialog</u>.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

Finally, click **Next** in the button bar to proceed to the **Mixes** step (Mixes step).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click it to open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the \frown \frown buttons below the table:

- 1. Click the row of the liquid to be moved.
- Click on the up and down arrows to move the currently selected row up or down. 2.

Use this function for a better overview. The report will list the liquids in this order.

Ac	Actions				
0	L	×			

The buttons in the **Actions** column

Button	Action To edit a liquid	Description The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.
		If you intend to change the liquid later, e.g., the liquid has already been used in a mix in the Mixes step, or, if the liquid is a ready-made reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed. In this case, remove assignments or reaction mixes to enable changes for defined input liquids.
L	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.
×	To delete a liquid	Deletes the row in the table.

7.2.6.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you want to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values for a liquid.

Add liquid	
Liquid name Dilution buffer Tube type 1.5 ml tapered tube (QIAGEN)	 ● Input liquid ○ Ready-made mix Cap color
Liquid class Diluent for dilution	Liquid type Diluent/Water
Define advanced pipetting parameters	/_/ III
	OK Cancel
Add liquid dialog	

- 1. Define the name of the liquid. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, e.g. when defining reaction mixes, standards or controls, and in the loading instructions of the Worktable step.
- 2. Define whether the liquid is an input liquid or a ready-made mix. Initially, the option **Input liquid** is selected, because it fits to most liquids.

Select the option **Ready-made mix** only, if the liquid is a final reaction/master mix that has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In this case, in the **Liquid type** field the value **Ready-made mix** is automatically selected and the liquid class menu is removed (the liquid class is defined later in the Mixes step). Additionally, when entering the later Mixes step, the reaction (master) mix is already listed.

If the liquid is a mix that has already been prepared by the user, but will be part of a more complex mix that will be created during the run by the QIAgility, select the option **Input liquid**.

3. Click **Tube type** to select the tube that will be used to place the liquid on the QIAgility. The tube types used in the last experiment are listed at the top of the list. To select one of the listed tube types, click the respective entry.

Tube type	
Select	▼
Select	
1.5 ml tapered tube (QIAGEN)	
5.0 ml flat-base tube (QIAGEN)	
Choose other labware	
Any available	

Select the value **Any available**, if the liquid does not need to be provided in a special tube, e.g. coming from a kit. In this case, later, during worktable planning, the software will automatically select a tube of the required size, with minimal dead volume, from the list of available tubes that has been defined in the Labware Manager. (To define available tubes in the Labware Manager, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.) Use this option to give the software more flexibility during worktable planning.

If the required tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.

Filter options Category	Labware Select the item that most closely matches the labware to be us	ed.		
Tubes	1.5 ml tapered screw cap tube (QIAGEN) 1.5 ml tapered tube (QIAGEN) 2.0 ml self standing screw cap tube (QIAGEN) 200 µl PCR tube (QIAGEN) 5.0 ml flat-base tube (QIAGEN)	40	Catalog number 990552 Name 5.0 ml flat-base tube (QIAGEN) Dead Volume 26 µl	L,
Tube / Well size	Labware adapter Select the adapter to be used.			
Reset filter Apply filter	Select and everynes to be used.		Catalog number Name	

In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If you edit the liquid later and the **Tube type** is marked yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type or make the tube available in the Labware Manager again.

4. Click **Cap color** to select the color of the tube's cap.



5. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid**

Classes step to get more details.

In case the same liquid will be used for different purposes (e.g. nuclease-free water used for setting up a standard dilution series and for non-template control) it is highly recommended to define multiple input liquids to allow precise definition of liquid class and liquid type.

Note: If you edit the liquid later and the **Liquid class** is marked yellow, this indicates that the selected liquid class is no longer available, e.g. if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

6. Select the **Liquid type**. The liquid type defines how the liquid will be used. In later steps during experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection, e.g. when defining controls or standards. In case a defined input liquid is not visible in later screens (e.g. during control definition) check if the liquid type has been set correctly.

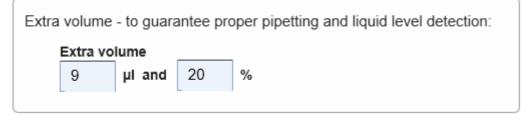
Liquid type	Description
Reagent	Use this type, if the liquid is a reagent that will be added to a reaction/master mix, either to a final reaction mix or to an intermediate reaction mix.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.
Pos./neg. control	Use this type, if the liquid is a positive or negative control (incl. NTCs).
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a control.
Standard	Use this type, if the liquid is a single standard as part of a user prepared standard series, or if the liquid is the standard stock for a standard series that shall be created by the QIAgility.
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a standard series that shall be created by the QIAgility.
Diluent/Water	Use this type, if the liquid is a diluent or water.
	Later in the experiment setup, a liquid of this type is available, e.g. for content definition of a reaction/master mix in the Mixes step, or in the Controls/Standards step as diluent for defining a control or standard series that shall be created by the QIAgility.
Ready-made mix	Use this type, if the liquid is a ready-made reaction/master mix prepared by the user. This type is preselected by the software, if the option Ready-made mix has been selected for the liquid.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.

- 7. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, these data will be present in the report.
- 8. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

Define advanced pipetting parameters



 In the dialog, click OK to confirm your values and to close the dialog. Otherwise, click Cancel to close the dialog and discard all values. In this case, no new liquid is defined, or – in case of editing an existing liquid –the liquid is not changed.

7.2.6.1.2 Liquid classes

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

The liquid classes that are available for experiments for **Generic PCR setup**, **Generic PCR setup** with **Normalization**, **PCR setup with serial dilution** or experiments having **Other workflows**, are listed in a table in the settings section of the QIAgility Setup Manager Software. Click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step.

Column	Description
Name	The name of the liquid class. This name is listed in the dialog were a liquid class has to be selected.
Version	The version of the liquid class.
	Liquid classes will be improved over time and new versions of the QIAgility Setup Manager Software may come with newer versions of liquid classes. These new

versions of liquid classes will apply to new experiments. If you use existing templates or experiments that have been created before, they still use the older versions of liquid classes, as a snapshot of the liquid classes is saved within the experiments and templates. This ensures that existing templates run as before, after a software update. If you would like the new versions of liquid classes to be applied to your existing templates, open the templates, and for every liquid defined reselect the liquid class. Then, save the template again.

Description Information for which liquids the liquid class should be used.

All pre-defined liquid classes make use of the liquid level sensor. For more information refer to <u>Liquid-level</u> <u>tracking</u>.

7.2.6.2 Mixes step

The **Mixes** step is used to define all kinds of mixes that are used for an experiment. These are, for example:

- all variants of reaction (master) mixes, i.e., final reaction (master) mixes that
 - o have already been prepared by the user and are ready to be used,
 - o will be generated by the QIAgility during run,
 - o and will be distributed to the output labware.
- all kinds of intermediate mixes. These are generic pre-mixes that will be used to create new (more specific) reaction (master) mixes.

The screen lists all mixes that have already been defined in a table. User prepared reaction (master) mixes are automatically listed, if the corresponding liquid has been defined in the previous Liquids step. The rows for these mixes are initially highlighted in yellow to indicate that the mixes need further specification.

s .	Define your mixes						
>	Define reaction (master) mix(es) r pipetting/tip handling task for distr screen). Adv. parameters: Mixing settings						
ls / Standards	mixing repetition can be changed For more information, press F1 ke	l if required. ey when editing mix(es).					
	Name	Reaction mix		Volume / reaction [µl]	Total volume / reaction [µl]	Liquid class	Actions
s / Labware	Mix	•	Reaction mix		9 19	Reaction (maste	0 🖪 🗙
		•	Primer mix		9	reaction (master	
able							
ible							
ble							
ble							
ble							
ble							
ble							

Defining a new mix or editing a listed mix:

1. To define a new mix, click the **Add mix** button. The **Add new mix** dialog opens.

To edit a listed mix, click in the **Actions** column in the corresponding table row. The **Edit mix** dialog opens. It displays the values that have been entered before in the **Add mix** dialog or that have been automatically pre-filled by the software for a ready-made reaction (master) mix.

Note: If no final reaction (master) mix has been defined, the **Add mix** button is highlighted in yellow to indicate that the definition of a final reaction (master) mix is still missing.

- 2. Define the general properties of the mix. Details are described in <u>Add new mix dialog</u>.
- 3. In the dialog, click **Next** to proceed to the **Input** screen.
- 4. In the dialog **Input** screen define the volume per reaction and the content of the mix; as described in <u>Add new mix dialog input</u>.
- 5. For final reaction/master mixes, the optional **Target** screen is available. This screen allows the user to define target type and reports/quenchers. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import into the next instrument, e.g. a cycler. In this case, click **Next** to proceed to the **Target** screen. Details are described in <u>Add new mix dialog target</u>.

If you do not require target information in the report or for the next instrument, proceed.

- 6. Click **Finish** to finish the definition of the mix and to close the dialog.
- 7. The defined mix will be listed in the table in the Mixes screen.

Repeat the steps above until all final reaction (master) mixes have been defined.

Finally, click **Next** in the button bar to proceed to the **Controls/Standards** step, described in <u>Define</u> <u>controls and standards step</u>.

Note: The Next button is disabled, if a row in the table is highlighted yellow. User prepared reaction

(master) mixes are initially highlighted yellow, if the volume per reaction has not yet been defined. Edit(the mix and enter the volume per reaction in the Input screen of the dialog. The **Next** button is also disabled, if the definition of a final reaction (master) mix is still missing.

The mixes in the table can be reordered using the \checkmark buttons below the table. The report will list the mixes in this order. An intermediate mix (generic pre-mix) needs to be listed before the (more specific) reaction (master) mix of which it is part of. It will not be possible to move an intermediate mix below its reaction (master) mix.

- 1. Select the row of the mix to be moved by clicking on it.
- 2. Click on the up and down arrows to move the currently selected row up or down.

The butto	The buttons in the Actions column of the table have the following functions:						
Button	Action	Description					
0	To edit a mix	The Edit mix dialog will open, displaying the values that have been entered in the Add mix dialog.					
		If you intend to change a mix that is already in use as input for another mix, or if it is a final reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed anymore.					
	To duplicate a mix	The Duplicate mix dialog will open, displaying the values that have been entered for the original mix. The mix name will initially display a copy of the name of the original mix.					
×	To delete a mix	Deletes the row in the table.					

7.2.6.2.1 Add new mix dialog

In the **Add new mix** dialog a new mix can be defined. If you would like to change the values for an existing mix later, the **Edit mix** dialog will open. Both dialogs show the values of a mix.

Define the basic properties of the mix in the **General** screen of the dialog.

	Edit mix
General >	Name
Input	Test-mix Final reaction mix (ready-made) Intermediate mix (ready-made) Test-mix
Target	Final reaction mix already on output (ready-made) Liquid class Reaction (master) mix (final) to output
	Note: Make sure that ready-made mix is homogeneous and mixed manually and avoid foam.
	Define advanced parameters
	Back Next Finish Cancel

Edit mix dialog

	Add new mix
General >	Name
Input	Mix Final reaction mix (system prepared) Intermediate mix (system prepared) Final reaction mix (system prepared)
Target	 ▶ Final reaction mix already on output ■ Reaction (master) mix (final) to output ■ Define advanced parameters
	Back Next Finish Cancel Add new mix dialog - General

1. Define the name of the mix. The name must be unique. The name is used to refer to the mix in later steps of the experiment setup, e.g. when defining more specific mixes, and in the loading instructions of the Worktable step.

For a ready-made mix, the name is already defined from the liquid dialog definition.

2. Select the type of mix. For ready-made mixes and other mixes different options are available:

Option	Description
Final reaction mix (ready-made)	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
	Use this option, if this mix has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In the later Assignment step, the mix will be available for selection. During the run, the QIAgility will pipette the mix to the positions on the output labware as assigned in the Assignment step.

		Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QIAgility as QIAgility will not perform any mixing steps.
	Intermediate mix (ready- made)	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
		Use this option if this mix has already been prepared by the user and will be part of a more specific mix to be created during the run by the QIAgility. During the run, the QIAgility will pipette the mix into the tube, where the more specific mix is created.
		Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QIAgility as QIAgility will not perform any mixing steps.
	Final reaction mix already on output	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
(read	(ready-made)	Use this option only if this mix has already been distributed on the output labware before the run. In the later Worktable step, you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QIAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.
		The volume of the reaction mix must be specified. This value will be used to calculate the dispense height and the mixing volume.
		If the option is selected, the Input screen of the dialog will be skipped.
	Final reaction mix (system prepared)	Use this option if during the run the mix will be created first by the QIAgility and then distributed to the output labware.
	Intermediate mix (system prepared)	Use this option if this mix will be created by the QIAgility during the run, but will be part of a more specific mix. During the run, the mix will be created first by the QIAgility. Then the QIAgility will pipette the mix into the tube where the more specific mix is created.
	Final reaction mix already on output	Use this option only if this mix has already been distributed onto the output labware before the run. In the later Worktable step you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QIAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.
		If the option is selected, the Input screen of the dialog will be skipped.

3. The liquid class that will be used to distribute the reaction (master) mix during the run is preselected by the software. Change the liquid class only if it is really required. In this case, select the **Liquid class** that fits best to transfer the reaction (master) mix.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

4. Optionally, define advanced parameters. If you tick the box, additional parameters become visible to define extra volume and mixing options.

For reaction (master) mixes that will be created by the QIAgility, mixing options are displayed. Mixing is performed after the last reagent has been added to the reaction (master) mix, before the mix will be transferred. The mixing settings are defined in the **Liquid class for mixing**. This liquid class is already preselected by the software and should only be changed if really required. The **Mixing repetitions** – which are pre-defined by the selected liquid class for mixing – can be increased, if needed. The mixing cycles have been optimized to allow proper mixing for final reaction (master) mixes in which no pure Enzyme has been added as reagent. If your setup uses large volume final reaction (master) mixes or pure Enzymes as reagents, it is recommended to increase the mixing repetition to 15 or more.

Define advanced parameters	
Extra volume - to guarantee proper pipetting and	liquid level detection:
Extra volume Extra volume per reactive 39 µl and 2.2 µl Mix of system prepared final reaction mix:	ion
Liquid class for mixing	Repetitions (Min = 3)
Mix of reaction (master) mix (final)	▼ 10

For ready-made reaction (master) mixes the QIAgility does not perform a mixing step before transfer to output labware. Make sure the reaction (master) mix has been mixed properly before placing it onto the QIAgility.

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases, displayed loading volume is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover sample amount depending variations (e.g. volume loss per tip) and is added per single transfer. However, if you face any liquid level detection issues, increase **Extra volume per assignment** if issues are for higher sample amounts only, or increase absolute **Extra volume** if issues are not related to the sample amount.

5. Proceed with step 3 in <u>Mixes step</u>.

7.2.6.2.2 Add new mix dialog input

The **Input** screen of the **Add/Edit mix** dialog lists all input liquids/reagents of the mix and their volume per reaction in a table.

Add new mix							
General	Conter	nts of this mix					
Input >	reagen	all liquids/reagents and volume per reaction for respective reaction (n ts in "Liquids" screen can be selected.					
Target	volume	commended to sort the reagents of reaction (master) mix by volume p to ensure proper pipetting for final reaction mix. Use "Sort" button to ing to volume per reaction.	automatically sort reagents				
	Seq.	Name	Volume / reaction [µl]				
	1	Reaction mix V	9 🗙				
	2	Primer mix 🔹	9 🗙				
	Z Pliffiel flix 3 ✓ 3 X ✓ Soft Calculated reaction mix volume/reaction: 18 µl Add Input						
		Back Next	Finish Cancel				

Add new mix dialog - Input

For a **ready-made mix** (i.e., a user prepared mix), the input liquid is already defined from the liquid dialog definition. Enter the volume per reaction in μ l into the **Volume/reaction** field in the table. Left-click the field and then enter the value. Initially, the field is highlighted in yellow to indicate that input is required.

For a **system prepared mix**, i.e., a mix that will be created by the QIAgility, define the composition of the mix:

- 1. Click the **Add input** button below the table. A new row is added to the table. Initially, the fields are highlighted in yellow to indicate that input is required.
- 2. In the **Name** field of the table, select the input liquid. The menu lists intermediate mixes and all liquids that are of liquid type **Reagent** or **Diluent/Water**. If a required liquid is not listed as expected, check

if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.

- 3. Define the volume per reaction for the input liquid. Left-click the **Volume/reaction** field and then enter the value. The total volume per reaction of the mix is displayed below the table. It summarizes the volumes per reaction of the listed reagents. For not integer volumes, use a point as a decimal delimiter.
- 4. Repeat steps 1–3, until all components of the mix are listed.
- 5. It is recommended to sort the reagents of the mix by volume per reaction, starting with the highest volume, to ensure proper pipetting for the final reaction mix. Later, during the run, the QIAgility will pipette the reagents into the tube where the mix is created in the order listed in the table.

Click the **Sort** button below the table, to automatically sort the list by volume per reaction, starting with the highest volume.

Alternatively, use the 🗠 💟 buttons below the table to sort the reagents individually. Left-click the row of the reagent to be moved. Click on the up and down arrows to move the currently selected reagent up or down.

Proceed with step 5 in <u>Mixes step</u>.

7.2.6.2.3 Add new mix dialog target

The **Target** screen of the Add/Edit mix dialog is optional. It is available for final reaction (master) mixes to define all assay targets and allow easy transfer of defined targets to respective PCR cycling software. If defined, targets are used in later steps for control, standard and sample definition. The information will also be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g. a PCR cycler.

		Add new mi	ix		
General					
Input	Define all assay targets to allow easy transfer of defined targets to respective PCR cycling software. If defined, targets are used in later steps for control, standard and sample definition. Target type: Test: Amplicon of interest (e.g., HIV); Reference: Amplicon used for data normalization (e.g., house-keeping gene used for delta Ct calculations); IC: Internal control sequence to monitor assay performance (e.g., inhibition of sample purification efficiency) – consider kit manual for assay-specific details Reporter: Enter fluorescence dye name (e.g., FAM) per target. Quencher: If applicable, enter quencher name (e.g., TAMRA) per target. Ensure correct notation (case sensitive) of reporter and quencher as required by the PCR cycling software. Target definition, optional (List the individual targets of the mix here.)				
Target					
	Target name	Target type	Reporter	Quencher	
	Human	Test V	FAM	None 🛛	
	Male	Test 🔍	Cy5	None 🛛	
	IC	IC V	VIC	None	×
				Add	target
Back Next Finish Cancel					

Add new mix dialog - Target

If you do not need target information in the report or for the next instrument, proceed with step 6 in <u>Mixes</u> <u>step</u>.

To define targets:

- 1. Click the **Add target** button in the dialog. A new row will be displayed in the table.
- 2. Click into the **Target name** field and enter the name of the target. The name must be unique. It must be different to defined liquids, mixes, standards and controls.
- 3. Click on the menu in the **Target type** column and select a target type:

Target type	
Test 🔹	
Test	ľ
Reference	
IC	

Target type

Description

Test	Amplicon of interest (e.g., HIV)
Reference	Amplicon used for data normalization (e.g., house-keeping gene used for delta CT calculations)
IC	Internal control sequence to monitor assay performance (e.g., inhibition of sample purification efficiency) – consider kit manual for assay specific details)

Optional step: Click on the menu in the Reporter column and select an appropriate reporter dye for 4. the target.

Note: To use the QIAgility Wizard in the Q-Rex software for the Rotor-Gene Q, check the flourophore mapping in the Q-Rex software.

Reporter SYBR Green 1 SYBR Green 1 Cy5 Crimson ROX SYBR		
Green	Yellow	Orange
FAM	JOE	ROX
SYBRGREEN1	VIC	CALFLUORRED610
SYBRGREENI	HEX	CY35
FLUORESCEIN	TET	TEXASRED
ALEXAFLUOR488	MAX	ALEXAFLUOR568
	CALFLUOR	
	GOLD540	
	YAKIMAYELLOW	
Red	Blue	Crimson
CY5	MARINABLUE	QUASAR705
QUASAR670	EDANS	ALEXAFLUOR680
ALEXAFLUOR633	BOTHELLBLUE	LIGHTCYCLERRED705
LIGHTCYCLERRED640	ALEXAFLUOR350	
	AMCAX	

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section <u>Entering data</u>.

Note: Initially, the menu is empty.

5. **Optional step:** If applicable, click on the menu in the **Quencher** column and select an appropriate quencher for the target.

Use this option for automated selection of the appropriate quencher for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported quenchers. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the quencher can be left blank, if it is not required for the assay.

Note: The quenchers are listed in an editable menu. If the quencher you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the quencher as required by the PCR cycling software. The last entered quenchers will be available for selection for future experiments. For more details about editable menus, refer to the section <u>Entering data</u>.

- 6. Repeat the steps above until all individual targets of the mix have been defined.
- 7. Proceed with step 6 in <u>Mixes step</u>.

7.2.6.3 Controls/Standards step

This optional step is used to define controls and standard series. If you do not intend to use controls or standards for the run, proceed by clicking **Next**.

The screen contains two tables: **Controls** and **Standard series**. Both tables have an **Actions** column with interactive buttons to manage the controls and standard series.

Note: When distributing defined controls (NTC and NC) and standards to the output labware during the run, the QIAgility will transfer the same volume as defined for samples/template in the later Samples Labware step.

Adding a control or standard series

- 1. Click on Add control or Add standard series.
- 2. The **Add control** or **Add standard series** dialog will open. For details, refer to <u>Add control</u> or <u>Add</u> <u>standard series</u>, respectively.
- 3. The control will be added to the **Controls** table, and the standard series will be added to the **Standard series** table.
- 4. Repeat steps 1–3 until all controls and standard series have been defined.
- 5. Finally, click **Next** to proceed.

² *Experiment 0 ×								
Liquids	Define controls and standards							
	Controls							
Mixes	Seq.	Name	Reaction mix	Target	Target type	Control type	# of Replicates	Actions
	1-2	NTC	Mix	IC	IC	NTC	2	🖉 🖪 🗙
Controls / Standards >			·					
Samples / Labware								-
Assignment	Output labware order Add control							
Worktable	Standard	series						
	Seq.	Name	Reaction mix	Target	Target type		# of Replicates	Actions
_	1-12	Series	Mix	Human	Test		2	2 🖪 🗙
Run		Selies	WILX	Male	Test		2	
								Ŧ
	Output labware order 🔊 🔽 Add standard series							

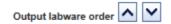
Controls / Standards step

The buttons in the **Actions** column of the **Controls** table and the **Standard series** table have the following functions:

Button	Action	Description
0	To edit a control or standard series	The Edit control or Edit standard series dialog will open, displaying the values that have been entered in the Add control or Add standard series dialog.
e	To duplicate a control or standard series	The Duplicate control or Duplicate standard series dialog will open, displaying the values that have been entered for the original control or standard series. The control or standard series names will initially display as a copy of the control or standard series.
×	To delete a control or standard series	Deletes the row in the Controls or Standard series table.

Changing the order of the output labware

You can change the order of the controls and standard series using the up and down arrows below each table.



Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.6.3.1 Add control

In the **Add control** dialog a new control can be defined. If you would like to change the values for an existing control at a later timepoint, the **Edit control** dialog will open. Both dialogs show the values of a control.

Add contro	bl
Control	
NTC	
iquid	
Water	▼
Reaction mix	
🛑 qPCR Master Mix	▼
Control type	
NTC	▼
	Target
Use specific volume	

Add control dialog

1. Enter a control name in the **Control name** field. The name must be unique (not similar to the name entered in the liquid screen). The name is used to refer to the control in later steps of the experiment setup, e.g. in the Assignment step, and in the loading instructions of the Worktable step.

- Select the input liquid from the Liquid menu. The menu lists all liquids that have been defined with liquid type Pos./neg. control or Diluent/Water. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 3. Select the reaction mix from the **Reaction mix** menu. The menu lists all mixes that have been defined with options as **Final reaction mix (ready-made)**, **Final reaction mix (system prepared)**, **Final reaction mix in output (ready-made)** or **Final reaction mix in output**.
- 4. Select the control type using the drop-down menu in the **Control type** column:

Control type	
Select control type	•
Select control type	
PC	
NC	
NTC	

Option	Description
--------	-------------

- PC Is a positive control for the intended assay.
- NTC Is a non-template control for the intended assay.
- NC Is a negative control for the intended assay.

During run, the QIAgility will pipet the selected liquid to the output labware considering defined replicates and the sample volume which will be defined in the Samples/Labware step.

5. If targets have been defined for the selected reaction mix, the software lists all defined targets in a table below. Tick the box next to a target name if the target is valid for the control. Tick the box in the table header if you want to select all targets.

Note: If you intend to save an output file after run for import into a cycler software, e.g. Rotor-Gene© Q PCR cycler with Q-Rex software, always select all targets.

6. If the PC control type has been chosen, you can specify a custom volume for the control. Tick the **Use** specific volume box, type in a value in the **Volume** field, and select a diluent from the **Diluent (used** to fill up to sample volume) drop-down menu.

Add control		
Control		
Liquid		
Water 🔻		
Reaction mix		
qPCR Master Mix		
Control type		
PC 🗸		
Target		
✓ Use specific volume		
Volume Diluent (used to fill up to sample volume) 1 µI Diluent		
OK Cancel		

Specifying control volume

- 7. Click **OK** to confirm and close the **Add control** dialog.
- 8. The control will be added to the **Controls** table.

7.2.6.3.2 Add standard series

In the **Add standard series** dialog a new standard series can be defined. If you would like to change the values for an existing standard series at a later timepoint, the **Edit standard series** dialog will open. Both dialogs show the values of a standard series. Define the basic properties of the standard series in the **General** screen.

Add standard series					
General >	Standard series name	Tube type			
Dilution Stone	Standard 161	Any available			
Dilution Steps	Standard Tor				
	Reaction mix	Use custom minimal volume for standard series.			
		-			
	PCR Master Mix	Use advanced pipetting parameters.			
	Use ready-made standard series				
	Standard stock liquid	Diluent			
	Standard 161	Diluent			
	Target and stock concentration				
	Target	Stock conc. Concentration unit			
	n/a 🛛	7 copies/µl 🔹 🗙			
		Define Std. stock			
	Back Next	Finish Cancel			
Add standard series dialog - General					

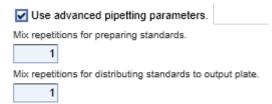
- 1. Enter a name for the series in the **Standard series name** field.
- 2. Select labware from the **Tube type** drop-down menu.
- 3. Select a reaction (master) mix, or multiple mixes, from the **Reaction mix** table by ticking the box next to the relevant mix.
- 4. Check the **Use custom minimal volume for standard series** option, if you intend to define a minimal volume value for the standard series. Enter the volume in the **Minimal volume per standard**

dilution field.

Use custom minimal volume for standard series.

Minimal volume per standard dilution

5. Check the **Use advanced pipetting parameters** option, if you would like to define the number of mix repetitions for preparing standards and distributing standards to the output plate. Enter the relevant values in the **Mix repetitions for preparing standards** and **Mix repetitions for distributing standards to output plate** fields.



6. Check the option **Use ready-made standard series**, if standards for a user prepared standard series have been defined in the **Liquids** step.

Uncheck the option if you plan to load one tube containing the standard stock and would like for the QIAgility to automatically create the dilutions for the standard series during the run.

- 7. If the option **Use ready-made standard series** has not been checked, select the appropriate liquid from the **Standard stock liquid** menu. The menu lists all liquids that have been defined with liquid type **Standard**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- If the option Use ready-made standard series has not been checked, select the appropriate diluent from the Diluent menu. The menu lists all liquids that have been defined with liquid type Diluent/Water. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 9. **Optional step.** Define the stock concentration, if you intend to define the standards of the series with their concentration. Otherwise, the standards of the series can be defined by dilution ratio, only.

If targets have been defined for the selected reaction mix, stock concentrations can be entered individually per target. This information will then also be part of the report and the output file, which can be saved after the run. If you intend to use the output file for import to a downstream instrument, e.g. a PCR cycler, define the concentrations for all available targets. For targets with no stock concentration, enter concentration value 0.

Target and stock concentration

Target	Stock conc.	Concentration unit	
Human 🛛	20	ng/µl	×
Select target	`	Select a concentratio	×
Select target Human			
Male			
IC			
			-
		Define S	Std. stock

Defining stock concentration per target.

If no targets have been defined for the mix, a common stock concentration can be defined, only.

Target and stock concentration

Target	Stoc	k conc.	Concentration unit		
n/a		20	Select a concentratio	×	
					ų.
			Define S		_

Defining common stock, if no targets have been defined for the mix.

Click the Define Std. stock button to add a stock concentration. A new row is displayed in the Targets table:

Column Target	Description Click the menu in the Targets field to select the appropriate target. If targets have been defined for the reaction mix, the menu lists all available targets. Otherwise, N/A is selected.
Stock conc.	Stock concentration. This column is available only if the option Use ready-made standard series has not been checked.
	Enter the concentration that is present in the stock liquid for the target.
Concentration unit	Click the menu in the Concentration unit field to select the concentration unit for the target. This unit is used in the report and the output file, which can be saved after run.

10. In the dialog click **Next** to proceed to the **Dilution steps** screen.

Add standard series dilutions 7.2.6.3.3

Use the **Dilution steps** screen to define the dilution steps and dilution ratio for the serial dilution series. The dilution steps are displayed in a table.

Add standard series				
General Define dilution steps and dilution ratio for serial dilution series. In "Dilution ratio" column, the total dilution ratio based on the standard stock is displayed, but QIAgility will prepare a serial dilution series.				
Dilution Steps >	A dilution ratio of 1:1 means "no dilution".			
	Dilution s 6 V Dilution r 1:4	teps Use Standard series stock as first standard atio between two standards (optional) Update dilution ratios		
	Seq.	Name of standard	Dilution ratio	
	1	Control DNA	1:1	
	2	Standard Series 1	1:4	
	3	Standard Series 2	1 : 16	
	4	Standard Series 3	1:64	
	5	Standard Series 4	1 : 256	
	â	Standard Series 5		
Back Next Finish Cancel Add standard series dialog - Dilution steps				

Add standard series dialog - Dilution steps

1. Select the number of dilution steps from the **Dilution steps** menu.



The number of rows in the table is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering</u> <u>data</u>.

2. If the option **Use ready-made standard series** has not been checked in the previous **General** screen, the additional option **Use Standard series stock as first standard** is available. Check the option in order to use the actual stock as the first standard. An additional row is automatically added to the table, showing a dilution ratio of 1:1 (meaning "no dilution"). During the run the QIAgility will directly use the stock as template for the reaction points of this standard.

Dilution steps

6 🔻

Use Standard series stock as first standard

Dilution ratio between two standards (optional)

Update dilution ratios

Seq.	Name of standard	Dilution ratio
1	Control DNA	1:1
2	Standard Series 1	1

Option "Use standard series stock as first standard" selected.

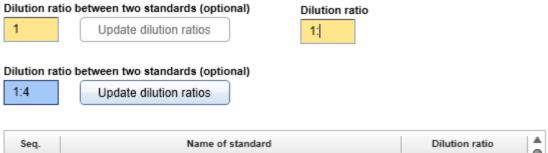
3. For each dilution step, if the option **Use ready-made standard series** has been checked in the previous **General** screen, select the appropriate liquid from the corresponding menu in the **Name of standard** cell.

Seq.	Name of standard	
1	Control DNA 🔹	
2	Standard1	
3	Select standard liquid	
4	Select standard liquid Control DNA Standard1	
5	Standard2	
6	Standard3 Standard4 Standard5	
4	Standard6	•



If the option **Use ready-made standard series** has not been checked in the previous **General** screen, optionally, enter a meaningful name into the **Name of standard** cell. This is especially important if you plan to define more than one standard series. The name must be unique. The name is used to refer to the standard in later steps of the experiment setup, e.g., in the Assignment step, and in the loading instructions of the Worktable step.

4. If an equal dilution ratio will be used throughout the standard series, define the dilution ratio in the **Dilution ratio** field above the dilution steps and then click **Update dilution ratios** to apply it to the series.



1	Control DNA	1:1	
2	Standard Series 1		
3	Standard Series 2		
	-	-	

Entering the dilution ratio, e.g. 1:4

Dilution ratio between two standards (optional)

- 1	:4	
	.4	

Update dilution ratios	
------------------------	--

Seq.	Name of standard	Dilution ratio
1	Control DNA	1:1
2	Standard Series 1	1:4
3	Standard Series 2	1 : 16

After button "Update dilution ratios" has been clicked.

Alternatively, for each dilution step, enter either a dilution ratio or a concentration for the respective standard. The other value will be calculated automatically by the software.

Dilution ratio between two standards (optional)

|--|

	Name of standard	Dilution ratio	Concentration i
DNA	٦	1:1	20
d Series 1	1	1:4	5
d Series 2	1	`	
d Series 3		`	
d Series 4	•		
d Sariao 5	`	`	Enter Concen

Entering dilution ratio for second dilution step.

In the **Dilution ratio** column the total dilution ratio based on the standard stock is displayed. However – if the option **Use ready-made standard series** has not been checked – during the run the QIAgility will prepare a standard dilution series using the previous standard to create the subsequent standard.

If several targets have been defined in the previous **General** screen, concentrations are listed individually per target. If you enter one of the concentrations or the dilution ratio for a standard, all other values will be calculated automatically by the software.

lame of standard	Dilution ratio		Concentration i Human	Concentration i Male	
	1 : 16	٦	1.25	0.76875	ſ
	1:64	٦	0.3125	0.1921875	
	1 : 256	1	0.078125	0.048046875	
	1:1024	1	0.01953125	0.01201171875	
	1:4096	1	0.0048828125	0.0030029296875	l
•					

Concentrations listed individually per target

Note: Concentration values for dilution steps are available only if stock concentration has been defined in the previous **General** screen.

Note: This step is optional if the option **Use ready-made standard series** has been checked and no stock concentration has been defined in the previous **General** screen. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g., a PCR cycler.

- 5. In the dialog, click **Finish** to complete the definition of the standard series and to close the dialog.
- 6. Proceed with step 3 in <u>Controls/Standards step</u>.

7.2.6.4 Samples/Labware step

Use the Labware Selection screen to select the labware for sample input and run output and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file. The QIAgility Setup Manager Software supports up to three input labwares.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side. Define the sample pipetting below the input labware panel.

_	Define samples and labware	
	Input labware	Output labware
	Samples will be provided on one single plate or adapter	Distribute assay points to single plate or adapter
	 Samples will be provided on multiple plates or adapters (up to 3) 	 Distribute assay points to multiple plates or adapters (up to 3)
andards		
	 Use a new input labware and enter sample names Load sample input from file 	 Use a new output labware Reuse a partially used output labware by loading an import file
bware >		
	Load sample input from file	Select file
	Labware	Output labware type
	QIAGEN Elution Microtubes RS	QIAGEN 96 Well Microplate RB
	Use cooling block Samples are covered with top-elute	Use cooling block Output labware is pre-filled with reagents
	Labware identifier	Labware identifier
	Input2_20190730_132026 Print barcode	Output1_20190730_132014 Print barcod
	Auto-generate sample IDs for all samples	Output Converter
	Define samples Import data from CSV file	Select 🔻
	Sample Pipetting	
	Sample volume Liquid class	
	10 µl Sample to output ▼ Edit parameters	
		J

Note: For Generic PCR experiments, four positions are available on the worktable. You can use them in different configurations, i.e. you can use three input adapters and one output, two input and two output adapters, or one input adapter and three output adapters. Proceed as follows:

1. Select the input labware and define the samples.

If the samples will be on a single plate or adapter, check the option **Samples will be provided on one single plate or adapter** on the left side of the screen and follow the steps in <u>Define an input</u> <u>labware</u>.

If the samples will be on several plates or adapters, check the option **Samples will be provided on multiple plates or adapters** and follow the steps in <u>Defining up to three input labware</u>.

2. Select the output labware.

Sample Dipatting

If the output will be on a single plate or adapter, select the **Distribute assay points to single plate or adapter** option, and follow the steps in <u>Define the output labware</u>.

If the output will be on several plates or adapters, select the **Distribute assay points to multiple plates or adapters** option, and follow the steps in <u>Define up to three output labware</u>.

3. Enter the sample volume for the run into the **Sample volume** field in the **Sample pipetting** panel. The value must be specified in μ l.

Sample volume	Liquid class	Use custom pipetting parameters
2 µl	Sample to output	Edit parameters

- 4. The liquid class used for sample transfer is predefined by default. If required, change the liquid class to be used for sample transfer by clicking the **Liquid class** dropdown menu. It defines the pipetting and tip handling for distribution of samples from the input labware to the output labware.
- 5. **Optional:** If required, use custom pipetting parameters for samples. If you tick the box, the **Edit parameters** button becomes active. If you click the button, the **Define advanced pipetting parameters** dialog opens, providing parameters for extra sample volume and mixing repetitions. For a detailed description refer to <u>Advanced sample pipetting</u>.

Note: Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. Change the parameters only if you face any liquid level detection issues.

6. Click **Next** to proceed.

The next step is the Assignment step.

7.2.6.4.1 Define an input labware

Note: For Generic PCR experiments, four positions are available on the worktable. You can use them in different configurations, ie. you can use three input adapters and one output, two input and two output adapters, or one input adapter and three output adapters.

If the option **Samples will be provided on one single plate or adapter** has been selected, define the input labware according to the following steps:

 If you would like to enter sample names manually, or, if you would like to auto-generate sample names, select Use a new input labware and enter sample names and proceed to step 2.
 If you would like to load a sample input file in csv format, select Use a new input labware and enter sample names and proceed to step 2.

Use a new input labware and enter sample names

O Load sample input from file

If you would like to load a sample input file in a QIAGEN specific xml file format (QDEF), select **Load** sample input from file and proceed to step 11.

If you would like to load a sample input file in txt format, select **Load sample input from file** and proceed to step 11.

- 2. Click on the Labware drop-down menu on the left side of the screen.
- 3. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

Labware

1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapere
1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered-Base Tubes
Choose other labware

4. Select your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

i Labware browser			
Filter options Category Standard 96 Well ▼ Shows available labware only	Labware Select the item that most closely matches the labware to be used QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name	
Advanced options Name of labware		QIAGEN S-Block 96 Well Dead Volume 40 µl	
Part number Manufacturer All			■ 000000000000000000000000000000000000
Tube / Well size	Labware adapter Select the adapter to be used.	Catalog number	
Reset filter Apply filter		Name	
	v		

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

5. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

7. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 9. Define the sample names. Check the option Auto-generate sample IDs for all samples if you want the software to automatically generate sample names for all positions on the labware. Alternatively, click the Define samples button to enter the sample names manually. It is also possible to edit the automatically generated sample names. For details about the sample editor refer to Edit samples.

Auto-generate sample IDs for all samples

Define samples ... Import data from CSV file...

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: It is also possible to define sample names first and then to import concentration values only from a csv file. In this case, define the sample names first, as described above, and then click **Import data from CSV file**.

10. Proceed with step 2 in <u>Samples/Labware step</u>.

To define samples by using a sample input file in QIAGEN specific xml (QDEF) format, follow these steps:

11. If the option **Load sample input from file** has been checked, the **Load sample input from file** and **Scan barcode** buttons will be enabled.



Note: Files in txt format must adhere to the syntax described in File dialog.

12. Click the **Load sample input from file** button and select the desired sample file. For details, refer to <u>File dialog</u>.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

The software will load the sample information from the sample file.

If the sample information file also contains the labware type, it will automatically be selected in the Labware drop-down menu. In this case, the software will automatically display the sample editor. Proceed to step 16.

Note: This is only possible for files in QDEF format.

Note: If the **Labware** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

14. If the sample information file does not contain the labware type, select the labware manually from the **Labware** drop-down menu. If it is not listed, click **Choose other labware** to open the **Labware browser**.

(i) Labware browser			
Filter options Category Standard 96 Well ▼ ✓ Shows available labware only ▲ Advanced options Name of labware Part number Manufacturer All	Labware Select the item that most closely matches the labware to be QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	2 used Catalog number 19585 Name QIAGEN S-Block 96 Well Dead Volume 40 µl	1 6 12 A 000000000000000000000000000000000000
Tube / Well size All Reset filter Apply filter	Labware adapter Select the adapter to be used.	Catalog number Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

15. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

 Optional step: Edit the sample information. If the sample editor did not open automatically, click Define samples to open it. Click OK to confirm the sample information and to close the sample editor.

For details about the sample editor, refer to Editing sample names.

- If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
 Note: Whenever you select another labware, this option will become unchecked again.
- 18. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 20. Proceed with step 2 in <u>Samples/Labware step</u>.

7.2.6.4.2 Edit samples

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.

	Sample editor		
Sele	ect an input labware identifier t	o enter, edit or	view the samples provided on that labware
Lab	ware identifier		
In	put1_20161205_101957		
_		0 1 1 1	
Pos.	Sample ID	Conc. [ng/µl]	Description
A2	,		
A3		•	
A4			
A5			•
A6	`	•	•
A7	`		•
A8	1		
B1	`		
B2	`		
B3	`		
B4			U
B5			
B6			
B7			
B8			
C1			
C2			
C3			
C4			▼
			OK Cancel

Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.

Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column **Sample ID**. Press and hold the **SHIFT** key and left-click to select multiple adjacent cells, or press and hold the **CTRL** key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on **Auto-generate sample names**. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on **Auto-generate sample** names.

Сору	Ctrl+C
0000	04110
Paste	Ctrl+V
Auto-generate sample names	
Allo generale cample hamee	

Note: For more details how to enter information into a table refer to the section Entering data.

Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

7.2.6.4.3 Defining up to three input labware

Note: For Generic PCR experiments, four positions are available on the worktable. You can use them in different configurations, ie. you can use three input adapters and one output, two input and two output adapters, or one input adapter and three output adapters.

If the option **Samples will be provided on multiple plates or adapters (up to 3)** has been selected, you can define up to three input labware types.

Note: The first loaded input plate will be located in the C1 worktable position.

All defined input labware will be listed in the **Sample input labware** table. Initially, the table is empty.

Sample input labware

Seq	Labware type	Labware identifie	er 🔺			
1	QIAGEN Elution Microtu	ibe Input1_20180430_17	72704 🖉 🖪 🗙			
2	QIAGEN Elution Microtu	ibe Input2_20180430_17	72723 🖉 🖪 🗙			
Load sample input from file						
-						
<u> </u>						
	īne samples	Import data from CSV file				

1. If you would like to enter sample names manually or if you would like to auto-generate sample names, select **Add new** and proceed to step 3.

If you would like to load a sample input file in csv format, select **Add new** and proceed to step 3.

If you would like to load a sample input file in QIAGEN specific xml file format (QDEF), proceed to step 2.

If you would like to load a sample input file in txt format, proceed to step 2.

Note: Files in txt format must adhere to the syntax described in File dialog.

 Click the Load sample input from file button and select the desired sample file. For details, refer to File dialog. Click Open to close the file dialog. The software will load the sample information from the selected sample file.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

The software will load the sample information from the sample file.

If the sample file does not contain the labware type, the **Add new input labware** dialog opens. In this case, proceed with step 3. Otherwise, proceed with step 4.

- 3. The Add new input labware dialog opens. Follow the steps in Add input labware dialog.
- 4. The new labware will be listed in the **Sample input labware** table.
- 5. Repeat steps 1-4 until all input labware has been defined.
- 6. Define the sample names. The **Define samples** button will be colored yellow to indicate that this information is required.

Click the **Define samples** button to enter sample names manually. It is also possible to edit the automatically generated sample names. For details, refer to <u>Editing sample names</u>.

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: This step is optional if the sample names were already loaded from a file or automatically generated.

7. Proceed with step 2 in <u>Samples/Labware step</u>.

The buttons in the right column of the **Sample input labware** table have the following functions:

Button	Action	Description
0	To edit an input labware	The Edit input labware dialog will open, displaying the values that have been entered in the Add input labware dialog.
Ŀ	To duplicate an input labware	The Duplicate input labware dialog will open, displaying the values that have been entered for the original input labware. The input labware identifier will initially display as a copy of the input labware.
×	To delete an input labware	Deletes the row in the Sample input labware table.

7.2.6.4.4 Define output labware

Note: For Generic PCR experiments, four positions are available on the worktable. You can use them in different configurations, ie. you can use three input adapters and one output, two input and two output adapters, or one input adapter and three output adapters.

If the **Distribute assay points to single plate or adapter** option has been selected, define the output labware by following the steps below.

- 1. You can either **Use a new output labware** or **Reuse a partially used output labware by loading an import file**. The option **Use a new output labware** will be selected by default and can be used for most runs.
 - Use a new output labware
 - Reuse a partially used output labware by loading an import file

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case, select the option **Reuse a partially used output labware by loading an import file** and proceed to step 9.

2. Select the output labware from the **Output labware type** menu on the right. Click **Choose other labware** to open the **Labware browser**.

Output labware type	
Select output labware type	•
Select output labware type	
Choose other labware	

Iter options	Labware		
ategory Standard 96 Well T Shows available labware only	Select the item that most closely matches the lab QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name	
Advanced options Name of labware		QIAGEN S-Block 96 We Dead Volume 40 µl	1 6
Part number Manufacturer			
All Tube / Well size	Labware adapter		
	Select the adapter to be used.	Catalog number	
Reset filter Apply filter		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.
- 4. If the output labware is already pre-filled with liquid or dried reagents, indicate this by selecting the option **Output labware is pre-filled with reagents**. During the run, the QIAgility will consider this information and, e.g., not reuse tips.
- 5. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

6. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

7. **Optional:** If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to **Output converter** to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, **None** is the only entry in the list.

✓ Output Converter

 Select...
 ABI7500_QuantiplexHyres.xslt
 ABI7500_SDS_v1_4.xslt
 ABI7500_SDS_v2.3_HID_v1.2.xslt
 CE Loading (AL).xslt
 ConvertToCsv.xslt
 RGQ-SW_QREX.xslt

Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

8. Proceed with step 3 in <u>Samples/Labware step</u>.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

9. The **Select file** and **Scan barcode** buttons will be enabled if the **Load existing output from file** option has been selected.

Use an empty output labware
Load existing output from file
Select file...
Scan barcode ...

10. Click the **Select file** button and select the desired labware file. For details, refer to <u>File dialog</u>.

Alternatively, click the **Scan barcode** button. In the **Scan labware barcode** dialog, scan the barcode and confirm by clicking **OK**. For details, refer to <u>Scan labware barcode dialog</u>.

Using the information from the loaded file, the software will automatically select the output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

Note: If the **Output labware type** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

11. Proceed to step 3.

7.2.6.4.5 Define up to three output labware

Output labour

Note: For Generic PCR experiments, four positions are available on the worktable. You can use them in different configurations, ie. you can use three input adapters and one output, two input and two output adapters, or one input adapter and three output adapters.

If the option **Distribute assay points to multiple plates or adapters (up to 3)** has been selected, you can define up to three output labware types by following the steps below.

All defined output labware will be listed in the **Output labware** table. Initially, the table is empty.

beq	Labware type	Labware identifier	1
1	QIAGEN S-Block 96 Well	Output1_20190729_141	•
2	Eppendorf twin.tec PCR PI	Output2_20190729_141 🔽 🖌 🎑 🔳 🕻	•
3	QIAGEN S-Block 96 Well	Output3_20190729_141 📃 📃 💋 🔳 🔀	۲, I
^	V Load output labware f	from file Add new	I

Defining multiple output labware

If you would like to add output labware manually, click the Add new button and proceed to step 4.
 If you would like to load an output labware file, proceed to step 2.

If you would like to provide output labware information by scanning a barcode, proceed to step 3.

Click the Load output labware from file button and select the desired file. For details, refer to File dialog. Click Open to close the file dialog. The software will load the output information from the selected file.

If the file does not contain the labware type, the **Add new output labware** dialog opens. In this case, proceed to step 4. Otherwise, proceed to step 5.

- 3. Click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.
- 4. The **Add new output labware** dialog opens. Follow the steps in <u>Add output labware dialog</u>.
- 5. The new labware will be listed in the **Output labware** table.

If the labware is to be used with the cooling block, the box in the 🏁 column will be ticked. If the

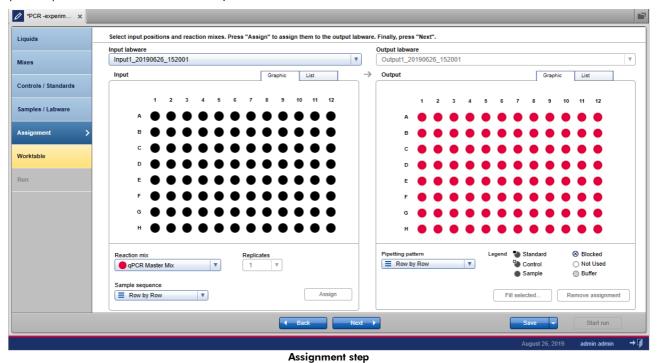
labware is pre-filled with reagents, the box in the **l** column will be ticked. To change these settings, edit the labware.

- 6. Repeat steps 1-5 until all labware has been defined.
- 7. Proceed with step 3 of the <u>Samples/Labware step</u>.

The buttons in the far right column of the **Output labware** table have the following functions:

Butto n	Action	Description
0	Edit the output labware	The Edit output labware dialog will open, displaying the values that were entered in the Add output labware dialog.
Ŀ	Duplicate the output labware	The Duplicate output labware dialog will open, displaying the values that have been entered for the original output labware. The output labware identifier will initially display as a copy of the input labware.
×	Delete the output labware	Deletes the row in the Sample output labware table.
7.2.6.	5 Assignment step	

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of sample-final reaction (master) mix combinations to the output labware.



Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware that have been selected in the **Input labware** menu. The meaning of the different symbols is described below:

	Name	Description
	Sample	Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been selected once for assay setup on output labware.
	Selected sample	Currently selected sample.
•		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
•		Note: It is possible to use the same sample multiple times to create reaction points on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. You cannot edit sample IDs that have already been assigned. You can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this sequence is used when assigning selected samples to the output labware.

If the option **Samples will be provided on multiple plates or adapters** has been selected, you can switch between the different input labwares using the box above the **Graphic** and **List** tabs.

At the bottom of the panel is a menu that lists all final reaction (master) mixes defined in the **Mixes** step, along with a **Replicates** menu, and a **Sample sequence** drop-down list.

In order to assign a sample for assay setup, select the sample position(s) and then the reaction (master) mix you would like to assign to the selected sample(s). Then set the number of replicates. When you click the **Assign** button, the selected samples and the reaction mix will be assigned to assay points created on the output labware. Follow the procedure described in <u>Assigning samples with reaction mixes to the output</u> <u>labware</u>.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. The creation of assay points containing samples, standards, and controls depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at

the input side. The assay points are colored with the reaction mixes. Standards and controls are indicated by an additional small symbol. If you hover with the mouse cursor over an output position, the software displays the sample ID and the reaction mix for that position.

Assay points can be removed. For details, refer to <u>Removing assay assignments</u>.

The **Sample sequence** menu at the bottom of the output panel can be used to set the pipetting pattern to either row by row or column by column.

It is possible to define blocked positions on the output labware by selecting the position(s) and then rightclicking on it.

In order to fill up unused positions on the output labware, e.g., with a buffer for CE loading, select the positions to be filled on the output labware and then click **Fill selected**. In the upcoming dialog select the liquid and the volume. For a detailed description refer to <u>Fill empty output positions</u>.

Use the **List** tab of the output panel to view the assay points that have been created.

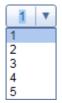
Outp	ut		G	raphic	List	
Pos.	Sample ID		Reaction mix			
A1	Liver 1		qPCR MasterMix			
B1	Liver 9		qPCR MasterMix			
C1	Liver 17		qPCR MasterMix			
D1	Lung 1		qPCR MasterMix			
E1	Lung 9		qPCR MasterMix			
F1	Lung 17		qPCR MasterMix			
G1	Brain 1		qPCR MasterMix			
H1	Brain 9		qPCR MasterMix			
A2	Brain 17		qPCR MasterMix			
B2	Liver 2		qPCR MasterMix			
C2	Liver 10		qPCR MasterMix			
D2	Liver 18		qPCR MasterMix			
Pipett	ting pattern	Lege	end 🍓 Standard	0	Blocked	
Ш	Column by Column		Control	(Not Used	
			Sample	(Ø Buffer	
			Fill selected	Re	move assignme	ent

The List tab of the Output panel

7.2.6.5.1 Assigning samples with reaction mixes to the output labware

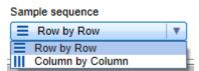
Proceed as follows:

- 1. From the **Input labware** menu on the left side of the screen, select an input labware or standards and controls that were defined in the previous **Labware selection** step. The software will display its defined samples below in the **Input** panel.
- 2. From the **Reaction mix** menu, select the reaction (master) mix you want to assign. The menu lists all final reaction (master) mixes that have been defined in the **Mixes** step.
- 3. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

4. From the **Sample sequence** drop-down list, select the sequence you want to use. You can choose from row by row and column by column.



Note: The sample sequence determines the order of the pipetting of the samples:

- If **Row by row** is selected, the first sample that is pipetted is the first sample in the first row, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the row and the second sample's replicates are pipetted and so on.
- If **Column by column** is selected, the first sample that is pipetted is the first sample in the first column, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the column and the second sample's replicates are pipetted and so on.
- 5. From the **Input panel**, select the sample position for which you would like to assign a reaction (master) mix. Initially, all previously defined positions are selected.

Note: To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions. Selecting in a rectangle is only possible on rectangular plates.

- 6. Click Assign.
- 7. The software creates assay points on the **Output** panel on the right side of the screen. The selected reaction (master) mix is assigned to the selected samples. If you have selected more than one replicate,

the sample positions are multiplied accordingly. The positioning of the created assay points on the output side follows the selections made for the **Pipetting pattern** and the sequence of samples from the sample sequence.

The samples are also listed in the **List** tab of the **Input** labware (in the **List** tab of the **Input** panel, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column or using the **Sample sequence** drop-down menu).

Note: The instrument follows a specified order when pipetting liquids. First, the master mix is pipetted, followed by the standards and controls, and then the samples.

- 8. Repeat the steps above until all positions of the output side have been defined for the planned run.
- 8. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 9. Close the lid.
- Click Next to enter the worktable step. If the instrument has not been used before, it will initialize.
 Note: While entering the Worktable step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.

7.2.6.5.2 Removing assay assignments

To clear a position on the output side, follow these steps:

 Select the desired position on the **Output** side. Selecting multiple positions is possible by pressing SHIFT and left-clicking with the mouse to select adjacent positions or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.

Note: If you select empty positions along with filled positions, you cannot remove their assignments. It is only possible to remove assignments from multiple positions if all positions are filled.

Click **Remove assignment**. The assignment will be removed, and the sample position will be cleared from the output side. Also, if the position was marked as filled with buffer, the position will be cleared.
 Note: The software will remove all replicates of the desired sample(s).

7.2.6.5.3 Assigning standards and controls to the output labware

The QIAgility Setup Manager Software allows you to assign the positions of standards and controls on the output plate.

Proceed as follows:

- 1. Select Standards and Controls from the Input labware drop-down list on the left side of the screen.
- 2. If you have defined one standard series, it will be automatically selected by the software, and a table with dilution steps and dilution ratios will be shown.

If you have defined multiple standard series, select one of them from the **Select standard series** dropdown list.

andards and Controls		
Select standard series	v	
Select standard series		
Standard 651		
Standard 161		

A table with dilution steps and dilution ratios will appear.

Standard 651			
	Standard 651	Dilution Ratio	
	Standard 651	1:1	
	Standard Stand	1:5	
	Standard Stand	1 : 25	
٦			Ţ

- 3. Tick the box next to one or more dilution steps in the table. To select all of them, tick the box in the table header.
- 4. Select a mix from the Reaction mix drop-down list.

Reaction mix			
qPCR MasterMix			

5. Select the number of replicates from the Replicates drop-down list.



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

6. Click Assign.

7. Tick the box next to one or more controls in the following table. To select all controls, tick the box in the table header.

✓	Controls	Туре	4
	NTC	NTC	
_			_

8. Select a mix from the Reaction mix drop-down list.

Reaction mix	
e qPCR MasterMix	•

9. Select the number of replicates from the Replicates drop-down list.



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

10. Click Assign.

11. Repeat steps 2 - 10 as needed, until you have defined all the positions for the planned run.

7.2.6.6 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

Click **Print loading instruction** to generate a PDF document with the loading instructions for the run.

Pos.	Content	Tube	Req.vol.	Loaded	
в	Reaction mix (Mix)	1.5 ml tapered tube (QIAGEN)	0 µl	~	
С	Reaction mix	1.5 ml tapered tube (QIAGEN)	295 µl	✓	
D	Primer mix	1.5 ml tapered tube (QIAGEN)	246 µl	V	
Е	Dilution buffer	1.5 ml tapered tube (QIAGEN)	157 µl	~	

M1 - Mix block

Table of tubes to be loaded to the worktable position M1.

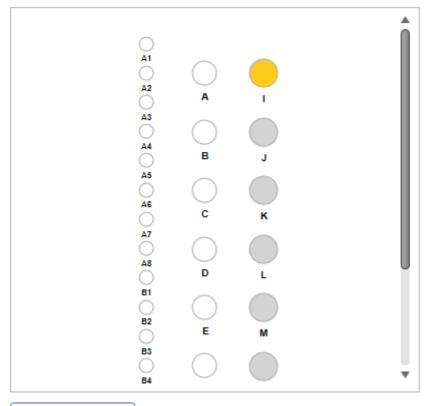
It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).

Tube	
200 µl PCR tube (QIAGEN)	N.
200 µl PCR tube (QIAGEN)	5
200 µI PCR tube (QIAGEN)	

Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware Manager**. Select one of the entries to change the tube type. Changing the tube type might result in a

replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.



Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

Calibrate labware ...

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

Click the **Labware** tab on the right to get an image and details about the input and output labware.

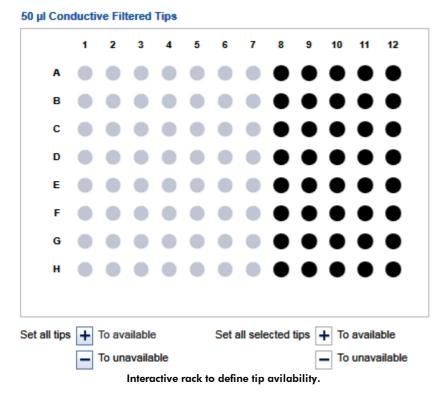
Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

Labware: 0.1 ml Strip Tube (QIAGEN) Adapter: Rotor-Gene Q, 72 x 0.1 ml Strip Tubes Adapter



Image and details for output labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To available** or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to Loading tips.

Note: To ensure optimal tip pickup, it is important to follow the instructions in Insert tips to tip rack

<u>holder</u>.

4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again.

5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to Loading tips.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 6. To confirm that the tip rack has been loaded, either check the box next to **Loaded** in the table or click on **Confirm all** to confirm. Refer to <u>Loading tips</u> for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

For worktable positions with samples:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

15. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

- 16. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 17. Ensure that samples are loaded using the correct labware type.
- 18. Load the samples in the correct positions as displayed in the table.
- Optional step: For sample tubes, click Validate sample loading to start sample loading validation using a hand-held barcode scanner and proceed with steps 20–23. Otherwise, proceed to step 24.
 Note: This button is only available if the corresponding option has been checked in the <u>User settings</u> for <u>Generic PCR experiments</u>.
- 20. The software will highlight the first sample position in the table. During validation, scanning is the only function available.

Note: To indicate that a user interaction is expected, a cursor with arrows in all four directions $\mathbf{\Phi}$ will be displayed for the entire validation.

- 21. Scan the barcode of the sample tube that has been loaded in the highlighted position.
- 22. If the barcode matches the sample's ID, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. If the barcode does not match the sample ID, a message is displayed. Click **Retry** in the message to scan the barcode again. If the barcode matches, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. To stop validation, click **Stop validating**, or, if the barcode did not match, click **Cancel** in the displayed message. In this case, proceed to step 24. There will be no documentation about sample loading validation in the report.
- 23. If all sample positions have been scanned successfully, a message is displayed. The successful sample loading validation will be documented in the report, which can be created after the run. Proceed to step 25.
- 24. Check that the samples are placed in the positions as displayed in the table.
- 25. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 26. Click **Next** to continue with the next worktable position.
- 27. Repeat steps 15–26 for all worktable positions with samples.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.6.7 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.6.7.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress

Task	Progress
Prepare standard series	
Prepare mixes	
Distribute mixes to output labware	
Distribute controls to output labware	· · · · · · · · · · · · · · · · · · ·
Distribute standards to output labware	· · · · · · · · · · · · · · · · · · ·
Distribute samples to output labware	
overall progress	
start time 5:12	Approximate end tim 15:1
J. 12	15.1

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.6.7.2 Pausing or canceling the run

A run can be paused or canceled at any time from the Run step of the Experiment environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	Pause
Overall progress		*
Start time 13:05	Paused at 13:21	Resume

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.6.7.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.

Run info	
Operator	
John Smith	
Comment	
	Ů
Output labware	
Print barcode	Save output file
	Create report

Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Save output file	An output file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>User</u> <u>settings</u> to define a standard directory.
	The file may serve as a sample input file for the Rotor-Gene Q operated with the Q-Rex Software. The .xml file can also be used by an LIMS (Laboratory Information Management System). A format description is available at <u>www.qiagen.com/goto/QIAgility</u> . You can find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QIAGEN\QIAgility\Examples.
	If an output converter has been selected in the Samples/Labware step, the software also creates a file in the format as defined by the output converter. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension (e.g., .txt for the ABI cycler), and will also be saved in the specified directory. In case the file extension is also .xml , the file name will be enhanced. The available output converters can be found in the output file converter directory, as specified in the <u>User settings</u> . Contact QIAGEN Technical Services if you need another file format.
	Note: Use this option also in case the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Samples/Labware step that you are Reusing a partially used output labware by loading an import file by selecting that option, and load the file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.
Print barcode	You can print a barcode for the output labware.
	Note: The option is available only if a printer has been defined in the Global Settings tab of the Configuration environment.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section <u>Cleaning the QIAgility</u>.

7.2.6.8 Report

The Generic PCR Plug-in offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results
- Exceptions
- Detailed run information

The **Advanced report** contains:

- General information
- Comment
- Reaction results
- Exceptions/Pauses
- Sample information
- Worktable information
- Detailed run information
- Advanced instrument information

Descriptions of the sections that are part of both report types are provided below.

General information

Some general information is provided at the beginning the report. This includes information such as the experiment file and the output file (if any has been saved), the instrument serial number, the software and plug-in version that has been used for the run and the date, time and duration of the run.

Comment

The comment that has been entered on the **Run** screen.

Reaction results

For regular rectangular output plates, an overview graphic is shown, visualizing each position on the output plate:

Symbol		Description
•	ok	The position was processed without any exceptions.
0	warning	The position was processed with exceptions that allowed further processing (e.g., tip failure, liquid level detection error). These exceptions are listed in the Exceptions chapter below.
•	error	The position could not be processed as required due to an exception that did not allow further processing (i.e., the run was stopped due to an error). The exception is listed in the Exceptions chapter below.
0	empty	The position is empty. The position has not been used during the run.
	not used	This symbol is only used if an output file has been loaded as a starting point for the output labware (e.g., when using several runs to fill the output labware). The symbol represents a position that was not part of this run, although it is not empty.

Reaction results

Input plate ID:	Input1_20161205_104738	1 2 3 4 5 6 7 8 9 10 11 12
	Input2_20161205_104747	A 000000000000000000000000000000000000
Output plate ID:	Output_20161205_103805	
		E 000000000000000000000000000000000000
		G 000000000000000000000000000000000000
		ноооооооооо

For your reference, the labware identifiers for input and output labware will be listed even if the graphic is not shown. If multiple input labwares were used for the run, the labware identifiers will be listed in the same order in which they were defined on the **Labware selection** step.

empty

A table listing the reaction result for each processed position on the output labware is shown:

Column	Description		
Output	The position on the output labware.		
Identifier	Specifies the sample ID or the name of the particular standard or control.		
Туре	Sample, Standard or for controls, the Control type.		
Input	The corresponding position on the input labware. Valid for samples only. Empty for standards and controls. If multiple input labwares were used for the run, the position is enhanced by a short input labware reference (e.g., A1/I1 refers to position A1 of the 1 st Input labware, where "I" indicates Input, and "1" indicates the 1st input labware in the identifier list).		
Actual content	Lists the components, i.e., the liquids that have been transferred into the position during the run.		
Initial sample conc. /Std conc.	For samples, the initial concentration is listed (only if the sample's concentration has been entered in the sample editor or loaded with a sample input file); otherwise, this is empty.		
	For standards, the concentration of the standard is listed. If standards have been defined individually per target, the concentrations are enhanced by a short target reference (e.g., (1) 5 ng/ μ l refers to the 1st target). A target legend is provided below the table.		
	Always empty for controls or NTC.		
xNA content	For samples, the final concentration is listed (only if the sample's concentration has been entered in the sample editor or loaded with a sample input file); otherwise, this is empty.		
	For standards, the concentration of the standard is listed.		
	Always empty for controls or NTC.		
Flag	Shows the status of the position: either ok, warning or error, corresponding to the symbols of the graphic		

Note: The table contains only those positions on the output labware that were subject to this run. Empty or unused positions are listed for reference below the table.

Exceptions

This section lists all events, if any, in a table:

Column	Description
Exception	The event that has been displayed during the run.
Position	Lists the positions that were affected by the exception.
	First, the position is listed for which the exception occurred. After "Destination," all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.
	Example: "M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which might have impacted the output positions D1–D8 (row-wise).
	Note: If an output position is flagged OK in the reaction results table, even though it is listed here as potentially affected by an exception, then you can trust that the output position was completed successfully.
	Example: If the "not enough liquid" liquid level detection error occurs in the final reaction (master) mix tube while serving the last output position with the mix, all output positions that were designated to contain this final reaction (master) mix will be listed here as potentially affected. However, most of these positions have already been served successfully and will be flagged OK in the reaction results table. You can trust an 'OK' flag in the reaction results table.
User response	A short form, which option (button) of the message dialog has been pressed.

Note: The **Basic report** does not list pauses. If you want to have also pauses listed, use the **Advanced report**, instead.

Detailed run information

This section lists experiment definitions and the task list of the run.

Defined mixes:

Column	Description
Name [Targets]	Name of the mix. If defined, targets are listed additionally.
Input	Lists the content of the mix per reaction.
Total volume	The total volume of the mix, given in μ l.
Liquid class	The liquid class used to distribute the mix, including version number.
Advanced pipetting parameters	The advanced pipetting parameters that have been used for the mix: extra volumes, liquid class for mixing incl. version number, mix repetitions after the last input liquid has been added.
	Note: Advanced pipetting parameters are always listed. If they have not been defined by user, the parameters inherited from the liquid class are shown.

Input liquids:

Column	Description
Name	Name of the liquid.
Lot number/Expiry date	Lot number and expiry date, if defined for the liquid.
Liquid class	The liquid class used to distribute the liquid, including version number.
Advanced pipetting parameters	The extra volumes that have been used for the liquid.
	Note: Advanced pipetting parameters are always listed. If they have not been defined by user, the parameters inherited from the liquid class are shown.

The definitions for sample pipetting:

Column	Description
Volume	Sample volume used for the run.
Liquid class	The liquid class used to distribute samples to the output labware, including version number.
Advanced pipetting parameters	The advanced pipetting parameters that have been used for samples: extra volumes, mix repetitions after the sample has been distributed.
	Note: Advanced pipetting parameters are always listed. If they have not been defined by user, the parameters inherited from the liquid class are shown.

Standard series, if defined in the experiment:

Column	Description
Name [Mix]	Name of the standard series and the related reaction (master) mix.
Standards	Name of the standard and the concentration, if defined the related target is listed.
Advanced pipetting parameters	The advanced pipetting parameters that have been used for samples: extra volumes, mix repetitions after the sample has been distributed.
	Note: Advanced pipetting parameters are always listed. If they have not been defined by user, the parameters inherited from the liquid class are shown.

Finally, this section lists all tasks of the run as they were displayed in the **Run** step.

Descriptions of the sections that are only included in the **Advanced** report are below.

Exceptions/Pauses of the Advanced report

In contrast to **Basic** report, this section not only lists all events but also any pauses that may have occurred during the run in a table:

Column	Description
Exception	The event or pause that has been displayed during run.
Position/Duration	For an event:
	Lists the positions that were affected by the exception.
	First, the position is listed for which the exception occurred. After "Destination," all positions are listed that might not have been completed as a result of the exception, especially all potentially affected positions on the output labware.
	The @ symbol is used to address positions. Consecutive positions are listed in the used pipetting order.
	Example: "M1 @ A Destination: output labware @ D1–D8": The exception occurred on the Mix block position M1 at position A, which might have impacted the output positions D1–D8 (row-wise).
	Note: If an output position is flagged OK in the reaction results table, even though it is listed here as potentially affected by an exception, then you can trust that the output position was completed successfully.
	For a pause: the duration of the pause.
User response	A short form, which option (button) of the message dialog has been pressed.

Sample information

Lists the sample information for each input labware as it was entered in the sample editor, loaded by the sample input file, or automatically generated by the software, together with the identifier of the input labware.

Worktable information

Lists for each worktable position the liquids that have been placed there.

Note: For tip racks of the same tip type, the overall required, and the overall available number of tips is shown, similar to the information in the worktable screens.

Advanced instrument information

This section lists information about HEPA filter usage, service due dates and volume calibration.

Note: The series numbers of the QIAgility and the pipettor head are part of section **General information** at the beginning of the report.

7.2.6.9 Samples only experiments

The Generic PCR Plug-in allows to setup a run to distribute samples to the output labware, only.

Such experiments are created by clicking **Generic PCR setup** from the **Home** screen and then defined in the **Experiment** environment of the software.

The experiment wizard has 7 steps that guide you through the run setup process. Use the **Next** and **Back** buttons at the bottom to navigate between steps. The setup uses the screens for <u>generic PCR setup</u>. We recommend that you familiarize yourself with the features of the generic PCR setup first.

To setup a run to distribute samples to the output labware, only:

1. Skip the **Liquids** step by clicking **Next** to proceed to the **Mixes** step.

Note: However, in the case that you need to define liquids for the run, follow the steps described in <u>Liquids step</u>.

2. In the Mixes step add at least one mix. In general, follow the instructions described in <u>Mixes step</u>, however, in the Add mix dialog select the option Final reaction mix already on output from the General screen, to indicate that the mix will not be created nor distributed. The Target screen of the Add mix dialog is optional in order to allow easy transfer of defined assay targets to respective PCR cycling software.

Click **Next** to proceed.

Skip the Controls/Standards step by clicking Next to proceed to the Samples/Labware step.
 Note: It is possible to use standards and controls in this workflow, too. In this case, define the needed liquids in the Liquids step. For details on how to define standards and controls refer to Controls/Standards step.

4. In the Samples/Labware step follow the instructions described in <u>Samples/Labware step</u>. For the output labware, the option Output labware is prefilled with reagents is preselected. Keep this option selected, because pipetting behavior will be adjusted accordingly. Click Next to proceed.

Note: It is recommended to use sample volumes of at least 5 μ l, as it is technically not reliable to transfer less than 5 μ l volume into an empty labware.

- 5. In the **Assignment** step follow the instructions described in <u>Assignment step</u>.
- 6. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 7. Close the lid.
- Click Next to enter the worktable step. If the instrument has not been used before, it will initialize.
 Note: While entering the Worktable step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.
- 9. In the **Worktable** step, follow the loading instructions and the instructions to start the run described in <u>Worktable step</u>.
- 10. Refer to <u>Run</u> for information about the **Run** step. After run, follow the instructions in <u>Run completion</u>.

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is ***.qsmrx**, and for templates ***.qsmrt** (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is ***.qsmrqt** (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.7 Generic PCR setup with normalization experiments

The Generic PCR Plug-in enables easy and convenient qPCR setup including sample normalization.

For pathogen testing and gene expression experiments using dedicated QIAGEN kits, refer to sections <u>Working with experiments for pathogen detection</u> and <u>Working with gene expression experiments</u>, respectively. For generic PCR setup experiments without sample normalization refer to section <u>Generic PCR</u> <u>experiments</u>. For PCR setup with serial dilution experiments, refer to the <u>PCR setup with serial dilution</u> <u>experiments</u> section. It is also possible to set up a run for sample normalization only. In this case, refer to section <u>Normalization only experiments</u>.

Samples can be normalized based on mass or concentration. Ensure that the correct selection has been made in the <u>User settings for Generic PCR experiments</u> before the experiment is created. Changing the setting will take effect for new experiments, only.

New experiments for generic PCR setup, including sample normalization, are created by clicking **Generic PCR setup with Normalization** from the **Home** screen, and then defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and **Mixes** step will be enabled, initially. Subsequent steps will become available, once the required information has been entered into the **Liquids** and **Mixes** step. The **Mixes** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 8 steps that guide you through the run setup process:

- 1. <u>Liquids</u>
- 2. <u>Mixes</u>
- 3. <u>Controls/standards</u>
- 4. <u>Samples/Labware</u>
- 5. Assignment
- 6. <u>Normalization</u>
- 7. <u>Worktable</u>
- 8. <u>Run</u>

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is ***.qsmrx**, and for templates ***.qsmrt** (**Exp**eriment, and **T**emplate, respectively). The file extension for generic PCR quick order templates is ***.qsmrqt** (Quick Order **T**emplate).The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.7.1 Liquids step

The **Liquids** step is used to define all input liquids required for your PCR setup that are to be loaded onto the instrument's worktable, besides your samples. These are, for example:

- all kinds of reagents, that will , e.g. be part of reaction (master) mixes
- all kinds of controls and standards (stock)
- all kinds of diluents/water
- ready-made reaction mixes, i.e., final reaction (master) mixes that have already been prepared by the user and are ready to be used and distributed to the output labware by the QIAgility.

Detailed information for (final) reaction (master) mixes will be defined later in the **Mixes** step. Samples will be defined later in the **Samples/Labware** step.

The **Liquids** step also includes information about the kit that is used during the run.

GEN Home	Experiment Service Configur					Instrument
PCR and nor ×						
uids >	Define input liquids					
es	Define required input liquids (reagent (master) mix, Standards (stock) and of Predefined liquid classes (LC) define	controls) for assay setup. the pipetting/tip handling	Kit information Kit Name	Lot number	Comment	^
trols / Standards	task for liquid transfer (refer to LC table in configuration screen). LC-naming: Liquid type + pipetting destination/intended use For more information, press F1 key when editing liquids.		Product number	Kit expiry date		T
nples / Labware	Name	Liquid type Liqui	d class	Taka Area		Actions
ignment	Wasser		ous liquid to reaction (master) mix	Tube type 5.0 ml flat-base tube (QIAGEN)		
-9	MasterMix	Reagent Visco	ous liquid to reaction (master) mix	2.0 ml screw cap micro tube (Sarstedt)		
nalization	PrimerMix	Reagent Prime	er-Probe or IC to reaction (master	2.0 ml screw cap micro tube (Sarstedt)		
ktable	Std Plasmid	Standard Cont	rol or Std. to output or for dilution	2.0 ml screw cap micro tube (Sarstedt)		
	Std Plasmid 2	Standard Cont	rol or Std. to output or for dilution	2.0 ml screw cap micro tube (Sarstedt)		
1	Diluent	Diluent/Water Dilue	ent for dilution	5.0 ml flat-base tube (QIAGEN)		
					(New liquid
			Back Next		Save	Start run

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

,

- 1. Click the **New liquid** button below the table.
- 2. The Add liquid dialog opens.
- 3. Define the liquid as described in <u>Add liquid dialog</u>.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

Finally, click **Next** in the button bar to proceed to the **Mixes** step (<u>Mixes step</u>).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the \frown \frown buttons below the table:

- 1. Click the row of the liquid to be moved.
- 2. Click on the up and down arrows to move the currently selected row up or down.

Use this function for a better overview. The report will list the liquids in this order.

The buttons in the Actions column of the table have the following functions:				
Button	Action	Description		
0	To edit a liquid	The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.		
		If you intend to change the liquid later, e.g., the liquid has already been used in a mix in the Mixes step, or, if the liquid is a ready-made reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed. In this case, remove assignments or reaction mixes to enable changes for defined input liquids.		
Ŀ	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.		
×	To delete a liquid	Deletes the row in the table.		

7.2.7.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you want to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values for a liquid.

Add liquid				
Liquid name Dilution buffer	 Input liquid Ready-made mix 			
Tube type 1.5 ml tapered tube (QIAGEN)	Cap color			
Liquid class	Liquid type			
Lot number	Expiry date			
Define advanced pipetting parameters				
Add liquid dialog	OK Cancel			

- 1. Define the name of the liquid. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, e.g. when defining reaction mixes, standards or controls, and in the loading instructions of the Worktable step.
- 2. Define whether the liquid is an input liquid or a ready-made mix. Initially, the option **Input liquid** is selected, because it fits to most liquids.

Select the option **Ready-made mix** only, if the liquid is a final reaction/master mix that has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In this case, in the **Liquid type** field the value **Ready-made mix** is automatically selected and the liquid class menu is removed (the liquid class is defined later in the Mixes step). Additionally, when entering the later Mixes step, the reaction (master) mix is already listed.

If the liquid is a mix that has already been prepared by the user, but will be part of a more complex mix that will be created during the run by the QIAgility, select the option **Input liquid**.

3. Click **Tube type** to select the tube that will be used to place the liquid on the QIAgility. The tube types used in the last experiment are listed at the top of the list. To select one of the listed tube types, click the respective entry.

Tube type		
Select	▼	
Select		
1.5 ml tapered tube (QIAGEN)		
5.0 ml flat-base tube (QIAGEN)		
Choose other labware		
Any available		

Select the value **Any available**, if the liquid does not need to be provided in a special tube, e.g. coming from a kit. In this case, later, during worktable planning, the software will automatically select a tube of the required size, with minimal dead volume, from the list of available tubes that has been defined in the Labware Manager. (To define available tubes in the Labware Manager, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.) Use this option to give the software more flexibility during worktable planning.

If the required tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.

Filter options Category	Labware Select the item that most closely matches the labware to be used	sed.
Advanced options Advanced options Name of labware Part number Manufacturer	1.5 mi tapered screw cap tube (QIAGEN) 1.5 mi tapered tube (QIAGEN) 2.0 mi self standing screw cap tube (QIAGEN) 200 µI PCR tube (QIAGEN) 5.0 mi flat-base tube (QIAGEN)	Catalog number 990552 Name 5.0 mil flat-base tube (QIAGEN) Dead Volume 26 µl
All Tube / Well size	Labware adapter	V
Reset filter Apply filter	Select the adapter to be used.	Catalog number Name

In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If you edit the liquid later and the **Tube type** is marked yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type or make the tube available in the Labware Manager again.

4. Click **Cap color** to select the color of the tube's cap.



5. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid**

Classes step to get more details.

In case the same liquid will be used for different purposes (e.g. nuclease-free water used for setting up a standard dilution series and for non-template control) it is highly recommended to define multiple input liquids to allow precise definition of liquid class and liquid type.

Note: If you edit the liquid later and the **Liquid class** is marked yellow, this indicates that the selected liquid class is no longer available, e.g. if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

6. Select the **Liquid type**. The liquid type defines how the liquid will be used. In later steps during experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection, e.g. when defining controls or standards. In case a defined input liquid is not visible in later screens (e.g. during control definition) check if the liquid type has been set correctly.

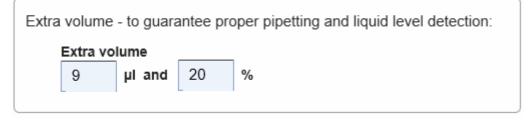
Liquid type	Description
Reagent	Use this type, if the liquid is a reagent that will be added to a reaction/master mix, either to a final reaction mix or to an intermediate reaction mix.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.
Pos./neg. control	Use this type, if the liquid is a positive or negative control (incl. NTCs).
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a control.
Standard	Use this type, if the liquid is a single standard as part of a user prepared standard series, or if the liquid is the standard stock for a standard series that shall be created by the QIAgility.
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a standard series that shall be created by the QIAgility.
Diluent/Water	Use this type, if the liquid is a diluent or water.
	Later in the experiment setup, a liquid of this type is available, e.g. for content definition of a reaction/master mix in the Mixes step, or in the Controls/Standards step as diluent for defining a control or standard series that shall be created by the QIAgility.
Ready-made mix	Use this type, if the liquid is a ready-made reaction/master mix prepared by the user. This type is preselected by the software, if the option Ready-made mix has been selected for the liquid.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.

- 7. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, these data will be present in the report.
- 8. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

Define advanced pipetting parameters



 In the dialog, click OK to confirm your values and to close the dialog. Otherwise, click Cancel to close the dialog and discard all values. In this case, no new liquid is defined, or – in case of editing an existing liquid –the liquid is not changed.

7.2.7.2 Mixes step

The **Mixes** step is used to define all kinds of mixes that are used for an experiment. These are, for example:

- all variants of reaction (master) mixes, i.e., final reaction (master) mixes that
 - o have already been prepared by the user and are ready to be used,
 - o will be generated by the QIAgility during run,
 - o and will be distributed to the output labware.
- all kinds of intermediate mixes. These are generic pre-mixes that will be used to create new (more specific) reaction (master) mixes.

The screen lists all mixes that have already been defined in a table. User prepared reaction (master) mixes are automatically listed, if the corresponding liquid has been defined in the previous Liquids step. The rows for these mixes are initially highlighted in yellow to indicate that the mixes need further specification.

<pre>*Experiment_0 ×</pre>							
Liquids	Define your mixes						
Mixes >	Define reaction (master) mix(es) require pipetting/tip handling task for distribution screen). Adv. parameters: Mixing settings for sys	n of reaction (master) n stem-prepared reaction	nix (refer to LC table in configuration				
Controls / Standards	mixing repetition can be changed if requ For more information, press F1 key whe	uired. en editing mix(es).					
	Name	Reaction mix	Input	Volume / reaction [µl]	Total volume / reaction [µl]	Liquid class	Actions
Samples / Labware	Mix	~	Fast Reaction Mix Primer Mix	7.5	10	Reaction (maste	2 🖪 🗙
Assignment		I	1	1	I		
Normalization							
Worktable							
Run							
							Add mix
			Mixes step				

Defining a new mix or editing a listed mix:

1. To define a new mix, click the Add mix button. The Add new mix dialog opens.

To edit a listed mix, click in the **Actions** column in the corresponding table row. The **Edit mix** dialog opens. It displays the values that have been entered before in the **Add mix** dialog or that have been automatically pre-filled by the software for a ready-made reaction (master) mix.

Note: If no final reaction (master) mix has been defined, the **Add mix** button is highlighted in yellow to indicate that the definition of a final reaction (master) mix is still missing.

- 2. Define the general properties of the mix. Details are described in <u>Add new mix dialog</u>.
- 3. In the dialog, click **Next** to proceed to the **Input** screen.
- 4. In the dialog **Input** screen define the volume per reaction and the content of the mix; as described in<u>Add new mix dialog input</u>.
- 5. For final reaction/master mixes, the optional **Target** screen is available. This screen allows the user to define target type and reports/quenchers. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import into the next instrument, e.g. a cycler. In this case, click **Next** to proceed to the **Target** screen. Details are described in<u>Add new mix dialog target</u>.

If you do not require target information in the report or for the next instrument, proceed.

- 6. Click **Finish** to finish the definition of the mix and to close the dialog.
- 7. The defined mix will be listed in the table in the Mixes screen.

Repeat the steps above until all final reaction (master) mixes have been defined.

Finally, click Next in the button bar to proceed to the Controls/Standards step, described in

Note: The Next button is disabled, if a row in the table is highlighted yellow. User prepared reaction

(master) mixes are initially highlighted yellow, if the volume per reaction has not yet been defined. Edit() the mix and enter the volume per reaction in the Input screen of the dialog. The **Next** button is also disabled, if the definition of a final reaction (master) mix is still missing.

The mixes in the table can be reordered using the \bigtriangleup buttons below the table. The report will list the mixes in this order. An intermediate mix (generic pre-mix) needs to be listed before the (more specific) reaction (master) mix of which it is part of. It will not be possible to move an intermediate mix below its reaction (master) mix.

- 1. Select the row of the mix to be moved by clicking on it.
- 2. Click on the up and down arrows to move the currently selected row up or down.

A	ction	s	
0	L	×	

The buttons in the **Actions** column

Button	Action	Description
0	To edit a mix	The Edit mix dialog will open, displaying the values that have been entered in the Add mix dialog.
		If you intend to change a mix that is already in use as input for another mix, or if it is a final reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed anymore.
Ŀ	To duplicate a mix	The Duplicate mix dialog will open, displaying the values that have been entered for the original mix. The mix name will initially display a copy of the name of the original mix.
×	To delete a mix	Deletes the row in the table.

7.2.7.2.1 Add new mix dialog

In the **Add new mix** dialog a new mix can be defined. If you would like to change the values for an existing mix later, the **Edit mix** dialog will open. Both dialogs show the values of a mix.

Define the basic properties of the mix in the **General** screen of the dialog.

	Edit mix
General >	Name
Input	Test-mix Final reaction mix (ready-made) Intermediate mix (ready-made) Test-mix
Target	Final reaction mix already on output (ready-made) Liquid class Reaction (master) mix (final) to output
	Note: Make sure that ready-made mix is homogeneous and mixed manually and avoid foam.
	Define advanced parameters
	Back Next Finish Cancel

Edit mix dialog

	Add new mix
General >	Name
Input	Mix Final reaction mix (system prepared) Intermediate mix (system prepared) Final reaction mix (system prepared)
Target	 ▶ Final reaction mix already on output ■ Reaction (master) mix (final) to output ■ Define advanced parameters
	Back Next Finish Cancel Add new mix dialog - General

1. Define the name of the mix. The name must be unique. The name is used to refer to the mix in later steps of the experiment setup, e.g. when defining more specific mixes, and in the loading instructions of the Worktable step.

For a ready-made mix, the name is already defined from the liquid dialog definition.

2. Select the type of mix. For ready-made mixes and other mixes different options are available:

Option	Description
Final reaction mix (ready-made)	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
	Use this option, if this mix has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In the later Assignment step, the mix will be available for selection. During the run, the QIAgility will pipette the mix to the positions on the output labware as assigned in the Assignment step.

		Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QIAgility as QIAgility will not perform any mixing steps.
	Intermediate mix (ready- made)	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
		Use this option if this mix has already been prepared by the user and will be part of a more specific mix to be created during the run by the QIAgility. During the run, the QIAgility will pipette the mix into the tube, where the more specific mix is created.
		Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QIAgility as QIAgility will not perform any mixing steps.
alre	Final reaction mix already on output	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
	(ready-made)	Use this option only if this mix has already been distributed on the output labware before the run. In the later Worktable step, you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QIAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.
		The volume of the reaction mix must be specified. This value will be used to calculate the dispense height and the mixing volume.
		If the option is selected, the Input screen of the dialog will be skipped.
	Final reaction mix (system prepared)	Use this option if during the run the mix will be created first by the QIAgility and then distributed to the output labware.
	Intermediate mix (system prepared)	Use this option if this mix will be created by the QIAgility during the run, but will be part of a more specific mix. During the run, the mix will be created first by the QIAgility. Then the QIAgility will pipette the mix into the tube where the more specific mix is created.
	Final reaction mix already on output	Use this option only if this mix has already been distributed onto the output labware before the run. In the later Worktable step you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QIAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.
		If the option is selected, the Input screen of the dialog will be skipped.

3. The liquid class that will be used to distribute the reaction (master) mix during the run is preselected by the software. Change the liquid class only if it is really required. In this case, select the **Liquid class** that fits best to transfer the reaction (master) mix.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

4. Optionally, define advanced parameters. If you tick the box, additional parameters become visible to define extra volume and mixing options.

For reaction (master) mixes that will be created by the QIAgility, mixing options are displayed. Mixing is performed after the last reagent has been added to the reaction (master) mix, before the mix will be transferred. The mixing settings are defined in the **Liquid class for mixing**. This liquid class is already preselected by the software and should only be changed if really required. The **Mixing repetitions** – which are pre-defined by the selected liquid class for mixing – can be increased, if needed. The mixing cycles have been optimized to allow proper mixing for final reaction (master) mixes in which no pure Enzyme has been added as reagent. If your setup uses large volume final reaction (master) mixes or pure Enzymes as reagents, it is recommended to increase the mixing repetition to 15 or more.

Define advanced p	arameters
Extra volume - to gua	rantee proper pipetting and liquid level detection:
Extra volume	Extra volume per reaction
39 µl a	and 2.2 µl
Mix of system prepar	ed final reaction mix:
Liquid class for	mixing Repetitions (Min = 3)
Mix of reaction	n (master) mix (final) 🔹 🔹 10

For ready-made reaction (master) mixes the QIAgility does not perform a mixing step before transfer to output labware. Make sure the reaction (master) mix has been mixed properly before placing it onto the QIAgility.

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases, displayed loading volume is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover sample amount depending variations (e.g. volume loss per tip) and is added per single transfer. However, if you face any liquid level detection issues, increase **Extra volume per assignment** if issues are for higher sample amounts only, or increase absolute **Extra volume** if issues are not related to the sample amount.

5. Proceed with step 3 in <u>Mixes step</u>.

7.2.7.2.2 Add new mix dialog input

The **Input** screen of the **Add/Edit mix** dialog lists all input liquids/reagents of the mix and their volume per reaction in a table.

Add new mix				
General	Conter	nts of this mix		
Input >	reagen	all liquids/reagents and volume per reaction for respective reaction (n ts in "Liquids" screen can be selected.		
Target	volume	commended to sort the reagents of reaction (master) mix by volume p to ensure proper pipetting for final reaction mix. Use "Sort" button to ing to volume per reaction.	automatically sort reagents	
	Seq.	Name	Volume / reaction [µl]	
	1	Reaction mix V	9 🗙	
	2	Primer mix 🔹	9 🗙	
2 Primer mix 9 X Soft Calculated reaction mix volume/reaction: 18 µl Add Input				
		Back Next	Finish Cancel	

Add new mix dialog - Input

For a **ready-made mix** (i.e., a user prepared mix), the input liquid is already defined from the liquid dialog definition. Enter the volume per reaction in μ l into the **Volume/reaction** field in the table. Left-click the field and then enter the value. Initially, the field is highlighted in yellow to indicate that input is required.

For a **system prepared mix**, i.e., a mix that will be created by the QIAgility, define the composition of the mix:

- 1. Click the **Add input** button below the table. A new row is added to the table. Initially, the fields are highlighted in yellow to indicate that input is required.
- 2. In the **Name** field of the table, select the input liquid. The menu lists intermediate mixes and all liquids that are of liquid type **Reagent** or **Diluent/Water**. If a required liquid is not listed as expected, check

if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.

- 3. Define the volume per reaction for the input liquid. Left-click the **Volume/reaction** field and then enter the value. The total volume per reaction of the mix is displayed below the table. It summarizes the volumes per reaction of the listed reagents. For not integer volumes, use a point as a decimal delimiter.
- 4. Repeat steps 1–3, until all components of the mix are listed.
- 5. It is recommended to sort the reagents of the mix by volume per reaction, starting with the highest volume, to ensure proper pipetting for the final reaction mix. Later, during the run, the QIAgility will pipette the reagents into the tube where the mix is created in the order listed in the table.

Click the **Sort** button below the table, to automatically sort the list by volume per reaction, starting with the highest volume.

Alternatively, use the \bigtriangleup buttons below the table to sort the reagents individually. Left-click the row of the reagent to be moved. Click on the up and down arrows to move the currently selected reagent up or down.

Proceed with step 5 in Mixes step.

7.2.7.2.3 Add new mix dialog target

The **Target** screen of the Add/Edit mix dialog is optional. It is available for final reaction (master) mixes to define all assay targets and allow easy transfer of defined targets to respective PCR cycling software. If defined, targets are used in later steps for control, standard and sample definition. The information will also be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g. a PCR cycler.

Add new mix						
General						
Input	Define all assay targets to allow easy transfer of defined targets to respective PCR cycling software. If defined, targets are used in later steps for control, standard and sample definition. Target type: Test: Amplicon of interest (e.g., HIV); Reference: Amplicon used for data normalization (e.g.,					
Target	house-keeping gene used for delta Ct calculations); IC: Internal control sequence to monitor assay performance (e.g., inhibition of sample purification efficiency) – consider kit manual for assay-specific details Reporter: Enter fluorescence dye name (e.g., FAM) per target. Quencher: If applicable, enter quencher name (e.g., TAMRA) per target. Ensure correct notation (case sensitive) of reporter and quencher as required by the PCR cycling software.					
	Target name	Target type	Reporter	Quencher		
	Human	Test V	FAM	None 🛛		
	Male	Test 🔍	Cy5	None 🛛		
	IC	IC V	VIC	None	×	
Add target						
Back Next Finish Cancel						

Add new mix dialog - Target

If you do not need target information in the report or for the next instrument, proceed with step 6 in <u>Mixes</u> <u>step</u>.

To define targets:

- 1. Click the **Add target** button in the dialog. A new row will be displayed in the table.
- 2. Click into the **Target name** field and enter the name of the target. The name must be unique. It must be different to defined liquids, mixes, standards and controls.
- 3. Click on the menu in the **Target type** column and select a target type:

Target type			
Test	•		
Test			
Reference	9		
IC			

Target type

Description

Test	Amplicon of interest (e.g., HIV)
Reference	Amplicon used for data normalization (e.g., house-keeping gene used for delta CT calculations)
IC	Internal control sequence to monitor assay performance (e.g., inhibition of sample purification efficiency) – consider kit manual for assay specific details)

4. **Optional step:** Click on the menu in the **Reporter column** and select an appropriate reporter dye for the target.

Note: To use the QIAgility Wizard in the Q-Rex software for the Rotor-Gene Q, check the flourophore mapping in the Q-Rex software.

Reporter SYBR Green 1 SYBR Green 1 Cy5 Crimson ROX SYBR		
Green	Yellow	Orange
FAM	JOE	ROX
SYBRGREEN1	VIC	CALFLUORRED610
SYBRGREENI	HEX	CY35
FLUORESCEIN	TET	TEXASRED
ALEXAFLUOR488	MAX	ALEXAFLUOR568
	CALFLUOR	
	GOLD540	
	YAKIMAYELLOW	
Red	Blue	Crimson
CY5	MARINABLUE	QUASAR705
QUASAR670	EDANS	ALEXAFLUOR680
ALEXAFLUOR633	BOTHELLBLUE	LIGHTCYCLERRED705
LIGHTCYCLERRED640	ALEXAFLUOR350	
	AMCAX	

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section <u>Entering data</u>.

Note: Initially, the menu is empty.

5. **Optional step:** If applicable, click on the menu in the **Quencher** column and select an appropriate quencher for the target.

Use this option for automated selection of the appropriate quencher for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported quenchers. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the quencher can be left blank, if it is not required for the assay.

Note: The quenchers are listed in an editable menu. If the quencher you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the quencher as required by the PCR cycling software. The last entered quenchers will be available for selection for future experiments. For more details about editable menus, refer to the section <u>Entering data</u>.

- 6. Repeat the steps above until all individual targets of the mix have been defined.
- 7. Proceed with step 6 in <u>Mixes step</u>.

7.2.7.3 Controls/Standards step

This optional step is used to define controls and standard series. If you do not intend to use controls or standards for the run, proceed by clicking **Next**.

The screen contains two tables: **Controls** and **Standard series**. Both tables have an **Actions** column with interactive buttons to manage the controls and standard series.

Note: When distributing defined controls (NTC and NC) and standards to the output labware during the run, the QIAgility will transfer the same volume as defined for samples/template in the later Samples Labware step.

Adding a control or standard series

- 1. Click on Add control or Add standard series.
- 2. The **Add control** or **Add standard series** dialog will open. For details, refer to <u>Add control</u> or <u>Add</u> <u>standard series</u>, respectively.
- 3. The control will be added to the **Controls** table, and the standard series will be added to the **Standard series** table.
- 4. Repeat steps 1–3 until all controls and standard series have been defined.
- 5. Finally, click **Next** to proceed.

*Experiment_0 ×	_					_	_	
Liquids	Define controls and standards							
	Controls							
Mixes	Seq.	Name	Reaction mix	Target	Target type	Control type	# of Replicates	Actions
	1-1	Pos	Mix			PC	1	🖉 🖪 🗙
Controls / Standards >	2-2	NTC	Mix			NTC	1	
Samples / Labware		1		I				Ţ
Assignment	ment Output labware order 🔊 🗸							
Normalization	nalization Standard series							
	Seq.	Reaction mix	Target	Target type	# of Replicates Acti	ons		A
Worktable								
Run								
	Output lab	ware order \land ∨					Add star	idard series

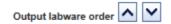
Controls / Standards step

The buttons in the **Actions** column of the **Controls** table and the **Standard series** table have the following functions:

Button	Action	Description
D	To edit a control or standard series	The Edit control or Edit standard series dialog will open, displaying the values that have been entered in the Add control or Add standard series dialog.
Ľ	To duplicate a control or standard series	The Duplicate control or Duplicate standard series dialog will open, displaying the values that have been entered for the original control or standard series. The control or standard series names will initially display as a copy of the control or standard series.
×	To delete a control or standard series	Deletes the row in the Controls or Standard series table.

Changing the order of the output labware

You can change the order of the controls and standard series using the up and down arrows below each table.



Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.7.3.1 Add control

In the **Add control** dialog a new control can be defined. If you would like to change the values for an existing control at a later timepoint, the **Edit control** dialog will open. Both dialogs show the values of a control.

Add control	
Control	
NTC	
Liquid	
Water	•
Reaction mix	
🛑 qPCR Master Mix	v
Control type	
	Target
Use specific volume	
	OK Cancel
اداد ۵	control dialog

1. Enter a control name in the **Control name** field. The name must be unique (not similar to the name entered in the liquid screen). The name is used to refer to the control in later steps of the experiment setup, e.g. in the Assignment step, and in the loading instructions of the Worktable step.

- Select the input liquid from the Liquid menu. The menu lists all liquids that have been defined with liquid type Pos./neg. control or Diluent/Water. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 3. Select the reaction mix from the **Reaction mix** menu. The menu lists all mixes that have been defined with options as **Final reaction mix (ready-made)**, **Final reaction mix (system prepared)**, **Final reaction mix in output (ready-made)** or **Final reaction mix in output**.
- 4. Select the control type using the drop-down menu in the **Control type** column:

Control type				
Select control type	•			
Select control type				
PC				
NC				
NTC				

Option	Description
--------	-------------

- PC Is a positive control for the intended assay.
- NTC Is a non-template control for the intended assay.
- NC Is a negative control for the intended assay.

During run, the QIAgility will pipet the selected liquid to the output labware considering defined replicates and the sample volume which will be defined in the Samples/Labware step.

5. If targets have been defined for the selected reaction mix, the software lists all defined targets in a table below. Tick the box next to a target name if the target is valid for the control. Tick the box in the table header if you want to select all targets.

Note: If you intend to save an output file after run for import into a cycler software, e.g. Rotor-Gene© Q PCR cycler with Q-Rex software, always select all targets.

 If the PC control type has been chosen, you can specify a custom volume for the control. Tick the Use specific volume box, type in a value in the Volume field, and select a diluent from the Diluent (used to fill up to sample volume) drop-down menu.

Add control
Control
Liquid
Water 🔻
Reaction mix
qPCR Master Mix
Control type
PC 🔻
Target
✓ Use specific volume
Volume Diluent (used to fill up to sample volume) 1 µI Diluent
OK Cancel

Specifying control volume

- 7. Click **OK** to confirm and close the **Add control** dialog.
- 8. The control will be added to the **Controls** table.

7.2.7.3.2 Add standard series

In the **Add standard series** dialog a new standard series can be defined. If you would like to change the values for an existing standard series at a later timepoint, the **Edit standard series** dialog will open. Both dialogs show the values of a standard series. Define the basic properties of the standard series in the **General** screen.

Add standard series					
General >	Standard series name	Tube type			
Dilution Stone	Standard 161	Any available			
Dilution Steps	Standard Tor				
	Reaction mix	Use custom minimal volume for standard series.			
		-			
	PCR Master Mix	Use advanced pipetting parameters.			
	Use ready-made standard series				
	Standard stock liquid	Diluent			
	Standard 161	Diluent			
	Target and stock concentration				
	Target	Stock conc. Concentration unit			
	n/a 🔻	7 copies/µl 🔍 🗙			
		Define Std. stock			
	Back Next	Finish Cancel			
	Add standard series dialog -	General			

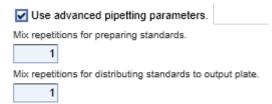
- 1. Enter a name for the series in the **Standard series name** field.
- 2. Select labware from the **Tube type** drop-down menu.
- 3. Select a reaction (master) mix, or multiple mixes, from the **Reaction mix** table by ticking the box next to the relevant mix.
- 4. Check the **Use custom minimal volume for standard series** option, if you intend to define a minimal volume value for the standard series. Enter the volume in the **Minimal volume per standard**

dilution field.

Use custom minimal volume for standard series.

Minimal volume per standard dilution

5. Check the **Use advanced pipetting parameters** option, if you would like to define the number of mix repetitions for preparing standards and distributing standards to the output plate. Enter the relevant values in the **Mix repetitions for preparing standards** and **Mix repetitions for distributing standards to output plate** fields.



6. Check the option **Use ready-made standard series**, if standards for a user prepared standard series have been defined in the **Liquids** step.

Uncheck the option if you plan to load one tube containing the standard stock and would like for the QIAgility to automatically create the dilutions for the standard series during the run.

- 7. If the option **Use ready-made standard series** has not been checked, select the appropriate liquid from the **Standard stock liquid** menu. The menu lists all liquids that have been defined with liquid type **Standard**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- If the option Use ready-made standard series has not been checked, select the appropriate diluent from the Diluent menu. The menu lists all liquids that have been defined with liquid type Diluent/Water. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 9. **Optional step.** Define the stock concentration, if you intend to define the standards of the series with their concentration. Otherwise, the standards of the series can be defined by dilution ratio, only.

If targets have been defined for the selected reaction mix, stock concentrations can be entered individually per target. This information will then also be part of the report and the output file, which can be saved after the run. If you intend to use the output file for import to a downstream instrument, e.g. a PCR cycler, define the concentrations for all available targets. For targets with no stock concentration, enter concentration value 0.

Target and stock concentration

Target	Stock conc.	Concentration unit	
Human 🛛	20	ng/µl	×
Select target	`	Select a concentratio	×
Select target Human			
Male			
IC			
		Define S	td. stock

Defining stock concentration per target.

If no targets have been defined for the mix, a common stock concentration can be defined, only.

Target and stock concentration

Target		k conc.	Concentration unit		
n/a		20	Select a concentratio	×	
					ų.
			Define S		_

Defining common stock, if no targets have been defined for the mix.

Click the Define Std. stock button to add a stock concentration. A new row is displayed in the Targets table:

Column Target	Description Click the menu in the Targets field to select the appropriate target. If targets have been defined for the reaction mix, the menu lists all available targets. Otherwise, N/A is selected.
Stock conc.	Stock concentration. This column is available only if the option Use ready-made standard series has not been checked.
	Enter the concentration that is present in the stock liquid for the target.
Concentration unit	Click the menu in the Concentration unit field to select the concentration unit for the target. This unit is used in the report and the output file, which can be saved after run.

10. In the dialog click **Next** to proceed to the **Dilution steps** screen.

Add standard series dilutions 7.2.7.3.3

Use the **Dilution steps** screen to define the dilution steps and dilution ratio for the serial dilution series. The dilution steps are displayed in a table.

Add standard series						
General	Define dilution steps and dilution ratio for serial dilution series. In "Dilution ratio" column, the total dilution ratio based on the standard stock is displayed, but QIAgility will prepare a serial dilution series.					
Dilution Steps >	A dilution ratio of 1:1 means "no dilution".					
Dilution steps 6 Image: Standard series stock as first standard Dilution ratio between two standards (optional) 1:4 Update dilution ratios						
	Seq.	Name of standard	Dilution ratio			
	1	Control DNA	1:1			
	2	Standard Series 1	1:4			
	3	Standard Series 2	1:16			
	4	Standard Series 3	1:64			
	5	Standard Series 4	1:256			
	•	Standard Sarias 5				
Back Next Finish Cancel						

Add standard series dialog - Dilution steps

1. Select the number of dilution steps from the **Dilution steps** menu.



The number of rows in the table is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering</u> <u>data</u>.

2. If the option **Use ready-made standard series** has not been checked in the previous **General** screen, the additional option **Use Standard series stock as first standard** is available. Check the option in order to use the actual stock as the first standard. An additional row is automatically added to the table, showing a dilution ratio of 1:1 (meaning "no dilution"). During the run the QIAgility will directly use the stock as template for the reaction points of this standard.

Dilution steps

6 🔻

Use Standard series stock as first standard

Dilution ratio between two standards (optional)

Update dilution ratios

Seq.	Name of standard	Dilution ratio
1	Control DNA	1:1
2	Standard Series 1	1

Option "Use standard series stock as first standard" selected.

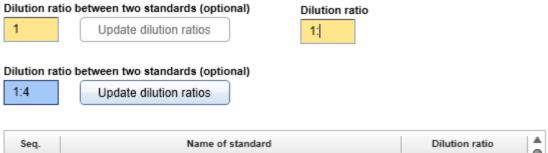
3. For each dilution step, if the option **Use ready-made standard series** has been checked in the previous **General** screen, select the appropriate liquid from the corresponding menu in the **Name of standard** cell.

Seq.	Name of standard	
1	Control DNA 🔹	
2	Standard1	
3	Select standard liquid	
4	Select standard liquid Control DNA Standard1	
5	Standard2	
6	Standard3 Standard4 Standard5	
4	Standard6	•



If the option **Use ready-made standard series** has not been checked in the previous **General** screen, optionally, enter a meaningful name into the **Name of standard** cell. This is especially important if you plan to define more than one standard series. The name must be unique. The name is used to refer to the standard in later steps of the experiment setup, e.g., in the Assignment step, and in the loading instructions of the Worktable step.

4. If an equal dilution ratio will be used throughout the standard series, define the dilution ratio in the **Dilution ratio** field above the dilution steps and then click **Update dilution ratios** to apply it to the series.



1	Control DNA	1:1
2	Standard Series 1	`
3	Standard Series 2	
	-	-

Entering the dilution ratio, e.g. 1:4

Dilution ratio between two standards (optional)

- 1	4
	4

Seq.	Name of standard	Dilution ratio
1	Control DNA	1:1
2	Standard Series 1	1:4
3	Standard Series 2	1 : 16

After button "Update dilution ratios" has been clicked.

Alternatively, for each dilution step, enter either a dilution ratio or a concentration for the respective standard. The other value will be calculated automatically by the software.

Dilution ratio between two standards (optional)

		Update dilution ratios
--	--	------------------------

	Name of standard	Dilution ratio	Concentration i
DNA	٦	1:1	20
d Series 1		1:4	5
d Series 2	1	`	
d Series 3		`	
d Series 4	•		
d Sariao 5	`	`	Enter Concen

Entering dilution ratio for second dilution step.

In the **Dilution ratio** column the total dilution ratio based on the standard stock is displayed. However – if the option **Use ready-made standard series** has not been checked – during the run the QIAgility will prepare a standard dilution series using the previous standard to create the subsequent standard.

If several targets have been defined in the previous **General** screen, concentrations are listed individually per target. If you enter one of the concentrations or the dilution ratio for a standard, all other values will be calculated automatically by the software.

lame of standard	Dilution ratio	0	Concentration i Human	Concentration i Male	
	1:16	1	1.25	0.76875	ſ
	1:64	٦	0.3125	0.1921875	
	1 : 256	1	0.078125	0.048046875	
	1:1024	1	0.01953125	0.01201171875	
	1:4096	1	0.0048828125	0.0030029296875	ļ
•	·				

Concentrations listed individually per target

Note: Concentration values for dilution steps are available only if stock concentration has been defined in the previous **General** screen.

Note: This step is optional if the option **Use ready-made standard series** has been checked and no stock concentration has been defined in the previous **General** screen. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g., a PCR cycler.

- 5. In the dialog, click **Finish** to complete the definition of the standard series and to close the dialog.
- 6. Proceed with step 3 in <u>Controls & standards steps with normalization</u>.

7.2.7.4 Samples/Labware step

Use the Labware Selection screen to select the labware for sample input and run output and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file. The QIAgility Setup Manager Software supports up to three input labwares.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side. Define the sample pipetting below the input labware panel.

	Define samples and labware	
	Input labware	Output labware
	Samples will be provided on one single plate or adapter Samples will be provided on multiple plates or adapters (up to 2)	Distribute assay points to single plate or adapter Distribute assay points to multiple plates or adapters (up to 2)
Standards	Use a new input labware and enter sample names	Use a new output labware
.abware 🗲	Load sample input from file Load sample input from file	Reuse a partially used output labware by loading an import file Select file
t	Labware	Output labware type
on	QIAGEN Elution Microtubes RS ▼ Use cooling block □ Samples are covered with top-elute	QIAGEN 96 Well Microplate RB Use cooling block Output labware is pre-filled with reagents
	Labware identifier Input1_20190730_125953 Print barcode	Labware identifier Output1_20190730_130019 Print barcode
	Auto-generate sample IDs for all samples	Output Converter
	Define samples Import data from CSV file	Select
	Sample Pipetting Sample volume Liquid class Use custom pipetting parameters	
	Sample volume Liquid class Use custom pipeting parameters 10 µl Sample to output ▼ Edit parameters	
		, ,

Proceed as follows:

1. Select the input labware and define the samples.

If the samples will be on a single plate or adapter, check the option **Samples will be provided on one single plate or adapter** on the left side of the screen and follow the steps in <u>Define an input</u> <u>labware</u>.

If the samples will be on several plates or adapters, check the option **Samples will be provided on multiple plates or adapters** and follow the steps in <u>Defining up to two input labware</u>.

2. Select the output labware.

Sample Pinetting

If the output will be on a single plate or adapter, select the **Distribute assay points to single plate or adapter** option, and follow the steps in <u>Define output labware</u>.

If the output will be on several plates or adapters, select the **Distribute assay points to multiple plates or adapters** option, and follow the steps in <u>Define up to two output labware</u>.

3. Enter the sample volume for the run into the **Sample volume** field in the **Sample pipetting** panel. The value must be specified in μ l.

Sample volume	Liquid class		Use custom pipetting parameters
2 µI	Sample to output	•	Edit parameters

4. The liquid class used for sample transfer is predefined by default.

If required, change the liquid class to be used for sample transfer by clicking the **Liquid class** dropdown menu. It defines the pipetting and tip handling for distribution of samples from the input labware to the output labware.

5. **Optional:** If required, use custom pipetting parameters for samples. If you tick the box, the **Edit parameters** button becomes active. If you click the button, the **Define advanced pipetting parameters** dialog opens, providing parameters for extra sample volume and mixing repetitions. For a detailed description refer to <u>Advanced sample pipetting</u>.

Note: Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. Change the parameters only if you face any liquid level detection issues.

6. Click **Next** to proceed.

The next step is the <u>Assignment step</u>.

7.2.7.4.1 Define an input labware

If the option **Samples will be provided on one single plate or adapter** has been selected, define the input labware according to the following steps:

1. If you would like to enter sample names manually, or, if you would like to auto-generate sample names, select **Use a new input labware and enter sample names** and proceed to step 2.

If you would like to load a sample input file in csv format, select **Use a new input labware and enter** sample names and proceed to step 2.

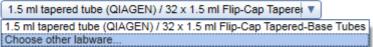
Use a new input labware and enter sample names
 Load sample input from file

If you would like to load a sample input file in a QIAGEN specific xml file format (QDEF), select **Load** sample input from file and proceed to step 11.

If you would like to load a sample input file in txt format, select **Load sample input from file** and proceed to step 11.

- 2. Click on the Labware drop-down menu on the left side of the screen.
- 3. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

Labware



4. Select your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

() Labware browser			
Filter options Category Standard 96 Well ▼ Standard 96 Well ▼	Labware Select the liem that most closely matches the labware to be used QIAGEN 96 Well Microfubes RS QIAGEN Elution Microfubes RS QIAGEN Selicet 98 Well	Catalog number 19585 Name	
Advanced options		QIAGEN S-Block 96 Well Dead Volume 40 µl	1 6 12
Part number			A 00000000000 0000000000000000000000000
All V Tube / Well size All	Labware adapter Select the adapter to be used.		
Reset filter Apply filter	A	Catalog number Name	
	T		

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

5. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

7. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 9. Define the sample names. Check the option Auto-generate sample IDs for all samples if you want the software to automatically generate sample names for all positions on the labware. Alternatively, click the Define samples button to enter the sample names manually. It is also possible to edit the automatically generated sample names. For details about the sample editor refer to Edit samples.

Auto-generate sample IDs for all samples

Define samples ... Import data from CSV file...

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: It is also possible to define sample names first and then to import concentration values only from a csv file. In this case, define the sample names first, as described above, and then click **Import data** from CSV file.

10. Proceed with step 2 in <u>Samples/Labware step</u>.

To define samples by using a sample input file in QIAGEN specific xml (QDEF) format, follow these steps:

11. If the option **Load sample input from file** has been checked, the **Load sample input from file** and **Scan barcode** buttons will be enabled.

Use a new input labware and enter sample names
 Load sample input from file
 Load sample input from file...

Note: Files in txt format must adhere to the syntax described in File dialog.

12. Click the **Load sample input from file** button and select the desired sample file. For details, refer to <u>File dialog</u>.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

The software will load the sample information from the sample file.

If the sample information file also contains the labware type, it will automatically be selected in the Labware drop-down menu. In this case, the software will automatically display the sample editor. Proceed to step 16.

Note: This is only possible for files in QDEF format.

Note: If the **Labware** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

14. If the sample information file does not contain the labware type, select the labware manually from the **Labware** drop-down menu. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Labware browser Filter options Category Standard 96 Well	Labware Select the item that most closely matches the labwar QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS	e to be used Catalog number 19585	
Shows available labware only Advanced options Name of labware Part number Manufacturer All V	QIAGEN S-Block 96 Well	Name QIAGEN S-Block 96 Well Dead Volume 40 µl	1 6 12 A 0 0 0 D 0 0 0 0 D 0 0 0 0 0 D 0
Tube / Well size All Reset filter Apply filter	Labware adapter Select the adapter to be used.	Catalog number Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

15. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

 Optional step: Edit the sample information. If the sample editor did not open automatically, click Define samples to open it. Click OK to confirm the sample information and to close the sample editor.

For details about the sample editor, refer to Edit samples.

- If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
 Note: Whenever you select another labware, this option will become unchecked again.
- 18. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 20. Proceed with step 2 in <u>Samples/Labware step</u>.

7.2.7.4.2 Edit samples

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.

	Sample editor		
Sele	ect an input labware identifier t	o enter, edit or	view the samples provided on that labware
Lab	ware identifier		
In	put1_20161205_101957		
_		0 1 1 1	
Pos. A1	Sample ID	Conc. [ng/µl]	Description
A2	,		
A3		•	
A4			
A5			•
A6	`	•	•
A7	`		•
A8	1		
B1	`		
B2	`		
B3	`		
B4			U
B5			
B6			
B7			
B8			
C1			
C2			
C3			
C4			▼
			OK Cancel

Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.

Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column **Sample ID**. Press and hold the **SHIFT** key and left-click to select multiple adjacent cells, or press and hold the **CTRL** key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on **Auto-generate sample names**. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on **Auto-generate sample** names.

Сору	Ctrl+C
0000	04110
Paste	Ctrl+V
Auto-generate sample names	
Allo generale cample hamee	

Note: For more details how to enter information into a table refer to the section Entering data.

Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

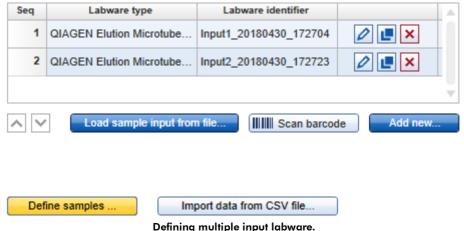
- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

7.2.7.4.3 Defining up to two input labware

If the option **Samples will be provided on multiple plates or adapters (up to 2)** has been selected, you can define up to two input labware types.

All defined input labware will be listed in the **Sample input labware** table. Initially, the table is empty.

Sample in	put labware
-----------	-------------



1. If you would like to enter sample names manually or if you would like to auto-generate sample names, select **Add new** and proceed to step 3.

If you would like to load a sample input file in csv format, select **Add new** and proceed to step 3.

If you would like to load a sample input file in QIAGEN specific xml file format (QDEF), proceed to step 2.

If you would like to load a sample input file in txt format, proceed to step 2.

Note: Files in txt format must adhere to the syntax described in File dialog.

 Click the Load sample input from file button and select the desired sample file. For details, refer to File dialog. Click Open to close the file dialog. The software will load the sample information from the selected sample file.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

The software will load the sample information from the sample file.

If the sample file does not contain the labware type, the **Add new input labware** dialog opens. In this case, proceed with step 3. Otherwise, proceed with step 4.

- 3. The Add new input labware dialog opens. Follow the steps in Add input labware dialog.
- 4. The new labware will be listed in the **Sample input labware** table.
- 5. Repeat steps 1–4 until all input labware has been defined.
- 6. Define the sample names. The **Define samples** button will be colored yellow to indicate that this information is required.

Click the **Define samples** button to enter sample names manually. It is also possible to edit the automatically generated sample names. For details, refer to <u>Editing sample names</u>.

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: This step is optional if the sample names were already loaded from a file or automatically generated.

7. Proceed with step 2 in <u>Samples/Labware step</u>.

The buttons in the right column of the **Sample input labware** table have the following functions:

Button	Action	Description
0	To edit an input labware	The Edit input labware dialog will open, displaying the values that have been entered in the Add input labware dialog.
e	To duplicate an input labware	The Duplicate input labware dialog will open, displaying the values that have been entered for the original input labware. The input labware identifier will initially display as a copy of the input labware.
×	To delete an input labware	Deletes the row in the Sample input labware table.

7.2.7.4.4 Define output labware

If the **Distribute assay points to single plate or adapter** option has been selected, define the output labware by following the steps below.

1. You can either **Use a new output labware** or **Reuse a partially used output labware by loading an import file**. The option **Use a new output labware** will be selected by default and can be used for most runs.

Use a new output labware

Reuse a partially used output labware by loading an import file

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case, select the option **Reuse a partially used output labware by loading an import file** and proceed to step 9.

2. Select the output labware from the **Output labware type** menu on the right. Click **Choose other labware** to open the **Labware browser**.

Output labware type	
Select output labware type	•
Select output labware type	
Choose other labware	

Iter options	Labware		
ategory Standard 96 Well T Shows available labware only	Select the item that most closely matches the lab QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name	
Advanced options Name of labware		QIAGEN S-Block 96 We Dead Volume 40 µl	1 6
Part number Manufacturer			
All Tube / Well size	Labware adapter		
	Select the adapter to be used.	Catalog number	
Reset filter Apply filter		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.
- 4. If the output labware is already pre-filled with liquid or dried reagents, indicate this by selecting the option **Output labware is pre-filled with reagents**. During the run, the QIAgility will consider this information and, e.g., not reuse tips.
- 5. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

6. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

7. **Optional:** If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to **Output converter** to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, **None** is the only entry in the list.

✓ Output Converter

 Select...
 ABI7500_QuantiplexHyres.xslt
 ABI7500_SDS_v1_4.xslt
 ABI7500_SDS_v2.3_HID_v1.2.xslt
 CE Loading (AL).xslt
 ConvertToCsv.xslt
 RGQ-SW_QREX.xslt

Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

8. Proceed with step 3 in <u>Samples/Labware step</u>.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

9. The **Select file** and **Scan barcode** buttons will be enabled if the **Load existing output from file** option has been selected.

Use an empty output labware
Load existing output from file
Select file...
Scan barcode ...

10. Click the **Select file** button and select the desired labware file. For details, refer to <u>File dialog</u>.

Alternatively, click the **Scan barcode** button. In the **Scan labware barcode** dialog, scan the barcode and confirm by clicking **OK**. For details, refer to <u>Scan labware barcode dialog</u>.

Using the information from the loaded file, the software will automatically select the output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

Note: If the **Output labware type** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

11. Proceed to step 3.

7.2.7.4.5 Define up to two output labware

If the option **Distribute assay points to multiple plates or adapters (up to 2)** has been selected, you can define up to two output labware types.

All defined output labware will be listed in the Output labware table. Initially, the table is empty.

Outp	ut labware		
⊙ Di	istribute assay points to single p istribute assay points to multiple ut labware)
Seq	Labware type	Labware identifier	*
1	Eppendorf twin.tec PCR PI	Output1_20190730_102	 Image: A state of the state of t
2	QIAGEN S-Block 96 Well	Output2_20190730_102	
	Load output labware t	from file)	Add new

Defining multiple output labware

1. If you would like to add output labware manually, click the **Add new** button and proceed to step 4. If you would like to load an output labware file, proceed to step 2.

If you would like to provide output labware information by scanning a barcode, proceed to step 3.

Click the Load output labware from file button and select the desired file. For details, refer to File dialog. Click Open to close the file dialog. The software will load the output information from the selected file.

If the file does not contain the labware type, the **Add new output labware** dialog opens. In this case, proceed to step 4. Otherwise, proceed to step 5.

- 3. Click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.
- 4. The Add new output labware dialog opens. Follow the steps in Add output labware dialog.
- 5. The new labware will be listed in the **Output labware** table.

If the labware is to be used with the cooling block, the box in the 🗱 column will be ticked. If the labware is pre-filled with reagents, the box in the 🔺 column will be ticked. To change these settings, edit the labware.

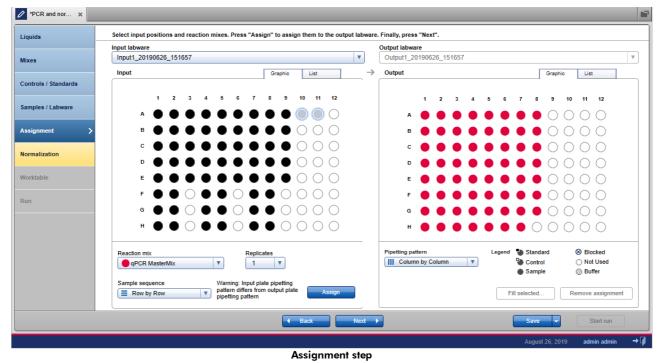
- 6. Repeat steps 1-5 until all labware has been defined.
- 7. Proceed with step 3 of the <u>Samples/Labware step</u>.

The buttons in the far right column of the **Output labware** table have the following functions:

Butto n	Action	Description
0	Edit the output labware	The Edit output labware dialog will open, displaying the values that were entered in the Add output labware dialog.
Ŀ	Duplicate the output labware	The Duplicate output labware dialog will open, displaying the values that have been entered for the original output labware. The output labware identifier will initially display as a copy of the input labware.
×	Delete the output labware	Deletes the row in the Sample output labware table.

7.2.7.5 Assignment step

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of sample-final reaction (master) mix combinations to the output labware.



Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware that have been selected in the **Input labware** menu. The meaning of the different symbols is described below:

	Name Sample	Description Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been selected once for assay setup on output labware.
	Selected sample	Currently selected sample.
•		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
•		Note: It is possible to use the same sample multiple times to create reaction points on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. You cannot edit sample IDs that have already been assigned. You can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this sequence is used when assigning selected samples to the output labware.

If the option **Samples will be provided on multiple plates or adapters** has been selected, you can switch between the different input labwares using the box above the **Graphic** and **List** tabs.

At the bottom of the panel is a menu that lists all final reaction (master) mixes defined in the **Mixes** step, along with a **Replicates** menu, and a **Sample sequence** drop-down list.

In order to assign a sample for assay setup, select the sample position(s) and then the reaction (master) mix you would like to assign to the selected sample(s). Then set the number of replicates. When you click the **Assign** button, the selected samples and the reaction mix will be assigned to assay points created on the output labware. Follow the procedure described in <u>Assigning samples with reaction mixes to the output</u> <u>labware</u>.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. The creation of assay points containing samples, standards, and controls depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at the input side. The assay points are colored with the reaction mixes. Standards and controls are indicated by an additional small symbol. If you hover with the mouse cursor over an output position, the software displays the sample ID and the reaction mix for that position.

Assay points can be removed. For details, refer to <u>Removing assay assignments</u>.

The **Sample sequence** menu at the bottom of the output panel can be used to set the pipetting pattern to either row by row or column by column.

Furthermore, it is possible to define blocked positions on the output labware by selecting the position(s) and then right-clicking on it.

In order to fill up unused positions on the output labware, e.g., with a buffer for CE loading, select the positions to be filled on the output labware and then click **Fill selected**. In the upcoming dialog select the liquid and the volume. For a detailed description refer to <u>Fill empty output positions</u>.

Use the **List** tab of the output panel to view the assay points that have been created.

Outp	ut		Grap	hic List	
Pos.	Comple ID		Reaction mix		
POS.	Sample ID				
A1	Liver 1	•	qPCR MasterMix		
B1	Liver 9		qPCR MasterMix		
C1	Liver 17		qPCR MasterMix		
D1	Lung 1		qPCR MasterMix		
E1	Lung 9		qPCR MasterMix		
F1	Lung 17		qPCR MasterMix		
G1	Brain 1		qPCR MasterMix		
H1	Brain 9		qPCR MasterMix		
A2	Brain 17		qPCR MasterMix		
B2	Liver 2		qPCR MasterMix		
C2	Liver 10		qPCR MasterMix		
D2	Liver 18		qPCR MasterMix		
Pipett	ting pattern	Leg	end 🐌 Standard	Blocked	
III	Column by Column	_	Control	O Not Used	
			Sample	⊘ Buffer	
		ſ	Fill selected	Remove assignmer	nt

The List tab of the Output panel

7.2.7.5.1 Assigning samples with reaction mixes to the output labware

Proceed as follows:

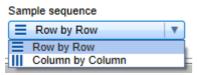
1. From the **Input labware** menu on the left side of the screen, select an input labware or standards and controls that were defined in the previous **Labware selection** step. The software will display its defined samples below in the **Input** panel.

- 2. From the **Reaction mix** menu, select the reaction (master) mix you want to assign. The menu lists all final reaction (master) mixes that have been defined in the **Mixes** step.
- 3. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

4. From the **Sample sequence** drop-down list, select the sequence you want to use. You can choose from row by row and column by column.



Note: The sample sequence determines the order of the pipetting of the samples:

- If **Row by row** is selected, the first sample that is pipetted is the first sample in the first row, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the row and the second sample's replicates are pipetted and so on.
- If **Column by column** is selected, the first sample that is pipetted is the first sample in the first column, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the column and the second sample's replicates are pipetted and so on.
- 5. From the **Input panel**, select the sample position for which you would like to assign a reaction (master) mix. Initially, all previously defined positions are selected.

Note: To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions. Selecting in a rectangle is only possible on rectangular plates.

- 6. Click Assign.
- 7. The software creates assay points on the **Output** panel on the right side of the screen. The selected reaction (master) mix is assigned to the selected samples. If you have selected more than one replicate, the sample positions are multiplied accordingly. The positioning of the created assay points on the output side follows the selections made for the **Pipetting pattern** and the sequence of samples from the sample sequence.

The samples are also listed in the **List** tab of the **Input** labware (in the **List** tab of the **Input** panel, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column or using the **Sample sequence** drop-down menu).

Note: The instrument follows a specified order when pipetting liquids. First, the master mix is pipetted, followed by the standards and controls, and then the samples.

8. Repeat the steps above until all positions of the output side have been defined for the planned run.

7.2.7.5.2 Removing assay assignments

To clear a position on the output side, follow these steps:

 Select the desired position on the **Output** side. Selecting multiple positions is possible by pressing SHIFT and left-clicking with the mouse to select adjacent positions or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.

Note: If you select empty positions along with filled positions, you cannot remove their assignments. It is only possible to remove assignments from multiple positions if all positions are filled.

Click Remove assignment. The assignment will be removed, and the sample position will be cleared from the output side. Also, if the position was marked as filled with buffer, the position will be cleared.
 Note: The software will remove all replicates of the desired sample(s).

7.2.7.5.3 Assigning standards and controls to the output labware

The QIAgility Setup Manager Software allows you to assign the positions of standards and controls on the output plate.

Proceed as follows:

- 1. Select Standards and Controls from the Input labware drop-down list on the left side of the screen.
- 2. If you have defined one standard series, it will be automatically selected by the software, and a table with dilution steps and dilution ratios will be shown.

If you have defined multiple standard series, select one of them from the **Select standard series** dropdown list.

andards and Controls			
Select standard series Select standard series	v		
Standard 651			
Standard 161			

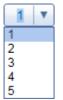
A table with dilution steps and dilution ratios will appear.

	•
Dilution Ratio	
1 1:1	
and 1:5	
and 1 : 25	

- 3. Tick the box next to one or more dilution steps in the table. To select all of them, tick the box in the table header.
- 4. Select a mix from the Reaction mix drop-down list.

Reaction mix		
epcR MasterMix	•	

5. Select the number of replicates from the Replicates drop-down list.



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

6. Click Assign.

7. Tick the box next to one or more controls in the following table. To select all controls, tick the box in the table header.



8. Select a mix from the Reaction mix drop-down list.

Reaction mix	
e qPCR MasterMix	•

9. Select the number of replicates from the Replicates drop-down list.

1	•
1	
2	
3	
4	
5	

Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

10. Click Assign.

- 11. Repeat steps 2 10 as needed, until you have defined all the positions for the planned run.
- 7.2.7.6 Normalization step

In the **Normalization** step define the parameters for normalization. The QIAgility Setup Manager Software provides the option to normalize samples based on sample mass or sample concentration. When normalizing to a specific concentration, the sample will be diluted to the chosen target value and afterwards transferred to the output labware. In case normalization of a sample is not directly possible in the output position, the sample will be pre-diluted on a dilution labware. The software pre-calculates for each sample, if the target can be reached. Based on this calculation you can decide, if you want to include or exclude samples.

To configure if normalization is based on target sample mass or sample concentration, click **Configuration** in the main tool bar, click the **Settings** tab and then select the **Generic PCR user settings** step. For details refer to <u>settings</u>.

Experiment 0 ×		
Liquids	Define normalization parameters	
Mixes	Normalization parameters Target sample mass in output plate Max. sample vol. used for pre-dilution 0.1 ng 30 µl	Within configuration, it can be defined if normalization for the assay is based on target mass or concentration. In case normalization of sample is not directly
Controls / Standards	Diluent Vater	possible in output well, the sample will be pre-diluted on a dilution labware. "Max sample vol. used for pre-dilution". Maximal template vol. used in dilution labware (doesn't affect template vol. used for final assay). Only input liquids defined as diluent / water can be selected as diluent.
Samples / Labware	Dilution labware	Advanced pipetting parameters (change only if required): Liquid classes for normalization and no. of mixes after addition of template to dilution buffer are
Assignment	Labware 1.5 mi tapered tube (QIAGEN) / 32 x 1.5 mi Flip-Cap Cooling block required	preselected. Max.vol. in dilution well: Restricts volume to guarantee homogenous mixing. Min. vol. in dilution well: Used to guarantee proper pipetting of dil. sample. Min diuent / sample transfer volume: Guarantees proper pipetting of diluent or
Normalization	Labware identifier Dilution_1_20180502_215959 Print barcode	sample. In case data reveal non-homogeneous mixing or inaccurate pipetting, parameters can be adjusted.
Worktable	Advanced pipetting parameters	
Run		
	View dilution details	
	Normalization step	

To define sample normalization:

1. Enter the **Target sample mass** or **concentration** that shall be reached in the output labware.

Note: When normalizing to target sample mass, the unit is predefined as "ng".

2. Enter into the field **Max. sample vol. used for pre-dilution** the maximal sample volume that shall be used in maximum for pre-dilution, e.i. the maximal template volume that will be used in the dilution labware to pre-dilute a sample.

Note: The higher the maximal sample volume for pre-dilution is set, the more flexible the software can calculate possible normalization strategies.

- 3. Click **Diluent** to select the diluent that shall be used for sample normalization. The menu lists all liquids that have been defined with liquid type **Diluent/Water** in the **Liquids** step. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 4. Click **Labware** to select the labware that shall be used to pre-dilute samples. All previously selected labware types will be listed. The **Choose other labware** option will be listed last. Select your dilution labware from the drop-down list. If it is not listed, click **Choose other labware** to open the Labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

er options	Labware		
egory tandard 96 Well T	Select the item that most closely matches the labw QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name QIAGEN S-Block S	96 Well
Advanced options ame of labware		Dead Volume 40 µl	
art number			
All Transformed and the state of the state o	Labware adapter	Ť	
Reset filter Apply filter	Select the adapter to be used.	Catalog number	
		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

- 5. **Optional:** If you would like to use the cooling block for the dilution labware, check the option **Cooling block required** next to **Labware**.
- 6. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 8. **Optional:** Define advanced pipetting parameters. If you tick the box, parameters become visible. In case the initial sample concentration is too high or too near to target, parameters can be adjusted.

ſ	Liquid class for pipetting diluent on	to dilution labware		
	Diluent to dilution	v		
	Liquid class for pipetting sample on	to dilution laburaro	No. of mix repetitions on dilution	an lahwara
	Liquid class for pipeting sample on	ito dilution labware		Jiliabwale
	Sample for dilution	T	7	
	Max. volume in dilution well	Min. volume in dilution well	Min. diluent transfer volume	Min. sample transfer volume
	1400 µl	20 µl	5 µl	2 µl

(C	p	ti	O	n	
÷	•		•			

Advanced pipetting parameters

....

1.1

Description

. . .

Liquid class for pipetting diluent on	o Preselected.
dilution labware	Defines the pipetting and tip handling for pipetting the diluent to the dilution labware.
Liquid class for pipetting sample or	to Preselected.
dilution labware	Defines the pipetting and tip handling for pipetting the sample to the dilution labware. Sample (template) will be pipetted after the diluent has been distributed.
No. of mix repetitions on dilution	The value is predefined by the selected liquid class.
labware	Defines the mix repetitions after the sample (template) has been added to the diluent on the dilution labware.
	Mix repetitions have been developed to guarantee proper mixing of water based diluents and samples for the default value of maximal volume in dilution well. In case maximal volume in dilution well is manually increased, it is recommended to increase mix repetitions.
Max. volume in dilution well	Predefined.

	Restricts the volume in the dilution positions in order to guarantee homogeneous mixing.
	In case the maximal volume in dilution well is manually iincreased, it is recommende to increase mix repetitions. If the total volume of the selected dilution labware is smaller than the setting of this parameter, the total volume of the dilution labware will be used.
Min. volume in dilution well	Predefined.
	Ensures proper creation of pre-dilution and pipetting of the diluted sample to the output labware.
Min. diluent transfer volume	Predefined.
	Guarantees presize pipetting of diluent for pre-dilution. A setting $< 2\mu$ l is not recommended.
Min. sample transfer volume	Predefined.
	Guarantees presize pipetting of sample (template) for pre- dilution. A setting $< 1 \ \mu$ l is not recommended.

- Click View dilution details to open a list with the results of pre-calculation for the samples. The button is colored yellow, if the normalization target will not be reached for all samples.
 Follow the steps described in <u>Dilution details</u>.
- 10. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 11. Close the lid.
- 12. Click Next to enter the worktable step. If the instrument has not been used before, it will initialize. Note: While entering the Worktable step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.

7.2.7.6.1 Dilution details

The dialog lists the normalization pre-calculation for all samples. It is possible to enter individual normalization targets for output positions.

nclude	Exclude	Pos.	Sample ID	Target mass	Output Pos.	Labware Identifier	Orig.Con	Dilution strategy	Used sample volume
•	0	A1	Liver 1	50	A1	Output1_20190626_151657	518	Dilution uses 1 dilution position.	2
•	0	B1	Liver 2	50	B2	Output1_20190626_151657	475	Dilution uses 1 dilution position.	2
•	0	C1	Liver 3	50	C3	Output1_20190626_151657	440	Dilution uses 1 dilution position.	2
•	0	D1	Liver 4	50	D4	Output1_20190626_151657	438	Dilution uses 1 dilution position.	2
•	0	E1	Liver 5	50	E5	Output1_20190626_151657	439	Dilution uses 1 dilution position.	2
•	0	F1	Liver 6	50	F6	Output1_20190626_151657	668	Dilution uses 1 dilution position.	2
•	0	G1	Liver 7	50	D7	Output1_20190626_151657	580	Dilution uses 1 dilution position.	2
•	0	H1	Liver 8	50	B8	Output1_20190626_151657	668	Dilution uses 1 dilution position.	2
•	0	A2	Liver 9	50	B1	Output1_20190626_151657	488	Dilution uses 1 dilution position.	2
•	0	B2	Liver 10	50	C2	Output1_20190626_151657	548	Dilution uses 1 dilution position.	2
۲	0	C2	Liver 11	50	D3	Output1_20190626_151657	2	Orig. concentration too low. Reachable mass in output = 16 ng.	8
•	0	D2	Liver 12	50	E4	Output1_20190626_151657	487	Dilution uses 1 dilution position.	2
•	0	E2	Liver 13	50	F5	Output1_20190626_151657	480	Dilution uses 1 dilution position.	2
•	0	F2	Liver 14	50	G6	Output1_20190626_151657	492	Dilution uses 1 dilution position.	2
•	0	G2	Liver 15	50	E7	Output1_20190626_151657	470	Dilution uses 1 dilution position.	2
•	0	H2	Liver 16	50	C8	Output1_20190626_151657	494	Dilution uses 1 dilution position.	2
•	0	A3	Liver 17	50	C1	Output1_20190626_151657	521	Dilution uses 1 dilution position.	2
•	0	B3	Liver 18	50	D2	Output1_20190626_151657	468	Dilution uses 1 dilution position.	2

Include all

Reasons why a sample cannot be diluted: Too less / high original conc. or original conc. is too near to target for chosen normalization parameter. Manually included sample will be transferred undiluted to final assay well using user-defined sample volume. Excluded sample will be unassigned and won't be pipetted at all. If sample-specific target mass or concentration is required, change target value accordingly.



Dilution details

- 1. Check the calculation. Find a description of the table columns, below.
- 2. **Optional:** You can enter an individual normalization target for positions on the output labware. Click into the **Target mass / concentration** field of the corresponding output position row and enter the new target value. The calculation for this position will automatically be updated.
- 3. For each sample that has yellow colored columns **Include/Exclude**, decide whether you want to include the sample into the run or not. Check the corresponding option. The sample position will no longer be colored yellow. To include all samples, click the Include all button below the table. For details refer to the description of the **Include** and **Exclude** columns, below.
- 4. **Optional:** Samples that have no yellow color indication in **Include/Exclude** column do not have any restrictions in regards to normalization, but can also be excluded, if needed.

- 5. If no sample has yellow colored **Include/Exclude** column, the **OK** button will be enabled.
- 6. Click **OK** to confirm your selections and to close the dialog.

Proceed with step 10 in Normalization step.

The table contains all samples from the input labware. Samples that have not been previously assigned in the Assignemt step are listed last.

Column	Description
Include	If checked, the sample will be included in the run.
	All samples, previously assigned in the Assignment step, which have no restriction to reach the normalization target, are automatically included. Otherwise, the columns Include/Exclude are highlighted in yellow to indicate that a decision by the user is required.
	Check the option to include the sample.
Exclude	If checked, the sample will not be used in the run and will be automatically unassigned.
	All samples, previously assigned in the Assignment step, which have a restriction to reach the normalization target, are marked in yellow to indicate that a decision (include or exclude) by the user is required.
	After confirming the dialog with OK, the sample gets automatically unassigned. The following positions on the output labware will be filled up according to the selected Pipetting pattern and Pipetting order from the Assignment step.
Pos.	The sample position on the input labware.
Sample ID	The sample ID.
	If the sample ID is too long, it is cut off. To view the whole sample ID, hover the mouse over the field.
Target mass/ Target conc.	The normalization target. Initially, the value that has been entered into the Normalization step is shown.
	However, you can edit the value if you need an individual normalization target for the corresponding output position. If no target value has been entered, or if the target value is too high or too low, the field is highlighted in yellow to indicate that a valid target needs to be entered.
Output pos.	The position on the output labware.
Orig. conc.	The original sample concentration as imported from the sample input file or entered in the sample editor.
Dilution strategy	The dilution strategy is automatically selected by the software and cannot be changed by the user.
	If the normalization target cannot be reached, the reason is listed. Decide whether to include or exclude the sample. For details, refer to the description below.

Note: It is possible to influence the dilution strategies by changing normalization parameters. The software always used the dilution strategy for which the lowest reagent volumes are required.

Used sample volume The sample volume used during normalization.

The dilution strategy depends on

- the value for the normalization target
- the original concentration of the sample
- the setting for maximum volume to be used for pre-dilution of a sample in the dilution labware
- the setting for minimum volume in the dilution well
- the sample volume for the run as specified in Samples/Labware
- the selected dilution labware (total volume and among of dilution positions)
- the maximum volume allowed in the dilution labware (advanced pipetting parameters for normalization)
- the minimum sample transfer volume (advanced pipetting parameters for normalization)
- the minimum diluent transfer volume (advanced pipetting parameters for normalization)

Dilution strategy	Description
Dilution in output position.	It is sufficient to dilute the sample directly in the output position within the defined sample volume for the assay setup.
Dilution uses 1 dilution position.	The sample will be diluted in one position of the dilution labware. Then the defined sample volume for the run will be transferred from the dilution labware to the output labware position.
Dilution uses 2 dilution positions.	The sample will be diluted using two positions of the dilution labware. Then the defined sample volume for the run will be transferred from the second dilution position on the dilution labware to the output labware position.
Dilution uses 1 dilution position and output position.	The sample will be pre-diluted in one position of the dilution labware. The last dilution step will be performed in the output position within the defined sample volume for the assay setup.
Original concentration too high.	The sample cannot be normalized to the given normalization target, because the original sample concentration is too high. The required dilution ratio cannot be created using the defined normalization parameters. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will be diluted to this value.
Original concentration too low.	The original sample concentration is lower than the normalization target. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will

be use	l undiluted.

Original concentration too close to target.	The sample cannot be normalized to the given normalization target, because the defined normalization parameters do not allow such a small sample dilution ratio. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will be used undiluted. Otherwise, the normalization parameters have to be changed (e.g., increase the Max. sample
	volume used for pre-dilution value).

7.2.7.7 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



Worktable selector

- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

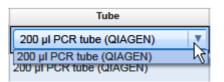
Click Print loading instruction to generate a PDF document with the loading instructions for the run.

644		Mix	ы	lock
IVI-1	-	IVILX.	D	OCK

B Reaction mix (Mix) 1.5 ml tapered tube (QIAGEN) 0 μl Image: Comparison of the state of tube (QIAGEN) C Reaction mix 1.5 ml tapered tube (QIAGEN) 295 μl Image: Comparison of tube of tube (QIAGEN) D Primer mix 1.5 ml tapered tube (QIAGEN) 246 μl Image: Comparison of tube of tub				Content	os.
D Primer mix 1.5 ml tapered tube (QIAGEN) 246 µl	\checkmark	0 µl	1.5 ml tapered tube (QIAGEN)	Reaction mix (Mix)	в
	~	295 µl	1.5 ml tapered tube (QIAGEN)	Reaction mix	С
E Dilution buffer 1.5 ml tapered tube (QIAGEN) 157 ul	~	246 µl	1.5 ml tapered tube (QIAGEN)	Primer mix	D
	~	157 µl	1.5 ml tapered tube (QIAGEN)	Dilution buffer	Е
		157 µl	1.5 ml tapered tube (QIAGEN)	Dilution buffer	E

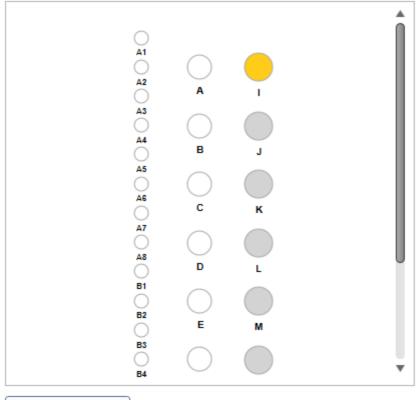
Table of tubes to be loaded to the worktable position M1.

It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware Manager**. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.



Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

Calibrate labware ...

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

Click the **Labware** tab on the right to get an image and details about the input and output labware.

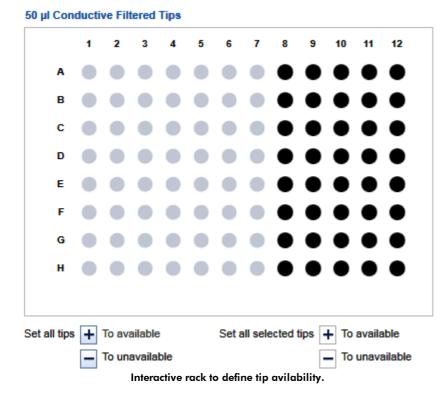
Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

Labware: 0.1 ml Strip Tube (QIAGEN) Adapter: Rotor-Gene Q, 72 x 0.1 ml Strip Tubes Adapter



Image and details for output labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To available** or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to Loading tips.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> <u>holder</u>.

4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again.

5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 6. To confirm that the tip rack has been loaded, either check the box next to Loaded in the table or click on Confirm all to confirm. Refer to Loading tips for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

For worktable positions with samples:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

15. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

- 16. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 17. Ensure that samples are loaded using the correct labware type.
- 18. Load the samples in the correct positions as displayed in the table.
- Optional step: For sample tubes, click Validate sample loading to start sample loading validation using a hand-held barcode scanner and proceed with steps 20–23. Otherwise, proceed to step 24.
 Note: This button is only available if the corresponding option has been checked in the <u>User settings</u> for <u>Generic PCR experiments</u>.
- 20. The software will highlight the first sample position in the table. During validation, scanning is the only function available.

Note: To indicate that a user interaction is expected, a cursor with arrows in all four directions $\mathbf{\Phi}$ will be displayed for the entire validation.

- 21. Scan the barcode of the sample tube that has been loaded in the highlighted position.
- 22. If the barcode matches the sample's ID, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. If the barcode does not match the sample ID, a message is displayed. Click **Retry** in the message to scan the barcode again. If the barcode matches, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. To stop validation, click **Stop validating**, or, if the barcode did not match, click **Cancel** in the displayed message. In this case, proceed to step 24. There will be no documentation about sample loading validation in the report.
- 23. If all sample positions have been scanned successfully, a message is displayed. The successful sample loading validation will be documented in the report, which can be created after the run. Proceed to step 25.
- 24. Check that the samples are placed in the positions as displayed in the table.
- 25. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 26. Click **Next** to continue with the next worktable position.
- 27. Repeat steps 15–26 for all worktable positions with samples.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.7.8 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.7.8.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress

Task	Progress
Prepare dilution plate	
Prepare mixes	
Distribute mixes to output labware	
Distribute controls to output labware	
Distribute dilution liquid to output labware	· · · · · · · · · · · · · · · · · · ·
Distribute samples to output labware	
Overall progress	
Start time	Approximate end tim
15:12	15:1
Run ta	isks

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.7.8.2 Pausing or canceling the run

A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	Pause
Overall progress		*
Start time 13:05	Paused at 13:21	Resume

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.7.8.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.

Run info	
Operator	
John Smith	
Comment	
	Ļ
Output labware	
Print barcode	Save output file
Save dilution plate	
	Create report
Options af	ter the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Save output file	An output file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>User</u> <u>settings</u> to define a standard directory.
	The file may serve as a sample input file for the Rotor-Gene Q operated with the Q-Rex Software. The .xml file can also be used by an LIMS (Laboratory Information Management System). A format description is available at www.giagen.com/goto/QIAgility . You can find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QIAGEN\QIAgility\Examples.
	If an output converter has been selected in the Samples/Labware step, the software also creates a file in the format as defined by the output converter. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension (e.g., .txt for the ABI cycler), and will also be saved in the specified directory. In case the file extension is also .xml , the file name will be enhanced. The available output converters can be found in the output file converter directory, as specified in the <u>User settings</u> . Contact QIAGEN Technical Services if you need another file format.
	Note: Use this option also in case the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Samples/Labware step that you are Reusing a partially used output labware by loading an import file by selecting that option, and load the file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.
Save dilution plate	A file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the dilution labware and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file.
Print barcode	You can print a barcode for the output labware.
	Note: The option is available only if a printer has been defined in the Global Settings tab of the Configuration environment.

After the run has finished, follow these steps:

1. Remove the labware from the worktable.

- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

7.2.7.9 Report

The Generic PCR Plug-in offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results
- Exceptions
- Detailed run information (incl. dilution labware)

The **Advanced report** contains:

- General information
- Comment
- Reaction results
- Exceptions/Pauses
- Sample information (incl. dilution strategy)
- Worktable information
- Detailed run information (incl. dilution labware)
- Advanced instrument information

The sections of both report types are described in <u>Report</u>. Additionally, the report contains information about normalization parameters and dilution labware (both report types). Information about the dilution strategies used for the samples is available in the **Advanced** report, section **Sample information**.

Additional information is described below.

Detailed run information

In addition to the information described in <u>Report</u>, this section contains further information for both report types. It lists the normalization parameters as defined in the **Normalization** step, and contains a table that describes the dilution labware:

Column	Description
Dilution position	The position on the dilution labware.
Identifier	Specifies the sample ID of the originating sample.
Туре	Sample.
Input	The corresponding position on the input labware. If multiple input labwares were used for the run, the position is enhanced by the input labware identifier.
Actual content	Lists the volume and the components.
Concentration	The sample concentration in the dilution labware position.
Flag	Shows the status of the position: either ok, warning or error.

Sample information

The normalization strategy for each sample is provided, in addition.

7.2.8 PCR setup with serial dilution experiments

The Generic PCR Plug-in enables easy and convenient qPCR setup including serial dilution.

For pathogen testing and gene expression experiments using dedicated QIAGEN kits, refer to sections <u>Working with experiments for pathogen detection</u> and <u>Working with gene expression experiments</u>, respectively. For generic PCR setup experiments without sample normalization, refer to section <u>Generic PCR</u> <u>experiments</u>. For generic PCR setup experiments with sample normalization, refer to the <u>Generic PCR setup</u> <u>with normalization experiments</u>. It is also possible to set up a run for sample normalization only. In this case, refer to section <u>Normalization only experiments</u>.

New experiments for generic PCR setup with serial dilution, are created by clicking **PCR Setup with Serial Dilution** from the **Home** screen, and then defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and **Mixes** step will be enabled, initially. Subsequent steps will become available, once the required information has been entered into the **Liquids** and **Mixes** step. The **Mixes** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the <u>Experiment</u> environment.

The experiment wizard has eight steps that will guide you through the setup process:

- 1. Liquids
- 2. <u>Mixes</u>
- 3. Controls/Standards
- 4. <u>Samples/Labware</u>
- 5. Assignment

- 6. <u>Dilution</u>
- 7. <u>Worktable</u>
- 8. <u>Run</u>

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is ***.qsmrx**, and for templates ***.qsmrt** (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is ***.qsmrqt** (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.8.1 Liquids step

The **Liquids** step is used to define all input liquids required for your PCR setup that are to be loaded onto the instrument's worktable, besides your samples. These are, for example:

- all kinds of reagents, that will , e.g. be part of reaction (master) mixes
- all kinds of controls and standards (stock)
- all kinds of diluents/water
- ready-made reaction mixes, i.e., final reaction (master) mixes that have already been prepared by the user and are ready to be used and distributed to the output labware by the QIAgility.

Detailed information for (final) reaction (master) mixes will be defined later in the **Mixes** step. Samples will be defined later in the **Samples/Labware** step.

The **Liquids** step also includes information about the kit that is used during the run.

*Experiment_0 ×						
quids >	Define input liquids					
xes	Define required input liquids (reagents (master) mix, Standards (stock) and co Predefined liquid classes (LC) define th task for liquid transfer (refer to LC table	ontrols) for assay setup. he pipetting/tip handling	Kit information Kit Name Product number	Lot number	Comment	Â
ntrols / Standards	screen). LC-naming: Liquid type + pipetting des For more information, press F1 key wh					
mples / Labware	Name	Liquid type Liqu	uid class	Tube type		Actions
signment	Water	Diluent/Water Aqu	ueous liquid to reaction (master) mix	5.0 ml flat-base tube (QIAGEN)		
	Mastermix	Reagent Vise	cous liquid to reaction (master) mix	2.0 ml screw cap micro tube (Sarstedt)		
ution	Primer Mix	Reagent Prin	mer-Probe or IC to reaction (master	2.0 ml screw cap micro tube (Sarstedt)		🖉 🖪 🗙
orktable	Standard 1	Standard Cor	ntrol or Std. to output or for dilution	2.0 ml screw cap micro tube (Sarstedt)		2 🖪 🗙
	Standard 2	Standard Cor	ntrol or Std. to output or for dilution	2.0 ml screw cap micro tube (Sarstedt)		0 🗉 🗙
in	Diluent	Diluent/Water Dilu	uent for dilution	5.0 ml flat-base tube (QIAGEN)		0 🖪 🗙
						New liquid
	~ v					New liquid

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The Add liquid dialog opens.
- 3. Define the liquid as described in Add liquid dialog.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

Finally, click **Next** in the button bar to proceed to the **Mixes** step (<u>Mixes step</u>).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the \frown \frown buttons below the table:

1. Click the row of the liquid to be moved.

2. Click on the up and down arrows to move the currently selected row up or down.

Use this function for a better overview. The report will list the liquids in this order.



The buttons in the **Actions** column

Button	Action To edit a liquid	Description The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.
		If you intend to change the liquid later, e.g., the liquid has already been used in a mix in the Mixes step, or, if the liquid is a ready-made reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed. In this case, remove assignments or reaction mixes to enable changes for defined input liquids.
L	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.
×	To delete a liquid	Deletes the row in the table.

7.2.8.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you want to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values for a liquid.

Add liquid	
Liquid name Dilution buffer	 Input liquid Ready-made mix
Tube type 1.5 ml tapered tube (QIAGEN)	Cap color
Liquid class	Liquid type
Lot number	Expiry date
Define advanced pipetting parameters	
Add liquid dialog	OK Cancel

- 1. Define the name of the liquid. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, e.g. when defining reaction mixes, standards or controls, and in the loading instructions of the Worktable step.
- 2. Define whether the liquid is an input liquid or a ready-made mix. Initially, the option **Input liquid** is selected, because it fits to most liquids.

Select the option **Ready-made mix** only, if the liquid is a final reaction/master mix that has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In this case, in the **Liquid type** field the value **Ready-made mix** is automatically selected and the liquid class menu is removed (the liquid class is defined later in the Mixes step). Additionally, when entering the later Mixes step, the reaction (master) mix is already listed.

If the liquid is a mix that has already been prepared by the user, but will be part of a more complex mix that will be created during the run by the QIAgility, select the option **Input liquid**.

3. Click **Tube type** to select the tube that will be used to place the liquid on the QIAgility. The tube types used in the last experiment are listed at the top of the list. To select one of the listed tube types, click the respective entry.

Tube type	
Select	▼
Select	
1.5 ml tapered tube (QIAGEN)	
5.0 ml flat-base tube (QIAGEN)	
Choose other labware	
Any available	

Select the value **Any available**, if the liquid does not need to be provided in a special tube, e.g. coming from a kit. In this case, later, during worktable planning, the software will automatically select a tube of the required size, with minimal dead volume, from the list of available tubes that has been defined in the Labware Manager. (To define available tubes in the Labware Manager, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.) Use this option to give the software more flexibility during worktable planning.

If the required tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.

Filter options	Labware Select the item that most closely matches the labware to be used	sed.
Advanced options Name of labware Part number Manufacturer	1.5 mi tapered screw cap tube (QIAGEN) 1.5 mi tapered tube (QIAGEN) 2.0 mi self standing screw cap tube (QIAGEN) 200 µI PCR tube (QIAGEN) 5.0 mi flat-base tube (QIAGEN)	Catalog number 990552 Name 5.0 mil flat-base tube (QIAGEN) Dead Volume 26 µl
All Tube / Well size	Labware adapter	V
Reset filter Apply filter	Select the adapter to be used.	Catalog number Name

In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If you edit the liquid later and the **Tube type** is marked yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type or make the tube available in the Labware Manager again.

4. Click **Cap color** to select the color of the tube's cap.



5. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid**

Classes step to get more details.

In case the same liquid will be used for different purposes (e.g. nuclease-free water used for setting up a standard dilution series and for non-template control) it is highly recommended to define multiple input liquids to allow precise definition of liquid class and liquid type.

Note: If you edit the liquid later and the **Liquid class** is marked yellow, this indicates that the selected liquid class is no longer available, e.g. if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

6. Select the **Liquid type**. The liquid type defines how the liquid will be used. In later steps during experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection, e.g. when defining controls or standards. In case a defined input liquid is not visible in later screens (e.g. during control definition) check if the liquid type has been set correctly.

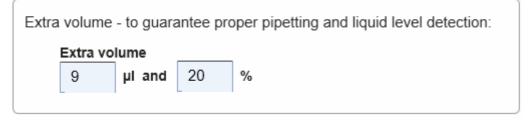
Liquid type	Description
Reagent	Use this type, if the liquid is a reagent that will be added to a reaction/master mix, either to a final reaction mix or to an intermediate reaction mix.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.
Pos./neg. control	Use this type, if the liquid is a positive or negative control (incl. NTCs).
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a control.
Standard	Use this type, if the liquid is a single standard as part of a user prepared standard series, or if the liquid is the standard stock for a standard series that shall be created by the QIAgility.
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a standard series that shall be created by the QIAgility.
Diluent/Water	Use this type, if the liquid is a diluent or water.
	Later in the experiment setup, a liquid of this type is available, e.g. for content definition of a reaction/master mix in the Mixes step, or in the Controls/Standards step as diluent for defining a control or standard series that shall be created by the QIAgility.
Ready-made mix	Use this type, if the liquid is a ready-made reaction/master mix prepared by the user. This type is preselected by the software, if the option Ready-made mix has been selected for the liquid.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.

- 7. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, these data will be present in the report.
- 8. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

Define advanced pipetting parameters



 In the dialog, click OK to confirm your values and to close the dialog. Otherwise, click Cancel to close the dialog and discard all values. In this case, no new liquid is defined, or – in case of editing an existing liquid –the liquid is not changed.

7.2.8.2 Mixes step

The **Mixes** step is used to define all kinds of mixes that are used for an experiment. These are, for example:

- all variants of reaction (master) mixes, i.e., final reaction (master) mixes that
 - o have already been prepared by the user and are ready to be used,
 - o will be generated by the QIAgility during run,
 - o and will be distributed to the output labware.
- all kinds of intermediate mixes. These are generic pre-mixes that will be used to create new (more specific) reaction (master) mixes.

The screen lists all mixes that have already been defined in a table. User prepared reaction (master) mixes are automatically listed, if the corresponding liquid has been defined in the previous Liquids step. The rows for these mixes are initially highlighted in yellow to indicate that the mixes need further specification.

*PCR -experim	PCR and nor	0	*Experiment_0	* 🖉 *Exp	eriment_0					
quids	Define your mi									
cs >	pipetting/tip han screen). Adv. parameters mixing repetition	ndling task fo s: Mixing se n can be cha	or distribution of re- ttings for system-p anged if required.	action (master) r repared reaction	uid class (pre-selected) del nix (refer to LC table in cor n (master) mix(es) are pred	nfiguration				
ntrols / Standards	For more inform	nation, press	F1 key when editi	ng mix(es). Reaction mix	Input	V	olume / reaction [µl]	Total volume / reaction [µ]	Liquid class	Actions
nples / Labware	Master Mix			~	Water Mastermix		10)	Reaction (maste	 Image: A state of the state of
signment					Primer Mix		2	2		
tion										
table										
										Ţ
orktable un										

Defining a new mix or editing a listed mix:

1. To define a new mix, click the **Add mix** button. The **Add new mix** dialog opens.

To edit a listed mix, click in the **Actions** column in the corresponding table row. The **Edit mix** dialog opens. It displays the values that have been entered before in the **Add mix** dialog or that have been automatically pre-filled by the software for a ready-made reaction (master) mix.

Note: If no final reaction (master) mix has been defined, the **Add mix** button is highlighted in yellow to indicate that the definition of a final reaction (master) mix is still missing.

- 2. Define the general properties of the mix. Details are described in Add new mix dialog.
- 3. In the dialog, click **Next** to proceed to the **Input** screen.
- 4. In the dialog **Input** screen define the volume per reaction and the content of the mix; as described in<u>Add new mix dialog input</u>.
- 5. For final reaction/master mixes, the optional **Target** screen is available. This screen allows the user to define target type and reports/quenchers. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import into the next instrument, e.g. a cycler. In this case, click **Next** to proceed to the **Target** screen. Details are described in<u>Add new mix dialog target</u>.

If you do not require target information in the report or for the next instrument, proceed.

- 6. Click **Finish** to finish the definition of the mix and to close the dialog.
- 7. The defined mix will be listed in the table in the Mixes screen.

Repeat the steps above until all final reaction (master) mixes have been defined.

Finally, click Next in the button bar to proceed to the Controls/Standards step, described in

Note: The Next button is disabled, if a row in the table is highlighted yellow. User prepared reaction

(master) mixes are initially highlighted yellow, if the volume per reaction has not yet been defined. Edit(the mix and enter the volume per reaction in the Input screen of the dialog. The **Next** button is also disabled, if the definition of a final reaction (master) mix is still missing.

The mixes in the table can be reordered using the \checkmark buttons below the table. The report will list the mixes in this order. An intermediate mix (generic pre-mix) needs to be listed before the (more specific) reaction (master) mix of which it is part of. It will not be possible to move an intermediate mix below its reaction (master) mix.

- 1. Select the row of the mix to be moved by clicking on it.
- 2. Click on the up and down arrows to move the currently selected row up or down.

	Actions	
The buttons in the Actions column		of the table have the following functions:

Button	Action To edit a mix	Description The Edit mix dialog will open, displaying the values that have been entered in the Add mix dialog.	
		If you intend to change a mix that is already in use as input for another mix, or if it is a final reaction mix and has already been assigned to samples in the Assignment step, some restrictions apply. Not all values can be changed anymore.	
L	To duplicate a mix	The Duplicate mix dialog will open, displaying the values that have been entered for the original mix. The mix name will initially display a copy of the name of the original mix.	
×	To delete a mix	Deletes the row in the table.	

7.2.8.2.1 Add new mix dialog

In the **Add new mix** dialog a new mix can be defined. If you would like to change the values for an existing mix later, the **Edit mix** dialog will open. Both dialogs show the values of a mix.

Define the basic properties of the mix in the **General** screen of the dialog.

Edit mix				
General >	Name			
Input	Test-mix Final reaction mix (ready-made) Intermediate mix (ready-made) Test-mix			
Target	Final reaction mix already on output (ready-made) Liquid class Reaction (master) mix (final) to output			
	Note: Make sure that ready-made mix is homogeneous and mixed manually and avoid foam.			
	Define advanced parameters			
	Back Next Finish Cancel			

Edit mix dialog

Add new mix				
General >	Name			
Input	Mix Final reaction mix (system prepared) Intermediate mix (system prepared) Final reaction mix (system prepared) 			
Target	 ▶ Final reaction mix already on output ■ Reaction (master) mix (final) to output ■ Define advanced parameters 			
	Back Next Finish Cancel Add new mix dialog - General			

1. Define the name of the mix. The name must be unique. The name is used to refer to the mix in later steps of the experiment setup, e.g. when defining more specific mixes, and in the loading instructions of the Worktable step.

For a ready-made mix, the name is already defined from the liquid dialog definition.

2. Select the type of mix. For ready-made mixes and other mixes different options are available:

Option	Description
Final reaction mix (ready-made)	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
	Use this option, if this mix has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In the later Assignment step, the mix will be available for selection. During the run, the QIAgility will pipette the mix to the positions on the output labware as assigned in the Assignment step.

		Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QIAgility as QIAgility will not perform any mixing steps.
	Intermediate mix (ready- made)	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
		Use this option if this mix has already been prepared by the user and will be part of a more specific mix to be created during the run by the QIAgility. During the run, the QIAgility will pipette the mix into the tube, where the more specific mix is created.
		Ensure that the reaction (master) mix is homogenously mixed before it is loaded on the QIAgility as QIAgility will not perform any mixing steps.
	Final reaction mix already on output	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
(ready-made)	Use this option only if this mix has already been distributed on the output labware before the run. In the later Worktable step, you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QlAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.	
		The volume of the reaction mix must be specified. This value will be used to calculate the dispense height and the mixing volume.
		If the option is selected, the Input screen of the dialog will be skipped.
	Final reaction mix (system prepared)	Use this option if during the run the mix will be created first by the QIAgility and then distributed to the output labware.
	Intermediate mix (system prepared)	Use this option if this mix will be created by the QIAgility during the run, but will be part of a more specific mix. During the run, the mix will be created first by the QIAgility. Then the QIAgility will pipette the mix into the tube where the more specific mix is created.
	Final reaction mix already on output	Use this option only if this mix has already been distributed onto the output labware before the run. In the later Worktable step you will not be prompted to load a tube with this ready-made mix. Instead, during the run, the QlAgility will not pipette the mix, but assume that the positions on the output labware already contain the mix. Output positions will be defined in the later Assignment step.
		If the option is selected, the Input screen of the dialog will be skipped.

3. The liquid class that will be used to distribute the reaction (master) mix during the run is preselected by the software. Change the liquid class only if it is really required. In this case, select the **Liquid class** that fits best to transfer the reaction (master) mix.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

4. Optionally, define advanced parameters. If you tick the box, additional parameters become visible to define extra volume and mixing options.

For reaction (master) mixes that will be created by the QIAgility, mixing options are displayed. Mixing is performed after the last reagent has been added to the reaction (master) mix, before the mix will be transferred. The mixing settings are defined in the **Liquid class for mixing**. This liquid class is already preselected by the software and should only be changed if really required. The **Mixing repetitions** – which are pre-defined by the selected liquid class for mixing – can be increased, if needed. The mixing cycles have been optimized to allow proper mixing for final reaction (master) mixes in which no pure Enzyme has been added as reagent. If your setup uses large volume final reaction (master) mixes or pure Enzymes as reagents, it is recommended to increase the mixing repetition to 15 or more.

Define advanced p	arameters
Extra volume - to gua	rantee proper pipetting and liquid level detection:
Extra volume	Extra volume per reaction
39 µl a	and 2.2 µl
Mix of system prepar	ed final reaction mix:
Liquid class for	mixing Repetitions (Min = 3)
Mix of reaction	n (master) mix (final) 🔹 🔹 10

For ready-made reaction (master) mixes the QIAgility does not perform a mixing step before transfer to output labware. Make sure the reaction (master) mix has been mixed properly before placing it onto the QIAgility.

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases, displayed loading volume is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover sample amount depending variations (e.g. volume loss per tip) and is added per single transfer. However, if you face any liquid level detection issues, increase **Extra volume per assignment** if issues are for higher sample amounts only, or increase absolute **Extra volume** if issues are not related to the sample amount.

5. Proceed with step 3 in <u>Mixes step</u>.

7.2.8.2.2 Add new mix dialog input

The **Input** screen of the **Add/Edit mix** dialog lists all input liquids/reagents of the mix and their volume per reaction in a table.

Add new mix					
General	General Contents of this mix				
Input >	reagen	all liquids/reagents and volume per reaction for respective reaction (r ts in "Liquids" screen can be selected. commended to sort the reagents of reaction (master) mix by volume p			
Target	volume	to ensure proper pipetting for final reaction mix. Use "Sort" button to ing to volume per reaction.	automatically sort reagents		
	Seq.	Name	Volume / reaction [µl]		
	1	Reaction mix 🔹	9 ×		
	2	Primer mix 🔹	9 8		
2 Primer mix ♥ 9 X					
Back Next Finish Cancel					

Add new mix dialog - Input

For a **ready-made mix** (i.e., a user prepared mix), the input liquid is already defined from the liquid dialog definition. Enter the volume per reaction in μ l into the **Volume/reaction** field in the table. Left-click the field and then enter the value. Initially, the field is highlighted in yellow to indicate that input is required.

For a **system prepared mix**, i.e., a mix that will be created by the QIAgility, define the composition of the mix:

- 1. Click the **Add input** button below the table. A new row is added to the table. Initially, the fields are highlighted in yellow to indicate that input is required.
- 2. In the **Name** field of the table, select the input liquid. The menu lists intermediate mixes and all liquids that are of liquid type **Reagent** or **Diluent/Water**. If a required liquid is not listed as expected, check

if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.

- 3. Define the volume per reaction for the input liquid. Left-click the **Volume/reaction** field and then enter the value. The total volume per reaction of the mix is displayed below the table. It summarizes the volumes per reaction of the listed reagents. For not integer volumes, use a point as a decimal delimiter.
- 4. Repeat steps 1–3, until all components of the mix are listed.
- 5. It is recommended to sort the reagents of the mix by volume per reaction, starting with the highest volume, to ensure proper pipetting for the final reaction mix. Later, during the run, the QIAgility will pipette the reagents into the tube where the mix is created in the order listed in the table.

Click the **Sort** button below the table, to automatically sort the list by volume per reaction, starting with the highest volume.

Alternatively, use the 🗠 💟 buttons below the table to sort the reagents individually. Left-click the row of the reagent to be moved. Click on the up and down arrows to move the currently selected reagent up or down.

Proceed with step 5 in <u>Mixes step</u>.

7.2.8.2.3 Add new mix dialog target

The **Target** screen of the Add/Edit mix dialog is optional. It is available for final reaction (master) mixes to define all assay targets and allow easy transfer of defined targets to respective PCR cycling software. If defined, targets are used in later steps for control, standard and sample definition. The information will also be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g. a PCR cycler.

Add new mix						
General						
Input	Define all assay targets to allow easy transfer of defined targets to respective PCR cycling software. If defined, targets are used in later steps for control, standard and sample definition. Target type: Test: Amplicon of interest (e.g., HIV); Reference: Amplicon used for data normalization (e.g., house-keeping gene used for delta Ct calculations); IC: Internal control sequence to monitor assay performance (e.g., inhibition of sample purification efficiency) – consider kit manual for assay-specific details Reporter: Enter fluorescence dye name (e.g., FAM) per target. Quencher: If applicable, enter quencher name (e.g., TAMRA) per target. Ensure correct notation (case sensitive) of reporter and quencher as required by the PCR cycling software.					
Target						
	Target name	Target type	Reporter	Quencher		
	Human	Test V	FAM V	None	×	
	Male	Test V	Cy5 🗸	None	×	
	IC		VIC	None	×	
Add target						
Back Next Finish Cancel						

Add new mix dialog - Target

If you do not need target information in the report or for the next instrument, proceed with step 6 in <u>Mixes</u> <u>step</u>.

To define targets:

- 1. Click the **Add target** button in the dialog. A new row will be displayed in the table.
- 2. Click into the **Target name** field and enter the name of the target. The name must be unique. It must be different to defined liquids, mixes, standards and controls.
- 3. Click on the menu in the **Target type** column and select a target type:

Target type		
Test 🔻		
Test		
Reference		
IC		

Target type

Description

Test	Amplicon of interest (e.g., HIV)
Reference	Amplicon used for data normalization (e.g., house-keeping gene used for delta CT calculations)
IC	Internal control sequence to monitor assay performance (e.g., inhibition of sample purification efficiency) – consider kit manual for assay specific details)

4. **Optional step:** Click on the menu in the **Reporter column** and select an appropriate reporter dye for the target.

Note: To use the QIAgility Wizard in the Q-Rex software for the Rotor-Gene Q, check the flourophore mapping in the Q-Rex software.

Reporter		
SYBR Green 1 SYBR Green 1 Cy5 Crimson ROX		
SYBR		2
Green	Yellow	Orange
FAM	JOE	ROX
SYBRGREEN1	VIC	CALFLUORRED610
SYBRGREENI	HEX	CY35
FLUORESCEIN	TET	TEXASRED
ALEXAFLUOR488	MAX	ALEXAFLUOR568
	CALFLUOR	
	GOLD540	
	YAKIMAYELLOW	
Red	Blue	Crimson
CY5	MARINABLUE	QUASAR705
QUASAR670	EDANS	ALEXAFLUOR680
ALEXAFLUOR633	BOTHELLBLUE	LIGHTCYCLERRED705
LIGHTCYCLERRED640	ALEXAFLUOR350	
	AMCAX	

Use this option for automated selection of the appropriate acquisition channel (fluorescence dye name, e.g., FAM) for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported reporter dyes. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the reporter can be left blank. In this case, the acquisition channel has to be selected manually in the cycler's instrument software.

Previously used reporters are listed in an editable menu. If the reporter you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the reporter as required by the PCR cycling software, e.g. in case of FAMTM, enter FAM, not FAM TM; when using SYBR Green I and RGQ PCR cycler with Q-Rex software, enter SYBR Green 1. The last entered reporters will be available for selection for future experiments. For more details about editable menus, refer to the section <u>Entering data</u>.

Note: Initially, the menu is empty.

5. **Optional step:** If applicable, click on the menu in the **Quencher** column and select an appropriate quencher for the target.

Use this option for automated selection of the appropriate quencher for your target in your cycler instrument software. Refer to the instructions for use for your cycler for more information regarding supported quenchers. After the run has been completed, <u>save the output</u> file and import the generated cycler file into your cycler instrument software.

Alternatively, the quencher can be left blank, if it is not required for the assay.

Note: The quenchers are listed in an editable menu. If the quencher you need is not listed, or if the list is empty, you can type it into the menu field. Ensure correct notation (case sensitive) of the quencher as required by the PCR cycling software. The last entered quenchers will be available for selection for future experiments. For more details about editable menus, refer to the section <u>Entering data</u>.

- 6. Repeat the steps above until all individual targets of the mix have been defined.
- 7. Proceed with step 6 in <u>Mixes step</u>.

7.2.8.3 Controls/Standards step

This optional step is used to define controls and standard series. If you do not intend to use controls or standards for the run, proceed by clicking **Next**.

The screen contains two tables: **Controls** and **Standard series**. Both tables have an **Actions** column with interactive buttons to manage the controls and standard series.

Note: When distributing defined controls (NTC and NC) and standards to the output labware during the run, the QIAgility will transfer the same volume as defined for samples/template in the later Samples Labware step.

Adding a control or standard series

- 1. Click on Add control or Add standard series.
- 2. The **Add control** or **Add standard series** dialog will open. For details, refer to <u>Add control</u> or <u>Add</u> <u>standard series</u>, respectively.
- 3. The control will be added to the **Controls** table, and the standard series will be added to the **Standard series** table.
- 4. Repeat steps 1–3 until all controls and standard series have been defined.
- 5. Finally, click **Next** to proceed.

Controls / Standards Control Samples / Labware Output Dilution Standards Worktable 1- Run 4-	Experiment Service							
Controls / Standards Control Samples / Labware Output Dilution Standards Worktable 1- Run 4-								
Mixes Seq Controls / Standards > Samples / Labware Assignment Output Dilution Stand Seq 1- 1- Worktable 4- Run	Define controls and st	tandards						
Controls / Standards > Controls / Standards > Controls / Standards > Controls / Standards > Controls / Labware Controls / Labwa	Controls							
Controls / Standards > Samples / Labware Assignment Dutput Stand See 1 Vorktable 4 Cun	Seq. Name		Reaction mix	Target		Target type	Control type	Actions
iamples / Labware Signment Vilution Vilution Vorktable Lun	1-1 NTC		Master Mix				NTC	0 🖪 🗙
n Seq	Dutput labware order 🖉							Add control
in 1-	Standard series							
Vorktable 4-	-	Reactio		rget	Target type	Tube type		Actions
un						Any available		
	4-8 Standard 2	Master	rMix			Any available		2 🖪 🗙
	Dutput labware order 🖉						Add sta	ndard series
			Back	Next >			Save 🗸	Start

Controls / Standards step

The buttons in the **Actions** column of the **Controls** table and the **Standard series** table have the following functions:

Button	Action To edit a control or standard series	Description The Edit control or Edit standard series dialog will open, displaying the values that have been entered in the Add control or Add standard series dialog.
Ľ	To duplicate a control or standard series	The Duplicate control or Duplicate standard series dialog will open, displaying the values that have been entered for the original control or standard series. The control or standard series names will initially display as a copy of the control or standard series.
×	To delete a control or standard series	Deletes the row in the Controls or Standard series table.

Changing the order of the output labware

You can change the order of the controls and standard series using the up and down arrows below each table.

Output labware order

Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.8.3.1 Add control

In the **Add control** dialog a new control can be defined. If you would like to change the values for an existing control at a later timepoint, the **Edit control** dialog will open. Both dialogs show the values of a control.

Add control		
Control		
NTC		
Liquid		
Water	▼	
Reaction mix		
e qPCR Master Mix	▼	
Control type		
NTC	•	
	Target	
Use specific volume		
	ок	ancel

Add control dialog

1. Enter a control name in the **Control name** field. The name must be unique (not similar to the name entered in the liquid screen). The name is used to refer to the control in later steps of the experiment setup, e.g. in the Assignment step, and in the loading instructions of the Worktable step.

- Select the input liquid from the Liquid menu. The menu lists all liquids that have been defined with liquid type Pos./neg. control or Diluent/Water. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 3. Select the reaction mix from the **Reaction mix** menu. The menu lists all mixes that have been defined with options as **Final reaction mix (ready-made)**, **Final reaction mix (system prepared)**, **Final reaction mix in output (ready-made)** or **Final reaction mix in output**.
- 4. Select the control type using the drop-down menu in the **Control type** column:

Control type				
Select control type	•			
Select control type				
PC				
NC				
NTC				

Option	Description
--------	-------------

- PC Is a positive control for the intended assay.
- NTC Is a non-template control for the intended assay.
- NC Is a negative control for the intended assay.

During run, the QIAgility will pipet the selected liquid to the output labware considering defined replicates and the sample volume which will be defined in the Samples/Labware step.

5. If targets have been defined for the selected reaction mix, the software lists all defined targets in a table below. Tick the box next to a target name if the target is valid for the control. Tick the box in the table header if you want to select all targets.

Note: If you intend to save an output file after run for import into a cycler software, e.g. Rotor-Gene© Q PCR cycler with Q-Rex software, always select all targets.

6. If the PC control type has been chosen, you can specify a custom volume for the control. Tick the **Use specific volume** box, type in a value in the **Volume** field, and select a diluent from the **Diluent (used to fill up to sample volume)** drop-down menu.

Add control
Control
PC
Liquid
Water T
Reaction mix
e qPCR Master Mix
Control type
PC V
Target
✓ Use specific volume
Volume Diluent (used to fill up to sample volume)
□ µI Diluent ▼
OK Cancel

Specifying control volume

- 7. Click **OK** to confirm and close the **Add control** dialog.
- 8. The control will be added to the **Controls** table.

7.2.8.3.2 Add standard series

In the **Add standard series** dialog a new standard series can be defined. If you would like to change the values for an existing standard series at a later timepoint, the **Edit standard series** dialog will open. Both dialogs show the values of a standard series. Define the basic properties of the standard series in the **General** screen.

Add standard series						
General >	Standard series name	Tube type				
Dilution Stone	Standard 161	Any available				
Dilution Steps	Standard Tor					
	Reaction mix	Use custom minimal volume for standard series.				
		-				
	PCR Master Mix	Use advanced pipetting parameters.				
	Use ready-made standard series					
	Standard stock liquid	Diluent				
	Standard 161	Diluent				
	Target and stock concentration					
	Target	Stock conc. Concentration unit				
	n/a 🛛	7 copies/µl 🔹 🗙				
		Define Std. stock				
	Back Next	Finish Cancel				
Add standard series dialog - General						

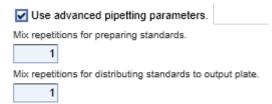
- 1. Enter a name for the series in the **Standard series name** field.
- 2. Select labware from the **Tube type** drop-down menu.
- 3. Select a reaction (master) mix, or multiple mixes, from the **Reaction mix** table by ticking the box next to the relevant mix.
- 4. Check the **Use custom minimal volume for standard series** option, if you intend to define a minimal volume value for the standard series. Enter the volume in the **Minimal volume per standard**

dilution field.

Use custom minimal volume for standard series.

Minimal volume per standard dilution

5. Check the **Use advanced pipetting parameters** option, if you would like to define the number of mix repetitions for preparing standards and distributing standards to the output plate. Enter the relevant values in the **Mix repetitions for preparing standards** and **Mix repetitions for distributing standards to output plate** fields.



6. Check the option **Use ready-made standard series**, if standards for a user prepared standard series have been defined in the **Liquids** step.

Uncheck the option if you plan to load one tube containing the standard stock and would like for the QIAgility to automatically create the dilutions for the standard series during the run.

- 7. If the option **Use ready-made standard series** has not been checked, select the appropriate liquid from the **Standard stock liquid** menu. The menu lists all liquids that have been defined with liquid type **Standard**. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- If the option Use ready-made standard series has not been checked, select the appropriate diluent from the Diluent menu. The menu lists all liquids that have been defined with liquid type Diluent/Water. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.
- 9. **Optional step.** Define the stock concentration, if you intend to define the standards of the series with their concentration. Otherwise, the standards of the series can be defined by dilution ratio, only.

If targets have been defined for the selected reaction mix, stock concentrations can be entered individually per target. This information will then also be part of the report and the output file, which can be saved after the run. If you intend to use the output file for import to a downstream instrument, e.g. a PCR cycler, define the concentrations for all available targets. For targets with no stock concentration, enter concentration value 0.

Target and stock concentration

Target	Stock conc.	Concentration unit	
Human 🛛	20	ng/µl	×
Select target	`	Select a concentratio	×
Select target Human			
Male			
IC			
			-
		Define S	Std. stock

Defining stock concentration per target.

If no targets have been defined for the mix, a common stock concentration can be defined, only.

Target and stock concentration

Target	Stoc	k conc.	Concentration unit		
n/a		20	Select a concentratio	×	
					ų.
			Define S		_

Defining common stock, if no targets have been defined for the mix.

Click the Define Std. stock button to add a stock concentration. A new row is displayed in the Targets table:

Column	Description
Target	Click the menu in the Targets field to select the appropriate target. If targets have been defined for the reaction mix, the menu lists all available targets. Otherwise, N/A is selected.
Stock conc.	Stock concentration.
	This column is available only if the option Use ready-made standard series has not been checked.
	Enter the concentration that is present in the stock liquid for the target.
Concentration unit	Click the menu in the Concentration unit field to select the concentration unit for the target. This unit is used in the report and the output file, which can be saved after run.

10. In the dialog click **Next** to proceed to the **Dilution steps** screen.

Add standard series - dilutions 7.2.8.3.3

Use the **Dilution steps** screen to define the dilution steps and dilution ratio for the serial dilution series. The dilution steps are displayed in a table.

Add standard series						
General	General Define dilution steps and dilution ratio for serial dilution series. In "Dilution ratio" column, the total dilution ratio based on the standard stock is displayed, but QIAgility will prepare a serial dilution series.					
Dilution Steps >	A dilution ratio of 1:1 means "no dilution".					
	Dilution s 6 v Dilution r 1:4	teps ✓ Use Standard series stock as first standard atio between two standards (optional) Update dilution ratios				
	Seq.	Name of standard	Dilution ratio			
	1	Control DNA	1:1			
	2	Standard Series 1	1:4			
	3	Standard Series 2	1:16			
	4	Standard Series 3	1:64			
	5	Standard Series 4	1:256			
	<u>م</u>	Standard Series 5				
Back Next Finish Cancel						

Add standard series dialog - Dilution steps

1. Select the number of dilution steps from the **Dilution steps** menu.



The number of rows in the table is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering</u> <u>data</u>.

2. If the option **Use ready-made standard series** has not been checked in the previous **General** screen, the additional option **Use Standard series stock as first standard** is available. Check the option in order to use the actual stock as the first standard. An additional row is automatically added to the table, showing a dilution ratio of 1:1 (meaning "no dilution"). During the run the QIAgility will directly use the stock as template for the reaction points of this standard.

Dilution steps

6 🔻

Use Standard series stock as first standard

Dilution ratio between two standards (optional)

Update dilution ratios

Seq.	Name of standard	Dilution ratio
1	Control DNA	1:1
2	Standard Series 1	1

Option "Use standard series stock as first standard" selected.

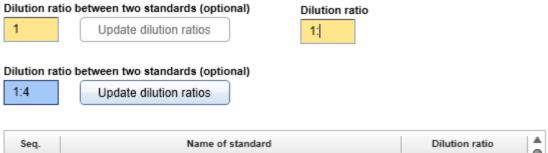
3. For each dilution step, if the option **Use ready-made standard series** has been checked in the previous **General** screen, select the appropriate liquid from the corresponding menu in the **Name of standard** cell.

Seq.	Name of standard	
1	Control DNA 🔹	
2	Standard1	
3	Select standard liquid	
4	Select standard liquid Control DNA Standard1	
5	Standard2	
6	Standard3 Standard4 Standard5	
4	Standard6	•



If the option **Use ready-made standard series** has not been checked in the previous **General** screen, optionally, enter a meaningful name into the **Name of standard** cell. This is especially important if you plan to define more than one standard series. The name must be unique. The name is used to refer to the standard in later steps of the experiment setup, e.g., in the Assignment step, and in the loading instructions of the Worktable step.

4. If an equal dilution ratio will be used throughout the standard series, define the dilution ratio in the **Dilution ratio** field above the dilution steps and then click **Update dilution ratios** to apply it to the series.



1	Control DNA	1:1	
2	Standard Series 1		
3	Standard Series 2		
	-	-	

Entering the dilution ratio, e.g. 1:4

Dilution ratio between two standards (optional)

- 4	:4	
	.4	

Update dilution ratios	s
------------------------	---

Seq.	Name of standard	Dilution ratio
1	Control DNA	1:1
2	Standard Series 1	1:4
3	Standard Series 2	1 : 16

After button "Update dilution ratios" has been clicked.

Alternatively, for each dilution step, enter either a dilution ratio or a concentration for the respective standard. The other value will be calculated automatically by the software.

Dilution ratio between two standards (optional)

|--|

	Name of standard	Dilution ratio	Concentration i
DNA	٦	1:1	20
d Series 1	1	1:4	5
d Series 2	1	`	
d Series 3		`	
d Series 4	•		
d Sariao 5	`	`	Enter Concen

Entering dilution ratio for second dilution step.

In the **Dilution ratio** column the total dilution ratio based on the standard stock is displayed. However – if the option **Use ready-made standard series** has not been checked – during the run the QIAgility will prepare a standard dilution series using the previous standard to create the subsequent standard.

If several targets have been defined in the previous **General** screen, concentrations are listed individually per target. If you enter one of the concentrations or the dilution ratio for a standard, all other values will be calculated automatically by the software.

lame of standard	Dilution ratio		Concentration i Human	Concentration i Male	
	1 : 16	٦	1.25	0.76875	ſ
	1:64	٦	0.3125	0.1921875	
	1 : 256	1	0.078125	0.048046875	
	1:1024	1	0.01953125	0.01201171875	
	1:4096	1	0.0048828125	0.0030029296875	l
•					

Concentrations listed individually per target

Note: Concentration values for dilution steps are available only if stock concentration has been defined in the previous **General** screen.

Note: This step is optional if the option **Use ready-made standard series** has been checked and no stock concentration has been defined in the previous **General** screen. If defined, this information will be part of the report and the output file, which can be saved after the run to be used for import to a downstream instrument, e.g., a PCR cycler.

- 5. In the dialog, click **Finish** to complete the definition of the standard series and to close the dialog.
- 6. Proceed with step 3 in <u>Controls/Standards step</u>.

7.2.8.4 Samples/Labware step

Use the Labware Selection screen to select the labware for sample input and run output and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file. The QIAgility Setup Manager Software supports up to three input labwares.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side. Define the sample pipetting below the input labware panel.

QIAGEN	Experiment Service Configuration	Instrument
PCR -experim		
Liquids	Define samples and labware	
Mixes	Input labware Output labware Samples will be provided on one single plate or adapter Samples will be provided on one single plate or adapter	
Controls / Standards	Samples will be provided on multiple plates or adapters (up to 2) Distribute assay points to single plates or adapters (up to 2)	
Samples / Labware 🗲	O Use a new input labware and enter sample names O Load sample input from file Load sample input from file Load sample input from file Select file	
Assignment	Labware Output labware type	
Dilution	QIAGEN Elution Microtubes CL T Use cooling block Samples are covered with top-elute Use cooling block Output labware is pre-filled with reagents	
Worktable		rint barcode
Run	Auto-generate sample IDs for all samples Output Converter Define samples Import data from CSV file	
	Sample Pipetting Sample volume Liquid class Use custom pipetting parameters B µt Sample to output ▼ Edit parameters Define dilutions	
	← Back Next → Save ▼	Start run
	August 30, 2019	admin admin 🛛 🔿 🗍

Samples / Labware step

Proceed as follows:

1. Select the input labware and define the samples.

If the samples will be on a single plate or adapter, check the option **Samples will be provided on one single plate or adapter** on the left side of the screen and follow the steps in <u>Define input</u> <u>labware</u>.

If the samples will be on several plates or adapters, check the option **Samples will be provided on multiple plates or adapters** and follow the steps in <u>Defining up to two input labware</u>.

2. Select the output labware.

If the output will be on a single plate or adapter, select the **Distribute assay points to single plate or adapter** option, and follow the steps in <u>Define output labware</u>.

If the output will be on several plates or adapters, select the **Distribute assay points to multiple plates or adapters** option, and follow the steps in <u>Define up to two output labware</u>.

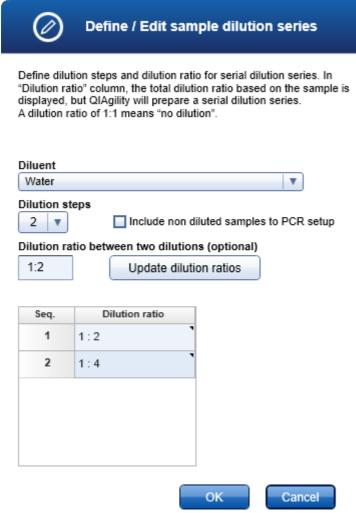
3. Enter the sample volume for the run into the **Sample volume** field in the **Sample pipetting** panel. The value must be specified in μ l.

Sample Pipetting		
Sample volume	Liquid class	Use custom pipetting parameters
8 µl	Sample to output	Edit parameters
Define dilution	s	

- 4. The liquid class used for sample transfer is predefined by default. If required, change the liquid class to be used for sample transfer by clicking the **Liquid class** dropdown menu. It defines the pipetting and tip handling for distribution of samples from the input labware to the output labware.
- 5. To define sample dilutions, click the **Define dilutions** button. The **Define/Edit sample dilution** series dialog will open. For details, refer to <u>Define sample dilution series</u>.
- 6. **Optional:** If required, use custom pipetting parameters for samples. If you tick the box, the **Edit parameters** button becomes active. If you click the button, the **Define advanced pipetting parameters** dialog opens, providing parameters for extra sample volume and mixing repetitions. For a detailed description refer to <u>Advanced sample pipetting</u>.
- 7. **Note:** Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. Change the parameters only if you face any liquid level detection issues.
- 8. Click **Next** to proceed.

7.2.8.4.1 Define/Edit sample dilution series dialog

The **Define/Edit sample dilution series** dialog is used for defining and editing dilution steps and the dilution ratio for serial dilution series.



- 1. Select a diluent from the **Diluent** drop-down menu.
- 2. Select the number of dilution steps from the **Dilution steps** drop-down list.

Dilution steps			
2]		
1	atio b		
2			
2 3			
4	۲		
5			
6			
7			
8	1::		
9			
10	1:-		

Note: The **Dilution steps** menu is an editable menu. If you want to work with more than 10 steps, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

- 3. To include non-diluted samples in the setup, tick the box next to **Include non diluted samples to PCR setup**.
- 4. To specify the dilution ratio, type in a ratio in the **Dilution ratio between two dilutions (optional)** field and click the **Update dilution ratios** button, or type in a ratio for each sequence in the **Dilution ratio** column.
- 5. Click **OK** and proceed with step 6 in <u>Samples/Labware step</u>.
- 7.2.8.4.2 Define input labware

If the option **Samples will be provided on one single plate or adapter** has been selected, define the input labware according to the following steps:

 If you would like to enter sample names manually, or, if you would like to auto-generate sample names, select Use a new input labware and enter sample names and proceed to step 2.
 If you would like to load a sample input file in csv format, select Use a new input labware and enter sample names and proceed to step 2.

Output laboration of the second se

O Load sample input from file

If you would like to load a sample input file in a QIAGEN specific xml file format (QDEF), select **Load** sample input from file and proceed to step 11.

If you would like to load a sample input file in txt format, select **Load sample input from file** and proceed to step 11.

- 2. Click on the Labware drop-down menu on the left side of the screen.
- 3. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

Labware
1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapere
1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered-Base Tubes
Choose other labware

4. Select your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

() Labware browser			
Filter options Category Standard 96 Well ▼ Standard 96 Well ▼	Labware Select the item that most closely matches the labware to be used QIAGEN 96 Weil Microplate RB QIAGEN Elution Microplate RS QIAGEN Selicet 98 Weil	Catalog number 19585 Name	
Advanced options		QIAGEN S-Block 96 Well Dead Volume 40 µl	1 6 12
Part number			A 00000000000 0000000000000000000000000
All V Tube / Well size All	Labware adapter Select the adapter to be used.		
Reset filter Apply filter	A	Catalog number Name	
	T		

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

5. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

7. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 9. Define the sample names. Check the option Auto-generate sample IDs for all samples if you want the software to automatically generate sample names for all positions on the labware. Alternatively, click the Define samples button to enter the sample names manually. It is also possible to edit the automatically generated sample names. For details about the sample editor refer to Edit samples.

Auto-generate sample IDs for all samples

Define samples ... Import data from CSV file...

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: It is also possible to define sample names first and then to import concentration values only from a csv file. In this case, define the sample names first, as described above, and then click **Import data** from CSV file.

10. Proceed with step 2 in <u>Samples/Labware step</u>.

To define samples by using a sample input file in QIAGEN specific xml (QDEF) format, follow these steps:

11. If the option **Load sample input from file** has been checked, the **Load sample input from file** and **Scan barcode** buttons will be enabled.

Use a new input labware and enter sample names
 Load sample input from file
 Load sample input from file...

Note: Files in txt format must adhere to the syntax described in File dialog.

12. Click the **Load sample input from file** button and select the desired sample file. For details, refer to <u>File dialog</u>.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

The software will load the sample information from the sample file.

If the sample information file also contains the labware type, it will automatically be selected in the Labware drop-down menu. In this case, the software will automatically display the sample editor. Proceed to step 16.

Note: This is only possible for files in QDEF format.

Note: If the **Labware** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

14. If the sample information file does not contain the labware type, select the labware manually from the **Labware** drop-down menu. If it is not listed, click **Choose other labware** to open the **Labware browser**.

(i) Labware browser			
Filter options Category Standard 96 Well ▼ ✓ Shows available labware only ▲ Advanced options Name of labware Part number Manufacturer All	Labware Select the item that most closely matches the labware to be QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	2 used Catalog number 19585 Name QIAGEN S-Block 96 Well Dead Volume 40 µl	1 6 12 A 000000000000000000000000000000000000
Tube / Well size All Reset filter Apply filter	Labware adapter Select the adapter to be used.	Catalog number Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

15. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

 Optional step: Edit the sample information. If the sample editor did not open automatically, click Define samples to open it. Click OK to confirm the sample information and to close the sample editor.

For details about the sample editor, refer to Edit samples.

- If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
 Note: Whenever you select another labware, this option will become unchecked again.
- 18. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 20. Proceed with step 2 in <u>Samples/Labware step</u>.

7.2.8.4.3 Edit samples

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.

	Sample editor		
Sele	ct an input labware identifier t	o enter, edit or	view the samples provided on that labware
Lab	ware identifier		
In	put1_20161205_101957		v
Pos.	Sample ID	Conc. [ng/µl]	Description
A1			in
A2			
A3			
A4			
A5			
A6			
A7			
A8			
B1			
B2			
B3			
B4			
B5	`		
B6			
B7			
B8			
C1			
C2	`		
C3			
C4	1		۲
			OK

Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.

Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column **Sample ID**. Press and hold the **SHIFT** key and left-click to select multiple adjacent cells, or press and hold the **CTRL** key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on **Auto-generate sample names**. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on **Auto-generate sample** names.

Сору	Ctrl+C
Paste	Ctrl+V
1 date	our v
Auto-generate sample names	

Note: For more details how to enter information into a table refer to the section Entering data.

Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

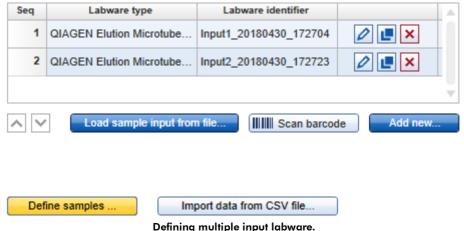
- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

7.2.8.4.4 Define up to two input labware

If the option **Samples will be provided on multiple plates or adapters (up to 2)** has been selected, you can define up to two input labware types.

All defined input labware will be listed in the **Sample input labware** table. Initially, the table is empty.





1. If you would like to enter sample names manually or if you would like to auto-generate sample names, select **Add new** and proceed to step 3.

If you would like to load a sample input file in csv format, select **Add new** and proceed to step 3.

If you would like to load a sample input file in QIAGEN specific xml file format (QDEF), proceed to step 2.

If you would like to load a sample input file in txt format, proceed to step 2.

Note: Files in txt format must adhere to the syntax described in File dialog.

 Click the Load sample input from file button and select the desired sample file. For details, refer to File dialog. Click Open to close the file dialog. The software will load the sample information from the selected sample file.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

The software will load the sample information from the sample file.

If the sample file does not contain the labware type, the **Add new input labware** dialog opens. In this case, proceed with step 3. Otherwise, proceed with step 4.

- 3. The Add new input labware dialog opens. Follow the steps in Add input labware dialog.
- 4. The new labware will be listed in the **Sample input labware** table.
- 5. Repeat steps 1–4 until all input labware has been defined.
- 6. Define the sample names. The **Define samples** button will be colored yellow to indicate that this information is required.

Click the **Define samples** button to enter sample names manually. It is also possible to edit the automatically generated sample names. For details, refer to <u>Editing sample names</u>.

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: This step is optional if the sample names were already loaded from a file or automatically generated.

7. Proceed with step 2 in <u>Samples/Labware step</u>.

The buttons in the right column of the **Sample input labware** table have the following functions:

Button	Action	Description
0	To edit an input labware	The Edit input labware dialog will open, displaying the values that have been entered in the Add input labware dialog.
e	To duplicate an input labware	The Duplicate input labware dialog will open, displaying the values that have been entered for the original input labware. The input labware identifier will initially display as a copy of the input labware.
×	To delete an input labware	Deletes the row in the Sample input labware table.

7.2.8.4.5 Define output labware

If the **Distribute assay points to single plate or adapter** option has been selected, define the output labware by following the steps below.

1. You can either **Use a new output labware** or **Reuse a partially used output labware by loading an import file**. The option **Use a new output labware** will be selected by default and can be used for most runs.

Use a new output labware

Reuse a partially used output labware by loading an import file

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case, select the option **Reuse a partially used output labware by loading an import file** and proceed to step 9.

2. Select the output labware from the **Output labware type** menu on the right. Click **Choose other labware** to open the **Labware browser**.

Output labware type	
Select output labware type	•
Select output labware type	
Choose other labware	

Iter options	Labware		
ategory Standard 96 Well T Shows available labware only	Select the item that most closely matches the labware QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name QIAGEN S-Block 96 Well	
Advanced options Name of labware		Dead Volume 40 μl	1 6
Part number			A 000000000000000000000000000000000000
All Tube / Well size		•	H 000000000000
All T	Labware adapter Select the adapter to be used.		
Reset filter Apply filter		Catalog number	
		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.
- 4. If the output labware is already pre-filled with liquid or dried reagents, indicate this by selecting the option **Output labware is pre-filled with reagents**. During the run, the QIAgility will consider this information and, e.g., not reuse tips.
- 5. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

6. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

7. **Optional:** If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to **Output converter** to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, **None** is the only entry in the list.

✓ Output Converter

 Select...
 ▼

 Select...
 ABI7500_QuantiplexHyres.xslt

 ABI7500_SDS_v1_4.xslt
 ABI7500_SDS_v2.3_HID_v1.2.xslt

 CE Loading (AL).xslt
 ConvertToCsv.xslt

 RGQ-SW_QREX.xslt
 RGQ-SW_QREX.xslt

Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

8. Proceed with Step 3 of the Samples/Labware step.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

9. The **Select file** and **Scan barcode** buttons will be enabled if the **Load existing output from file** option has been selected.

Use an empty output labware
Load existing output from file
Select file...

10. Click the Select file button and select the desired labware file. For details, refer to File dialog.

Alternatively, click the **Scan barcode** button. In the **Scan labware barcode** dialog, scan the barcode and confirm by clicking **OK**. For details, refer to <u>Scan labware barcode dialog</u>.

Using the information from the loaded file, the software will automatically select the output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

Note: If the **Output labware type** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 11. Proceed to step 3.
- 7.2.8.4.6 Define up to two output labware

If the option **Distribute assay points to multiple plates or adapters (up to 2)** has been selected, you can define up to two output labware types.

All defined output labware will be listed in the **Output labware** table. Initially, the table is empty.

Output labware

pe	Labware type	Labware identifier	繳	٨	
1	Eppendorf twin.tec PCR PI	Output1_20190730_102	~		0 🗉 🗙
2	QIAGEN S-Block 96 Well	Output2_20190730_102			0 🗉 🗙
	V Load output labware f	from file IIII Scan bar	code		Add new
				_	

Defining multiple output labware

1. If you would like to add output labware manually, click the **Add new** button and proceed to step 4. If you would like to load an output labware file, proceed to step 2.

If you would like to provide output labware information by scanning a barcode, proceed to step 3.

Click the Load output labware from file button and select the desired file. For details, refer to File dialog. Click Open to close the file dialog. The software will load the output information from the selected file.

If the file does not contain the labware type, the **Add new output labware** dialog opens. In this case, proceed to step 4. Otherwise, proceed to step 5.

- 3. Click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.
- 4. The Add new output labware dialog opens. Follow the steps in Add output labware dialog.
- 5. The new labware will be listed in the **Output labware** table.

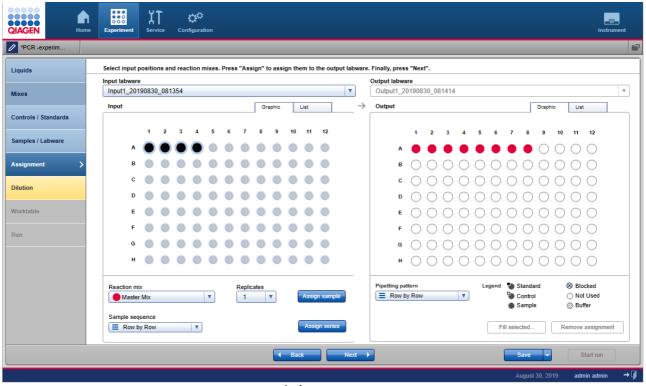
If the labware is to be used with the cooling block, the box in the ³ column will be ticked. If the labware is pre-filled with reagents, the box in the column will be ticked. To change these settings, edit the labware.

- 6. Repeat steps 1-5 until all labware has been defined.
- 7. Proceed with step 3 of the <u>Samples/Labware step</u>.

The buttons in the far right column of the **Output labware** table have the following functions:

Butto n	Action	Description
0	Edit the output labware	The Edit output labware dialog will open, displaying the values that were entered in the Add output labware dialog.
L	Duplicate the output labware	The Duplicate output labware dialog will open, displaying the values that have been entered for the original output labware. The output labware identifier will initially display as a copy of the input labware.
×	Delete the output labware	Deletes the row in the Sample output labware table.
7.2.8.	5 Assignment step	

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of sample-final reaction (master) mix combinations to the output labware.



Assignment step

Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware that have been selected in the **Input labware** menu. The meaning of the different symbols is described below:

•	Name Sample	Description Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been selected once for assay setup on output labware.
	Selected sample	Currently selected sample.
•		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
•		Note: It is possible to use the same sample multiple times to create reaction points on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. You cannot edit sample IDs that have already been assigned. You can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this sequence is used when assigning selected samples to the output labware.

If the option **Samples will be provided on multiple plates or adapters** has been selected, you can switch between the different input labwares using the box above the **Graphic** and **List** tabs.

At the bottom of the panel is a menu that lists all final reaction (master) mixes defined in the **Mixes** step, along with a **Replicates** menu, and a **Sample sequence** drop-down list.

In order to assign a sample for assay setup, select the sample position(s) and then the reaction (master) mix you would like to assign to the selected sample(s). Then set the number of replicates. When you click the **Assign** button, the selected samples and the reaction mix will be assigned to assay points created on the output labware. Follow the procedure described in <u>Assigning samples with reaction mixes to the output</u> <u>labware</u>.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. The creation of assay points containing samples, standards, and controls depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at the input side. The assay points are colored with the reaction mixes. Standards and controls are indicated by an additional small symbol. If you hover with the mouse cursor over an output position, the software displays the sample ID and the reaction mix for that position.

Assay points can be removed. For details, refer to <u>Removing assay assignments</u>.

The **Sample sequence** menu at the bottom of the output panel can be used to set the pipetting pattern to either row by row or column by column.

It is possible to drag assigned samples, controls and standards within the output labware if a specific layout is required. Blocked positions cannot be used. If you drag a sample, control or standard to a position that is already in use, the respective sample/control/standard at that position and all following are automatically shifted to the next position, depending on the defined pipetting pattern.

Furthermore, it is possible to define blocked positions on the output labware by selecting the position(s) and then right-click on it.

In order to fill up unused positions on the output labware, e.g., with a buffer for CE loading, select the positions to be filled on the output labware and then click **Fill selected**. In the upcoming dialog select the liquid and the volume. For a detailed description refer to <u>Fill empty output positions</u>.

Sample ID Liver 1 Liver 9 Liver 17 Lung 1 Lung 9		Reaction mix qPCR MasterN qPCR MasterN qPCR MasterN qPCR MasterN	Mix Mix		
iver 1 iver 9 iver 17 ung 1 ung 9		qPCR MasterN qPCR MasterN qPCR MasterN	Mix Mix		
iver 9 iver 17 .ung 1 .ung 9	• • • •	qPCR Master	Mix Mix		_(
.iver 17 .ung 1 .ung 9	•	qPCR Master	Mix		
.ung 1 .ung 9	•	•			
ung 9	•	qPCR Master	vix		
-					
upg 17		qPCR Master	Nix		
ung 17		qPCR Master	Vix		
Brain 1		qPCR Master	Vix		
Brain 9		qPCR Master	Vix		
Brain 17		qPCR Master	Vix		
iver 2		qPCR Master	Mix		
iver 10		qPCR Master	Mix		
iver 18	٠	qPCR Master	Mix		
g pattern	Lege	end 🐌 Stand	ard (Blocked	
olumn by Column	-	Contro	ol (○ Not Used	
				<u> </u>	
	ſ	Fill selected	Re	move assignme	ent
	g pattern	g pattern	iver 10 g pattern g pattern plumn by Column	arain 1 Image: Provide the standard st	irain 1 qPCR MasterMix qPCR MasterMix qPCR MasterMix qPCR MasterMix qPCR MasterMix qPCR MasterMix iver 2 qPCR MasterMix iver 10 qPCR MasterMix iver 18 qPCR MasterMix QPCR MasterMix gpattern Legend Standard Column by Column V Legend Standard Standard Standard Standard Blocked Standard Blocked Blocked Blocked Buffer

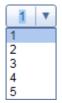
Use the **List** tab of the output panel to view the assay points that have been created.

The List tab of the Output panel

7.2.8.5.1 Assigning samples with reaction mixes to the output labware

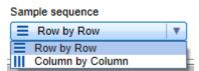
Proceed as follows:

- 1. From the **Input labware** menu on the left side of the screen, select an input labware or standards and controls that were defined in the previous **Labware selection** step. The software will display its defined samples below in the **Input** panel.
- 2. From the **Reaction mix** menu, select the reaction (master) mix you want to assign. The menu lists all final reaction (master) mixes that have been defined in the **Mixes** step.
- 3. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

4. From the **Sample sequence** drop-down list, select the sequence you want to use. You can choose from row by row and column by column.



Note: The sample sequence determines the order of the pipetting of the samples:

- If **Row by row** is selected, the first sample that is pipetted is the first sample in the first row, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the row and the second sample's replicates are pipetted and so on.
- If **Column by column** is selected, the first sample that is pipetted is the first sample in the first column, followed by its replicates, regardless of their placement on the output plate. The replicates are pipetted in the order specified in the Pipetting pattern (see step 7). After the replicates are pipetted, the second sample in the column and the second sample's replicates are pipetted and so on.
- 5. From the **Input panel**, select the sample position for which you would like to assign a reaction (master) mix. Initially, all previously defined positions are selected.

Note: To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions. Selecting in a rectangle is only possible on rectangular plates.

- 6. Click Assign.
- 7. The software creates assay points on the **Output** panel on the right side of the screen. The selected reaction (master) mix is assigned to the selected samples. If you have selected more than one replicate,

the sample positions are multiplied accordingly. The positioning of the created assay points on the output side follows the selections made for the **Pipetting pattern** and the sequence of samples from the sample sequence.

The samples are also listed in the **List** tab of the **Input** labware (in the **List** tab of the **Input** panel, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column or using the **Sample sequence** drop-down menu).

Note: The instrument follows a specified order when pipetting liquids. First, the master mix is pipetted, followed by the standards and controls, and then the samples.

8. Repeat the steps above until all positions of the output side have been defined for the planned run.

7.2.8.5.2 Removing assay assignments

To clear a position on the output side, follow these steps:

 Select the desired position on the **Output** side. Selecting multiple positions is possible by pressing SHIFT and left-clicking with the mouse to select adjacent positions or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.

Note: If you select empty positions along with filled positions, you cannot remove their assignments. It is only possible to remove assignments from multiple positions if all positions are filled.

- Click **Remove assignment**. The assignment will be removed, and the sample position will be cleared from the output side. Also, if the position was marked as filled with buffer, the position will be cleared.
 Note: The software will remove all replicates of the desired sample(s).
- 7.2.8.5.3 Assigning standards and controls to the output labware

The QIAgility Setup Manager Software allows you to assign the positions of standards and controls on the output plate.

Proceed as follows:

- 1. Select **Standards and Controls** from the **Input labware** drop-down list on the left side of the screen.
- 2. If you have defined one standard series, it will be automatically selected by the software, and a table with dilution steps and dilution ratios will be shown.

If you have defined multiple standard series, select one of them from the **Select standard series** dropdown list.

Input labware

Standards and Controls

ect standard series	
indard 651	1
indard 161	

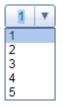
A table with dilution steps and dilution ratios will appear.

Sta	andard 651		•
	Standard 651	Dilution Ratio	
	Standard 651	1:1	
	Standard Stand	1:5	
	Standard Stand	1 : 25	
٦			•

- 3. Tick the box next to one or more dilution steps in the table. To select all of them, tick the box in the table header.
- 4. Select a mix from the Reaction mix drop-down list.



5. Select the number of replicates from the Replicates drop-down list.



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

- 6. Click Assign.
- 7. Tick the box next to one or more controls in the following table. To select all controls, tick the box in the

table header.

Controls	; Туре	
	NTC	
	I	

8. Select a mix from the Reaction mix drop-down list.

Reaction mix	
epcR MasterMix	•

9. Select the number of replicates from the Replicates drop-down list.



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

- 10. Click Assign.
- 11. Repeat steps 2 10 as needed, until you have defined all the positions for the planned run.

7.2.8.6 Dilution step

The **Dilution** step screen shows a summary of the dilution ratios defined in the **Samples/Labware** step and allows you to specify dilution labware.

	Internet Service Configuration	Instrument
PCR -experim		2
Liquids	Dilution Parameters	
Mixes	The following dilution ratios have been defined on the Samples / Labware screen: Seq. Dilution ratio	
Controls / Standards	1 1:2 2 1:4	
Samples / Labware		
Assignment	Dilution labware	
Dilution >	Labware QIAGEN S-Block 96 Well ▼ □ Cooling block required	
Worktable	Labware identifier Dilution_1_20190830_142817 Print barcode	
Run	Advanced pipetting parameters	
		art run
	August 30, 2019 adm	in admin 🛛 🔿 🚺

To choose the labware, proceed with the steps below:

1. Select the labware from the **Labware** drop-down menu. If it is not listed, click **Choose other labware** to open the **Labware browser**.

r options gory	Labware Select the item that most closely matches the labwa	re to be used	
andard 96 Well	QIAGEN 96 Well Microplate RB QIAGEN Elution Microfubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name QIAGEN S-Block 96 Well	
dvanced options me of labware		Dead Volume 40 µl	1 6
rt number			A
nufacturer		v	H 00000000000
All T	Labware adapter Select the adapter to be used.		
Reset filter Apply filter		Catalog number	
		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

2. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.

To define advanced pipetting parameters, tick the box next to Advanced pipetting parameters.

Advanced pipetting para	meters			
Liquid class for pipetting diluer	t onto dilution labware			
Diluent to dilution	▼			
Liquid class for pipetting samp	e onto dilution labware	No. of mix repetitions on dilute	1 labware	
Max. volume in dilution well 1650 µl	Min. volume in dilution well 20 µl	Min. diluent transfer volume 5 µl	Min. sample transfer volume	

You can specify the liquid class for pipetting the diluent or the sample onto dilution labware, the number of mix repetitions on the dilution labware, the maximal and minimal volumes in the dilution well, the minimal diluent transfer volume, and the minimal sample transfer volume.

If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).

Close the lid.

Click Next to enter the worktable step. If the instrument has not been used before, it will initialize.

Note: While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.

7.2.8.7 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- Worktable selector
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

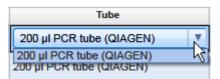
Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position. Click **Print loading instruction** to generate a PDF document with the loading instructions for the run.

0 µl 🔽 95 µl 🔽
95 ul 🔽
46 µl 🔽
57 µl 🔽

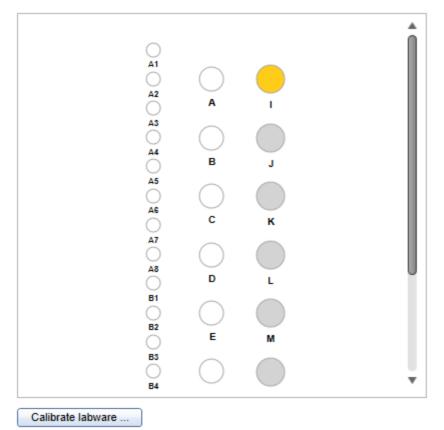
Table of tubes to be loaded to the worktable position M1.

It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware Manager**. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.



Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

Click the **Labware** tab on the right to get an image and details about the input and output labware.

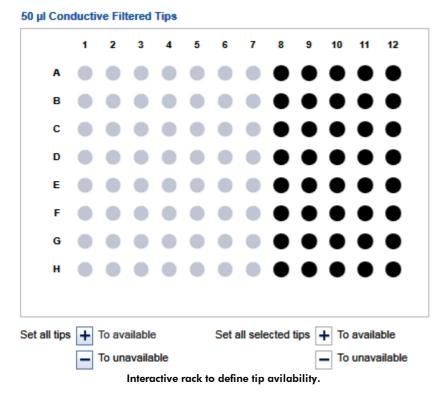
Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

Labware: 0.1 ml Strip Tube (QIAGEN) Adapter: Rotor-Gene Q, 72 x 0.1 ml Strip Tubes Adapter



Image and details for output labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To available** or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to Loading tips.

Note: To ensure optimal tip pickup, it is important to follow the instructions in Insert tips to tip rack

<u>holder</u>.

4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again.

5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to Loading tips.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 6. To confirm that the tip rack has been loaded, either check the box next to Loaded in the table or click on Confirm all to confirm. Refer to Loading tips for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

For worktable positions with samples:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

15. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

- 16. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 17. Ensure that samples are loaded using the correct labware type.
- 18. Load the samples in the correct positions as displayed in the table.
- Optional step: For sample tubes, click Validate sample loading to start sample loading validation using a hand-held barcode scanner and proceed with steps 20–23. Otherwise, proceed to step 24.
 Note: This button is only available if the corresponding option has been checked in the <u>User settings</u> for <u>Generic PCR experiments</u>.
- 20. The software will highlight the first sample position in the table. During validation, scanning is the only function available.

Note: To indicate that a user interaction is expected, a cursor with arrows in all four directions $\mathbf{\Phi}$ will be displayed for the entire validation.

- 21. Scan the barcode of the sample tube that has been loaded in the highlighted position.
- 22. If the barcode matches the sample's ID, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. If the barcode does not match the sample ID, a message is displayed. Click **Retry** in the message to scan the barcode again. If the barcode matches, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. To stop validation, click **Stop validating**, or, if the barcode did not match, click **Cancel** in the displayed message. In this case, proceed to step 24. There will be no documentation about sample loading validation in the report.
- 23. If all sample positions have been scanned successfully, a message is displayed. The successful sample loading validation will be documented in the report, which can be created after the run. Proceed to step 25.
- 24. Check that the samples are placed in the positions as displayed in the table.
- 25. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 26. Click **Next** to continue with the next worktable position.
- 27. Repeat steps 15–26 for all worktable positions with samples.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.8.8 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.8.8.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Task	Progress
Prepare standard series	
Prepare dilution plate	
Prepare mixes	
Distribute mixes to output labware	
Distribute controls to output labware	
Distribute standards to output labware	
Distribute samples to output labware	
	▼
Overall progress	
Start time 14:52	Approximate end time 14:57
Run progr	ess



The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.8.8.2 Pausing or canceling the run

A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	
Overall progress	¥	
Start time 13:05	Paused at Resume 13:21	

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.8.8.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.

Run info	
Operator	
admin admin	
Comment	
	ļ
Output labware	
Print barcode	Save output file
Save dilution plate)
	Create report

Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Save output file	An output file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>User</u> <u>settings</u> to define a standard directory.
	The file may serve as a sample input file for the Rotor-Gene Q operated with the Q-Rex Software. The .xml file can also be used by an LIMS (Laboratory Information Management System). A format description is available at <u>www.qiagen.com/goto/QIAgility</u> . You can find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QIAGEN\QIAgility\Examples.
	If an output converter has been selected in the Samples/Labware step, the software also creates a file in the format as defined by the output converter. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension (e.g., .txt for the ABI cycler), and will also be saved in the specified directory. In case the file extension is also .xml , the file name will be enhanced. The available output converters can be found in the output file converter directory, as specified in the <u>User settings</u> . Contact QIAGEN Technical Services if you need another file format.
	Note: Use this option also in case the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Samples/Labware step that you are Reusing a partially used output labware by loading an import file by selecting that option, and load the file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.
Save dilution plate	You can save an XML file with the contents of the dilution labware.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section <u>Cleaning the QIAgility</u>.

7.2.9 Other workflow experiments

The Generic PCR Plug-in provides further workflows: Normalization only

	and transferred to the output labware without transferring the reaction (master) mix.
Prepare reaction (master) mix	Use this workflow if only a reaction (master) mix will be created for later reuse.
Prepare and distribute reaction (master) mix	Use this workflow if a reaction (master) mix will be created and transferred to the output labware without transferring the samples.
Prepare standards	Use this workflow if only standards will be created for future use.

Use this workflow if samples will be normalized

7.2.10 Normalization only experiments

The Generic PCR Plug-in enables easy and convenient setup of runs for sample normalization only.

For generic PCR setup experiments, including sample normalization, refer to section <u>Generic PCR with</u> <u>normalization experiments</u>.

Samples can be normalized based on mass or concentration. Ensure that the correct selection has been made in the <u>User settings for Generic PCR experiments</u> before the experiment is created. Changing the setting will take effect for new experiments, only.

New experiments for sample normalization only are created by clicking **Other workflows** from the **Home** screen and then selecting **Normalization only**. The experiment will then be defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and the **Samples/Labware** step will be enabled initially. Subsequent steps will become available once the required information has been entered into the **Liquids** and **Samples/Labware** step. The **Samples/Labware** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 6 steps that guide you through the run setup process:

- 1. Liquids
- 2. <u>Samples/Labware</u>
- 3. Assignment
- 4. Normalization
- 5. <u>Worktable</u>
- 6. <u>Run</u>

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is ***.qsmrx**, and for templates ***.qsmrt** (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is ***.qsmrqt** (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.10.1 Liquids step

The **Liquids** step is used to define the diluent that will be used for normalization. Samples will be defined later in the **Samples/Labware** step. Optionally, liquids of type diluent/water can be defined that will be used to fill empty positions in the output labware later in the **Assignment** step.

🚊 QIAgility Setup Manager						
File Edit View Help 🖛 🦟	24					0
QIAGEN Home	Experiment Service Configu					 Instrument
Contraction of the second seco						
Liquids >	Define input liquids					
Mixes	Define required input liquids (reagent (master) mix, Standards (stock) and Predefined liquid classes (LC) define	controls) for assay setup. the pipetting/tip handling	Kit Name	Lot number 23	Some comment	A
Controls / Standards	task for liquid transfer (refer to LC tat screen). LC-naming: Liquid type + pipetting de For more information, press F1 key w	stination/indented use	Product number 123	Kit expiry date		v
Samples / Labware	Name		uid class	Tube type		Actions
Assignment	[] L1			Any available		
Worktable Run						Y
						New liquid
			Back Next		Save 👻	Start run
					August 21, 2019	admin admin → 🗍

Liquids step

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The Add liquid dialog opens.
- 3. Define the liquid as described in Add liquid dialog.
- In the dialog, click OK to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the Liquids screen.
 Note: Repeat the step until all needed liquids are listed.

Finally, click **Next** in the button bar to proceed to the **Samples/Labware** step (<u>Samples/Labware step</u>).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click 2 to open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the \frown buttons below the table:

- 1. Click the row of the liquid to be moved.
- 2. Click on the up and down arrows to move the currently selected row up or down.

Use this function for a better overview. The report will list the liquids in this order.

The buttons in the Actions column				
Button	Action	Description		
0	To edit a liquid	The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.		
		If you intend to change the liquid later, some restrictions apply. Not all values can be changed. In this case, remove assignments to enable changes for defined input liquids.		
Ŀ	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.		
×	To delete a liquid	Deletes the row in the table.		

7.2.10.1.1 Add liquid dialog

In the **Add liquid** dialog, a new input liquid of type diluent/water can be defined. If you would like to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values of a liquid.

Add liquid	
Liquid name Dilution buffer Tube type 1.5 ml tapered tube (QIAGEN) ▼ Liquid class Diluent for dilution ▼ Lot number	 Input liquid Ready-made mix Cap color Liquid type Diluent/Water Expiry date
Define advanced pipetting parameters	_/_/ 📰
Add liquid dialog	

- 1. Define the liquid's name. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, when defining normalization, and in the loading instructions of the **Worktable** step.
- 2. Click **Tube type** to select the tube that will be used to place this liquid on the QlAgility. The last used tube types are listed at the top of the list. To select one of the listed tube types, click the respective entry. Select the value **Any available**, if the liquid is not required to be provided in a special tube, e.g., coming from a kit. In this case, later, during worktable planning the software will automatically select a tube of the required size with minimal dead volume from the list of available tubes that has been defined in the Labware Manager (To define available tubes in the Labware Manager, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.). Use this option to give the software more flexibility during worktable planning.

If the required tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.

Filter options	Labware Select the item that most closely matches the labware to be used	sed.
Advanced options Name of labware Part number Manufacturer	1.5 mi tapered screw cap tube (QIAGEN) 1.5 mi tapered tube (QIAGEN) 2.0 mi self standing screw cap tube (QIAGEN) 200 µI PCR tube (QIAGEN) 5.0 mi flat-base tube (QIAGEN)	Catalog number 990552 Name 5.0 mil flat-base tube (QIAGEN) Dead Volume 26 µl
All Tube / Well size	Labware adapter	V
Reset filter Apply filter	Select the adapter to be used.	Catalog number Name

In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If you edit the liquid later and the **Tube type** is marked yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type, or make the tube available in the Labware Manager again.

3. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

In case the same liquid will be used for different purposes (e.g., a diluent used for normalization and to fill empty positions of the output labware) it is highly recommended to define multiple input liquids to allow precise definition of liquid class.

Note: If you edit the liquid later and the **Liquid class** is marked in yellow, this indicates that the selected liquid class is no longer available, e.g., if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

- 4. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, this data will be present in the report.
- 5. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

 In the dialog, click OK to confirm your values and to close the dialog. Otherwise, click Cancel to close the dialog and discard all values. In this case, a new liquid is defined, or, in case of editing an existing liquid, the liquid is not changed.

7.2.10.2 Samples/Labware step

Use the Labware Selection screen to select the labware for sample input and run output and to define the sample information (sample ID, concentration, description). Enter the sample IDs manually using the **Sample editor** or add the sample information by importing it from a sample input file. The QIAgility Setup Manager Software supports up to three input labwares.

The screen is split into two similar panels: define the input labware on the left side, and define the output labware on the right side. Define the sample pipetting below the input labware panel.

	Define samples and labware	
	Input labware	Output labware
abware >	Samples will be provided on one single plate or adapter	Distribute assay points to single plate or adapter
	Samples will be provided on one single plate or adapter Samples will be provided on multiple plates or adapters (up to 2)	 Distribute assay points to single plate or adapter Distribute assay points to multiple plates or adapters (up to 2)
	 Use a new input labware and enter sample names Load sample input from file 	 Use a new output labware Reuse a partially used output labware by loading an import file
	Load sample input from file	Select file
	Labware	Output labware type
	QIAGEN Elution Microtubes RS	QIAGEN 96 Well Microplate RB
	Use cooling block Samples are covered with top-elute	Use cooling block Output labware is pre-filled with reagents
	Labware identifier	Labware identifier
	Input2_20190730_122308 Print barcode	Output1_20190730_122256 Print barcod
	Auto-generate sample IDs for all samples	Output Converter
	Define samples Import data from CSV file	Select
	Output Pipetting	
	Output Volume Liquid class	
	10 µl Sample to output V Edit parameters	

Proceed as follows:

1. Select the input labware and define the samples.

If the samples will be on a single plate or adapter, check the option **Samples will be provided on one single plate or adapter** on the left side of the screen and follow the steps in <u>Define an input</u> <u>labware</u>.

If the samples will be on several plates or adapters, check the option **Samples will be provided on multiple plates or adapters** and follow the steps in <u>Defining up to 3 input labware</u>.

2. Select the output labware.

If the output will be on a single plate or adapter, select the **Distribute assay points to single plate or adapter** option, and follow the steps in <u>Define the output labware</u>.

If the output will be on several plates or adapters, select the **Distribute assay points to multiple plates or adapters** option, and follow the steps in <u>Define up to two output labware</u>.

3. Enter the output volume for the run into the **Output volume** field in the **Output pipetting** panel. The value must be specified in μ l.

Output	Pipetting
--------	-----------

Output Volume	Liquid class		Use custom pipetting parameters
5 µl	Sample to output	•	Edit parameters

Entering output volume

Note: It is recommended to use sample volumes of at least 5 μ l, as it is technically not reliable to transfer less than 5 μ l volume into an empty labware.

- 4. The liquid class used for sample transfer is predefined by default. If required, change the liquid class to be used for sample transfer by clicking the **Liquid class** dropdown menu. It defines the pipetting and tip handling for distribution of samples from the input labware to the output labware.
- 5. **Optional:** If required, use custom pipetting parameters for samples. If you tick the box, the **Edit parameters** button becomes active. If you click the button, the **Define advanced pipetting parameters** dialog opens, providing parameters for extra sample volume and mixing repetitions. For a detailed description refer to <u>Advanced sample pipetting</u>.

Note: Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. Change the parameters only if you face any liquid level detection issues.

6. Click **Next** to proceed.

The next step is the Assignment step.

7.2.10.2.1 Define an input labware

If the option **Samples will be provided on one single plate or adapter** has been selected, define the input labware according to the following steps:

 If you would like to enter sample names manually, or, if you would like to auto-generate sample names, select Use a new input labware and enter sample names and proceed to step 2.
 If you would like to load a sample input file in csv format, select Use a new input labware and enter sample names and proceed to step 2.

O Use a new input labware and enter sample names

O Load sample input from file

If you would like to load a sample input file in a QIAGEN specific xml file format (QDEF), select **Load** sample input from file and proceed to step 11.

If you would like to load a sample input file in txt format, select **Load sample input from file** and proceed to step 11.

- 2. Click on the Labware drop-down menu on the left side of the screen.
- 3. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

Labware

 1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Taperei

 1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap Tapered-Base Tubes Choose other labware...

 4. Select your input labware from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

() Labware browser			
Filter options Category Standard 96 Well ▼ Standard 96 Well ▼	Labware Select the liem that most closely matches the labware to be used QIAGEN 96 Well Microfubes RS QIAGEN Elution Microfubes RS QIAGEN Selicet 98 Well	Catalog number 19585 Name	
Advanced options		QIAGEN S-Block 96 Well Dead Volume 40 µl	1 6 12
Part number			A 00000000000 0000000000000000000000000
All V Tube / Well size All	Labware adapter Select the adapter to be used.		
Reset filter Apply filter	A	Catalog number Name	
	T		

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

5. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.

Note: Whenever you select another labware, this option will become unchecked again.

7. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 9. Define the sample names. Check the option Auto-generate sample IDs for all samples if you want the software to automatically generate sample names for all positions on the labware. Alternatively, click the Define samples button to enter the sample names manually. It is also possible to edit the automatically generated sample names. For details about the sample editor refer to Edit samples.

Auto-generate sample IDs for all samples

Define samples ... Import data from CSV file...

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: It is also possible to define sample names first and then to import concentration values only from a csv file. In this case, define the sample names first, as described above, and then click **Import data** from CSV file.

10. Proceed with step 2 in <u>Samples/Labware step</u>.

To define samples by using a sample input file in QIAGEN specific xml (QDEF) format, follow these steps:

11. If the option **Load sample input from file** has been checked, the **Load sample input from file** and **Scan barcode** buttons will be enabled.

Use a new input labware and enter sample names
 Load sample input from file
 Load sample input from file...

Note: Files in txt format must adhere to the syntax described in File dialog.

12. Click the **Load sample input from file** button and select the desired sample file. For details, refer to <u>File dialog</u>.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

The software will load the sample information from the sample file.

If the sample information file also contains the labware type, it will automatically be selected in the Labware drop-down menu. In this case, the software will automatically display the sample editor. Proceed to step 16.

Note: This is only possible for files in QDEF format.

Note: If the **Labware** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

14. If the sample information file does not contain the labware type, select the labware manually from the **Labware** drop-down menu. If it is not listed, click **Choose other labware** to open the **Labware browser**.

(i) Labware browser			
Filter options Category Standard 96 Well ▼ ✓ Shows available labware only ▲ Advanced options Name of labware Part number Manufacturer All	Labware Select the item that most closely matches the labware to be QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	2 used Catalog number 19585 Name QIAGEN S-Block 96 Well Dead Volume 40 µl	1 6 12 A 000000000000000000000000000000000000
Tube / Well size All Reset filter Apply filter	Labware adapter Select the adapter to be used.	Catalog number Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

15. **Optional step:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

 Optional step: Edit the sample information. If the sample editor did not open automatically, click Define samples to open it. Click OK to confirm the sample information and to close the sample editor.

For details about the sample editor, refer to Editing sample names.

- If your samples are covered with TopElute Fluid (to prevent samples from evaporation), indicate this using the corresponding check box. The QIAgility will pipet the samples accordingly.
 Note: Whenever you select another labware, this option will become unchecked again.
- 18. **Optional step:** If you would like to use the cooling block for the input labware, check the option **Use cooling block** next to **Labware**.

Note: Whenever you select another labware, this option will become unchecked again. If the newly selected labware cannot be used with the cooling block, the option will be disabled.

- Optional step: Click the Print barcode button to print a barcode of the labware identifier.
 Note: This option is only available if a printer has been defined in the Global Settings tab of the Configuration environment.
- 20. Proceed with step 2 in <u>Samples/Labware step</u>.

7.2.10.2.2 Edit samples

To define samples manually:

- 1. The **Sample editor** will be opened for the input labware indicated in the **Labware identifier** menu at the top.
- 2. If the samples have not yet been defined, for example, using the option **Auto-generate sample IDs** for all samples, the **Sample ID** fields and the **Labware identifier** will be colored yellow to indicate that this information is required.

	Sample editor		
Sele	ct an input labware identifier t	o enter, edit or	view the samples provided on that labware
Laby	ware identifier		
In	out1_20161205_101957		V
Pos.	Sample ID	Conc. [ng/µl]	Description
A1			
A2			
A3			
A4			
A5			
A6	1		
A7		`	
A8			
B1	1		
B2			
B3			
B4			0
B5			
B6			
B7			
B8			
C1			
C2			
C3			
C4	1	•	
			OK

Note: If you are using more than one input plate, at least one sample must be defined on each plate.

3. Enter each sample ID and optionally, enter the concentration and sample description.

Alternatively, you can automatically generate sample IDs using the context menu. Select the relevant cells in column **Sample ID**. Press and hold the **SHIFT** key and left-click to select multiple adjacent cells, or press and hold the **CTRL** key and left-click to select multiple non-adjacent cells. Then right-click in one of the selected cells to open the context menu and click on **Auto-generate sample names**. In order to use a common prefix for all generated sample IDs you can enter the prefix in the first cell for which you intend to generate sample IDs before selecting cells. Then right-click to open the context menu and click on **Auto-generate sample** names.

Сору	Ctrl+C
0000	04110
Paste	Ctrl+V
Auto-generate sample names	
Allo generale cample hamee	

Note: For more details how to enter information into a table refer to the section Entering data.

Note: If you return later to the sample editor, samples that have already been assigned in the Assignment step cannot be edited anymore. To edit those samples, remove their assignments first.

- 4. If you are using multiple input labware, select the next labware identifier from the **Labware identifier** menu at the top, and then repeat steps 3 and 4.
- 5. If samples are defined for each defined labware, the **Labware identifier** field will no longer be colored yellow, and the **OK** button will be enabled. If the Labware **identifier** field is yellow, that means that samples have not been defined for at least one labware. Go back to step 4 and define at least one sample for all labware.
- 6. Click **OK** to close the **Sample editor**.

7.2.10.2.3 Defining up to two input labware

If the option **Samples will be provided on multiple plates or adapters (up to 2)** has been selected, you can define up to two input labware types.

All defined input labware will be listed in the **Sample input labware** table. Initially, the table is empty.

Seq	Labware type	Labware identifier	
1	QIAGEN Elution Microtube	. Input1_20180430_172704	🖉 🖪 🗙
2	QIAGEN Elution Microtube	. Input2_20180430_172723	🖉 🖪 🗙
	Load sample input fro	om file	de Add new
	Load sample input fro	om file	de Add new
	Load sample input fro	om file	de Add new
<u> </u>		m file Scan barco	de Add new

1. If you would like to enter sample names manually or if you would like to auto-generate sample names, select **Add new** and proceed to step 3.

If you would like to load a sample input file in csv format, select **Add new** and proceed to step 3.

If you would like to load a sample input file in QIAGEN specific xml file format (QDEF), proceed to step 2.

If you would like to load a sample input file in txt format, proceed to step 2.

Note: Files in txt format must adhere to the syntax described in File dialog.

 Click the Load sample input from file button and select the desired sample file. For details, refer to File dialog. Click Open to close the file dialog. The software will load the sample information from the selected sample file.

Alternatively, if you would like to load the sample information based on the barcode on the labware, click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.

The software will load the sample information from the sample file.

If the sample file does not contain the labware type, the **Add new input labware** dialog opens. In this case, proceed with step 3. Otherwise, proceed with step 4.

- 3. The Add new input labware dialog opens. Follow the steps in Add input labware dialog.
- 4. The new labware will be listed in the **Sample input labware** table.
- 5. Repeat steps 1–4 until all input labware has been defined.
- 6. Define the sample names. The **Define samples** button will be colored yellow to indicate that this information is required.

Click the **Define samples** button to enter sample names manually. It is also possible to edit the automatically generated sample names. For details, refer to <u>Editing sample names</u>.

Alternatively, to load sample data from a file in csv format, click **Import data from CSV file**. The **Import data** dialog opens. Follow the steps in <u>Import data dialog</u>.

Note: This step is optional if the sample names were already loaded from a file or automatically generated.

7. Proceed with step 2 in <u>Samples/Labware step</u>.

The buttons in the right column of the **Sample input labware** table have the following functions:

Button	Action	Description
0	To edit an input labware	The Edit input labware dialog will open, displaying the values that have been entered in the Add input labware dialog.
Ŀ	To duplicate an input labware	The Duplicate input labware dialog will open, displaying the values that have been entered for the original input labware. The input labware identifier will initially display as a copy of the input labware.
×	To delete an input labware	Deletes the row in the Sample input labware table.

7.2.10.2.4 Define output labware

If the **Distribute assay points to single plate or adapter** option has been selected, define the output labware by following the steps below.

1. You can either **Use a new output labware** or **Reuse a partially used output labware by loading an import file**. The option **Use a new output labware** will be selected by default and can be used for most runs.

Use a new output labware

Reuse a partially used output labware by loading an import file

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case, select the option **Reuse a partially used output labware by loading an import file** and proceed to step 9.

2. Select the output labware from the **Output labware type** menu on the right. Click **Choose other labware** to open the **Labware browser**.

Output labware type			
Select output labware type	•		
Select output labware type			
Choose other labware			

Iter options	Labware		
ategory Standard 96 Well T Shows available labware only	Select the item that most closely matches the labware QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name QIAGEN S-Block 96 Well	
Advanced options Name of labware		Dead Volume 40 μl	1 6
Part number			A 000000000000000000000000000000000000
All Tube / Well size		•	H 000000000000
All T	Labware adapter Select the adapter to be used.		
Reset filter Apply filter		Catalog number	
		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.
- 4. If the output labware is already pre-filled with liquid or dried reagents, indicate this by selecting the option **Output labware is pre-filled with reagents**. During the run, the QIAgility will consider this information and, e.g., not reuse tips.
- 5. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

6. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

7. **Optional:** If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to **Output converter** to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, **None** is the only entry in the list.

✓ Output Converter

 Select...
 ABI7500_QuantiplexHyres.xslt
 ABI7500_SDS_v1_4.xslt
 ABI7500_SDS_v2.3_HID_v1.2.xslt
 CE Loading (AL).xslt
 ConvertToCsv.xslt
 RGQ-SW_QREX.xslt

Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

8. Proceed with step 3 in <u>Samples/Labware step</u>.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

9. The **Select file** and **Scan barcode** buttons will be enabled if the **Load existing output from file** option has been selected.

Use an empty output labware
Load existing output from file
Select file...
Scan barcode ...

10. Click the **Select file** button and select the desired labware file. For details, refer to <u>File dialog</u>.

Alternatively, click the **Scan barcode** button. In the **Scan labware barcode** dialog, scan the barcode and confirm by clicking **OK**. For details, refer to <u>Scan labware barcode dialog</u>.

Using the information from the loaded file, the software will automatically select the output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

Note: If the **Output labware type** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

11. Proceed to step 3.

7.2.10.2.5 Define up to two output labware

If the option **Distribute assay points to multiple plates or adapters (up to 2)** has been selected, you can define up to two output labware types.

All defined output labware will be listed in the Output labware table. Initially, the table is empty.

Output labware					
 Distribute assay points to single plate or adapter Distribute assay points to multiple plates or adapters (up to 2) Output labware 					
Seq	Labware type	Labware identifier	*		
1	Eppendorf twin.tec PCR PI	Output1_20190730_102	 Image: A state of the state of t		
2	QIAGEN S-Block 96 Well				
			Ŧ		
^	Load output labware t	from file	rcode Add new		

If you would like to add output labware manually, click the Add new button and proceed to step 4.
 If you would like to load an output labware file, proceed to step 2.

If you would like to provide output labware information by scanning a barcode, proceed to step 3.

Click the Load output labware from file button and select the desired file. For details, refer to <u>File</u> dialog. Click Open to close the file dialog. The software will load the output information from the selected file.

If the file does not contain the labware type, the **Add new output labware** dialog opens. In this case, proceed to step 4. Otherwise, proceed to step 5.

- 3. Click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.
- 4. The **Add new output labware** dialog opens. Follow the steps in <u>Add output labware dialog</u>.
- 5. The new labware will be listed in the **Output labware** table.

If the labware is to be used with the cooling block, the box in the ²⁰ column will be ticked. If the labware is pre-filled with reagents, the box in the ¹⁰ column will be ticked. To change these settings, edit the labware.

6. Repeat steps 1-5 until all labware has been defined.

7. Proceed with step 3 of the <u>Samples/Labware step</u>.

The buttons in the far right column of the **Output labware** table have the following functions:

Butto n	Action	Description
0	Edit the output labware	The Edit output labware dialog will open, displaying the values that were entered in the Add output labware dialog.
L	Duplicate the output labware	The Duplicate output labware dialog will open, displaying the values that have been entered for the original output labware. The output labware identifier will initially display as a copy of the input labware.
×	Delete the output labware	Deletes the row in the Sample output labware table.
7.2.10).3 Assignment step	

The **Assignment step** screen shows a graphic visualization of the input labware on the left and the output labware on the right. It is designed to support flexible and intuitive assignment of samples to the output labware.

-	Select input position and reaction mixes. Press	- •		Output labware				
abware	Input1_20190827_091713		•	Output1_201908	27_092544			
	Input	Graphic List		Output			Graph	ic List
t >			_		9	17 ()	25	33 ()
n	1 2 3 4 5 6 7	7 8 9 10 11 1	2		•••	17 🔿	25	33 ()
	▲ ● ● ● ○ ○ ○ () 0 0 0 0 0 0		2	● ¹⁰ ○	18 🔾	26 🔾	34 🔾
	B 0 0 0 0 0 0 0	$) \circ \circ \circ \circ \circ \circ$		3	● 11 O	19 🔾	27 🔿	35 🔿
	c () () () () () () () () () () () () ()	000000		4	12 ()	20 🔿	28	36 ()
		00000		5	13	21	29	0
		00000				0	\bigcirc	
	FOOOOO			6	● ¹⁴ ○	22 🔾	30 🔾	
	9000000			7) ¹⁵ ()	23 🔾	31 🔾	
	0000000			8 (→ ¹⁶ →	24	32 🔾	
	Replicates			Pipetting pattern		Legend	Sample	8 Blocked
	2 🔻			Column by C	olumn 🔻			O Not Used

Assignment step

Input

The **Graphic** tab of the **Input** panel on the left side of the screen shows all sample positions on the input labware that have been selected in the **Input labware** menu. The meaning of the different symbols is described below:

•	Name Sample	Description Position for which a sample ID has been defined (either using the Sample editor or from a sample file).
\bigcirc	Empty position	Position for which no sample IDs have been defined. The position cannot be used for the run.
•	Assigned sample	The sample has already been assigned once to output labware.
	Selected sample	Currently selected sample.
•		Note: It is possible to select samples and samples that have been already assigned at the same time.
	Selected assigned sample	Currently selected, already assigned sample.
•		Note: It is possible to use the same sample multiple times to create a dedicated layout on the output labware.

Use the **List** tab of the input panel to view or edit the sample definition again. In the **List** tab, you can change the sequence of samples for the input labware by clicking the column header **Pos.** of the position column. This might be required to guarantee a certain sample sequence on output labware, because this sequence is used when assigning selected samples to the output labware.

If the option **Samples will be provided on multiple plates or adapters** has been selected, you can switch between the different input labwares using the box above the **Graphic** and **List** tabs.

At the bottom of the panel is a **Replicates** menu and a **Sample sequence** drop-down list.

In order to assign a sample to the output labware, set the number of replicates, choose a sample sequence and select the sample position(s). When you click the **Assign** button, the selected samples will be assigned to the output labware. Follow the procedure described in <u>Assigning samples to the output labware</u>.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. Positioning of samples depends on the assignments you make in this step, and the output side will be updated when you confirm an assignment at the input side. If you hover with the mouse cursor over an output position, the software displays the sample ID for that position.

Samples can be removed from the output labware. For details, refer to Removing assignments.

The **Sample sequence** menu at the bottom of the output panel can be used to set the pipetting pattern to either row by row or column by column.

Furthermore, it is possible to define blocked positions on the output labware by selecting the position(s) and then right-clicking on it.

In order to fill up unused positions on the output labware, e.g., with a buffer for CE loading, select the positions to be filled on the output labware and then click **Fill selected**. In the upcoming dialog select the liquid and the volume. For a detailed description refer to <u>Fill empty output positions</u>.

Use the List tab of the output panel to view the samples on the output labware.

7.2.10.3.1 Assigning samples to the output labware

Proceed as follows:

- 1. From the **Input labware** menu on the left side of the screen, select an input labware that was defined in the previous **Labware selection** step. The software will display its defined samples below in the **Input** panel.
- 2. Click on the down arrow of the **Replicates** drop-down menu to define the number of replicates:



Note: The replicates menu is an editable menu. If you want to work with more than 5 replicates, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

3. From the **Input** panel, select the sample position that will be assigned to the output labware. Initially, all previously defined positions are selected.

Note: To select multiple samples, either press **SHIFT** and left-click with the mouse to select adjacent positions, **CTRL** and left-click with the mouse to select multiple, non-adjacent positions, or drag the mouse to select adjacent samples in a rectangle. Selecting in a rectangle is only possible on rectangular plates.

- 4. Click Assign.
- 5. The software displays the assigned samples on the **Output** panel on the right side of the screen. If you have selected more than one replicate, the sample positions are multiplied accordingly. The positioning on the output side follows the selection made for the **Sample sequence** in the **List** tab of the **Input** panel. You can also change the sequence of samples for the input labware by clicking the **Pos.** column header in the table in the **List** tab.
- 6. Repeat the steps above until all positions of the output side have been defined for the planned run.

7.2.10.3.2 Removing assignments

To clear a position on the output side, follow these steps:

- Select the desired position on the **Output** side. Selecting multiple positions is possible by pressing SHIFT and left-clicking with the mouse to select adjacent positions or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.
- Click **Remove assignment**. The assignment will be removed, and the sample position will be cleared from the output side. Also, if the position was marked as filled with buffer, the position will be cleared.
 Note: The software will remove all replicates of the desired sample(s).

7.2.10.4 Normalization step

In the **Normalization** step define the parameters for normalization. The QIAgility Setup Manager Software provides the option to normalize samples based on sample mass or sample concentration. When normalizing to a specific concentration, the sample will be diluted to the chosen target value and afterwards transferred to the output labware. In case normalization of a sample is not directly possible in the output position, the sample will be pre-diluted on a dilution labware. The software pre-calculates for each sample, if the target can be reached. Based on this calculation you can decide, if you want to include or exclude samples.

To configure if normalization is based on target sample mass or sample concentration, click **Configuration** in the main tool bar, click the **Settings** tab and then select the **Generic PCR user settings** step. For details refer to <u>settings</u>.

✓ *Experiment 0 ×		
Liquids	Define normalization parameters	
Mixes	Normalization parameters Target sample mass in output plate Max. sample vol. used for pre-dilution 0.1 ng 30 µl	 Within configuration, it can be defined if normalization for the assay is based on target mass or concentration. In case normalization of sample is not directly
Controls / Standards	Diluent Vater	possible in output well, the sample will be pre-diluted on a dilution labware. "Max sample vol. used for pre-dilution". Maximal template vol. used in dilution labware (doesn't affect template vol. used for final assay). Only input liguids defined as dilutent / vater can be selected as dilutent.
Samples / Labware	Dilution labware	Advanced pipetting parameters (change only if required): Liquid classes for normalization and no. of mixes after addition of template to dilution buffer are
Assignment	Labware 1.5 ml tapered tube (QIAGEN) / 32 x 1.5 ml Flip-Cap) Cooling block required	preselected. Max. vol. in dilution well: Restricts volume to guarantee homogenous mixing. Min. vol. in dilution well: Used to guarantee proper pipetting of dil. sample. Min diluent / sample transfer volume: Guarantees proper pipetting of diluent or
Normalization	Labware identifier Dilution_1_20180502_215959 Print barcode	sample. In case data reveal non-homogeneous mixing or inaccurate pipeting, parameters can be adjusted.
Worktable	Advanced pipetting parameters	
Run		
	View dilution details	
	Normalization step	

To define sample normalization:

- Enter the Target sample mass or concentration that shall be reached in the output labware.
 Note: When normalizing to target sample mass, the unit is predefined as "ng".
- 2. Enter into the field **Max. sample vol. used for pre-dilution** the maximal sample volume that shall be used in maximum for pre-dilution, e.i. the maximal template volume that will be used in the dilution labware to pre-dilute a sample.

Note: The higher the maximal sample volume for pre-dilution is set, the more flexible the software can calculate possible normalization strategies.

3. Click **Diluent** to select the diluent that shall be used for sample normalization. The menu lists all liquids that have been defined with liquid type **Diluent/Water** in the **Liquids** step. If a required liquid is not

listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.

4. Click **Labware** to select the labware that shall be used to pre-dilute samples. All previously selected labware types will be listed. The **Choose other labware** option will be listed last. Select your dilution labware from the drop-down list. If it is not listed, click **Choose other labware** to open the Labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

Filter options Category Standard 96 Well ✓ Shows available labware only Advanced options Name of labware Part number Manufacturer	Labware Select the item that most closely matches the labv QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well		Catalog number 19585 Name QIAGEN S-Block 96 Well Dead Volume 40 µl	
All Tube / Well size All	Labware adapter	¥		
Reset filter Apply filter	Select the adapter to be used.		Catalog number Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the Labware drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

- 5. **Optional:** If you would like to use the cooling block for the dilution labware, check the option **Cooling block required** next to **Labware**.
- 6. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

7. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

8. **Optional:** Define advanced pipetting parameters. If you tick the box, parameters become visible. In case the initial sample concentration is too high or too near to target, parameters can be adjusted.

Advanced pipetting param	eters		
Liquid class for pipetting diluent of Diluent to dilution	nto dilution labware		
Liquid class for pipetting sample Sample for dilution	onto dilution labware	No. of mix repetitions on dilution	on labware
Max. volume in dilution well	Min. volume in dilution well	Min. diluent transfer volume	Min. sample transfer volume

Option	Description		
Liquid class for pipetting diluent onto	Preselected.		
dilution labware	Defines the pipetting and tip handling for pipetting the diluent to the dilution labware.		
Liquid class for pipetting sample onto	Preselected.		
dilution labware	Defines the pipetting and tip handling for pipetting the sample to the dilution labware. Sample (template) will be pipetted after the diluent has been distributed.		
No. of mix repetitions on dilution	The value is predefined by the selected liquid class.		
labware	Defines the mix repetitions after the sample (template) has been added to the diluent on the dilution labware.		
	Mix repetitions have been developed to guarantee proper mixing of water based diluents and samples for the default value of maximal volume in dilution well. In case maximal volume in dilution well is manually increased, it is recommended to increase mix repetitions.		
Max. volume in dilution well	Predefined.		
	Restricts the volume in the dilution positions in order to guarantee homogeneous mixing.		
	In case the maximal volume in dilution well is manually iincreased, it is recommende to increase mix repetitions. If the total volume of the selected dilution labware is smaller than the setting of this parameter, the total volume of the dilution labware will be used.		
Min. volume in dilution well	Predefined.		
	Ensures proper creation of pre-dilution and pipetting of the		

	diluted sample to the output labware.
Min. diluent transfer volume	Predefined.
	Guarantees presize pipetting of diluent for pre-dilution. A setting $< 2\mu$ l is not recommended.
Min. sample transfer volume	Predefined.
	Guarantees presize pipetting of sample (template) for pre- dilution. A setting < 1 μ l is not recommended.

- Click View dilution details to open a list with the results of pre-calculation for the samples. The button is colored yellow, if the normalization target will not be reached for all samples.
 Follow the steps described in <u>Dilution details</u>.
- 10. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 11. Close the lid.
- 12. Click Next to enter the worktable step. If the instrument has not been used before, it will initialize. Note: While entering the Worktable step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.

7.2.10.4.1 Dilution details

The dialog lists the normalization pre-calculation for all samples. It is possible to enter individual normalization targets for output positions.

nclude	Exclude	Pos.	Sample ID	Target mass	Output Pos.	Labware Identifier	Orig.Con	Dilution strategy	Used sample volume
•	0	A1	Liver 1	50	A1	Output1_20190626_151657	518	Dilution uses 1 dilution position.	2
•	0	B1	Liver 2	50	B2	Output1_20190626_151657	475	Dilution uses 1 dilution position.	2
•	0	C1	Liver 3	50	C3	Output1_20190626_151657	440	Dilution uses 1 dilution position.	2
•	0	D1	Liver 4	50	D4	Output1_20190626_151657	438	Dilution uses 1 dilution position.	2
•	0	E1	Liver 5	50	E5	Output1_20190626_151657	439	Dilution uses 1 dilution position.	2
•	0	F1	Liver 6	50	F6	Output1_20190626_151657	668	Dilution uses 1 dilution position.	2
•	0	G1	Liver 7	50	D7	Output1_20190626_151657	580	Dilution uses 1 dilution position.	2
•	0	H1	Liver 8	50	B8	Output1_20190626_151657	668	Dilution uses 1 dilution position.	2
•	0	A2	Liver 9	50	B1	Output1_20190626_151657	488	Dilution uses 1 dilution position.	2
•	0	B2	Liver 10	50	C2	Output1_20190626_151657	548	Dilution uses 1 dilution position.	2
•	0	C2	Liver 11	50	D3	Output1_20190626_151657	2	Orig. concentration too low. Reachable mass in output = 16 ng.	8
•	0	D2	Liver 12	50	E4	Output1_20190626_151657	487	Dilution uses 1 dilution position.	2
•	0	E2	Liver 13	50	F5	Output1_20190626_151657	480	Dilution uses 1 dilution position.	2
•	0	F2	Liver 14	50	G6	Output1_20190626_151657	492	Dilution uses 1 dilution position.	2
•	0	G2	Liver 15	50	E7	Output1_20190626_151657	470	Dilution uses 1 dilution position.	2
•	0	H2	Liver 16	50	C8	Output1_20190626_151657	494	Dilution uses 1 dilution position.	2
•	0	A3	Liver 17	50	C1	Output1_20190626_151657	521	Dilution uses 1 dilution position.	2
•	0	B3	Liver 18	50	D2	Output1_20190626_151657	468	Dilution uses 1 dilution position.	2

Include all

Reasons why a sample cannot be diluted: Too less / high original conc. or original conc. is too near to target for chosen normalization parameter. Manually included sample will be transferred undiluted to final assay well using user-defined sample volume. Excluded sample will be unassigned and won't be pipetted at all. If sample-specific target mass or concentration is required, change target value accordingly.



Dilution details

- 1. Check the calculation. Find a description of the table columns, below.
- 2. **Optional:** You can enter an individual normalization target for positions on the output labware. Click into the **Target mass / concentration** field of the corresponding output position row and enter the new target value. The calculation for this position will automatically be updated.
- 3. For each sample that has yellow colored columns **Include/Exclude**, decide whether you want to include the sample into the run or not. Check the corresponding option. The sample position will no longer be colored yellow. To include all samples, click the Include all button below the table. For details refer to the description of the **Include** and **Exclude** columns, below.
- 4. **Optional:** Samples that have no yellow color indication in **Include/Exclude** column do not have any restrictions in regards to normalization, but can also be excluded, if needed.

- 5. If no sample has yellow colored **Include/Exclude** column, the **OK** button will be enabled.
- 6. Click **OK** to confirm your selections and to close the dialog.

Proceed with step 10 in Normalization step.

The table contains all samples from the input labware. Samples that have not been previously assigned in the Assignemt step are listed last.

Column	Description
Include	If checked, the sample will be included in the run.
	All samples, previously assigned in the Assignment step, which have no restriction to reach the normalization target, are automatically included. Otherwise, the columns Include/Exclude are highlighted in yellow to indicate that a decision by the user is required.
	Check the option to include the sample.
Exclude	If checked, the sample will not be used in the run and will be automatically unassigned.
	All samples, previously assigned in the Assignment step, which have a restriction to reach the normalization target, are marked in yellow to indicate that a decision (include or exclude) by the user is required.
	After confirming the dialog with OK, the sample gets automatically unassigned. The following positions on the output labware will be filled up according to the selected Pipetting pattern and Pipetting order from the Assignment step.
Pos.	The sample position on the input labware.
Sample ID	The sample ID.
	If the sample ID is too long, it is cut off. To view the whole sample ID, hover the mouse over the field.
Target mass/ Target conc.	The normalization target. Initially, the value that has been entered into the Normalization step is shown.
	However, you can edit the value if you need an individual normalization target for the corresponding output position. If no target value has been entered, or if the target value is too high or too low, the field is highlighted in yellow to indicate that a valid target needs to be entered.
Output pos.	The position on the output labware.
Orig. conc.	The original sample concentration as imported from the sample input file or entered in the sample editor.
Dilution strategy	The dilution strategy is automatically selected by the software and cannot be changed by the user.
	If the normalization target cannot be reached, the reason is listed. Decide whether to include or exclude the sample. For details, refer to the description below.

Note: It is possible to influence the dilution strategies by changing normalization parameters. The software always used the dilution strategy for which the lowest reagent volumes are required.

Used sample volume The sample volume used during normalization.

The dilution strategy depends on

- the value for the normalization target
- the original concentration of the sample
- the setting for maximum volume to be used for pre-dilution of a sample in the dilution labware
- the setting for minimum volume in the dilution well
- the sample volume for the run as specified in Samples/Labware
- the selected dilution labware (total volume and among of dilution positions)
- the maximum volume allowed in the dilution labware (advanced pipetting parameters for normalization)
- the minimum sample transfer volume (advanced pipetting parameters for normalization)
- the minimum diluent transfer volume (advanced pipetting parameters for normalization)

Dilution strategy	Description
Dilution in output position.	It is sufficient to dilute the sample directly in the output position within the defined sample volume for the assay setup.
Dilution uses 1 dilution position.	The sample will be diluted in one position of the dilution labware. Then the defined sample volume for the run will be transferred from the dilution labware to the output labware position.
Dilution uses 2 dilution positions.	The sample will be diluted using two positions of the dilution labware. Then the defined sample volume for the run will be transferred from the second dilution position on the dilution labware to the output labware position.
Dilution uses 1 dilution position and output position.	The sample will be pre-diluted in one position of the dilution labware. The last dilution step will be performed in the output position within the defined sample volume for the assay setup.
Original concentration too high.	The sample cannot be normalized to the given normalization target, because the original sample concentration is too high. The required dilution ratio cannot be created using the defined normalization parameters. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will be diluted to this value.
Original concentration too low.	The original sample concentration is lower than the normalization target. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will

The sample cannot be normalized to the given normalization target, because the defined normalization parameters do not allow such a small sample dilution ratio. Additionally, the mass/concentration that could be reached if you decide to include the sample, is listed. In this case the sample will be used undiluted. Otherwise, the normalization parameters have to be changed (e.g., increase the Max. sample
parameters have to be changed (e.g., increase the Max. sample volume used for pre-dilution value).

7.2.10.5 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



Worktable selector

- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

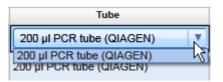
The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

Click Print loading instruction to generate a PDF document with the loading instructions for the run.

M1 - Mix block

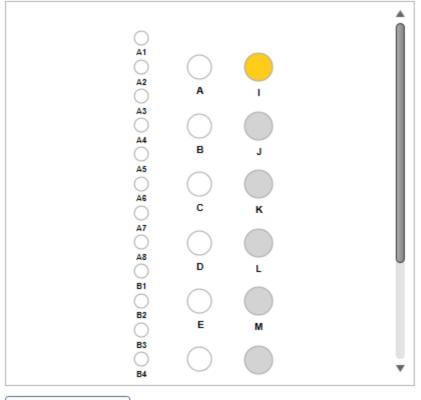
Pos.	Content	Tube	Req.vol.	Loaded
в	Diluent	1.5 ml tapered tube (QIAGEN)	1052 µl	✓
с	Diluent	1.5 ml tapered tube (QIAGEN)	1052 µl	~

It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware Manager**. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.



Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

Calibrate labware ...

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

Click the **Labware** tab on the right to get an image and details about the input and output labware.

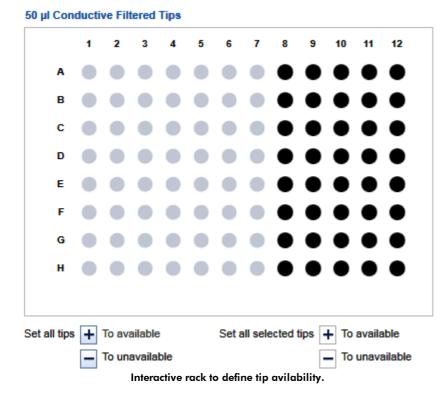
Note: In the image, the cooling block is not shown. If tubes or plates are used with an adapter and cooling block, the image will only show the tubes or plates and the adapter.

Labware: 0.1 ml Strip Tube (QIAGEN) Adapter: Rotor-Gene Q, 72 x 0.1 ml Strip Tubes Adapter



Image and details for output labware

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To available** or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> <u>holder</u>.

4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again.

5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 6. To confirm that the tip rack has been loaded, either check the box next to Loaded in the table or click on Confirm all to confirm. Refer to Loading tips for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

For worktable positions with samples:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

15. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

- 16. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 17. Ensure that samples are loaded using the correct labware type.
- 18. Load the samples in the correct positions as displayed in the table.
- Optional step: For sample tubes, click Validate sample loading to start sample loading validation using a hand-held barcode scanner and proceed with steps 20–23. Otherwise, proceed to step 24.
 Note: This button is only available if the corresponding option has been checked in the <u>User settings</u> for <u>Generic PCR experiments</u>.
- 20. The software will highlight the first sample position in the table. During validation, scanning is the only function available.

Note: To indicate that a user interaction is expected, a cursor with arrows in all four directions $\mathbf{\Phi}$ will be displayed for the entire validation.

- 21. Scan the barcode of the sample tube that has been loaded in the highlighted position.
- 22. If the barcode matches the sample's ID, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. If the barcode does not match the sample ID, a message is displayed. Click **Retry** in the message to scan the barcode again. If the barcode matches, the software highlights the next sample position in the table. Proceed to step 21 for the next sample. To stop validation, click **Stop validating**, or, if the barcode did not match, click **Cancel** in the displayed message. In this case, proceed to step 24. There will be no documentation about sample loading validation in the report.
- 23. If all sample positions have been scanned successfully, a message is displayed. The successful sample loading validation will be documented in the report, which can be created after the run. Proceed to step 25.
- 24. Check that the samples are placed in the positions as displayed in the table.
- 25. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 26. Click **Next** to continue with the next worktable position.
- 27. Repeat steps 15–26 for all worktable positions with samples.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.10.6 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the

current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

Monitoring the run 7.2.10.6.1

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress

Task	Progress
Prepare dilution plate	
Distribute dilution liquid to output labware	· · · · · · · · · · · · · · · · · · ·
Distribute samples to output labware	
	Y
Overall progress	
Start time	Approximate end time
15:12	15:16
Run progress	

Run progress

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.10.6.2 Pausing or canceling the run

A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	Pause
Overall progress		*
Start time 13:05	Paused at 13:21	Resume

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.10.6.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report or to save the output file.

Run info	
Operator	
John Smith	
Comment	
	Ļ
Output labware	
Print barcode	Save output file
Save dilution plate	
	Create report
Options af	ter the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Save output file	An output file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the output labware, the run, and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file. Refer to <u>User</u> <u>settings</u> to define a standard directory.
	The file may serve as a sample input file for the Rotor-Gene Q operated with the Q-Rex Software. The .xml file can also be used by an LIMS (Laboratory Information Management System). A format description is available at www.giagen.com/goto/QIAgility . You can find examples of output files in the "Examples" folder provided with the software: C:\Users\Public\QIAGEN\QIAgility\Examples.
	If an output converter has been selected in the Samples/Labware step, the software also creates a file in the format as defined by the output converter. This file will have the same name as the .xml file (i.e., the same name entered in the file dialog), but with another file extension (e.g., .txt for the ABI cycler), and will also be saved in the specified directory. In case the file extension is also .xml , the file name will be enhanced. The available output converters can be found in the output file converter directory, as specified in the <u>User settings</u> . Contact QIAGEN Technical Services if you need another file format.
	Note: Use this option also in case the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. Save the output file after the first run. To set up the second run properly, indicate in the Samples/Labware step that you are Reusing a partially used output labware by loading an import file by selecting that option, and load the file from the first run as a starting point for the output labware of the second run. After the second run, the information from both runs can be saved in an output file.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.
Save dilution plate	A file will be saved in a QIAGEN specific xml format, in particular the QDEF format, containing information about the dilution labware and the content of each labware position, e.g., the sample ID.
	A file dialog opens to select directory and file name of the .xml file.
Print barcode	You can print a barcode for the output labware.
	Note: The option is available only if a printer has been defined in the Global Settings tab of the Configuration environment.

After the run has finished, follow these steps:

1. Remove the labware from the worktable.

- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

7.2.10.7 Report

The Generic PCR Plug-in offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results
- Exceptions
- Detailed run information (incl. dilution labware)

The **Advanced report** contains:

- General information
- Comment
- Reaction results
- Exceptions/Pauses
- Sample information (incl. dilution strategy)
- Worktable information
- Detailed run information (incl. dilution labware)
- Advanced instrument information

The sections of both report types are described in <u>Report</u>. In addition, the report contains information about normalization parameters and dilution labware (both report types). Information about the dilution strategies used for the samples is available in the **Advanced** report, section **Sample information**. As the experiment setup does not contain mixes, standard series and controls, the corresponding information is not present in the report.

Additional information is described below.

Detailed run information

In addition to the information described in <u>Report</u>, this section contains further information for both report types. It lists the normalization parameters as defined in the **Normalization** step, and contains a table that describes the dilution labware:

Column	Description
Dilution position	The position on the dilution labware.
Identifier	Specifies the sample ID of the originating sample.
Туре	Sample.
Input	The corresponding position on the input labware. If multiple input labwares were used for the run, the position is enhanced by the input labware identifier.
Actual content	Lists the volume and the components.
Concentration	The sample concentration in the dilution labware position.
Flag	Shows the status of the position: either ok, warning or error.

Sample information

The normalization strategy for each sample is provided, in addition.

7.2.11 Prepare reaction mix experiments

The QIAgility allows you to setup a run to create a reaction (master) mix for later reuse.

In case the reaction (master) mix will also be distributed to output labware, refer to <u>Prepare and distribute</u> reaction mix experiments, instead.

New experiments for creating a reaction (master) mix only are created by clicking **Other workflows** from the **Home** screen and then selecting **Prepare reaction mix**. The experiment will then be defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and the **Mixes** step will be enabled initially. Subsequent steps will become available once the required information has been entered into the **Liquids** and the **Mixes** step. The **Mixes** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 4 steps that guide you through the run setup process:

- 1. Liquids
- 2. <u>Mixes</u>
- 3. <u>Worktable</u>
- 4. <u>Run</u>

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is ***.qsmrx**, and for templates ***.qsmrt**

(Experiment, and Template, respectively). The file extension for generic PCR quick order templates is *.gsmrgt (Quick Order Template).The Start run button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.11.1 Liquids step

The **Liquids** step is used to define all input liquids required to be loaded onto the instrument's worktable. These are all kind of reagents that will be part of the reaction (master) mix(es). In this step, you can also provide information about the kit that is used during the run.

QIAGEN	Experiment Service	Configuration				Instrument
*Experiment_1 ×						
Liquids >	Define input liquids					
Mixes	(master) mix, Standards Predefined liquid classes	uids (reagents or ready-made rea (stock) and controls) for assay se s (LC) define the pipetting/tip han efer to LC table in configuration	dling	Lot number	Comment	-
Worktable	screen). LC-naming: Liquid type	+ pipetting destination/intended u ess F1 key when editing liquids.	Se Product number	Kit expiry date		v
Run	Name	Liquid type	Liquid class	Tube type		Actions
	Fast reaction mix	Reagent	Viscous liquid to reaction (master) mix	Any available		
	Primer mix	Reagent	Primer-Probe or IC to reaction (master	Any available		
						New liquid
(Back Next) Save V Start run						
October 31, 2019 admin admin → I						
			Liquids step		000000101,2019	

Liquids step

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- Click the **New liquid** button below the table. 1.
- 2. The Add liquid dialog opens.

- 3. Define the liquid as described in <u>Add liquid dialog</u>.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

Finally, click Next in the button bar to proceed to the Mixes step (Mixes step).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click it to open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the \bigtriangleup buttons below the table:

- 1. Click the row of the liquid to be moved.
- 2. Click on the up and down arrows to move the currently selected row up or down.

Use this function for a better overview. The report will list the liquids in this order.

Actions

The buttons in the Actions column				
Button	Action	Description		
0	To edit a liquid	The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.		
		If you intend to change the liquid later,some restrictions apply. Not all values can be changed. In this case, remove the liquid from the reaction mixes to enable changes for defined input liquids.		
L	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.		
×	To delete a liquid	Deletes the row in the table.		

7.2.11.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you would like to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values of a liquid.

Add liquid	
Liquid name Dilution buffer	 Input liquid Ready-made mix
Tube type 1.5 ml tapered tube (QIAGEN)	Cap color
Liquid class Diluent for dilution	Liquid type Diluent/Water
Lot number	Expiry date
Define advanced pipetting parameters	
	OK Cancel
Add liquid dialog	

- 1. Define the liquid's name. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, e.g., when defining reaction mixes, and in the loading instructions of the **Worktable** step.
- The option Input liquid is automatically selected and cannot be changed.
 All liquids are input liquids, also if the liquid is a mix that has already been prepared by the user, but will be part of a more complex mix.
- 3. Click **Tube type** to select the tube that will be used to place this liquid on the QIAgility. The last used tube types are listed at the top of the list. To select one of the listed tube types, click the respective entry.

Select the value **Any available**, if the liquid is not required to be provided in a special tube, e.g., coming from a kit. In this case, later, during worktable planning the software will automatically select a tube of the required size with minimal dead volume from the list of available tubes that has been defined in the Labware Manager (To define available tubes in the Labware Manager, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.). Use this option to give the software more flexibility during worktable planning.

If the needed tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.

ter options itegory	Labware Select the item that most closely matches the labware to	be used.		
Tubes Tubes Advanced options	1.5 ml tapered screw cap tube (QIAGEN) 1.5 ml tapered tube (QIAGEN) 2.0 ml self standing screw cap tube (QIAGEN) 200 µl PCR tube (QIAGEN) 50 µl for the QIAGEN)		Catalog number 990552 Name 5.0 ml flat-base tube (QIAGEN)	1
Name of labware	5.0 ml flat-base tube (QIAGEN)	T	Dead Volume 26 µl	
Tube / Well size	Labware adapter			
	Select the adapter to be used.	A	Catalog number	
Reset filter Apply filter			Name	

In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If you edit the liquid later and the **Tube type** is marked in yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type, or make the tube available in the Labware Manager again.

4. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

Note: If you edit the liquid later and the **Liquid class** is marked in yellow, this indicates that the selected liquid class is no longer available, e.g., if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

5. Select the **Liquid type**. The liquid type defines how the liquid will be used.

Liquid type	Description
Reagent	Use this type, if the liquid is a reagent.
Diluent/Water	Use this type, if the liquid is a diluent or water.

- 6. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, this data will be present in the report.
- 7. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

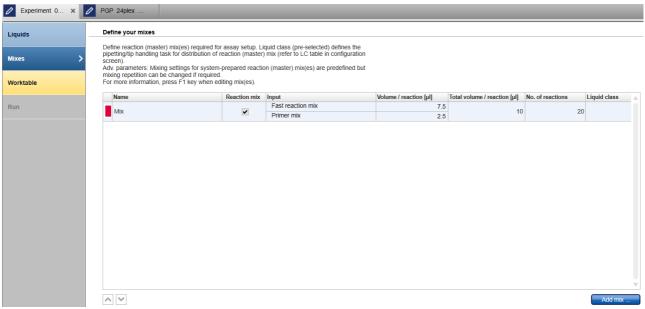
 In the dialog, click OK to confirm your values and to close the dialog. Otherwise, click Cancel to close the dialog and discard all values. In this case, no new liquid is defined, or, in case of editing an existing liquid, the liquid is not changed.

7.2.11.2 Mixes step

The **Mixes** step is used to define all kinds of mixes that are used for the experiment. These are, for example:

- all kinds of intermediate mixes. These are generic pre-mixes that will be used to create new (more specific) reaction (master) mixes.
- all variants of reaction (master) mixes, i.e., final reaction (master) mixes that will be generated by the QIAgility during the run.

The screen lists all mixes that have already been defined in a table.



Mixes step

Repeat the steps 1–7 until all final reaction (master) mixes have been defined. At least one final reaction (master) mix needs to be defined.

1. To define a new mix, click the Add mix button. The Add new mix dialog opens.

To edit a listed mix, click in the **Actions** column in the corresponding table row. The **Edit mix** dialog opens. It displays the values that have been entered before in the **Add mix** dialog.

Note: If no final reaction (master) mix has been defined, the **Add mix** button is highlighted in yellow to indicate that the definition of a final reaction (master) mix is still missing.

- 2. Define general properties of the mix. Details are described in Add new mix dialog.
- 3. In the dialog, click **Next** to proceed to the **Input** screen.
- 4. In the dialog **Input** screen define the volume per reaction and the content of the mix as described in <u>Add new mix input</u>.
- 5. Click **Finish** to finish the definition of the mix and to close the dialog.
- 6. The defined mix will be listed in the table in the Mixes screen.
- 7. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 8. Close the lid.

you enter the Worktable step.

Click Next to enter the worktable step. If the instrument has not been used before, it will initialize.
 Note: While entering the Worktable step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time

Note: The **Next** button is disabled, if the definition of a final reaction (master) mix is still missing.

The mixes in the table can be reordered using the \checkmark buttons below the table. The report will list the mixes in this order. An intermediate mix (generic pre-mix) needs to be listed before the (more specific) reaction (master) mix, of which it is part of. It will not be possible to move an intermediate mix below its reaction (master) mix.

- 1. Click the row of the mix to be moved to select it.
- 2. Click on the up and down arrows to move the currently selected row up or down.

The buttons in the Actions column				
Button	Action	Description		
0	To edit a mix	The Edit mix dialog will open, displaying the values that have been entered in the Add mix dialog.		
		If you intend to change a mix that is already used as input for another mix, some restrictions apply. Not all values can be changed anymore.		
L	To duplicate a mix	The Duplicate mix dialog will open, displaying the values that have been entered for the original mix. The mixes name will initially display a copy of the name of the original mix.		
×	To delete a mix	Deletes the row in the table.		

7.2.11.2.1 Add new mix dialog

In the **Add new mix** dialog a new mix can be defined. If you would like to change the values for an existing mix later, the **Edit mix** dialog will open. Both dialogs show the values of a mix.

Define the basic properties of the mix in the **General** screen of the dialog.

Add new mix					
General >	Name				
Input	Mix		nal reaction mix (system prepared) termediate mix (system prepared)		
		No. o	f reactions		
	A Back	Next	Finish	Cancel	
		Add mix dialog - Gene			

- 1. Define the mix's name. The name must be unique. The name is used to refer to the mix in later steps of the experiment setup, e.g., when defining more specific mixes, and in the loading instructions of the **Worktable** step.
- 2. Select, which kind of mix you define. Different options are available depending on the selection:

Option	Description
Final reaction mix	Use this option for the final mix that will be created by the QIAgility.
(system prepared)	If selected, the field No.of reactions will be visible. Proceed to step 3.
Intermediate mix (system prepared)	Use this option, if this mix shall be created by the QIAgility during the run, but will be part of a more specific mix. During the run, this mix will be created by the QIAgility first. Then the QIAgility will pipette the mix into the tube where the more specific mix is created.
	Proceed to step 4.

- 3. For final reaction (master) mixes, only: enter the number of reactions for which the reaction mix shall be prepared into the field **No. of reactions**. Proceed to step 6.
- 4. For intermediate mixes, only: the liquid class that will be used to distribute the reaction (master) mix during the run is preselected by the software. Change the liquid class only, if it is really required. In this case, select the **Liquid class** that fits best to transfer the reaction (master) mix.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

5. For intermediate mixes, only: optionally, define advanced parameters. If you tick the box, additional parameters become visible to define extra volume and mixing options.

Mixing is performed after the last reagent has been added to the reaction (master) mix, before the mix will be transferred. The mixing settings are defined in the **Liquid class for mixing**. This liquid class is already preselected by the software and should only be changed if really required. The **Mixing repetitions** – which are pre-defined by the selected liquid class for mixing – can be increased, if needed. The mixing cycles have been optimized to allow proper mixing for final reaction (master) mixes in which no pure Enzyme has been added as a reagent. If your setup uses large volume final reaction (master) mixes or pure Enzymes as reagents, it is recommended to increase the mixing repetition to 15 or more.

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases, displayed loading volume is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover sample amount depending variations (e.g. volume loss per tip) and is added per single transfer. However, if you face any liquid level detection issues, increase **Extra volume per assignment** if issues are for higher sample amounts only, or increase absolute **Extra volume** if issues are not related to the sample amount.

6. Proceed with step 3 in <u>Mixes step</u>.

7.2.11.2.2 Add new mix input

The **Input** screen of the Add/Edit mix dialog lists all input liquids/reagents of the mix and their volume per reaction in a table.

	Add new mix						
General	ntents of this mix						
rea It is vol	Define all liquids/reagents and volume per reaction for respective reaction (master) mix. All liquids defined as reagents in "Liquids" screen can be selected. It is recommended to sort the reagents of reaction (master) mix by volume per reaction, starting with highest volume to ensure proper pipetting for final reaction mix. Use "Sort" button to automatically sort reagents according to volume per reaction.						
S	eq. Name	Volume / reaction [µl]					
1	Fast reaction mix	▼ 7.5 ×					
2	Primer mix	▼ 2.5 [°] ×					
	Sort Calculated reaction mix volume/	reaction: 10 μl Add Input					
	Add mix dialog - Input						

To define the composition of the mix:

- 1. Click the **Add input** button below the table. A new row is added to the table. Initially, the fields are yellow to indicate that input is required.
- 2. In the **Name** field of the table, select the input liquid. The menu lists intermediate mixes and all liquids that are of liquid type **Reagent** or **Diluent/Water**.
- 3. Define the volume per reaction for the input liquid. Left-click the **Volume/reaction** field and then enter the value. The total volume per reaction of the mix is displayed below the table. It summarizes the volumes per reaction of the listed reagents. For non-integer volumes use a point as a decimal delimiter.
- 4. Repeat the steps 1–3 above, until all components of the mix are listed.
- 5. It is recommended to sort the reagents of the mix by volume per reaction, starting with the highest volume, to ensure proper pipetting for the final reaction mix. Later, during the run, the QIAgility will pipette the reagents into the tube where the mix is created in the order as listed in this table.

Click the **Sort** button below the table, to automatically sort the list by volume per reaction, starting with the highest volume first.

Alternatively, use the souther below the table to sort the reagents individually. Left-click the row of the reagent to be moved. Click on the up and down arrows to move the currently selected reagent up or down.

Proceed with step 5 in Mixes step.

7.2.11.3 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.

Worktable selector

• M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

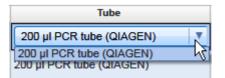
Click **Print loading instruction** to generate a PDF document with the loading instructions for the run.

M1 - M	ix b	lock
--------	------	------

Pos.	Content	Tube	Req.vol.	Loaded
в	Reaction mix (Mix)	1.5 ml tapered tube (QIAGEN)	0 µI	✓
с	Fast reaction mix	1.5 ml tapered tube (QIAGEN)	223 µl	✓
D	Primer mix	1.5 ml tapered tube (QIAGEN)	67 µl	~

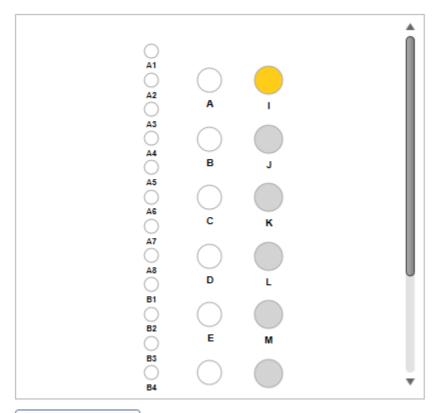
Table of tubes to be loaded to the worktable position M1.

It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware Manager**. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.

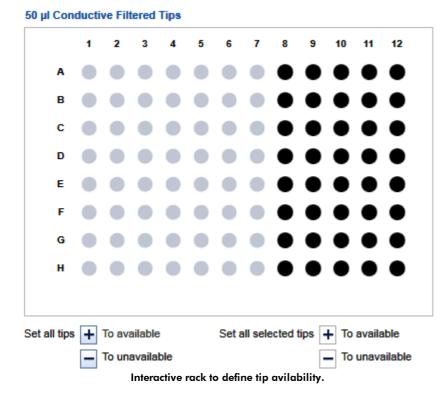


Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

Calibrate labware ...

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To available** or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> <u>holder</u>.

4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again.

5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 6. To confirm that the tip rack has been loaded, either check the box next to Loaded in the table or click on Confirm all to confirm. Refer to Loading tips for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

 Optional step: A yellow Calibrate labware button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.11.4 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.11.4.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress

Approximate end
1

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.11.4.2 Pausing or canceling the run

A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	
Overall progress	¥	
Start time 13:05	Paused at Resume 13:21	

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.11.4.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, the option to create a report is available on the right side of the **Run** step screen. This option will still be available if you reopen the experiment later.

Run info	
Operator	
John Smith	
Comment	
	$\overline{\mathbf{v}}$
Output labware	

Print barcode	Save output file
	Create report

Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

7.2.11.5 Report

The Generic PCR Plug-in offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

- General information
- Comment
- Reaction results (This section is empty.)
- Exceptions
- Detailed run information

The Advanced report contains:

- General information
- Comment
- Reaction results (This section is empty.)
- Exceptions/Pauses
- Sample information (This section is empty.)
- Worktable information
- Detailed run information
- Advanced instrument information

The sections of both report types are described in <u>Report</u>. As the experiment setup does not contain samples, standard series and controls, the corresponding information is not present in the report.

7.2.12 Prepare and distribute reaction mix experiments

The QIAgility allows to setup a run to create a reaction (master) mix and distribute it to up to three output labware, or to distribute a reaction (master) mix to the output labware only.

In case the reaction (master) mix shall only be created for later reuse, but not distributed to an output labware, refer to <u>Prepare reaction mix experiments</u>, instead.

New experiments for creating and distributing a reaction (master) mix are created by clicking **Other workflows** from the **Home** screen and then selecting **Prepare and distribute reaction mix**. The experiment will then be defined in the **Experiment** environment of the software. When a new experiment is created, only the **Liquids** and the **Mixes** step will initially be enabled. Subsequent steps will become available once the required information has been entered in the **Liquids** and the **Mixes** step. The **Mixes** step is colored yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the **Experiment** environment.

The experiment wizard has 6 steps that guide you through the run setup process:

- 1. Liquids
- 2. Mixes
- 3. Output labware
- 4. Assignment
- 5. <u>Worktable</u>
- 6. <u>Run</u>

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is ***.qsmrx**, and for templates ***.qsmrt** (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is ***.qsmrqt** (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.12.1 Liquids step

The **Liquids** step is used to define all input liquids required to be loaded onto the instrument's worktable required for your setup. These are, for example:

- all kinds of reagents or diluents/water, that will be part of reaction (master) mixes
- ready-made reaction mixes, i.e., final reaction (master) mixes that have already been prepared by the user and are ready to be used and distributed to the output labware by the QIAgility.

Detailed information for (final) reaction (master) mixes will be defined later in the Mixes step.

	Home		eriment	XT Service	Configura							Instrument
*Experiment_1 ×												
Liquids	>	Defi	ne input lie	quids								
Mixes	Define required input liquids (reagents or ready-made reaction					tup.	Kit information Kit Name		Lot number	Comment	^	
Output Labware		scre LC-r	en). naming: Liq	uid type + p	ipetting des	stination/intended us hen editing liquids.	e	Product number		Kit expiry date		×
Assignment			Name			Liquid type	Liquid	alace	Tub	be type		Actions
Worktable			Fast reacti	on mix		Reagent		us liquid to reaction (master) mix	-	ny available		
Run			Primer mix			Reagent	Prime	r-Probe or IC to reaction (master	Ar	ny available		
			V									New liquid
								Back Next	Þ		Save	Start run
											October 31, 2019	admin admin 🛛 🔿 🗍

Liquids step

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The Add liquid dialog opens.
- 3. Define the liquid as described in <u>Add liquid dialog</u>.
- 4. In the dialog, click **OK** to confirm your values and to close the dialog. The defined liquid will be listed as a new row in the table in the **Liquids** screen.

Note: Repeat the step until all needed liquids are listed.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

Finally, click **Next** in the button bar to proceed to the **Mixes** step (Mixes step).

Note: The **Next** button is disabled, if a row in the table is highlighted in yellow. Click open the **Edit liquid** dialog. In the dialog, the field is highlighted in yellow, which indicates the necessity for user action. If the field is not yellow anymore, click **OK** in the dialog to confirm the changes and to close it.

The liquids in the table can be reordered using the \frown \frown buttons below the table:

- 1. Click the row of the liquid to be moved.
- 2. Click on the up and down arrows to move the currently selected row up or down.

Use this function for a better overview. The report will list the liquids in this order.

A	tion	5
2		×

The buttons in the **Actions** column

Button	Action To edit a liquid	Description The Edit liquid dialog will open, displaying the values that have been entered in the Add liquid dialog.
		If you intend to change the liquid later, if, for example the liquid has already been used in a mix in the Mixes step, or, if the liquid is a ready-made reaction mix and has already been assigned to positions on the output labware in the Assignment step, some restrictions apply. Not all values can be changed. In this case, remove assignments or reaction mixes to enable changes for defined input liquids.
Ŀ	To duplicate a liquid	The Duplicate liquid dialog will open, displaying the values that have been entered for the original liquid. The liquid names will initially display a copy of the liquid.
×	To delete a liquid	Deletes the row in the table.

7.2.12.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you later want to change the values for an existing liquid, the **Edit liquid** dialog will open. Both dialogs show the same values of a liquid.

Add liquid	
Liquid name Dilution buffer Tube type 1.5 ml tapered tube (QIAGEN)	 Input liquid Ready-made mix Cap color
Liquid class Diluent for dilution	Liquid type Diluent/Water
Lot number	Expiry date
Define advanced pipetting parameters	
	OK Cancel

Add liquid dialog

- 1. Define the liquid's name. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup, when defining reaction mixes, and in the loading instructions of the Worktable step.
- 2. Select, if the liquid is an input liquid or a ready-made reaction/master mix. Initially, the option **Input liquid** is selected, because it fits to most liquids.

Select the option **Ready-made reaction/master mix** only, if the liquid is a final reaction/master mix that has already been prepared by user and is ready to be used and distributed to the output labware by the QlAgility. In this case, in the **Liquid type** field the value **Ready-made mix** is automatically selected and the liquid class menu is removed (the liquid class is defined later in the Mixes step). Additionally, when entering the later Mixes step, the reaction (master) mix is already listed. If the liquid is a mix that has already been prepared by user, but will be part of a more complex mix that shall be created during run by the QlAgility, select the option **Input liquid**.

3. Click **Tube type** to select the tube that will be used to place this liquid on the QIAgility. The last used tube types are listed at the top of the list. To select one of the listed tube types, click the respective entry.

Select the value **Any available**, if the liquid is not required to be provided in a special tube, e.g., coming from a kit. In this case, later, during worktable planning the software will automatically select a tube of the required size with minimal dead volume from the list of available tubes that has been defined in the Labware Manager (To define available tubes in the Labware Manager, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.). Use this option to give the software more flexibility during worktable planning.

If the needed tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.

Iter options	Labware		
Ategory Tubes Tube	Select the item that most closely matches the labware to 1.5 ml tapered screw cap tube (QIAGEN) 1.5 ml tapered tube (QIAGEN) 2.0 ml self standing screw cap tube (QIAGEN) 200 µl PCR tube (QIAGEN) 5.0 ml flat-base tube (QIAGEN)	Catalog number 990562 Name 5.0 ml flat-base tube (QIAGEN) Dead Volume 26 µl	
All Tube / Well size All Reset filter Apply filter	Labware adapter Select the adapter to be used.	Catalog number Name	

In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If you edit the liquid later and the **Tube type** is marked in yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type or make the tube available in the Labware Manager again.

4. Select the **Liquid class** that will be used to pipette the liquid during he run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

In case the same liquid is intended to be used for different purposes it is highly recommended to define multiple input liquids to allow precise definition of liquid class and liquid type.

Note: If you edit the liquid later and the **Liquid class** is marked in yellow, this indicates that the selected liquid class is no longer available, e.g., if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

5. Select the **Liquid type**. The liquid type defines how the liquid will be used. In later steps during experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection, e.g., when defining reaction (master) mixes.

Liquid type	Description
Reagent	Use this type, if the liquid is a reagent that will be added to a reaction/master mix, either to a final reaction mix or to an intermediate reaction mix.
Diluent/Water	Use this type, if the liquid is a diluent or water.
	Later in the experiment setup, a liquid of this type is available for content definition of a reaction/master mix in the Mixes step, or in the Controls/Standards step as diluent for defining a standard series that shall be created by the QIAgility.
Ready-made mix	Use this type, if the liquid is a ready-made reaction/master mix prepared by user. This type is preselected by the software, if the option Ready-made mix has been selected for the liquid.
	Later in the Mixes step, when you define the content for a mix, a liquid of this type is available for selection.

- 6. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, this data will be present in the report.
- 7. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

 In the dialog, click OK to confirm your values and to close the dialog. Otherwise, click Cancel to close the dialog and discard all values. In this case, no new liquid is defined, or --in case of editing an existing liquid-- the liquid is not changed.

7.2.12.2 Mixes step

The **Mixes** step is used to define all kinds of mixes that are used for an experiment. These are, for example:

- all variants of reaction (master) mixes, i.e., final reaction (master) mixes that
 - o have already been prepared by the user and are ready to be used,
 - o will be generated by the QIAgility during run,
 - o and will be distributed to the output labware.
- all kinds of intermediate mixes. These are generic pre-mixes that will be used to create new (more specific) reaction (master) mixes.

The screen lists all mixes that have already been defined in a table. User prepared reaction (master) mixes are automatically listed, if the corresponding liquid has been defined in the previous Liquids step. The rows for these mixes are initially highlighted in yellow to indicate that the mixes need further specification.

*Experiment 0 ×							_
quids	Define your mixes						
ixes >	Define reaction (master) mix(es) required for pipetting/tip handling task for distribution of screen). Adv. parameters: Mixing settings for system						
utput Labware	mixing repetition can be changed if require For more information, press F1 key when e	ed.					
	Name	Reaction mix	Input	Volume / reaction [µl]	Total volume / reaction [µl]	Liquid class	Actions
signment	Mix		Fast reaction mix	7.5	40	Deastion (maste	
	IVIIX	~	Primer mix	2.5	10	Reaction (maste	
1							
							Add mix
			Mixes step				

Defining a new mix or editing a listed mix:

1. To define a new mix, click the Add mix button. The Add new mix dialog opens.

To edit a listed mix, click in the **Actions** column in the corresponding table row. The **Edit mix** dialog opens. It displays the values that have been entered before in the **Add mix** dialog or that have been automatically pre-filled by the software for a ready-made reaction (master) mix.

Note: If no final reaction (master) mix has been defined, the **Add mix** button is highlighted in yellow to indicate that the definition of a final reaction (master) mix is still missing.

- 2. Define the general properties of the mix. Details are described in <u>Add new mix dialog</u>.
- 3. In the dialog, click **Next** to proceed to the **Input** screen.
- 4. In the dialog **Input** screen define the volume per reaction and the content of the mix; as described in <u>Add new mix input</u>.
- 5. Click **Finish** to finish the definition of the mix and to close the dialog.
- 6. The defined mix will be listed in the table in the **Mixes** screen.

Repeat the steps above until all final reaction (master) mixes have been defined.

Finally, click **Next** in the button bar to proceed to the **Output Labware** step, described in <u>Output labware</u> step.

Note: The Next button is disabled, if a row in the table is highlighted yellow. User prepared reaction

(master) mixes are initially highlighted yellow, if the volume per reaction has not yet been defined. Edit() the mix and enter the volume per reaction in the Input screen of the dialog. The **Next** button is also disabled, if the definition of a final reaction (master) mix is still missing.

The mixes in the table can be reordered using the \bigtriangleup buttons below the table. The report will list the mixes in this order. An intermediate mix (generic pre-mix) needs to be listed before the (more specific) reaction (master) mix of which it is part of. It will not be possible to move an intermediate mix below its reaction (master) mix.

- 1. Click the row of the mix to be moved to select it.
- 2. Click on the up and down arrows to move the currently selected row up or down.

The buttor	The buttons in the Actions column			
Button	Action	Description		
0	To edit a mix	The Edit mix dialog will open, displaying the values that have been entered in the Add mix dialog.		
		If you intend to change a mix that is already used as input for another mix, or if it is a final reaction mix and has already been assigned to positions on the output labware in the Assignment step, some restrictions apply. Not all values can be changed anymore.		
Ŀ	To duplicate a mix	The Duplicate mix dialog will open, displaying the values that have been entered for the original mix. The mixes name will initially display a copy of the name of the original mix .		
×	To delete a mix	Deletes the row in the table.		

7.2.12.2.1 Add new mix dialog

In the **Add new mix** dialog a new mix can be defined. If you later want to change the values for an existing mix, the **Edit mix** dialog will open. Both dialogs show the values of a mix.

Define the basic properties of the mix in the **General** screen of the dialog.

-		Add new mix		
General 📏	Name			
Input	Міх	 Final reaction mix Intermediate mix 		
		No. of reactions		
		Next	Finish	Cancel
	Ad	d mix dialog - General		

1. Define the mix's name. The name must be unique. The name is used to refer to the mix in later steps of the experiment setup, e.g., when defining more specific mixes, and in the loading instructions of the Worktable step.

For a ready-made mix, the name is already defined from the liquid dialog definition.

2. Select, which kind of mix you define. For ready-made mixes and other mixes different options are available:

Option	Description
Final reaction mix (ready-made)	Only available for a mix which has been defined as ready-made mix within the Liquid step.
	Use this option, if this mix has already been prepared by the user and is ready to be used and distributed to the output labware by the QIAgility. In the later Assignment step, the mix will be available for selection. During the run, the QIAgility will pipette the mix to the positions on the output labware as assigned in the Assignment step.
	Ensure that the reaction (master) mix is homogenously mixed before it is loaded to the QIAgility as the QIAgility will not perform any mixing steps.
Intermediate mix (ready- made)	Only available for a mix that has been defined as a ready-made mix within the Liquid step.
	Use this option if this mix has already been prepared by the user and will be part of a more specific mix that will be created during the run by the QIAgility. During the run, the QIAgility will pipette the mix into the tube where the more specific mix is created.
	Ensure that the reaction (master) mix is homogenously mixed before it is loaded to the QIAgility as the QIAgility will not perform any mixing steps.
Final reaction mix (system prepared)	Use this option, if during the run the mix will be created by the QIAgility first, and then distributed to the output labware.
Intermediate mix (system prepared)	Use this option, if this mix will be created by the QIAgility during the run, but will be part of a more specific mix. During the run, this mix will be created by the QIAgility first. Then the QIAgility will pipette the mix into the tube where the more specific mix is created.

3. The liquid class that will be used to distribute the reaction (master) mix during the run is preselected by the software. Change the liquid class only if it is really required. In this case, select the **Liquid class** which fits best to transfer the reaction (master) mix.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

4. Optionally, define advanced parameters. If you tick the box, additional parameters become visible to define extra volume and mixing options.

For reaction (master) mixes that will be created by the QIAgility, mixing options are displayed. Mixing is performed after the last reagent has been added to the reaction (master) mix, before the mix is transferred. The mixing settings are defined in the **Liquid class for mixing**. This liquid class is already preselected by the software and should only be changed if really required. The **Mixing repetitions** – that are pre-defined by the selected liquid class for mixing – can be increased, if needed. The mixing cycles have been optimized to allow proper mixing for final reaction (master) mixes in which no pure Enzyme has been added as reagent. If your setup uses large volume final reaction (master) mixes or pure Enzymes as reagents it is recommended to increase the mixing repetition to 15 or more.

For ready-made reaction (master) mixes the QIAgility does not perform a mixing step before transfer to output labware. Make sure the reaction (master) mix has been mixed properly before placing it onto the QIAgility.

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

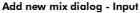
Extra volumes are set conservatively. Therefore, in most cases, displayed loading volume is sufficient. In contrast to absolute **Extra volume**, the **Extra volume per assignment** shall cover sample amount depending variations (e.g. volume loss per tip) and is added per single transfer. However, if you face any liquid level detection issues, increase **Extra volume per assignment** if issues are for higher sample amounts only, or increase absolute **Extra volume** if issues are not related to the sample amount.

5. Proceed with step 3 in <u>Mixes step</u>.

7.2.12.2.2 Add new mix input

The **Input** screen of the **Add/Edit mix** dialog lists all input liquids/reagents of the mix and their volume per reaction in a table.

		Add new mix				
General	Conte	nts of this mix				
Input >	reagen	Define all liquids/reagents and volume per reaction for respective reaction (master) mix. All liquids defined as reagents in "Liquids" screen can be selected. It is recommended to sort the reagents of reaction (master) mix by volume per reaction, starting with highest				
Target	It is recommended to sort the reagents of reaction (master) mix by volume per reaction, starting with highest volume to ensure proper pipetting for final reaction mix. Use "Sort" button to automatically sort reagents according to volume per reaction.					
	Seq.	Name	Volume / reaction [µl]			
	1	Reaction mix V	9 🗙			
	2	Primer mix V	9 ×			
		Sort Calculated reaction mix volume/reac	tion: 18 µl Add Input			
		Back Next	Finish Cancel			



For a **ready-made mix** (i.e., a user prepared mix), the input liquid is already defined from the liquid dialog definition. Enter the volume per reaction in μ l into the **Volume/reaction** field in the table. Left-click the field and then enter the value. Initially, the field is highlighted in yellow to indicate that input is required.

For a **system prepared mix**, i.e., a mix that will be created by the QIAgility, define the composition of the mix:

- 1. Click the **Add input** button below the table. A new row is added to the table. Initially, the fields are highlighted in yellow to indicate that input is required.
- In the Name field of the table, select the input liquid. The menu lists intermediate mixes and all liquids that are of liquid type Reagent or Diluent/Water. If a required liquid is not listed as expected, check if the liquid has already been defined in the Liquid step and if the correct liquid type has been selected.

- 3. Define the volume per reaction for the input liquid. Left-click the **Volume/reaction** field and then enter the value. The total volume per reaction of the mix is displayed below the table. It summarizes the volumes per reaction of the listed reagents. For not integer volumes, use a point as a decimal delimiter.
- 4. Repeat steps 1–3, until all components of the mix are listed.
- 5. It is recommended to sort the reagents of the mix by volume per reaction, starting with the highest volume, to ensure proper pipetting for the final reaction mix. Later, during the run, the QIAgility will pipette the reagents into the tube where the mix is created in the order listed in the table.

Click the **Sort** button below the table, to automatically sort the list by volume per reaction, starting with the highest volume.

Alternatively, use the buttons below the table to sort the reagents individually. Left-click the row of the reagent to be moved. Click on the up and down arrows to move the currently selected reagent up or down.

Proceed with step 5 in Mixes step.

7.2.12.3 Output labware step

Use the **Output Labware** screen to select the output labware for the run. You can distribute assay points to one plate or adapter or up to three plates or adapters. To define output labware on a single plate or adapter, follow the steps in <u>Define one output labware</u>. To define multiple labware, follow the steps in <u>Define up to three output labware</u>.

iquids	Define Output Labware
Aixes	Distribute assay points to single plate or adapter
Output Labware	 Distribute assay points to single plate or adapter Distribute assay points to multiple plates or adapters (up to 3)
Assignment	Use a new output labware Reuse a partially used output labware by loading an import file Select file
Vorktable	Output labware type
un	200 µl PCR tube (QIAGEN) / 36 x 0.2 ml PCR Tubes ▼ □ Use cooling block □ Output labware is pre-filled with reagents
	Labware identifier Output1 20190827 131029 Print barcode
	Output Converter
	Select

Output Labware step

7.2.12.3.1 Define one output labware

If the **Distribute assay points to single plate or adapter** option has been selected, define the output labware by following the steps below.

1. You can either **Use a new output labware** or **Reuse a partially used output labware by loading an import file**. The option **Use a new output labware** will be selected by default and can be used for most runs.

Use a new output labware
 Reuse a partially used output labware by loading an import file

In some cases, the sample input is limited, and one setup run is not sufficient to completely fill the output labware. In these situations, you can perform more than one run to completely fill the output labware. When doing this, the output file needs to be saved after the first run. Then, the output file from the first run can be loaded and used as a starting point for the second run. All used positions from previous runs will be blocked for usage in this run. Later in Assignment step, new samples will be added without gap. After the second run, the information from both runs can be saved in the output file. To set up the second run properly, you must indicate that you are reusing a partially used output labware by loading an import file and also load the respective output file from the first run. In this case, select the option **Reuse a partially used output labware by loading an import file** and proceed to step 9.

2. Select the output labware from the **Output labware type** menu on the right. Click **Choose other labware** to open the **Labware browser**.

Output labware type	
Select output labware type	•
Select output labware type	
Choose other labware	

Iter options	Labware		
ategory Standard 96 Well T Shows available labware only	Select the item that most closely matches the lab QIAGEN 96 Well Microplate RB QIAGEN Elution Microtubes RS QIAGEN S-Block 96 Well	Catalog number 19585 Name	
Advanced options Name of labware		QIAGEN S-Block 96 We Dead Volume 40 µl	1 6
Part number Manufacturer			
All Tube / Well size	Labware adapter		
	Select the adapter to be used.	Catalog number	
Reset filter Apply filter		Name	

In the filter options, select the labware category from the **Category** drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the input labware from the list at the top. If the labware requires an adapter, then select the adapter below. Click **OK** to close the **Labware** browser.

The selected labware will be displayed and added to the **Labware** drop-down menu.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 3. **Optional:** If you would like to use the cooling block for the output labware, check the option **Use cooling block** next to the **Output labware type**.
- 4. If the output labware is already pre-filled with liquid or dried reagents, indicate this by selecting the option **Output labware is pre-filled with reagents**. During the run, the QIAgility will consider this information and, e.g., not reuse tips.
- 5. **Optional:** If you would like to change the labware identifier that was automatically generated by the software, you can edit it in the **Labware identifier** field. This identifier will be used in the report and in the sample output file.

Note: Whenever you select another labware, this identifier will be automatically generated again by the software.

6. **Optional:** Click the **Print barcode** button to print a barcode of the labware identifier.

Note: This option is only available if a printer has been defined in the **Global Settings** tab of the **Configuration** environment.

7. **Optional:** If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, check the box next to **Output converter** to select a converter from the drop-down menu. The menu lists all converters that are currently available in the directory for converters as specified in the <u>User settings</u>. If no converter is available, **None** is the only entry in the list.

✓ Output Converter

 Select...
 ▼

 Select...
 ABI7500_QuantiplexHyres.xslt

 ABI7500_SDS_v1_4.xslt
 ABI7500_SDS_v2.3_HID_v1.2.xslt

 CE Loading (AL).xslt
 ConvertToCsv.xslt

 RGQ-SW_QREX.xslt
 RGQ-SW_QREX.xslt

Later, when saving the output file after the run, depending on the selected converter, the software also creates a file in the format that is defined by the converter. Contact QIAGEN Technical Services if you need another file format.

8. Proceed to step 12, below.

Alternatively, load a labware file for the output labware:

Note: It is required that you have saved the output file after the previous run of the output labware. If you have not yet saved the file, open the corresponding experiment in parallel, navigate to the **Run step** and click **Save output file**.

9. The **Select file** and **Scan barcode** buttons will be enabled if the **Load existing output from file** option has been selected.

Use an empty output labware
Load existing output from file
Select file...
Scan barcode ...

10. Click the **Select file** button and select the desired labware file. For details, refer to <u>File dialog</u>.

Alternatively, click the **Scan barcode** button. In the **Scan labware barcode** dialog, scan the barcode and confirm by clicking **OK**. For details, refer to <u>Scan labware barcode dialog</u>.

Using the information from the loaded file, the software will automatically select the output labware type. In addition, the positions that were used with this labware for a previous run will be blocked.

Note: If the **Output labware type** selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

- 11. Proceed to step 3.
- 12. Click **Next** to proceed.

The next step is the <u>Assignment</u> step.

7.2.12.3.2 Define up to three output labware

If the option **Distribute assay points to multiple plates or adapters (up to 3)** has been selected, you can define up to three output labware types by following the steps below.

All defined output labware will be listed in the Output labware table. Initially, the table is empty.

Seq	Labware type	Labware identifier	*	٨	
1	200 µl PCR tube (QIAGEN	Output1_20190828_114		~	🖉 🔳 🗙
2	QIAGEN Collection Microt	Output2_20190828_114			🖉 🔳 🗙
3	2.0 ml screw-cap tube with	Output3_20190828_114			0 🗉 🗙
^	Load output labware t	from file	code		Add new

If you would like to add output labware manually, click the Add new button and proceed to step 4.
 If you would like to load an output labware file, proceed to step 2.

If you would like to provide output labware information by scanning a barcode, proceed to step 3.

Click the Load output labware from file button and select the desired file. For details, refer to File dialog. Click Open to close the file dialog. The software will load the output information from the selected file.

If the file does not contain the labware type, the **Add new output labware** dialog opens. In this case, proceed to step 4. Otherwise, proceed to step 5.

- 3. Click the **Scan barcode** button. The **Scan labware barcode** dialog opens. Scan the labware barcode and click **OK** to confirm. For details, refer to <u>Scan labware barcode dialog</u>.
- 4. The Add new output labware dialog opens. Follow the steps in Add output labware dialog.
- 5. The new labware will be listed in the **Output labware** table.

If the labware is to be used with the cooling block, the box in the ²⁰ column will be ticked. If the labware is pre-filled with reagents, the box in the ¹⁰ column will be ticked. To change these settings, edit the labware.

- 6. Repeat steps 1-5 until all labware has been defined.
- 7. Click **Next** to proceed.

The next step is the <u>Assignment</u> step.

The buttons in the far right column of the **Output labware** table have the following functions:

Butto n	Action	Description
0	Edit the output labware	The Edit output labware dialog will open, displaying the values that were entered in the Add output labware dialog.
Ŀ	Duplicate the output labware	The Duplicate output labware dialog will open, displaying the values that have been entered for the original output labware. The output labware identifier will initially display as a copy of the input labware.
×	Delete the output labware	Deletes the row in the Sample output labware table.
7.2.12	2.4 Assignment step	

The **Assignment step** screen shows a graphic visualization of the output labware on the right. It is designed to support flexible and intuitive assignment of final reaction (master) mix(es) to the output labware.

*Experiment 0 ×	
Liquids	Select input position and reaction mixes. Press "Assign" to assign them to the output labware. Finally, press "Next".
Mixes	
Output Labware	→ Output Graphic List 1 ● 17 ● 33 ○ 49 ○ 65 ○
Assignment >	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
Worktable	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$
Run	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$
	$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$
	Reaction mix Reactions ● Mix ▼ 20 ▼ Pipetting pattern Legend Sample Selocked ○ Not Used ② Buffer
	Assign Fill selected Remove assignment
	Assignment step

At the bottom on the left is a menu that lists all final reaction (master) mixes defined in the **Mixes** step, along with a **Reactions** menu.

In order to assign a final reaction (master) mix to the output labware, select the reaction (master) mix. Then set the number of reactions. When you click the **Assign** button, the defined number of reactions for the selected mix will be created on the output labware. Follow the procedure described in <u>Assigning reaction</u> mixes to the output labware.

Output

The **Graphic** tab of the **Output** panel on the right side of the screen shows a graphic visualization of the output labware. The graphic will be updated when you confirm an assignment. The reactions are colored with the reaction mixes. If you hover with the mouse cursor over an output position, the software displays the reaction mix for that position.

Reactions can be removed. For details, refer to <u>Removing assignments</u>.

The menu at the bottom of the output panel can be used to set the **Pipetting pattern** (e.g., row by row vs. column by column).

Furthermore, it is possible to define blocked positions on the output labware by selecting the position(s) and then right-clicking on it.

In order to fill up unused positions on the output labware, e.g., with a buffer for CE loading, select the positions to be filled on the output labware and then click **Fill selected**. In the upcoming dialog select the liquid and the volume. For a detailed description refer to <u>Fill empty output positions</u>.

Use the List tab of the output panel to view the reaction (master) mixes for the output positions.

7.2.12.4.1 Assigning reaction mixes to the output labware

Proceed as follows:

- 1. From the **Reaction mix** menu, select the reaction (master) mix you want to assign. The menu lists all final reaction (master) mixes that have been defined in the **Mixes** step.
- 2. Click on the down arrow of the **Reactions** drop-down menu to define the number of reactions for the mix:



Note: The reactions menu is an editable menu. If you want to work with more than 5 reactions, just type the number into the field. For details about editable menus, refer to <u>Entering data</u>.

- 3. Click **Assign**.
- 4. The software creates the defined number of reactions for the selected reaction mix on the **Output** panel on the right side of the screen. The positioning follows the selection made for the **Pipetting pattern**.

Note: To create a desired output layout you can use the **Assign** button multiple times with different selections for reaction mix and number of reaction.

- 5. Repeat the steps above until all positions of the output side have been defined for the planned run.
- 6. If the QIAgility instrument is not yet connected, switch it on now. Wait until the instrument has connected with the software (indicated by the instrument icon at the upper right corner of the screen).
- 7. Close the lid.
- Click Next to enter the worktable step. If the instrument has not been used before, it will initialize.
 Note: While entering the Worktable step, the software automatically plans the worktable and checks the instrument calibration status. This may take some time. Worktable planning is repeated every time you enter the Worktable step.
- 7.2.12.4.2 Removing assignments

To clear a position on the output side, follow these steps:

- Select the desired position on the **Output** panel. Selecting multiple positions is possible by pressing SHIFT and left-clicking with the mouse to select adjacent positions or **CTRL** and left-clicking with the mouse to select multiple, non-adjacent positions.
- 2. Click **Remove assignment**. The assignment will be removed, and the position will be cleared from the output side. Also, if the position was marked as filled with buffer, the position will be cleared. The following positions will be shifted to fill the gap.

7.2.12.5 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:



Worktable selector

- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

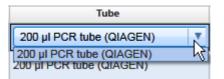
Click **Print loading instruction** to generate a PDF document with the loading instructions for the run.

M1 - Mix block

Pos.	Content	Tube	Req.vol.	Loaded
в	Reaction mix (Mix)	1.5 ml tapered tube (QIAGEN)	0 µl	✓
с	Fast reaction mix	1.5 ml tapered tube (QIAGEN)	223 µl	✓
D	Primer mix	1.5 ml tapered tube (QIAGEN)	67 µl	✓

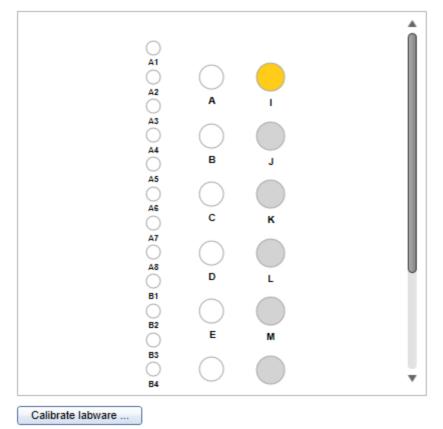
Table of tubes to be loaded to the worktable position M1.

It is possible to change the tube type for a liquid to be loaded (with certain limitations, see below).



Click into a tube field to activate a drop-down menu. The drop-down menu provides a list of all tube types that fit to the adapter position, that have also been marked as available in the laboratory in the **Labware Manager**. Select one of the entries to change the tube type. Changing the tube type might result in a replanning of the worktable. Click outside the field if you do not want to change the tube type.

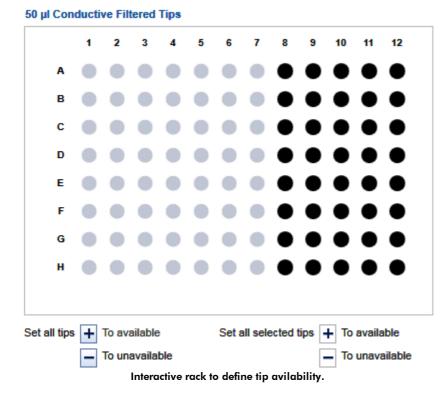
For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.



Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To available** or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to Loading tips.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> <u>holder</u>.

4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the Calibrate labware button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section Calibrate labware.

After the calibration steps have been completed, the worktable screen will be displayed again.

5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to <u>Loading tips</u>.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 6. To confirm that the tip rack has been loaded, either check the box next to Loaded in the table or click on Confirm all to confirm. Refer to Loading tips for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

10. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.

- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click **Confirm all** to confirm all positions.
- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.12.6 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.12.6.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress

Task	Progress
Prepare mixes	
Distribute mixes to output labware	

Start time		Approximate end time
15:12		15:16
13.12	_	15.16
	Run progress.	

Note: The displayed times are an approximation.

The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.12.6.2 Pausing and canceling the run

A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Every pause of a run will be recorded and listed in the Exceptions section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	Pause
Overall progress		¥
Start time 13:05	Paused at 13:21	Resume

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.12.6.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report or to print a barcode.

Run info	
Operator John Smith	
Comment	_
Output labware	
Print barcode Save output file	

Create report... Options after the run.

Option	Description
Comment	You can enter a comment into the Comment field. It will be part of the report. You may enter the comment already during the run.
Create report	A <u>report</u> can be created for documentation purposes. Refer to the <u>user settings</u> to configure the report options.
Print barcode	You can print a barcode for the output labware.
	Note: The option is available only if a printer has been defined in the Global Settings tab of the Configuration environment.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

7.2.12.7 Report

The Generic PCR Plug-in offers two types of reports: a basic report that gives a short overview and an advanced report that contains all the details.

The **Basic report** contains:

• General information

- Comment
- Reaction results
- Exceptions
- Detailed run information

The **Advanced report** contains:

- General information
- Comment
- Reaction results
- Exceptions/Pauses
- Sample information (This section is empty.)
- Worktable information
- Detailed run information
- Advanced instrument information

The sections of both report types are described in <u>Report</u>. As the experiment setup does not contain samples, standard series and controls, the corresponding information is not present in the report. The section **Reaction results** lists the actual content of the output positions, only.

7.2.13 Prepare standards experiments

The QIAgility allows you to prepare standards without PCR, so that they can be used in your future PCR experiments.

New experiments for preparing standards are created by clicking **Other worfkflows** from the **Home** screen and then selecting **Prepare standards**. The experiment will then be defined in the Experiment environment of the software. When a new experiment is created, only the **Liquids** and **Controls/Standards** steps will be initially enabled. Subsequent steps will become available once the required information has been entered into the **Liquids** and **Controls/Standards** step. The **Controls/Standards** step is highlighted in yellow as a visual reminder that required information still needs to be entered. This is a general principle when working in the <u>Experiment</u> environment.

The experiment wizard has four steps that guide you through the run setup process:

- 1. <u>Liquids</u>
- 2. Controls/Standards
- 3. <u>Worktable</u>
- 4. <u>Run</u>

Use the **Next** and **Back** buttons at the bottom to navigate between steps.

An experiment can be saved at any time using the **Save** or **Save as** buttons in the button bar. You will be given the opportunity to save your experiments as a template, but also reuse your definitions for similar experiments. The file extension for generic PCR experiments is ***.qsmrx**, and for templates ***.qsmrt** (Experiment, and Template, respectively). The file extension for generic PCR quick order templates is ***.qsmrqt** (Quick Order Template). The **Start run** button will be disabled, as long as there are incomplete or invalid steps, or missing data entries.

For more information about templates and experiments refer to the Experiments and templates concept.

7.2.13.1 Liquids step

The Liquids step is used to define standards and diluents.

Standard only x * Standard only x * Standard only x * Outrols / Standards * Outrols / Standards <th>🚊 QIAgility Setup Manager</th> <th></th> <th></th> <th></th> <th></th> <th></th> <th>– 🗆 X</th>	🚊 QIAgility Setup Manager						– 🗆 X
	File Edit View Help 🖛 😋	<u>4</u>					9
Controls / Standards Workhable Run							Instrument
Controls / Standards Worktable Worktable Run Number Image: Init Standards Number: Init Standards Number: Init Standards Image: Init Standards Number: Init Standards Number: Init Standards Image: Init Standard Image: Init Init Standard Image: Init Init Init Init Init Init Init Init	Liquids >	Define input liquids					
Name Liquid type Stock1 Standard Aqueous liquid to reaction (master) mix Any available dituent Dituent/Water Aqueous liquid to reaction (master) mix Any available Image: Contract of the type Image	Controls / Standards	(master) mix, Standards (stock) and Predefined liquid classes (LC) define task for liquid transfer (refer to LC tat screen). LC-naming: Liquid type + pipetting de	controls) for assay setup. the pipetting/tip handling ble in configuration estination/intended use	Kit Name	Kit expiry date	Comment	Ì
Stock1 Standard diluent Diluent/Water Aqueous liquid to reaction (master) mix Any available Image: Control of the standard Image: Control of the standard <td>Run</td> <td>Name</td> <td>Liquid type Liq</td> <td>guid class</td> <td>Tube type</td> <td></td> <td>Actions</td>	Run	Name	Liquid type Liq	guid class	Tube type		Actions
Image: Start run July 4, 2019 admin admin		diluent Diluent/Water Aque		queous liquid to reaction (master) mix	Any available		
July 4, 2019 admin admin →							V New liquid
				Back Next		Save	Start run
Liquids step						July 4, 2019	admin admin 🔫 🌵

The screen lists all input liquids that have already been defined in a table. In addition, an optional Kit information pane, as well as a comment field are available.

To define a new liquid:

- 1. Click the **New liquid** button below the table.
- 2. The **Add liquid** dialog opens.
- 3. Define the liquid as described in <u>Add liquid dialog</u>.

To specify kit information, enter the **Kit Name**, **Lot number**, **Product number** and **Kit expiry date** in the **Kit information** pane.

7.2.13.1.1 Add liquid dialog

In the **Add liquid** dialog a new input liquid can be defined. If you want to change the values for an existing liquid later, the **Edit liquid** dialog will open. Both dialogs show the same values for a liquid.

Add liquid	
Liquid name Dilution buffer	 Input liquid Ready-made mix
Tube type 1.5 ml tapered tube (QIAGEN)	Cap color
Liquid class	Liquid type
Diluent for dilution	Diluent/Water 🔹
Lot number	Expiry date
Define advanced pipetting parameters	
	OK Cancel
م ما ما بنا ما ما م	/

Add liquid dialog

1. Define the name of the liquid. The name must be unique. The name is used to refer to the liquid in later steps of the experiment setup.

2. Click **Tube type** to select the tube that will be used to place the liquid on the QIAgility. The tube types used in the last experiment are listed at the top of the list. To select one of the listed tube types, click the respective entry.

Tube type	
Select	•
Select	
1.5 ml tapered tube (QIAGEN)	
5.0 ml flat-base tube (QIAGEN)	
Choose other labware	
Any available	

Select the value **Any available**, if the liquid does not need to be provided in a special tube, e.g. coming from a kit. In this case, later, during worktable planning, the software will automatically select a tube of the required size, with minimal dead volume, from the list of available tubes that has been defined in the Labware Manager. (To define available tubes in the Labware Manager, click **Configuration** in the main tool bar, select the **Labware Manager** tab and then select the **Tubes** step.) Use this option to give the software more flexibility during worktable planning.

If the required tube type is not listed, select the value **Choose other labware** to select a tube from the Labware Browser.

ilter options	Labware			
Tubes Tubes Tubes Tubes Shows available labware only Advanced options Name of labware Part number Manufacturer All	Select the item that most closely matches the labware to be 1.5 ml tapered screw cap tube (QIAGEN) 1.5 ml tapered tube (QIAGEN) 2.0 ml self standing screw cap tube (QIAGEN) 200 µl PCR tube (QIAGEN) 5.0 ml flat-base tube (QIAGEN)	used.	Catalog number 990552 Name 5.0 ml flat-base tube (QIAGEN) Dead Volume 26 µl	k
Tube / Well size	Labware adapter			
Reset filter Apply filter	Select the adapter to be used.		Catalog number Name	

In the filter options, click **Apply filter** to show all available tubes. In the middle of the screen, select the tube from the list at the top. Click **OK** to close the **Labware browser**.

The selected tube will be displayed and added to the **Tube type** drop-down menu.

Note: The labware category Tubes and the option Shows available labware only are preselected.

Note: For more details about the Labware browser, refer to the section Using the labware browser.

Note: If you edit the liquid later and the **Tube type** is marked yellow, this indicates that the selected tube type is no longer marked as available in the Labware Manager. Either select another available tube type or make the tube available in the Labware Manager again.

3. Select the **Liquid class** that will be used to pipette the liquid during the run. Select the liquid class that fits best.

Liquid classes defined by QIAGEN define the pipetting and tip handling for respective liquid transfers. The names of the liquid classes contain information about:

- the liquid or liquid group to be transferred
- the destination or usage for the liquid/liquid group

For information about a liquid class, hover the mouse over a liquid class name to get a short explanation, or click **Configuration** in the main tool bar, select the **Settings** tab and then select the **Generic PCR Liquid Classes** step to get more details.

In case the same liquid will be used for different purposes (e.g. nuclease-free water used for setting up a standard dilution series and for non-template control) it is highly recommended to define multiple input liquids to allow precise definition of liquid class and liquid type.

Note: If you edit the liquid later and the **Liquid class** is marked yellow, this indicates that the selected liquid class is no longer available, e.g. if the experiment was defined on another computer where a customized liquid class was used. Select an available liquid class.

4. Select the **Liquid type**. The liquid type defines how the liquid will be used. In later steps during experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection, e.g. when defining controls or standards. In case a defined input liquid is not visible in later screens (e.g. during control definition) check if the liquid type has been set correctly.

Liquid type	Description
Standard	Use this type, if the liquid is a single standard as part of a user prepared standard series, or if the liquid is the standard stock for a standard series that shall be created by the QIAgility.
	Later in the Controls/Standards step, a liquid of this type is available for selection while defining a standard series that shall be created by the QIAgility.
Diluent/Water	Use this type, if the liquid is a diluent or water.
	Later in the experiment setup, a liquid of this type is available, e.g. for content definition of a reaction/master mix in the Mixes step, or in the Controls/Standards step as diluent for defining a control or standard series that shall be created by the QIAgility.

- 5. Optionally, enter the **Lot number** and/or the **Expiry date** of the liquid for documentation purposes. If entered, these data will be present in the report.
- 6. Optionally, define advanced pipetting parameters. If you tick the box, parameters become visible to define extra volume for the liquid.

During worktable planning, the QIAgility Setup Manager Software calculates the required volume for each

liquid. Extra volumes required for proper pipetting/liquid level detection are predefined and shall only be changed if required.

Extra volumes are set conservatively. Therefore, in most cases displayed loading volume is sufficient. In contrast to absolute extra volume, the percentile extra volume shall cover sample amount depending variations (e.g. volume loss per tip) and is a percentile addition of the transfer volume. However, if you face any liquid level detection issues increase the percentile extra volume if issues are for higher sample amounts only, or increase absolute extra volume if issues are not related to sample amount.

Define advanced pipetting parameters

Extra volume - to guarantee proper pipetting and liquid level detection:			
Extra v	olume		
9	µl and	20	%
_			

 In the dialog, click OK to confirm your values and to close the dialog. Otherwise, click Cancel to close the dialog and discard all values. In this case, no new liquid is defined, or – in case of editing an existing liquid –the liquid is not changed.

7.2.13.2 Controls/Standards step

This step is used to define standard series.

The screen contains one table: **Standard series**. The table has an **Actions** column with interactive buttons to manage the standard series.

Adding a standard series

- 1. Click on Add standard series.
- 2. The Add standard series dialog will open. For details, refer to Add standard series.
- 3. The standard will be added to the **Standard series** table.
- 4. Repeat steps 1–3 until all standard series have been defined.
- 5. Click **Next** to proceed.

The buttons in the **Actions** column of the **Standard series** table have the following functions:

Button	Action	Description
0	To edit a standard series	The Edit standard series dialog will open, displaying the values that have been entered in the Add standard series dialog.
Ŀ	To duplicate a standard series	The Duplicate standard series dialog will open, displaying the values that have been entered for the original standard series. The standard series names will initially display as a copy of the standard series.
×	To delete a standard series	Deletes the row in the Standard series table.

Changing the order of the output labware

You can change the order of the standard series using the up and down arrows below each table. Use this option to change the order of several controls within the controls section or the order of several standard series within the standard series section. The order of the output labware will be based on the order indicated in these tables.

Output labware order

Click on the up and down arrows to move the currently selected control or standard series up or down.

7.2.13.2.1 Add standard series

In the **Add standard series** dialog, a new standard series can be defined. If you would like to change the values for an existing standard series at a later timepoint, the **Edit standard series** dialog will open. Both dialogs show the values of a standard series. Define the basic properties of the standard series in the **General** screen.

	Add standard s	eries
General >	Standard series name	Tube type
Dilution Steps	Standard series 1	Any available
	Minimal volume per standard dilution	
	Standard stock liquid	Diluent
	Stock1	diluent 🔍
	Target and stock concentration	
	Target	Stock conc. Concentration unit
	n/a v	100 µg/µl 🔻 🗙
		•
		Define Std. stock
	Back Next	Finish Cancel

- Add standard series dialog General
- 1. Enter a name for the series in the **Standard series name** field.
- 2. Click on the **Tube type** drop-down menu, which is on the right side of the **Standard series name** field.
- 3. All previously selected labware types will be listed. The **Choose other labware** option will be listed last.

Tube type

Any available	•
1.5 ml tapered tube (QIAGEN)	
Any available	
Choose other labware	

4. Select the tube type from the drop-down list. If it is not listed, click **Choose other labware** to open the **Labware browser**.

Note: If the selection is yellow, you are required to mark the labware or an appropriate adapter as available in the **Labware manager**.

() Labware browser		
Filter options Category Tubes ✓ Advanced options Name of labware Part number	Labware Select the item that most closely matches the labware to be used 1.5 ml tapered screw cap tube (QIAGEN) 2.0 ml self standing screw cap tube (QIAGEN) 200 µl PCR tube (QIAGEN) 5.0 ml flat-base tube (QIAGEN)	Catalog number Name 1.5 ml tapered tube (QIAGEN) Dead Volume 8 µl
Manufacturer All Tube / Well size All	Labware adapter Select the adapter to be used.	
Reset filter Apply filter		Catalog number Name
		OK Cancel

In the filter options, select the labware **Category** from the drop-down menu. Select the option **Shows available labware only**. Use **Apply filter** to show all available labware for the selected category. In the middle of the screen, select the labware from the list at the top. If the labware requires an adapter, select the adapter from the **Labware adapter** list. Click **OK** to close the **Labware browser**.

The selected labware will be displayed and added to the **Tube type** drop-down menu.

- 5. Specify a minimal volume per standard dilution in the Minimal volume per standard dilution field.
- 6. Select the appropriate liquid from the **Standard stock liquid** drop-down menu. The menu lists all liquids that have been defined with liquid type **Standard**. If a required liquid is not listed as expected,

check if the liquid has already been defined in the Liquids step and if the correct liquid type has been selected.

- Select the appropriate diluent from the **Diluent** drop-down menu. The menu lists all liquids that have 7. been defined with liquid type **Diluent/Water**. If a required liquid is not listed as expected, check if the liquid has already been defined in the **Liquids** step and if the correct liquid type has been selected.
- Optional step. Define the stock concentration, if you intend to define the standards of the series with 8. their concentration.

	Stock conc.	Concentration unit	
T	20	Select a concentratio	×

not and atack as a saturation

Defining common stock

Click the Define Std. stock button to add a stock concentration. A new row is displayed in the Targets table:

Description
N/A is selected.
Stock concentration.
This column is available only if the option Use ready-made standard series has not been checked.
Enter the concentration that is present in the stock liquid for the target.
Click the menu in the Concentration unit field to select the concentration.

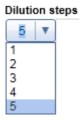
9. In the dialog, click Next to proceed to the **Dilution steps** screen.

Add standard series dilutions 7.2.13.2.2

For general information about the Controls/Standards step, refer to Control/Standards step. For information about the **General** screen of this dialog, refer to Add standard series. Use the **Dilution steps** screen to define the dilution steps and dilution ratio for the serial dilution series. The dilution steps are displayed in a table.

		Add standard series					
bas	ed on the s	steps and dilution ratio for serial dilution series. In "Dilution ratio" standard stock is displayed, but QIAgility will prepare a serial dilution of 1:1 means "no dilution".	column, the total dilution ratio on series.				
Dilu 5	ution steps	B Use Standard series stock as first standard					
	Dilution ratio between two standards (optional) 1:10 Update dilution ratios						
	Seq.	Name of standard	Dilution ratio				
	1 St	andard series-1	1:10				
	2 St	andard series-2	1:100				
	3 St	andard series-3	1:1000				
	4 St	andard series-4	1 : 10000				
	5 St	andard series-5	1 : 100000				
	 Back 	Next 🕨	-inish Cancel				
		Add standard series dialog - Dilution steps					

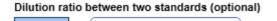
1. Select the number of dilution steps from the Dilution steps menu.



The number of rows in the table is dynamically correlated with the number of dilution steps you select in this step.

Note: The number of dilution steps are listed in an editable menu. If the number you need is not listed, you can type it into the menu field. For more details about editable menus, refer to the section <u>Entering</u> <u>data</u>.

- 2. Enter a meaningful name into the **Name of standard** cell of each row. This is especially important if you plan to define more than one standard series. The name must be unique. The name is used to refer to the standard in later steps of the experiment setup, e.g., in the loading instructions of the Worktable step.
- 3. If an equal dilution ratio will be used throughout the standard series, define the dilution ratio in the Dilution ratio field above the dilution steps and then click **Update dilution ratios** to apply it to the series.



Update dilution ratios

1:4

Seq.	Name of standard	Dilution ratio	
1	Standard series-1		
2	Standard series-2		1

Entering the dilution ratio, e.g. 1:4

Dilution ratio between two standards (optional)

1:4	Update dilution ratios
-----	------------------------

ion ratio	1
`	
٦	٦

After the "Update dilution ratios" button has been clicked

Alternatively, for each dilution step, enter either a dilution ratio or a concentration for the respective standard.

Dilution ratio between two standards (optional)

Update dilution ratios

Seq.	Name of standard	Dilution ratio	
1	Standard series-1	1:4	
2	Standard series-2		
	•		

Entering dilution ratio for dilution step

In the **Dilution ratio** column the total dilution ratio based on the standard stock is displayed.

- 4. In the dialog, click **Finish** to complete the definition of the standard series and to close the dialog.
- 5. Proceed with step 4 in <u>Controls/Standards step</u>.

7.2.13.3 Worktable step

Note: The instrument must be switched on and connected to the software before entering the **Worktable** step.

While entering the **Worktable** step, the software automatically plans the worktable and checks the instrument calibration status.

The Worktable step supports you in loading the worktable, step-by-step, for each worktable position.

The **Worktable selector** at the top shows a visualization of the worktable positions to be loaded. To navigate, use the **Next** and **Back** buttons, or just click on a position in the **Worktable selector**.

The Worktable selector displays the status of each worktable position:

M1	A1	A2
	B1	B2
R1	C1	C2

- R1 (black border): worktable position for which the loading details are displayed.
- R1, C1, C2 (yellow): worktable positions that still need to be loaded.
- A1, A2 (blue): worktable positions that have already been loaded.
- Worktable selector
- M1, B1, B2 (greyed out): worktable positions that are not used for the run.

Note: The run cannot be started if any worktable position remains yellow.

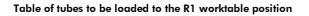
Find a step-by-step loading procedure below in this chapter.

The software displays the labware and required minimum volume of liquids to be loaded in a table located in the middle of the screen. Displayed volumes include extra volumes required for liquid level detection and pipetting. If several tubes need to be loaded to the worktable position, the table will display one row for each tube. You must confirm that the labware has been loaded by checking the box in the **Loaded** column. Alternatively, click **Confirm all** below the table to confirm that all labware and liquids have been loaded onto the worktable position.

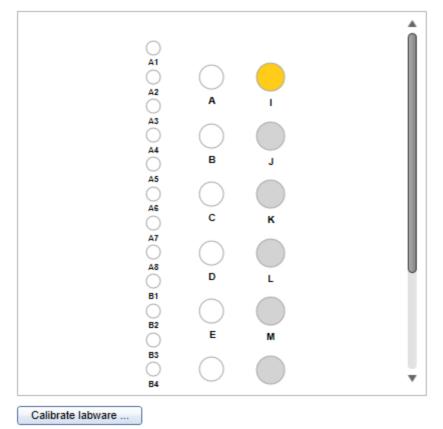
Click **Print loading instruction** to generate a PDF document with the loading instructions for the run.

		_				
R1	-	Rea	ag	en	tb	lock

Pos.	Content	Tube	Req.vol.	Loaded	
Т.	Stock1	1.5 ml tapered tube (QIAGEN)	25 µl	V	
J	Standard series 1-2	1.5 ml tapered tube (QIAGEN)	0 µl	✓	
к	Standard series 1-3	1.5 ml tapered tube (QIAGEN)	0 µl	~	
L	Standard series 1-4	1.5 ml tapered tube (QIAGEN)	0 µl	~	
м	Standard series 1-5	1.5 ml tapered tube (QIAGEN)	0 µl	~	
N	Standard series 1-6	1.5 ml tapered tube (QIAGEN)	0 µl	✓	
					ļ
					-
		Print loading instruction	Confirm all		



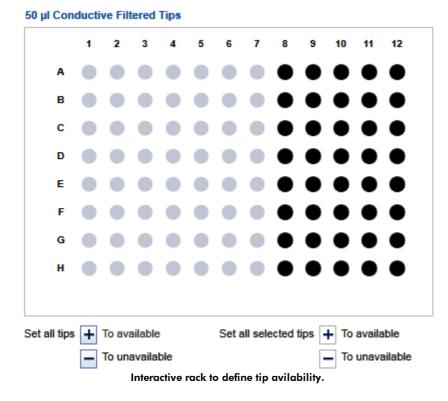
For better orientation, a schematic graphic at the right of the screen highlights the positions to be loaded. If a cap color has been defined for a liquid in the **Liquids** step, the corresponding position is displayed with that color.



Adapter: Reagent Block, 16 x 0.2 ml PCR, 8 x 2 ml Flat, 8 x 1.5 ml Tapered Tubes

Schematic graphic for R1. Cap color has been defined for the liquid in position I.

For tip rack positions, an interactive rack is displayed. The software tracks the tip usage during operation and displays the tip availability accordingly. However, the software cannot detect whether tip racks have been replaced or removed in the meantime. Therefore, you must set singular, multiple or all tips **To available** or **To unavailable** in the interactive tip rack, according to the available tips on the tip rack on the worktable. For details, refer to <u>Loading tips</u>.



If a labware type has not yet been calibrated on the required worktable position, you must first perform a labware calibration. This will be indicated by a yellow **Calibrate labware** button.

Preparing the worktable

- 1. **Optional step:** Generate the loading instructions by clicking on **Print loading instruction**. A PDF will be generated containing the loading instructions for the run. Use this option, if, for example, liquids need to be prepared in another room.
- 2. Open the instrument lid.

The worktable preparation starts with the positions for tip racks:

3. Check that the correct tip type is loaded and that the number of tips is sufficient. If not, load additional tips. For details, refer to <u>Loading tips</u>.

Note: To ensure optimal tip pickup, it is important to follow the instructions in <u>Insert tips to tip rack</u> <u>holder</u>.

4. Optional step: A yellow Calibrate labware button below the schematic indicates that the tip rack has

not yet been calibrated on the worktable position. It must be calibrated before the run can be started. Click the **Calibrate labware** button. The software starts the calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again.

5. Check that the interactive graphic on the right side of the screen shows that the tips available are the same as the tips present on the tip rack on the worktable. If not, adjust the tip availability in the interactive graphic. For details, refer to Loading tips.

Note: For an accurate run, the software requires that the actual tips on the worktable are the same as those displayed in the interactive graphic.

- 6. To confirm that the tip rack has been loaded, either check the box next to Loaded in the table or click on Confirm all to confirm. Refer to Loading tips for information about how to indicate that the tip rack should not be loaded for the run.
- 7. Click **Next** to continue with the next worktable position.
- 8. Repeat steps 3–7 for all tip rack positions.

For worktable positions with reagents and empty labware:

Note: To ensure run accuracy, it is important to follow the instructions in <u>Loading adapters and labware</u> and <u>Loading reagents and samples</u>.

9. **Optional step:** A yellow **Calibrate labware** button below the schematic indicates that the required labware has not yet been calibrated on the worktable position. The labware must be calibrated before the run can be started.

Before clicking the button, remove all filled labware from the worktable position. Then click the **Calibrate labware** button. The software automatically prepares a labware calibration worklist containing all labware that is required for that worktable position for the current run and starts the labware calibration wizard. Follow the instructions of the wizard. For details, refer to the sub-chapters of section <u>Calibrate labware</u>.

After the calibration steps have been completed, the worktable screen will be displayed again. Load (or reload) all positions that are displayed in the table as described in the next steps. Tip rack loading has to be checked and confirmed again.

Note: Ensure that you have the empty labware available for the calibration.

- 10. **Optional step:** If an adapter is required for this worktable position, the adapter name will be displayed above the graphic on the right side of the screen. Load the adapter to the worktable position.
- 11. Perform the following steps for each row in the table:
 - For reagents: Check that the correct amount of the listed liquid is provided in the correct tube type. Load the tube in the correct position of the adapter. For orientation, refer to the graphic on the right side of the screen.
 - For empty labware: Place the labware in the correct position. For orientation, refer to the graphic on the right side of the screen.
- 12. Confirm that the position has been loaded by checking the box in the **Loaded** column, or click

Confirm all to confirm all positions.

- 13. Click **Next** to continue with the next worktable position.
- 14. Repeat steps 9–13 for all worktable positions with reagents and empty labware.

After you have confirmed that all worktable positions have been loaded, the **Start run** button will be activated.

Note: If any labware has not yet been calibrated, or if the loading of any position has not yet been confirmed, the run cannot be started, and the **Start run** button will remain deactivated. The software will indicate the affected worktable position with a yellow button in the worktable selector.

Starting the run

- 1. Close the lid.
- 2. Ensure that the tip disposal box is empty.
- 3. Click the **Start run** button to start the run. The software automatically saves the experiment and then starts the actual run.

Note: If you have not yet saved the experiment, you will be prompted to enter an experiment name and to select the directory where the experiment should be saved.

4. The **Run** step will be displayed.

7.2.13.4 Run step

The run progress can be monitored in the **Run** step. During the run, this is the only available step of the experiment. A run can be paused or canceled at any time. After the run, several options are provided.

During a run, it is possible to set up and save another experiment. However, only one experiment can be run at the same time. Therefore, the worktable screen and the run screen will not be available while the current run is still in progress. While preparing the next experiment, you can return to the run page at any time by selecting the experiment's tab in the **Experiment** environment or by clicking the instrument icon.

7.2.13.4.1 Monitoring the run

The run progress can be monitored in the **Run** step of the **Experiment** environment. Single tasks and their progress are listed in a table. The tasks listed are different for different experiment setups.

The instrument icon in the main toolbar, as well as the progress indicator on the run step, show the overall run progress.

Run progress

Task	Progress
Prepare standard series	
Overall progress	
Start time 8:22	Approximate end ti 08
0.22	08



The name of the operator and a comment field are displayed on the right. This comment field will be part of the report. You may enter the comment during or after the run.

7.2.13.4.2 Pausing or canceling a run

A run can be paused or canceled at any time from the **Run** step of the **Experiment** environment.

Every pause of a run will be recorded and listed in the **Exceptions** section of the report.

To pause a run

- 1. Click the **Pause** button.
- 2. The instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

When a run is paused, the **Pause** button will change its label to **Resume**.

Overall progress		
Start time 13:05	Approximate end time 13:23	
Overall progress	¥	
Start time 13:05	Paused at Resume 13:21	

To resume the run

- 1. Ensure that the lid is closed.
- 2. Click the **Resume** button.
- 3. The **Resume** button will change back to **Pause**. The run continues.

If a run is stopped, it cannot be continued or recovered. It is not possible to restart the run from the point at which it was stopped.

To stop a run

- 1. Click the **Stop** button in the button bar.
- 2. A confirmation message will be displayed asking whether you really want to stop the run now. Click **OK** to confirm to stop the run now. Otherwise, click **Cancel** to keep the run running.
- 3. If you clicked **OK**, the instrument will complete the current movement, which may take up to 10 seconds. Before opening the lid, wait until the movement has finished.

7.2.13.4.3 Run completion

After the run has finished, the progress indicator bar on the run screen shows the run completion time, and the progress indicator below the instrument icon disappears. If a notification has been configured in the plug-in user settings, a sound or e-mail notification occurs.

After the run, several options are available in the right side of the **Run** step screen. The options will still be available if you reopen the experiment later. It is still possible to create a report.

After a run is completed, it is possible to create a <u>report</u> for documentation purposes. Refer to the <u>user</u> <u>settings</u> to configure the report options.

Run info	
Operator	
John Smith	
Comment	
	ļ
Output labware	
Print barcode	Save output file
	Create report

Options after the run.

After the run has finished, follow these steps:

- 1. Remove the labware from the worktable.
- 2. Empty the tip disposal box.
- 3. Clean the worktable as described in section Cleaning the QIAgility.

7.3 Administrative tasks

This section describes various administrative tasks for the QIAgility.

7.3.1 Configuring QG

To set up the QIAgility, follow the instructions in the following sections, in the order listed below:

- 1. <u>Unpacking and installation</u>
- 2. Software installation

- 3. <u>Managing users</u>
- 4. Customizing settings
- 5. Labware manager
- 6. Instrument calibration

7.3.2 Viewing software version

When you contact QIAGEN Technical Services for support purposes, you may be asked to provide the software version number of your installation. To see the version number, click on **Help** from the main menu and select the **About** command. The **About** dialog will be displayed, and the current version number will be displayed in the top right of the dialog.

	QIAGEN	QIAgilit	y Setup Manager 1.4.0.19
💂 QIAgility Setup Manager	About		Loaded plug-ins
File Edit View Help Help Help About Home Experiment	Copyright © 20	15-2021 QIAG	EN GmbH, Hilden, Germany
			Close

Click on **Loaded plug-ins** for information about the installed plug-ins.

7.3.3 View system audit trail

The audit trail is a versatile tool to keep track of events that occur in the QIAgility Setup Manager Software. You can filter for specific events using the date or date ranges and user criteria. Results matching the criteria are displayed and can be exported to a PDF file.

Note: This function is only available for users with administrator user role.

Relevant events are:

- Starting a run
- Completion of a run
- Stopping a run

- Creating a report
- Saving calibration data

📃 QIAgility Setup Manager



The audit trail is placed as separate tab in the "Service" environment.

You can apply filters from the **Filter options** group box on the left to find audit trail entries that meet specific criteria. Criteria you can use are specific dates, date ranges or specific users. All audit trail entries that meet the criteria are listed in the **Result table** on the right of the screen. You can also generate a PDF file of the results.

QIAgility Setup Ma	-			- 0	×
Cleaning Procedure	🔺 🔛 🏌	Service Configuration		Listrumer	
Filter options Date & time Date from 03/28/2019 Date to	Time from 0:0:0:00 Time to 2:3:59:59	Result table	User 785681b4-a74d-4edc-b839-9409e04a5bd2 785881b4-a74d-4edc-b839-9409e04a5bd2 785881b4-a74d-4edc-b839-9409e04a5bd2 785881b4-a74d-4edc-b839-9409e04a5bd2 785881b4-a74d-4edc-b839-9409e04a5bd2 785881b4-a74d-4edc-b839-9409e04a5bd2	Message OlAgility version 1.4.0.23941 started. 7a5381b4-a74d-4edc-b339-9409e04a5bd2 with role Administrator is logged in successful 7a5381b4-a74d-4edc-b339-9409e04a5bd2 logged out successfully. OLAgility version 1.4.0.23941 is shutting down. OLAgility version 1.4.0.23941 started. 7a5381b4-a74d-4edc-b339-9409e04a5bd2 with role Administrator is logged in successful Software OLAgility version 1.4.0.23941 installation completed successfully, labware librar;	

To filter for specific audit trail entries

- 1. Click on the Audit Trail tab in the Service environment.
- 2. The Audit Trail is displayed.
- From the Filter options group box on the left, select the filter criteria.
 Note: You can combine time/date filter and user filter for more advanced queries:

Time/date filter

Enter the desired date/time to the **Date from/Time from** and **Date to/Time to** fields:



Note: You can use the date picker () to open an interactive calendar for entering the dates

User filter Select one of the three options to filter for all users, the current user or a specific user. In the latter case, select a user ID from the **User id** drop-down menu below:

User	
 All users 	
O This user	
O Specific	
User id	

- 4. Click on Apply filter to apply the selected filter.
- 5. All entries in the audit trail that meet the selected filter will be displayed in the **Result table** on the right of the screen.

Exporting filter results

To export the results of a specific filter, click on the **Print to PDF** button at the bottom right. A PDF will created and opened, displaying the current result table.

Note: To view reports generated in PDF format, a PDF reader must be installed on the computer, see <u>Computer</u>.

Maintenance procedures

8 Maintenance procedures

There are two procedures required to carry out the maintenance. Following these procedures ensures optimum performance of your QIAgility instrument.

- Regular maintenance
- Periodic maintenance

Before undertaking any maintenance procedures, we recommend that you familiarize yourself with the safety information by referring to section <u>Maintenance safety</u>.

Important: Switch the instrument off and disconnect the line power cord from the power outlet before servicing.

	Risk of electric shock [W15]Do not open any panels on the QIAgility.Risk of personal injury and material damageOnly perform maintenance that is specifically described in this user manual.
--	---

8.1 Servicing

The QIAgility is supplied with a warranty that lasts for 1 year, beginning on the date of shipment. The warranty includes all repairs due to mechanical breakdown. Application development, software upgrades, accessories and disposable items are not included in the warranty.

QIAGEN offers comprehensive Service Support Agreements, including warranty extensions, full cover support agreements and instrument/application training, including on-site installation. Service Support Agreements maximize productivity and ensure high performance from your instrument. In addition, service histories are fully documented and all parts are certified and guaranteed.

Contact your local QIAGEN Field Service Specialist or your local distributor for more information about flexible Service Support Agreements from QIAGEN .

8.2 Regular maintenance

	Damage to the instrument [C4] Do not use solvents or reagents containing acids, alkalis or abrasives to clean the QIAgility.
--	---

WARNING/	Risk of electric shock [W15]
CAUTION	Do not open any panels on the QIAgility.
	Risk of personal injury and material damage Only perform maintenance that is specifically described in this user manual.

	Damage to the QIAgility lid [C5] Do not use alcohol, solvents, reagents containing acids, alkalis or abrasives to clean the instrument lid. Alcohol will damage the lid. To clean the lid, use
د نے	distilled water only.

	Damage to the QIAgility side panels [C6] Never clean the instrument side panels with alcohol or alcohol-based solutions.
--	---

Cleaning agents

The following disinfectants and detergents are recommended for cleaning the QIAgility.

Note: If using disinfectants different from those recommended, ensure that their compositions are similar to those described below.

General cleaning of the QIAgility:

- Mild detergents
- 70% ethanol (except for the QIAgility lid)
- Mikrozid[®] Liquid (Schülke & Mayr GmbH)

Disinfection

- Ethanol-based disinfectants can be used for disinfection of surfaces, such as the worktable: e.g., Mikrozid Liquid, Mikrozid Wipes. Do not use ethanol-based disinfectants to clean the lid.
- Disinfectants based on glyoxal and quaternary ammonium salt can be used for submerging worktable items, such as the adapter and tip ejector: e.g., 10 g glyoxal, 12 g lauryldimethylbenzylammonium chloride, 12 g myristyldimethylbenzylammonium chloride, and 5–15% nonionic detergent per 100 g liquid, Lysetol[®] AF (Gigasept[®] Instru AF in Europe, Schülke & Mayr GmbH, cat. no. 107410; or DECON-QUAT[®] 100, Veltek Associates, Inc., USA, cat. no. DQ100-06-167-01)

Removal of RNase contamination

5 PRIME RNaseKiller (5 PRIME, cat. no. 2500080) for cleaning surfaces and submerging worktable items

Removal of nucleic acid contamination

- DNA-ExitusPlus[™] (AppliChem, cat. no. A7089,0100) for cleaning surfaces and to submerge worktable items
- ANIGENE (formerly TriGene, Medichem International) for submerging worktable items

8.2.1 Cleaning the QIAgility

- Do not use spray bottles to spray cleaning or disinfectant liquids onto surfaces of the QIAgility workstation. Spray bottles should be used only for items that have been removed from the instrument.
- Follow manufacturer's safety instructions for handling cleaning agents.
- Follow manufacturer's instructions for soaking time and concentration of the cleaning agents. Immersing for longer than the recommended soak time can harm the instrument.

Cleaning the QIAgility:

- Remove all adapters and the tip ejector from the worktable. To clean the adapters, refer to <u>Cleaning accessories</u>. For instructions for removing and cleaning the tip ejector, refer to <u>Maintenance of the tip ejector</u>.
- 2. Clean the worktable inside the instrument using a moist, soft cloth.
- 3. Wipe the instrument with a damp cloth only.
- 4. Discard waste according to local safety regulations.

Note: Do not wipe the rails. These rails support the pipettor head and allow it to slide backwards and forwards easily. Wiping the rails will remove the grease and make them more susceptible to rust.

8.2.2 Cleaning the lid

The instrument lid is manufactured from a special UV-absorbent acrylic glass.

- Do not use alcohol, alcohol solvents or reagents containing acids, alkalis or abrasives to clean the instrument lid as this could damage the lid and its UV absorbtion.
- If solvents or saline, acidic or alkaline solutions are spilled on the QIAgility, wipe the spilled liquid away immediately.

Clean the instrument lid only with distilled water and a clean, soft cloth.

Alternatively, a cleaning product specifically designed to clean acrylic glass can be used. Any cleaning product should first be tested on a small, inconspicuous section of the instrument lid.

8.2.3 Cleaning accessories

Cleaning adapters

- Do not use a dishwasher to clean adapters as this will damage the anodization of the parts.
- Do not allow household bleach to come in contact with anodized parts.
- 1. Wash briefly in a quaternary ammonium-based solution.
- 2. Rinse with room-temperature, deionized water and allow to drain and dry.

Cleaning the guide rails

The X, Y and Z guide rails are the coated hardened steel rails supporting the linear bearings that allow the robotic arm to easily slide back and forth. Do not wipe these guide rails with a cloth as doing so removes the grease.

- 1. Check for splashed reagents daily.
- 2. Wipe only the areas which have splashed reagent on them and then move the robotic arm back and forth over the axis to redistribute the grease.

The linear bearings incorporate seals to keep the bearings free of dust or grit.

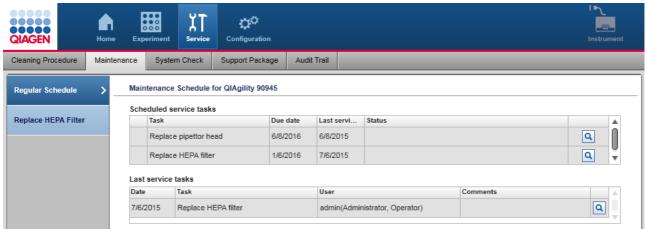
8.3 Periodic maintenance

In addition to the regular maintenance procedures which should be performed very frequently, there are also periodic maintenance tasks which need to be performed less often. These periodic tasks mainly focus on the exchange of hardware parts such as the HEPA filter, among others.

Periodic maintenance tasks are listed in the software in the <u>Maintenance schedule</u> in the **Service** environment.

8.3.1 Maintenance schedule

QIAgility Setup Manager Software provides a maintenance tracking tool to give you an overview of scheduled upcoming service tasks and recent tasks. You can find this tool in the **Regular Schedule** step of the **Maintenance** tab in the **Service** environment:



Scheduled tasks (upper table) and recent tasks (lower table)

The maintenance scheduler lists the periodic tasks for maintenance of the <u>pipettor head</u> and the <u>HEPA</u> <u>filters</u>, and consists of two tables which are described below:

- Scheduled service tasks
- Last service tasks

Scheduled service tasks table

Column	Description
First column	If the task is due, a maintenance symbol is displayed. If the task is due because the service period has been reached, a screwdriver will be displayed. If the task is due because the number of operations between services has been exceeded, a part of a large screwdriver will be displayed.
	Note : The number of operations is counted for the P-axis movements of the pipettor head only.
Task	Description of the task
Due date	Date the task is due
Last service date	Date the task was last performed
Status	Current status of the task. The cell is empty if the task is not due. If the task is due, the reason why the task is due will be displayed: because the service period has been exceeded, or if the number of operations between services has been exceeded, or both.
View icon (Opens the View service task dialog with detailed information; see below

Note: If one task is due, the software indicates this also with a screwdriver symbol on the instrument icon. Additionally, a message is shown if a task becomes due (the message is shown only once).

Last service tasks table

Column	Description
Date	Date the task is due
Task	Description of the task
User	Which user performed the task
Comments	Comment noted by the QIAGEN Field Service Specialist
View icon (🔍)	Opens the View service task dialog with detailed information; see below

The **View** icon ((a)) has the identical functionality in both tables: it opens the **View service task** dialog with detailed information.

Service task	Instrument
Replace pipettor head	90945
Scheduled due date	Period between services
6/8/2016	12 months
Operations since last service	Operations between services
Operations since last service 244 The service task has been succe The service task could not be co	300000 essfully completed.
244	300000 essfully completed.

Field	Description
Service task	Description of the task
Schedule due date	Date the task is due
Operations since last service	Number of operations (e.g., number of P-Axis movements of the pipettor head) that have been performed since the last service
	Note : This field is only displayed for the pipettor head maintenance task.
	Note: This field is not available from the Last service tasks table.
Instrument	Serial number of the instrument
Period between services	Proposed period between two service dates
Operations between services	Recommended number of operations between two services
	Note : This field is only displayed for the pipettor head maintenance task.
	Note: This field is not available from the Last service tasks table.
	The service task has been successfully completed.
	The service task could not be completed successfully.
	Result of the service task: whether or not the task has been completed successfully.
Comment	Comment noted by the QIAGEN Field Service Specialist
Acknowledge date	Date the task has been performed
Acknowledge by	User ID who has acknowledged the task

8.3.2 Maintenance of the QIAgility pipettor

QIAGEN recommends that a regular preventive maintenance service is carried out on the QIAgility. The service should be performed by a trained QIAGEN Field Service Specialist. Contact your local QIAGEN Field Service Specialist or your local distributor for more information.

We recommend regular servicing of the pipettor. The recommendation will be displayed by the maintenance schedule. As P-axis movements and the date of pipettor installation are tracked by the software, the software will remind you with a message. In addition, the instrument icon will display a screwdriver as long as the maintenance is due.

The pipettor head maintenance task can only be acknowledged by QIAGEN Field Service Specialist.

8.3.3 Maintenance of the QIAgility instrument

To remove DNA or RNA from the worktable and to eliminate cross-contamination, the QIAgility must be routinely decontaminated. Proceed as follows:

1. Remove all adapters and the tip ejector from the worktable. Wash the adapters in detergent and rinse with clean water.

For information how to remove the tip ejector, refer to Cleaning the tip ejector.

- 2. Soak the components in ANIGENE (formerly TriGene, Medichem International; for more information, see **www.medi-mark.co.uk**) or one of the tested cleaning agents listed in the section <u>Regular</u> <u>maintenance</u>, for 15–30 min.
- 3. Thoroughly rinse the components in nucleic acid-free water.
- 4. Briefly rinse with ethanol and dry with a soft paper towel.
- 5. Dampen the instrument worktable with ANIGENE or one of the tested worktable cleaning agents listed above, and allow to stand for 15–30 min. Wipe dry with clean paper towels.
- 6. Dampen the worktable with nucleic acid-free water and wipe dry with clean paper towels. Repeat this 3 times.
- 7. Dampen the worktable with ANIGENE or one of the tested worktable cleaning agents listed above, and allow to air dry.
- 8. Replace the tip ejector and close the lid.
- 9. Switch on UV lamp.

8.3.4 Using UV lamp

The UV lamp function is located in the Service environment under the Cleaning Procedure tab.





UV irradiation hazard [W9]

An electronic interlock ensures that the instrument lid must be closed for operation of the UV lamp. Under no circumstances should this interlock be disabled.

To use the UV lamp, follow the instructions below.

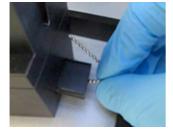
- 1. Click on the **Service** environment button. If not yet selected, click on the **Cleaning Procedure** tab header. There is only the UV lamp function.
- Empty the worktable!
 During UV irradiation, the robotic arm moves to ensure the worktable is fully exposed.
- Ensure that the tip ejector is in place!
 The tip ejector must be in place, to prevent exposure to UV irradiation outside the instrument.
- 4. Close the lid.
- 5. There are three options to start the UV lamp operation:
 - a) Click **Start** to switch on the UV lamp for the configured operation time. After the time has elapsed, the UV lamp will be switched off automatically.
 - b) Click **Start and shutdown application**, to switch on the UV lamp for the configured operation time. After the time has elapsed, the UV lamp will be switched off automatically and the software will automatically close. The instrument itself does not automatically switch off.
 - c) Click **Start and shutdown computer**, to switch on the UV lamp for the configured operation time. After the time has elapsed, the UV lamp will be switched off automatically and the computer will automatically shut down. The instrument itself does not automatically switch off.

Note: During UV irradiation, the pipettor head moves. All items must be removed from the worktable to ensure the worktable is fully exposed. The UV lamp does not irradiate certain areas, including the pipettor head, due to construction constraints.

Note: The operating time of the UV lamp can be configured by the administrator in the Settings.

8.3.5 Cleaning the tip ejector

1. Remove the retention by pulling the chain connected to the tip ejector.



2. Remove the tip ejector by carefully pulling the tip chute upwards.



Note: If it is stuck, slightly vibrate while pulling.

- 3. Soak it in ANIGENE (formerly TriGene, Medichem International; for more information, see **www.medi-mark.co.uk**) or one of the tested cleaning agents listed in section <u>Regular maintenance</u> above, for 15–30 min.
- 4. Thoroughly rinse the components in nucleic acid-free water.
- 5. Briefly rinse with ethanol and dry with a soft paper towel.
- 6. Replace the tip ejector by lowering it onto the keeper bracket on the worktable.
- 7. Press the sliding retention pin into the side slot opening.



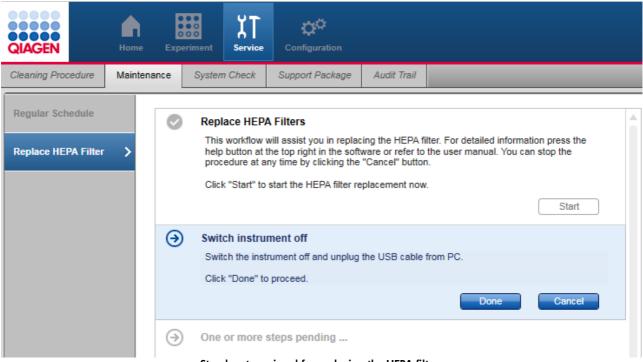
8.3.6 Replacing the HEPA filter

HEPA filters should be replaced on a regular basis. The QIAgility Setup Manager Software will remind you when it is time to replace the HEPA filters. Additionally, the instrument icon will display a screwdriver as long as the maintenance is due.

The software provides a step-by-step wizard to guide you through all steps to replace the HEPA filter. The wizard can be accessed via the **Replace HEPA filter** step in the **Maintenance** tab of the **Service** environment.

Follow the instructions displayed in every step. After you confirm that you have completed the instructions in a step, click **Done** to continue to the next step. The next step will only be displayed once you have successfully completed the instruction and no errors have occurred. While the wizard is active, all other parts of the software become unavailable.

To leave the wizard without completion, click **Cancel**.



Step-by-step wizard for replacing the HEPA filters

To exchange the HEPA filters:

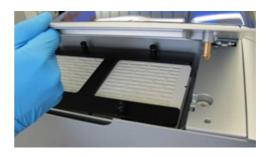
- 1. Click the **Service** button in the main toolbar to switch to the **Service** environment. Click on the **Maintenance tab** and then click on the **Replace HEPA filter** step.
- 2. Click **Start** to start the procedure now. The next step will be displayed.

3. If the instrument is still switched on, you will be prompted to switch it off. Empty the worktable. If the pipettor head is holding a tip, manually remove it. Then switch off the instrument and disconnect the line power cord from the power outlet. If the instrument is connected via USB cable, unplug the USB cable from the computer. Finally, click **Done** to proceed.

Note: When the instrument is switched off, the pipettor head will drop approximately 70 mm.

4. Lift the HEPA filter cover and remove it.

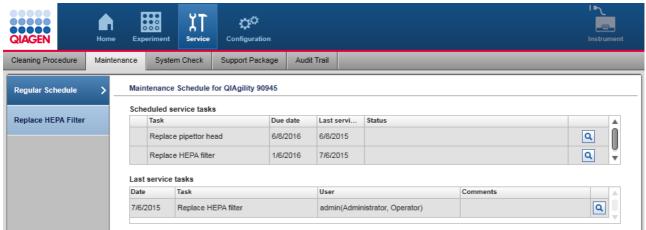




- 5. Unscrew the four thumb screws. See schematic in the step-by-step wizard.
- 6. Remove the H frame.
- 7. Remove the old HEPA filters.
- 8. Insert new HEPA filters with the arrows down.
- 9. Reassemble the H frame.
- 10. Screw the four thumb screws tight.
- 11. Re-assemble the cover onto the instrument.
- 12. Click **Done** to finish the task. The software automatically acknowledges the task as successfully completed. The task will be listed in the **Last service tasks** table of the **Regular Schedule**. In the task details the task will be marked as successfully completed.
- 13. Reconnect the instrument to the computer.

If the USB cable had been unplugged from the computer, plug it in again. Then switch on the instrument. It takes some time for the software to recognize the instrument again.

Note: If you leave the wizard by clicking **Cancel**, the task will still be listed in the **Last service tasks** table. However, in the task details, the task will be marked as not successfully completed. The service task remains due, indicated in the **Scheduled service tasks** table and on the instrument icon.



Performed HEPA filter exchange is listed in the "Last service tasks" table.

If you click the **View** button on the right side of the task, the detailed view opens:

ervice task	Instrument
Replace HEPA filter	90945
Scheduled due date	Period between services
12/8/2015	6 months
The service task could not be	
The service task has been suc The service task could not be Comment Acknowledge date	

Troubleshooting

9 Troubleshooting

If you need to contact QIAGEN Technical Services for technical assistance, note down the steps that occurred leading up to the issue. Alternatively, save or choose the applicable support package from the run, and then send this to QIAGEN Technical Services. This will help the QIAGEN Field Service Specialist resolve the issue.

It is also possible to <u>export support packages</u>, even if the instrument is powered down.

A warning will be issued when an error, such as insufficient tips, is encountered during the run; the error will also be listed in the report.

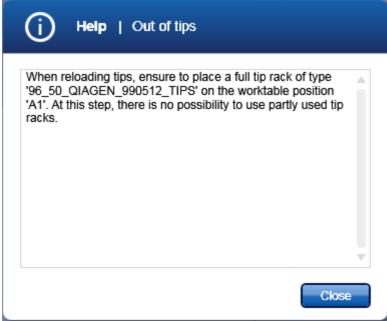
If you receive a software warning or error message that contains a **Help** button, click on it to get more information.

Example:



Run out of tip message during a run.

Click on the **Help** button in the message dialog.



After clicking the messages' Help button.

Click the **Close** button to return to the message dialog.

9.1 Support packages

Support packages are folders containing multiple files that help QIAGEN Technical Services to analyze issues that might have been observed. The function to export support packages is located in the **Support Package** tab in the **Service** environment:

ĺ		QIAgility												×	
	Eik	le Edit View He	elp 👘	1										0	
	Ğ	NAGEN	Home	Experiment	۲T Service								Instrument	:	
	С	leaning Procedure	Maintenar	nce System	Check	Support Pag	kage	Audit Trai	a						
	Γ	Filter options				Support packa	ges froi	m experimer	nts				_		
		Start date	End date			Date	Tin		xperiment name	•	 	 	Status		
		06/10/2015	06/17/2	015 📰		6/17/2015	10	:00 a	ab		 	 	Completed		
													Ţ		Packages from experiments
Filtor					:	Support packa	ges froi	m system ch	hecks				_		
Filter options –						Date Date	Tim	ne Te	lest name		Result		<u></u>		
															Packages from system checks
		Reset filte	r Ap	ply filter								(i) The log f	iles are automatically		
	H				1										
			_					_			 		cted package(s)		
Į												June 18, 2015	admin admin	→Ũ	

Screen to export support packages.

Use the filter options to search for support packages created between the start and the end date. The right part of the screen consists of two tables that list support packages created from experiment runs and support packages created from performed <u>system checks</u>. Click on **Export selected package(s)** to export the selected support packages to a definable directory.

Support packages are exported for reporting different scenarios, including:

- an issue during a run
- the results of performed system checks
- instrument issues

Note: Support packages older than 90 days will be automatically deleted.

Exporting a support package

- 1. Click on the Support Package tab in the Service environment.
- 2. The Support Package screen will be displayed.

3. To filter for support packages created within a specific date range:

a) Enter a start date and an end date, or use the 📰 icon to interactively select a date. Ensure that the range covers the date the run or the system test was performed.

b) Click on Apply filter.

- 4. All available support package information created within the selected date range will be displayed in the **Support packages from experiments** table and the **Support packages from system checks** table, listing the run experiments and the test results, respectively.
- 5. Activate the check boxes for the support package you want to export.

Activate the check box of the experiment(s) if you want to report issues during run. Activate the check box of the performed system test to report the results.

Note: If you don't select any experiment run or system test result, the support package will contain the currently available log files only.

- 6. Click on **Export selected packages**.
- 7. The **Select folder** file dialog is opened.
- 8. Select/create the target directory (where the files should be saved in) and click **OK**. **Note**: The target directory folder must have a **read/write** access.
- 9. The support package is exported to the selected directory.
- 10. Navigate to the newly created directory and send all files in it to QIAGEN Technical Services.

The directory contains the support packages of the selected experiments and/or system test results, including plug-in specific files, user information, settings, labware database and instrument specific data combined in a zip file. Additionally, current log files and the audit trail are copied to the destination directory (unzipped). Even if the software stops due to a program failure during a run, the support package will be completed when the software is restarted.

The file name of the support package file for an experiment run is generated from a time stamp, instrument and experiment data according to the following naming scheme:

Time stamp_Instrument type_Instrument serial number_Experiment name_Experiment status.zip

Log files are created for analysis by QIAGEN Technical Services only.

9.2 System setup

	Comments and suggestions
Instrument does not run	
a) Power cord not connected	Check the power connection.
b) Fuse blown	Replace the fuse according to the following procedure:
	1. Switch off the QIAgility using the main power switch.

	Comments and suggestions
	Unplug the power cord from the power outlet and from the rear of the instrument.
	 Use a small flat-bladed screwdriver to remove the fuse- carrier assembly located directly to the right of the area where the cord connector enters into the inlet.
	4. Replace the fuse. Replace only with type F5A250V.
	5. Reinsert the fuse-carrier assembly and plug in the power cord.
	6. Turn on the power and check the instrument for normal operation. If the instrument does not function normally, or continues to blow fuses, unplug the system and contact QIAGEN Technical Services.
c) Instrument does not initialize on software start	The instrument does not initialize on software start. It initializes just before use, e.g., before calibration or during worktable planning before a run.

Computer software cannot communicate with system

a) USB cable not connected between the computer and the instrument	Check the connection of the USB cable. See section <u>Connecting</u> <u>the computer to the QIAgility</u> .
b) Instrument is not switched on	Switch the instrument on. If the instrument is connected to the computer via the USB cable, the software automatically connects to the instrument. This can take a few seconds
c) A communication problem occurred	If the instructions of a) and b) have already been followed, but the software does not connect to the instrument, restart the computer. If the problem persists, collect the support package and contact QIAGEN Technical Services.

QIAgility Setup Manager Software does not launch

a) QIAgility Setup Manager Software not installed	Install the QIAgility Setup Manager Software. See section <u>Software installation</u> .
b) Old version of Microsoft Windows	The QIAgility Setup Manager Software operates with Windows 7 Professional (32- or 64-bit) and Windows 10 (64-bit). See section <u>Computer</u> .

QIAgility Setup Manager Software cannot be installed

a) Message during installation: Wrong SiLabs USB driver version has been	The wrong driver version may have been installed by another QIAGEN software, e.g the old QIAgility Software.
detected on the computer.	If the message shows up, allow the installer to update the driver to the version needed by QIAgility Setup Manager Software.
	Otherwise, QIAgility Setup Manager Software cannot be
	installed correctly. However, the other software may become

	Comments and suggestions			
	inoperable with this driver version. It is not possible to have both an old version of QIAgility software and the QIAgility Setup Manager Software installed on the same computer.			
b) Message during installation: .NET Framework needs to be installed has been answered with No	The appropriate version of the Microsoft .NET Framework will be installed during setup if it is not already present. In this case, another dialog opens. Click Yes to install the Microsoft .NET Framework. This may take some time. If you click No , the QIAgility Setup Manager Software will not be installed.			
c) Message during installation - Windows 7 only: Installation of .NET Framework failed.	On computers with Windows 7, ensure that the Windows Service Pack 1 has been installed. To check if the Service Pack 1 has been installed, click the Windows Start icon at the bottom left of the Windows screen. Then right-click on the entry Computer in the right bar and select Properties from the appearing context menu. Check that the Service Pack 1 is mentioned in the Windows edition section.			
d) Message during installation: SQL Server needs to be installed has been answered with No	The appropriate version of the Microsoft SQL Server will be installed during setup if it is not already present. In this case, another dialog opens. Click Yes to install the Microsoft SQL Server. This may take some time. If you click No , the www.qiagen.com/ will not be installed.			
e) Policy prevents software installation from an Unknown Publisher	Contact your local IT administrator.			
f) On Windows 10 only. An update installation fails at step "Stopping services."	On Windows 10 only, when updating from QIAgility Setup Manager Software 1.0 or 1.1. Please note that QIAgility Setup Manager Software version 1.2 is the first version that can be used on Windows 10.			
	To get the new version installed:			
	1. Take a backup of your experiments, reports and output files.			
	2. Uninstall the previous version.			
	3. Install the new version.			
Windows security message shown dur	ng the Device Driver Installation Wizard			
a) A Windows security confirmation	Click Install to install the QIAgility driver.			
message is shown during QIAgility Setup Manager Software driver installation: QIAGEN GmbH drivers	If you do not install the driver software, the QIAgility Setup Manager Software			
attempt to be installed	a) will not be able to operate the QIAgility if you install the QIAgility Setup Manager Software for the first time, or			
	b) might not be able to operate the QIAgility correctly in case of an update installation because an older driver version remains active on the computer.			

	Comments and suggestions				
b) A security warning message is shown during driver installation of the QIAgility Setup Manager Software:	If the message is shown in the context of an installation of the QIAgility Setup Manager Software, allow to install the driver anyway.				
Unknown publisher of driver software	If you do not install the driver software, the QIAgility Setup Manager Software				
	a) will not be able to operate the QIAgility if you install the QIAgility Setup Manager Software for the first time, or				
	 b) might not be able to operate the QIAgility correctly in case of an update installation because an older driver version remains active on the computer. 				
c) Policy prevents driver installation from an Unknown Publisher	Contact your local IT administrator.				
Device Driver Installation Wizard fails					

On Windows 10, the Secure Boot option If you use UEFI Boot, the Secure Boot option must be disabled. is used together with UEFI Boot

Operation 9.3

	Comments and suggestions
Experiment setup	
a) The Next button in the Sample/Labware screen is not available	An output converter that is not available has been selected. Change the output converter to an available one.
	An adapter for the output plate is not available.
b) Samples in the quick order are not assigned	Samples IDs used in a quick order must be unique.
c) The Calibrate labware button remains yellow	If a protocol uses the same plate/adapter combination with and without a cooling block, the calibration must be completed in the calibration wizard. For more information, refer to <u>Calibrate</u> <u>labware</u> .
possible to add a second output plate if	If the Rotor Disc 100 is used as input labware, and it should also be used as output labware, the Rotor Disc 100 must be selected I first, and then another output plate type can be added. If different output labware is added first, you cannot add the Rotor Disc 100 afterward.
e) Reporter is not mapped in Q-Rex v1.1	The reporte/fluorophore map is not aligned with Q-Rex v1.1. Refer to the mapping list below:

	Comments and suggestions
	Green: FAM, SYBRGREEN1, SYBRGREENI, FLUORESCEIN, ALEXAFLUOR488
	• Yellow: JOE, VIC, HEX, TET, MAX, CALFLUOR, GOLD540, YAKIMAYELLOW
	 Orange: ROX, CALFLUORRED610, CY35, TEXASRED, ALEXAFLUOR568
	• Red: CY5, QUASAR670, ALEXAFLUOR633, LIGHTCYCLERRED640
	 Blue: MARINABLUE, EDANS, BOTHELLBLUE, ALEXAFLUOR350, AMCAX
	 Crimson: QUASAR705, ALEXAFLUOR680, LIGHTCYCLERRED705
f) Labware on the worktable position has not yet been calibrated	The run can only be started if all labware is calibrated on the positions that have been planned for the run. The yellow Calibrate labware button indicates that the labware on the position has not yet been calibrated. Follow the instructions in <u>Starting a run</u> .
g) An additional tip rack is planned on B1 or B2 but has not yet been calibrated on that position	The software plans additional tip racks to support completely using previously started tip racks and to minimize the need of tip reloading during run. If a tip rack has not yet been calibrated on that additional position, the Calibrate labware button is yellow and the run cannot be started. The button will remain yellow even if you decide not to use this position for tips in this run (i.e., you define all tips on this position as unavailable and do not check the box next to Loaded). Click the button to calibrate the tip rack on this position. In general, we recommend that you also calibrate 50 μ l tip racks on worktable positions B1 and B2.
Running out of master mix	
a) Master mix remains in the tube after completion of the run, but insufficient volume of master mix was delivered to the last reactions aliquoted	Ensure that the height calibration of the master mix tube is set correctly.
b) Correct initial volume was supplied or made but all master mix is consumed before all reactions are made	The pipettor may be aspirating larger volumes than expected. Ensure that the volume calibration data are correct and have been configured properly.
c) Source reagent remains in the tube after completion of the run, but the master mix has run out before all reactions aliquoted	Ensure that the height calibration of all reagent wells used for construction of the master mix is set correctly.

Comments and suggestions

Uneven levels in reaction tubes

a) Air bubbles trapped in sample tubes or master mix tubes	The liquid-level sensor functions equally well on air bubbles or the liquid surface. Centrifuge the sample plate to remove air bubbles.
b) Liquid droplets appear on reaction tubes above the liquid surface	The tip is colliding with the base of the tube causing liquid ejected under pressure to spray when the tip is retracted. Height calibration needs to be raised to prevent tip contact with the tube base during dispense.
c) None of the above	Pipettor is operating erratically and may be clogged. Organize a service for the pipettor.

Defined mix is not created by the QIAgility

a) The reaction/master mix has not been	For the run, the software will create only reaction (master) mixes
assigned with samples	(with their components like intermediate mixes and single
	reagents) that have been assigned to samples in the Assignment
	step.

Software locks or crashes

a) Screen savers and power save	Disable screen savers and power save modes on the computer.
modes not disabled on the computer	

Z-axis not rising after power has been switched on

a) Power cords not securely plugged in	Ensure that all power cords are securely plugged in, then try switching the instrument on again.
b) An internal fault on the instrument has occurred	Contact QIAGEN Technical Services.

Replicates do not provide high reproducibility

a) Incorrect reagent volumes entered	Insert the correct volumes for each reagent.
b) Less than 5 μ l DNA template used in reactions	It is recommended to use at least 5 μ l DNA template in reactions.
c) Air bubbles trapped in sample tubes	Centrifuge the sample plate to remove air bubbles.
Software is very slow	

a) Virus scanners running on the	Refer to chapter <u>Computer</u> . Follow the instructions to minimize
computer	influence of other processes to the software.

Instrument pipets in mid-air or pipets no liquid

	Comments and suggestions
a) Level sensor malfunction	Static electricity on the plasticware may be interfering with operation of the liquid-level sensor. Generate a support package and contact QIAGEN Technical Services.
Liquid in source well, but received "In	sufficient Liquid" error message
a) Incorrect tip configuration	The instrument may have been configured to pick up tips that were not present in the Tip Rack Holder. Ensure that tip configuration is correct.
b) Incorrect Z-calibration	If the Z-calibration is too high, the tip cannot reach low enough into the tube to aspirate all the fluid. Ensure that Z-calibration is correct. Use automatic Z-calibration whenever possible.
c) Physical plate does not match model selected in software	The software uses models of tubes and wells to translate the detected liquid surface height into a volume. When the wells or tubes are physically different than the model chosen, the volume calculated may be much lower than the actual volume present, causing aspiration steps to fail. Ensure the plate chosen in the software matches the physical plate.
d) Master mix has been loaded with the required volume, but insufficient volume of master mix was detected	The software uses models of tubes to translate the detected liquid surface height into a volume. For highly viscous liquids, this might result in an "Insufficient Liquid" error, if only the minimum required volume has been loaded. For master mix, load the tubes provided with the QIAGEN kit. They already contain some extra liquid. Or, add an extra volume of 10%.

Error messages recorded in post-run report, but the run was completed

a) Volumes of reagents or samples	If liquid was not detected, an error is recorded in the post-run
below the minimum volume which is	report. Add an extra 20 μ l volume as required. Alternatively,
required for liquid-level sensing.	dilute samples and reagents and pipet larger volumes rather
	than pipetting small volumes of concentrated solutions.

An error message is received during worktable planning

a) The run cannot be planned, because no suitable labware has been found. Check the labware availability in the Labware Manager (Configuration environment). The software looks for tube and adapter combinations to place all liquids on the worktable, taking into account the required volumes for reagents, dilutions for standard series and intermediate mixes that need to be created along with the tubes' dead volumes. The software only considers the available labware. If one or more liquids or empty tubes cannot be placed, the software will try another tube/adapter combination. If no combination can be found for placing all required tubes, this error message will appear.

To overcome this issue, check and try the following:

a) Check that you have made tubes available so that all positions on the adapters for M1 and R1 can be used.

Comments and suggestions

- b) Check if different adapters for M1 and R1 are available to support either more small-sized tubes or fewer large-sized tubes.
- c) In the assignment step, reduce the number of assay points on the output labware. If the worktable can be planned, you can try to increase the assay points on the output labware stepwise.

A non-readable error message is received when the software is started

a) Correct plate definitions not installed	Uninstall and then reinstall the software. See section <u>Software</u> <u>installation</u> .
	Important: Copy all user files to another location outside C: \ Users \ Public \ QIAGEN \ QIAgility before uninstalling the software, because they will be removed during the uninstallation process.

An error message is shown on software start that the disc is nearly full

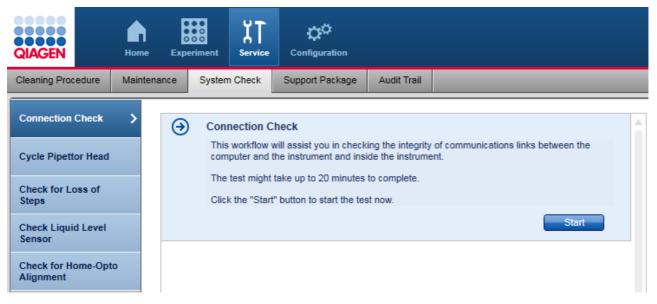
a) Not enough free hard disc capacity available	Follow the instructions in the message and remove files from the hard disc. Otherwise, it will not be possible to start a run.	
Output file in additional format is empty (for experiments created with Generic PCR Plug-in only)		
a) Targets have not been defined for final reaction (master) mix	Output converters rely on the target definition made in the experiment. If it is missing, the file in the additional format cannot be created and is empty.	
	When defining final reaction (master) mix(es) ensure to define all	

When defining final reaction (master) mix(es) ensure to define all targets. When defining controls ensure to select all targets. When defining standard series that shall be created by QIAgility ensure to define stock concentrations for all available targets; for targets with no stock concentration, enter concentration value 0.

9.4 Self-diagnostic tools

QIAgility Setup Manager Software provides several different checks to verify whether the most important instrument parts are working correctly. The tests are designed to collect information to help with troubleshooting. They are bundled in the **System Check** tab of the **Service** environment:

Note: The tests are only available if the instrument is connected.



Each check is carried out in a separate step, and the steps are independent from each other, so you can start any test you choose. The checks are built in a step-by-step wizard format. The initial step shows a description of the check, its duration and a **Start** button. Click the **Start** button to begin the check and to display the instructions for the next step. Follow the instructions on the screen. After you have finished, click **Done** to proceed to the next step. To cancel a test, click **Cancel** or **Stop**.

Check name	Description	Duration
Connection Check	Checks the connection between computer and instrument and within the instrument itself	20 min
Cycle Pipettor Head	Checks the cycling of the pipettor head	10–15 min
<u>Check for Loss of Steps</u>	Checks whether the pipettor head movement is working correctly	40–45 min
Check Liquid-Level Sensor	Checks whether the liquid-level detection is working correctly	90 min
<u>Check for Home-Opto</u> <u>Alignment</u>	Checks if the instrument can perform the homing routine correctly	5 min

Important: Before starting a test, read the details first by clicking the link in the table.

Note: These tests should only be performed by experienced users.

To perform a test:

- 1. Click the **Service** button in the main toolbar and then then click the **System Check** tab to switch to the **System Check** tab of the **Service** environment.
- 2. Click on the step marker of the test you would like to perform. The description of the test is shown.
- 3. Click **Start** to start the test. The next step is displayed.

a) Follow the instructions, if any, and then click **Done** to confirm and proceed.

b) Or, wait while the instrument performs an automatic step. The software will then proceed to the next step automatically.

Repeat this for all steps of the test.

4. If the test is finished, the software displays the test result as a last step. The test result is automatically saved.

To cancel the test, click **Cancel** or **Stop**. Wait until the instrument has stopped any movement, before opening the lid.

If the test failed, save the <u>Support package</u> now. Ensure that the performed test is listed in the table **Support packages from system tests** and checked. Click **Export selected packages** and contact QIAGEN Technical Services for further assistance.

Click **Restart** if you want to repeat the task. Click on another step marker to perform another test. Or, click to another part in the software for another task.

9.4.1 Connection check

This test checks the communications links between the computer and the instrument and inside the instrument.

- 1. In the step marker, click **Connection Check**. The description of the test is shown.
- 2. Click **Start** to start the test. The next step is displayed.
- 3. Wait while the instrument checks the connection links.
- 4. If the test finished, the software displays the test result as a last step. The test result is automatically saved.

To cancel the test, click **Cancel** or **Stop**.

If the test failed, save the <u>Support package</u> subsequently. Ensure that the performed test is listed in the table **Support packages from system tests** and checked. Click **Export selected packages** and contact QIAGEN Technical Services for further assistance.

Click **Restart** if you want to repeat the task. Click on another step marker to perform another test. Or, click to another part in the software for another task.

9.4.2 Cycle pipettor head

If the QIAgility has been unused for a long period of time, it is recommended to cycle the pipettor head. This helps to distribute the grease evenly.

- 1. In the step marker, click on **Cycle Pipettor** head. The description is shown.
- 2. Click **Start**. The next step is displayed.
- 3. Empty the worktable. Remove the tip ejector, all plates, tubes, adapters, accessories and tips. **Important:** Failure to do this may result in permanent damage to the pipettor head.
- 4. Close the lid.
- 5. Click **Done** to confirm and proceed.
- 6. Wait while the instrument cycles the pipettor head.
- 7. If the task finished, the software displays the last step. The task result is automatically saved. Click **Restart** if you want to repeat the task. Click on another step marker to perform another test. Click on the **Support Package** tab if you want to export the result, or click to another part in the software for another task.

To cancel the task, click **Cancel** or **Stop**. Wait until the instrument has stopped any movement, before opening the lid.

9.4.3 Check for loss of steps

This test establishes whether the instrument has reliable movement.

- 1. In the step marker, click on **Check for Loss of Steps**. The description of the test is shown.
- 2. Click **Start** to start the test. The next step is displayed.
- 3. Empty the worktable. Remove the tip ejector, all plates, tubes, adapters, accessories and tips. **Important:** Failure to do this may result in permanent damage to the pipettor head.
- 4. Close the lid.
- 5. Click **Done** to confirm and proceed.
- 6. Wait while the instrument moves the robotic arm with the pipettor head.
- 7. If the test is finished, the software displays the test result in the last step. The test result is automatically saved.

To cancel the test, click **Cancel** or **Stop**. Wait until the instrument has stopped any movement, before opening the lid.

If the test failed, save the <u>Support package</u> now. Ensure that the performed test is listed in the table **Support packages from system tests** and checked. Click **Export selected packages** and contact QIAGEN Technical Services for further assistance.

Click **Restart** if you want to repeat the task. Click on another step marker to perform another test, or click to another part in the software for another task.

9.4.4 Check liquid-level sensor

This test checks that the liquid sensor circuitry is functioning correctly, so that it only triggers when the conductive tip touches the liquid.

- 1. In the step marker, click on **Check Liquid-Level Sensor**. The description of the test is shown.
- 2. Click **Start** to start the test. The next step is displayed.
- 3. Prepare the worktable as displayed by the software.
- 4. Close the lid.
- 5. Click **Done** to confirm and proceed.
- 6. Wait while the instrument checks the sensor. This will be repeated several times.
- 7. If the test is finished, the software displays the test result in the last step. The test result is automatically saved.

To cancel the test, click **Cancel** or **Stop**. Wait until the instrument has stopped any movement, before opening the lid.

If the test failed, save the <u>Support package</u> now. Ensure that the performed test is listed in the table **Support packages from system tests** and checked. Click **Export selected packages** and contact QIAGEN Technical Services for further assistance.

Click **Restart** if you want to repeat the task. Click on another step marker to perform another test, or click on another part in the software for another task.

9.4.5 Check for home-opto alignment

This test checks if the instrument can perform the homing routine correctly.

- 1. In the step marker, click on **Check for Home-Opto Alignment**. The description of the test is shown.
- 2. Click **Start** to start the test. The next step is displayed.
- Empty the worktable. Remove the tip chute, all plates, tubes, adapters, accessories and tips.
 Important: Failure to do this may result in permanent damage to the pipettor head.
- 4. Close the lid.
- 5. Click **Done** to confirm and proceed.
- 6. Wait while the instrument moves the robotic arm.

7. If the test finished, the software displays the test result in the last step. The test result is automatically saved.

To cancel the test, click **Cancel** or **Stop**. Wait until the instrument has stopped any movement, before opening the lid.

If the test failed, save the <u>Support package</u> now. Ensure that the performed test is listed in the table **Support packages from system tests** and is checked. Click **Export selected packages** and contact QIAGEN Technical Services for further assistance.

Click **Restart** if you want to repeat the task. Click on another step marker to perform another test, or click to another part in the software for another task.

Glossary

10 Glossary

Glossary terms in alphabetical order

A	
Aspirate	To draw liquid up into the pipette.
Audit Trail	A record of actions. The audit trail tracks user actions and changes to the instrument.
В	
С	
Calibration	Before the instrument can be used, the locations of work items such as the tip ejector or labware must be determined. This process is termed calibration. All labware that will be used needs to be calibrated on the respective worktable positions.
Calibration report	The calibration report is a printed document delivered with QIAgility. It defines the P-axis movements to be performed for a given volume. The values must be entered when configuring QIAgility.
Conductive Tip	QIAgility uses conductive tips to detect the liquid level.
Control	Controls are used to verify the PCR process. There are negative controls (NTC, non-template control) and positive controls.
.csv file	A file format, which can be used to import sample data. In general, a .csv file represents comma separated values (CSV), like in a table, where each line in the file represents a row in the table and a comma (,) represents the cell border to the next column.
Cycler	The thermal cycler (also known as a Thermocycler, PCR Machine or DNA Amplifier) is a laboratory apparatus used to amplify segments of DNA via the polymerase chain reaction (PCR) process. Rotor-Gene Q is the cycler developed by QIAGEN.
D	
Dispense	To distribute the liquid out of the pipette.

Dilution labware	For experiments created with the Generic PCR Plug-in, only. Labware that is used during sample normalization to create predilutions.
Dilution position/well	Position/well on the dilution labware/plate.
E	
Expiry Date	The date after which QIAGEN can no longer guarantee that a kit performs according to its specification.
Extra Volume	Additional volume that is needed for pipetting and LLD. The software automatically calculates the extra volume needed for a run.
F	
Final reaction (master) mix	A liquid that contains all reagents for a PCR reaction, including target- specific components, and will be distributed to the output labware.
G	
GUI	Abbreviation for Graphical User Interface.
Н	
HEPA Filter	Abbreviation for High Efficiency Particulate Airfilter. Air filter to prevent airborne contamination underneath the instrument hood.
Home Position	The position where the pipettor head moves to reset all axis.
l Innert linerial	For supering the exacted with the Conserie PCP Plug is a shu
Input liquid	For experiments created with the Generic PCR Plug-in, only.
	A liquid, e.g., a reagent or water, which will be loaded on the QIAgility for the run or will be used by the QIAgility to create a mix.
	In the Add liquid dialog the options Input liquid and Ready-made mix are available. If the option Ready-made mix is selected in the Mixes step, a mix with the same name as the liquid is listed automatically. You need to select the option Ready-made mix , if the liquid is the final reaction (master) mix. Select the option Input liquid , if the liquid should not be listed as a mix in the Mixes step.

Intermediate mix	For experiments created with the Generic PCR Plug-in, only.
	A liquid that serves as a generic pre-mix that will be used to create more specific reaction (master) mixes, e.g., final reaction (master) mixes.
J	
К	
Kit	Ready-to-use composition of reagents and consumables for a given protocol.
L	
Labware Library	The labware library is delivered with the software and contains all labware to be used on the QIAgility instrument.
Labware Type	Labware is grouped into types such as tubes, 96-well or 384-well plates, to enhance customer convenience while looking for specific labware.
Lid	The instrument lid isolates the worktable, maintaining sample integrity and acting as a safety mechanism.
Liquid class	Liquid classes define the pipetting and tip handling for respective liquid transfers. Liquid classes are defined by QIAGEN.
Liquid-Level Detection (LLD)	Ability to determine the filled in volume in the labware.
Liquid type	For experiments created with the Generic PCR Plug-in, only.
	The liquid type defines how the liquid will be used. During experiment setup, the software uses the liquid type to present sub-sets of defined liquids for selection.
Loading instruction	For experiments created with the Generic PCR Plug-in, only.
	During worktable setup, printable loading instructions can be created. These list the worktable positions for the labware and the required minimum volumes of liquids to be loaded.
Log file	A log file contains information about (instrument) actions that were performed.

М

Master Mix	A master mix is a provided liquid that contains all reagents for a PCR reaction, except target-specific components.
Master Mix Block	Special adapter that can be placed on position M1. The Master Mix Block is intended to hold up to five tubes on the instrument worktable.
Ν	
Normalization	Based on the imported original sample concentration, samples will be normalized to reach the chosen target concentration/mass value. Samples can be normalized based on mass or concentration.
0	
Output converter	If the output file is going to be saved after the run for import to a downstream instrument, e.g., a PCR cycler, and the downstream instrument expects the file in a certain format, an output converter is needed. The output converter defines the required file format.
	For more detailed information, refer to the section Run completion of the respective experiment type, within the section <u>Working with experiments</u> .
Р	
P P-Axis	The direction of pipetting piston movement.
	The direction of pipetting piston movement. A passive reference is a fluorescence dye added to the reaction mix to provide the PCR cycler with the ability to normalize the fluorescence signals between all wells.
P-Axis	A passive reference is a fluorescence dye added to the reaction mix to provide the PCR cycler with the ability to normalize the fluorescence signals
P-Axis Passive reference	A passive reference is a fluorescence dye added to the reaction mix to provide the PCR cycler with the ability to normalize the fluorescence signals between all wells.
P-Axis Passive reference Pipette Order	A passive reference is a fluorescence dye added to the reaction mix to provide the PCR cycler with the ability to normalize the fluorescence signals between all wells. Defines the order of samples, controls and standards on the output plate.
P-Axis Passive reference Pipette Order Pipettor Head	A passive reference is a fluorescence dye added to the reaction mix to provide the PCR cycler with the ability to normalize the fluorescence signals between all wells. Defines the order of samples, controls and standards on the output plate. Part of the instrument that is directly involved in the transfer of liquid.
P-Axis Passive reference Pipette Order Pipettor Head Pipetting Piston	A passive reference is a fluorescence dye added to the reaction mix to provide the PCR cycler with the ability to normalize the fluorescence signals between all wells. Defines the order of samples, controls and standards on the output plate. Part of the instrument that is directly involved in the transfer of liquid. Part of the pipettor head. Labware with wells that are connected physically and arranged in a two dimensional way. Plates can be placed in an adapter or as standalone

	ready to use on the instrument.
	Same as Ready-made reaction mix .
Q	
QDEF	QIAGEN Data Exchange Format. XML files that describe plates as well as adapters containing tubes. This file format enables the seamless transfer of plates (or adapters with tubes) together with a corresponding list of samples from one instrument to the next, in a workflow.
	An output file in QDEF format can be saved after a run, describing the output labware. If sample normalization was part of the run, a file describing the dilution labware can also be created.
	A detailed description of the file format can be found at <u>www.giagen.com/goto/QIAgility</u> .
R	
Reagent	A reagent can be combined with other reagents to make a master mix or added individually to a reaction. Reagents are always dispensed into reaction wells before samples.
Reagent Block	Special adapter that can be placed on position R1. The Reagent Block is intended to hold up to three different tube types on the instrument worktable.
Reformatting	Reformatting means exclusive pipetting of a certain liquid from one input container into one or more defined target container.
Replicate	Number of identical reactions derived from the same control or standard sample.
Report	A report can be generated after each run and contains information on the well contents and run settings.
Ready-made reaction mix	A liquid that has already been prepared by the user, which contains all reagents for a PCR reaction, including target-specific components, and is ready to be loaded onto the QIAgility for distribution to the output labware.
	Same as Premade reaction mix.

Sample	Samples are source liquids. Samples may have unique identifiers. Each sample is used as an ingredient in a final PCR setup.
SBS format	Dimensional standard for labware to be used on QIAgility. Abbreviation for Society for Biomolecular Sciences.
Standard Series	A standard series is a dilution series with known concentrations.
Support Packages	A support package contains useful information about the last run which is important when contacting QIAGEN Technical Services for technical assistance.
т	
Target	The specific nucleic acid sequence to be amplified in a PCR reaction.
Тір	Tips are disposables that are used to pipette liquids. They are provided in the Tip Rack Holder and must be set to available or unavailable.
Tip Disposal Box	The tip disposal box collects used tips at the side of the instrument.
Tip Ejector	Used tips are ejected through the tip ejector into a tip disposal box at the side of the instrument.
Tip Rack Holder	A Tip Rack Holder supports tips on the instrument worktable.
Tube	Single container for liquids. A tube is usually placed in an adapter onto the instrument worktable.
U V	
v Volume Calibration	Volume calibration defines the number of units that the P-axis motor must move to accurately deliver a given volume of liquid. Refer to <u>Instrument</u> <u>calibration</u> .
W	
Well	A hole in a plate that can contain liquids.
Worktable	The instrument worktable supports tips racks, adapter plates and tubes.

Х	
XY Calibration	The exact xy-position of each item on the worktable has to be calibrated manually. Refer to <u>Instrument calibration</u> .
Y	
Z	
Z Calibration	The exact height of the tube/well bottoms above the worktable has to be calibrated. Refer to <u>Instrument calibration</u> .

Appendices

11 Appendices

11.1 Appendix A

11.1.1 Technical data

QIAGEN reserves the right to change specifications at any time.

11.1.2 Environmental conditions

Operating conditions

Power	100–240 V AC, 50–60 Hz, 250 VA
	Mains supply voltage fluctuations are not to exceed
	±10% of the nominal supply voltages.
Fuse	F5A 250 V fuse
Overvoltage categor	yll
Air temperature	15–30°C (59–86°F)
Relative humidity*	40–70%
Altitude*	Up to 2000 m (6500 ft.)
Place of operation	For indoor use only
Pollution degree	2
Environmental class	3K2 (IEC 60721-3-3)
	3M2 (IEC 60721-3-3)

*Conditions under which equipment is electrically safe to operate

Transportation conditions

Air temperature	-25°C to 60°C (–13°F to 140°F) in manufacturer's package
Relative humidity	Max. 75% (noncondensing)

Storage conditions

Air temperature	15°C to 30°C (59°F to 86°F) in manufacturer's package
Relative humidity	Max. 75% (noncondensing)

Mechanical data and hardware features

Dimensions	Width: 54 cm (21.3 in.)
	Depth: 63 cm (24.8 in.) without cables
	72 cm (28.3 in.) with cables
	Height: 45 cm (17.7 in.) lid closed
	86 cm (33.9 in) lid open
Mass	Instrument (unpacked): 51 kg (112.4 lb.) standard configuration
	Accessories (unpacked): 8 kg (17.6 lb.)
Capacity	Up to 96 samples per run

11.1.3 Waste Electrical and Electronical Equipment

This section provides information about disposal of waste electrical and electronic equipment by users.

The crossed-out wheeled bin symbol (see below) indicates that this product must not be disposed of with other waste; it must be taken to an approved treatment facility or to a designated collection point for recycling, according to local laws and regulations.

The separate collection and recycling of waste electronic equipment at the time of disposal helps to conserve natural resources and ensures that the product is recycled in a manner that protects human health and the environment.



Recycling can be provided by QIAGEN upon request at additional cost. In the European Union, in accordance with the specific WEEE recycling requirements and where a replacement product is being supplied by QIAGEN, free recycling of its WEEE-marked electronic equipment is provided.

To recycle electronic equipment, contact your local QIAGEN sales office for the required return form. Once the form is submitted, you will be contacted by QIAGEN either to request follow-up information for scheduling collection of the electronic waste or to provide you with an individual quote.

11.1.4 FCC declaration

The "United States Federal Communications Commission" (USFCC) (in 47 CRF 15. 105) declared that the users of this product must be informed of the following facts and circumstances.

This device complies with part 15 of the FCC. Operation is subject to the following two conditions: (1) This device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesired operation.

This Class A digital apparatus complies with Canadian ICES-003.

The following statement applies to the products covered in this manual, unless otherwise specified herein. The statement for other products will appear in the accompanying documentation.

NOTE: This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.



QIAGEN GmbH, Germany is not responsible for any radio television interference caused by unauthorized modifications of this equipment or the substitution or attachment of connection cables and equipment other than those specified by QIAGEN GmbH, Germany. The correction of interference caused by such unauthorized modification, substitution or attachment will be the responsibility of the user.

11.1.5 Declaration of conformity

Name and address of the legal manufacturer:

QIAGEN GmbH QIAGEN Strasse 1 40724 Hilden Germany

An up-to-date declaration of conformity can be requested from QIAGEN Technical Services.

11.2 Appendix B

11.2.1 Liability clause

QIAGEN shall be released from all obligations under its warranty in the event repairs or modifications are made by persons other than its own personnel, except in cases where the Company has given its written consent to perform such repairs or modifications. All materials replaced under this warranty will be warranted only for the duration of the original warranty period, and in no case beyond the original expiration date of original warranty unless authorized in writing by an officer of the Company. Read-out devices, interfacing devices and associated software will be warranted only for the period offered by the original manufacturer of these products. Representations and warranties made by any person, including representatives of QIAGEN, which are inconsistent or in conflict with the conditions in this warranty shall not be binding upon the Company unless produced in writing and approved by an officer of QIAGEN.

11.3 Appendix Kit Compatibility

The following QIAGEN kits are supported by the first release of the QIAgility Setup Manager Software Gene Expression Plug-in.

Product	Cat. no.
QuantiTect [®] Probe PCR Kit (1000 x 50 μ l)	204345
QuantiTect Probe PCR Kit (200)	204343
QuantiTect Multiplex PCR NoRox Kit (1000)	204745
QuantiTect Multiplex PCR Kit (1000)	204545
QuantiTect SYBR® Green PCR Kit (1000 x 50 μ l)	204145
QuantiTect SYBR Green PCR Kit (200)	204143
QuantiFast [®] SYBR Green PCR Kit (400)	204054
QuantiFast SYBR Green PCR Kit (4000)	204057
QuantiFast SYBR Green PCR Kit (2000)	204056
QuantiFast Multiplex PCR +R-Kit (400)	204754
QuantiFast Multiplex PCR +R-Kit (2000)	204756
QuantiFast Probe PCR Kit (400)	204254
QuantiFast Probe PCR Kit (2000)	204256
Rotor-Gene SYBR Green PCR Kit (2000)	204076
Rotor-Gene SYBR Green PCR Kit (400)	204074
Rotor-Gene Multiplex PCR Kit (400)	204774
Rotor-Gene Multiplex PCR Kit (80)	204772
Rotor-Gene Probe PCR Kit (400)	204374
QuantiNova® Probe PCR Kit (500)	208254
QuantiNova Probe PCR Kit (100)	208252
QuantiNova Probe PCR Kit (2500)	208256
QuantiNova SYBR Green PCR Kit (100)	208052
QuantiNova SYBR Green PCR Kit (500)	208054
QuantiNova SYBR Green PCR Kit (2500)	208056

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

The following QIAGEN kits are supported by the first release of the QIAgility Setup Manager Software Pathogen Detection Plug-in.

Product	Cat. no.
QuantiFast Pathogen PCR +IC Kit (400)	211354
QuantiFast Pathogen PCR +IC Kit (100)	211352
QuantiTect Virus Kit (50)	211011
QuantiTect Virus Kit +ROX Vial Kit (50)	211031
QuantiNova Pathogen +IC Kit (100)	208652
QuantiNova Pathogen +IC Kit (500)	208654
QuantiTect Virus Kit (200)	211013
QuantiTect Virus Kit (1000)	211015
QuantiTect Virus Kit +ROX Vial Kit (200)	211033
QuantiTect Virus Kit +ROX Vial Kit (1000)	211035

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

11.4 Appendix C Consignes de sécurité

Avant d'utiliser le QIAgility il est impératif de lire attentivement ce manuel et de porter une attention particulière aux consignes de sécurité. Afin de garantir un fonctionnement de l'appareil en toute sécurité et de maintenir l'appareil en bon état de marche, il est impératif de suivre les instructions et consignes de sécurité fournies dans le présent manuel d'utilisation.

Les types d'informations de sécurité suivants sont fournis tout u long du manuel.

Le terme AVERTISSEMENT signale des situations risquant d'entraîner des accidents corporels dont l'utilisateur, ou d'autres personnes, pourraient être victime.
Les détails concernant ces circonstances sont donnés dans un encadré identique à celui-ci.

Le terme ATTENTION signale des situations risquant d'entraîner des détériorations de l'appareil ou de tout autre matériel.
Les détails concernant ces circonstances sont donnés dans un encadré identique à celui-ci.

Les conseils donnés dans ce manuel ont pour but de venir compléter les exigences de sécurité habituelles en vigueur dans le pays de l'utilisateur, et non de s'y substituer.

11.4.1 Utilisation appropriée

AVERTISSEMENT/ ATTENTION	Risque d'accident corporel et de détérioration du matériel [W1] L'utilisation inappropriée du QIAgility peut entraîner des accidents corporels ou une détérioration de l'appareil.
	L'utilisation du QIAgility est exclusivement réservée au personnel qualifié ayant été convenablement formé. La maintenance du QIAgility doit uniquement être effectuée par des spécialistes du Service Après-Vente QIAGEN.
	spécialistes du Service Après-Vente QIAGEN.

AVERTISSEMENT/ ATTENTION Risque de surchauffe [W2] Afin de garantir une bonne ventilation, laisser un dégagement d'au moins 10 cm sur les côtés et à l'arrière du QIAgility. Les fentes et les ouvertures qui assurent la ventilation de l'appareil ne doivent pas êt obstruées.	
--	--

Risque d'accident corporel et de détérioration du matériel [W3]
Le QIAgility est trop lourd pour être soulevé par une seule personne. Afin d'éviter tout accident corporel et toute détérioration du matériel, ne pas soulever l'appareil seul.

AVERTISSEMENT/ ATTENTION	Atmosphère explosive [W5] Le QIAgility n'est pas conçu pour être utilisé dans une atmosphère explosive.
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AVERTISSEMENT/ ATTENTION	Risque d'explosion [W6] Le QIAgility a été conçu pour l'utilisation des réactifs et substances fournis par les kits QIAGEN.
	L'utilisation de réactifs et de substances autres que celles indiquées peut entrainer un risque d'incendie ou d'explosion.

	Détérioration de l'appareil [C1] La lumière directe du soleil peut décolorer les pièces de l'appareil et abîmer les éléments en plastique. Tenir le QIAgility à l'abri de la lumière directe du soleil.
--	---

Éviter de renverser de l'eau ou des produits chimiques sur le QIAgility. La détérioration de l'appareil dû au déversement de liquides annule la garantie.

	Risque d'accident corporel [W7] Pendant les étapes de calibration des positions, le bras robotisé se déplace alors que le capot de l'appareil est relevé. Ne jamais actionner ou cliquer sur aucunes touches tant que des parties du corps se trouvent dans l'enceinte de l'appareil.
--	--

En cas d'urgence, éteindre le QIAgility à l'aide de l'interrupteur d'alimentation situé à l'arrière de l'appareil et débrancher le cordon d'alimentation de la prise de courant.

11.4.2 Sécurité électrique

Avant l'entretien, débrancher le cordon d'alimentation de la prise de courant.

AVERTISSEMENT/ ATTENTION	Danger électrique [W8] Toute interruption du conducteur de protection (conducteur de terre/de masse) à l'intérieur ou à l'extérieur de l'appareil ou toute déconnexion de la borne du conducteur de protection est susceptible de rendre l'appareil dangereux. Toute interruption intentionnelle est interdite.
	Tensions mortelles à l'intérieur de l'appareil. Lorsque l'appareil est relié à l'alimentation, les bornes peuvent être sous tension et l'ouverture de capots de l'appareil ou le retrait de pièces risque d'exposer des éléments sous tension.

Danger d'irradiation UV [W9] Un dispositif électronique de verrouillage empêche la lampe UV de fonctionner tant que le capot de l'appareil est ouvert. Ce dispositif ne doit en ausur cas être désactivé
doit en aucun cas être désactivé.

Afin que le QIAgility fonctionne de manière satisfaisante et en toute sécurité, suivre les conseils suivants:

- Le cordon d'alimentation de l'appareil doit être branché sur une prise d'alimentation munie d'un conducteur de protection (terre/masse).
- Ne pas régler ni remplacer les pièces internes à l'instrument.
- Ne pas faire fonctionner l'appareil si des capots ou des pièces ont été retirés.
- En cas de déversement de liquides à l'intérieur de l'appareil, débrancher celui-ci de la prise d'alimentation et contacter les Services techniques de QIAGEN.

Si l'appareil présente un danger électrique, empêcher le reste du personnel de s'en servir et contacter les Services Techniques de QIAGEN. L'appareil peut présenter un danger électrique dans les cas suivants:

- Le cordon d'alimentation présente des signes de détérioration.
- L'appareil a été stocké pendant une longue période dans des conditions non conformes à celles énoncées dans l'Annexe A.
- L'appareil a subi des chocs sévères durant le transport.

11.4.3 Sécurité biologique

Les spécimens et les réactifs contenant des matières provenant d'êtres humains doivent être considérés comme potentiellement infectieux. Utilisez des procédures de laboratoire sûres comme décrit dans des publications telles que Biosafety in Microbiological and Biomedical Laboratories, HHS (www.cdc.gov/od/ohs/biosfty/biosfty.htm).

11.4.3.1 Échantillons

Samples may contain infectious agents. You should be aware of the health hazard presented by such agents, and should use, store and dispose of such samples according to the required safety regulations.

Samples containing infectious agents [W10] Certains échantillons utilisés avec cet appareil peuvent contenir des agents infectieux. Manipulez ces échantillons avec la plus grande précaution et conformément aux règles de sécurité nécessaires. Portez toujours des lunettes de protection, deux paires de gants et une blouse de laboratoire.
La personne responsable (par exemple, le directeur du laboratoire) doit prendre les précautions nécessaires afin de garantir que l'environnement de travail est sûr, que les opérateurs de l'appareil sont convenablement formés et ne sont pas exposés à des niveaux dangereux d'agents infectieux comme cela est défini dans les fiches techniques santé-sécurité (SDS) ou dans les documents de l'OSHA*, de l'ACGIH [†] ou du COSHH [‡] applicables.
L'évacuation des vapeurs et la mise au rebut des déchets doivent s'effectuer conformément à toutes les réglementations et lois nationales, régionales et locales relatives à la santé et à la sécurité.

* OSHA : Occupational Safety and Health Administration (États-Unis d'Amérique) (Administration pour la santé et la sécurité du travail).

[†] ACGIH : American Conference of Government Industrial Hygienists (États-Unis d'Amérique) (Conférence américaine des hygiénistes industriels gouvernementaux).

[‡] COSHH : Control of Substances Hazardous to Health (Royaume-Uni) (Contrôle des substances dangereuses pour la santé).

11.4.4 Produits chimiques

Produits chimiques [W11] Certains produits chimiques utilisés avec cet appareil peuvent être dangereux ou le devenir après l'exécution du cycle du protocole.
Toujours porter des lunettes de protection, des gants et une blouse de laboratoire.
La personne responsable (par exemple, le directeur du laboratoire) doit prendre les précautions nécessaires afin de garantir que le lieu de travail environnant est sûr et que les opérateurs de l'appareil ne sont pas exposés à des niveaux dangereux de substances (chimiques ou biologiques) toxiques comme cela est défini dans les fiches de données de sécurité (FDS) ou dans les documents de l'OSHA*, de l'ACGIH [†] ou du COSHH [‡] applicables.
L'évacuation des vapeurs et la mise au rebut des déchets doivent s'effectuer conformément à toutes les réglementations et lois nationales, régionales et locales relatives à la santé et à la sécurité.

* OSHA : Occupational Safety and Health Administration (États-Unis d'Amérique) (Administration pour la santé et la sécurité du travail).

[†] ACGIH : American Conference of Government Industrial Hygienists (États-Unis d'Amérique) (Conférence américaine des hygiénistes industriels gouvernementaux).

[‡] COSHH : Control of Substances Hazardous to Health (Royaume-Uni) (Contrôle des substances dangereuses pour la santé).

11.4.4.1 Vapeurs toxiques

Si vous travaillez avec des solvants volatils ou des substances toxiques, vous devez disposer d'un système de ventilation de laboratoire efficace afin d'évacuer les vapeurs qui peuvent être générées.

11.4.5 Traitement des déchets

Le matériel en plastique usagé peut contenir des produits chimiques dangereux. Ces déchets doivent être convenablement collectés et mis au rebut conformément aux réglementations de sécurité locales.

Pour plus d'informations sur la mise au rebut du QIAgility, voir l'Annexe A.

11.4.6 Dangers mécaniques

	Pièces mobiles [W12] Éviter le contact avec les pièces en mouvement lorsque le QlAgility est en marche. Ne jamais placer les mains sous le bras robotisé lorsque celui- ci s'abaisse. Ne pas tenter de déplacer des plaques ou des tubes lorsque le bras robotisé est en marche.
--	---

	 Pièces mobiles [W15] Pour éviter tout contact avec des pièces en mouvement pendant le fonctionnement du QIAgility, l'appareil doit être utilisé avec le couvercle fermé. Si le capteur de couvercle intégré ne fonctionne pas correctement, prendre contact avec les services techniques de QIAGEN.
--	---

Détérioration de l'appareil [C3] N'appliquer aucune force manuelle sur le bras Y (c'est-à-dire la tête de pipetage).
En cas de déplacement manuel accidentel du bras Y, il est fortement recommandé de redémarrer le système.

Afin que le QIAgility fonctionne de manière satisfaisante et en toute sécurité, suivre les conseils suivants

- Utiliser uniquement les tips et les supports de tips recommandés.
- Dans la mesure du possible, maintenir le capot de l'appareil fermé.
- Lors de la calibration des plaques capot ouvertes, toujours se tenir à l'écart de l'appareil au moment des réglages. Le dispositif de verrouillage est désactivé en mode de calibration. Par conséquent, ne pas cliquer avec la souris ni appuyer sur une des touches du clavier lors de l'observation de l'inspection visuelle de la calibration des plaques. Attendre l'arrêt complet du bras robotisé pour déplacer une plaque ou un tube.
- Ne rien poser sur le bras robotisé.

Avant de retirer des plaques ou des tubes de la table de travail, sélectionner « Pause » dans la barre d'outils du logiciel QIAgility pour mettre l'appareil en mode de pause. Attendre l'arrêt complet du bras robotisé. Cela peut prendre jusqu'à 10 s.

11.4.7 Sécurité relative à la maintenance

Procéder à la maintenance comme décrit à la <u>Maintenance procedures</u>. QIAGEN facture les réparations rendues nécessaires suite à une maintenance inappropriée.

	Risque d'accident corporel et de détérioration du matériel [W13] Effectuer uniquement la maintenance spécifiquement décrite dans ce manuel.
--	--

	Risque de décharge électrique [W14] Ne pas ouvrir les panneaux du QIAgility. Risque d'accident corporel et de détérioration du matériel Effectuer uniquement la maintenance spécifiquement décrite dans ce manuel.
--	--

ATTENTION	Détérioration de l'appareil [C4]
	Ne pas utiliser de solvants ni de réactifs contenant des acides, des bases ou des composés abrasifs pour nettoyer le QIAgility.

ATTENTION	Détérioration du capot du QIAgility [C5]
	Ne pas utiliser de solvants ni de réactifs contenant des acides, des bases ou des composés abrasifs pour nettoyer le capot de l'appareil. L'alcool peut endommager le capot. Employer uniquement de l'eau distillée.

ATTENTION	Détérioration des panneaux latéraux du QIAgility [C6]
	Ne jamais nettoyer le capot ou les panneaux latéraux de l'appareil à l'alcool ou avec une solution à base d'alcool.

ATTENTION	Réinstallation du QIAgility [C7]
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La pièce la plus importante de l'emballage est le support en bois qui immobilise le bras Y. Ce support doit être réutilisé si vous déplacez le QIAgility.

11.4.8 Symboles sur le QIAgility

Symbole	Emplacement	Description
	Table de travail et près de la plaque signalétique	Symbole de mise en garde
	Table de travail et près de la sortie des tips	Symbole de danger biologique
	Table de travail	Symbole de risque d'écrasement
CE	Plaque signalétique à l'arrière de l'appareil	Marque CE
	Plaquette à l'arrière de l'appareil	Marquage CSA pour le Canada et les Etats-Unis
	Plaquette à l'arrière de l'appareil	Fabricant légal
X	Plaque signalétique à l'arrière de l'appareil	Symbole DEEE
F©	Plaque signalétique à l'arrière de l'appareil	Label FCC de la Federal Communications Commission des États-Unis
\bigotimes	Plaque signalétique à l'arrière de l'appareil	Marque RCM (antérieurement marque C-Tick) pour l'Australie (identifiant du fournisseur N17965)
2 5	Plaque signalétique à l'arrière de l'appareil	Label RoHS pour la Chine (restriction de l'utilisation de certaines substances dangereuses dans l'équipement électrique et électronique))
ĺ	À l'arrière de l'appareil	Consulter les consignes d'utilisation

11.5 Appendix D Sicherheitshinweise

Lesen Sie dieses Handbuch sorgfältig durch, bevor Sie den QlAgility benutzen. Beachten Sie dabei insbesondere die Sicherheitshinweise. Die Gebrauchsanweisungen und Sicherheitshinweise im Handbuch müssen befolgt werden, um einen sicheren Betrieb des Geräts zu gewährleisten und das Gerät in einem sicheren Zustand zu erhalten. In diesem Handbuch werden die folgenden Kategorien von Sicherheitshinweisen verwendet:

WARNUNG	Der Begriff "WARNUNG" ("WARNING") weist Sie auf Situationen hin, in denen eine Verletzungsgefahr für Sie selbst oder andere Personen besteht.	
	Nähere Einzelheiten über diese Situationen werden in einem Textfeld wie diesem beschrieben.	

	Der Begriff "ACHTUNG" ("CAUTION") weist Sie auf Situationen hin, in denen das Gerät oder andere Geräte beschädigt werden könnten. Nähere Einzelheiten über diese Situationen werden in einem Textfeld wie diesem
<u> </u>	beschrieben.

Die in diesem Handbuch enthaltenen Hinweise stellen eine Ergänzung und keinen Ersatz der üblichen Sicherheitsanforderungen dar, die im jeweiligen Land gelten.

11.5.1 Sachgemäße Handhabung

	Verletzungsgefahr und Beschädigung des Geräts [W1] Die unsachgemäße Bedienung des QlAgility kann zu einer Verletzung des Benutzers oder zur Beschädigung des Geräts führen. Der QlAgility darf nur durch qualifiziertes Personal, das entsprechend geschult wurde, bedient werden. Die Instandhaltung des QlAgility darf nur durch einen Außendienst-Mitarbeiter des QIAGEN Service durchgeführt werden.
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	Überhitzungsgefahr [W2] Vergewissern Sie sich, dass zwischen Wand und QlAgility (Rückseite und Seitenteile) ein Mindestabstand von 10 cm eingehalten wird. Das gewährleistet eine ausreichende Belüftung des Gerätes. Die Lüftungsschlitze und Öffnungen,
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WARNUNG/ ACHTUNG Verletzungsgefahr und Beschädigung des Geräts [W3] Der QlAgility ist zu schwer, um von einer einzelnen Person gehoben zu we Zur Vermeidung einer Verletzung oder Beschädigung des Gerätes heben Gerät nicht alleine an.

	Explosionsfähige Atmosphären [W5] Der QlAgility ist nicht für den Gebrauch in explosionsfähigen Atmosphären vorgesehen.
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	Explosionsgefahr [W6] Der QlAgility ist ausschließlich mit Reagenzien und Substanzen aus den QlAGEN Kits zu benutzen. Die Benutzung von anderen Reagenzien oder Substanzen kann Feuer oder eine Explosion auslösen.
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ACHTUNG	Beschädigung des Geräts [C2]
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Vermeiden Sie es, Wasser oder Chemikalien auf der Oberfläche des QIAgility zu verschütten. Durch verschüttete Chemikalien oder verschüttetes Wasser verursachte Geräteschäden sind nicht durch die Garantie abgedeckt.

Schalten Sie im Notfall den QIAgility aus (der Netzschalter befindet sich auf der Geräterückseite), und ziehen Sie den Netzstecker aus der Steckdose.

11.5.2 Elektrische Sicherheit

Ziehen Sie das Netzanschlusskabel aus der Steckdose, bevor Sie Wartungsarbeiten am Gerät vornehmen.

Gefahr durch Stromschlag [W8] Jede Unterbrechung des Schutzleiters (Erdungs- bzw. Masseleiter) im Gerät oder außerhalb des Geräts und jede Abtrennung des Schutzleiters am Anschluss der Netzleitung erhöht die Gefahr eines Stromschlags. Eine absichtliche Unterbrechung der Schutzleiterverbindung ist verboten. Gefährliche Spannung im Gerät Wenn das Gerät an die Stromversorgung angeschlossen ist, sind die Anschlussstellen spannungsführend. Durch das Öffnen der Abdeckungen oder das Entfernen von Gehäuseteilen können spannungsführende Komponenten
das Entfernen von Gehäuseteilen können spannungsführende Komponenten freigelegt werden.

WARNUNG	Gefahr durch UV-Strahlung [W9]
⚠	Eine elektronisch gesteuerte Verriegelung stellt sicher, dass die Gerätehaube geschlossen sein muss, bevor die UVLampe eingeschaltet werden kann. Diese Verriegelung sollte auf keinen Fall deaktiviert werden.

Um einen zufriedenstellenden und sicheren Betrieb des QIAgility zu gewährleisten, befolgenden Sie bitte die nachstehenden Richtlinien:

- Das Netzkabel muss an eine Wechselstrom-Steckdose mit Schutzleiter (Erdungs-/ Masseleiter) angeschlossen werden.
- Nehmen Sie im Geräteinneren keine Einstellungen an Teilen vor und wechseln Sie keine Teile aus.
- Nehmen Sie das Gerät nicht in Betrieb, wenn Abdeckungen oder Teile entfernt worden sind.
- Falls Flüssigkeit auf dem Gerät verschüttet wird und in das Gerät läuft, dann schalten Sie es sofort aus, trennen Sie es von der Netzspannung (Stecker ziehen!) und setzen Sie sich mit dem Technischen Service von QIAGEN in Verbindung.

Falls die elektrische Sicherheit bei der Bedienung des Geräts nicht mehr gewährleistet werden kann, muss das Gerät gegen unbefugte oder unabsichtliche Benutzung gesichert werden. Kontaktieren Sie anschließend den Technischen Service von QIAGEN. Die elektrische Sicherheit des Geräts ist nicht mehr gegeben, wenn:

- das Netzkabel beschädigt ist;
- das Gerät längere Zeit unter ungünstigen Bedingungen, d. h. unter anderen Bedingungen als in Anhang A angegeben, gelagert wurde;
- das Gerät unsachgemäß transportiert worden ist.

11.5.3 Biologische Sicherheit

Bei Substanzen und Reagenzien, die humanes Untersuchungsmaterial enthalten, sollte immer von einer möglichen Infektionsgefahr ausgegangen werden. Wenden Sie nur sichere Laborverfahren an, wie sie z. B. in Veröffentlichungen wie Biosafety in Microbiological and Biomedical Laboratories (HHS, www.cdc.gov/biosafety/publications) beschrieben werden.

11.5.3.1 Proben

Proben können infektiöse Erreger enthalten. Sie sollten sich der Gesundheitsgefahr bewusst sein, die von diesen Erregern ausgeht, und derartige Proben gemäß den erforderlichen Sicherheitsbestimmungen handhaben, lagern und entsorgen.

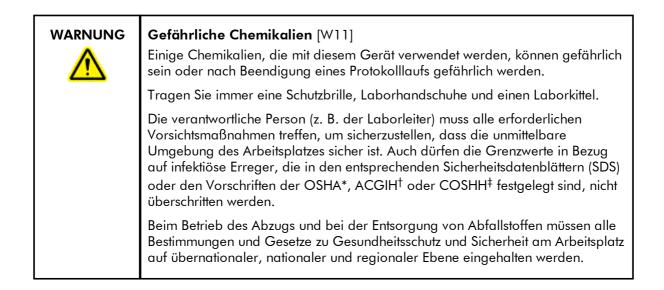
Proben, die infektiöse Erreger enthalten [W10] Einige Proben, die mithilfe dieses Geräts verarbeitet werden, können infektiöse Erreger enthalten. Gehen Sie beim Umgang mit diesen Proben mit der größtmöglichen Vorsicht und gemäß den erforderlichen Sicherheitsbestimmungen vor.
Tragen Sie immer eine Schutzbrille, zwei Paar Laborhandschuhe und einen Laborkittel.
Die verantwortliche Person (z. B. der Laborleiter) muss alle erforderlichen Vorsichtsmaßnahmen treffen, um sicherzustellen, dass die unmittelbare Umgebung des Arbeitsplatzes sicher ist und die Bediener des Geräts ausreichend geschult sind. Außerdem dürfen die Grenzwerte in Bezug auf infektiöse Erreger, die in den entsprechenden Sicherheitsdatenblättern (SDS) oder den Vorschriften der OSHA,* ACGIH [†] oder COSHH [‡] festgelegt sind, nicht überschritten werden.
Beim Betrieb des Abzugs und bei der Entsorgung von Abfallstoffen müssen alle Bestimmungen und Gesetze zu Gesundheitsschutz und Sicherheit am Arbeitsplatz auf übernationaler, nationaler und regionaler Ebene eingehalten werden.

* OSHA: Occupational Safety and Health Administration (Vereinigte Staaten von Amerika)

[†] ACGIH: American Conference of Government Industrial Hygienists (Vereinigte Staaten von Amerika)

[‡] COSHH: Control of Substances Hazardous to Health (Vereinigtes Königreich)

11.5.4 Chemikalien



- * OSHA: Occupational Safety and Health Administration (Vereinigte Staaten von Amerika)
- [†] ACGIH: American Conference of Government Industrial Hygienists (Vereinigte Staaten von Amerika) [‡] COSHH: Control of Substances Hazardous to Health (Vereinigtes Königreich)

11.5.4.1 Giftige Dämpfe

Alle Arbeiten mit flüchtigen Lösungsmitteln oder toxischen Substanzen müssen unter einem funktionierenden Laborabzugssystem durchgeführt werden, damit die möglicherweise entstehenden Dämpfe abziehen können.

11.5.5 Abfallentsorgung

Benutzte Kunststoff-Laborartikel können gefährliche Chemikalien enthalten. Derartige Abfälle müssen gemäß den geltenden regionalen Sicherheitsbestimmungen gesammelt und entsorgt werden.

Beachten Sie bei der Entsorgung des QIAgility die gesetzlichen Bestimmungen zur Entsorgung von Elektround Elektronik-Altgeräten (siehe Anhang A).

11.5.6 Gefahren durch mechanische Teile

WARNUNG	Sich bewegende Geräteteile [W12]		
	Vermeiden Sie jeglichen Kontakt mit sich bewegenden Geräteteilen, während der QlAgility in Betrieb ist. Auf gar keinen Fall dürfen sich Ihre Hände unter dem Roboterarm befinden, wenn dieser herunterfährt. Versuchen Sie nicht, Reaktionsgefäße oder Platten zu entnehmen oder zu bewegen, während der Roboterarm arbeitet.		

Wenn der integrierte Deckelsensor nicht ordnungsgemäß funktioniert, wenden Sie sich an den Technischen Service von QIAGEN.
--

	Beschädigung des Geräts [C3] Bewegen Sie den Y-Arm (den Pipettierkopf) nicht von Hand.
<u> </u>	Wir empfehlen dringend, einen Neustart des Systems durchzuführen, falls der Y- Arm versehentlich von Hand bewegt wurde.

Um einen zufriedenstellenden und sicheren Betrieb des QIAgility zu gewährleisten, befolgen Sie bitte die nachstehenden Richtlinien:

- Verwenden Sie nur die empfohlenen Pipettenspitzen und -Racks.
- Halten Sie die Gerätehaube möglichst immer geschlossen.
- Wenn bei geöffneter Gerätehaube die Plattenpositionen kalibriert werden, achten Sie darauf, dass Sie nicht in den Arbeitsbereich des Roboterarms kommen, wenn Sie Justierungen vornehmen. Im Kalibrier-Modus ist die Verriegelung der Gerätehaube deaktiviert. Achten Sie deshalb darauf, nicht mit der Maus zu klicken oder eine der Bedienungstasten auf der Tastatur zu drücken, wenn Sie die Kalibrierungen der Plattenpositionen überprüfen. Warten Sie, bis die Bewegungsabläufe des Roboterarms beendet sind, bevor Sie Reaktionsgefäße oder Platten entnehmen oder bewegen.
- Stellen Sie keine Gegenstände auf den Roboterarm.

Wählen Sie die Option "Pause" in der Werkzeugleiste der QIAgility Software, um das Gerät anzuhalten, bevor Sie Reaktionsgefäße oder Platten von der Arbeitsplattform entfernen. Warten Sie, bis der Roboterarm die gerade ablaufende Bewegung beendet hat – dies kann bis zu 10 Sekunden dauern.

11.5.7 Sicherheitshinweise – Wartungsarbeiten

Führen Sie alle Wartungsarbeiten gemäß den Anweisungen in Abschnitt durch. QIAGEN stellt alle Reparaturen in Rechnung, die nachweislich auf eine inkorrekte Wartung zurückzuführen sind.

ACHTUNG	Verletzungsgefahr und Beschädigung des Geräts [W13] Führen Sie nur Wartungsarbeiten durch, die ausdrücklich in dieser Gebrauchsanweisung beschrieben sind.
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	Gefahr durch Stromschlag [W14] Öffnen Sie keine Gehäuseteile des QIAgility. Verletzungsgefahr und Beschädigung des Geräts Führen Sie nur Wartungsarbeiten durch, die ausdrücklich in dieser Gebrauchsanweisung beschrieben sind.
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ACHTUNG	Beschädigung des Geräts [C4]	
	Verwenden Sie weder Lösungsmittel noch Reagenzien, die Säuren, Laugen oder Abrasivstoffe enthalten, um das QIAgility zu reinigen.	

ACHTUNG	Beschädigung der QlAgility Gerätehaube [C5] Verwenden Sie weder Alkohol noch Lösungsmittel oder Reagenzien, die Säuren, Laugen oder Abrasivstoffe enthalten, um die QlAgility Haube zu reinigen. Alkohol schädigt die Gerätehaube. Verwenden Sie zum Reinigen der Gerätehaube ausschließlich destilliertes Wasser.	

ACHTUNG	CHTUNG Beschädigung der QlAgility Seitenwände [C6]	
	Reinigung sie niemals die Gerätehaube und Seitenwände mit Alkohol oder alkoholhaltigen Lösungen.	

ACHTUNG
حنے

Standortwechsel des QIAgility [C7]

Der wichtigste Bestandteil der Verpackung ist die Holzstütze, die den Y-Arm fixiert. Diese Stütze muss verwendet werden, wenn der QlAgility an einen anderen Standort verlagert wird.

11.5.8	Symbole auf dem QIAgility
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Symbol	Ort	Beschreibung
	Arbeitsplattform und nähe Typenschild	Symbol "Achtung"
	Auf der Arbeitsplattform und nähe "Tip ejector" Ausgang	Symbol "Biogefährdung"
	Arbeitsplattform	Symbol "Quetschgefahr"
CE	Typenschild auf der Rückseite des Instruments	CE-Kennzeichnung
	Plakette auf der Rückseite des Gerätes	CSA-Zeichen für Kanada und die USA
	Typenschild auf der Rückseite des Instruments	Hersteller i. S. d. Gesetzes
X	Typenschild auf der Rückseite des Instruments	WEEE-Kennzeichnung (gemäß europäischer Richtlinien bzw. Elektround Elektronik-Altgeräte- Verordnung)
F©	Typenschild auf der Rückseite des Instruments	FCC-Kennzeichen der Federal Communications Commission der Vereinigten Staaten
	Typenschild auf der Rückseite des Instruments	RCM-Kennung (ehemals C-Tick-Kennzeichen) für Australien (Herstellerkennung: N17965)
(25)	Typenschild auf der Rückseite des Instruments	Markierung gemäß RoHSRichtlinie für China (Beschränkung der Verwendung bestimmter gefährlicher Stoffe in Elektro- und Elektronikgeräten)
ĺ	Auf der Rückseite des Instruments	Bedienungsanleitung beachten

11.6 Appendix F

11.6.1 License Terms

Xceed license

QIAgility Setup Manager Software uses the Xceed library version 4.2.0, which is licensed under the Xceed Software License Agreement.

Xceed Software License Agreement Revised January 23rd, 2007

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11.6.2 Version history

Description
Previous version
Updated the Normalization and Serial Dilution workflow screen to include Kit Information.
Updated the <u>Symbols on the QIAgility</u> section.
Updated the Environmental conditions section.
Added the Sample to output include premix on the <u>Generic PCR Liquid</u> <u>Classes</u> .
Updated the weight information of the QIAgility.
Updated the laptop specifications on the <u>Computer</u> section.
Added a note on multiple sections to explain the new cursor $igoplus$.

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