iontorrent

Genexus™ Integrated Sequencer

USER GUIDE

for use with Genexus™ Software 6.6.2

Catalog Number A45727

Publication Number MAN0017910

Revision F.0

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

Life Technologies Holdings Pte Ltd |
Block 33 |
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#07-06, Singapore 739256

Products manufactured at this site:

Genexus™ Integrated Sequencer

Life Technologies Corporation |
200 Oyster Point Blvd |
South San Francisco, California 94080 USA

Products manufactured at this site:

Genexus™ Software

Life Technologies Corporation |
7335 Executive Way |
Frederick, Maryland 21704 USA

Products manufactured at this site:

- GX5™ Chip and Genexus™ Coupler
- Genexus™ Library Strips 1 and 2-AS
- Genexus™ Library Strips 1 and 2-HD
- Genexus™ Templating Strips 3-GX5™ and 4
- Genexus™ Templating Strips 3B-GX5™ and 4
- Genexus™ Barcodes 1–96 AS
- Genexus™ Barcodes 1–32 HD
- Genexus™ Primer Pool Tubes
- Genexus™ Pipette Tips
- Genexus™ Sequencing Kit
- Genexus™ Controls
- Genexus™ Conical Bottles
- Genexus™ Filter
- Genexus™ GX5™ Starter Pack-AS
- Genexus™ GX5™ Starter Pack-HD
- Oncomine™ GX assays

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: MAN0017910 F.0 (English)

Revision	Date	Description
F.0	18 October 2022	• Updated for Genexus™ Software 6.6.2.1 • Added procedural guidelines and best practices for reagent handling and for preventing sample and control contamination. See “Guidelines for panel and reagent use and handling” on page 33 and “Guidelines for preventing contamination” on page 34. • Added support for use of the Genexus™ Strip Centrifuge Adapter. See “Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter” on page 227. • Updated recommended procedure for cleaning and decontaminating the sequencer. See “Clean or decontaminate the sequencer” on page 194. • Added guidance for consumables loading (pipette tip box loading, Genexus™ Bottle 2 installation) in “Load the sequencer and start a run” on page 100. • Moved required reagents and supplies from Chapter 1, “Product information” to a new Chapter 2, “Reagents, supplies, and required materials” for ease of use. • Moved sequencer maintenance information from Appendix F, “Supplemental information” to a new Appendix B, “Maintain the sequencer” for ease of use. • Moved Library QC Archive information from Appendix F, “Supplemental information” to a new Appendix E, “Library QC Archive: recover library preparations from the Genexus™ Integrated Sequencer for reuse” for ease of use. • Updated guidance for antivirus software. See “Antivirus software” on page 42.
E.0	9 November 2021	New revision of the user guide for early access users including the following updates: • Updated for Genexus™ Software 6.6. • Run planning updated to include BAM to Result runs. See “Plan a BAM to Result run” on page 90. • Antivirus software guidance added. See “Antivirus software” on page 42.
D.0	25 November 2020	Corrected an error in Genexus™ Software version number in “Software compatibility and requirements” on page 13.
C.0	29 October 2020	• Updated the contents of the Genexus™ Installation and Training Kit. See “Genexus™ Integrated Sequencer”. • Corrected guidance on reuse of Genexus™ Barcodes plates. See “Guidelines for Genexus™ Integrated Sequencer operation” on page 35. • Added the topic “Guidelines for expired reagents and chips” on page 36. • Changed the recommended concentration for manually prepared libraries from 125 pM to 200 pM.

Revision	Date	Description
C.0 *(continued)*	29 October 2020	• Clarified use of "Do Force Clean" checkbox. See “Load the sequencer and start a run” on page 100. • Added screenshots for chip verification and leak checking in “Load the sequencer and start a run” on page 100. • Updated “Options for an expired sequencer initialization” and moved to Chapter 7. • Updated recommended actions for instrument error and warning messages in “Genexus™ Integrated Sequencer error and warning messages” on page 184. • Performance qualification results table updated with removal of MAPD metric to align with a new version of the Performance Qualification Assay (v1.4.0).
B.0	7 July 2020	• Updated for Genexus™ Software 6.2. • Updated for library and template strip part numbers. • Updated the components of the Genexus™ Installation and Training Kit. • Added guidance for using inline controls for troubleshooting. • Added the troubleshooting section #unique_173/unique_173_Connect_42_GUID-43D2DE91-971D-4FC2-BED0-2823DC853B50. • Added the topic #unique_49/unique_49_Connect_42_GUID-A193553D-2F33-4194-8074-008BF88FA6DE on page 195. • Added the section #unique_204/unique_204_Connect_42_GUID-BDCC9371-0C11-4BDA-ADE4-BD0EF4CD0C10 on page 0 .
A.0	13 November 2019	New user guide for the Genexus™ Integrated Sequencer.

The information in this guide is subject to change without notice.

Contents

About this guide

IMPORTANT! Before using this product, read and understand the information in the "Safety" appendix in this document.

Purpose of this guide

This user guide provides detailed instructions for operating the Genexus™ Integrated Sequencer, as well as product information, troubleshooting, instrument maintenance, and other instrument information. In addition, the guide provides basic instructions for assay creation, sample entry, run planning, and data review in Genexus™ Software 6.6.2.1.

For detailed instructions for using Genexus™ Software for sample management, assay creation, data analysis, and system management, see the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953), or the software help system.

For detailed instructions for using the Genexus™ Purification Instrument for sample purification, see the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475).

For a list of Oncomine™ GX assay-specific user guides, see "Related documentation" on page 245.

Prerequisites

Category	Prerequisites
Instrument	• Genexus™ Integrated Sequencer • Reagents and supplies for operating the Genexus™ Integrated Sequencer
Software	Genexus™ Software 6.6.2.1 or later
Functional knowledge and understanding	• Key steps in a next-generation sequencing (NGS) workflow • Main functions of the Genexus™ Integrated Sequencer • Main features of the Genexus™ Software 6.6.2.1 or later

1 Product information

Product description

The Ion Torrent™ Genexus™ Integrated Sequencer is a next-generation sequencing (NGS) system that integrates library preparation, template preparation, and sequencing into a single-day, single-instrument automated run. The instrument supports sample-to-results, nucleic acid-to-results, and library-to-results sequencing runs (up to 32 DNA or RNA samples/run). Ion Torrent™ Genexus™ Software streamlines the NGS workflow by integrating the setup-to-report workflow within a single software system. Key features include:

- Go from nucleic acid to report in a single day
- Flexible and cost-effective run planning making use of a multi-lane, multi-run sequencing chip: the Ion Torrent™ GX5™ Chip
- Automated library preparation, including cDNA synthesis, for up to 400 base-read libraries using either standard Ion AmpliSeq™ or Ion AmpliSeq™ HD library chemistry
- Automated Ion Sphere™ Particle (ISP)-loading and template preparation with on-chip amplification
- Support for up to four compatible assays on a GX5™ Chip in a single run, with an output of 12–15 million reads from each lane.
- As little as five minutes total hands-on time required per run
- Real-time consumables tracking by the instrument to guide consumable loading during run setup
- Consumables that are usable for up to 14 days after loading
- On-instrument sequencing data analysis requiring no external server

Genexus™ Integrated Sequencer

The Genexus™ Integrated Sequencer includes the following components.

Components	Cat. No.
Genexus™ Integrated Sequencer	A45727
Genexus™ Installation and Training Kit	A40278[1]

[1] Not available for separate purchase.

The Ion Torrent™ Genexus™ Installation and Training Kit (Cat. No. A40278) is available to first-time owners of a Genexus™ Integrated Sequencer and is shipped with the instrument. The kit contains the following reagents, supplies, and controls that are used during the installation, training, and operation of the instrument. Catalog numbers that appear as links open the web pages for those products.

For information on the reagents and supplies needed for general operation of the sequencer, see Chapter 2, "Reagents, supplies, and required materials".

Genexus™ Installation and Training Kit

Contents	Part No.	Quantity	Storage
Genexus™ Controls	A40267	1 kit	–30°C to –10°C
Genexus™ Strip 1	A46812	8 strips	2°C to 8°C
Genexus™ Strip 2-AS	A46813	8 strips	–30°C to –10°C
Genexus™ Barcodes 1–32 AS	A40258	1 plate	15°C to 30°C
Genexus™ Strip 3-GX5™	A46815	8 strips	2°C to 8°C
Genexus™ Strip 4	A46816	8 strips	–30°C to –10°C
Genexus™ Cartridge	A40272	2 cartridges	–30°C to –10°C
Genexus™ Bottle 2	A40273	4 bottles	15°C to 30°C
Genexus™ Bottles 1 and 3	A40274	2 bottles each	
Genexus™ Pipette Tips	A40266	12 racks	
Genexus™ Conical Bottles	A40275	2 sets of 5 bottles	
Genexus™ Filter	A40302	1 filter	
GX5™ Chip and Genexus™ Coupler	A40269	2 each	
Adhesive PCR Plate Foils	AB0626	1 box (100 foils)	

Software compatibility and requirements

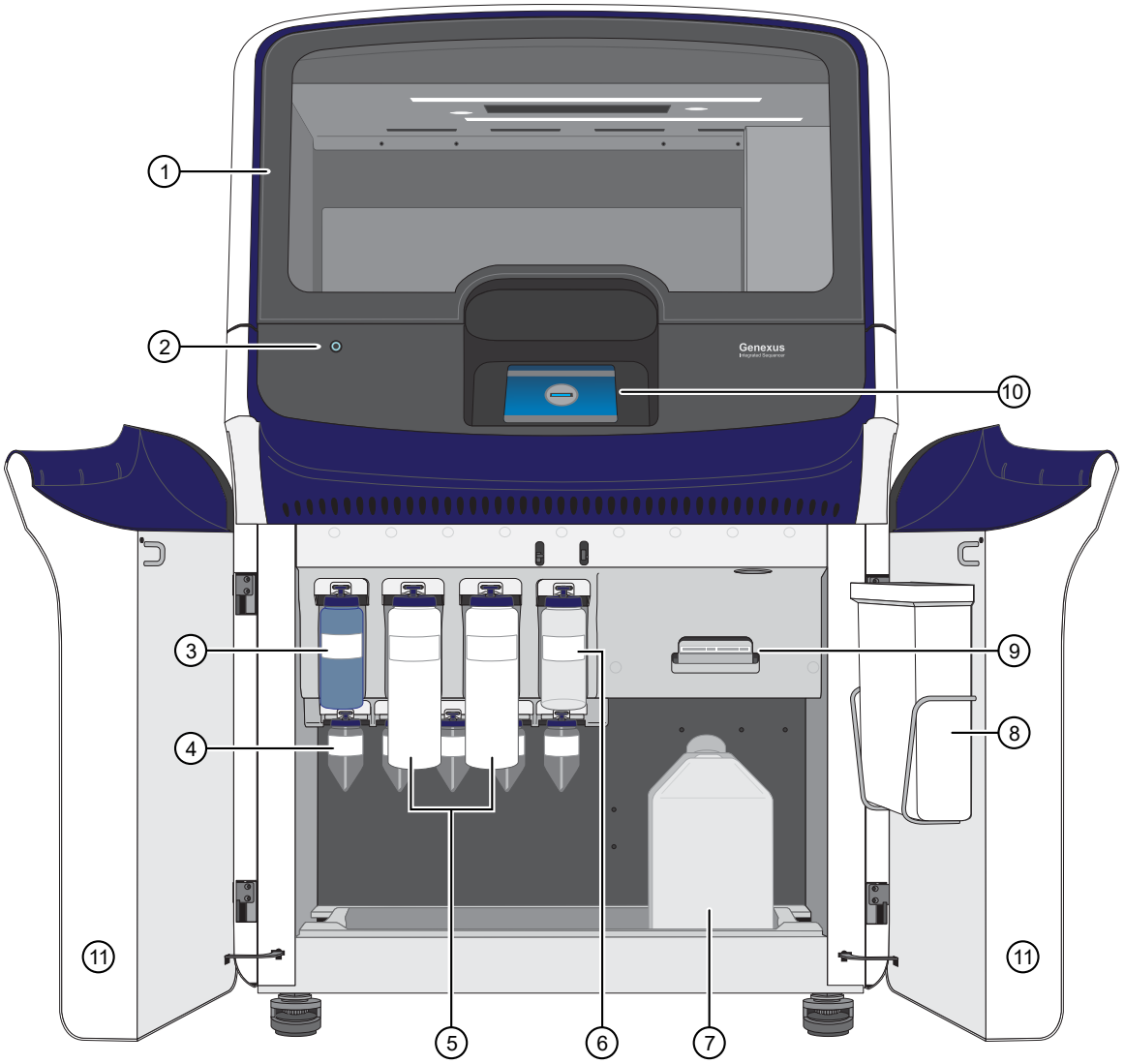
The procedures in this guide are designed for use with Genexus™ Software 6.6.2.1 or later. Version-specific information is provided in the software release notes for your version of the software. An administrator-level user can view the software version in the ⚙ **(Settings) / Software Updates** screen.

Genexus™ Software is supported on Google Chrome™ browser version 90 and later and is best viewed with 1440 × 900 screen resolution. Google Chrome™ browser is recommended for use with the software.

The operating system of the sequencer is Ubuntu™ 18.04.1 LTS.

For more information, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

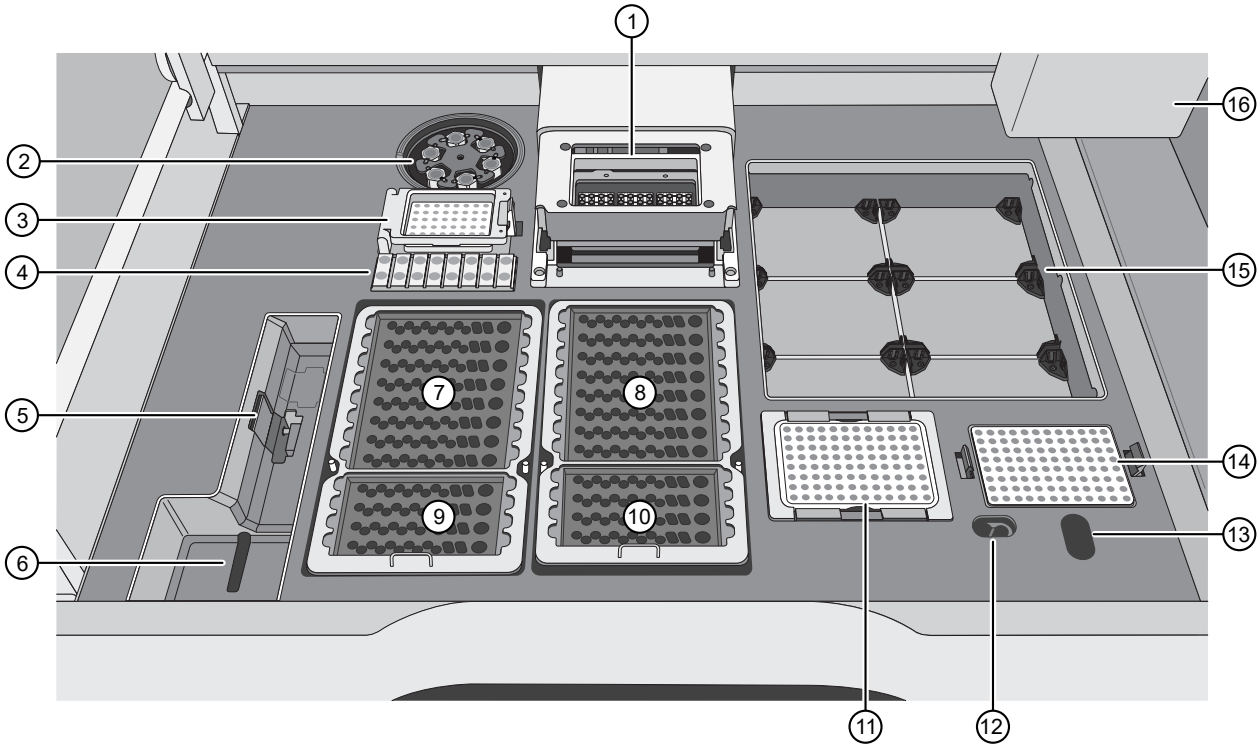
Genexus™ Integrated Sequencer components



Major features and components of the exterior and sequencing reagents bay of the Genexus™ Integrated Sequencer

① Door to deck chamber. The door is locked in the closed position during an instrument run.
② Power button
③ Genexus™ Bottle 1 (Chemical Waste)
④ Genexus™ Conical Bottles (Reusable conical bottles for Genexus™ Cartridge reagent dilution)
⑤ Genexus™ Bottle 2 (Sequencing Solution)
⑥ Genexus™ Bottle 3 (Cleaning Solution)
⑦ Waste carboy
⑧ Waste pipette tip bin
⑨ Genexus™ Cartridge
⑩ Touchscreen
⑪ Sequencing reagents bay door. Doors are locked in the closed position during an instrument run.

Genexus™ Integrated Sequencer deck stations

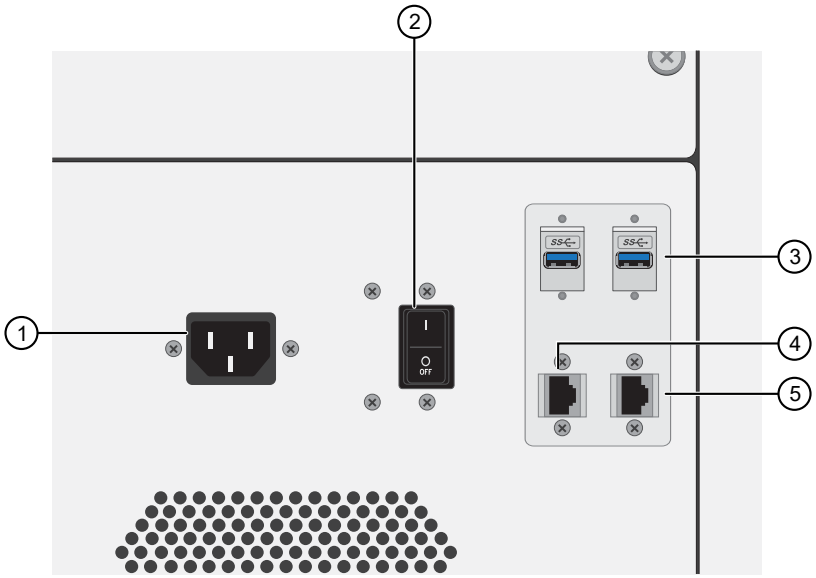


Interior Genexus™ Integrated Sequencer deck components and stations

1. PCR amplification station
2. Microcentrifuge
3. Genexus™ Barcodes plate station
4. Genexus™ Primer Pool Tube station
5. Genexus™ Coupler station
6. Chip install station
7. Zone 1 station (Genexus™ Strip 1)
8. Zone 2 station (Genexus™ Strip 2-AS or Genexus™ Strip 2-HD, depending on your assay)
9. Zone 3 station (Genexus™ Strip 3-GX5™)
10. Zone 4 station (Genexus™ Strip 4)
11. Enrichment plate station
12. Liquid waste disposal port
13. Waste pipette tip disposal port
14. Sample plate station
15. Genexus™ Pipette Tips station
16. Robotic pipettor

Genexus™ Integrated Sequencer input and output connections

The connection panel, power port, and an on/off switch are located on the right side of the rear panel of the instrument.



① Power port—100–240VAC port that provides power to the instrument.
② On/off switch—Power switch, where the states are on (|) or off (O).
③ USB ports—Connects a USB device to the instrument.
④ Ethernet port—An RJ45 port that provides Ethernet (Gigabit) communication between the sequencer and a local area network.
⑤ Ethernet port—An RJ45 port that provides Ethernet (Gigabit) communication between the sequencer and an accessory instrument such as the Genexus™ Purification Instrument.

Nucleic Acid to Result workflow

Nucleic Acid to Result workflow using the Genexus™ Integrated Sequencer

Create an assay (page 43)

System installed assays that are specifically configured for each sample type are available in Genexus™ Software. If you need to modify assay settings, copy a system-installed assay, then edit the settings as needed.

Enter samples (page 60)

Enter samples in Genexus™ Software to assign sample names and provide information such as collection date, gender, type, and disease category.

Plan a Nucleic Acid to Result run (page 76)

Runs planned in Genexus™ Software contain all of the settings that are used in sample purification, library preparation, templating, sequencing, and analysis, including sample information and plate location, assays, and barcodes.

Dilute the samples and load the sample plate (page 92)

Dilute the nucleic acid samples, if needed, then load the sample plate.

Load the sequencer and start a run (page 95)

Follow the step-by-step instructions on the sequencer touchscreen to load the sample plate and consumables.

Monitor the run (page 112)

Monitor the run in Genexus™ Software in real time.

Review data and results (page 115)

Review data and results in Genexus™ Software, or analyze data with an analysis workflow in Ion Reporter™ Software.

Library to Result workflow

Library to Result workflow using the Genexus™ Integrated Sequencer

Create an assay (page 43)

System installed assays that are specifically configured for each sample type are available in Genexus™ Software. If you need to modify assay settings, copy a system-installed assay, then edit the settings as needed.

Prepare a library batch (page 68)

Prepare or import a library batch in Genexus™ Software to assign samples, Library Batch ID, and the barcodes that were used to prepare the sample libraries.

Plan a Library to Result run (page 84)

Runs planned in Genexus™ Software contain all of the settings that are used in templating, sequencing, and analysis, including sample information and plate location, assays, and barcodes.

Dilute the libraries and load the sample plate (page 94)

Dilute libraries, if needed, then load the sample plate.

Load the sequencer and start a run (page 95)

Follow the step-by-step instructions on the sequencer touchscreen to load the sample plate and consumables.

Monitor the run (page 112)

Monitor the run in Genexus™ Software in real time.

Review data and results (page 115)

Review data and results in Genexus™ Software, or analyze data with an analysis workflow in Ion Reporter™ Software.

2 Reagents, supplies, and required materials

This chapter lists the reagents, supplies, and materials needed to operate the Genexus™ Integrated Sequencer, and provides consumables ordering and storage information. Available Oncomine™ GX assays, and recommended products for nucleic acid isolation and purification are also provided.

Reagents and supplies—Ion AmpliSeq™ library chemistry

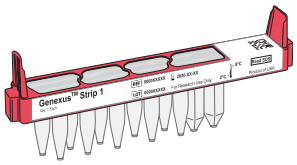
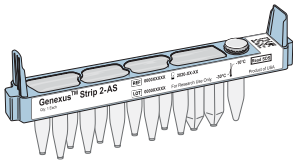
Genexus™ Integrated Sequencer reagents and supplies can be ordered in convenient combination kits and starter packs, but most consumables can also be ordered individually as your needs require. The following tables provide information on the various ordering options that are available for Ion AmpliSeq™ library chemistry.

Note:

- Consumables that have catalog numbers are orderable. Components that have part numbers cannot be ordered individually.
- Reagents that are specific to Ion AmpliSeq™ library chemistry have an AS suffix.

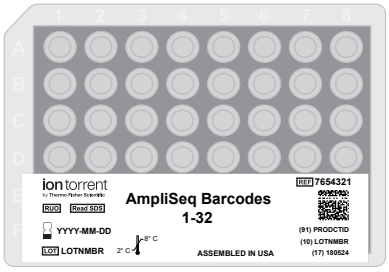
Genexus™ Library Strips 1 and 2-AS

Ion Torrent™ Genexus™ Library Strips 1 and 2-AS (Cat. No. A40252) for standard Ion AmpliSeq™ library-based chemistry are ordered as kits with eight pairs of strips/kit.

Component	Carrier color	Part No.	Quantity per kit	Storage
Genexus™ Strip 1 	Light red	A46812	8 strips	2°C to 8°C
Genexus™ Strip 2-AS 	Light blue	A46813	8 strips	–30°C to –10°C

Genexus™ Barcodes AS

Ion Torrent™ Genexus™ Barcodes AS are supplied in plates containing 32 dual barcodes per plate. The barcodes can be ordered as a set of three plates (Cat. No. A40257), or ordered individually.

Item	Label color	Cat. No.	Quantity	Storage
Genexus™ Barcodes 1–96 AS	Blue	A40257	3 plates	15°C to 30°C
Genexus™ Barcodes 1–32 AS 	Blue	A40258	1 plate	
Genexus™ Barcodes 33–64 AS	Blue	A40259	1 plate	
Genexus™ Barcodes 65–96 AS	Blue	A40260	1 plate	

Genexus™ GX5™ Starter Pack-AS

Ion Torrent™ Genexus™ GX5™ Starter Pack-AS (Cat. No. A40279) supplies the following components for Ion AmpliSeq™ library preparation and sequencing using a Genexus™-ready assay.

Note: For custom assays, Genexus™ Primer Pool Tubes (Cat. No. A40262) must be ordered separately.

Component	Part or Cat. No.	Quantity	Storage
Genexus™ Strip 1	A46812	8 strips	2°C to 8°C
Genexus™ Strip 2-AS	A46813	8 strips	–30°C to –10°C
Genexus™ Strip 3-GX5™	A46815	8 strips	2°C to 8°C
Genexus™ Strip 4	A46816	8 strips	–30°C to –10°C
Genexus™ Barcodes 1–32 AS	A40258	1 plate	15°C to 30°C
Genexus™ Pipette Tips	A40266	12 racks	
Genexus™ Cartridge	A40272	2 cartridges	–30°C to –10°C
Genexus™ Bottle 2	A40273	4 bottles	15°C to 30°C
Genexus™ Bottles 1 and 3	A40274	2 bottles each	

Reagents and supplies—Ion AmpliSeq™ HD library chemistry

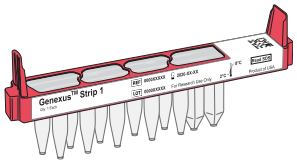
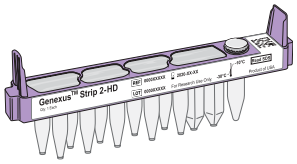
Genexus™ Integrated Sequencer reagents and supplies can be ordered in convenient combination kits and starter packs, but most consumables can also be ordered individually as your needs require. The following tables provide information on the various ordering options that are available for Ion AmpliSeq™ HD library chemistry.

Note:

- Consumables that have catalog numbers are orderable. Components that have part numbers cannot be ordered individually.
- Reagents that are specific to Ion AmpliSeq™ HD library chemistry have an HD suffix.

Genexus™ Library Strips 1 and 2-HD

Ion Torrent™ Genexus™ Library Strips 1 and 2-HD (Cat. No. A40255) for Ion AmpliSeq™ HD library-based chemistry are ordered as kits with eight pairs of strips/kit.

Component	Carrier color	Part No.	Quantity per kit	Storage
Genexus™ Strip 1 	Light red	A46812	8 strips	2°C to 8°C
Genexus™ Strip 2-HD 	Violet	A46814	8 strips	–30°C to –10°C

Genexus™ Barcodes 1–32 HD

Ion Torrent™ Genexus™ Barcodes 1–32 HD are supplied in a plate containing 32 dual barcodes.

Item	Label color	Cat. No.	Quantity	Storage
Genexus™ Barcodes 1–32 HD	Purple	A40261	1 plate	15°C to 30°C

Genexus™ GX5™ Starter Pack-HD

Ion Torrent™ Genexus™ GX5™ Starter Pack-HD (Cat. No. A40280) supplies the following components for Ion AmpliSeq™ HD library preparation and sequencing using a Genexus™-ready assay.

Note: For custom assays, Genexus™ Primer Pool Tubes (Cat. No. A40262) must be ordered separately.

Component	Part or Cat. No.	Quantity	Storage
Genexus™ Strip 1	A46812	8 strips	2°C to 8°C
Genexus™ Strip 2-HD	A46814	8 strips	–30°C to –10°C
Genexus™ Strip 3-GX5™	A46815	8 strips	2°C to 8°C
Genexus™ Strip 4	A46816	8 strips	–30°C to –10°C
Genexus™ Barcodes 1–32 HD	A40261	1 plate	15°C to 30°C
Genexus™ Pipette Tips	A40266	12 racks	
Genexus™ Cartridge	A40272	2 cartridges	–30°C to –10°C
Genexus™ Bottle 2	A40273	4 bottles	15°C to 30°C
Genexus™ Bottles 1 and 3	A40274	2 bottles each	

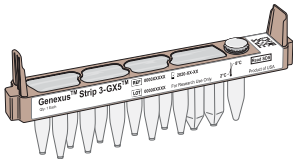
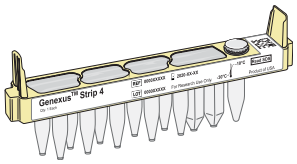
Shared reagents and supplies

The following reagents and supplies are used in both Ion AmpliSeq™ library chemistry and Ion AmpliSeq™ HD library chemistry runs.

Note: Consumables that have catalog numbers are orderable. Components that have part numbers cannot be ordered individually.

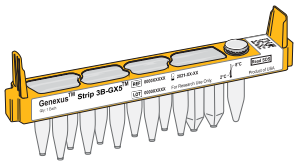
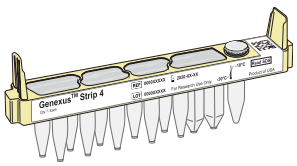
Genexus™ Templating Strips 3-GX5™ and 4

Ion Torrent™ Genexus™ Templating Strips 3-GX5™ and 4 (Cat. No. A40263) are ordered as kits with 8 pairs of strips per kit.

Component	Carrier color	Part No.	Quantity per kit	Storage
Genexus™ Strip 3-GX5™ 	Brown	A46815	8 strips	2°C to 8°C
Genexus™ Strip 4 	Yellow	A46816	8 strips	–30°C to –10°C

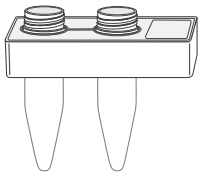
Genexus™ Templating Strips 3B-GX5™ and 4

Ion Torrent™ Genexus™ Templating Strips 3B-GX5™ and 4 (Cat. No. A49782) are ordered as kits with 8 pairs of strips per kit. The Genexus™ Strip 3B-GX5™ is optimized for use with the Oncomine™ Myeloid Assay GX v2.

Component	Carrier color	Part No.	Quantity per kit	Storage
Genexus™ Strip 3B-GX5™ 	Orange	A47712	8 strips	2°C to 8°C
Genexus™ Strip 4 	Yellow	A46816	8 strips	–30°C to –10°C

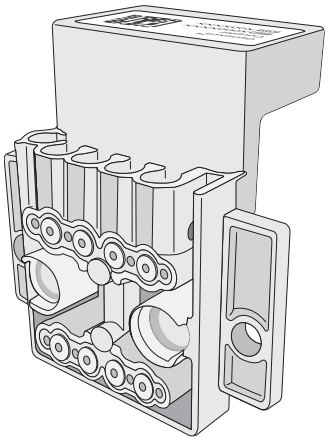
Genexus™ Primer Pool Tubes and Pipette Tips

Genexus™ Primer Pool Tubes and Genexus™ Pipette Tips can be ordered individually. Genexus™ Primer Pool Tubes are required for custom assays.

Item	Cat. No.	Quantity	Storage
Genexus™ Primer Pool Tubes 	A40262	50 assemblies (2 tubes/assembly) Bag of 100 caps	15°C to 30°C
Genexus™ Pipette Tips	A40266	12 racks	

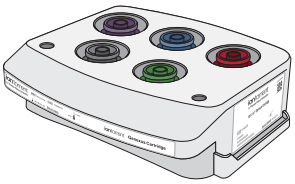
GX5™ Chip and Genexus™ Coupler

The GX5™ Chip and Genexus™ Coupler (Cat. No. A40269) are ordered as a set that contains 2 chips and 2 couplers, sufficient for up to 8 sequencing runs.

Component	Part No.	Quantity	Storage
GX5™ Chip 	100081364	2 chips	15°C to 30°C
Genexus™ Coupler 	100081252	2 couplers	

Genexus™ Sequencing Kit

The Ion Torrent™ Genexus™ Sequencing Kit (Cat. No. A40271) provides reagents and solutions sufficient to sequence up to 2 full chips.

Component	Part No.	Quantity	Storage
Genexus™ Cartridge 	A40272	2 cartridges	–30°C to –10°C
Genexus™ Bottle 2	A40273	4 bottles	15°C to 30°C
Genexus™ Bottles 1 and 3	A40274	2 bottles each (4 bottles total)	

Genexus™ Conical Bottles

Genexus™ Conical Bottles (Cat. No. A40275) are installed in the sequencing reagents bay and serve as reservoirs for nucleotide reagent dilutions. For information on when and how to replace the bottles, see “Replace the Genexus™ Conical Bottles” on page 195.

Component	Quantity	Storage
Genexus™ Conical Bottles	5 bottles	15°C to 30°C

Genexus™ Filter

The Genexus™ Filter (Cat. No. A40302) is installed in the liquid waste disposal port on the instrument deck to prevent liquid waste line blockage. For information on installation, see “Replace the Genexus™ Filter” on page 194.

Component	Quantity	Storage
Genexus™ Filter	2 filters	15°C to 30°C

Genexus™ Controls

The Ion Torrent™ Genexus™ Controls kit (Cat. No. A40267) provides sufficient Genexus™ Control Library-AS to perform four **Library to Result** runs. The kit also provides sufficient Genexus™ Control Panel-AS and Genexus™ DNA Control to perform eight **Nucleic Acid to Result** runs.

IMPORTANT! Genexus™ Strip 2-AS is required for sequencing Genexus™ Controls. For ordering information, see "Genexus™ Library Strips 1 and 2-AS" on page 20.

Note: The Genexus™ Control Library-AS is barcoded with IonCode™ 0101.

Component	Quantity	Storage
Genexus™ Control Library-AS	1 tube	–30°C to –10°C
Genexus™ Control Panel-AS	8 carriers (white)	
Genexus™ DNA Control	2 tubes	

Oncomine™ GX assays

Ion Torrent™ Oncomine™ GX assays are Genexus™-ready assays sufficient for 32 reactions, and are supplied in pre-measured ready-to-load Genexus™ Primer Pool Tubes. Assays are provided with Genexus™ Library Strips 1 and 2-AS (Cat. No. A40252) or Genexus™ Library Strips 1 and 2-HD (Cat. No. A40255) in the amount listed.

Note:

- Assays using Ion AmpliSeq™ chemistry have primer pools that are loaded in capped tubes in position 1 of the primer pool tube carriers. Empty uncapped tubes are loaded in position 2.
- Assays using Ion AmpliSeq™ HD chemistry have FWD and REV primer pools that are loaded in capped tubes in both positions of the primer pool tube carrier.

Contents	Carrier color	Pool	Carriers per kit	Part No.	Storage
Oncomine™ Comprehensive Assay v3 GX (Cat. No. A46296)					
Oncomine™ Comprehensive Assay v3 DNA GX	Magenta	DNA Pool 1	4	A40281	–30°C to –10°C
	Pale green	DNA Pool 2	4		
Oncomine™ Comprehensive Assay v3 RNA GX	Pale orange	RNA Pool 1	4	A44351	
	Blue	RNA Pool 2	4		
Genexus™ Strip 1	Light red	—	2 × 8	A46812	2°C to 8°C
Genexus™ Strip 2-AS	Light blue	—	2 × 8	A46813	–30°C to –10°C
Oncomine™ Precision Assay GX (Cat. No. A46291)					
Oncomine™ Precision Assay GX (panel only)	Magenta	OPA Pool 1 (FWD and REV primers)	8	A44350	–30°C to –10°C
Genexus™ Strip 1	Light red	—	8	A46812	2°C to 8°C
Genexus™ Strip 2-HD	Violet	—	8	A46814	–30°C to –10°C
Oncomine™ TCR Beta-LR Assay GX (Cat. No. A46297)					
Oncomine™ TCR Beta-LR Assay GX (panel only)	Magenta	RNA Pool 1	8	A40282	–30°C to –10°C
Genexus™ Strip 1	Light red	—	8	A46812	2°C to 8°C
Genexus™ Strip 2-AS	Light blue	—	8	A46813	–30°C to –10°C

(continued)

Contents	Carrier color	Pool	Carriers per kit	Part No.	Storage
Oncomine™ Myeloid Assay GX v2 (Cat. No. A47857)					
Oncomine™ Myeloid Assay GX v2 (panel only)	Magenta	DNA pool 1	2 × 4	A44354	–30°C to –10°C
	Pale green	DNA pool 2	2 × 4		
	Pale orange	RNA pool 1	8	A44355	
Genexus™ Strip 1	Light red	—	3 × 8	A46812	2°C to 8°C
Genexus™ Strip 2-AS	Light blue	—	3 × 8	A46813	–30°C to –10°C
Oncomine™ BRCA Assay GX (Cat. No. A47912)					
Oncomine™ BRCA Assay GX (panel only)	Magenta	DNA pool 1	2 × 4	A44353	–30°C to –10°C
	Pale green	DNA pool 2	2 × 4		
Genexus™ Strip 1	Light red	—	2 × 8	A46812	2°C to 8°C
Genexus™ Strip 2-AS	Light blue	—	2 × 8	A46813	–30°C to –10°C

Required materials—general laboratory equipment and supplies

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier. Catalog numbers that appear as links open the web pages for those products.

Item	Source
MicroAmp™ EnduraPlate™ Optical 96-Well Clear Reaction Plates with Barcode	4483352, 4483354
Adhesive PCR Plate Foils	AB0626
20-, 200-, and 1,000-µL pipettors and appropriate filtered tips	**MLS**
Microcentrifuge tubes, 1.5-mL or 1.7-mL (low retention for nucleic acids)	**MLS**
Vortex mixer with a rubber platform	**MLS**
Gloves, powder-free nitrile	**MLS**
Ice buckets and ice	—
Nuclease-free water, molecular biology grade	AM9932
Isopropyl alcohol, 70% solution	**MLS**
Wipes, disposable lint-free	**MLS**
(Optional) Uninterruptible Power Supply (UPS)[1]	**MLS**
(Optional) Sorvall™ ST 8 Small Benchtop Centrifuge[2], with Thermo Scientific™ M10 Microplate Swinging Bucket Rotor, and Sealed Bucket; Capacity: 4 Standard or 2 Midi-Deepwell plates (Set of 2) (or equivalent)	75007200 75005706 75005721

[1] For laboratories that experience frequent power outages or line voltage fluctuations, we recommend that you use an uninterruptible power supply that is compatible with 2500 W output or higher.

[2] For centrifuging library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter.

Recommended materials for nucleic acid isolation and quantification

Unless otherwise indicated, all materials are available through **thermofisher.com**. Catalog numbers that appear as links open the web pages for those products.

Item	Source
Genexus™ Purification System reagent kits for automated nucleic acid isolation and quantification[1]	
Ion Torrent™ Genexus™ FFPE DNA/RNA Purification Kit	A45539
Ion Torrent™ Genexus™ Cell-Free Total Nucleic Acid Purification Kit	A45542
Ion Torrent™ Genexus™ Multisample DNA Purification Kit	A45540
Ion Torrent™ Genexus™ Total RNA Purification Kit	A45541
Nucleic acid isolation—manual (Nucleic Acid to Result runs)	
Ion AmpliSeq™ Direct FFPE DNA Kit	A31133, A31136
RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE	AM1975
RecoverAll™ Multi-Sample RNA/DNA Workflow	A26069
MagMAX™ FFPE DNA/RNA Ultra Kit	A31881
PureLink™ Genomic DNA Mini Kit	K1820-00
MagMAX™ Cell-Free DNA Isolation Kit	A29319
MagMAX™ Cell-Free Total Nucleic Acid Isolation Kit	A36716
RNase*Zap*™ RNase Decontamination Solution	AM9780
Nucleic acid quantification—manual	
TaqMan™ RNase P Detection Reagents Kit (Recommended for DNA only)	4316831
Qubit™ 4 Fluorometer[2]	Q33238
One or more of the following kits for use with the Qubit™ 4 Fluorometer: · Qubit™ dsDNA HS Assay Kit (High-sensitivity DNA) · Qubit™ dsDNA BR Assay Kit (Broad range DNA) · Qubit™ RNA HS Assay Kit (High-sensitivity RNA) · Qubit™ RNA BR Assay Kit (Broad range RNA)	 Q32851, Q32854 Q32850, Q32853 Q32852, Q32855 Q10210, Q10211
Library quantification (Library to Result runs only)	
Ion Library TaqMan™ Quantitation Kit	4468802

[1] Used with the Genexus™ Purification System. See the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475) for detailed information on the Genexus™ Purification System and its consumables.

[2] Qubit™ 2.0 Fluorometer and later are supported.

3 Before you begin

Precautions

Avoid nucleic acid contamination

IMPORTANT! A primary source of contamination is spurious nucleic acid fragments from previous sample processing steps. Do not introduce amplified DNA into the work area where the instrument is located.

Avoid strong electromagnetic radiation

WARNING! Do not use the instrument in close proximity to sources of strong electromagnetic radiation (for example, unshielded intentional RF sources), as these sources can interfere with proper operation.

Protection by equipment

WARNING! The protection that is provided by the equipment can be impaired if the instrument is operated outside the environment and use specifications, the user provides inadequate maintenance, or the equipment is used in a manner that is not specified by the manufacturer (Thermo Fisher Scientific).

Guidelines for panel and reagent use and handling

- Use only the reagents and supplies that have been recommended in "Required materials—general laboratory equipment and supplies" on page 30 and "Recommended materials for nucleic acid isolation and quantification" on page 31.
- Keep panel tubes capped until immediately before loading in the Genexus™ Integrated Sequencer.
- If using, thaw positive controls on ice for 30 minutes. After the positive controls are completely thawed, vortex, the tubes, then centrifuge to collect tube contents. Return to ice before loading into sample plate.

  IMPORTANT! Ensure that contents of the control tubes are completely thawed before adding to the sample plate.

- Equilibrate Genexus™ Strip 1 and Genexus™ Strip 3-GX5™ (or Genexus™ Strip 3B-GX5™) at room temperature for 30 minutes before loading in the sequencer.
- Thaw Genexus™ Strip 2-AS (or Genexus™ Strip 2-HD) and Genexus™ Strip 4 on ice for 30 minutes.

  IMPORTANT! Ensure that strip contents are completely thawed before loading in the sequencer.

- Thawed library and templating strips can be vortexed on a platform vortexer to dissolve precipitate or dislodge air bubbles. If you vortex, you must centrifuge the strips to collect tube contents using the Genexus™ Strip Centrifuge Adapter to hold strips during centrifugation.

  For information on obtaining and using the Genexus™ Strip Centrifuge Adapter, see "Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter" on page 227.
- Keep thawed panel, Genexus™ Strip 2-AS (or Genexus™ Strip 2-HD), Genexus™ Strip 4, and the sample plate on ice or at 4°C until ready to load in the sequencer.
- Do not freeze-thaw the panel. Thaw only the number of panel tubes that are required for an instrument run. Discard unused panel tubes after they are thawed. Store panel tubes at –30°C to –10°C.
- If you are using an assay that uses Ion AmpliSeq™ HD chemistry, do not combine the contents of panel tubes. Forward and reverse primers must remain separate, until they are combined by the sequencer as part of the on-instrument library preparation workflow.
- Do not store primer pool tubes or reagent strips on the sequencer for more than 24 hours before starting an instrument run.

Guidelines for preventing contamination

We recommend following these guidelines to prevent cross-contamination of samples and controls between and within sequencing runs.

Personal protective equipment

- Wear a lab coat that is reserved for sequencing work and is laundered frequently. If possible, change to a fresh lab coat before setting up a run, or use new sleeve covers.
- Wear fresh gloves to load the instruments, including during the loading of the sequencing chip and coupler. Do not remove gloves to install the sequencing chip.
- If you are using positive controls and a no-template control (NTC) in your run, change gloves between dispensing these controls, and if applicable, between dispensing samples and controls in sample plate wells.

Equipment and instrument cleaning

- If you are preparing samples and sample plates in a hood (recommended), illuminate the hood with UV light for 15 minutes before use.
- Before use, wipe working surfaces of the hood or bench where samples or sample plates are handled, and other equipment such as vortexers, microcentrifuges, and pipettors with lint-free wipes moistened with DNA*Zap*™ decontamination solutions. Wipe with solution 1 first, then follow with solution 2. Alternatively, a 10% solution of commercial bleach can be used. Follow with wiping of bench and equipment surfaces with wipes moistened with 70% isopropanol or 70% ethanol.
- Before and after a run, sequentially wipe the deck surface of the Genexus™ Purification Instrument and Genexus™ Integrated Sequencer with lint-free wipes moistened with the two DNA*Zap*™ decontamination solutions. Follow with wiping of deck surfaces with wipes moistened with 70% isopropanol or 70% ethanol. The robotic pipettor arm of each instrument can also be cleaned in this manner.

  IMPORTANT! Do not spray decontamination solution or alcohol solution directly onto deck surfaces or into deck openings. Instead, use a lint-free wipe moistened with solution to clean surfaces. Do not use bleach to clean instrument surfaces.

- We recommend that you centrifuge the sample plate after sealing. Ensure that the centrifuge has been wiped down and cleaned before centrifuging the plate.

Workflow tips

- After dispensing a positive control or sample (if applicable) in a sample plate well, do not pass the end of the used tip over wells intended for other samples or NTC. This practice minimizes the chance of depositing microdroplets in adjacent wells.
- If you dilute samples on the sample plate and vortex the plate after sealing, apply the foil seal carefully between wells with an applicator before vortexing to ensure that the seal is complete and contamination between wells does not occur.
- If possible, have a pipettor and tips reserved for dispensing only NTC.
- Before disposal, close or cap used sample and positive control tubes to avoid creation of aerosols.
- Avoid touching the foil seals of the reagent strips, barcode plate, and sample plate.

- When loading the sequencer deck for a run, install the sample plate last.
- After a run, seal the PCR amplification plate with a foil seal before removing the plate from the PCR amplification station. Sealing the plate before removal helps prevent contamination of libraries if libraries are recovered for reuse.

Guidelines for Genexus™ Integrated Sequencer operation

- Follow guidance that is provided by Genexus™ Software when you plan a run to determine which consumables must be loaded and which consumables can be reused from a previous run.
- Follow guidance that is provided by the software when you plan a run to determine how many samples can be run with a given assay or assays in an instrument run. The number of samples that can be included in a sequencing run depends on multiple factors.

Limiting factor	Description
The number of available barcodes in the barcode plate	The maximum number of available barcodes per run is 32. **IMPORTANT!** When libraries are prepared on the Genexus™ Integrated Sequencer, each target amplification reaction for a sample requires a unique barcode.
Maximum number of target amplification reactions per run	One library strip pair has the reagents necessary for 4 target amplification reactions, or 4 barcodes. With a maximum of 8 library strip pairs loaded, a maximum of 32 samples can be run using an assay with one primer pool.
The number of primer pools per assay	Given the limits of 32 target amplification reactions, and 32 available barcodes, the number of samples in a run multiplied by the total number of primer pools in the assays that are used in a run cannot exceed 32. For one single-pool assay, a maximum of 32 samples can be run on a single chip. If you are using 2 assays with two primer pools each, you can sequence a maximum of 8 samples in a run. Similarly, for one assay with 4 primer pools, you can sequence a maximum of 8 samples in a run, if the minimum read count per sample allows it.
The number of unused lanes on an installed chip	
The minimum read count per sample for an assay	The minimum read count per sample parameter is set during assay creation.

- Two assays cannot share a chip lane, so a maximum of 4 assays can be run per chip.
- The assays that are used in a single run must use the same chemistry (Ion AmpliSeq™ or Ion AmpliSeq™ HD), and have compatible cycling parameters to allow amplification in the instrument thermal cycler. The thermal cycler has two independently controlled heating zones. After you select an assay, Genexus™ Software restricts the list of available assays to use in the run to those that are compatible with the selected assay or assays.
- One library strip pair is needed for each primer tube position 1–8 that is filled in a run.
- One template strip pair is needed for each chip lane that is used in a run.
- Consumables are configured to support sample batch sizes in multiples of four samples. The most efficient use of consumables occurs when samples are run in multiples of four.

- If a chip installed in a sequencer has unused lanes, do not remove it unless you are sure that you want to replace it with a new chip. After a partially used chip has been removed from the sequencer, it cannot be reinserted and reused. The sequencer cannot track lane usage after chip removal.
- You can remove a chip in one of the following situations.
  - After all the lanes of a chip are used in a run, the chip shuttles to the install position and you are asked to remove the used chip.
  - When you select a run plan that requires more lanes than are available on the installed chip, you are asked to remove the partially used chip, and the sequencer performs a post-chip clean. In addition, you must clear consumables from the lower sequencing reagents bay, even if only a single lane of the chip was used.
- The Genexus™ Integrated Sequencer can track used and unused barcodes in barcode plates in Genexus™ Software 6.2.0 and later, enabling you to swap plates between runs if needed, and reload a partially used barcode plate for a run if a sufficient number of barcodes is available on the plate.
- After loading in the sequencer, reusable consumables, such as barcode plate, chips, and sequencing reagents bay components, must be used within 14 days for optimal results.
- An assay that is selected in a Library to Result run cannot include library batches that share a library with the same barcode. However, two different assays in a run can include a barcode in common, because assays are run in separate lanes of a chip.

Guidelines for expired reagents and chips

Follow these guidelines for using reagents and sequencing chips that are at or near expiration. It is not recommended to use components past the expiration date, but under specific circumstances, a sequencer warning for expired reagents can be overridden to allow a sequencing run to proceed.

- For all reagents, including the barcode plate, the instrument bypasses an expired reagent warning after you tap the **Help** button in the sequencer screen. However, the sequencer must be able to detect and read the 2D barcode on the expired reagent for the bypass to proceed.
- In Genexus™ Software 6.6 and later, if a chip is expiring in a given month, and you start a run in the next calendar month with the same chip installed, the sequencer allows the run to proceed. In addition, in Genexus™ Software 6.6 and later you can use expired chips for post-chip cleans or forced instrument cleans.

Guidelines for nucleic acid isolation and quantification—Nucleic Acid to Result runs

These are general guidelines for manual isolation and quantification of DNA and RNA for Nucleic Acid to Result runs. For assay-specific guidelines, see the assay user guide. If you do not dilute sample concentrations manually to the target concentration of the assay, quantify sample nucleic acid concentrations ahead of time so concentrations are available to enter during run planning.

- See "Recommended materials for nucleic acid isolation and quantification" on page 31 for recommended kits for isolating DNA and RNA.
- We recommend the TaqMan™ RNase P Detection Reagents Kit (Cat. No. 4316831) for quantifying amplifiable human genomic DNA (see *Demonstrated Protocol: Sample Quantification for Ion AmpliSeq™ Library Preparation Using the TaqMan™ RNAse P Detection Reagents Kit* (Pub. No. MAN0007732) available at **thermofisher.com**).
- The Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851 or Q32854) can also be used for quantification, particularly for formalin-fixed, paraffin-embedded (FFPE) DNA, and highly degraded DNA samples. See "Quantify FFPE DNA with the Qubit™ Fluorometer" on page 226 for a detailed procedure for quantifying FFPE DNA.
- We recommend the Qubit™ RNA HS Assay Kit (Cat. No. Q32852 or Q32855) for quantifying RNA.
- Quantification methods such as densitometry (for example, using a NanoDrop™ spectrophotometer) are not recommended, because they are not specific for DNA or RNA. Use of these methods can lead to gross overestimation of the concentration of sample nucleic acid, under-seeding of the target amplification reaction, and low library yields.
- The Ion AmpliSeq™ Direct FFPE DNA Kit bypasses nucleic acid isolation when preparing libraries from FFPE sections on slides. See the *Ion AmpliSeq™ Direct FFPE DNA Kit User Guide* (Pub. No. MAN0014881) for a protocol for using this kit to prepare gDNA from FFPE tissue.
- The Direct FFPE DNA preparation can be stored for up to 6 months at –30°C to –10°C before library preparation.

Power the Genexus™ Integrated Sequencer on or off

Power on

If the touchscreen is unresponsive, check the power switch on the back of the instrument to ensure that the switch is in the on (|) position. If the power switch is in the off (O) position, proceed with step 1. If the power switch is already in the on position, proceed to step 2.

1. Turn the power switch on the back of the instrument to the on (|) position.
2. Press the power button on the front of the instrument.
   The button illuminates.
3. In the **Sign In** screen, enter the username and password created by the field service engineer when the instrument was set up, or the unique username and password set up for you as an instrument user.
   When the instrument home screen appears, the instrument is ready for use.

Power off

It is not necessary to power off the instrument overnight or over the weekend. If the instrument or Genexus™ Software will not be used for more than 3 days, power off the instrument as follows:

1. In the home screen, tap **Settings** ▸ **System Tools** ▸ **Shut down**.
2. Select either **Shutdown** or **Reboot**.
3. If you select **Shutdown**, a confirmation message appears. Select **Yes** to power off the instrument.

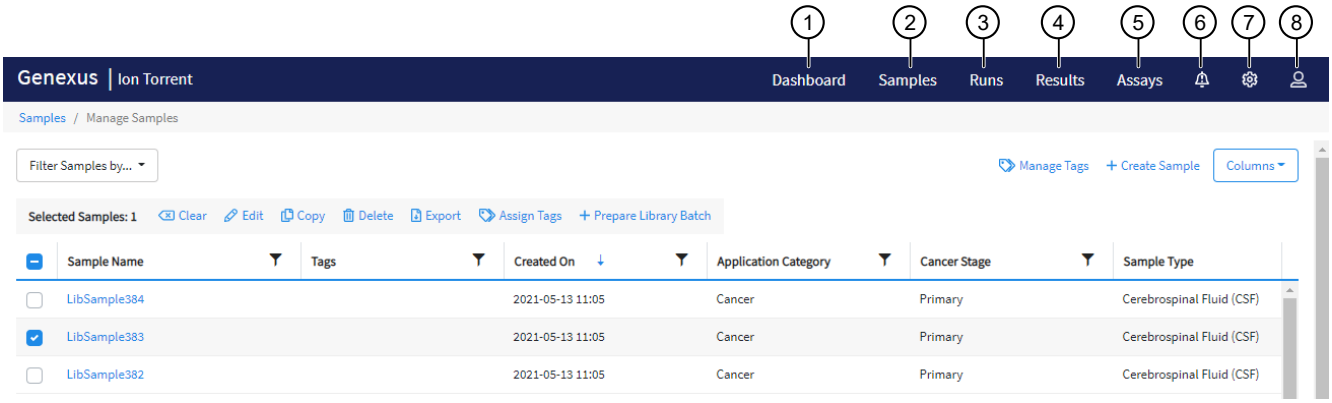
Note: If you power off the sequencer with a partially used chip installed, the chip and consumables status is saved. When you power back on, the saved chip and consumable information enables you to use the chip for up to 14 days after the chip was installed.

IMPORTANT! Do *not* press the power button during a run. Interrupting power to the instrument during a run can result in run failure and loss of sample.

Get started with Genexus™ Software

About Genexus™ Software

Genexus™ Software provides menus to help you add, select, and manage samples, libraries, runs, and assays. You can also view and manage sequencing results, monitor Genexus™ Integrated Sequencer runs in progress, and manage software settings.



① **Dashboard**—View recent run history, and current purification or sequencing run status.

② **Samples**—Add new samples, import samples, prepare library batches, import library batches and manage attributes.

③ **Runs**—Plan a run starting from a sample, a nucleic acid sample, a BAM sample, or a library. View, edit, and manage runs. Sample to result runs are for nucleic acid isolation on a Genexus™ Purification Instrument followed by sequencing on a Genexus™ Integrated Sequencer.

④ **Results**—View sample results, run results, and verification results.

⑤ **Assays**—Manage, create, and import assays. Manage assay preset parameters and panels.

⑥ **Notifications**—Receive alerts and messages for password expiration, system critical service failures, system backup failures, available software updates, and full disk space.

⑦ **Settings**—Access audit records and run logs, configure network settings, manage backup settings, restore runs, manage gene lists, link to Connect user accounts and Ion Reporter™ Software accounts, check for software updates, and manage data archiving, disk space, and users. Field Service Engineers access verification templates during sequencer installation.

⑧ **Profile**—Access the Help system, manage and edit user profile settings, configure an SSH key (system administrator only), and sign out.

User-access levels

Users at this level...	Can...
Operator	• Add and import samples. • Prepare library batches. • Plan and save runs. • Monitor runs. • View results and reports.

(continued)

Users at this level...	Can...
Manager	Operator functions plus: • Create and edit sample attributes. • Delete runs. • Create and import assays. • Manage reference sequences and panel, hotspot, and other sequence files. • Access services information.
Administrator	Operator and manager functions plus: • View, export, and print audit records. • Configure network settings. • View and manage software updates. • Configure data archive and storage settings. • Manage instrument and software log files. • Add and manage users.

System tracking

The system tracks and checks user, sample, workflow, reagents and QC metrics for auditable records. If the software detects an error at any step—for example, a scanned barcode is inconsistent with the information given for the run—the software alerts the user and does not proceed with the run.

Request and sign in to a new account

Only administrator-level users can create user accounts.

After account creation, the Genexus™ Integrated Sequencer automatically sends an email to the new user with the username and password information.

- To request a new account, contact your local administrator.
- To sign in to a new account for the first time:
  a. Open the Genexus™ Software, then enter your username and password.
  b. Press **Enter**, or click **Sign In**.
  c. Click **Accept** to accept the End User Software License Agreement.

d. In the **Change Password** screen, enter your temporary password in the **Current Password** field. Type a new password in the **New Password** field, then confirm the password.
   - Passwords must be between 6 and 10 alphanumeric characters (0–9, Aa–Zz) with no spaces or special characters.
   - Passwords must contain at least one alphabetic character (Aa–Zz).
   - Passwords must contain at least one numeric character (0–9).
   - Passwords are case-sensitive.

e. Click **Change**.

Sign in

1. Open the software home page.
2. Select your preferred language from the dropdown list in the upper right corner of the page.
3. Enter your username and password, then press **Enter** or click **Sign In**.

   IMPORTANT! Your username and password must be unique and not shared with other users.

   The software opens to the **Manage Samples** screen.

Network and password security requirements

Network configuration and security

The network configuration and security settings (for example, firewalls, antivirus software, network passwords) of your laboratory or facility are the sole responsibility of your facility administrators and IT and security personnel. Genexus™ Software does not provide any network or security configuration files, utilities, or instructions.

If external or network drives are connected to the sequencer, it is the responsibility of your IT personnel to ensure that such drives are configured and secured correctly to prevent data corruption or loss.

If a LIMS connection is set up, you can use the LIMS API to get the list of files generated for the assay. For more information, see the software help system, or the *Genexus™ Software 6.6 User Guide*.

Note: If a LIMS system is configured to retrieve files from the sequencer, you have the option to manually set up an FTP directory or drive mapping. This configuration is not provided as part of sequencer installation, and must be set up by your LIMS system integration or IT group.

Password security

Best practice is to maintain unique passwords for all accounts in use in Genexus™ Software. All passwords must be reset the first time a user signs into the software. Change passwords according to your organization's password policy.

It is the sole responsibility of your IT personnel to develop and enforce secure use of passwords.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

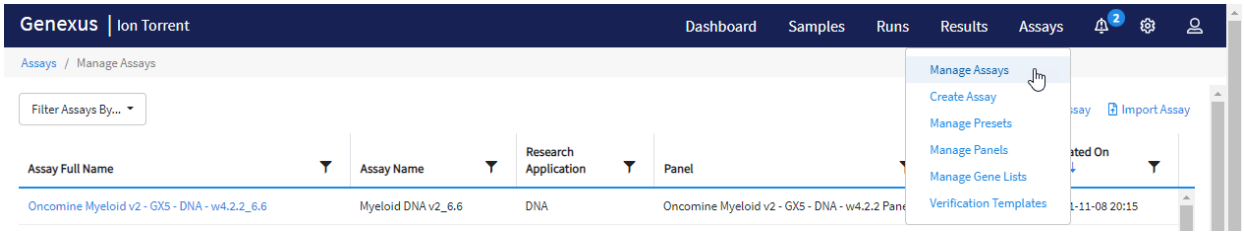
Antivirus software

Thermo Fisher Scientific has tested Genexus™ Software with the following antivirus software products and found them compatible as antivirus solutions:

- Bitdefender GravityZone™ Business Security
- Kaspersky™ Endpoint Security for Linux

Note: Antivirus Software definition files are updated frequently, sometimes daily. Definition file updates for antivirus software can bring additional settings or updates to the system which may affect the function of Genexus™ Software.

4 Create and manage assays (manager/administrator)



Manager- and administrator-level users can create and manage assays in Genexus™ Software. This chapter describes the system-installed assays and how to copy them to create a custom assay. If you are using a system-installed assay without change, proceed to Chapter 5, “Enter samples and libraries”.

Assays in Genexus™ Software

Assays contain the settings and parameters for library preparation, templating, controlling the sequencing run, analyzing, and reporting the results. Assays also define the panels, kits, and chips that are used in a run, and specify the reference files and threshold values for quality control and variant detection. The software files that contain the assay settings and parameters are packaged in a ZIP file called an assay definition file (ADF).

An assay is a reusable experimental design that contains predefined settings appropriate for use with common types of research applications. An assay can be used to plan many runs and plays an important role in enabling rapid throughput across the sequencing instrument. Assays help reduce the chance of errors, because information is stored and then applied to runs instead of entered manually for each run.

Custom assays can be created from system-installed templates, and you can copy a locked assay to create an assay, or copy and edit custom assays. You can also create custom versions of the system-installed presets that you can add to assays, including annotation sets, and copy number baselines, sequence variant baselines, exon tile assay baselines, and report templates.

Before you can create a custom assay, you must add a panel file, and hotspot and copy number baseline files (if needed for the assay), to the software. Custom assays are for advanced users. For help, contact a Field Service Engineer.

The software provides to tools to:

- Create, import, and manage assays.
- Create and manage annotation sets, report templates, filter chains, and copy number baselines (**Manage Presets**).
- Add and manage panels (**Manage Panels**).

System-installed assays

Genexus™ Software includes system-installed assays that are preconfigured for use with Oncomine™ GX assays. System-installed assays are available for download at **Software Updates** in the **(Settings)** menu. System-installed assays are locked and cannot be changed, but the assays can be copied, then edited.

Manage assays

You can view assay details, view the audit trail of an assay, and download assay parameters in Genexus™ Software.

In addition, manager- and administrator-level users can create or import an assay, export a locked assay, edit, lock, or delete a draft assay, edit the QC parameters of a locked assay, and obsolete a locked assay in the software.

In the menu bar, click **Assays** ▸ **Manage Assays** to open the **Assays / Manage Assays** screen.

The following tools are available in this screen.

To	Do the following
Review and download the assay audit trail	1. In the **Assays / Manage Assays** screen, place the pointer over the row of an assay of interest, then click **Audit**. 2. In the **Audit Trail** screen, click **Download** to download an audit details PDF file to your local drive.
Edit a draft assay	When a manager- or administrator-level user creates an assay, the status of the assay is draft. While the assay is in draft status, it can be edited. 1. Place the pointer In the row of a draft assay, then click **Edit**. The **Edit Assay** workflow opens. 2. Edit the options on each assay step as needed, then click **Save**.
Lock a draft assay	When a manager- or administrator-level user creates an assay, the status of the assay is draft. To use the assay in a run, you must lock it. Place the pointer In the row of a draft assay, then click **Lock**. Locked assays cannot be edited or deleted.

(continued)

To	Do the following
Copy an assay to create a new assay	Only locked assays can be copied. 1. Place the pointer In the row of a locked assay, then click **Copy**. The **Copy Assay** workflow opens. 2. Edit the options at each assay step as needed, enter a new name for the assay, then click **Save**.
Delete an assay	Only draft assays can be deleted. When an assay is locked, it can be removed from use in the software by designating it obsolete. Place the pointer In the row of a draft assay, then click **Delete**, then confirm the deletion.
Remove a locked assay	A manager-or administrator-level user can remove a locked assay from use in the software by designating it obsolete. The assay is not deleted and a record of it is maintained in the audit trail. The results for any runs already performed with the assay remain on the sequencer. 1. Place the pointer In the row of a locked assay, then click **Obsolete**. 2. Click **Yes** to confirm the operation.
Export an assay	An assay can be exported, for example if you want to use that assay in another Genexus™ Integrated Sequencer in your lab. Only locked assays can be exported. Place the pointer in the row of a locked assay, then click **Export**. The assay parameter files are downloaded to your local drive as a ZIP folder and are available for import to another sequencer. Panel reference files are not included in the exported folder.
Download parameters	Place the pointer In the row of the assay, then click **Download Parameters**. Assay parameter files are downloaded to your local drive as a ZIP folder containing assay parameter JSON files.

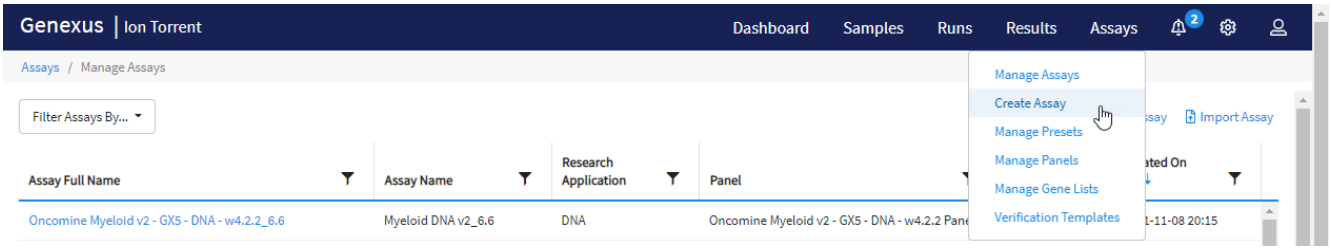
Create a new assay (manager/administrator)

Manager- and administrator-level users can create a new assay.

Assays can be copied from an existing system-installed assay or other assay, then modified as needed. For more information, see “Copy an assay (manager/administrator)” on page 58. For detailed information on how to create and manage assays, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

To create a new assay from an assay template, follow these steps.

1. In the menu bar, click **Assays** ▸ **Create Assay**.

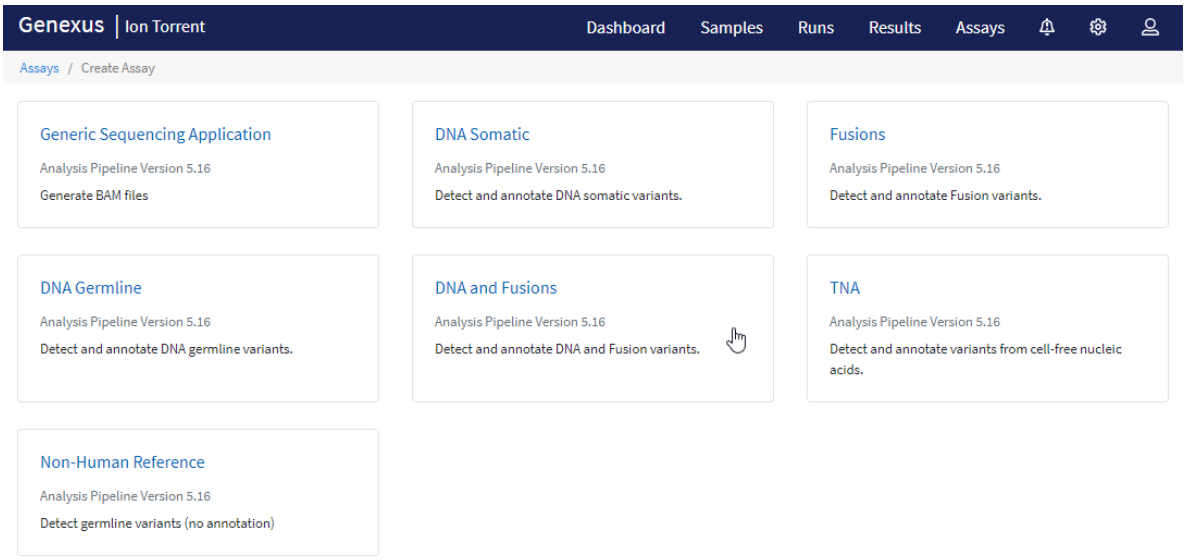


The **Create Assay** screen opens. Five oncology, one generic sequencing, and one non-human reference assay templates are available. The templates have assay-specific configuration steps that are pre-populated with default settings and parameters.

- Generic Sequencing Application
- DNA Germline
- Non-Human Reference
- DNA Somatic
- DNA and Fusions
- Fusions
- TNA

Note: Clicking **+ Create Assay** takes you to the same screen.

2. Select the assay template that you want to use, then click anywhere inside its box.

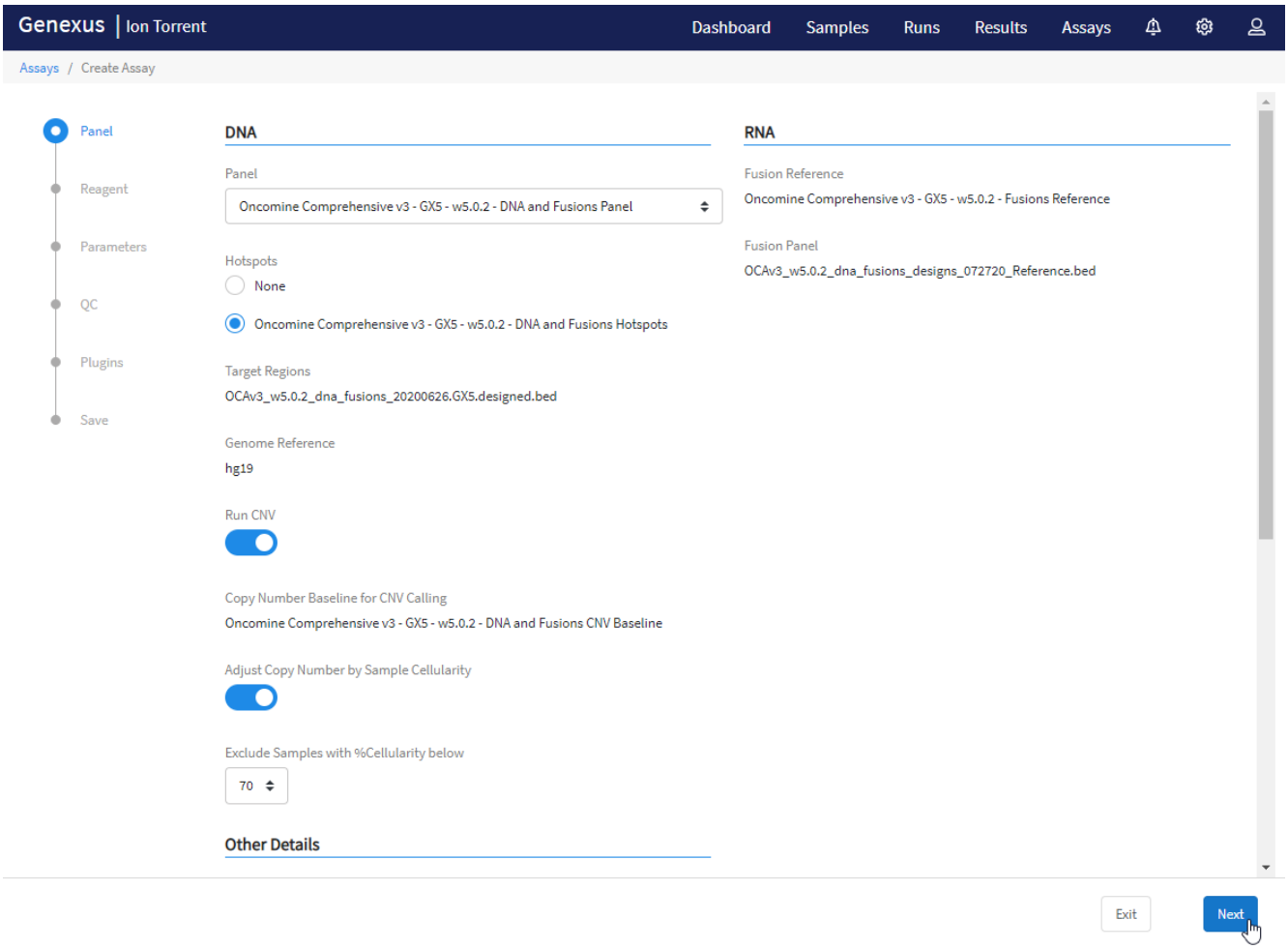


Note:

- Although each assay template has a specific set of steps in the setup wizard, the setup procedures for all are similar. The following is an example of assay creation after selecting the **DNA and Fusions** template.
- If you are using a custom assay, see “Guidelines for using custom assays with the Genexus™ Integrated Sequencer” on page 228 for guidelines for setting parameters for Minimum Read Count Per Sample and target amplification in the **Panel** and **Parameters** steps.

3. In the **Panel** step, make or confirm the following selections, then click **Next**.
   a. The panel for the assay from the **Panel** list.

> **Note:** To add a new panel, click **Assays** ▸ **Manage Panels**, then click **+ Add New**.



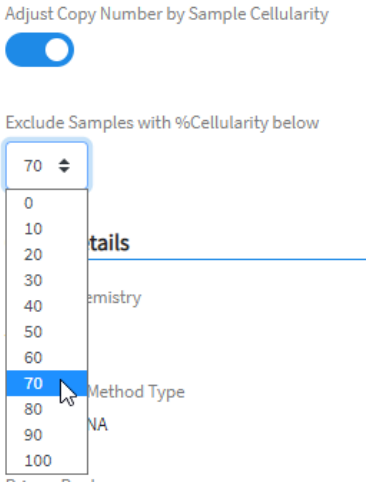
   b. The hotspot file for the panel.

> **Note:** To add a hotspot file, click **Assays** ▸ **Manage Panels** ▸ **Hotspots**, then click **+ Add New**.

   c. Click the **Run CNV** toggle to include copy number variant (CNV) analysis in the assay. The Copy Number Baselines that are associated with the panel are displayed. If multiple baselines are listed, select the desired baseline. Deselect **Run CNV** toggle if you are not performing CNV analysis in the assay.

> **Note:** To add a CNV baseline, click **Assays** ▸ **Manage Presets** ▸ **Copy Number Baselines**, then click **+ Add New** or **Import Copy Number Baseline**.

d. *(Optional)* If you selected **Run CNV**, and you want the CNV analysis to adjust for heterogenous tumor content in your samples, click the **Adjust Copy Number for CNV Calling** toggle. Select a value from the **Exclude Samples with %Cellularity below** dropdown menu. Samples with %cellularity below this value when the sample was created in the software are excluded. If you do not want the assay to adjust for cellularity, leave the toggle deselected. If you want to adjust for sample cellularity, but not exclude any samples, select **0** in the dropdown menu.



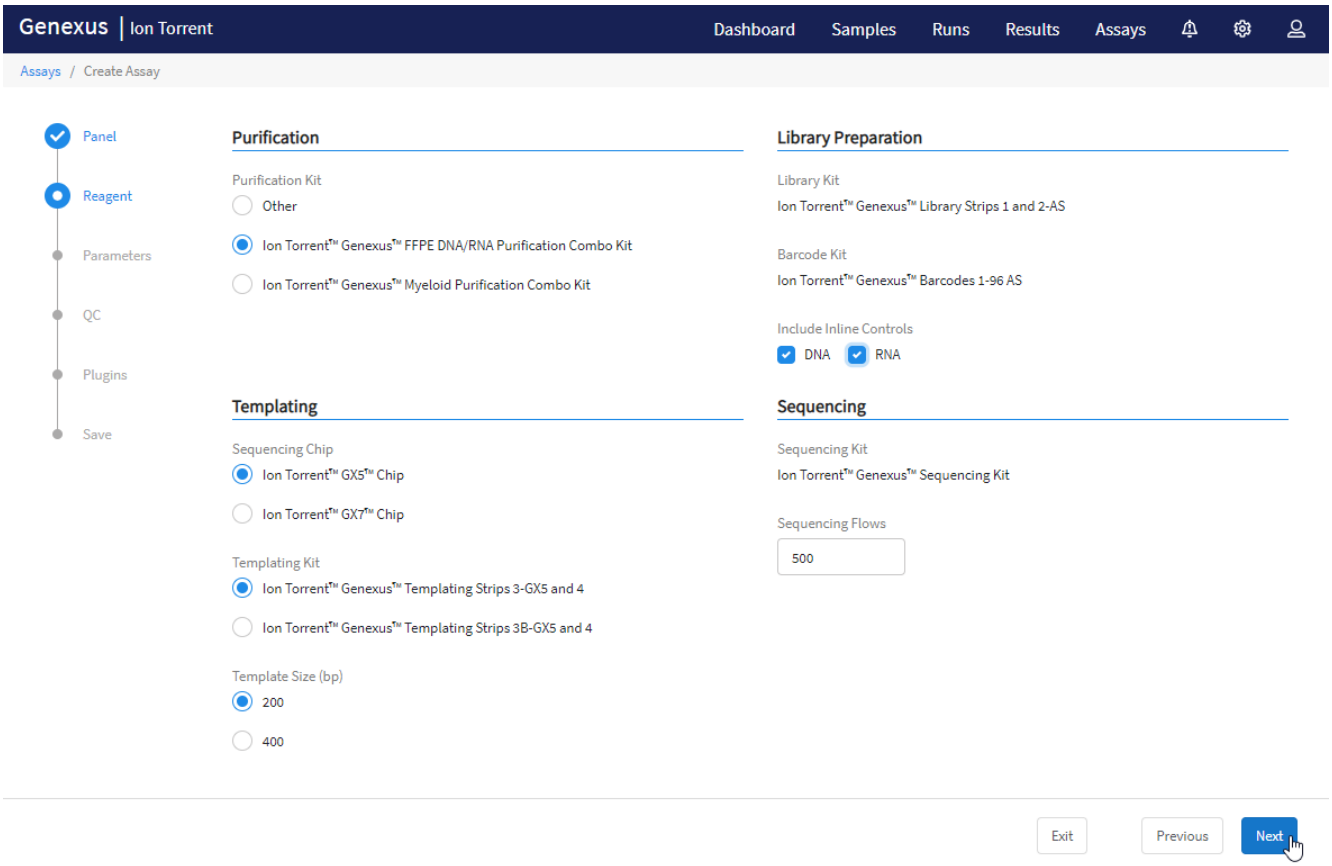
e. The minimum read count per sample (DNA and/or RNA) coverage settings (required).

f. The annotation set to use in variant reporting.

Note: To add an annotation set, click **Assays** ▸ **Manage Presets** ▸ **Annotation Sets**, then click **+ Add New**.

4. In the **Reagent** step, make the following selections or entries, then click **Next**. Confirm or select names of the purification, library, barcode, templating, and sequencing kits, select the sequencing chip, and inline control check boxes, then select or enter values for template size and sequencing flows, if different from the pre-populated values. Click **Next**.
   a. If applicable, select the purification kit to be used. If not applicable, leave as **Other**.

b. Confirm or select names of library, barcode, templating, and sequencing kits.



Note: If you select **Other AmpliSeq** from the **Library Kit** list, the **Barcode Kit** dropdown list includes barcodes sets, such as IonCode™ and Ion Xpress™ barcodes, that are compatible with manually or Ion Chef™-prepared libraries.

c. Select one or both of the DNA and RNA inline control check boxes if you want to include inline controls in the quality control analysis.

d. Select the sequencing chip.

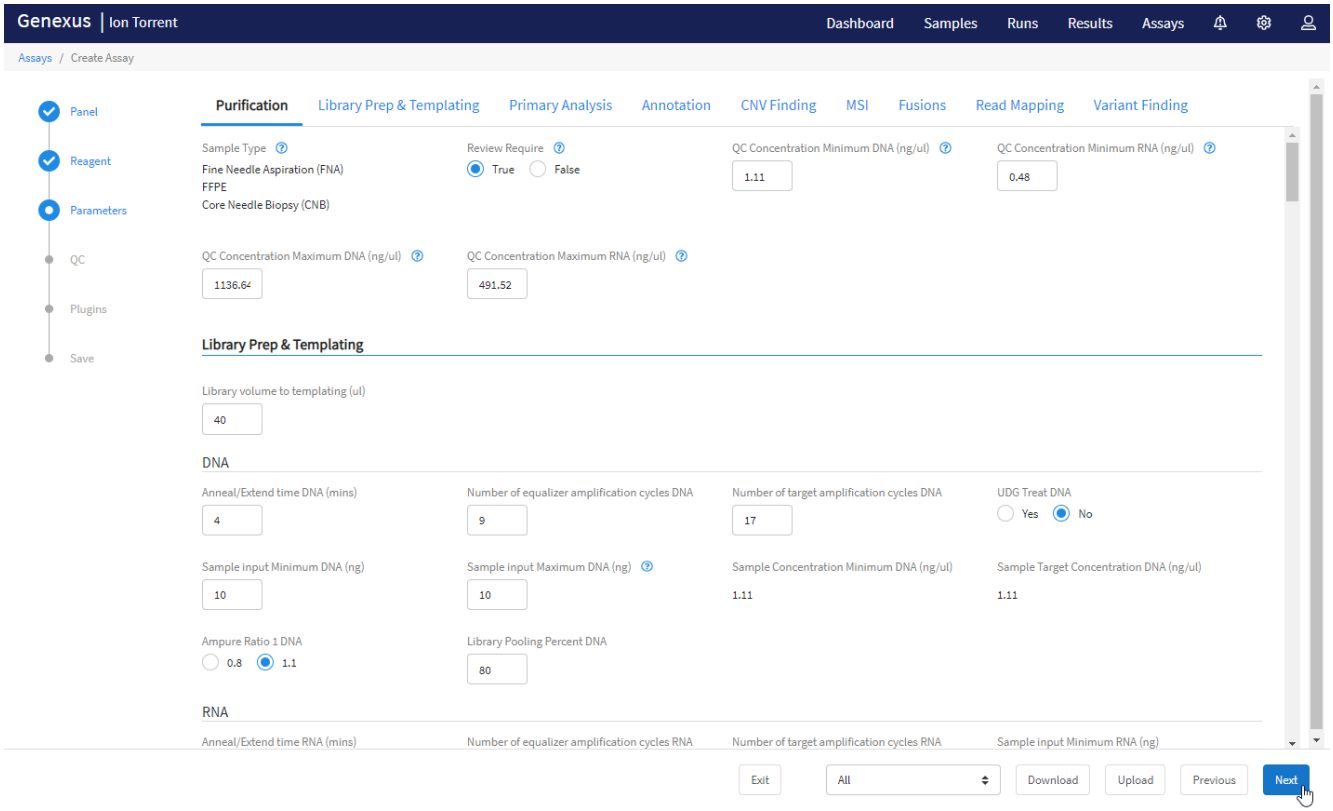
e. Select or enter values for template size and sequencing flows, if different from the pre-populated values.

5. In the **Parameters** step, review the pre-populated analysis settings, then adjust if needed. Click **Next** when finished.

Note:

- Not all parameters are adjustable. To modify primary analysis parameters, select the **Customize Parameters** checkbox.
- Select **Yes** under **UDG Treat DNA** in the **Library Prep & Templating Parameters** section to include uracil DNA glycosylase (UDG) treatment of DNA during library preparation. Removal of uracil residues can increase sequencing quality for FFPE samples that have undergone significant cytosine deamination.

  We recommend using multiple samples in runs that include UDG treatment. Single sample runs can result in low read number.



Scrolling from the top, the settings are grouped in the following categories, depending on the assay template selected:

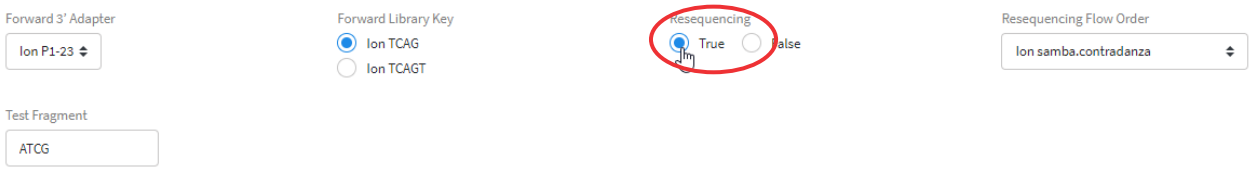
- **Purification** (if applicable)
- **Library Prep & Templating**

  If needed, change the default settings for the cycling and input parameters used in library and template preparation.
- **Primary Analysis**
- **Annotation**
- **CNV Finding**
- **MSI** (microsatellite instability analysis parameters)
- **Fusions**
- **Read Mapping**
- **Variant Finding**

A parameter category can be quickly brought to the top of the screen by selecting it in the category list along the top of the screen.

Parameters can also be set by uploading an **Advanced Parameter Configuration** file, which overrides default settings. Click **Upload** at the bottom of the screen, then click **Select File** in the **Upload Parameters** dialog box to navigate to and select this file on your drive. Click **Upload** to upload the parameters settings as a JSON file to your hard drive.

Note: To enable resequencing in the assay, click the **Resequencing = True** setting in the **Primary Analysis** parameter category. We recommend that you keep the Resequencing parameter set to False unless you have spoken with a Field Bioinformatics Specialist (FBS) and understand how the change can affect results.



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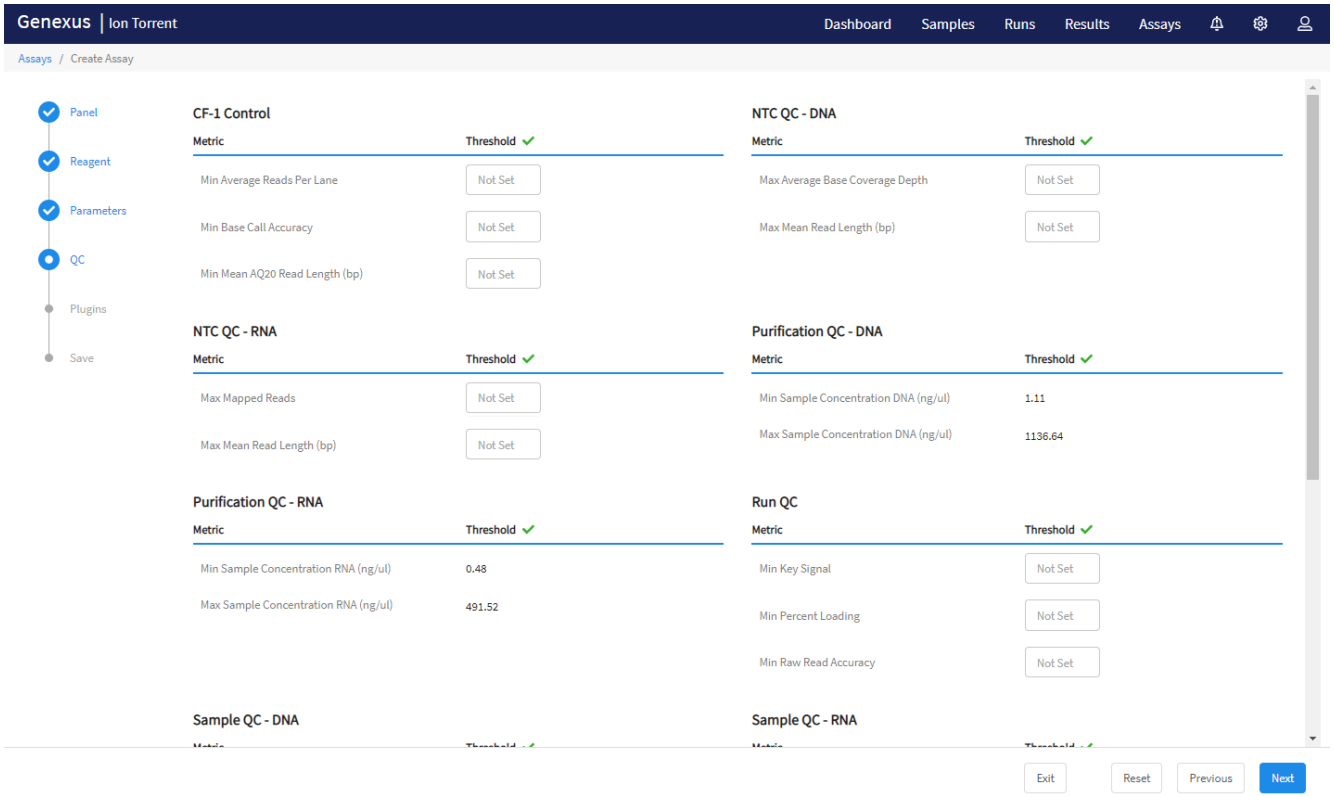
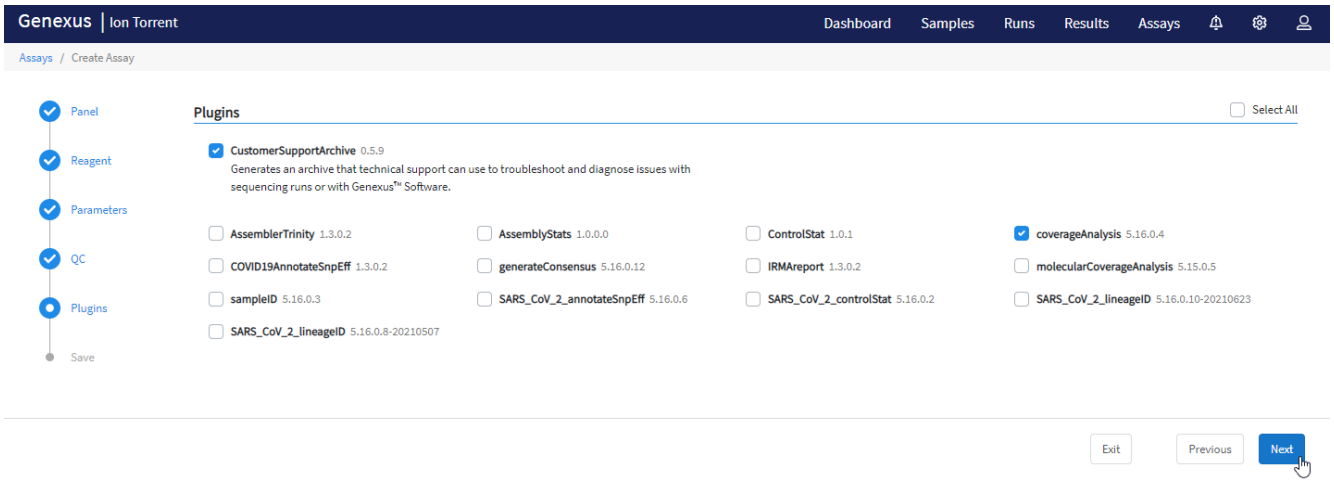
6. In the **QC** step, enter parameters in the **CF-1 Control** and **Run QC** sections, and in the NTC (no template control) QC and Sample QC fields that are appropriate to the sample types specified in the assay: **NTC QC - DNA**, **NTC QC - RNA**, **Sample QC - DNA**, and **Sample QC - RNA**. Leave parameters that are not applicable to your assay as **Not Set**. Click **Next** when finished.

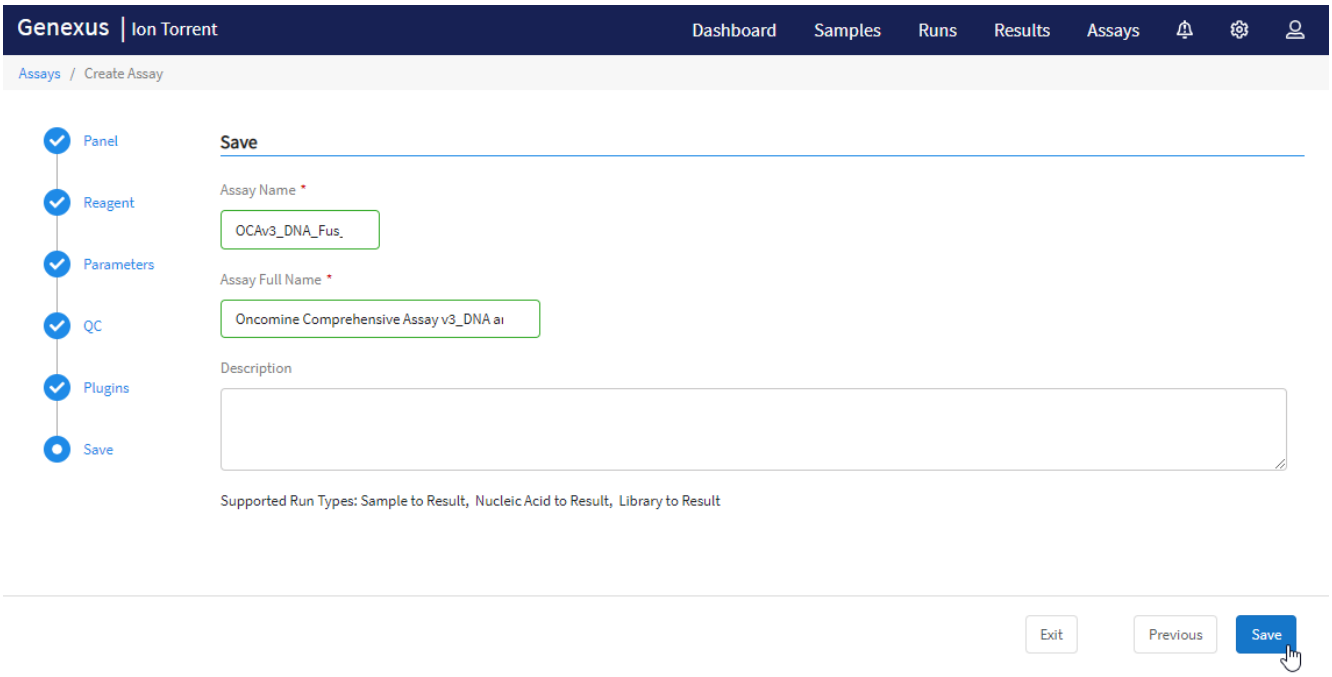


For descriptions of QC parameters, see “QC step assay options” on page 54.

7. In the **Plugins** step, select plugins that you want to include in the sequencing data analysis from the plugin list, then click **Next**.



8. In the **Save** step, enter a name and a short name for the assay, an optional description, then click **Save**.



The assay appears in the **Manage Assays** screen with the name you entered.

9. In the **Manage Assays** screen, click **Lock** in the **Actions** column of the assay to prevent changes to the assay.

QC step assay options

You can specify Quality Control (QC) metric values to use in an assay in the **QC** step of the **Create Assay** workflow.

The following tables list options that are available in the **QC** step. The available options depend on the type of assay.

CF-1 Control

Options	Description
Min Average Reads Per Lane	The average number of CF-1 reads per chip lane. You can specify the minimum threshold value required for the CF-1 (templating Control Fragment-1) to pass the QC metric.
Min Base Call Accuracy	The percentage of accuracy of the CF-1 calls aligned to the CF-1 reference. You can specify the minimum threshold value required for the CF-1 to pass the QC metric.
Min Mean AQ20 Read Length	The mean length of CF-1 reads having ≥99% accuracy at each position. You can specify the minimum threshold value required for the CF-1 to pass the QC metric.

NTC QC - DNA

Options	Description
Max Average Base Coverage Depth	The average number of reads of all targeted reference bases. You can specify the maximum threshold value required for the DNA no template control to pass the QC metric.
Max Mean Read Length (bp)	The average length, in base pairs, of called reads. You can specify the maximum threshold value required for the DNA no template control to pass the QC metric.

NTC QC - RNA

Options	Description
Max Mapped Reads	The total number of reads in the no template control mapping to a fusion reference sequence. You can specify the maximum threshold value required for the RNA no template control to pass the QC metric. **Mapped Reads** can be configured in the **QC** step or in the **Parameters** step in the **Fusions** section as **Minimum Total Valid mapped reads**. The values configured in the **Parameters** step overwrite those configured in the **QC** step.
Max Mean Read Length (bp)	The average length, in base pairs, of called reads in the no template control. You can specify the maximum threshold value required for the RNA no template control to pass the QC metric. **Mean Read Length** can be configured in the **QC** step or in the **Parameters** step in the **Fusions** section as **Minimum mean read length for valid sample QC**. The values configured in the **Parameters** step overwrite those configured in the **QC** step.

Purification QC - DNA

Options	Description
Max Sample Concentration DNA (ng/µl)	The maximum threshold value for the sample concentration of purified DNA is shown. This value cannot be changed in the **QC** step, but can be changed in the **Parameters** step.
Min Sample Concentration DNA (ng/µl)	The minimum threshold value for the sample concentration of purified DNA is shown. This value cannot be changed in the **QC** step, but can be changed in the **Parameters** step.

Purification QC - RNA

Options	Description
Max Sample Concentration RNA (ng/µl)	The maximum threshold value for the sample concentration of purified RNA is shown. This value cannot be changed in the **QC** step, but can be changed in the **Parameters** step.
Min Sample Concentration RNA (ng/µl)	The minimum threshold value for the sample concentration of purified RNA is shown. This value cannot be changed in the **QC** step, but can be changed in the **Parameters** step.

Purification QC - TNA

Options	Description
Max Sample Concentration TNA (ng/µl)	The minimum threshold value for the sample concentration of purified TNA is shown. This value cannot be changed in the **QC** step, but can be changed in the **Parameters** step.
Min Sample Concentration TNA (ng/µl)	The maximum threshold value for the sample concentration of purified TNA is shown. This value cannot be changed in the **QC** step, but can be changed in the **Parameters** step.

Run QC

Options	Description
Min Key Signal	The average signal after software processing for all ISPs that identically match the library key (TCAG). A measure of the efficiency of template amplification. You can specify the minimum threshold value required for a run to pass QC for the key signal metric.
Min Percent Loading	The percentage of addressable wells on a chip lane that are loaded with an ISP. You can specify the minimum threshold value required for a run to pass QC for the percent loading metric.
Min Raw Read Accuracy	The percentage of raw reads mapping to the reference sequence. You can specify the minimum threshold value required for a run to pass QC for the raw read accuracy metric.

Sample QC - DNA

Options	Description
Min Deamination score	Deamination is reported as the estimated SNP proportion consistent with deamination (low allele frequency C:G>T:A SNVs). The deamination score can be used to determine the quality of an FFPE sample. You can specify the minimum threshold value required for a DNA sample to pass the QC metric.
Max MAPD	The Median of the Absolute values of all Pairwise Differences; a quality metric that estimates coverage variability between adjacent amplicons in copy number variant (CNV) analyses. A MAPD value ≤0.5 generally indicates an acceptable level of coverage variability in the DNA Library or DNA Control. You can specify the maximum threshold value required for a DNA sample to pass the QC metric.
Min Mapped Reads	The total number of bases mapped to target amplicons. You can specify the minimum threshold value required for a DNA sample to pass the QC metric.

Sample QC - DNA *(continued)*

Options	Description
Min Mean AQ20 Read Length (bp)	The mean length of sample reads aligned to a reference sequence that have ≥99% accuracy at each position. You can specify the minimum threshold value required for a DNA sample to pass the QC metric.
Min Mean Read Length (bp)	The mean length of all sample reads. You can specify the minimum threshold value required for a DNA sample to pass the QC metric.
Min Number of bases used in calculating TMB	The number of exonic bases or all of the genomic bases that are covered by the panel. Only bases with sufficient base coverage are used in the calculation, as defined in the workflow parameters. By default, the TMB calculation uses total exonic bases with ≥60 bp coverage. In the TMB parameter settings, you can select between exonic bases or all of the genomic bases. You can also modify the sufficient coverage value. You can specify the minimum threshold value required for a DNA sample to pass the QC metric.
Min Number of variant calls	The number of somatic variants that are identified in the sample. This value is reported in the statistic.txt file as Total Somatic Filtered Variants Count (numerator for TMB calculation) and Variant Count. You can specify the minimum threshold value required for a DNA sample to pass the QC metric.
Min Uniformity of Amplicon Coverage	The percentage of reads showing a depth of coverage ≥20% of the mean base coverage. You can specify the minimum threshold value required for a DNA sample to pass the QC metric.

Sample QC - RNA

Options	Description
Min Mapped Reads	The total number of reads aligned to a fusion reference sequence. You can specify the minimum threshold value required for an RNA sample to pass the QC metric. The best practice to achieve consistent results is to use the same value in both the **QC** step and the **Parameters** step.
Min Mean AQ20 Read Length (bp)	The average length, in base pairs, at which the accuracy rate is ≥99% for reads of a library. You can specify the minimum threshold value required for an RNA sample to pass the QC metric.

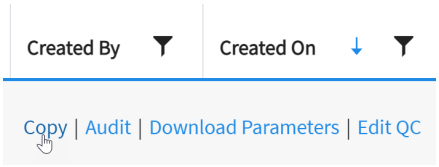
Sample QC - RNA *(continued)*

Options	Description
Min Mean Read Length (bp)	The average length, in base pairs, of called reads. You can specify the minimum threshold value required for an RNA sample to pass the QC metric.
Min RNA Expression Ctrls Detected	The number of expression control genes detected for the sample. This metric measures the RNA input integrity, input amount, and the fidelity of the reverse transcriptase that was used in library preparation. Fusion panels include primer pairs that cover seven control housekeeping genes. For Oncomine™ Precision Assay GX to pass QC, cfTNA samples require 2 out of 7 control genes to be detected and FFPE RNA samples require 5 out of 7 control genes to be detected. You can specify the minimum threshold value required for an RNA sample to pass the QC metric. This option is available only for cfTNA panels.

Copy an assay (manager/administrator)

Manager- and administrator-level users can create a new assay by copying an existing system-installed assay or other custom assay and modifying it if needed. Only locked assays can be copied.

1. In the menu bar, click **Assays** ▸ **Manage Assays**.
2. In the **Manage Assays** screen, place the pointer over the row of the assay that you want to copy, then click **Copy**.

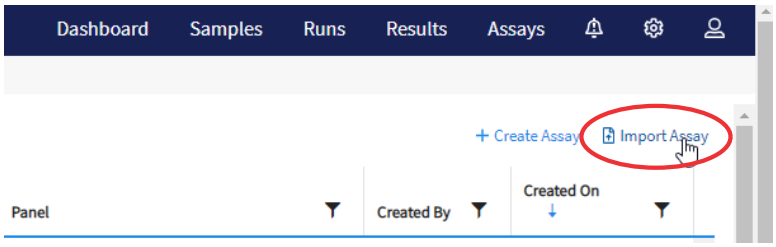


   The **Copy Assay** screen opens to the **Panel** step. The assay settings can be modified for the new assay.
3. Proceed through the workflow steps, and modify assay settings if needed.
4. In the **Save** step, enter a new assay name, an assay full name, and an optional description, then click **Save**.
   The newly created assay is added to the list of assays in the **Assays** / **Manage Assays** screen. The **Assay Full Name** is followed by **Draft** to indicate that the assay is in draft status. You can plan a run with the draft assay. The assay remains in draft status until it is locked. You can lock the draft assay to ensure that the assay cannot be edited. For more information, see “Manage assays” on page 44.

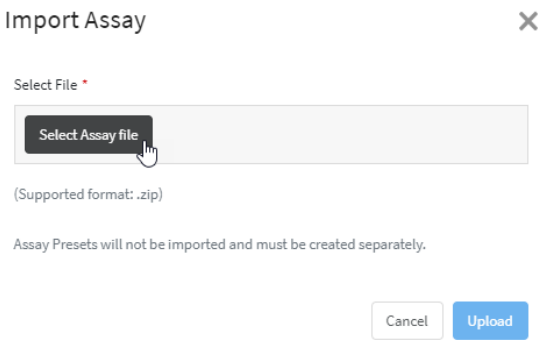
Import an assay (manager/administrator)

A manager- or administrator-level user can import an assay from another Genexus™ Integrated Sequencer if the assay has been first exported to a local drive.

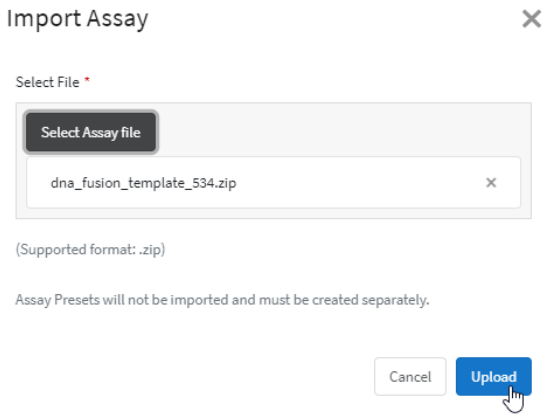
1. In the menu bar, click **Assays** ▸ **Manage Assays**.
2. In the **Manage Assays** screen, click **Import Assay**.



3. In the **Import Assay** dialog box, click **Select Assay file**, then go to the folder on the computer that contains the exported assay and select the ZIP file for the assay.

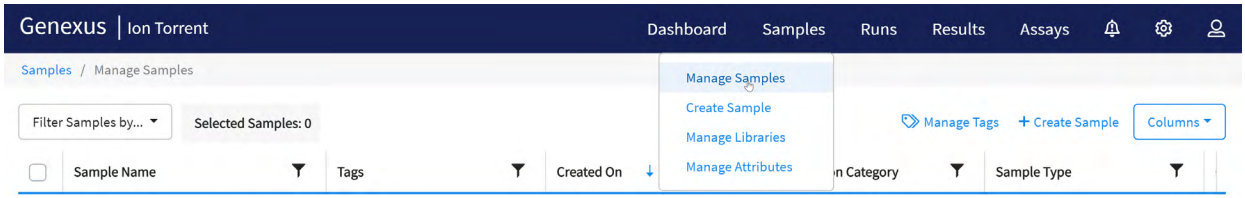


4. Click **Upload**.



The assay appears in the list of assays in the **Assays** ▸ **Manage Assays** screen.

5 Enter samples and libraries



Before you plan a run in Genexus™ Software, you must first enter sample information in the software to assign sample names and provide other information.

From the **Samples** menu, you can add samples in three ways. You can enter sample information for individual samples, you can import sample information from a file to create multiple samples, or you can upload a BAM file to create samples.

About samples and library batches

In Genexus™ Software, the data and attributes that characterize genomic data are called samples. A sample can be isolated nucleic acid, a specimen that requires nucleic acid isolation, or the sequencing data that are created from a BAM file that contains sample reads. Before you can plan a run to sequence or analyze a sample, you must add the information that characterizes each sample in the software.

A library batch is a group of samples that are sequenced in a Library to Result run. You can create library batches from samples you have previously added or uploaded to the software. During library batch preparation, you identify the barcode adapters that were used to prepare the libraries. After you create a library batch, you can plan and start a run to sequence and analyze the samples in the library.

Enter samples in the software

There are two types of samples in Genexus™ Software.

Sample	Description
Sample	A sample of isolated nucleic acid that is ready for library preparation, sequencing, and analysis or a specimen sample that requires nucleic acid isolation on a Genexus™ Purification System in integrated configuration before sequencing.
BAM sample	A sample created from a BAM file that contains sample reads. BAM samples can be uploaded and analyzed by the software. BAM samples are denoted with `BAM` after the sample name in the list of samples.

You can add samples to the software in three ways.

- Enter information for a single sample or multiple samples into the software.
- Create a file of information for a group of samples and import that file.
- Enter information and import a BAM or BAM files for a single sample or multiple samples.

If you enter the information for samples in the software, you can select from system-installed sample attributes that are available in the software. For more information, see “Create a new sample” on page 61.

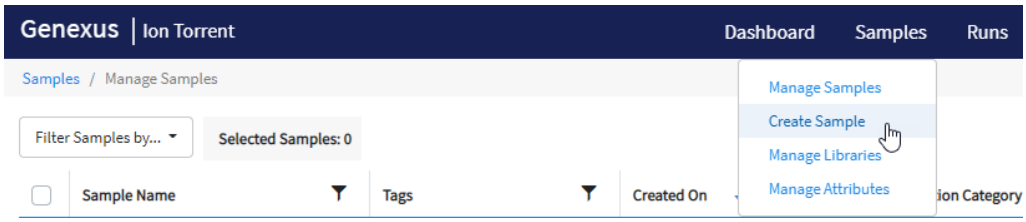
System-installed and custom sample attributes are also available in the example file that you can use for importing samples. For more information, see “Download a template file for sample creation” on page 66.

Samples are listed in the **Manage Samples** screen.

Create a new sample

You can enter information individually for each sample that you add in the software. Complete this procedure to make a sample available for use in a run, or to add the sample to a library batch. If you have many samples to add, see instead “Create multiple samples” on page 62 and “Import samples” on page 63.

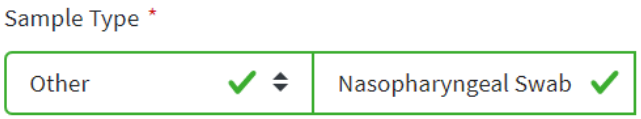
1. In the menu bar, click **Samples** ▸ **Create Sample**.



2. In the **Samples/Create Sample** screen, enter a name for the sample, select an **Application Category**, then complete the required fields, and optional fields if needed.
   - In the **Tags** field, enter a full or partial tag name of at least 3 characters, then select one or more tags of interest to assign a tag to the sample.
   - If the tag that you want to assign to the sample does not exist in the software, a manager-or administrator-level user can enter a new tag name in the **Tags** field to create a new tag and assign it to the sample.

   The fields for sample attributes change based on the **Application Category**. Attributes identified with a red asterisk (*) are required.

   If you select **Other** as the **Sample Type**, you must enter a name for the custom sample type in the text box that is shown. For example, enter ***Nasopharyngeal Swab***.



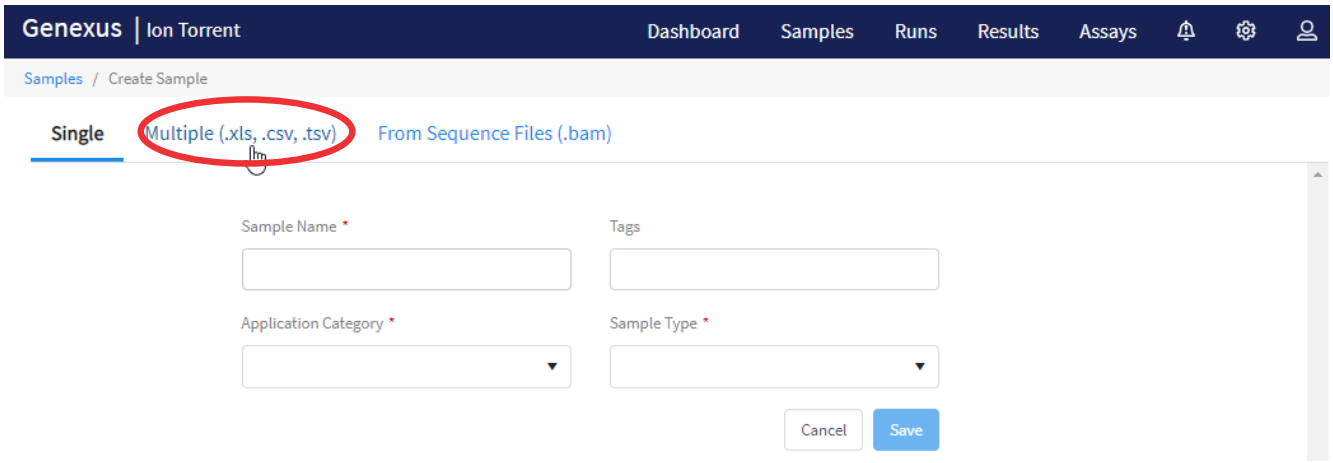
3. Click **Save**.
   The new sample is listed in the **Manage Samples** screen and is available to use in a run plan.

Create multiple samples

There are two ways you can create multiple new samples in Genexus™ Software.

- You can enter sample information for multiple samples in a table format in the software.
- You can import a file that contains sample data. For more information, see instead “Import samples” on page 63.

1. In the menu bar, click **Samples** ▸ **Create Sample**.
2. In the **Create Sample** screen, click the **Multiple (.xls, .csv, .tsv)** tab.



3. In **Application Category**, select the application category for the samples.
   A table with columns that are specific for the selected application category appears.

4. *(Optional)* Click **Columns** in the upper right corner of the screen to customize the optional attributes for the samples that you want to create.
   - Select the checkbox for a sample attribute to add a column to the table. Click the row to view fields into which you can enter attribute information.
   - Deselect the checkbox for a sample attribute to remove the attribute from the table.

5. Enter the information for a sample.
   Attributes identified with a red asterisk (*) are required.

6. Click **Add Row**, then enter the information in the new row for each new sample.

7. Repeat step 6 for each new sample.

8. Select the checkbox in the row for each sample in the list that you want to create. To select all samples, select the checkbox in the column heading row.

   IMPORTANT! The information for samples that are not selected is not retained by the software. Ensure that you select the checkbox for every sample that you will create.

9. Click **Save**.
   The new samples are listed in the **Manage Samples** screen and are available to use in a run plan.

Import samples

You can enter sample information for multiple samples directly in Genexus™ Software. When you want to create more than a few samples, an easy and fast way to add multiple samples in the software is to create a file of information for a group of samples and import that file.

Sample data files can be used to capture, manage, and edit sample data. You can import sample data files in TSV, XLS, XLSX, or CSV file formats. For a list of the sample attributes that are included in the import file, see “System-installed sample attributes” on page 67. For ease of use, you can download a Microsoft™ Excel™ template file to create an import file.

You must create custom attributes before importing sample and run information for the attributes to be propagated through to output files. All attributes that are included in the file that you use to import samples must be either system-installed attributes or custom attributes that exist in the software. Other file content is not transferred with the sample.

You can use a file to import sample information into the software. You can create the file, or use a file that is exported from external LIMS software. Before you import a file from LIMS software, you must first map the sample attribute names that are named differently in the LIMS file to the attribute used in

Genexus™ Software. For more information, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

1. In the menu bar, click **Samples** ▸ **Create Sample**.
2. In the **Create Sample** screen, click the **Multiple (.xls, .csv, .tsv)** tab.
3. In the **Application Category** dropdown list, select the application category for the samples.

   Tip: Use the search field to search for the application category of interest.

4. Set up a sample file using one of two options.

Option	Description
Download a template file, then edit it to create a new file.	For more information, see "Download a template file for sample creation" on page 66. Upload the edited file using the **Browse** button. **Note:** When you select **Other** as the **Application Category**, you must enter text in the **Application Category** text box, then click anywhere in the screen in order for the template file to be available.
Upload the sample data from an existing file.	1. Click **Browse**. 2. Navigate to the file, then click **Open**. The data contained in the file populates the table in the screen.

5. In the sample file, edit the sample table and data, if needed.

Option	Description
Remove an attribute column.	Click **Columns**, then deselect the column name.
Add an attribute column to the table.	Click **Columns**, then select the column name. The column names that are listed are **Application Category**-specific.
Edit sample data.	Click the sample row of interest, then edit the text field or dropdown list for the sample.
Add more samples to the table.	Click **Add Row**, then enter the sample data.
Remove a sample from the table.	Select the checkbox for the row, then click **Remove Row**.

6. Select the checkbox in the row for each sample to create. To select all samples, select the checkbox in the column heading row.

   IMPORTANT! The information for samples that are not selected is not retained by the software. Ensure that you select the checkbox for every sample you intend to create.

7. Click **Save**.

   You can place the pointer over ⓘ **(alert)** to view more information if needed.

The new samples are listed in the **Manage Samples** screen and are available to assign to a run plan.

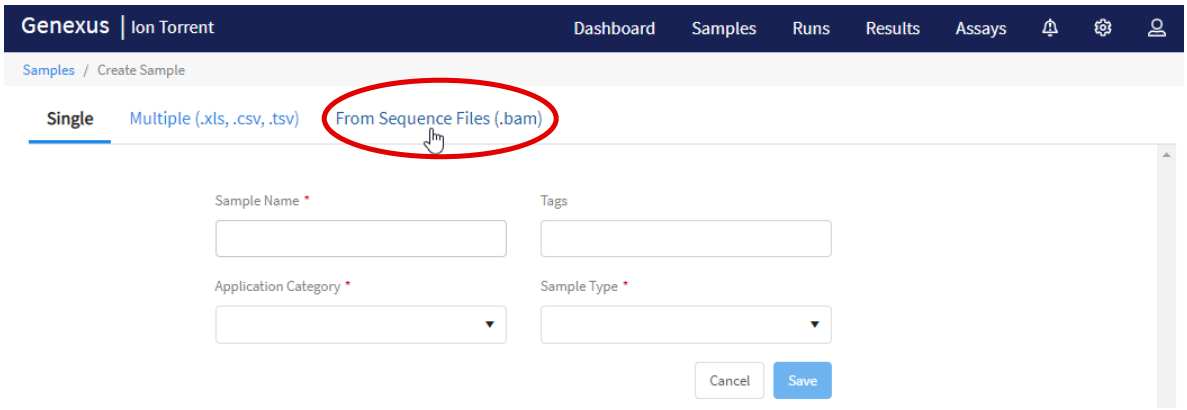
Upload a BAM file to create a sample or samples

You can upload BAM files to create samples in Genexus™ Software. You can also redefine existing BAM files as new samples. That is, you can upload a BAM file and create a new sample name for the BAM file and enter the same or different values for the sample attributes that are associated with the BAM file.

If the BAM file is not on the Genexus™ Integrated Sequencer, you can upload a BAM file from the computer that is used to access Genexus™ Software. You can upload the BAM file directly from the computer or from a USB drive connected to the computer.

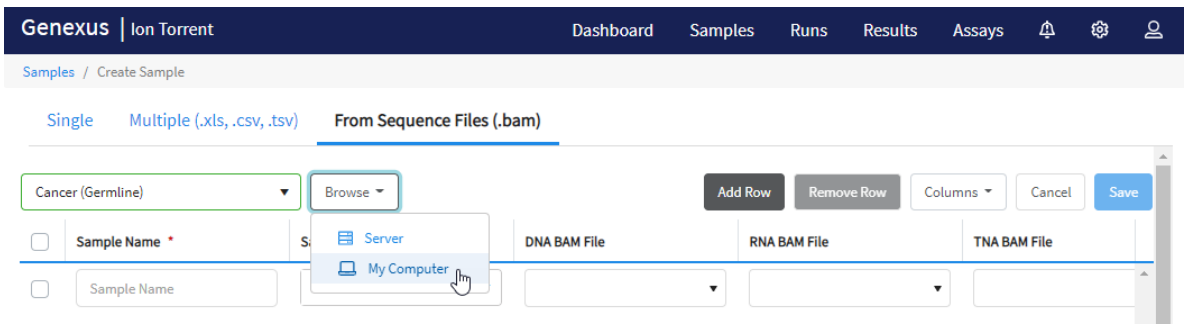
BAM files can be analyzed in the software. For more information, see “Plan a BAM to Result run” on page 90.

1. In the menu bar, click **Samples** ▸ **Create Sample**.
2. In the **Create Sample** screen, click the **From Sequence Files (.bam)** tab.



3. In **Application Category**, select the application category for the samples.
4. Click **Browse**, then navigate to the file.

Option	Description
Find the file on the instrument.	Click **Server**, then navigate to the file.
Find the file on the computer used to access the software.	Click **My Computer**, then in the **Browse** dialog box that appears, navigate to the file.



5. Navigate to the file, then click **Select**.
   The file is now available to select and assign to a sample. The BAM file name is visible in the dropdown lists in the **DNA BAM File**, **RNA BAM File**, and **TNA BAM File** columns.
6. Enter the information for the sample.
   Attributes identified with a red asterisk (*) are required.
7. In one or more of the **DNA BAM File**, **RNA BAM File**, and **TNA BAM File** dropdown lists for the sample, select the BAM file that you want to assign to the sample.
8. To add more samples, click **Add Row**.
9. Repeat step 4 to step 8 for each extra BAM sample you want to create.
10. Select the checkbox in the row for each sample that you want to create. To select all samples, select the checkbox in the column heading row.
11. Click **Save**.

The new samples are listed in the **Manage Samples** screen, are denoted with **BAM** after the sample name, and are available to assign to a BAM to Result run plan.

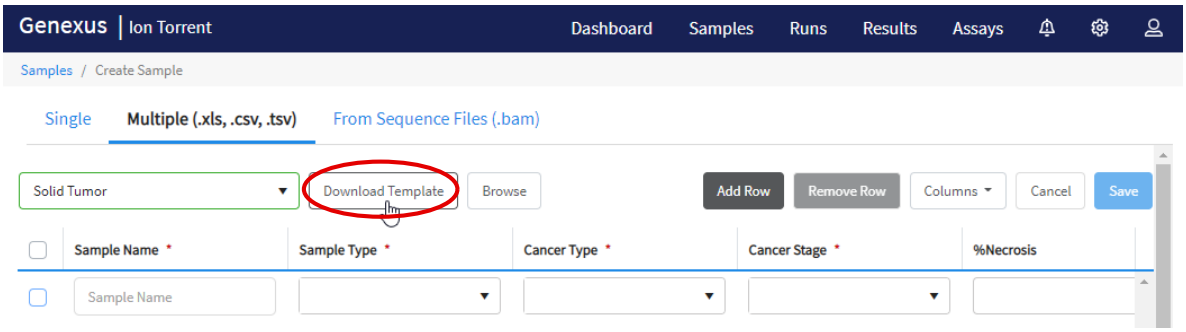
Download a template file for sample creation

You can download a template file, add sample information to it, then use the file to import the sample data for multiple samples.

Template files contain two tabs. The **Instruction** tab in the spreadsheet lists and indicates required and optional attributes, which are the column headings in the **Sample Details** tab. Use the **Sample Details** tab to enter sample information.

Template files contain both the system-installed and the custom sample attributes as column headings.

1. In the menu bar, click **Samples** ▸ **Create Sample**.
2. In the **Create Sample** screen, click the **Multiple (.xls, .csv, .tsv)** tab.
3. In **Application Category**, select the application category for the samples.
   Template files are specific for each application category.
4. Click **Download Template** to download the Microsoft™ Excel™ template file.



The template file contains default sample attributes as columns. If custom sample attributes have been configured in the software, the custom attributes are added to the template file.

5. Save the file to the computer, then open the file, enter sample data in the **Sample Details** tab.

6. When you are finished adding sample information to the file, save the file.

You can now import the file to Genexus™ Software.

System-installed sample attributes

The following table lists and describes system-installed sample attributes. System-installed sample attributes cannot be edited.

Note: Custom sample attributes are not listed in this table.

Sample attribute	Description
Sample Name[1]	A unique identifier representing the sample. The sample name can contain only alphanumeric characters (0–9, Aa–Zz), periods (.), underscores (_), or hyphens (-), cannot contain spaces, and is limited to a maximum of 20 characters. **IMPORTANT!** To prevent erroneous sample selection during run planning, ensure that you assign a unique and distinguishable sample name for each sample. **Note:** • Samples that have been used in a run cannot be deleted. • To prevent duplication, the software checks all sample names and returns an error message if a non-unique sample name is detected.
Collection Date[1]	The date that the sample was collected. Click **Calendar** to select the date in the correct format.
Gender[1]	The biological sex of the sample: **Female**, **Male**, or **Unknown**. **IMPORTANT!** **Male** or **Female** must be selected for proper measurement of AR CNV.
Sample Type[1]	A term that describes the sample, for example, FFPE, DNA, DNA & RNA. You can also select **Other**, then enter a custom sample type.
Application Category[1]	The sample application category, such as **Cancer (Germline)** or **Solid Tumor**. **Note:** If you select an oncology application category in this list, the **Cancer Stage**, **Cancer Type**, **% Cellularity**, and **% Necrosis** attributes listed below become available in the **Add New Sample** dialog box.
Cancer Type[1]	The type of cancer that is represented by the sample. Select the type of solid or hematologic cancer. If cancer type is unknown, select **Unknown Primary Origin**.

(continued)

Sample attribute	Description
Cancer Stage[1]	The stage of the cancer from which the sample was collected. Select **Stage 0–IV**, or **Primary**, **Unknown**, or **Other**.
% Cellularity	The percentage of tumor cells over normal cells in the sample. This is a whole number between 1 and 100. The **% Cellularity** attribute is applicable only to FFPE samples. **IMPORTANT!** • If this value is not set, **% Cellularity** is assumed to be 100% in calculations that use this attribute. • **% Cellularity** is a required attribute for CNV analyses. Do not leave the field blank. • *(FFPE samples only)* If **% Cellularity** value is set to <100, then the magnitude of copy number gain or loss can be decreased. For more information, see "CNVs table" on page 152.
% Necrosis	The percentage of cellular necrosis in the sample. This is a whole number between 1 and 100.
Notes	An open-entry field for more sample information.

[1] Required attribute

Manage samples, sample attributes, and sample tags

You can find tools for searching, sorting, editing, deleting, and exporting samples, and for viewing the sample history in the **Samples / Manage Samples** screen. In addition, you can find tools for creating and managing sample attributes and sample tags. For more information, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

Prepare or import a library batch

A library batch is a group of prepared libraries that are sequenced in the same Library to Result run. If you are planning a run starting from libraries that you have already prepared manually, you must first create a library batch in Genexus™ Software from samples. You can enter samples in the software or import a sample file. For more information see, "Enter samples in the software" on page 61.

Select the library batch when you plan the run. If you are planning a run starting from nucleic acid samples, skip this step and proceed to "Plan a Nucleic Acid to Result run" on page 76.

Note:

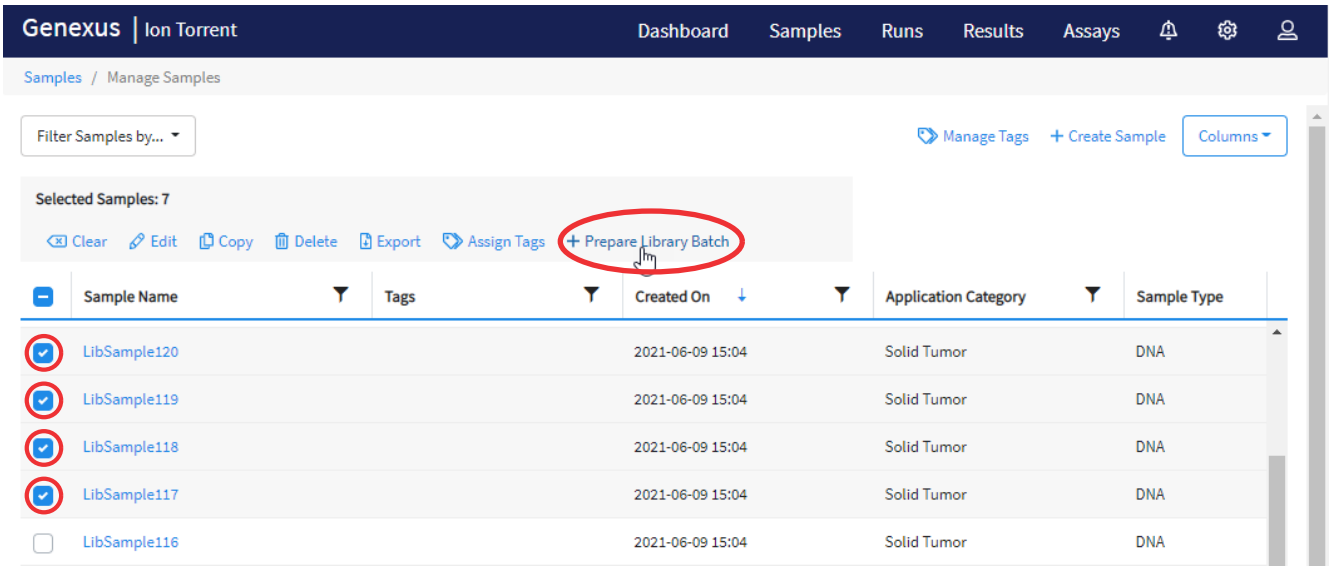
- Each library in a library batch must have a unique library name.
- Fields identified with a red asterisk (*) are required.

Prepare a library batch

A library batch is a group of prepared libraries that are sequenced in the same library run. If you are planning a run starting from libraries that you have already prepared manually, you must first create a library batch in Genexus™ Software from samples that you have added. If you are planning a run starting from nucleic acid samples, skip this step and proceed to "Plan a Nucleic Acid to Result run" on page 76.

1. In the menu bar, click **Samples ▸ Manage Samples**.
2. In the **Manage Samples** screen, in the **Filter Samples by** dropdown menu, apply the **To Be Prepared** filter to limit the displayed samples to those samples that have not been placed in a library batch.

   For more information about how to filter the list of samples, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).
3. Select samples in the list by clicking the checkbox to the left of each sample, then click + **Prepare Library Batch**.



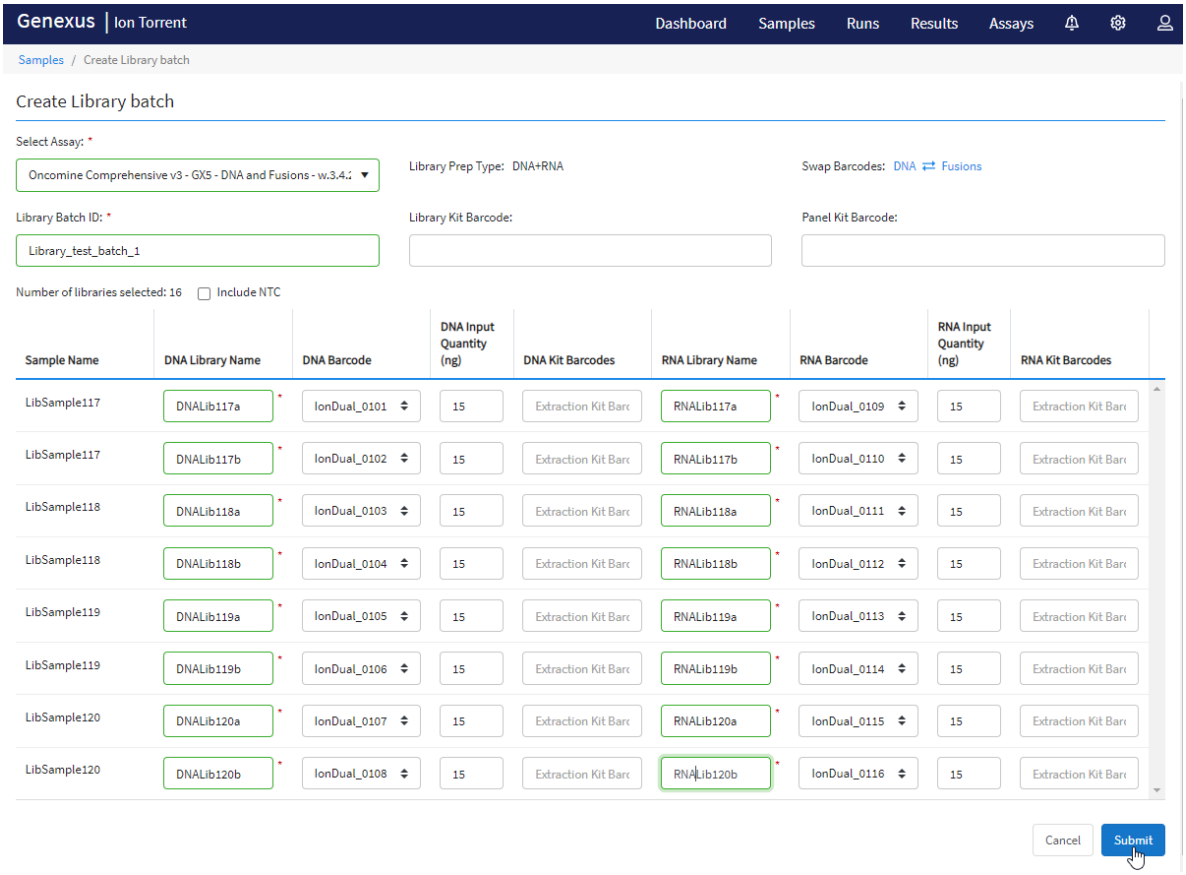
4. In the **Create Library batch** screen, select the assay that you want to use in the run plan from the **Select Assay** dropdown menu.

   The assay determines parameter selections for the run, including any required controls and post-run data analysis settings. If you select an assay that includes one library prep type, the library preparation type for the assay listed in the **Library Prep Type:** automatically fills for the nucleic acid type specified by the assay you selected: **DNA, RNA, DNA+RNA, or TNA**.

   a. For assays that include more than one library prep type, select the library prep type, such as **DNA, RNA, DNA+RNA, or TNA** for the library batch.

5. In the expanded screen, in **Library Batch ID**, enter a unique identifier for the library batch.

Note: Library Batch IDs can contain only alphanumeric characters (0–9, Aa–Zz), period (.), underscore (_), and hyphen (-). Required fields are indicated with a red asterisk (*).



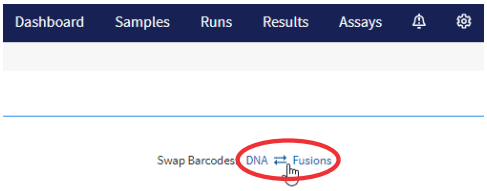
6. *(Optional)* Enter the **Library Kit Barcode** and the **Panel Kit Barcode**.

7. Select the **Include NTC** checkbox to add no template control sample processing and reporting to the library batch.

8. Enter a unique library name for each DNA and/or RNA library in the appropriate field.
   - Library names can contain only alphanumeric characters (0–9, Aa–Zz), period (.), underscore (_), and hyphen (-).
   - If your assay requires specific controls, they are automatically listed in the dialog box. These controls each require a unique barcode ID within the library batch, but do not require library names.

9. Select the barcode ID of the adapter used to prepare each library. If appropriate, swap the default barcodes in the dialog box between DNA, RNA, and Fusions by clicking the **Swap Barcodes** swap image.

   For example, click DNA ⇄ Fusions to swap barcodes.



Each library in a library batch must have a different barcode ID. When preparing the physical libraries, best practice is to swap barcodes between DNA and RNA libraries in consecutive sequencing runs to prevent carryover contamination. The barcodes that are listed in the **DNA Barcode** or **RNA Barcode** dropdown list belong to the barcode set that was selected when the assay was created.

IMPORTANT! Ensure that the barcodes that you used to create the libraries match the barcodes that you enter in the **Create Library batch** screen.

10. Enter the DNA, RNA, DNA+RNA, or TNA **Input Quantity** for each library.

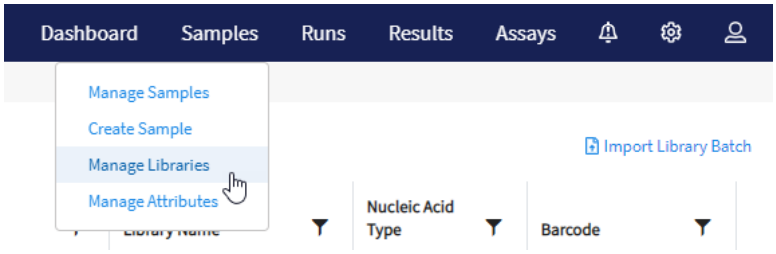
11. In the **DNA**, **RNA**, or **TNA Kit Barcodes** columns, enter the extraction kit barcode for each sample and no template control, if applicable.

12. Click **Submit** to save and submit your selections.
The **Samples / Manage Libraries** screen opens, listing the library batch that you created. Libraries that are prepared in the same batch have the same **Library Batch ID**.

Import a library batch

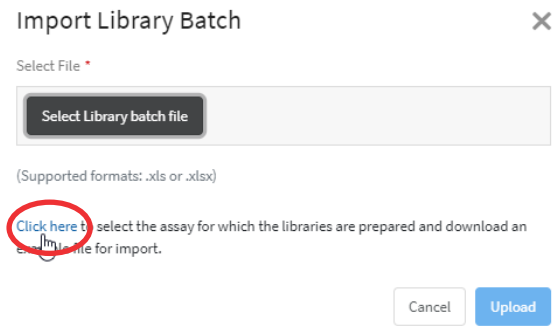
You can import library batch information in the form of an .XLS or .XLSX file. The import file must include all required library and kit information.

1. In the menu bar, click **Samples** ▸ **Manage Libraries**.



2. In the **Manage Libraries** screen, click **Import Library Batch**.

3. In the **Import Library Batch** dialog box, click **Click here** to select an assay for which the libraries are prepared, and to download an example file for import.



If the assay includes more than one library preparation type, a library preparation type dropdown list appears.

a. Select the library preparation type, such as **DNA, RNA, DNA+RNA, or TNA** for the library batch.

4. Select an assay from the list, then click **Download**.
   The assay name is auto-populated in the Microsoft™ Excel™ template file that downloads to your drive.

5. In the template file, enter or confirm the library batch information.

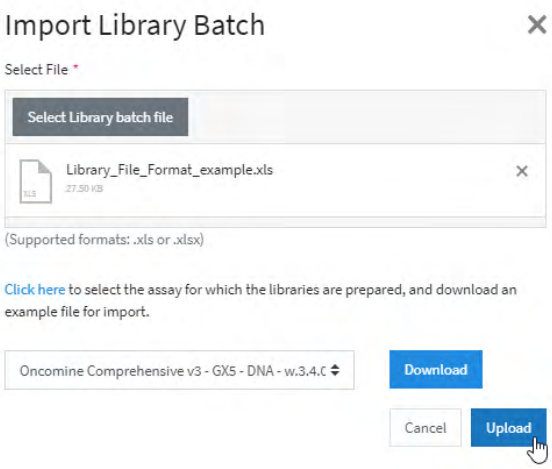
Template item	Description
Reagents tab	
Assay Name	Auto-populated when assay is selected in step 4 (required)
Extraction Method Type	Auto-populated when assay and library prep type, if applicable, is selected in step 4 (required)
Library Batch ID	Must be alphanumeric (0–9, Aa–Zz), period (.), underscore (_), and hyphen (-) (required)
Library Kit Barcode	For example, Genexus™ Library Strips 1 and 2-AS barcode (optional)
Panel Kit Barcode	For example, Oncomine™ Comprehensive Assay v3 GX barcode (optional)
Libraries tab	
Sample Name	Must be alphanumeric (0–9, Aa–Zz), period (.), underscore (_), and hyphen (-) (required)
Library Name	Must be alphanumeric (0–9, Aa–Zz), period (.), underscore (_), and hyphen (-) (required)
Barcode	Barcodes used for each sample and control library preparation (required)
Nucleic Acid Type	DNA+RNA, or TNA (required) DNA, RNA, or TNA (required)
Input Quantity	Library input quantity (optional)
No Template Control	To Include a no template control, add a row with **Sample Name** as ***NTC***, **Library Name** as ***NA***, and **Barcode** and **Nucleic Acid Type** similar to sample rows (optional)
Control Kit Barcode	The barcode for the kit used for nucleic acid extraction of no template controls (optional)
Extraction Kit Barcode	The barcode for the kit used for nucleic acid extraction of a sample (optional)

IMPORTANT! For DNA and Fusions assays, the DNA library and RNA libraries must be listed in sequential order per pool for each sample. For example, for a 1-pool DNA and Fusions assay, order should be DNA, RNA for sample 1, DNA, RNA for sample 2. For a 2-pool DNA and Fusions assay, library order should be DNA, RNA (pool 1), DNA, RNA (pool 2) for sample1, then DNA, RNA (pool 1), DNA, RNA (pool 2) for sample 2.

6. Save the file.

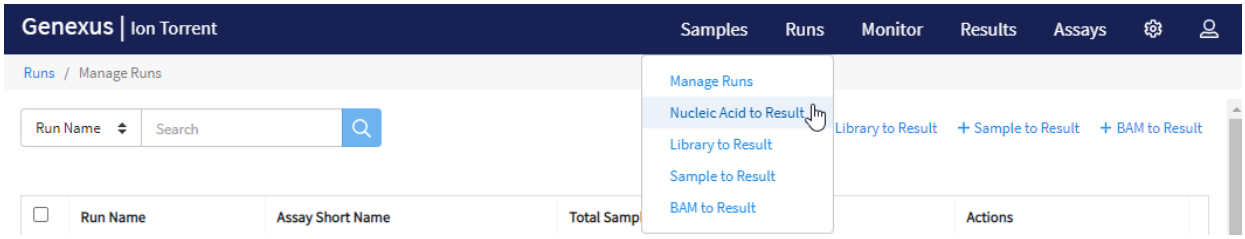
7. In the **Import Library Batch** dialog box, click **Select Library batch file**, navigate to the saved file, then select it.

8. Click **Upload**.



A progress bar followed by an import report displays. If the import process fails, an error message indicates the reason for failure (for example, an invalid character was used). For additional troubleshooting support, see “Library batch import fails” on page 191.

6 Plan a run



Before you can sequence samples on the Genexus™ Integrated Sequencer, you must create a run plan to specify the assay (or assays), the samples, and any additional parameters needed for the run. In Genexus™ Software 6.4 and later, there are four types of runs that you can plan for use on the Genexus™ Integrated Sequencer:

- Sample to Result—start from unprocessed samples and isolate nucleic acid on the Genexus™ Purification Instrument, then load the nucleic acid samples in the Genexus™ Integrated Sequencer
- Nucleic Acid to Result—start from purified nucleic acid samples
- Library to Result—start from prepared libraries, either prepared manually or prepared previously on the Genexus™ Integrated Sequencer
- BAM to Result—start from a BAM sequence file and analyze using the assay you specify

Before you plan a run

The following conditions must be met before you plan a run in Genexus™ Software.

- Sample information is accurate in the software and the name assigned to each sample is unique. For more information, see “Enter samples in the software” on page 61.
- A Genexus™ Purification Instrument is integrated for use with the system before you plan a **Sample to Result** run.
- Ensure that the following conditions are met for a **Library to Result** run.
  - Library batches are prepared and each batch uses a unique library batch ID.
  - Each sample library in a library batch is prepared with and assigned a unique barcode or barcode pair.

  For more information, see “Prepare or import a library batch” on page 68.

The software returns an error message when any of the conditions are not met.

Resequencing

When you copy and edit or create an assay in Genexus™ Software, the custom assay that you create can include an option to sequence and resequence samples in the same run. The option to resequence samples is available only in assays that specify a 200-bp template size. Runs that include the resequencing option have longer run times.

IMPORTANT! When you create an assay, keep the **Resequencing** parameter set to **False** unless you have spoken with a Field Bioinformatics Specialist (FBS) and understand how the change can affect results.

Resequencing settings are available in the **Primary Analysis** section of the **Parameters** step when you create an assay. Template size is specified in the **Templating** section of the **Reagent** step.

About Sample to Result runs

A **Sample to Result** run is an integrated run for sequential and automated nucleic acid purification and sequencing in Genexus™ Software 6.6 and later. You can create **Sample to Result** runs for the following sample types:

- Blood (Buffy Coat)
- Blood (Plasma)
- Blood (Whole)
- Bone marrow
- Core Needle Biopsy (CNB)
- Formalin-fixed, paraffin-embedded (FFPE) tissue
- Fine Needle Aspiration (FNA)
- Fresh Frozen Tissue

When you plan a **Sample to Result** run with multiple assays, the nucleic acid isolation is performed in a separate batch for each assay. Nucleic acid isolation is also performed in a separate batch for each sample type, except for FFPE and FNA samples, which are grouped together in a single batch for each assay. The Genexus™ Purification Instrument can run one nucleic acid isolation batch at a time. You

can run different nucleic acid isolation batches simultaneously on multiple purification instruments or sequentially on a single instrument.

You can choose to sequence all or some of the samples after nucleic acid isolation.

- Sequence all samples.
- Sequence only the samples that have a concentration within a specified threshold.
- Review the samples that do not have a concentration within a specified threshold, then choose which samples to sequence on a per sample-basis.

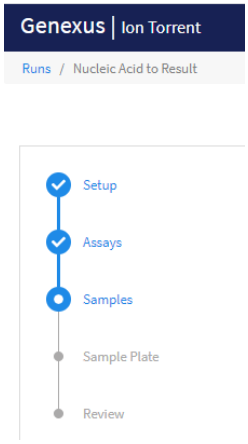
Note: The Genexus™ Purification Instrument is set up at installation to run in either the standalone configuration, or in the integrated configuration. In the standalone configuration, you must manually transfer samples from the archive plate to a sample plate for sequencing. In the integrated configuration, the **Sample to Result** run plan you set up in Genexus™ Software directs the Genexus™ Purification Instrument to load samples in an output plate that is ready to load in the sequencer.

For information on how to set up a run plan for a **Sample to Result** run for a given sample type, see the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475), the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub No. MAN0024953).

Plan a Nucleic Acid to Result run

You can plan a run for sequencing that starts with nucleic acid samples. In Genexus™ Software 6.6 and later, a run that starts with nucleic acid samples is called a **Nucleic Acid to Result** run.

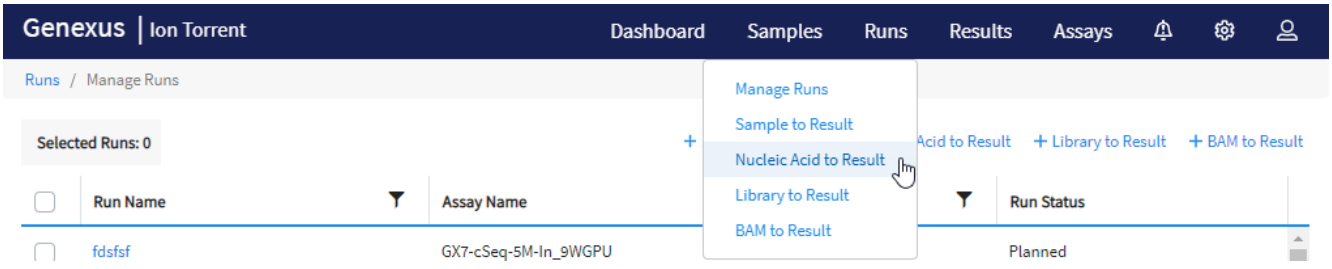
Planning a **Nucleic Acid to Result** run is organized into steps: **Setup**, **Assays**, **Samples**, **Sample Plate**, and **Review**. You can view progress through the steps in the upper left corner of the **Runs / Nucleic Acid to Result** screen.



Ensure that the following prerequisites are complete before you plan a **Nucleic Acid to Result** run.

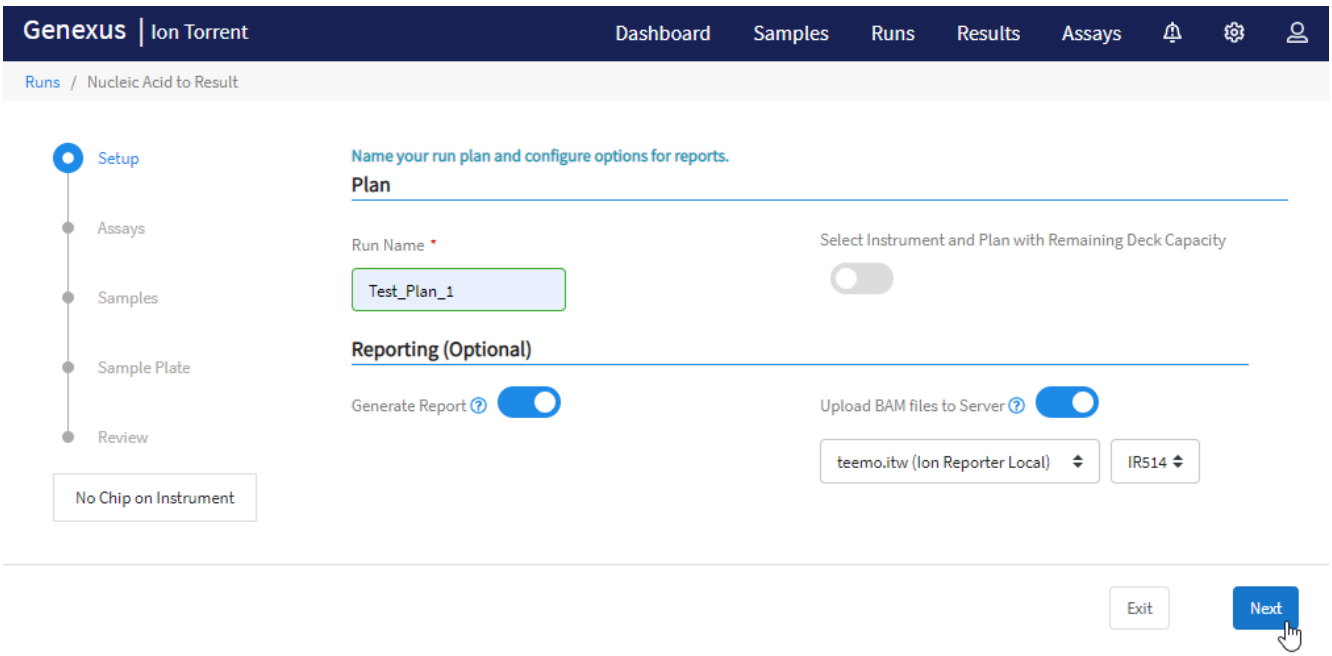
- Enter sample information into Genexus™ Software. For more information, see “Create a new sample” on page 61, or “Import samples” on page 63.
- Ensure that the assay that you want to use in the run exists in the software. For more information, see “Assays in Genexus™ Software” on page 43.
- Quantify sample concentration, if you do not intend to dilute sample concentrations manually to the target concentration of the assay.

1. In the menu bar, click **Runs** ▸ **Nucleic Acid to Result**.



Note: Alternatively, you can click + **Nucleic Acid to Result** in the **Runs / Manage Runs** screen.

2. In the **Setup** step, enter or make the following selections.
   a. In the **Plan** section, enter a unique name.
      The name is limited to 50 characters and no spaces are allowed.



   b. *(Optional)* In the **Reporting (Optional)** section, ensure that **Generate Report** is enabled to generate a variant report using the default report template.

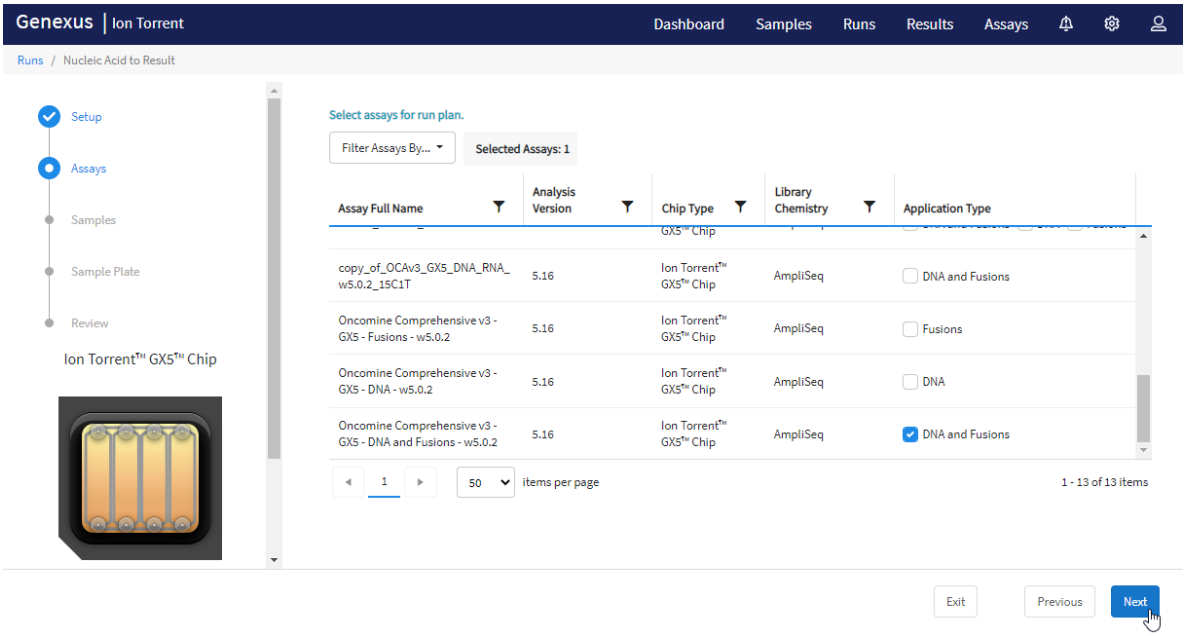
c. *(Optional)* In the **Reporting (Optional)** section, enable **Upload BAM files to Server** to upload BAM files to another server.

Option	Description
Upload BAM files to another Genexus™ Software server.	In the dropdown lists that appear, select the target Genexus™ Software server and version. To configure a Genexus™ Software account that you can use for data uploads, click **Set up Account**. For more information, see "Register Genexus™ Software accounts (manager/administrator)" on page 232.
Automatically upload data for further analysis with Ion Reporter™ Software.	In the dropdown lists that appear, select the Ion Reporter™ Software account and Ion Reporter™ Software version. To configure an Ion Reporter™ Server account that you can use for data uploads, click **Set up Account**.

Alternatively, you can upload BAM files to a server after a run. For more information, see "Upload results files to another Genexus™ Integrated Sequencer" on page 177.

d. Click **Next**.

3. In the **Assays** step, select one or more assays that you want to include in the run.



a. Use the ▼ **(Filter)** tools in table column headers to find assays of interest, if desired.

b. In the **Application Type** column for the assay of interest, select one or more application types, such as **DNA and Fusions** or **DNA**, to include each selected application type for the assay in the run plan.

After selecting an assay and the research application for the assay, the list is filtered to show compatible assays that can be selected and run at the same time.

Note: To create a new assay, see Chapter 4, "Create and manage assays (manager/administrator)".

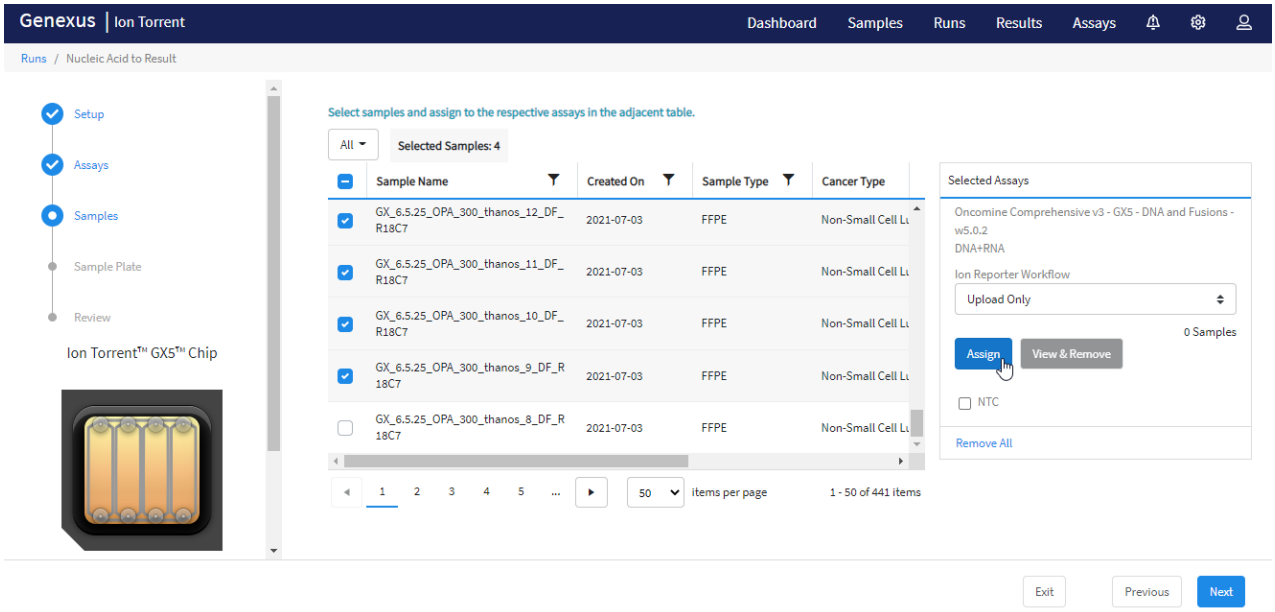
---

c. If more assays are included in the run, repeat substep 3b for each extra assay.

d. Click **Next**.

4. In the **Samples** step, select the samples that you want to run with each application type of each assay.

a. Select the checkbox next to each sample that you want to assign to the application type of an assay, then in the **Selected Assays** pane, for the assay and application type that you want to use for the selected samples, click **Assign**.



The **Chip View** updates to show the lanes used in the run. Lane usage is calculated based on the number of samples (including a no template control, if selected) and the minimum read counts per sample for the assay. Green denotes a chip lane in the run containing assigned samples within lane capacity.

If the

$$\textit{minimum reads per sample} \times \textit{number of samples}$$

exceeds the chip or lane well capacity, a dialog box appears after you click **Assign** asking you to confirm that you want to continue. After confirmation, the **Chip View** updates and shows the lane color as red instead of green. The run is allowed if the lane capacity is exceeded, but you may not achieve the required reads per sample to pass QC metrics.

Green lane color denotes lane usage and sample assignment within lane capacity.

Red lane color denotes sample assignment that exceeds lane capacity.

b. If you selected more than one application type or assay, repeat substep 4a for each application type for each assay in the run plan.

c. If needed, edit samples in one of the following ways.
   - Click **View & Remove**, make the selections, then click **Update**.
   - Click **Remove All**, to remove all sample assignments for all assays.

d. If you selected the **Upload BAM files to Server** reporting option in the **Setup** step, make the following selections from the **Genexus Workflow** or **Ion Reporter Workflow** dropdown list for each assay that you selected.
   - Select **Upload Only** to upload sample data to the selected server automatically after run completion.
   - Select the desired Genexus™ Software assay or Ion Reporter™ Software analysis workflow to upload sample data *and* perform an analysis in the target software automatically after run completion.

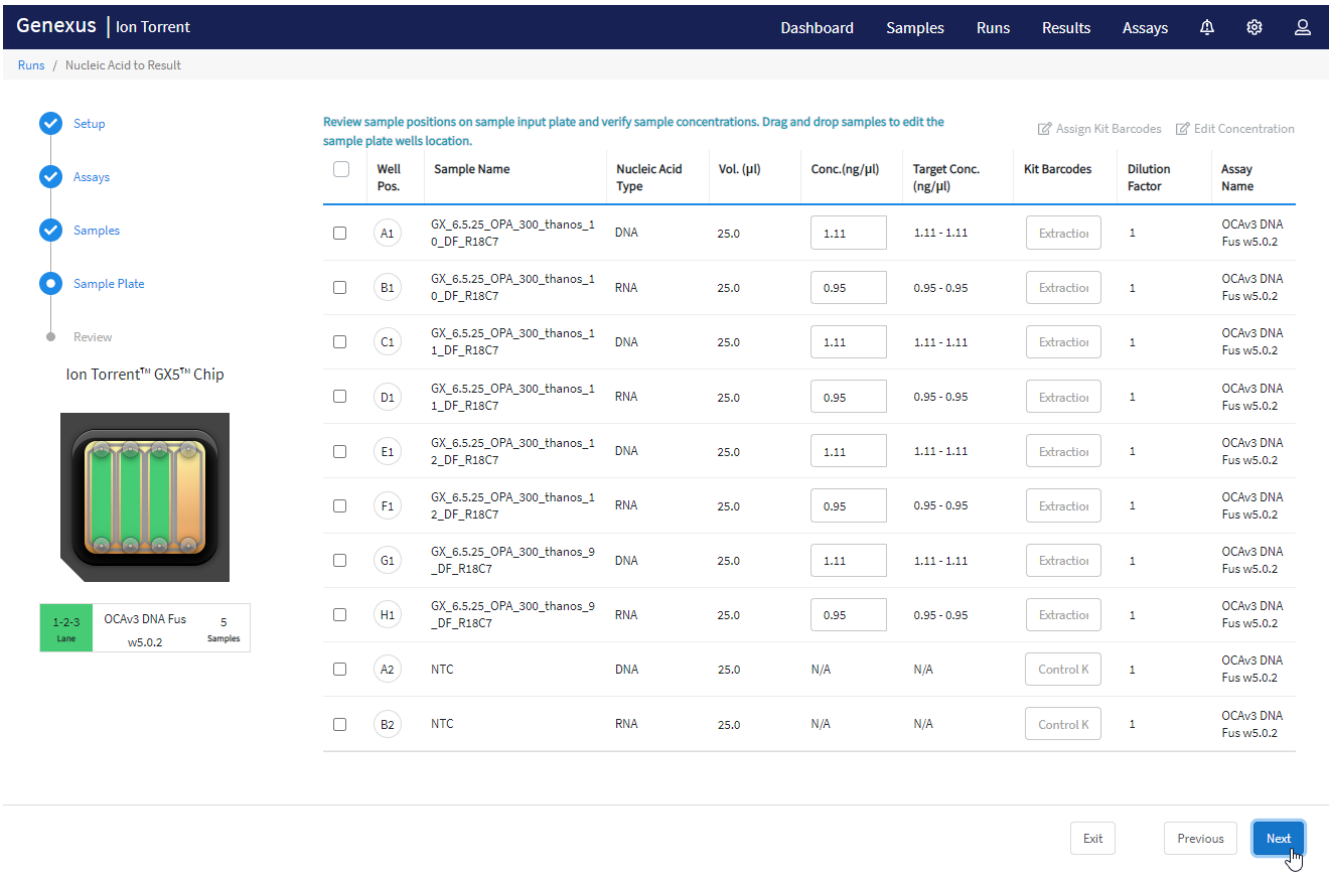
   Note: In order for the Ion Reporter™ Software analysis workflow to appear in the list, you must tag the analysis workflow for use with the IonReporterUploader plugin. For more information, see the *Ion Reporter™ Software 5.18 User Guide* (Pub. No. MAN0024776.

e. If desired, for each application type of each assay in the run plan, select **NTC** to include a no template control.
   The **Chip View** updates to show the lanes used in the run for the included no template controls.

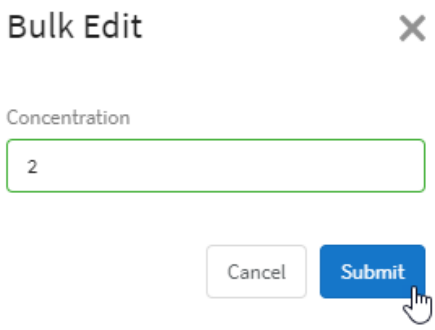
f. Click **Next**.

5. In the **Sample Plate** step, review position assignments in the sample plate. Drag-and-drop samples and no template controls to edit the location of samples and controls, if applicable.
   a. If desired, enter the extraction kit barcode for one or more samples or controls. For a single sample, in the row of the sample of interest, in the **Kit Barcodes** column, enter the extraction kit barcode or control kit barcode, if applicable. For multiple samples or controls, select the samples and controls, then click **Assign Kit Barcodes**. In the **Assign Kit Barcodes** dialog box, enter the extraction kit barcode for the samples, and if applicable, enter the barcode for the no template control.

b. Modify the concentration of samples, if needed. For a single sample, in the row of the sample of interest, in the **Conc. (ng/µl)** column, edit the concentration.



To modify the concentration of multiple samples, select the samples of interest, then click **Edit Concentration**. In the **Bulk Edit** dialog box, enter the concentration for all selected samples, then click **Submit**. The concentration for each selected sample is updated to the new value.



If a sample concentration is ≤1,024X of the target concentration for the assay, which is displayed as a default value for each sample in the **Sample Plate** screen, the sequencer automatically dilutes the sample to the target concentration during the run. If a sample concentration is greater than this value, you must manually dilute the sample to the target

concentration, or to a value within range for automated dilution before loading on the sample plate.

Note: The sample volume that is required for library preparation is not adjustable. The volume depends on the number of primer pools in the assay, sample type, and library chemistry. For more information, see "Dilute or concentrate the samples, if needed, then load the sample plate—Nucleic Acid to Result run" on page 92 for specific sample volumes to load on the sample plate.

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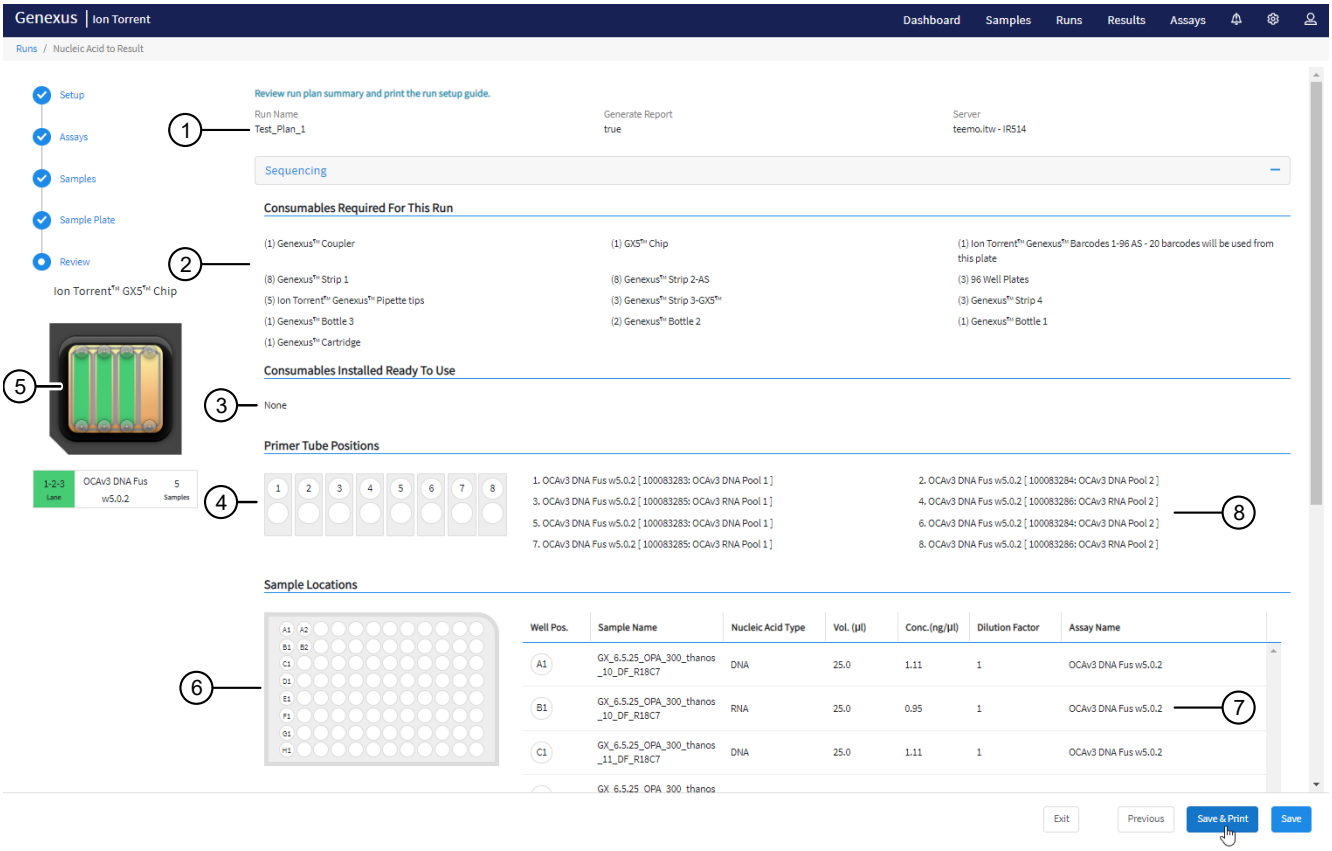
c. Ensure that sample plate information is correct, then click **Next**.

6. In the **Review** step, review the run plan summary, then click **Save & Print** to print the run setup guide, if desired. Click **Save** to save the run without printing.

Note: To print the run setup guide, you must allow pop-ups in the browser.

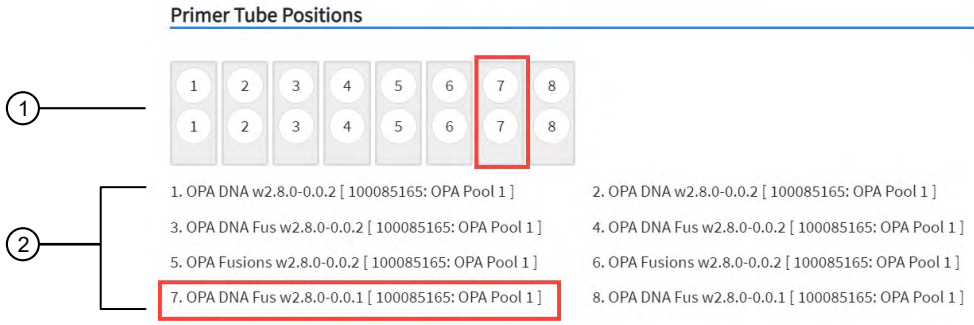
The run plan summary lists the following details.

- the consumables that are required for this run
- how much sample volume to load
- where to load samples and primer pool tubes
- details about the assay.



① Run information
② List of consumables required for the run
③ List of consumables that are installed on the sequencer and available for the run
④ Positions to load primer pool tubes
⑤ Chip view showing the lanes to be used in the run
⑥ Positions in the sample plate to load the samples
⑦ Table listing the sample plate position, sample type, volume to load, concentration, dilution factor, and assay for each sample
⑧ Description of each primer pool and its position

Note: If you are using an assay with Ion AmpliSeq™ HD library chemistry, the primer pool positions show that HD primer pools occupy both rows:



① The position of the primer tubes.
② The legend for the image.
In the example outlined in red, primer tube 7, which contains OPA Pool 1 for the OPA DNA Fusion assay, is in the seventh position from the left.

After saving, the run appears in the **Manage Runs** screen in the run list with the name you specified.

After you select the run and load the sequencer, the run is started on the sequencer screen.

Plan a Library to Result run

You can plan runs for sequencing that start with libraries. In Genexus™ Software 6.6 and later, this is called a **Library to Result** run. Before you can plan a **Library to Result** run, you must first enter sample information and prepare a library batch that associates an assay with the library batch. The library batch must be created with the assay to be used in the run. The assay specifies the barcode set that was used to prepare the sample libraries.

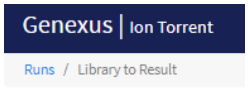
For more information, see “Prepare or import a library batch” on page 68.

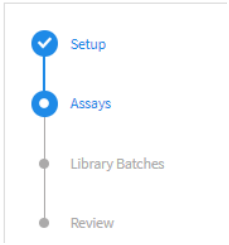
If the sample libraries were prepared using a barcode set that is not specified in an assay you want to use in the run, you must do the following:

- Create a new assay, or copy an existing assay, and specify the new barcode set in assay setup.
- Prepare a library batch that selects the new assay.

For more information, see “Create a new assay (manager/administrator)” on page 45, “Copy an assay (manager/administrator)” on page 58, and “Prepare a library batch” on page 69.

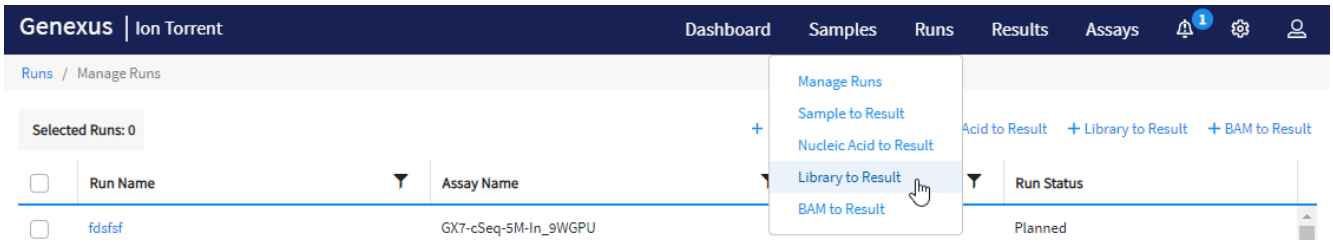
Genexus™ Software guides you through the four steps of planning a **Library to Result** run: **Setup**, **Assays**, **Library Batches**, and **Review**. You can view progress through the steps in the upper left corner of the **Runs / Library to Result** screen.



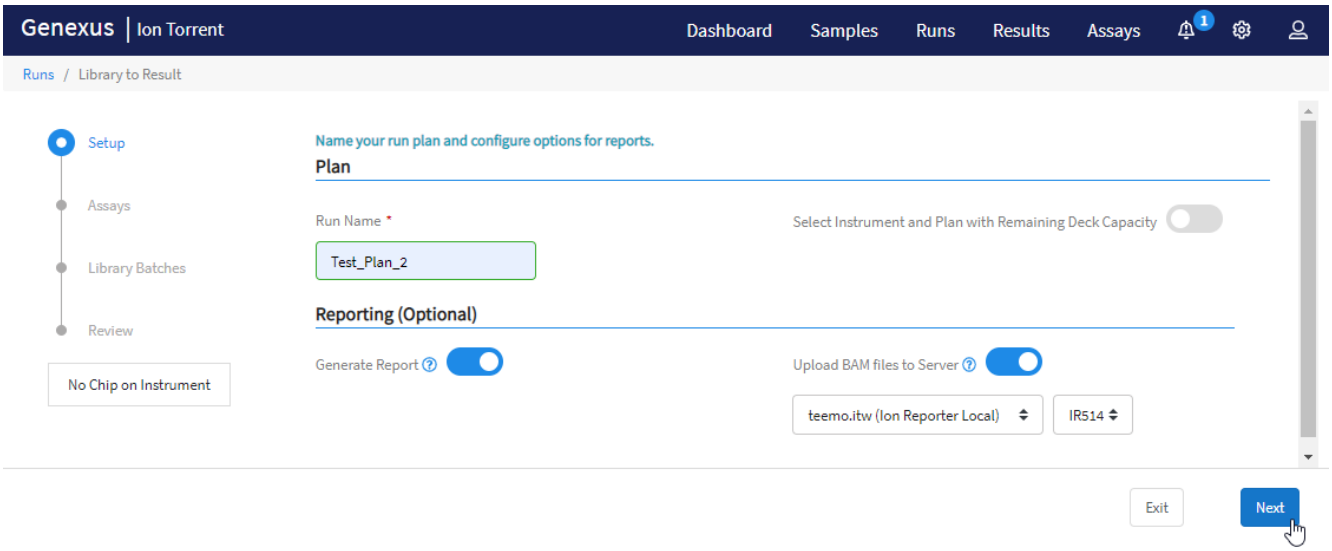


1. In the menu bar, click **Runs** ▸ **Library to Result**.

Note: You can also click + **Library to Result** in the **Runs / Manage Runs** screen.



2. In the **Setup** step, enter a name for the run, then configure the reporting options.
   a. In **Run Name**, enter a unique name.
      The name is limited to 50 characters and no spaces are allowed.



   b. *(Optional)* In the **Reporting (Optional)** section, ensure that **Generate Report** is enabled to generate a variant report using the default report template.

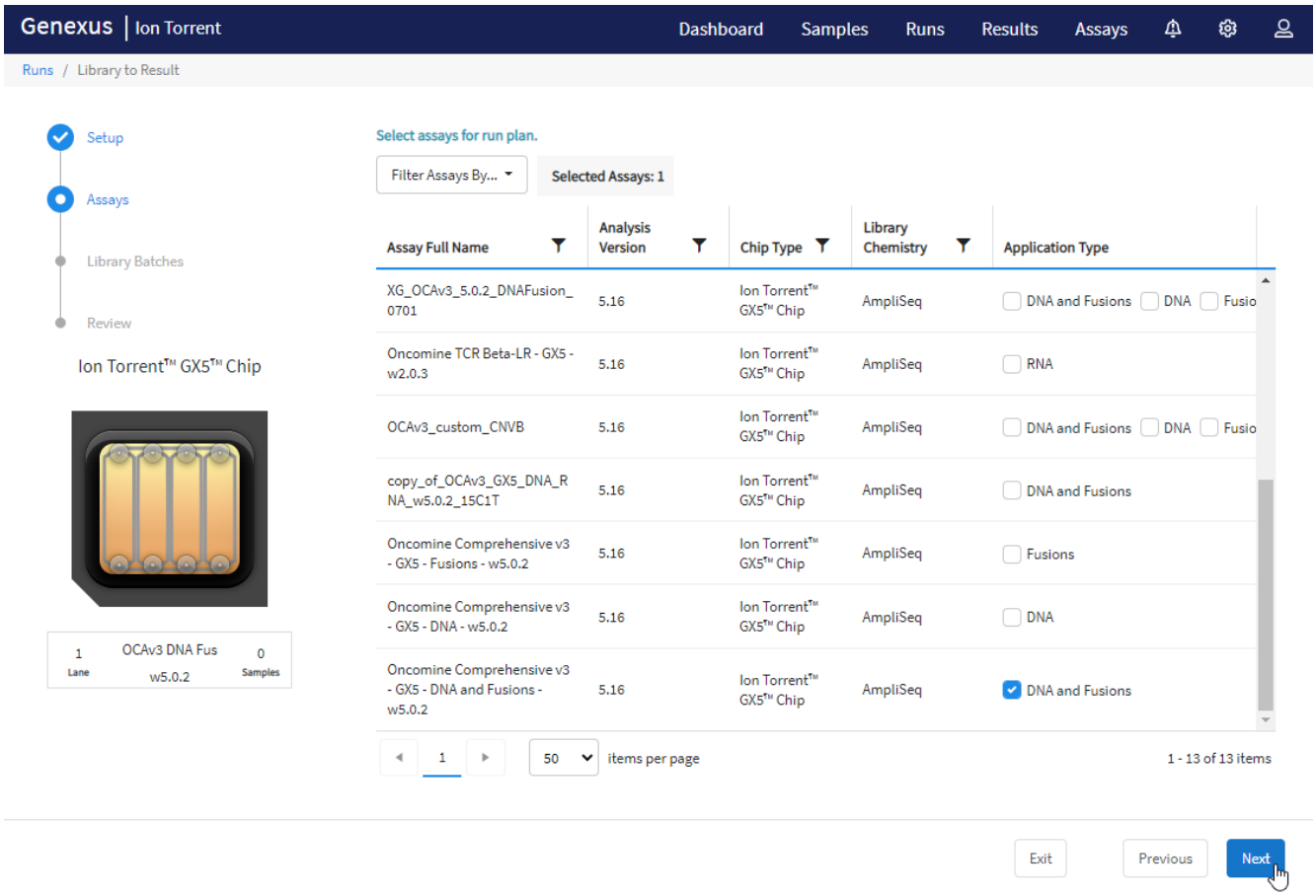
   c. *(Optional)* In the **Reporting (Optional)** section, enable **Upload BAM files to Server** to upload BAM files to another server.

Option	Description
Upload BAM files to another Genexus™ Software server.	In the dropdown lists that appear, select the target Genexus™ Software server and version. To configure a Genexus™ Software account that you can use for data uploads, click **Set up Account**. For more information, see "Register Genexus™ Software accounts (manager/administrator)" on page 232.
Automatically upload data for further analysis with Ion Reporter™ Software.	In the dropdown lists that appear, select the Ion Reporter™ Software account and Ion Reporter™ Software version. To configure an Ion Reporter™ Server account that you can use for data uploads, click **Set up Account**.

   Alternatively, you can upload BAM files to a server after a run. For more information, see "Upload results files to another Genexus™ Integrated Sequencer" on page 177.

   d. Click **Next**.
      If a chip is installed in the sequencer, the **Chip View** graphic in the lower left corner indicates the lanes that are available for sequencing.

3. In the **Assays** step, select one or more assays that you want to include in the run.



For the assay to be selectable at this step, you must have prepared a library batch that assigns the assay to the batch. The assay specifies the barcode set that was used to prepare the sample libraries.

a. Use the **(Filter)** tools in table column headers to find assays of interest, if desired.

b. In the **Application Type** column for the assay of interest, select one or more application types, such as **DNA and Fusions**, to include each selected application type for the assay in the run plan.

   After selecting an assay and the research application for the assay, the list is filtered to show compatible assays that can be selected and run at the same time.

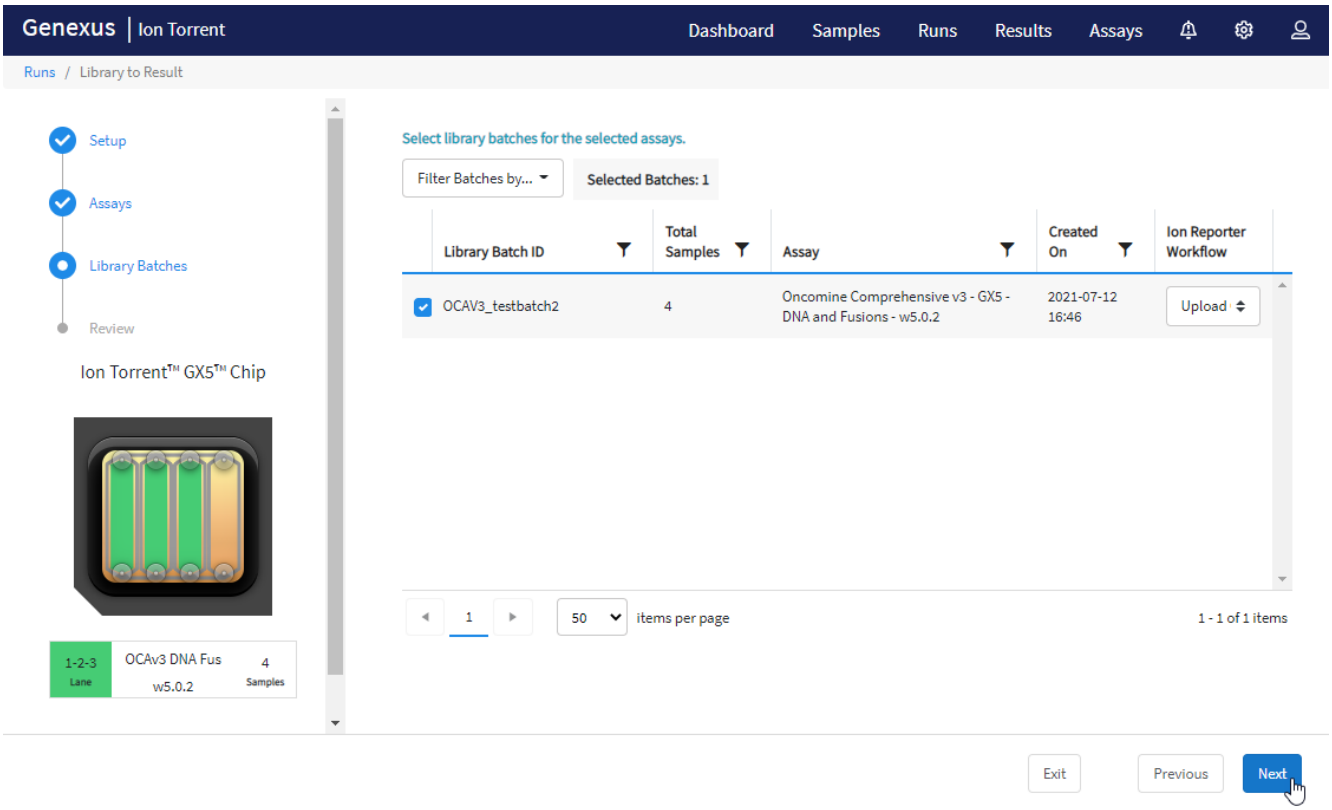
   Note: To create a new assay, see Chapter 4, "Create and manage assays (manager/administrator)".

c. If more assays are included in the run, repeat substep 3b for each extra assay.

d. Click **Next**.

4. In the **Library Batches** step, select the library batch that you want to use in the run, then click **Next**.

   Note: Only one library batch can be selected per assay. However, you can plan a library run that uses multiple assays if you select multiple compatible assays in the **Assays** step.



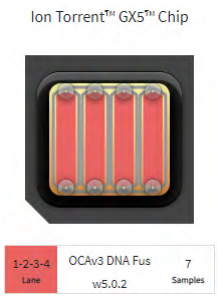
   a. If you selected the **Upload BAM files to Server** reporting option in the **Setup** step, make the following selections from the **Genexus Workflow** or **Ion Reporter Workflow** dropdown list.

      - Select **Upload Only** to upload sample data to the selected server automatically after run completion.
      - Select the desired Genexus™ Software assay or Ion Reporter™ Software analysis workflow to upload sample data *and* perform an analysis in the target software automatically after run completion.

        Note: In order for the Ion Reporter™ Software analysis workflow to appear in the list, you must tag the analysis workflow for use with the IonReporterUploader plugin. For more information, see the *Ion Reporter™ Software 5.18 User Guide* (Pub. No. MAN0024776.

The **Chip View** updates to show the lanes to be used in the run as green. Lane usage is calculated based on the number of samples and minimum reads per sample entered at assay setup.

Green lane color denotes lane usage and sample assignment within lane capacity.



Red lane color denotes sample assignment that exceeds lane capacity.

If the

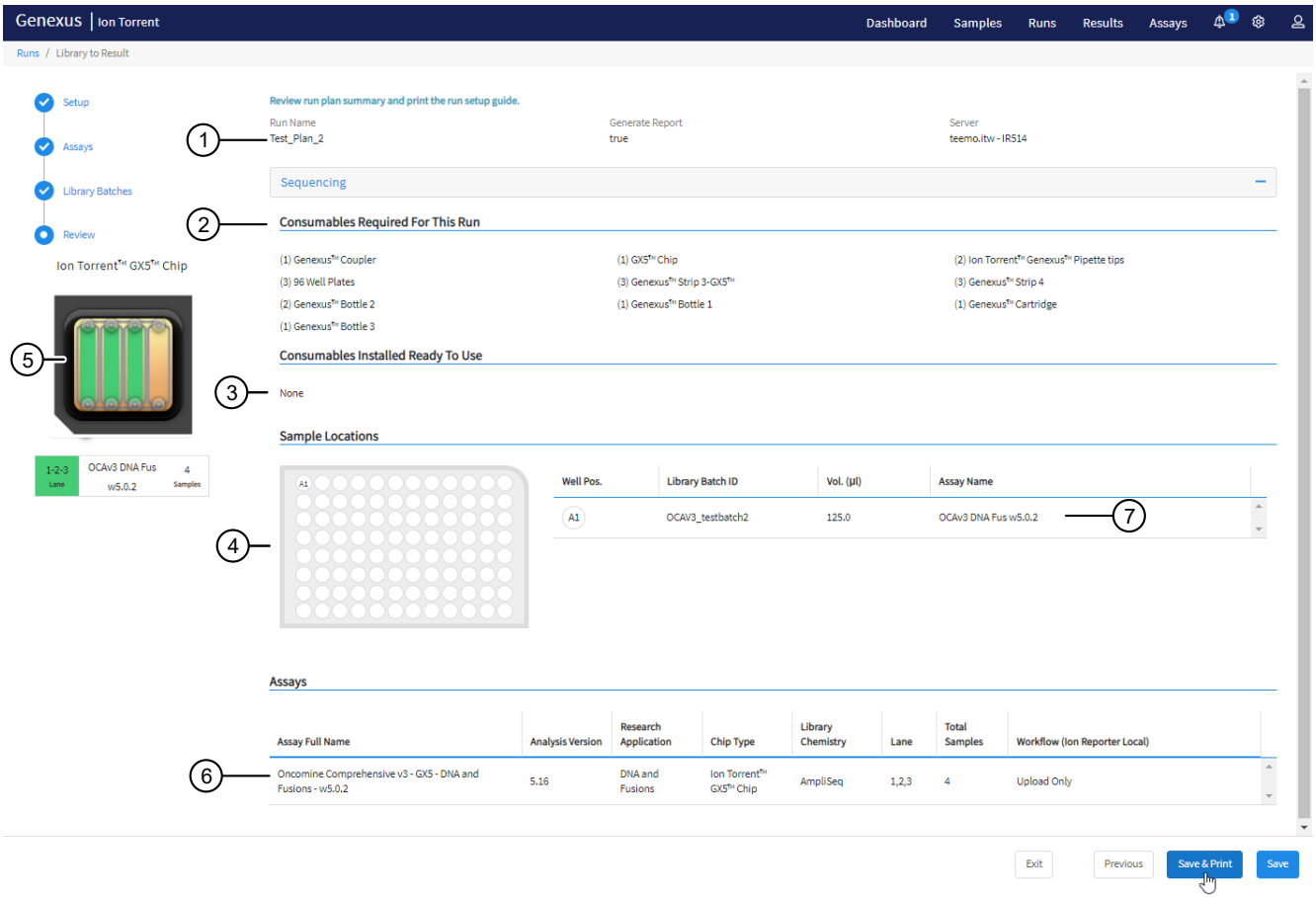
minimum reads per sample × *number of samples*

exceeds the chip or lane well capacity, a dialog box appears after you click **Next** asking you to confirm that you want to continue. After clicking **Yes**, the **Chip View** updates and shows the lane color as red instead of green. In the example shown at right, seven samples were included in a library batch instead of four. The run is allowed if the lane capacity is exceeded, but you may not achieve the required reads per sample to pass QC metrics.

5. In the **Review** step, review the run plan summary.
   - Click **Save and Print** to print the run setup guide.
   - Click **Save** to save the run without printing.

Note: To print the run setup guide, you must allow pop-ups in the browser.

The run plan summary lists the consumables that are required for the run, where to load the library batch on the sample plate, and how much library volume to load.



1. Run information
2. List of consumables required for the run
3. List of consumables installed on the sequencer and available for the run
4. Position(s) in the sample plate to load the library batch
5. Chip view showing the lanes to be used in the run
6. Table listing assay and run information
7. Table listing the well position, library batch ID, volume to load, and assay for each library batch

The run appears in the run list on the **Manage Runs** screen with the name you specified.

After you select the run and load the sequencer, the run is started on the sequencer screen.

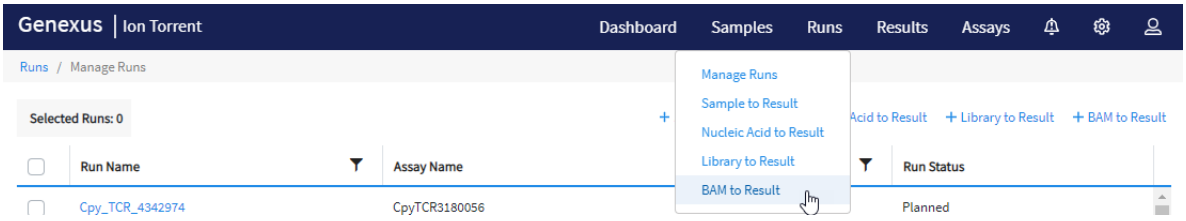
Plan a BAM to Result run

Before you can plan a **BAM to Result** run, you must enter BAM samples into Genexus™ Software. For more information, see “Upload a BAM file to create a sample or samples” on page 65.

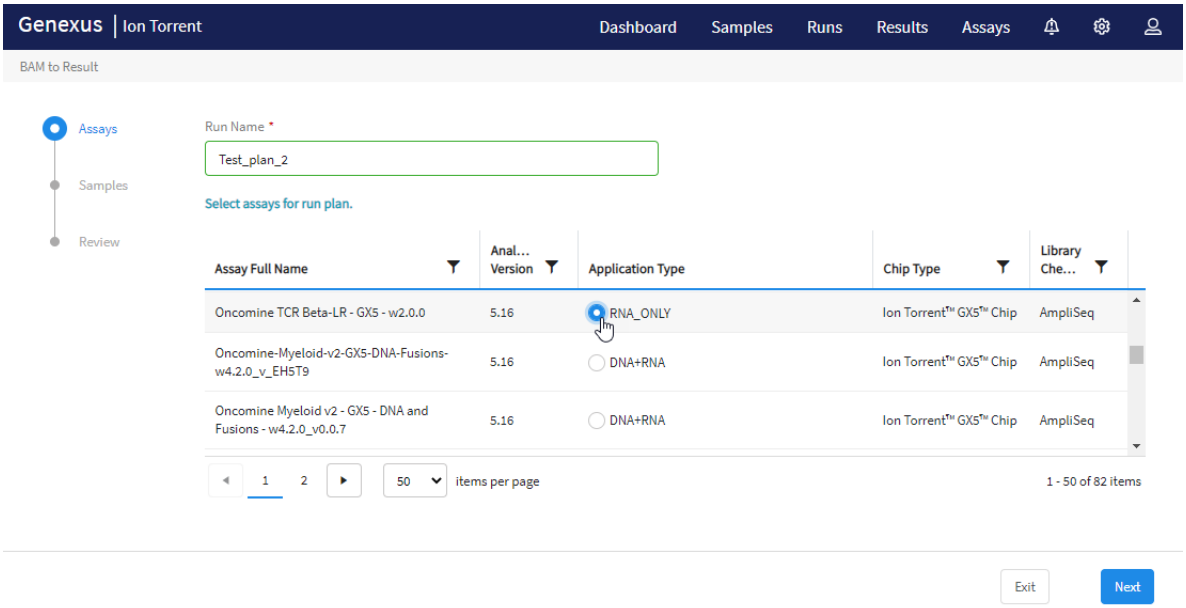
Planning a run to analyze BAM samples is organized into steps: **Assays**, **Samples**, and **Review**. You can view progress through the steps in the upper left corner of the **Runs / BAM to Result** screen.

1. In the menu bar, click **Runs** ▸ **BAM to Result**.

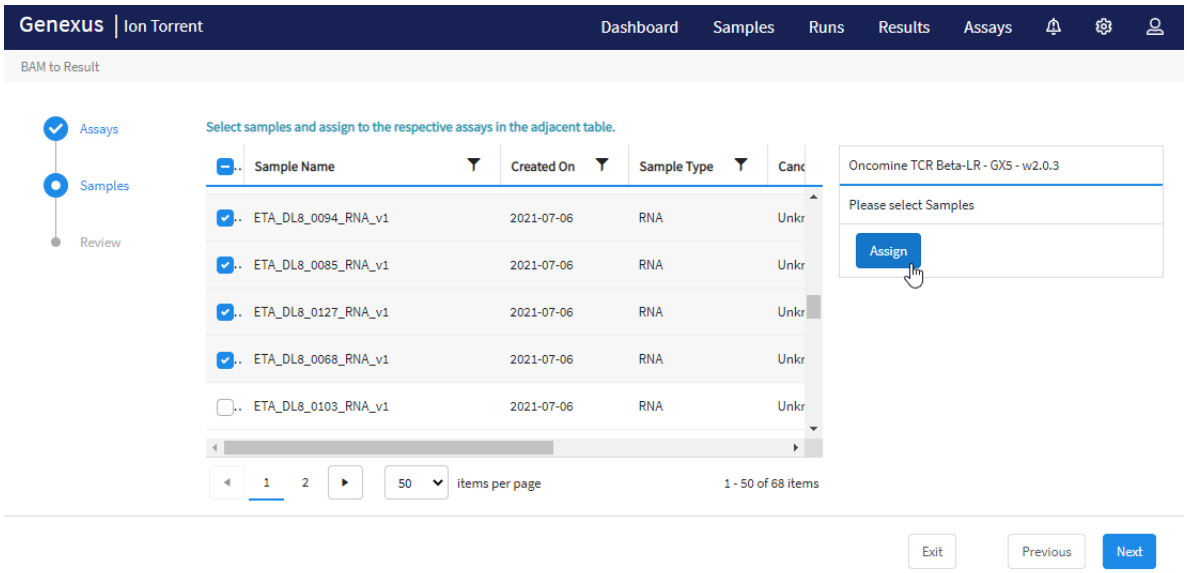
   Note: You can also click + **BAM to Result** in the **Runs / Manage Runs** screen.



2. In the **Assays** step, enter or make the following selections.
   a. Enter a unique name.
      The name is limited to 50 characters and no spaces are allowed.
   b. Use the ▼ **(Filter)** tools in table column headers to find assays of interest, if desired.
   c. In the **Application Type** column for the assay of interest, select the application type, such as **DNA and Fusions** or **DNA**, to include the selected application type for the assay in the run plan.
      You can select only one application type of an assay per run.
   d. Click **Next**.



3. In the **Samples** step, select the BAM samples from the list that you want to analyze, then click **Assign**.

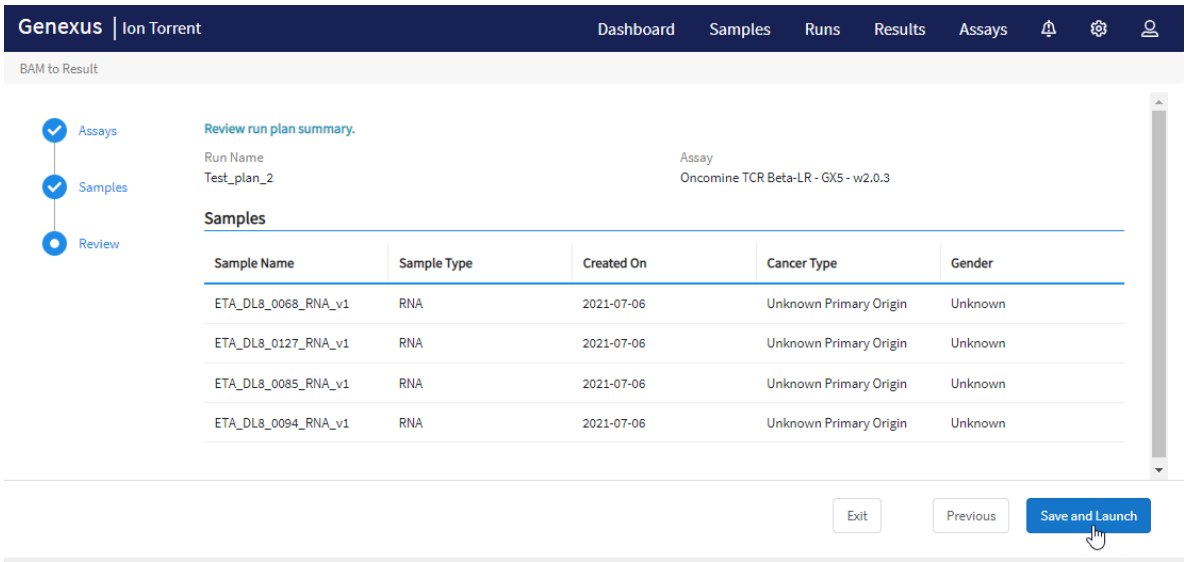


Note: Only BAM samples that are compatible with the selected assay are listed. Compatibility is determined by the sample type, such as DNA or DNA and Fusion.

   a. If needed, click ⊗ in an assigned sample tile to remove the sample from the plan.

   b. Click **Next**.

4. In the **Review** step, review the samples in the run plan summary, then click **Save and Launch**.



The run is listed in the **Results / Run Results** screen. The run is launched or the run is queued if other runs are in progress.

7 Load the sample plate

Before starting a Nucleic Acid to Result or Library to Result run on the instrument, you must quantify and dilute the samples or sample libraries, if needed, then load the sample plate.

For Sample to Result runs with the Genexus™ Purification Instrument in the integrated configuration, samples are quantified and loaded in the sample plate by the purification instrument. If you are using the Genexus™ Purification Instrument in the standalone configuration, you must manually transfer quantified samples from the 48-Well Nucleic Acid Archive Plate to the sequencer sample plate, following the run setup guide in the Nucleic Acid to Result run plan.

Dilute or concentrate the samples, if needed, then load the sample plate—Nucleic Acid to Result run

Isolate DNA and RNA samples using one of the procedures and kits that are recommended in “Recommended materials for nucleic acid isolation and quantification” on page 31.

Nucleic acid samples with concentrations up to 1,024X of the target concentration for an assay (displayed as default values in the **Sample Plate** step screen in run planning) are in range for automated dilution and require no manual dilution. Enter the concentrations during run planning at the **Sample Plate** step (see step 5 on page 80).

1. For samples with concentrations that are out of range for automated dilution, manually dilute the sample with nuclease-free water, or concentrate the sample to a concentration ≤1,024X of the target concentration. For samples that are in range, go to step 2.

If the sample concentration is…	Then…
<0.04 ng/µL	Concentrate the sample to greater than or equal to the target concentration.
≥0.04 ng/µL, but less than the target concentration	Run is allowed but sample concentration may not be optimal for library preparation. Concentrate the sample to greater than or equal to the target concentration.
≤1,024X of the target concentration	No manual dilution is necessary. The sequencer dilutes the sample to the target concentration automatically during the run.
>1,024X of the target concentration	Manually dilute to the target concentration based on assay type, or to a concentration in range for automated dilution by the sequencer.

Note:

- If you enter a concentration <0.04 ng/µL or >10,000 ng/µL, a warning that the concentration is out of range appears, and you are not allowed to proceed to the next step.
- If the concentration is ≤10,000 ng/µL, but >1,024X of the target concentration, you can proceed, but because the instrument cannot dilute samples more than 1,024-fold, the diluted sample concentration will be greater than the target concentration.

2. Add samples and controls, if used, to the sample plate at the volume and positions that are specified in the run setup guide.

   The sample volume is not adjustable and depends on sample type, the number of primer pools in the assay, and library chemistry. The following table also provides loading volume.

Sample type	Number of primer pools	Volume
Ion AmpliSeq™ chemistry		
DNA	1	15 µL
DNA	2	25 µL
RNA	1	15 µL
RNA	2	25 µL
Ion AmpliSeq™ HD chemistry		
DNA	1	20 µL
RNA	1	20 µL
TNA	1	20 µL

3. Seal the plate with a sheet of Adhesive PCR Plate Foils (Thermo Fisher Scientific Cat. No. AB0626).

   Note: The use of other plate seals may affect performance.

4. Keep the plate on ice until you are ready to load it in the sequencer.

Dilute and pool libraries, then load the sample plate—Library to Result run

1. Dilute each manually prepared and quantified sample library to 200 pM with nuclease-free water.

   Note: Each library must be barcoded with a unique barcode or barcode pair. Use this concentration as a starting point, then titrate up or down based on sequencing results, if needed.

2. Add equal volumes of each library to a new 1.5-mL low DNA retention tube so that the total volume is greater than the volume specified in the run setup guide provided by the software.

   Note: For information on combining DNA and RNA libraries recovered from Sample to Result or Nucleic acid to Result runs using assays that include DNA and fusions, see “Combine libraries” on page 225.

3. Mix well by pipetting up and down five times, then transfer the specified volume of each library batch to the sample plate position specified in the run setup guide.

4. Seal the plate with a sheet of Adhesive PCR Plate Foils (Thermo Fisher Scientific Cat. No. AB0626).

   Note: The use of other plate seals may affect performance.

5. Keep the plate on ice until you are ready to load it in the sequencer.

Guidelines for library quantification—Library to Result runs

- We recommend that you use libraries that are freshly quantified and diluted before pooling in a library batch.
- Pre-prepared libraries can be quantified by one of the following three methods:
  - Quantification using the Agilent™ 2100 Bioanalyzer™ instrument
  - Quantification using the Qubit™ Fluorometer
  - Quantification by qPCR using the Ion Library TaqMan™ Quantitation Kit

  See one of the following guides for specific procedures.
  - *Ion AmpliSeq™ Library Kit 2.0 User Guide* (Pub. No. MAN0006735)
  - *Ion AmpliSeq™ Library Kit Plus User Guide* (Pub. No. MAN0017003)
  - *Ion AmpliSeq™ HD Library Kit User Guide* (Pub. No. MAN0017392)

8 Load the sequencer and start a run

After you have planned a run in Genexus™ Software, use the run setup guide provided by the software to load samples in the sample plate, and to determine which consumables to load in the sequencer. Follow the step-by-step instructions in the sequencer touchscreen during run setup. The vision system of the sequencer tracks the addition of consumables in real-time and alerts you if a component is loaded in an incorrect position, or if an incorrect quantity is loaded.

Before you begin

Before setting up a sequencing run, review general procedural guidelines for handling panels, reagents, and samples to minimize the chance of contamination and ensure the success of the run. See “Guidelines for panel and reagent use and handling” on page 33 and “Guidelines for preventing contamination” on page 34.

1. Remove the library and templating strips from their boxes in the refrigerator or freezer, and prepare them for loading in the sequencer.
   - Genexus™ Strip 1 and Genexus™ Strip 3-GX5™ (or Genexus™ Strip 3B-GX5™): equilibrate to room temperature for 30 minutes.
   - Genexus™ Strip 2-AS or Genexus™ Strip 2-HD, depending on your assay and Genexus™ Strip 4: thaw on ice for 30 minutes. If you are delayed in loading, keep the thawed strips on ice or at 4°C until you load them in the sequencer.

     IMPORTANT! Confirm that the strip contents are completely thawed before installing in the sequencer.

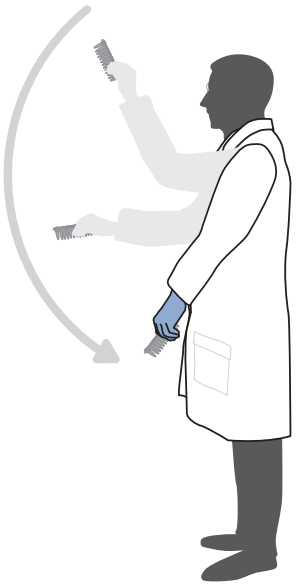
2. Visually check tube 3 of the Genexus™ Strip 2-HD for precipitation. If needed, flick the tube or gently vortex the strip to dissolve the precipitate.

   Note: You can also vortex the strip on a platform vortexer to dissolve precipitate if you have a Genexus™ Strip Centrifuge Adapter to hold the strip during the centrifugation. Centrifugation is needed after you vortex a strip. For more information on obtaining and using the adapter, see “Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter” on page 227.

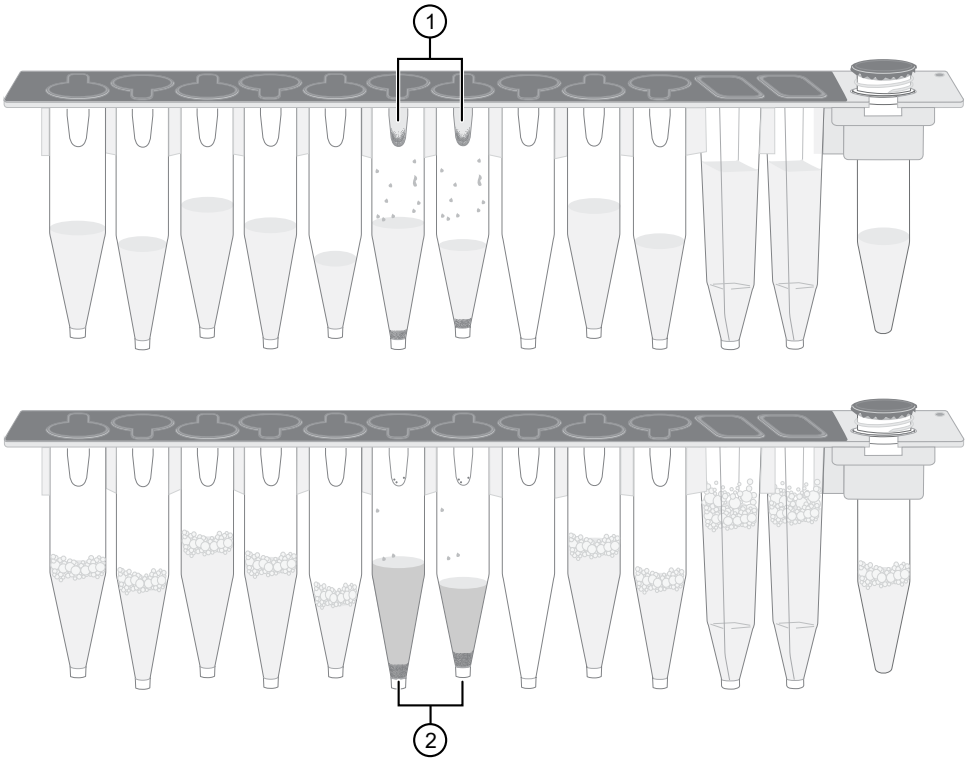
3. Remove primer pool tubes in tube carriers that are needed for the run from the freezer, then thaw at room temperature for 30 minutes. After thawing, gently tap the primer pool tube or tubes on a bench surface to ensure that contents are collected at the bottom of the tubes. Keep the tubes and carriers on ice or at 4°C until you load them in the sequencer.

4. If you are installing a new Genexus™ Cartridge, thaw the cartridge at room temperature for 30 minutes before installing in the sequencer.

5. Genexus™ Strip 1 and Genexus™ Strip 3-GX5™ contain magnetic beads in one or two positions, yellow or brown in color, that sometimes get trapped in the upper "keyhole" of the tube. Dislodge these beads from the keyhole before installing the strip in the sequencer. Use the following procedure for each strip.
   a. Invert the strip 3–4 times to dislodge beads that are trapped in the keyholes.
   b. To remove any remaining beads and liquid from the keyholes, grasp the strip at one end with the strip seal facing up, then swing the strip with a rapid, downward centrifugal arm motion, ending with a sharp wrist-flick.
   c. Grasp the strip at the other end, then repeat the centrifugal motion.



d. Check tube positions for significant amounts of beads that are still trapped in keyholes (see the following figure), then repeat the centrifugal motion, if needed. It is acceptable if a few beads remain in the keyhole or on the tube wall, but most should be either in suspension or in a pellet at the bottom of the tube.



Example Genexus™ Strip 3-GX5™ before (upper) and after (lower) inversion. The carrier has been removed to show tube contents more easily.

① Magnetic beads trapped in keyholes ② Magnetic beads dislodged from keyholes

Note:

- It is not necessary to resuspend the magnetic beads completely—it is only necessary to dislodge most of the beads that can be trapped in the keyhole. The instrument resuspends the beads during the run when needed.
- Fine bubbles can form above the liquid in some tubes after inversion. These bubbles do not affect the run.

6. Inspect all strips for large bubbles lodged under the surface of the liquid or at the bottom of each tube or well. Gently tap the strips on a benchtop to dislodge any bubbles without splashing the contents onto the upper tube walls. If tapping fails to dislodge a bubble, use the technique that is described in substep 5b until large bubbles are dislodged.

Note: If you have a Genexus™ Strip Centrifuge Adapter, you can vortex the strips on a platform vortexer, then centrifuge the strips using the adapter to dislodge air bubbles and magnetic beads. For more information, see “Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter” on page 227.

Review samples for Sample to Result runs

A **Sample to Result** run integrates nucleic acid purification and sequencing. When you create a **Sample to Result** run plan, you can include an option that allows samples to be reviewed before library preparation starts. Samples that do not meet a specified concentration threshold after purification have a run status of **Purification Review Required**.

For more information about **Sample to Result** runs, see “About Sample to Result runs” on page 75.

You can also apply this option when you create an assay. For more information, see "Custom assays for Sample to Result runs" in the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

1. In the menu bar, click **Results** ▸ **Run Results**.

   Note: Sample to Result runs that require review after purification have a **Run Status** of **Purification Review Required**.

2. In the **Run Results** screen, place the pointer over the row of the run plan of interest, then click **Review**.
   The **Purification Samples** screen opens.

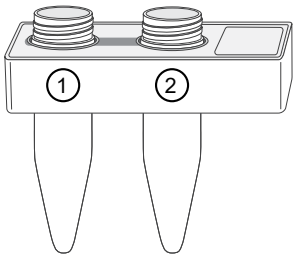
3. Review the concentration for each sample, then select the checkbox in the row for each sample you want to sequence.

4. Click **Submit**.

The samples are ready for sequencing. For more information, see the *Genexus™ Purification System User Guide* (Pub. No. MAN0018475).

Fill Genexus™ Primer Pool Tubes *(custom assays only)*

If you are using a custom assay, Genexus™ Primer Pool Tubes must be manually filled with the custom Ion AmpliSeq™ or Ion AmpliSeq™ HD panels at the appropriate volume and in the correct primer pool tube positions. For Ion AmpliSeq™ library panels, use one carrier per DNA or RNA assay primer pool. The two positions in the primer pool tube carrier are designated as shown in the following figure:



① Position 1 tube: Contains Ion AmpliSeq™ DNA, Ion AmpliSeq™ RNA, or Ion AmpliSeq™ HD FWD primer pool
② Position 2 tube: Contains Ion AmpliSeq™ HD REV primer pool

Note: When you order assays from Ion AmpliSeq™ Designer (**AmpliSeq.com**), be sure to order a sufficient amount of panel for your needs, and request the tube format, not the plate format. Library preparation on the Genexus™ Integrated Sequencer requires greater panel volume per sample than manual library preparation, or library preparation on the Ion Chef™ System.

1. Add primer pool at the indicated volume, appropriate to your assay type, to the Genexus™ Primer Pool Tubes using the following tables as a guide. Fill the number of tubes specified by the run plan summary.

Ion AmpliSeq™ DNA assays

Number of primer pairs per pool	Concentration	Volume in position 1	Volume in position 2
12–96	2X (400 nM)	140 µL	—
97–3,072	2X (100 nM)	140 µL	—
>3,072	2X ([3,072 / Number of primer pairs per pool] × 100 nM)[1]	140 µL	—

[1] For example, if a panel pool has 3,500 primer pairs, the 2X concentration is (3,072 / 3,500) × 100 nM = 87.8 nM.

Ion AmpliSeq™ RNA assays

Number of primer pairs per pool	Concentration	Volume in position 1	Volume in position 2
12-1,228	5X (250 nM)	75 µL	—
>1,228	5X ([1,228 / Number of primer pairs per pool] × 250 nM)[1]	75 µL	—

[1] For example, if a panel pool has 1,500 primer pairs, the 5X concentration is (1,228 / 1,500) × 250 nM = 205 nM.

Ion AmpliSeq™ HD assays

Primer pool type	Concentration	Volume in position 1	Volume in position 2
Ion AmpliSeq™ HD FWD	10X	50 µL	—
Ion AmpliSeq™ HD REV	10X	—	50 µL

IMPORTANT!

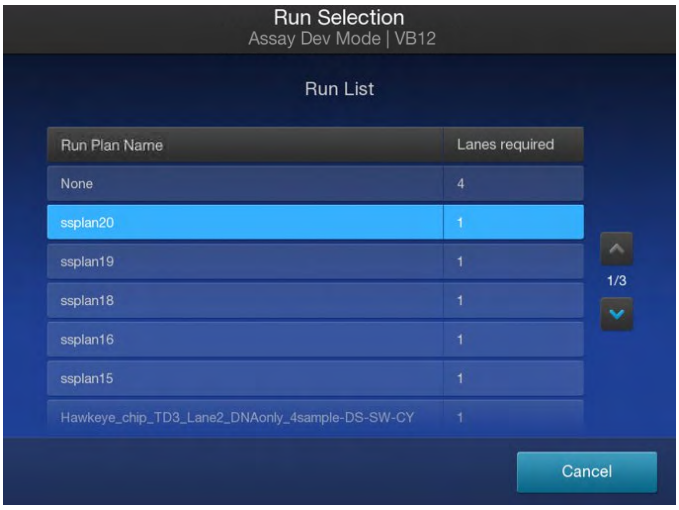
- If you are using Ion AmpliSeq™ library chemistry, leave the tube that is in position 2 empty and uncapped, but do not remove the tube from the carrier before loading in the sequencer. Do not add a second Ion AmpliSeq™ primer pool to the position 2 tube.
- If you are using Ion AmpliSeq™ HD library chemistry, add the FWD and REV primer pools to the appropriate tubes in the same carrier.
- Ensure that no bubbles are introduced at the bottom of the tube when adding the primer pool.

2. If you do not install the primer pool tube carriers in the sequencer immediately, cap the tubes that contain primer pools, then store the tube carriers on ice. Remember to uncap all tubes before installing.

Load the sequencer and start a run

1. Tap **Run** on the sequencer home screen to start the loading procedure.

2. In the **Run Selection** screen, select the run that you want to use from the list.



Note: If you select a run that requires more lanes than are available on a currently installed chip, a dialog appears giving you the option to install a new chip, or cancel. If you proceed with a new chip, a post-chip clean is performed, then the sequencer prompts you to perform the following steps:

- **Clear Deck**
- **UV Clean**
- **Load Deck**
- **Clear Sequencing Reagents**
- **Load Sequencing Reagents**

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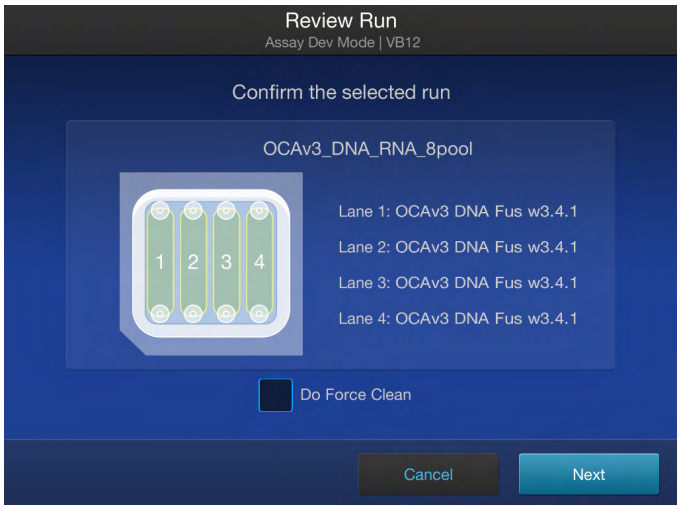
3. In the **Review Run** screen, confirm the run and assay selections, then tap **Next**.



The deck door opens automatically.

Note:

- If the instrument vision system detects consumables loaded on the deck, the sequencer prompts you to remove the consumables, then starts a UV Clean.
- Select the **Do Force Clean** checkbox if there will be an unused lane or lanes on the installed chip after the run, but you want to start your next run on a new chip after the current run. A force clean automatically cleans the instrument after the run, eliminating the need for an operator to execute the cleaning procedure between the completion of the current run and the next run. Selecting **Do Force Clean** renders all lanes of the installed chip unusable after the run.

---

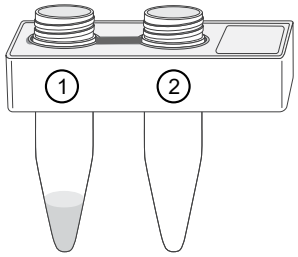
4. In the **Load Deck** screen, the sequencer instructs you step by step to load each required consumable in a highlighted position on the deck. The sequencer detects the loading of each consumable in real time and advances to the next component automatically.

IMPORTANT!

- Ensure that you remove the primer pool tube cap or caps before installing the tube carrier on the deck.
- Ensure that you load the correct type of barcode plate and library strip 2 for the type of run you are setting up. The sequencer displays a warning if you have installed consumables that are incompatible with the run you have selected, for example, a Genexus™ Barcodes AS plate or Genexus™ Strip 2-AS in an HD run.
- Ensure that you remove the lids from all of the boxes of Genexus™ Pipette Tips before you load the boxes in the sequencer.
- Load instrument plates, including PCR plate, barcode plate, enrichment plate, and sample plate, into position by pressing down firmly and evenly on all sides and corners of each plate.

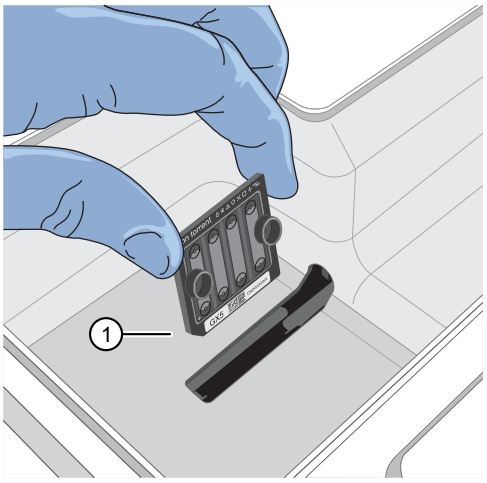
Note:

- A primer pool tube carrier can only be installed with the position 1 tube in the back row of the Primer Pool Tube Station. Follow the guidance in the run setup guide for loading the primer pool tube carrier or carriers in the correct position and order in the station.
- If the sequencer cannot read the correct loading of an unexpired consumable, tap **Help** in the lower left corner of the screen to override the block. After using this override, the name of the consumable will not appear in the run summary consumables list.



① Position 1
② Position 2

5. If prompted, insert a new and Genexus™ Coupler. Insert the chip into the chip install slot with the chip notch oriented down and toward the front of the instrument.



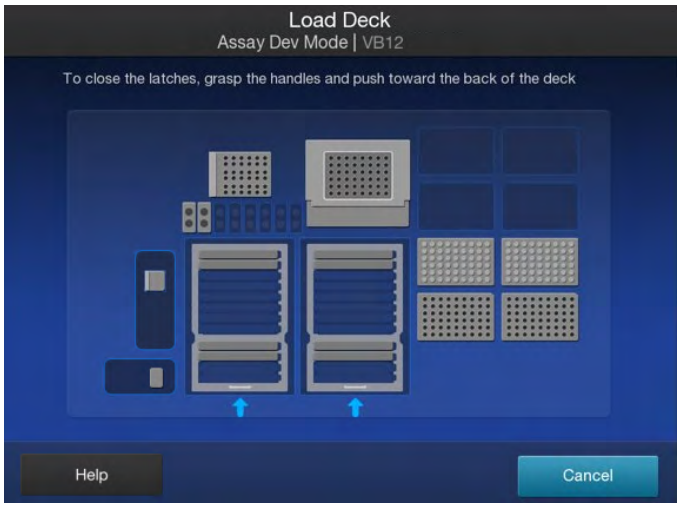
① Notched corner of chip

Note: A chip shuttle under the deck moves the installed chip to loading and sequencing positions during the run.

IMPORTANT! Insert the Genexus™ Coupler so that it is level to ensure it will properly align with the . A coupler that is installed at an angle or is not level will not align properly to the chip and can result in a failed run.

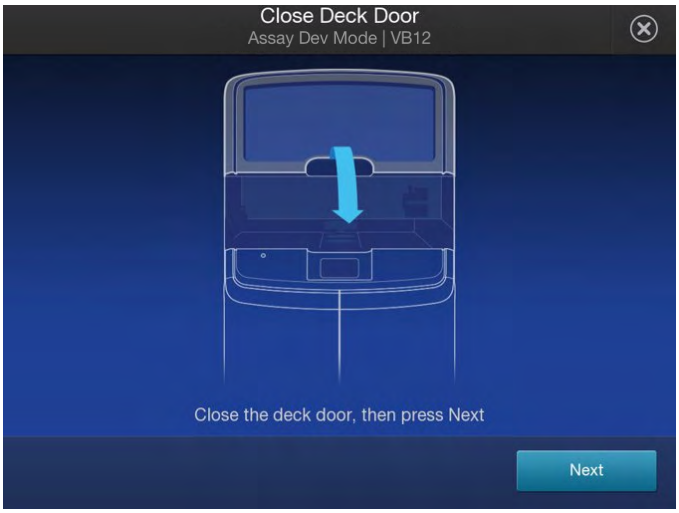
6. When the deck consumables have been loaded, lock the library and templating strips in place by sliding the latches toward the rear of the deck.

Note: We recommend that you load the sample plate last.



If a chip is detected and the strip latches are closed, the **Close Deck Door** screen appears.

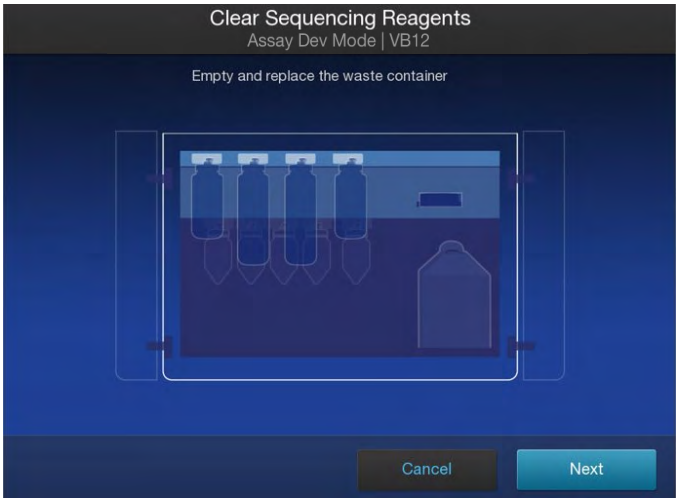
7. Close the deck door, then tap **Next**.



- If you installed a new chip in the sequencer, the sequencer prompts you to open the sequencing reagents bay doors to empty the waste and remove used sequencing reagents bay consumables. Proceed to step 8.
- If you are using a chip that was previously installed and has sufficient lane capacity for the run, the sequencer prompts you to start the run.

IMPORTANT! The cartridge and bottles in the sequencing reagents bay must be replaced every time that a new chip is installed, regardless of how many lanes were used in the previous chip.

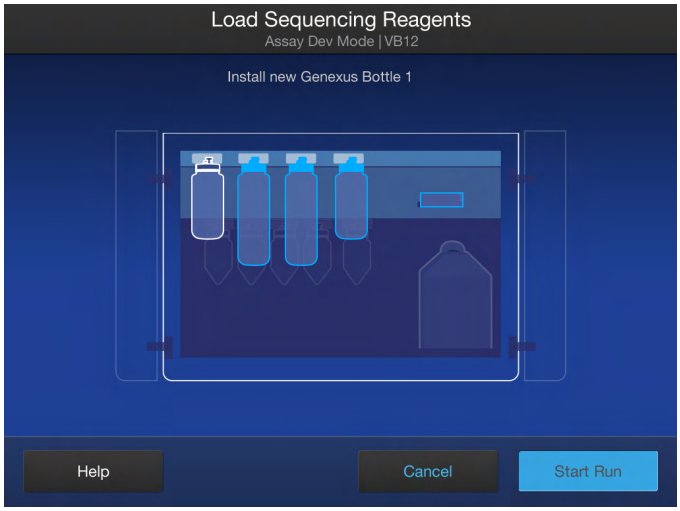
8. Follow on-screen instructions to empty the waste in the Waste carboy, remove waste pipette tips, remove the used Genexus™ Bottle 1, Genexus™ Bottle 2, Genexus™ Bottle 3, and Genexus™ Cartridge, then tap **Next**.



IMPORTANT!

- Ensure that you empty and replace the Waste carboy and the waste pipette tip bin.
- After replacing the emptied Waste carboy, ensure that you reinsert the waste tube into the carboy.
- Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of consumables and liquid waste.

9. Install a new Genexus™ Bottle 1, Genexus™ Bottle 2 (two required), Genexus™ Bottle 3, and Genexus™ Cartridge.

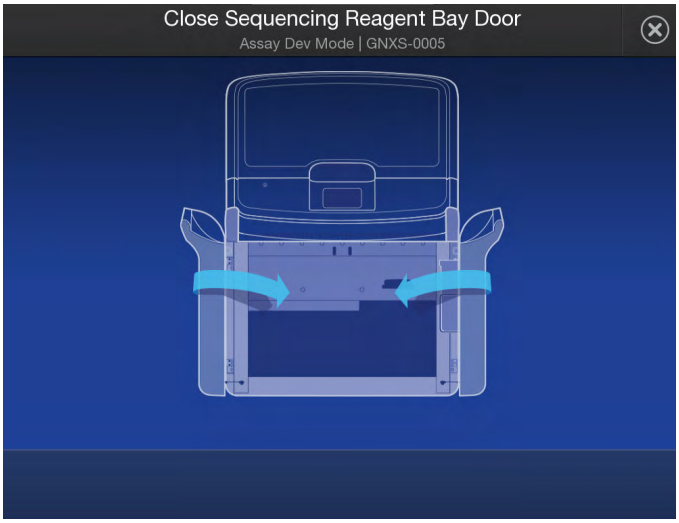


IMPORTANT!

- Before installing, *gently* invert each Genexus™ Bottle 2 five times to mix—avoid vigorous mixing. To avoid pinching or folding of the plastic nozzle, install the bottles straight-on, not at an angle.
- The installed reagents can be used for up to 14 days on the sequencer with full performance. After 14 days, you can observe reduced performance.

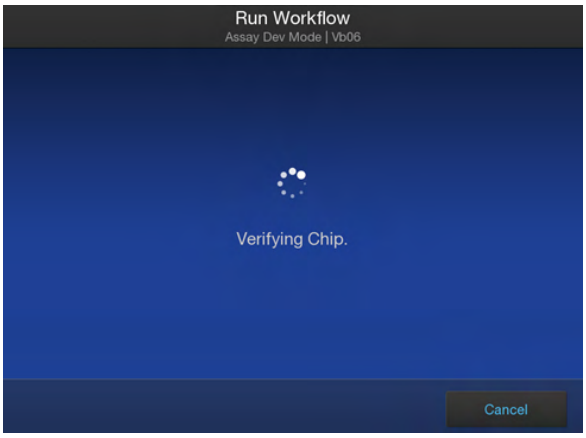
After reagents have been installed, the **Close Sequencing Reagent Bay Door** screen appears.

10. Close the sequencing reagents bay doors.



After the doors are closed, the sequencer automatically starts the run.

At the beginning of the run, the instrument verifies the chip, checks for leaks, then calculates run time.



A sequencing run encompasses the following stages:

1. Starting
2. Initializing
3. Library Prep
4. Templating
5. Pre-sequencing
6. Sequencing
7. Cleaning

At each stage, the instrument shows the time remaining on the touchscreen.

Note: The time remaining shown on the screen does not include run analysis time.

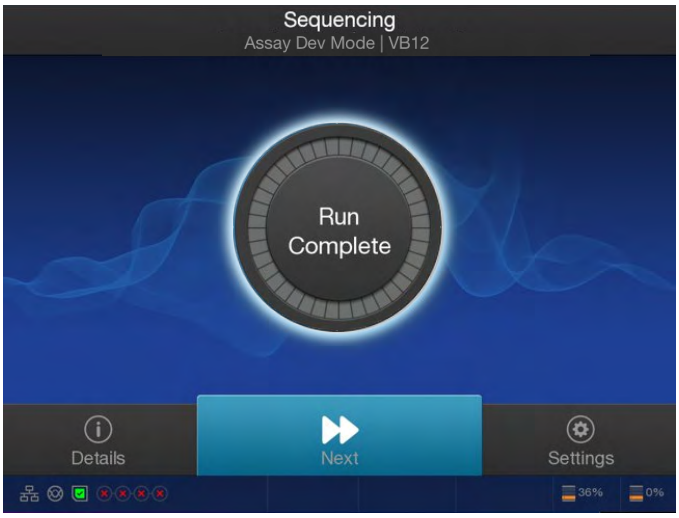
When the run finishes, the sequencer displays the **Run Complete** screen.

Note: If all the lanes of a chip are used, the chip shuttles to the install position. You are asked to remove the chip and coupler, and clear the sequencing reagents.

Clear the instrument deck and perform a UV Clean

After a run completes, remove used consumables from the deck and perform a **UV Clean** to ready the instrument for the next run.

1. In the **Run Complete** screen, tap **Next** to start removal of used consumables.

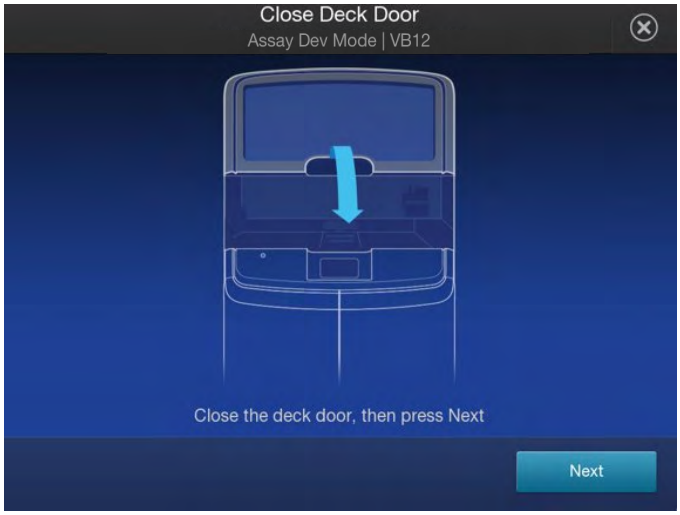


The deck door opens.

2. In the **Clear Deck** screen, the sequencer provides step-by-step instructions by highlighting the components to be removed. Unlock the library and templating strips by sliding the latches toward the front of the deck, then remove the used strips. Remove the remaining deck components specified by the sequencer.

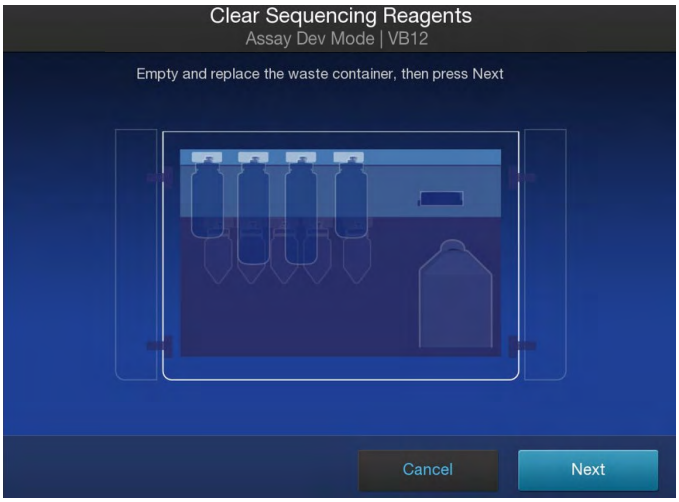
3. Inspect the Genexus™ Filter in the liquid waste disposal port and verify that no standing liquid is present. If standing liquid is present, manually remove the liquid with a pipette, then pull out the filter. Test the filter with water to determine if a clog is present.
   - If the Genexus™ Filter is clogged, replace it with a new filter. For more information, see "Replace the Genexus™ Filter" on page 194.
   - If the Genexus™ Filter does not appear to be clogged, a line clog downstream of the filter is implicated. Contact Technical Support and report a possible deck liquid waste line clog.

4. When finished, close the deck door, then tap **Next**.



A two-minute **UV Clean** starts.

5. After UV cleaning, if all the chip lanes were used, the sequencing reagents bay doors unlock. Open the doors, remove used components from the bay and empty the Waste carboy, then tap **Next**.



IMPORTANT! Do **not** discard or remove the conical bottles, unless alerted by the sequencer to replace the bottles after a conical bottle flow rate test. For more information, see "Replace the Genexus™ Conical Bottles" on page 195.

IMPORTANT! Follow all applicable local, state/provincial, and/or national regulations when recycling or disposing of Genexus™ Integrated Sequencer consumables and liquid waste.

CAUTION! The Genexus™ Bottle 1 (small waste bottle) contains small amounts of formamide. Dispose of this waste appropriately.

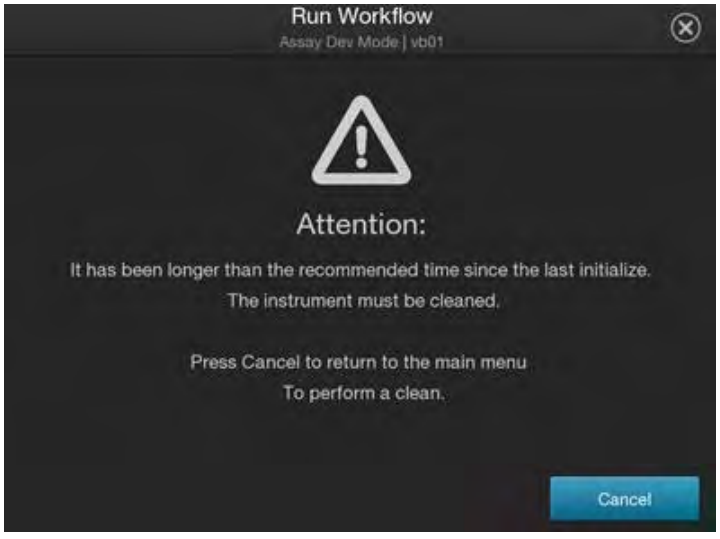
6. After removal of used components, close the sequencing reagents bay doors, then tap **Next**. The sequencer returns to the home screen.

Options for an expired sequencer initialization

A sequencer initialization is defined by the installation of a new chip, chip coupler, sequencing bottles, and reagent cartridge on the sequencer before a run. Reagents are stable on the sequencer for 14 days, after which you can experience reduced performance. After 14 days, the following on-instrument warning appears:

Users have the option to ignore the warning and proceed with an expired initialization by tapping **Ignore**, or to perform an instrument clean by tapping **Cancel**. After tapping **Cancel**, select **Settings** ▸ **Clean instrument** to clean the instrument before starting a run with a new chip. Lane assignment for a new run starts with lane 1.

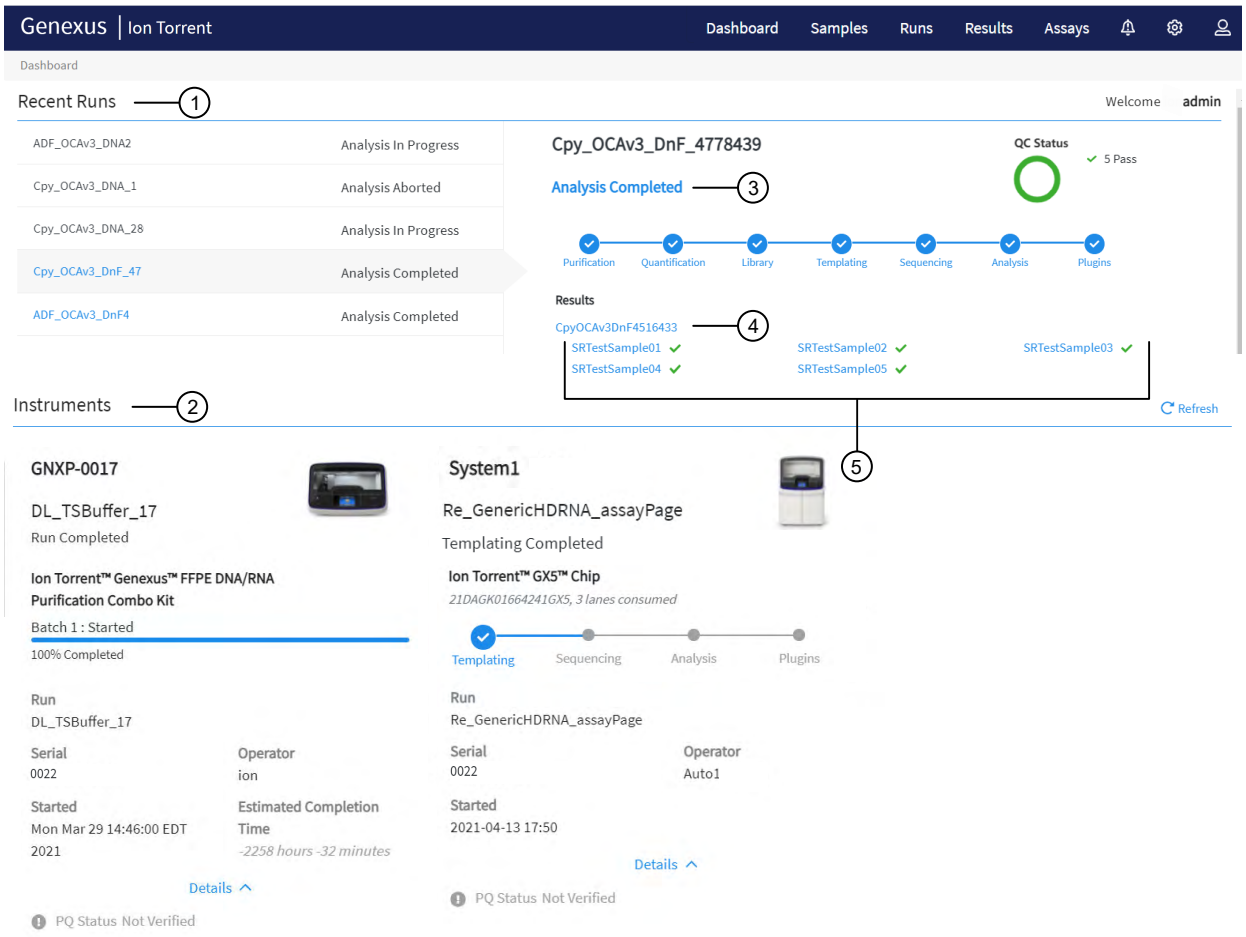
After 28 days, you can no longer proceed with an expired initialization. The following message appears:



Tap **Cancel** to return to the main menu to perform an instrument clean.

9 Monitor the run

In the **Dashboard** screen you can view instrument status and details for recent and current runs.



① The five most recent runs are listed in the left pane.

- You can click the row of a run of interest to view details for the run in the right pane.
- You can click the name of the run to leave the dashboard and view the results for the run.

② Instruments that are integrated with the software are shown.

③ In the details pane on the right, you can place the pointer over the status for the run to view the progress of the run.

④ For the recent run that is selected in the left pane, each assay for the run is shown. You can click the name of an assay to leave the dashboard and open the results for the run and view assay metrics.

⑤ For the recent run that is selected in the left pane, each sample in the run is shown. You can click a sample name to leave the dashboard and open the sample results.

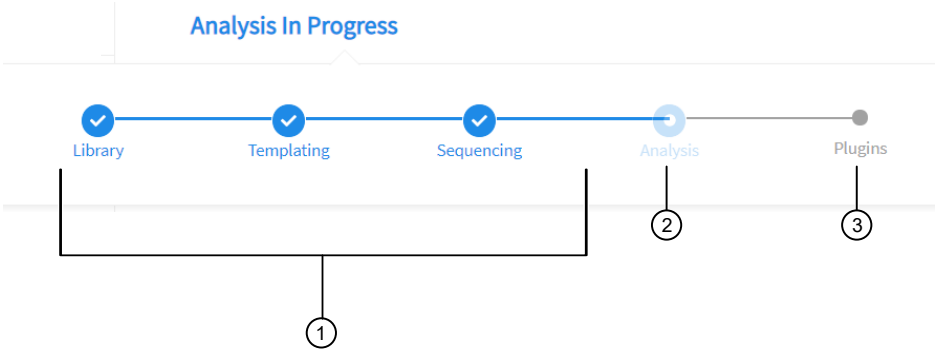
View run progress on the instrument

You can view details for the five most recent and current runs in the **Dashboard** in Genexus™ Software.

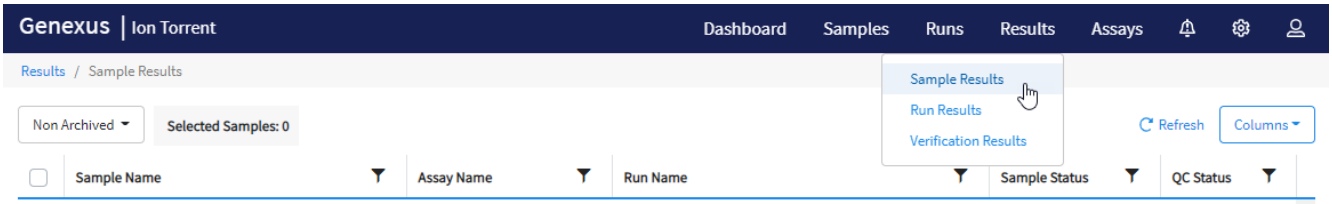
Runs that are reanalyzed are listed with ⟳ **(Reanalysis)** after the run name. For information about reanalyzing runs, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

1. In the menu bar, click **Dashboard**.
2. In the **Dashboard** screen, in the **Recent Runs** section, view information for recent runs.

Option	Description
View detailed run result information for completed runs.	In the left side of the screen, click the run name of interest. The **Run Summary** tab opens. For more information, see "The run summary" on page 122. In the menu bar, click **Dashboard** to return to the **Dashboard**.
View the quality control information.	In the left side of the screen, click the row of the run of interest. The QC status for the run and for the samples is shown in the details pane in the right side of the screen. For more information, see "QC results" on page 132.
View assay-level metrics and results for the run.	1. In the left side of the screen, click the row of the run of interest. 2. In the run details pane in the right side of the screen, click the assay name of interest. The **Assay Metrics** tab opens. For more information, see "Assay metrics and the run report" on page 125. In the menu bar, click **Dashboard** to return to the **Dashboard**.
View the sample results.	1. In the left side of the screen, click the row of the run of interest. 2. In the run details pane in the right side of the screen, click the sample name of interest. The **Key Findings** tab opens. For more information, see "Key Findings" on page 137. In the menu bar, click **Dashboard** to return to the **Dashboard**.

Option	Description
View the progress of the run.	1. In the left side of the screen, click the row of the run of interest. 2. In the run details pane in the right side of the screen, place the pointer over the status for the run. A progress bar for the run is shown. Steps in the run that are complete are listed with a check mark. Steps that are in progress are shown with a flashing circle. Steps that remain to be completed are listed in grey. If any failures occur, failed steps are also listed in grey.  ① Completed steps are shown with a check mark. In this example, **Library** preparation, **Templating**, and **Sequencing** are complete. ② Steps that are in progress are shown with a flashing circle. In this example, **Analysis** is in progress. ③ Steps that remain to be completed are shown in grey. In this example, the **Plugins** step remains to be started after **Analysis** is complete.

10 Review data and results



Use the **Results** menu to review results and data analysis, and to perform data management tasks. You can view results sorted by sample or by run.

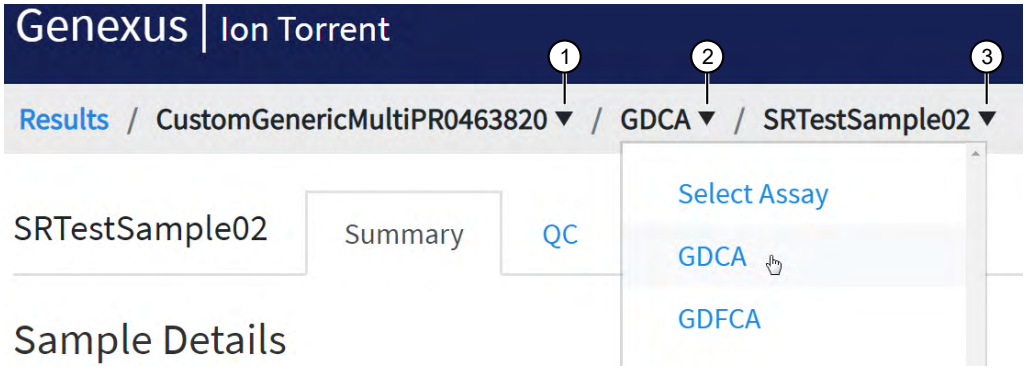
Selection	Description
Click **Results** ▸ **Sample Results**	Select this option to review completed sample results and reports.
Click **Results** ▸ **Run Results**	Select this option to review completed run results and reports by assay.
Click **Results** ▸ **Verification Results**	Select this option to review data from completed verification runs that were performed during sequencer installation or performance qualification.

Results navigation bar overview

After you select a run result or a sample result, a results navigation bar appears and allows you to easily toggle between different results screens for each run. You can quickly switch between run-level results, assay-level results, and sample-level results for the run. In addition, you can easily find samples or assays in the run with the search field that appears in each dropdown list.

Assay-level results include assay metrics, such as final read data.

Samples that are run with the same assay in the same run share the same run report. All other results are specific to the sample and are shown in the sample results.

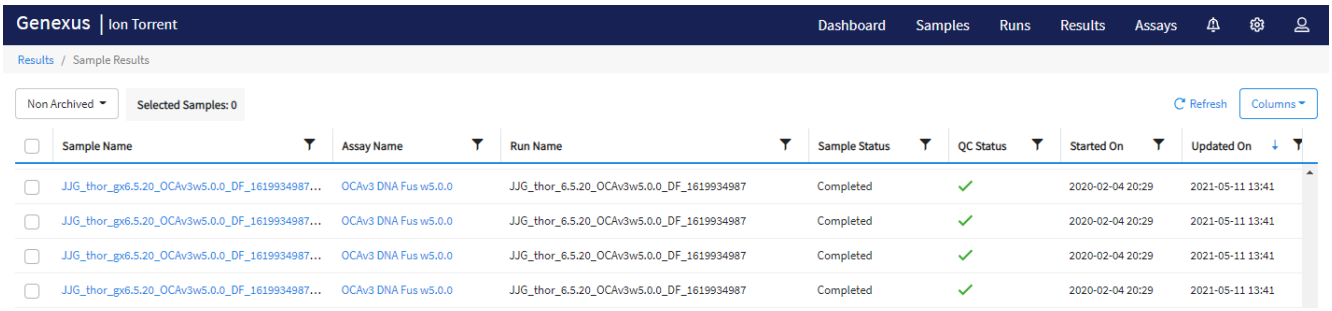


Callout	Dropdown menu	Description
1	**Run name**	The run name is listed. Multiple runs are listed only if the run has been reanalyzed.
2	**Select Assay**	You can click the assay name of interest to view assay metrics for the run. You can search for or toggle between different assays used in the run. If only one assay is associated with the run, only one assay name is listed. Some results from a plugin that is included in the run, such as the customer support archive plugin, apply to all of the samples for an assay in a run. In this case, the results from assay-level plugin are shown in the **Plugins** tab when you select an assay but do not select any samples for the run. For more information about plugins, see "Review coverageAnalysis plugin results" on page 174. You can select **Select Assay** from the dropdown menu to remove the assay selection. If you remove the assay and sample selections, the **Run Summary** tab opens with a summary of results for the run selected in the **Run Results** dropdown menu. Assay metrics are also shown in the **Run Report** tab. For more information, see "View assay metrics and the run report" on page 125.
3	**Select Sample**	You can click a sample name to view the sequencing results for the sample. You can click a different sample name to view other sample results for other samples in the run for the assay that is selected in **Select Assay**. The search field allows you to find samples in the run. For **Sample to Result** runs that include a positive control, results for the positive control are shown when you select **Control Sample**. You can select **Select Sample** from the dropdown menu to remove the sample selection. If you remove the sample selection, the **Assay metrics** tab for the selected assay opens. If you select **Select Assay** with **Select Sample** shown, the **Run Summary** tab opens. Assay metrics are also shown in the **Run Report** tab. For more information, see "View assay metrics and the run report" on page 125.

Review sample results

In the **Results / Sample Results** screen, samples that have been sequenced are listed by sample name.

You can search the list of results by **Sample Name**, **Run Name** or **Assay Name**. Enter a search term, then click **(Search)**.



The following information appears in the **Sample Results** screen.

Column	Description
Sample Name	The unique identifier created when the sample was entered into the software. Click the **Sample Name** to open the **Sample Details** screen for the sample. Use the tabs above the Sample Details to view the run summary, the run report, quality control, detailed variant results, and results for plugins that are associated with the selected assay, if any.
Sample Name followed by (Signed) or (Locked)	Manager- and administrator-level users can provide their electronic signature on sample results for completed runs. A sample name followed by (Signed) indicates that the report for the run has been signed electronically. The signature information appears in the variant report PDF file. A sample name followed by (Locked) indicates that the report for the run is locked. For more information, see "Sign off on the run results" on page 169.
Assay Name	The name of the assay used in the run plan.
Run Name	The unique name of the run given when it was created in the software.
Sample Status	The status of the run or sample (for example: Completed, Running, Failed, Terminated, Pending, Stalled).
QC Status	The QC status of a completed run. • (Passed) indicates the sample passed all QC metrics. • (Failed) indicates the sample failed a QC metric. • — (Not Calculated) indicates a sample did not undergo QC analysis.
Started On	The date and time when the run was started.
Updated On	The date and time when the last action was completed on the run.
Tags	Tags that are assigned to the sample are listed.

You can also perform the following actions in the **Sample Results** screen.

Option	Description
Actions	Available action links for a sample are shown when you place the pointer over the row of a sample. • **Report**—Download the report (available only for samples with a sample status of completed). • **Assign Tags**—Add or remove the tags assigned to a sample result. • **Audit**—View the audit trail for the run. • **Notes**—View, add, or delete notes for sample result. • **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting. • **Reanalyze**—Reanalyze a sample from the alignment step when a sample or reanalysis successfully completes the basecalling step. • **Variant Audit**—View the history of variant classifications.
Actions that you can perform when you select samples	More actions are shown when you select one or more samples. • **Assign Tags**—Assign a tag or tags to selected sample results in one action. The **Assign Tags** command appears above the **Sample Results** table after you select one or more samples. • **Compare**—Compare variant results between multiple samples, or from a single sample over time. The **Compare** command is not shown until you select at least two samples. **Note:** For more information on using the **Assign Tags** and **Compare** features, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

Review run results

In the **Results / Run Results** screen, runs that are pending, in progress, or completed are listed. Runs with a status of failed, aborted, or stalled are also listed.

You can search the list of results by run name or PCR plate number. Enter a search term, then click **(Search)**.

The following run information appears in the **Results / Run Results** screen.

Column	Description
Run Name	The unique name of the run given when it was created in the software. Click a run name to open the **Run Summary**. Runs that are reanalyzed are listed with **(Reanalysis)** after the run name. For more information, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).
Assay Name	The name of the assay selected for the run. You can view the **Assay Name** and corresponding **Assay Full Name** for all assays in the **Assays ▸ Manage Assays** screen.

(continued)

Column	Description
Run Status	The status of the run. For example, **Analysis In Progress**, **Executing Plugins**, **Analysis Completed**, **Terminated**, **Archival: In Progress**, **Purification In Progress**, or **Purification Completed**). For purification runs that have a status of **Purification In Progress**, **Purification Complete**, or for failed or aborted purification runs, you can place the pointer over the shaded number, in the **Status** column to view the status of each purification batch for the run. The shaded number, such as 1 or 2, represents a purification batch for the run. For example, a status can be **1 Completed**, **2 Started**, and **3 Planned**, which indicates that the first batch is complete, the second batch has started, and the third purification batch has not yet started, but is planned. For more information about purification batches, see "About Sample to Result runs" on page 75.
Total Samples	The total number of samples in a run.
PCR Plate Number	A unique identifier for the 96-well plate used for library preparation and templating. For more information, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).
Started On	The date and time when the run was started.
Updated On	The date and time when the last action was completed on the run.

You can also perform the following actions in the **Results / Run Results** screen.

Option	Description
Actions	Available action links for a run are shown when you place the pointer over the row of a run. The actions that are available depend on the type of run. • **Delete**—Delete the run. • **BAM Uploader**—Upload run information to another Genexus™ Integrated Sequencer or to Ion Reporter™ Software for further analysis. **BAM Uploader** is not available for BAM run results or for archived runs in which BAM files have been removed. For more information, see "Upload results files to another Genexus™ Integrated Sequencer" on page 177. • **Audit**—View the audit trail for the run. • **CSA**—Download customer support archive (CSA) log files for the run to help with troubleshooting. • **Assign PCR Plate**—Enter a unique identifier for the 96-well plate used for library preparation and templating.
Actions	These actions are available only for Sample to Result runs. • **View Plan**—View detailed run plan information. • **Review**—Review samples that do not have a concentration within a specified threshold after purification, but before library preparation. For more information, see "Review samples for Sample to Result runs" on page 98. • **Abort**—Allows you to abort a run after purification, but before sequencing. This action is available when the run status is **Purification Review Required**, or when the run status is **Purification Completed** and some purification samples have been excluded from sequencing.

View the run summary

The run summary provides an overview of the run. The information that is displayed includes the name of the assay used in the run, sample locations, information about the reagents used in the run, primer tube positions, and instrument information. Metrics from sample purification are also provided, if applicable.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Name** column, click the run name of interest.
   The **Run Summary** tab opens.
3. Review the run summary.

Action	Procedure
View the assay metrics.	In the **Assays** section, click the assay name of interest.
View the sample locations in an image of a 96-well sample plate.	In the **Sample Locations** section, click **PCR Plate View**.
Reanalyze a run with a new assay.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **Reanalyze**. For more information, see the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953), or the software help system.
Run plugins on the sequencing data after a sequencing run is complete.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **Run Plugin**.
Download customer support archive (CSA) log files for the run to help with troubleshooting.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **CSA**.
Upload results to another Genexus™ Integrated Sequencer or to Ion Reporter™ Software for further analysis.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **BAM Uploader**. For more information, see “Upload results files to another Genexus™ Integrated Sequencer” on page 177.
View the history of variant classifications.	In the upper right corner of the screen, click ··· **(More Options)** ▸ **Variant Audit**. The information is available only for results that include variant classifications.
View the run report.	Click the **Run Report** tab.

The run summary

The run summary displays run information, assays used in the run, sample locations, metrics from sample purification, information about the reagents used in the run, primer tube positions, and instrument information.

Depending on the type of the run and the selections made in the run plan, the run information can include the following items.

Run information

Item	Description
Run Name	The name of the run.
Started On	The date and time when the run was started.
Completed On	The date and time when the run was completed.
Run Status	The status of the run.
Report Template	If the option to generate a report was enabled in the run plan, the report template used is listed.
Chip Type	The semiconductor sequencing chip used in the run, such as the GX5™ Chip.
Library Chemistry	The type of library chemistry used in the run, such as Ion AmpliSeq™ HD.
Server	The Ion Reporter™ Software account or the Genexus™ Software account and respective software version that was selected for uploading BAM files when the run is complete.

Sequencing instrument information

Item	Description
Instrument Name	The name of the sequencer.
Instrument Serial Number	The serial number of the sequencer.
Status	The status of the sequencing portion of the run.
Operator	The name of the person who was signed in the Genexus™ Integrated Sequencer when the sequencing run was performed.
Start Date	The date and time when the run was started on the sequencer.
Completion Date	The date and time when the run was completed on the sequencer.
Sequencing Flows	The number of flows performed by the sequencer.

Purification instrument information

Item	Description
Purif. Instrument	The name of the purification instrument.
Instrument Serial Number	The serial number of the purification instrument.
Status	The status of the purification portion of the run for the batch.
Operator	The name of the person who was signed in the Genexus™ Purification Instrument when purification was performed.
Start Date	The date and time when the batch was started on the purification instrument.
Completion Date	The date and time when the batch was completed on the purification instrument.
Purif. Batch	The nucleic acid isolation batch.

Assays table

Item	Description
Assay Full Name	The name of the assay used in the run. Click the assay name to display the details of the assay.
Assay Name	An abbreviated name of the assay or assays used in the run.
Analysis Version	The version of the assay used for analysis.
Research Application	The research application for the assay, such as **DNA** or **DNA and Fusions**.
Lane	The chip lane or lanes used in the sequencing run for the assay.
Total Samples	The total number of samples sequenced for the assay. A single sample can correlate with multiple wells on the plate for some assays when multiple nucleic acid types are contained within the sample.

Purification Samples table

Item	Description
Batch ID	When the run includes more than one purification batch, the **Batch ID** is listed. The **Batch ID**, such as 1 or 2, indicates the purification group for each sample.
Sample Name	The unique identifier created when the sample was entered into the software.
Sample Type	A term that describes the sample, for example, FFPE or Blood (Plasma).
Nucleic Acid Type	DNA, RNA, or TNA.

Purification Samples table *(continued)*

Item	Description
Conc. (ng/µl)	The concentration of the sample measured by the purification instrument. In some instances, such as when purification is in progress or when the sample is a no-template control, the values is listed as N/A. If the concentration is not within the QC concentration range specified by the assay, a **Quantity Not Sufficient** (QNS) alert is displayed.
QC Conc. Range (ng/µl)	The QC concentration range for the assay.
Batch Status	The run status of a purification batch. The status can be N/A, Planned, Review, Started, Aborted, or Completed.
Archive Position	The sample positions in the archive plate. Sample positions can be in rows A to D and in columns 1 to 8.
Library Prep	An indicator of whether the sample is selected for sequencing. • ✔ – indicates that the sample is selected for sequencing. • ⊘ – indicates that the sample is not selected for sequencing. • N/A – indicates that purification has not started or is in progress.

Sample Locations table

Item	Description
PCR Plate View	Click to view the sample locations in an image of a 96-well sample plate
Well Pos.	The well position indicates the location of the sample on the plate.
Sample Name	The unique identifier created when the sample was entered into the software.
Nucleic Acid Type	The sample nucleic acid type, such as DNA, RNA, or TNA.
Vol. (µl)	The volume of the sample.
Conc. (ng/µl)	The concentration of the sample.
Dilution Factor	The dilution factor of the sample.
Kit Barcodes	The barcode for the kit used for nucleic acid extraction, if applicable.
Barcode	The name of the barcode adapter or adapters that are associated with the sample, for example, Genexus™ Barcodes 1–96 AS.
Assay Name	The assay that was used to sequence the sample for the indicated well position.
Library Position	The plate location of libraries. For completed runs that were planned using samples, the **Library Position** indicates the location of unused libraries that can be sequenced by planning and initiating a library run.

Reagents table

Item	Description
Consumable	Consumables used in the run, such as the **PanelKit** and **LibraryKit**, are listed.
Barcode	The consumable barcode, if applicable.
Part #	The consumable part number, if applicable.
Expiration Date	The consumable expiration date, if applicable.
Lot #	The consumable lot number, if applicable.

The **Primer Tube Positions** table shows an image that indicates the locations of the primer pool tubes.

View assay metrics and the run report

The run report provides detailed information about a run, such as the **Total Bases** and **Final Reads**. For runs with multiple assays, metrics are provided for each assay in the run. The run report contains the assay metrics for all assays in the run.

For a runs with only one assay, the run report and assay metrics show the same information for assay metrics. In addition to the metrics for the assay, the run report shows information about the run and the instruments used in the run.

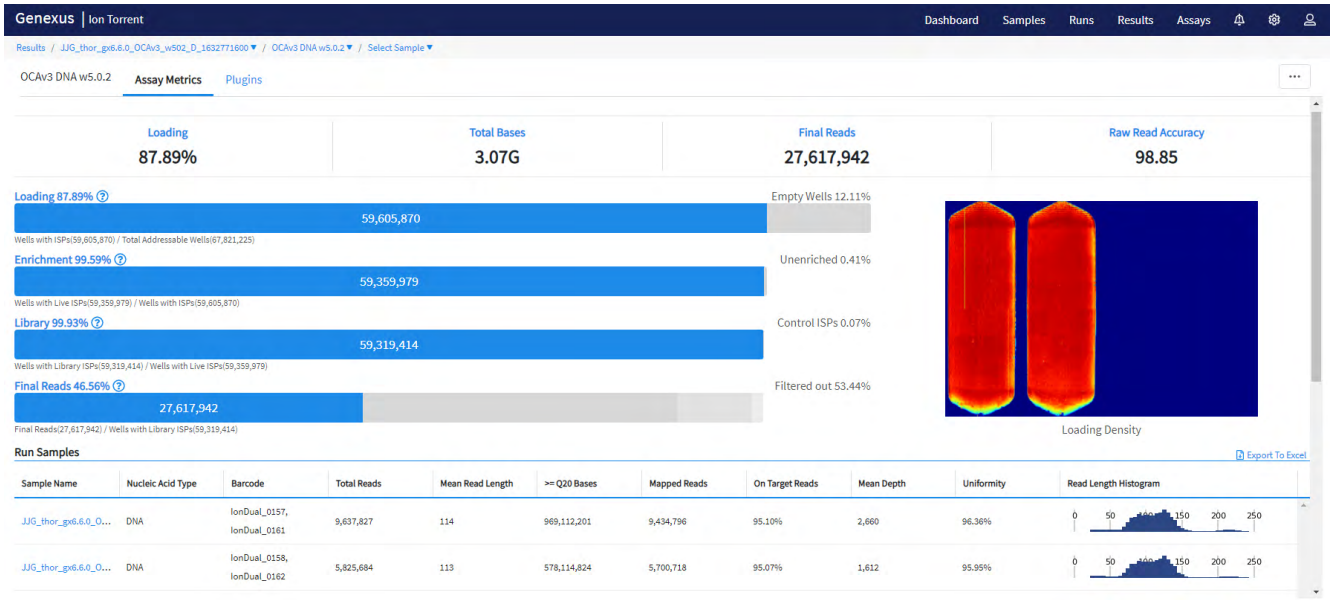
To view the **Run Report**, in the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest. In the **Run Summary** screen, click the **Run Report** tab.

The **Run Report** is assay-specific and cannot be viewed within the sample results screens. To view the **Run Report**, ensure that **Select Sample** is selected in the **Select Sample** dropdown menu.

Assay metrics and the run report

The run report provides detailed information, such as various chip metrics for the run, and well and Ion Sphere™ Particles (ISPs) statistics. For runs with multiple assays, metrics are provided for each assay in the run. Sequencing metrics are shown at the top of the screen, followed by sample-specific metrics in the **Run Samples** table. Read data for individual samples for the assay are listed in the **Run Samples** table.

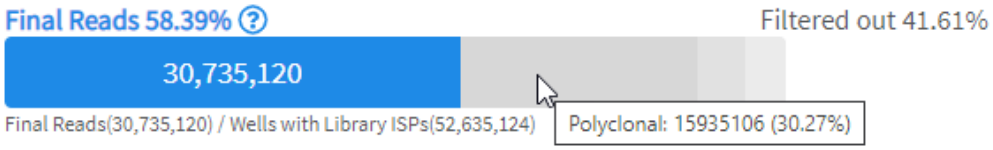
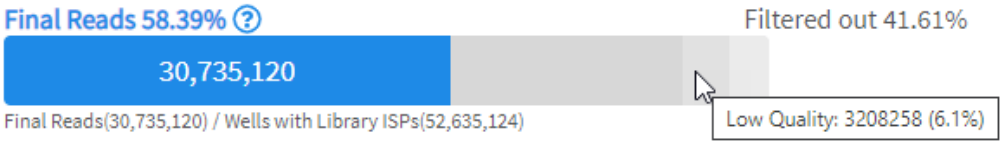
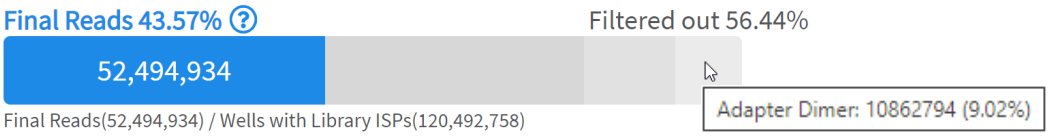
Barcode-specific metrics for barcodes that are included in the run are listed in the **Barcodes With Reads Reported** table, which follows the **Run Samples** table. The CSA file for the run contains information for barcodes that are not assigned to samples in the run. Information in the CSA file can help you troubleshoot results, if needed. For more information, see “Download a customer support archive” on page 180.



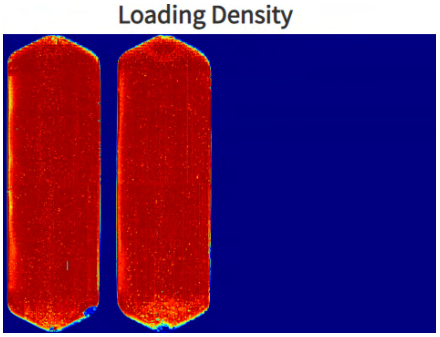
Run Report

Metric	Description
Loading	The number and percentage of total addressable wells on the chip that contain an ISP.
Enrichment	The number and percentage of wells ISPs that contain live ISPs.
Library	The number and percentage of wells with live ISPs that contain Library ISPs.
Final Reads	Library reads passing all filters that are recorded in the output BAM files. This value can be different from the total number of reads due to technicalities associated with read trimming beyond a minimal requirement.
Total Bases	The number of filtered and trimmed base pairs that are reported in the output BAM file.
Raw Read Accuracy	The raw read accuracy across each individual base position in a read calculated as, (1–[total errors in the sequenced reads]/[total bases sequenced]) × 100. Raw read accuracy is measured at each base across the length of the read and is based on 1x sequencing coverage; raw read accuracy is *not* based on consensus accuracy across multiple reads for the same base position.
Wells with ISPs	The number of wells that contain an ISP.
Unenriched	The number and percentage of wells with ISPs that do not contain live ISPs.
Total Addressable Wells	Wells on the chip that can be physically reached by a library.
Empty Wells	The percentage of total addressable wells on the chip that do not contain an ISP.
Wells with Live ISPs	Loaded wells with ISPs with a signal of sufficient strength and composition to be associated with the library or control fragment key.
Wells with Library ISPs	Loaded wells with live ISPs with a key signal that is identical to the library key signal.

Run Report *(continued)*

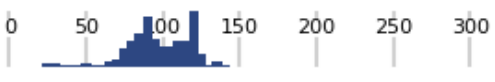
Metric	Description
Control ISPs	Loaded wells with live ISPs with a key signal that is identical to the control fragment key signal.
Filtered out	The total percentage of filtered reads, or the sum of the percentages of polyclonal, low quality, and adapter dimer reads.
Polyclonal	Wells with a live ISP that carries clones from two or more templates. To view polyclonal metrics, mouse over the first low quality portion (gray) of the **Final Reads** bar plot. 
Low Quality	Loaded wells with a low or unrecognizable signal. To view polyclonal metrics, mouse over the second low quality portion (gray) of the **Final Reads** bar plot. 
Adapter Dimer	Loaded wells with a library template of an insert size less than 8 bases. To view adapter dimer metrics, mouse over the lightest gray portion of the **Final Reads** bar plot.  **Note:** In assays using Ion AmpliSeq™ HD library chemistry, adapter dimer reads represent a small proportion of total reads and can be seen by hovering the pointer over the right end of the **Final Reads** bar.

Run Report *(continued)*

Metric	Description
Loading Density	A visual representation of chip loading. Red color indicates areas of higher density of loading. Blue color indicates areas of lower density of loading. The following example shows a sequencing experiment where two lanes on the chip are uniformly loaded with ISPs. 

Run Samples

The **Run Samples** table lists read data for each individual sample in the assay.

Column	Description
Sample Name	The unique identifier created when the sample was entered in the software.
Nucleic Acid Type	The sample nucleic acid type, such as DNA, RNA, or TNA.
Barcode	The unique identifiers of the dual barcode pair assigned to the DNA and/or RNA library for a sample.
Total Reads	The total number of filtered and trimmed reads with the listed dual barcodes assigned to the sample.
Mean Read Length	The average length, in base pairs, of usable library reads for each sample.
≥Q20 Bases	The total number of called bases that have ≥99% accuracy (or less than 1% error rate) aligned to the reference for the sample.
Mapped Reads	The number of reads that are mapped to the reference file.
On Target Reads	The percentage of sequencing reads mapped to any target region of the reference.
Mean Depth	The average number of reads of all targeted reference bases.
Uniformity	The percentage of bases in all targeted regions (or whole genome) with a depth of coverage ≥20% of the mean read coverage.
Read Length Histogram	A histogram that presents all filtered and trimmed library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). 

Barcodes with Reads Reported

The **Barcodes with Reads Reported** table lists barcode-specific metrics

Column	Description
Barcode	The unique identifiers of the dual barcode pair assigned to the DNA and/or RNA library for a sample.
Total Reads	The total number of filtered and trimmed reads with the listed dual barcodes assigned to the sample. The reads are independent of length reported in the output BAM file.
Mean Read Length	The average length, in base pairs, of usable library reads for each sample.
≥Q20 Bases	The total number of called bases that have ≥99% accuracy (or less than 1% error rate) aligned to the reference for the sample.
Read Length Histogram	A histogram that presents all filtered and trimmed library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). 

Download a run report

You can download a run report summary in PDF format. The run report includes assay metrics and the record of reagents that were used in a run. For information about the contents of the run report, see “View assay metrics and the run report” on page 125. If you entered extraction kit barcodes for samples when you prepared library batches or when you planned the run, the extraction kit barcodes are listed in the run report.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, click the sample name of interest.
3. Click the **Reports** tab.
   Multiple panes including a **Run Report** pane, a **Variant Report** pane, and any panes for customized reports that have been generated are shown.
4. In the **Run Report** pane, click **Download Report** to download a run report summary in PDF format.

Assign PCR Plate

Genexus™ Software allows you to track and associate a run with the PCR plate used in the run. The PCR plate is the 96-well plate that is used for library preparation and templating. You can assign a unique identifier, a **PCR Plate Number**, to runs that have a status of **Library Preparation Completed**, **Sequencing Completed**, or **Run Completed**. The PCR plate number that you enter is shown in the **Run Results** screen and if needed, can help you track libraries and troubleshoot. Later, if you sequence the remaining libraries in a different sequencing run and assign the PCR plate number to the run, you can easily search for and find all run results associated with the libraries in the PCR plate.

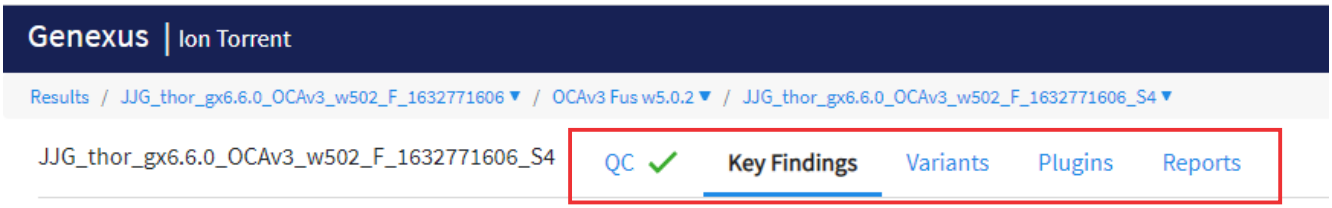
1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Results** screen, place the pointer over the row of a run of interest, then click **Assign PCR Plate number**.
3. In the **Assign PCR Plate Number** dialog box, confirm, edit, or enter the **PCR Plate Number**.

   The PCR plate number must be between 1 and 10 characters. Only alphanumeric characters (numbers 0 to 9 and letters A to Z), period (.), underscore (_), or hyphen (-) are allowed. Spaces are not allowed.
4. Click **Submit** to associate the PCR plate with the run.

View sequencing results

1. Click **Results** ▸ **Sample Results** to view sequencing results, including variant and fusion calls, for a particular sample.
2. In the **Sample Name** column, click a sample name.
3. In the **Results** screen, click the tabs to view the different types of sample-specific results and data.

Tab	Description
QC	The quality metrics for the sample sequenced in the run. For more information, see “QC results” on page 132.
Key Findings	An overview of the results for the sample, including **Sample Details**, **Key Metrics**, **Genes**, and **Coverage** graphs. For more information, see “Key Findings” on page 137.
Variants	Detailed variant results for SNVs/Indels, Fusions, and CNVs. For more information, see “View SNV/Indel results” on page 143.
Plugins	Results generated from the plugins associated with the assay used to analyze the sequenced sample. For more information, see “Review coverageAnalysis plugin results” on page 174.
Reports	You can download and generate summaries of run results. There are two types of reports: • Run reports – include assay metrics and the record of reagents that were used in a run. • Variant reports – include the variant results for each sample in a sequencing run, reagents used, and QC evaluation metrics. For more information, see “Download a run report” on page 129.



View the QC results

You can view the quality control (QC) metrics for each sample that was sequenced in a run in Genexus™ Software.

1. To view the **QC** screen, in the **Results / Sample Results** screen, click a sample name in the **Sample Name** column. In the **Results** screen, click the **QC** tab. The QC status for each metric is indicated beneath each QC test (Run QC, Templating Control QC–CF–1, Sample QC–DNA, and Sample QC–RNA).
2. Review the QC metrics.
   For more information, see “QC results” on page 132.

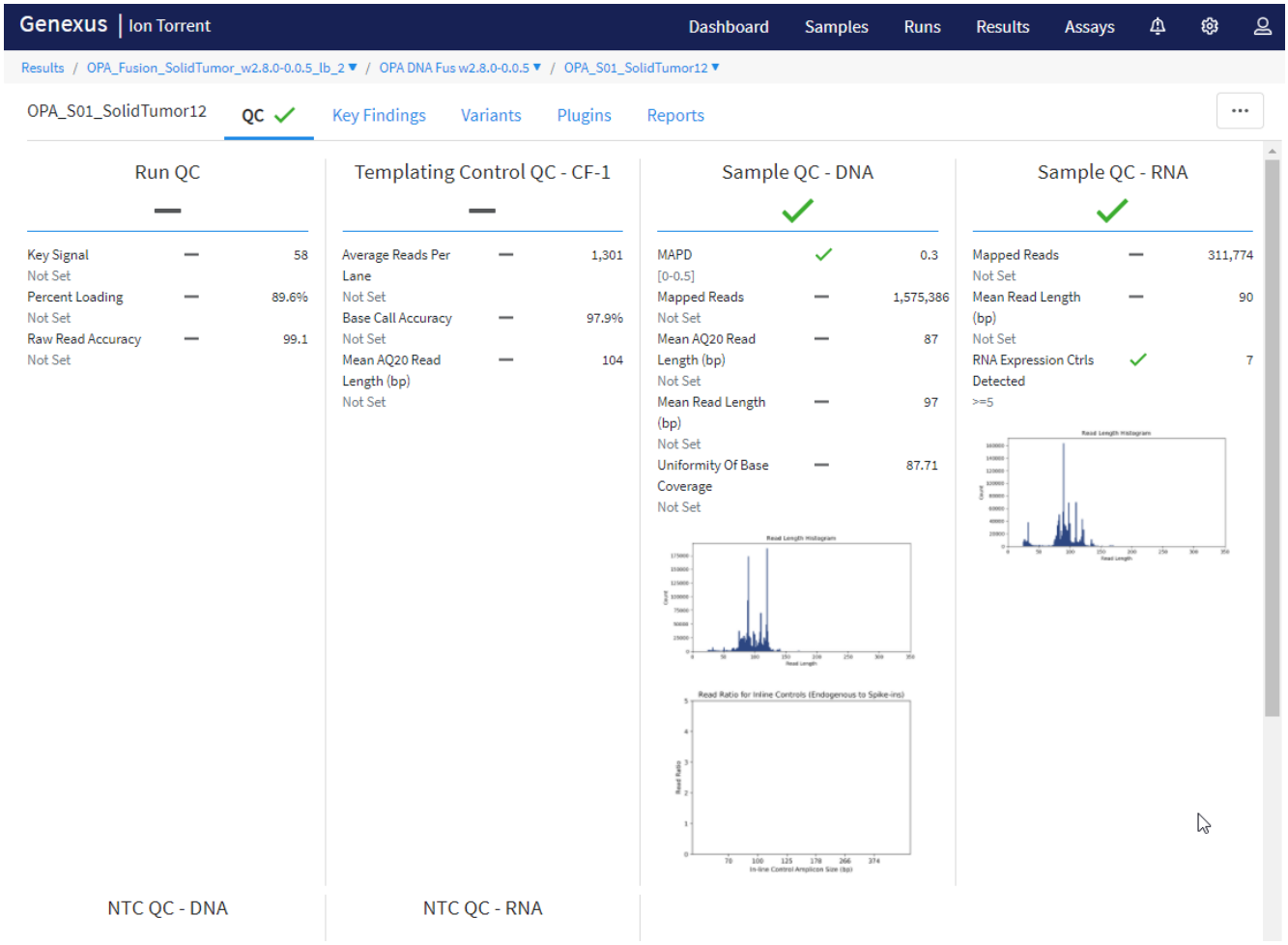
QC results

The quality control (QC) metrics for each sample that was sequenced in a run are displayed in Genexus™ Software.

If a sample fails a single test metric, the sample fails that QC test. A sample must meet all QC parameter thresholds of a particular QC test in order to pass. The QC status is divided into the following categories.

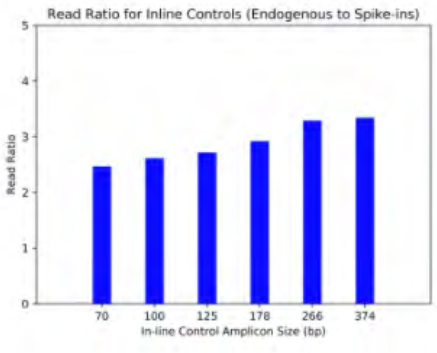
- ✔ (Passed) indicates the sample passed all QC metrics.
- ✘ (Failed) indicates the sample failed a QC metric.
- — (Not Calculated) indicates a sample did not undergo QC analysis.

The data displayed in the screen depend on the assay that was used in the run.

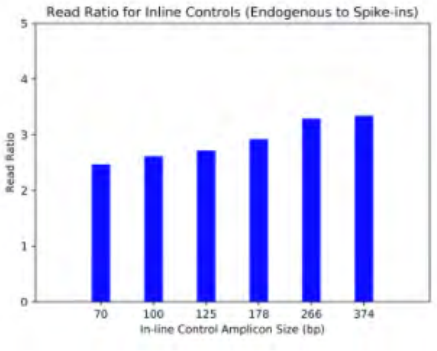


Metric[1]	Description
Purification QC	**Quality control information for nucleic acid extraction that is performed on a Genexus™ Purification Instrument.**
Sample Concentration RNA	The concentration of extracted RNA after purification.
Sample Concentration DNA	The concentration of extracted DNA after purification.
Sample Concentration TNA	The concentration of extracted TNA after purification.
Run QC	**General run quality control information.**
Key Signal	The average signal after software processing for library ISPs that identically match the library key (TCAG).
Percent Loading	The number of wells with ISPs divided by the number of the total addressable wells in a run.
Raw Read Accuracy	The average raw accuracy across each individual base position in a read, where raw read accuracy is calculated as 100 * (1 - (sum(per base error)/sum(per base depth))).
Templating QC—CF-1 Control	**Sequencing quality metrics of the control fragment. These metrics indicate templating success.**
Average Reads Per Lane	The number of CF-1 reads divided by the number of chip lanes used in the run.
Base Call Accuracy	The probability that a given base is called correctly. 1 – (total number of errors for all positions in CF-1) / (total number of CF-1 base reads).
Mean AQ20 Read Length (bp)	Average length, in base pairs, at which the accuracy rate is ≥99% for CF-1 reads.
Sample QC—DNA	**Sequencing quality metrics of the sample DNA library.**
MAPD	MAPD (Median of the Absolute values of all Pairwise Differences) is a quality metric that estimates coverage variability between adjacent amplicons in CNV analyses. A MAPD value of ≤0.5 indicates an acceptable level of coverage variability. High MAPD value typically translates to a lower coverage uniformity. Lower coverage uniformity can result in missed or erroneous CNV calls. If the MAPD QC threshold is not met, CNVs are not called. The MAPD metric does not affect SNVs/INDEL calls.
Mapped Reads	The total valid mapped reads.
Mean AQ20 Read Length (bp)	The average length, in base pairs, at which the accuracy rate is ≥99% for all aligned reads of a library.
Mean Read Cov	The number of average reads per amplicon. This metric is generated by the CoverageAnalysis plugin.
Molecular Uniformity	Uniformity of molecular coverage for all amplicons. This metric is generated from the MolecularCoverageAnalysis plugin.

(continued)

Metric[1]	Description
Mean Read Length (bp)	The average length, in base pairs, of final library reads for the sample.
Uniformity of Amplicon Coverage	The percentage of amplicons that had at least 20 percent of the average number of reads per amplicon. Cumulative coverage is linearly interpolated between nearest integer read depth counts.
Median Mol Cov	The median number of functional molecule reads per amplicon calculated over all amplicons in the assay. This metric is applicable to Ion AmpliSeq™ HD library chemistry only.
Uniformity of Base Coverage	The percentage of reads with a depth of coverage ≥20% of the mean read coverage at each position. This metric is applicable to Ion AmpliSeq™ HD library chemistry only.
Read Length Histogram	The histogram presents all filtered and trimmed DNA library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis). The shape of the histogram should closely resemble the library size distribution trace without the adapter sequences.
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of genomic sample reads to control reads for each inline control amplicon.  **Note:** The **Read Ratio** for each inline control amplicon is expected to be approximately 3 with 10 ng DNA input for both Ion AmpliSeq™ and Ion AmpliSeq™ HD chemistries.
Sample QC – RNA	**Sequencing quality metrics of the sample RNA library.**
Mapped Reads	The total valid mapped reads. That is, the mapped reads that pass the thresholds and filters across all of the RNA targets in the reference file. This number is a subset of the mapped reads shown in the **Run Samples** table in the **Assay Metrics**.
Mean Read Length (bp)	The average length, in base pairs, of the final library reads for the sample.
RNA Expression Ctrls Detected	The number of expression control genes detected for the sample. This metric measures the RNA input integrity, input amount, and the fidelity of the reverse transcriptase that was used in library preparation. Fusion panels include primer pairs that cover 5–7 control housekeeping genes.

(continued)

Metric[1]	Description
Mean AQ20 Read Length	The average length, in base pairs, at which the accuracy rate is ≥99% for all aligned reads of a library.
Read Length Histogram	The histogram presents all filtered and trimmed library reads that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis).
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of genomic sample reads to control reads for each inline control amplicon. You can use this metric to measure the level of contaminating genomic DNA in RNA libraries when you select the RNA checkbox for **Include Inline Controls** in assay set up. Using inline controls in RNA assays will reduce the total number of panel reads.  **Note:** The **Read Ratio** for each inline control amplicon is expected to be approximately 3 with 10 ng DNA input for both Ion AmpliSeq™ and Ion AmpliSeq™ HD chemistries.
NTC QC – DNA	**DNA Sequencing quality metrics of the no-template control.**
Average Base Coverage Depth	The average number of DNA reads of all targeted reference bases.
Mean Read Length (bp)	The average length, in base pairs, of final DNA library reads for the no template control.
Read Length Histogram	The histogram presents all filtered and trimmed reads for the no template control that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis).
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of no template control sample reads to control reads for each DNA inline control amplicon.
NTC QC – RNA	**RNA Sequencing quality metrics of the no template control.**
Mapped Reads	The total number of valid mapped reads for the NTC.
Mean Read Length (bp)	The average length, in base pairs, of final RNA library reads for the no template control.

(continued)

Metric[1]	Description
Read Length Histogram	The histogram presents all filtered and trimmed reads for the no template control that are reported in the output BAM file (Y-axis) and the mean read length in base pairs (X-axis).
Read Ratio for Inline Controls (Endogenous to Spike-ins)	The ratio of no template control sample reads to control reads for each RNA inline control amplicon.

[1] For **BAM to Result** runs, only the **Sample QC—DNA** or **Sample QC—RNA** metrics are shown.

MAPD copy number QC metric

The Median of the Absolute values of all Pairwise Differences (MAPD) score is reported on Aneuploidy run results and other runs that detect CNVs.

MAPD is one of the metrics that we define to assess whether the panel data is useful for copy number variation (CNV) run results.

MAPD is defined as the Median of the Absolute values of all Pairwise Differences between log2 ratios per tile for a given run. Tiles roughly correspond to amplicons in an Ion AmpliSeq™ assay. Each pair is defined as adjacent in terms of genomic distance. Tiles corresponding to copy number amplicons and other amplicons are being treated equally as no differences in variability are seen between these types. Then, any two log2 ratios that are adjacent on the genome are a pair. Except at the beginning and the end of a chromosome, every log2 ratio belongs to two pairs.

Formally, if `xi` is the log2 ratio at position `i`, with `i` ordered by genomic position:

```
MAPD = median ( | x(i - 1) - x(i) | )
```

MAPD is a per-sequencing run estimate of copy number variability, similar to standard deviation (SD). If one assumes the log2 ratios are distributed normally with mean 0 and a constant SD, then MAPD/0.95 is approximately equal to SD. However, unlike SD, using MAPD is robust against high biological variability in log2 ratios induced by known conditions such as cancer.

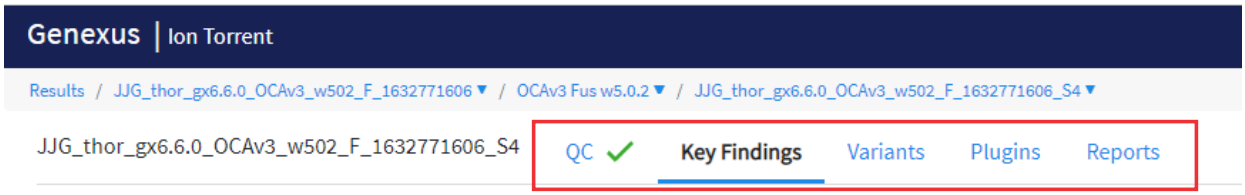
Regardless of the source of the variability, increased variability decreases the quality of CNV calls.

View key findings

You can view the **Key Findings** for a sample starting from either sample results or run results.

In the menu bar:

- Click **Results** ▸ **Sample Results**, then click a sample name.
- Click **Results** ▸ **Run Results**, then click a run name to open the **Results / Run Results** screen. In the **Run Name** column, click a run name to open the **Results** screen, then select a sample from the **Select Sample** dropdown list.



Key Findings

The **Key Findings** table shows details about the sample, a summary of key metrics for the run, and coverage plots for genes assayed in the run.

Note: The **Key Findings** table is by default the first view that is first shown for sample results.

The information that is displayed depends on the assay that was used in the run.

Sample Details

Section	Description
Sample Name	A unique identifier representing the sample.
Collection Date	The date that the sample was collected.
Gender	The biological sex of the sample: **Female**, **Male**, or **Unknown**.
Sample Type	A term that describes the sample, for example, FFPE, DNA, DNA & RNA.
Application Category	The application type of the sample.
Cancer Type	The type of cancer that is represented by the sample.
Cancer Stage	The stage of the cancer from which the sample was collected.
%Cellularity[1]	The percentage of tumor cellularity in the sample. This is a whole number between 1 and 100. The **% Cellularity** attribute is entered when a sample is created. The attribute is applicable to FFPE samples only.

[1] Metric is shown only for some assays.

Key Metrics

Section	Description
Target Coverage[1]	
Target base coverage at *N*x	The percentage of reference genome bases covered by at least *N* reads.
Amplicon Summary	
Average Base Coverage Depth	The average number of reads of all targeted reference bases. This is the total number of base reads on target divided by the number of targeted bases, and therefore includes any bases that had no coverage.
Uniformity Of Base Coverage	The percentage of bases in all targeted regions (or whole genome) with a depth of coverage ≥20% of the mean read coverage. Cumulative coverage is linearly interpolated between the nearest integer base read depths.
Percent Reads On Target	The percentage of filtered reads that are mapped to any targeted region relative to all reads mapped to the reference. A read is considered on target if at least one aligned base overlaps at least one target region. If no target regions (file) was specified, this value will be the percentage of reads passing uniquely mapped and/or non-duplicate filters, or 100% if no filters were specified.

[1] Metrics are shown only for analyses that run the **coverageAnalysis** plugin.

The Key Variants matrix

The **Key Variants** matrix provides a color-coded visual representation of the variant results. When the application category of the sample is **Cancer** or **Cancer (Germline)** the genes that are shown in the **Key Variants** matrix are determined by the filter chain and the gene list applied to the results. You can change the filters that are applied to expand or restrict the genes that are shown. For more information, see “View the Key Variants matrix” on page 139.

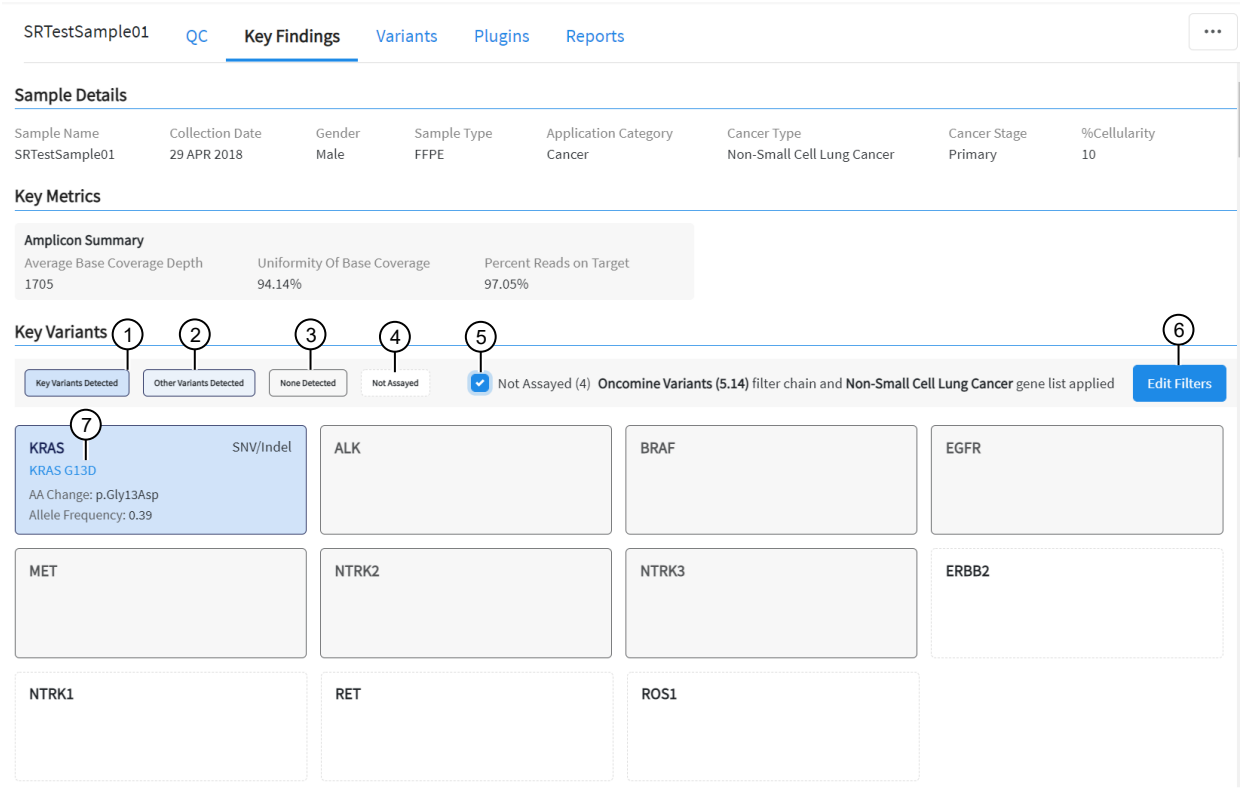
Each tile in the **Key Variants** matrix represents one gene and indicates whether a variant is detected or not. Note that a single gene is represented with multiple tiles when there are multiple mutations detected in that gene.

View the Key Variants matrix

You can view a color-coded visual summary of variant results in Genexus™ Software.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name.
   The **Results** screen opens to the **Key Findings** tab, which shows the **Sample Details**, **Key Metrics**, the **Key Variants**, and the **Coverage Graphs**.
3. Refine the genes that are shown in the **Key Variants** matrix.

Action	Procedure
Hide the genes that are not assayed.	Deselect the **Not Assayed** checkbox.
Refine the genes that are shown in the **Key Variants** matrix.	1. Click **Edit Filters**. 2. To refine or expand the genes, change one or both of the filters. • Select a different filter chain or no filter chain. For more information, see “Filter results” on page 163. • Select a different gene list or no gene list. 3. Click **Save**.



Example **Key Variants** matrix

① **Key Variants Detected** —A gene is present in the gene list, variants are called by the Oncomine™ Variant Annotator, and the variants are **Key Variants**. Genes that are called are listed in the Variants tab with **Present** in the **Call** column. **Key Variants** are listed in the **Variants** tab, with a value of **Yes** in the **Key Variant** column. For more information about gene lists, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

② **Other Variants Detected** —A gene is present in the gene list and variants are called by the Oncomine™ Variant Annotator, but the variants are not **Key Variants**. Genes that are called are listed in the Variants tab with **Present** in the **Call** column. Variants that are not **Key Variants** are listed in the **Variants** tab, with a value of **No** in the **Key Variant** column.

③ **None Detected** —A gene is present in the gene list but no variants are called by the Oncomine™ Variant Annotator.

④ **Not assayed** —A gene is present in the gene list, but is not included in the panel used in the assay.

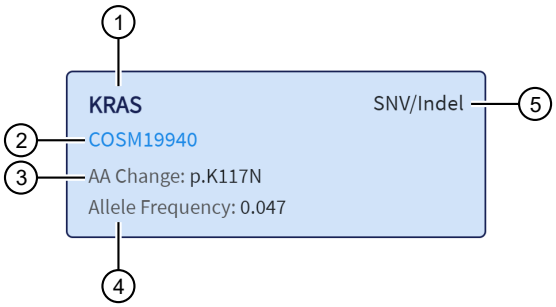
⑤ Checkbox to hide or show genes that are not assayed.

⑥ **Edit filters**—Allows you to select an available filter chain and gene list. Changes of the filter chain or gene list change the genes that appear in the **Key Variants** matrix.

⑦ **Variant Name** —The name of the variant. The **Variant Name** is a hyperlink to the pileup for the variant in the **Variants** tab.

Gene tiles in the Key Variants matrix

Each blue tile in the **Key Variants** matrix represents a variant and summarizes information for the variant.



1. The gene name.
2. The **Variant ID** – link to the variant details in the **Variants** tab.
3. The amino acid change using Human Genome Variation Society (HGVS) nomenclature.
4. The number of variant read counts divided by the total number of read counts for the sample.
5. The variant type.

View the amplicon coverage

You can view DNA exon amplicon coverage in Genexus™ Software to help you determine whether the sequencing reads across a gene are uniform and sufficient. The default genes that are shown are determined by the gene list that is designated as default for the cancer type of the sample. You can also view amplicon coverage graphs for more genes. These amplicon coverage graphs provide a high-level overview of coverage. More detailed coverage information is also available in the software.

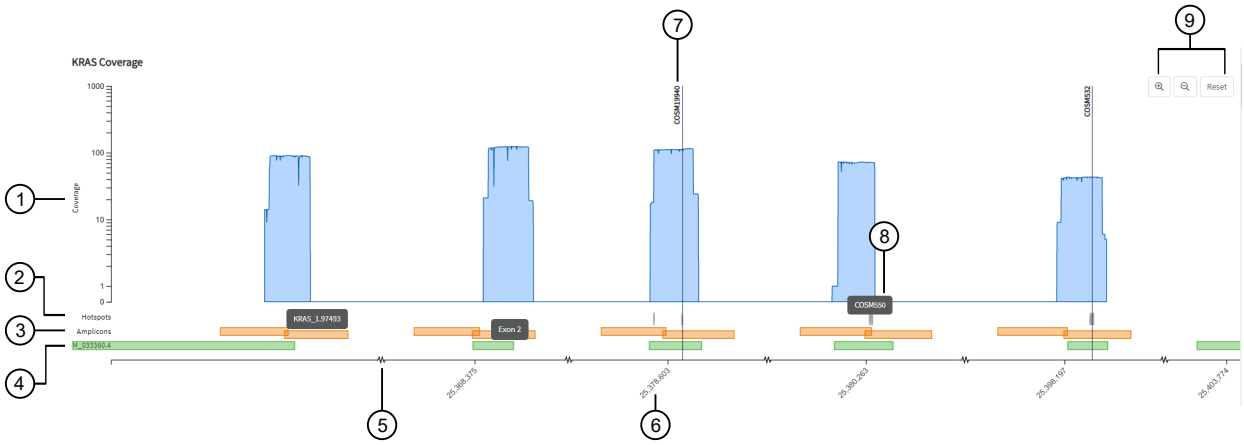
1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name to open the **Key Findings** tab in the **Results** screen.
3. Scroll to the amplicon **Coverage Graphs**.
4. Review the coverage graphs.
5. To show coverage graphs for more genes, scroll to the bottom of the screen. Click **Show Coverage for Gene**, then select the gene of interest.
6. You can adjust the amplicon coverage graph with the pointer and the buttons in the upper right corner of each graph.

Action	Procedure
Zoom in on a region of interest.	Click ⊕ one or multiple times.
Zoom out for an expanded view.	Click ⊖ one or multiple times.
Revert to the default view.	• Click ⊖ one or multiple times. • Click **Reset**.
Move the image left or right in the screen.	After you zoom in, click-drag at any position in the image.

The coverage graphs change to show coverage for the selected gene.

Example amplicon coverage graph

Here is an example of an amplicon coverage graph for the *KRAS* gene.



1. Base coverage is shown on the y-axis on a logarithmic scale.
2. The location of the known hotspots are denoted with gray bars.
3. The location and span of the amplicons are represented with orange bars. You can hover the mouse over an orange bar to view the amplicon name.
4. The location and span of the transcript track is denoted with green bars. You can hover the mouse over the green bar to view the exon number.
5. The line along the x-axis is broken to indicate that the graph is discontinuous to show exons.
6. The genome coordinate position is shown on the x-axis.
7. Called variants are indicated with a vertical line above the blue coverage plot. The variants noted in the graph depend on the filter chain applied to the results in the **Key Variants** matrix. For more information, see “View the Key Variants matrix” on page 139.
8. You can place the pointer over the hotspots that are represented with gray lines to view the hotspot name.
9. You can adjust the amplicon coverage graph. For more information, see “View the amplicon coverage” on page 141.

View variant results

You can view detailed variant results in the **Variants** table. In the default view, all variants are listed. You can also limit the types of variants that are shown and toggle between different variant results: SNVs/Indels, Fusions, and CNVs.

Variant Tables

Variant type	Description
SNVs/Indels	• **SNVs**: missense and nonsense single-nucleotide variants. Multi-nucleotide variants are also included. • **Indels**: insertion and deletion variants. For more information, see “View SNV/Indel results” on page 143.

Variant Tables *(continued)*

Variant type	Description
Fusions	Translocations of genetic material. For more information, see “View fusion results” on page 146.
CNVs	Copy number variations (CNVs) are variations of the number of copies of a given gene. For more information, see “View CNV results” on page 151.

View SNV/Indel results

The **SNVs/Indels** table lists the calls and other information for the SNV and Indel variants that are analyzed in each sample in a run.

To view the **SNVs/Indels** table for a sample, click **Results** ▸ **Sample Results** in the menu bar, then in the **Sample name** column, click the name of the sample of interest. In the **Variants** tab, click **SNVs/Indels** to display the data. To export the data in tabular format, click **Export** in the upper right corner of the screen.

SNVs/Indels table

The data displayed in the **SNVs/Indels** table depends on the assay that was used in the run.

You can filter the results list in the table using filtering tools and filter chains. For more information, see “Filter results” on page 163.

Column	Description
User Classifications	User-defined classification to select from the list. For more information, see “Create and assign variant classifications” on page 162.
Variant ID	The name of the hotspot as defined in the Browser Extensible Data (BED) file. Click the link to view more annotation information.
Variant Name	The name of the variant.
Key Variant	Indicates whether the variant is a key variant. Possible values are **Yes** or **No**. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Locus	The chromosome and position of the detected variant.
Oncomine Variant Class	The type of SNV or INDEL at the locus based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.

(continued)

Column	Description
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • **Gain-of-function**—The altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • **Loss-of-function**—The altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Gene	The gene name.
AA Change	Identification of the amino acid change using Human Genome Variation Society (HGVS) nomenclature.
Ref	The reference base or bases at that locus.
Alt	The alternate base or bases at that locus.
Type	The type of variant that is detected. • **snp** (single nucleotide polymorphism) • **mnp** (multi-nucleotide polymorphism) • **ins** (insertion) • **del** (deletion) • **complex** • **FLT3-ITD**
Call	Indicates the presence or absence of an SNV/Indel variant. When the default filter chain is applied, only the variant calls that are designated with **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants** (VCF) file (see "Results files" on page 172). • **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)**—Indicates a high confidence call that passes all filter thresholds at a given variant position. – When the default filter chain is applied, **PRESENT (HOMOZYGOUS)** or **PRESENT (HETEROZYGOUS)** indicates the presence of the ALT (alternative) allele. – When the **No Filter** option is applied or when viewing the **Variants** (VCF) file, **Present** does not imply the presence of the ALT (alternative) allele. To infer the presence of the ALT allele, see the **Alt** column. • **NO CALL**—Although some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call. • **ABSENT**—Indicates the presence of a variant at a given position that is not targeted by the assay and is not the reference allele.
Call Details	The reason why a variant is reported as **NO CALL**.

(continued)

Column	Description
Phred QUAL Score	The relative probability of either the "reference" hypothesis interval [0,cutoff], or the "variant" hypothesis interval [cutoff,1], Phred-scaled (-10*log10). A higher score means more evidence for the variant call.
Raw Read Depth	Total read coverage across amplicon containing SNV/Indel hotspot locations. Count of chip-level reads aligned at this locus that participate in variant calling.
Effective Read Depth	The number of reads covering the position.
Alt Allele Read Counts	The number of reads containing the alternate allele.
Coverage	The number of total reads at a locus, wild-type + alt allele.
Nuc Change	The position and identity of the nucleic acid change.
Allele Frequency	The number of variant read counts divided by the total number of read counts for the sample.
Allele Frequency (%)	The allele frequency, represented as a percentage.
Allele Ratio	The relative frequency of each allele.
Mut/WT	The ratio of mutant allele to wild-type allele.
Zygosity	Describes whether the variant is homozygous (0) or heterozygous (1).
Filtered Read Coverage	Coverage at the position considering only filtered reads.
Allele read Count	The number of reads detected for the allele. The Allele Read Count filter sets the minimum count of the genotype alleles.
Raw Alt Allele Read Counts	The number of unfiltered reads containing the alternate allele.
PPA	Possible Polyploidy Alleles (PPA). A value of **Yes** indicates variants that are PPA alleles. A value of **No** indicates variants that are not PPA alleles.
P-Value	The probability value for the detection of variant calls.
Mol Depth[1]	The reports number of interrogated DNA molecules containing target. This metric defines the limit of detection at a hotspot position in a particular run and sample. For reference calls, molecular depth provides a measurable metric that serves as confirmation of variant absence among a large number of interrogated molecules. For instance, if molecular depth is ≥1,500, you can have high confidence that no variant is present at ≥0.2% variant allele frequency. If molecular depth is ≥2,500, you can have high confidence that no variant is present at ≥0.1% variant allele frequency.
WT Mol Counts[1]	The number of detected molecules containing the wildtype allele.
Alt Allele Mol Counts[1]	The number of detected molecules containing the alternate allele.

(continued)

Column	Description
Mol Freq %[1]	Molecular frequency percentage. The percentage of alternate allele reads over total reads at the locus.
% LOD[1]	the limit of detection (LOD) of a variant allele expressed as a percentage of the WT allele. LOD is the lowest possible variant frequency in the sample that can be detected by the system with a true positive rate greater than 98% for FFPE samples or 95% for cfTNA samples. LOD is dependent on the molecular read depth at the locus. %LOD is reported when there are no variant calls for the gene.

[1] Column appears only in analyses of Ion AmpliSeq™ HD sequencing data.

View fusion results

You can view calls and other information for the fusions that are analyzed within each sample in a run.

To view the **Fusions** table for a sample, click **Results** ▸ **Sample Results** in the menu bar, then in the **Sample name** column, click the name of the sample of interest. In the **Variants** tab, click **Fusions** to display the data. To export the data in tabular format, click **Export** in the upper right corner of the screen.

Fusions table

The data displayed in the table depend on the assay that was used in the run.

Results in the table can be filtered using filtering tools. For more information, see “Filter results” on page 163.

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see “Create and assign variant classifications” on page 162.
Variant ID	The name of the fusion target as defined in the BED file.
Key Variant	Indicates whether the variant is a key variant. Possible values are **Yes** or **No**. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Locus	The chromosome positions in the reference genome that define the fusion junction.
Oncomine Variant Class	Oncomine variant class annotation that indicates fusion type based on Oncomine™ annotations. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay that is used in the run.

(continued)

Column	Description
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • **Gain-of-function**—the altered gene product has a new molecular function or pattern of gene expression compared to the wild-type gene • **Loss-of-function**—the altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Genes (Exon)	The name of fusion target and representative acceptor and donor exons.
Read Counts	The frequency that the fusion was detected in the sample.
Type	Assay type Fusion, RNA exon variant (exon skipping), RNAExon Tile, Proc Control.
Call	Indicates the presence or absence of a fusion or RNA exon variant. When the default filter chain is applied, only the fusion/RNA exon variant calls that are designated with **PRESENT** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants** (VCF) file (see “Results files” on page 172). • **PRESENT** – indicates a high confidence call that passes all filter thresholds at a given variant position. • **ABSENT** – indicates the absence of a fusion due to a variant call that falls below thresholds. • **NO CALL** – although some evidence for the presence of a fusion exists, the call does not pass one or more filters that are required for a high confidence fusion call.
Call Details	The reason for reporting a fusion as **NO CALL** or **ABSENT**.
Read Counts Per Million	The number of fusion read counts detected per million total reads.
Oncomine Driver Gene	The gene believed to be associated with increased oncogenic properties. The gene is inappropriately activated by the fusion.
Gene Isoform	The name of the fusion target as defined in the BED file.
Mol Cov. Mutant[1]	The median molecular coverage across a fusion amplicon.

(continued)

Column	Description
Imbalance Score[2]	Each fusion gene exhibits a characteristic **Imbalance Score** threshold. Scores that exceed the threshold value indicate a high likelihood of the presence of the fusion in the sample. Imbalance score calculation starts with the normalization of reads in the exon-tiling amplicons of the gene, followed by correction with a baseline that represents expression values in normal samples. Reads for observed imbalance scores come from samples. Baseline scores come from normal, fusion-negative samples and are stored in the exon tile assay baseline file for the assay. **Imbalance score** = Observed imbalance (samples) / Expected imbalance (baseline) where • Observed imbalance = 3' of breakpoint / 5' of breakpoint (in samples) where – 3' of breakpoint is the sum of the normalized reads of amplicons 3' of a predicted breakpoint for the gene in the sample. – 5' of breakpoint is the total normalized reads of the amplicons for the gene in the sample. • Expected imbalance = 3' of breakpoint / 5' of breakpoint (in the baseline) where – 3' of breakpoint is the sum of normalized baseline values of amplicons 3' of the breakpoint for the gene. – 5' of breakpoint is the total normalized baseline values of the amplicons for the gene.
Imbalance P-Value[2]	The statistical significance of measure of imbalance relative to a control gene.
Predicted Break-point Range[2]	The exonic range for predicted fusion break point in exon tiling assays.
Ratio To Wild Type[1]	The molecular ratio for exon skipping assays relative to wild type control amplicons.
Norm Count Within Gene[1]	*(Lung panel only)* Exon skipping assay coverage normalized to molecular coverage of wild type (WT) MET control amplicons.

[1] Column appears only in analyses of Ion AmpliSeq™ HD sequencing data.
[2] Column appears in analyses that use the exon tiling fusion detection method.

View RNA Exon Variants

The **RNA Exon Variant** data view displays a bar graph summary of intragenic exon rearrangements or fusions. The displayed RNA exon variants are defined in the BED file that is associated with an assay. The **RNA Exon Variant** data view is available for all RNA and fusion assays.

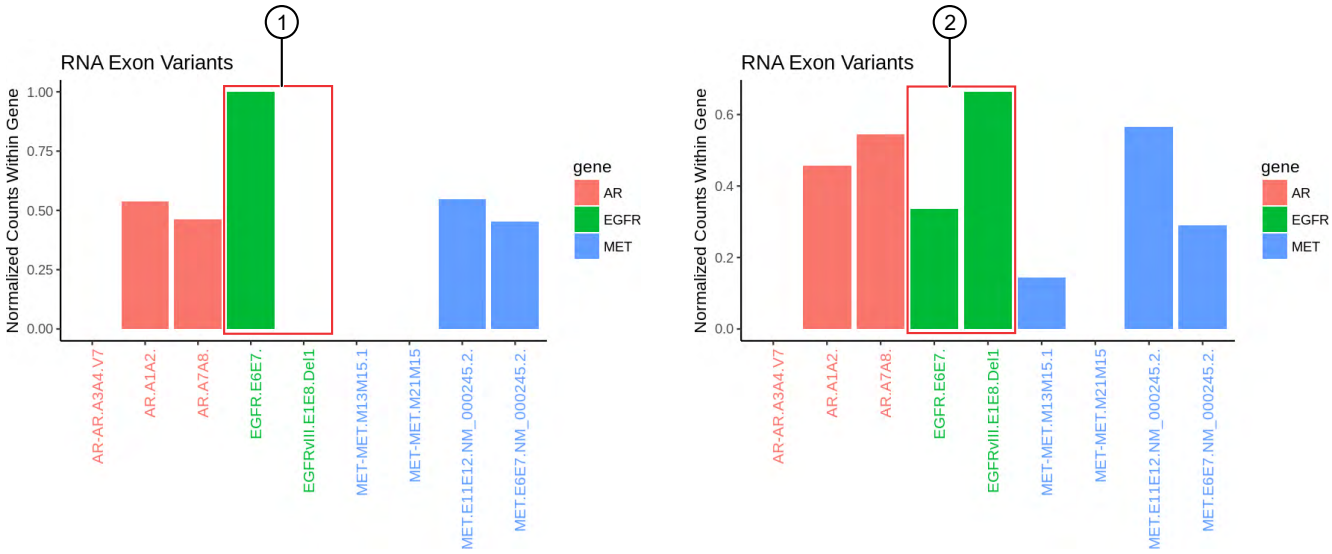
Note: To review **RNA Exon Tile Fusion Imbalance** analysis plots, see the user guide for your assay.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample name** column, click the name of the sample of interest.

3. Click the **Variants** tab, then click **Fusions**.
   The **Fusions** table opens to display fusions results. For more information, see “Fusions table” on page 146.

4. In the top right corner of the screen, click **Visualization** ▸ **RNA Exon Variant**, then review the **RNA Exon Variants** plot.

Representative RNA Exon Variant plots



The X-axis represents specific exon variants, where each variant is labeled with a gene ID followed by a sequence of adjacent exons. The Y-axis measures the read counts for each variant, normalized to the wild type.

(1) Example result where only the wild type EFGR (EFGR.E6E7) was detected.

(2) Example result where RNA exon 2–7 deletion occurred in the EFGR gene. The deletion of exons 2–7 resulted in an increase of normalized read counts for the EFGR variant that contains the intragenic fusion of exon 1 and exon 8 (EFGR.E1E8.Del1) and a decrease of normalized read counts for the wild type EFGR (EFGR.E6E7).

To return to the table view of fusions, click ✕ **(Remove)** next to the **Visualization** dropdown menu.

View RNA Exon Tile Fusion Imbalance

The **RNA Exon Tile Fusion Imbalance** data view provides a visual representation of the RNA fusion imbalance analyses.

Driver genes, for example ALK, RET, NTRK1, have amplicons that span exon-exon junctions to probe the expression difference between the 3’ and the 5’ regions of the gene.

An imbalance score is calculated for each driver gene in a sample to quantify the magnitude of the expression imbalance change between the two parts of the gene. For example, the 3’:5’ ratio in ALK; and the 5’:3’ ratio in FGFR2.

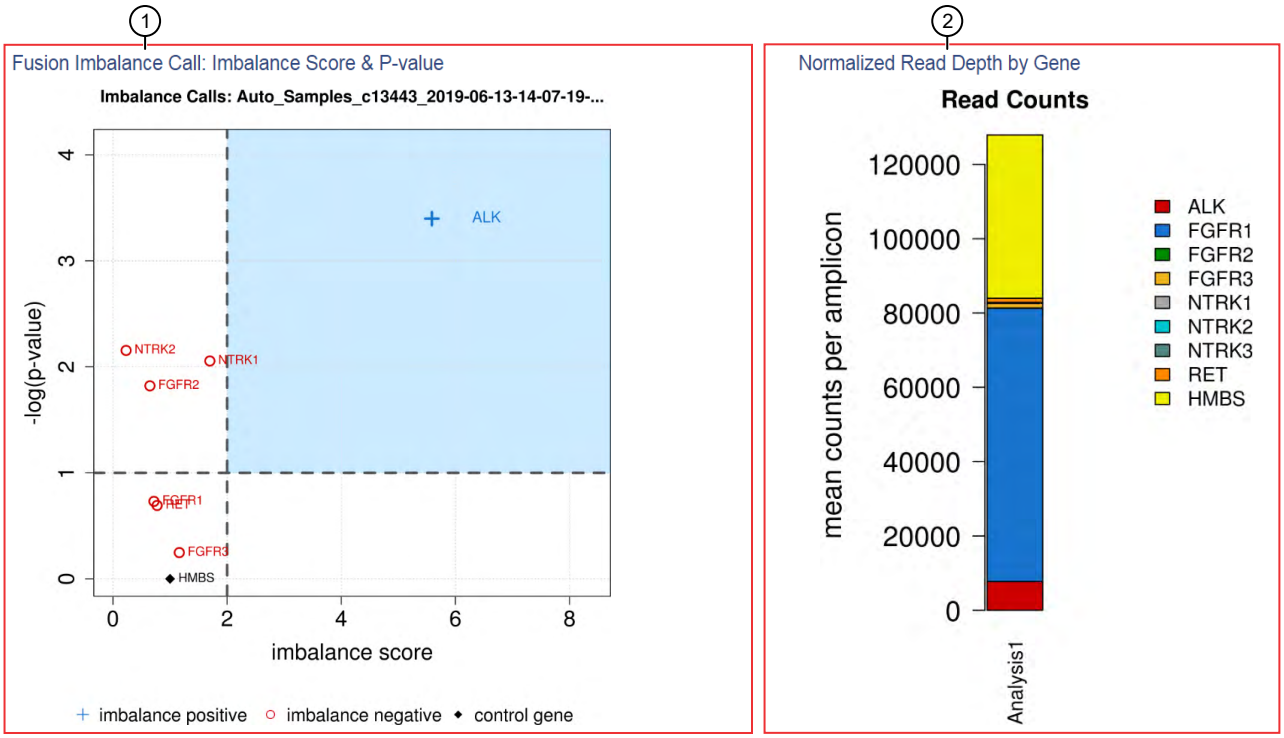
1. In the menu bar, click **Results** ▸ **Sample Results**.

2. In the **Sample name** column, click the name of the sample of interest.

3. Click the **Variants** tab, then click **Fusions**.
   The **Fusions** table opens to display fusions results. For more information, see “Fusions table” on page 146.

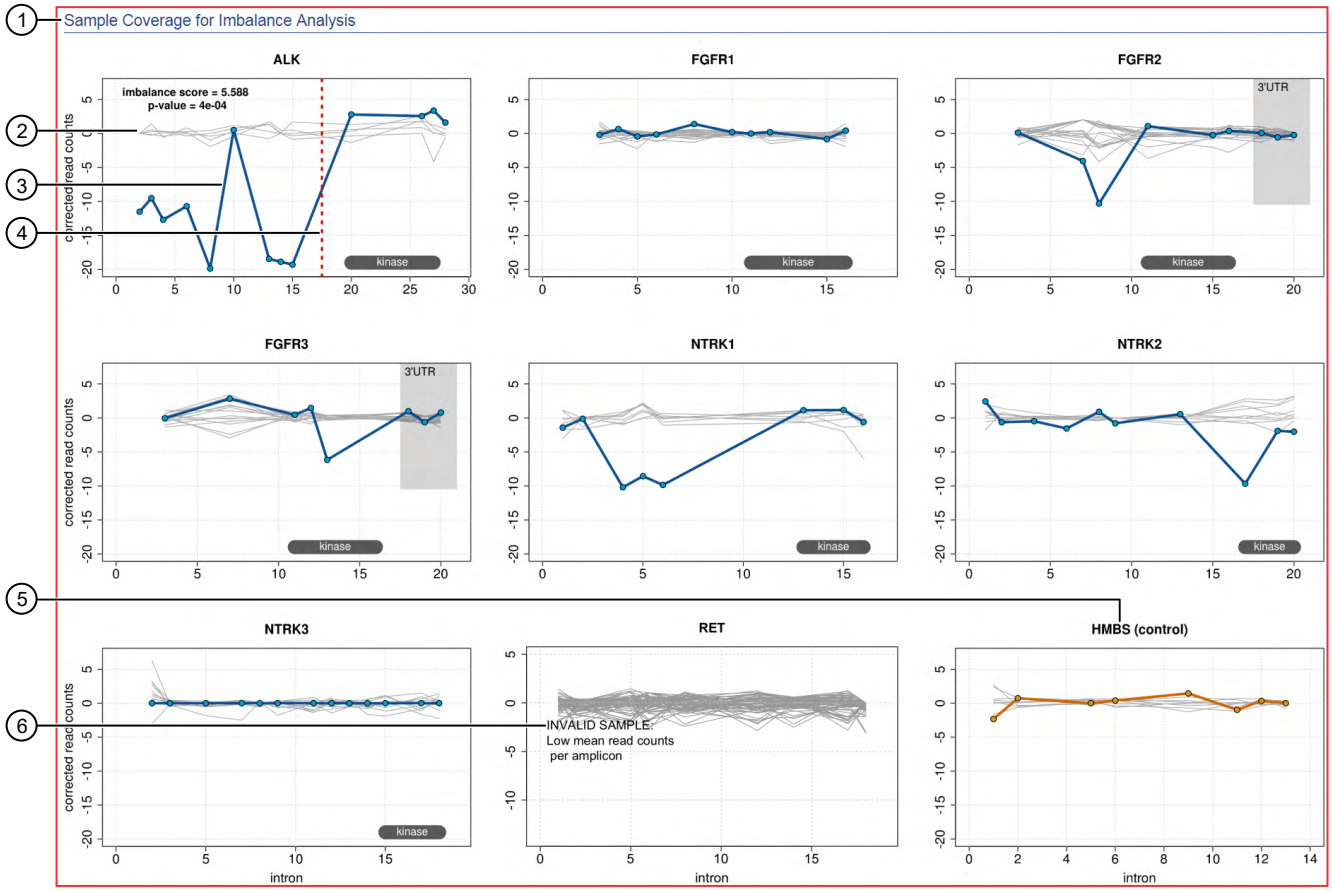
4. In the top right corner of the screen, click **Visualization** ▸ **RNA Exon Tile Fusion Imbalance**, then review the **RNA Exon Tile Fusion Imbalance** plots.

Representative RNA Exon Tile Fusion Imbalance plots



① The **Fusion Imbalance Call: Imbalance Score & P-value** plot shows the imbalance scores and p-values for all the genes in the selected sample. The dashed gray lines mark the threshold for an imbalance call, which is applied to all genes across all samples. Points that fall within the blue shaded area of the plot represent fusion-positive genes (+). All other points that are outside of the blue shaded area represent fusion-negative genes (○). Control genes are marked with ◆.

② The **Normalized Read Depth by Gene** plot shows the mean read counts of each gene that is captured on the chip for the selected sample. For each gene, the read counts are normalized to the number of amplicons.



1. The **Sample Coverage for Imbalance Analysis** plots show the expression profile for each exon-exon tiling amplicon for each gene. The Y-axis represents the corrected molecular counts. The X-axis represents individual exon-exon junctions, which are listed from 5′ to 3′. The **imbalance score** and **p-value** are listed in the panel of each gene that was called positive for fusion.
2. Baseline (a cluster of gray lines), generated from a fusion-negative sample.
3. Test sample corrected read coverage (blue line), normalized to the baseline. Each point on the line represents a unique exon-exon junction that was covered by the assay and normalized to the baseline.
4. Predicted range for the fusion break point for a fusion-positive gene (dashed red line).
5. Sample coverage profile for the control gene (orange line).
6. If the collected data are insufficient to determine an imbalance score, the **INVALID SAMPLE** message appears in the panel for that gene.

To return to the table view of fusions, click ✕ **(Remove)** next to the **Visualization** dropdown menu.

View CNV results

The **CNVs** table lists the calls and other information for the copy number variants (CNVs) analyzed in each sample in a run.

To view the **CNVs** table for a sample, click **Results** ▸ **Sample Results** in the menu bar, then in the **Sample name** column, click the name of the sample of interest. In the **Variants** tab, click **CNVs** to display the data. To export the data in tabular format, click **Export** in the upper right corner of the screen.

CNVs table

The data displayed in the table depend on the assay that was used in the run.

Results in the table can be filtered using the filtering tools. For more information, see “Filter results” on page 163.

IMPORTANT! *(FFPE samples only)* If the **%Cellularity** value for a sample is set to <100, then the magnitude of copy number gain or loss can be decreased.

Column	Description
User Classifications	A user-defined classification selected from the list. For more information, see “Create and assign variant classifications” on page 162.
Variant ID	The identifier of the CNV variant. Click the link to view more annotation information.
Key Variant	Indicates whether the variant is a key variant. Possible values are **Yes** or **No**. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Locus	The starting position of the first amplicon covering the CNV gene.
Oncomine Variant Class	Annotation that indicates whether CNV is an amplification or deletion. This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Oncomine Gene Class	The change in molecular function of the altered gene product due to the mutation, based on Oncomine™ annotations: • Gain-of-function—the altered gene product has a new molecular function or pattern of gene expression, compared to the wild-type gene • Loss-of-function—the altered gene product lacks the molecular function of the wild-type gene This information is available if the **Apply Oncomine Variant Annotations** checkbox is selected in the assay used in the run.
Gene	The gene name.
Copy Number	The copy number of a CNV gene locus per genome. This column is available when a positive call is made.

(continued)

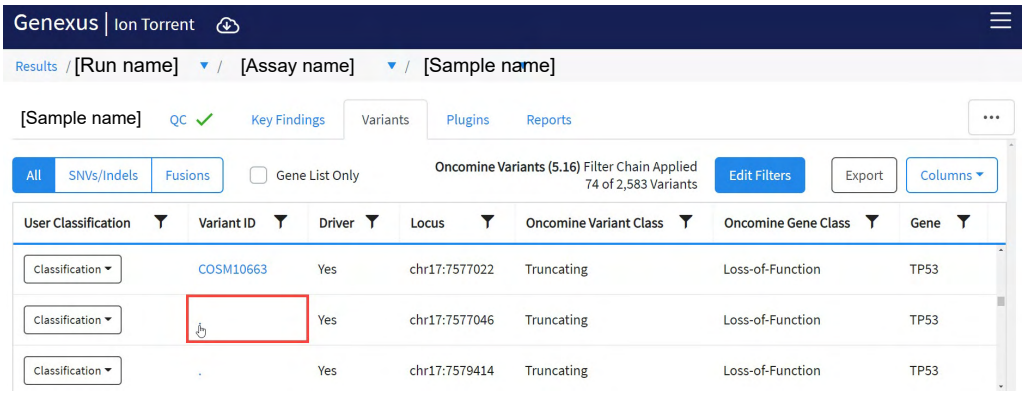
Column	Description
Call	Indicates the presence or absence of a CNV. When the default filter chain is applied, only the CNV-positive calls that are designated with **PRESENT** are displayed in the results table. To view all calls, including calls that do not pass the required filter thresholds, apply the **No Filter** option or download the **Variants** (VCF) file (see "Results files" on page 172). • **PRESENT** —indicates a high confidence call that passes all filter thresholds. • **PRESENT (GAIN)**—a CNV-positive call that indicates gene amplification; a high confidence variant call that passes all filter thresholds. • **PRESENT (LOSS)**—a CNV-positive call that indicates gene deletion; a high confidence variant call that passes all filter thresholds. • **ABSENT**—the absence of a variant; result is below the detection threshold for a CNV call. • **NO CALL**—although some evidence for the presence of a variant exists, the call does not pass one or more filters that are required for a high confidence variant call.
P-Value	The statistical significance of the CNV ratio measurement.
Call Details	The reason for reporting a CNV as NO CALL.
CNV Confidence	The CNV confidence interval associated with the call. The 5% lower confidence bound value is the ploidy estimate, where there is a 5% chance that the true ploidy is below that value. The 95% upper confidence bound is the ploidy estimate, where it is 95% certain that the true ploidy is below that value.
CNV Ratio	The ratio of measured CNV gene locus coverage relative to coverage of non-CNV loci.
Med Read Cov Gene	The median read coverage of targeted CNV gene.
Med Read Cov Ref	The median read coverage of non-CNV reference loci.
Valid CNV Amplicons	The number of amplicons spanning the CNV call.
Type	The type of variant that is detected. • CNV—copy number variant • LOH—loss of heterozygosity
Subtype	The CNV subtype. • BigDel—deletion of at least one exon. • BigDup—duplication of at least one exon. • GeneCNV—whole BRCA1/BRCA2 gene deletion or duplication. • NOCALL—read count differs from baseline by non-integer amount; evidence for a BigDel or BigDup call is weak. • REF—read count matches reference baseline. • ARM—aneuploidy of a chromosome arm.

View more annotations and annotation sources

In addition to the annotations that are included with Genexus™ Software, you can view annotations and annotation source information from public websites for each variant.

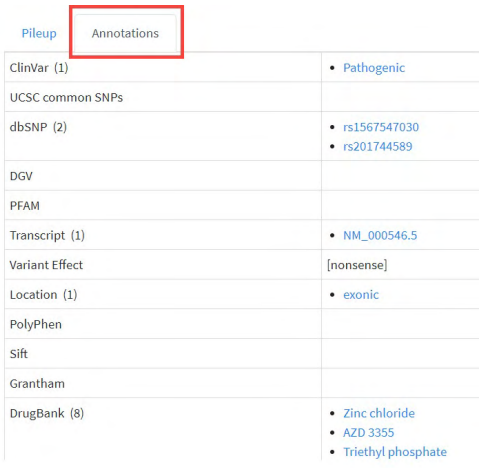
1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click the sample name in the **Sample Name** column in the row of a sample of interest.
3. Click the **Variants** tab.
4. In the **Variants** table, click the **Variant ID** of the variant of interest.

Note: You can click the row even if a **Variant ID** is not listed.



The **Pileup** view is shown below the variants table. For more information on the **Pileup** view, see *Genexus™ Software 6.6 Help*, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

5. Click the **Annotations** tab.
6. Review annotation information in the **Annotations** tab, and if available, click a link in the row of an annotation to open a website with information about the annotation.



Review the variant pileup view

You can review variant tracks with the pileup view in Genexus™ Software. The pileup view is a figure that the software generates to show each BAM track represented by a single bar, with the sequence variation or variations denoted in each track. The BAM tracks are aligned against a reference track. The variant pileup view can be used to identify potential variants.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name to open the **Results** screen.
3. Click the **Variants** tab.
4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.

The pileup view is shown below the variants table.

Change the view of the variant pileup

You can zoom and pan the variant pileup view and add or remove guides and track labels to more easily understand the data. You can also adjust the view of the individual pileup tracks. For more information, see “Adjust pileup tracks” on page 156.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name to open the **Results** screen.
3. Click the **Variants** tab.
4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.

Action	Procedure
Track the position as you place the pointer over the variant pileup.	Click **Cursor Guide**.
Mark the center of the variant pileup.	Click **Center Line**.
Remove the track labels.	Click **Track Labels**.
Zoom in to increase the magnification of the image.	Click ⊕ **(Zoom in)** one or more times, or double-click the image.
Zoom out to decrease the magnification of the image.	Click ⊖ **(Zoom out)** one or more times.
Pan across the image to move the view to another location on the screen with its current magnification.	Click and drag the pointer over the image.

Adjust pileup tracks

You can adjust the BAM read coverage track, the reference track, hotspot tracks, the target region track, and annotation tracks in Genexus™ Software to make it easier to view the data. When you adjust a track, it changes the amount of data that is shown in the plot. For example, you can see more tracks when you use the **Squish** setting.

Note: For best results, click the **Expanded** option to view details about variants and base calls that are shown in each read coverage track

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name to open the **Results** screen.
3. Click the **Variants** tab.
4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.
5. In the **Variants** tab, in the **Pileup** subtab, click ⚙ (Actions) next to a track, then select an option to adjust the view of the track.

Option	Description
Expanded	Select this option to view the tracks with the maximum visible height.
Squish	Select this option to view the tracks with the minimal height for each track.
Collapse	Select this option to view the tracks with overlapping transcripts shown along a single line. When **Collapse** is selected for BAM tracks, the BAM tracks are not shown; only the coverage density is shown.

Adjust the view of the reference track

You can adjust the view of the reference track in Genexus™ Software to view or hide the three-frame translation of the reference track sequence. You can also view the forward or reverse reference track sequence.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name to open the **Results** screen.
3. Click the **Variants** tab.
4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.

5. In the **Variants** tab, in the **Pileup** subtab, click ⚙ (Actions) next to the reference track.

Option	Description
View the reverse strand	Click **Reverse**. This option is available only when the forward strand is shown.
View the forward stand	Click **Forward**. This option is available only when the reverse strand is shown.
View the reference sequence translation	Click **Three-Frame Translate**. This option is available only when the amino acid sequence is not shown.
Hide the reference sequence translation	Click **Close Translation**. This option is available only when the amino acid sequence is shown.

Adjust the view of the BAM tracks

You can adjust the view of the BAM tracks in Genexus™ Software to dynamically customize the view of the data.

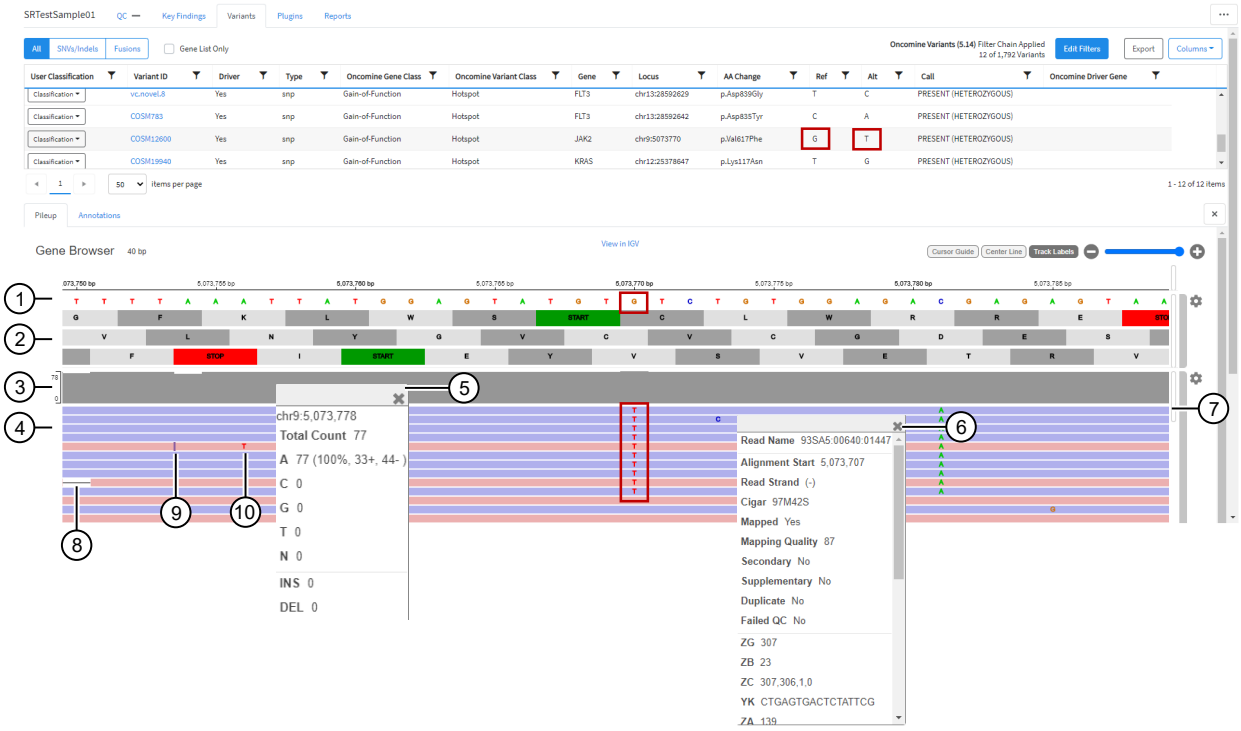
1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name to open the **Results** screen.
3. Click the **Variants** tab.
4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.

5. In the **Variants** tab, in the **Pileup** subtab, click (Actions) next to the BAM tracks.

Option	Description
View all BAM tracks in monochrome gray	In **Color By**, deselect the checkmark next to **Read Strand**. This option is available only when the BAM tracks are colored by read strand.
Distinguish between the direction of the BAM tracks with color	In **Color By**, click **Read Strand**. This option is available only when the BAM tracks are not colored by read strand.
Expand, squish or collapse the BAM tracks	For more information, see "Adjust pileup tracks" on page 156.
Show all the bases in the BAM tracks	Click **Show all bases**. This option is available only when all bases in the BAM tracks are not shown.
Change the minimum threshold for the visibility of BAM tracks	1. Click **Set visibility window**. 2. In the **Set visibility window** dialog box, enter the minimum number of bases needed to show the BAM tracks. When you zoom in and out to view the variant pileup, the scale of the tracks shown in the screen changes. For more information, see "Change the view of the variant pileup" on page 155. You can adjust the visibility window according to the scale shown in the screen.

Examples of a variant pileup view

The following figure is an example of a variant pileup view, shown in the **Gene Browser** in the **Variant** tab. The variant pileup view graphically represents the sequencing tracks, the reference track, and the annotation tracks, and can help you understand the variant data and analyze results. The reference allele and the alternate base for the selected variant are indicated in the image, both in the variant table and the in the pileup view. You can customize your pileup view. Reorder the tracks displayed by clicking a gray bar on the right edge of a track, then dragging the track to a different location. You can also click-drag the cursor at any position in the image to move the image left or right in the screen. For more information, see "Adjust pileup tracks" on page 156.



① The reference track. When zoomed out, the nucleotide sequence is no longer displayed, but the sequence is represented by colored bars.

② The three-frame translation is shown in this example (not by default). For more information, see “Adjust the view of the reference track” on page 156.

③ The coverage track.

④ The BAM track.

⑤ Click the coverage track to see detailed information, such as the total count, the number of reads, and the number of molecules.

⑥ Click a BAM track to see detailed information, such as the **Mapping Quality** and **Base Quality**.

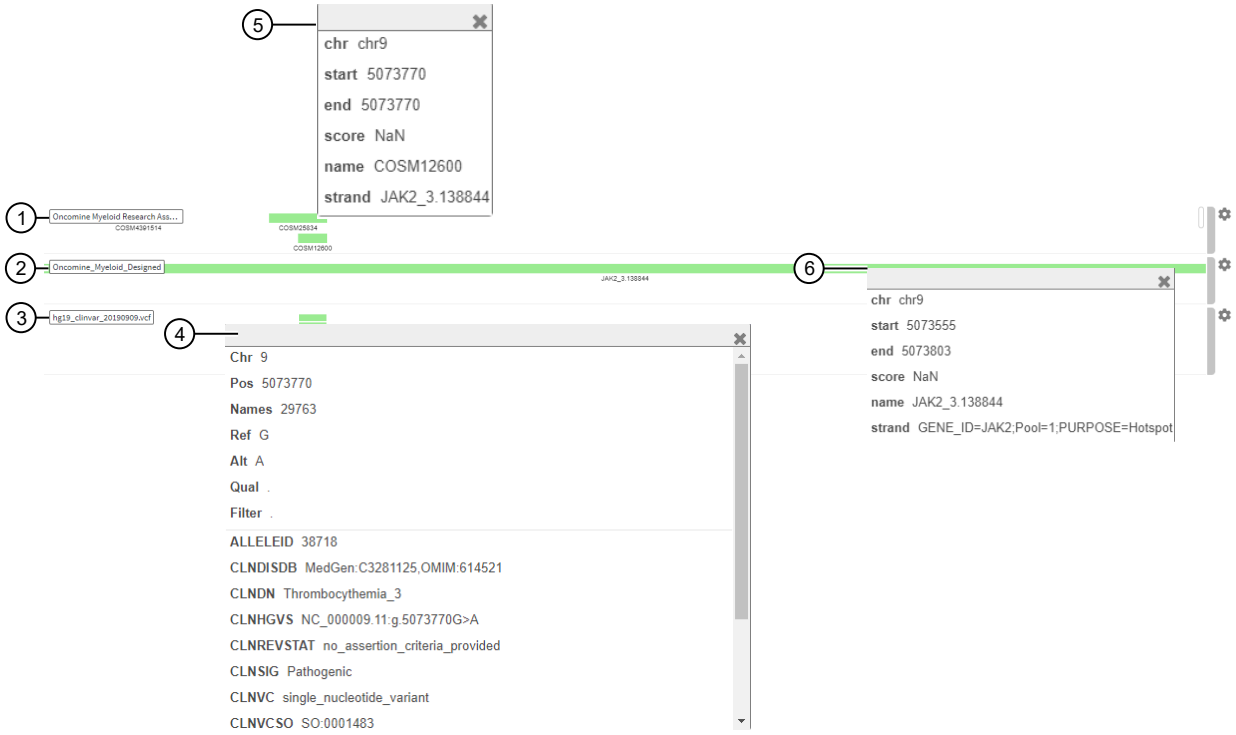
⑦ Use the sliders to view more tracks.

⑧ Deletion.

⑨ Insertion.

⑩ Alternate base in the BAM read coverage track.

The following figure is an example of the reference tracks and annotation tracks shown beneath the BAM tracks in the **Gene Browser**.



① The hotspot track.
② The target region track.
③ Annotation tracks (COSMIC and ClinVar). In this example, only ClinVar is shown.
④ Click the annotation track for additional details; for example the CLNHGVS (the top-level genomic HGVS expression for the variant).
⑤ Click a hotspot track for additional details, such as the start and end positions.
⑥ Click the target region track for additional details, such as the start and end positions.

Coverage histogram

When you zoom in on the pileup view, you see the coverage histogram and reads from the .bam track. The histogram in gray shows read depth at that location and reads from the .bam track show read direction and location of variants.

① Read depth
② Read location and direction of variants

View the variants from an Ion AmpliSeq™ HD chemistry run

You can view a summary of data about the identified variants, and toggle to other views that provide more details about the same variants.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name to open the **Results** screen.
3. Click the **Variants** tab.
4. In the variants table, in the **Variant ID** column, click the identifier of the variant of interest.
5. Review detailed variant data and read coverage tracks.

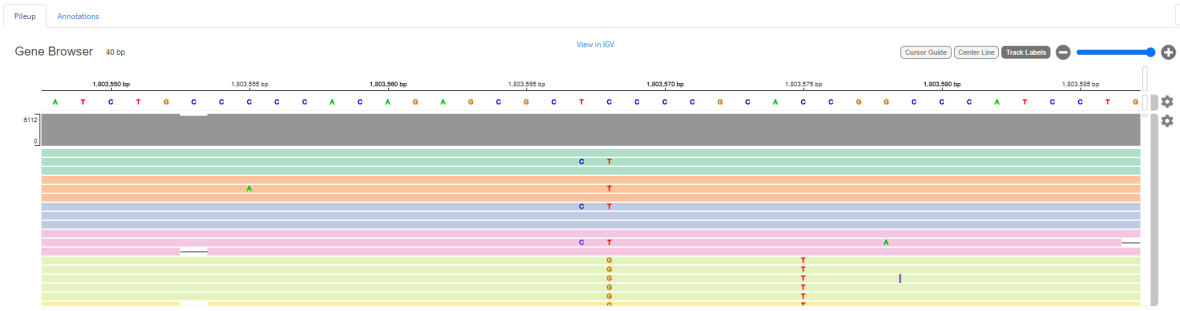


Figure 1 Example visualization

Ion AmpliSeq™ HD sequencing runs group consensus reads into families. A family is a group of reads that are associated with the same DNA molecule before library amplification. Each family is identified using the molecular tags, and consensus reads with the same molecular tags are grouped into the same family. The color of the consensus reads is used to indicate a family.

Within each read track, each nucleotide variant is indicated by a different color. T, A, C, and G are red, green, blue, and orange, respectively. An "I" denotes insertion, and white color with a dash indicates deletion.

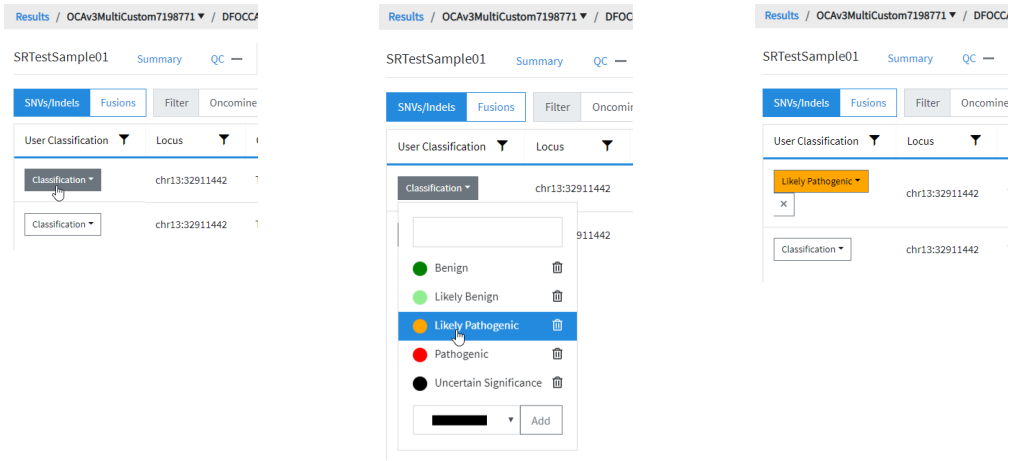
6. You can sort, adjust, and view details about variants and base calls that are visualized in each read coverage track.

Option	Description
View the read coverage tracks sorted by family	Click ⚙ **(Actions)** next to the read coverage track, then select **Color** ▸ **Molecule**.
Adjust the BAM tracks	Click ⚙ **(Actions)** next to the read coverage track, then select an option to adjust the view of the track. For more information, see “Adjust pileup tracks” on page 156.
Review detailed data about a BAM read	Single-click the read track to get information such as the mapping quality, the strand, and the read base.
Review distribution of base calls at a selected position	Click the density plot (the gray bar at the top of the read coverage tracks) to view information about the total count, total reads, and total number of molecules, the distribution of single nucleotides at that position, and the number of insertions and deletions.

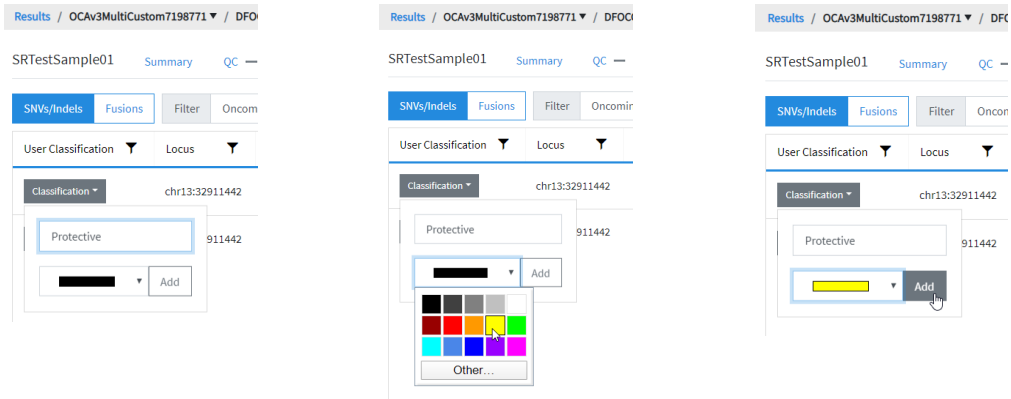
Create and assign variant classifications

You can create and assign user-defined variant classifications in the SNVs/Indels, Fusions, and CNVs tables in the **Variants** tab of the **Results** screen.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name in the **Sample Name** column in the row of a sample of interest to open the **Results** screen for the sample.
3. Click the **Variants** tab.
4. To refine the list of variants shown in the table, select a variant type to display: SNVs/Indels, Fusions, or CNVs.
5. In the **Variants** table, in the **User Classification** column, perform any of the following actions.
   - To assign an existing classification to a variant, select it from the list. The **Classification** menu changes to the name of the classification, as shown in this example.



   - To create a new classification, enter a name for the classification in the text box, select a color for the new classification, then click **Add**.



   - To remove a classification from a variant, click ✕ **(Remove)**.

- To delete a classification from the list, click **Delete** next to the classification name. The classification will be removed from all variants in all results.

Filter results

You can filter results in the **SNVs/Indels**, **Fusions**, and **CNV** tables in the **Variants** tab of the **Results** screen in two ways. You can apply filters to columns of information that appear in the screen. The filters, available at the top of each column, immediately narrow the list of information in any columns to which filters are applied.

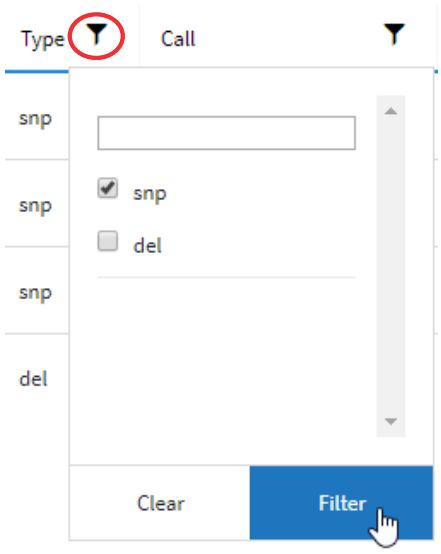
You can also apply a filter chain, a set of filters that Genexus™ Software uses to narrow the list of variants that are included in results. A manager-or administrator-level user creates filter chains from system-installed filters.

Search and filter the list of variants

You can search and filter to immediately narrow the list of results that are shown in the variant tables. You can apply filters to columns of information that appear in the screen. The filters, available at the top of each column, immediately narrow the list of information in any columns to which filters are applied.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. Click a sample name in the **Sample Name** column.
3. In the **Results** screen, click the **Variants** tab.
4. Select the variant class to display the results: **SNVs/Indels**, **Fusions**, or **CNVs**.
5. In the table of variants, in the column header of interest, click **(Filter)**.
   - In the search field, enter at least 3 characters, then click **Filter**.
   - Select the checkbox in the row of each filter that you want to apply, then click **Filter**.

   The options that are available depend on the column and variant class. For example, you can filter data in the **Type** column to show one specific variant type.
6. Click **Clear Filters** to remove all filters and view the full list of run results.



The column or columns to which you applied a filter change to reflect the filter and selected options.

Filter results with a filter chain

You can filter the results that are listed in the **Results** screen with a system-installed or a custom-designed filter chain. A filter chain is a set of filters that Genexus™ Software uses to narrow the list of variants that are included in results.

Select a filter chain to change the list of variants that are included in the results. You can apply the filter chain temporarily, then review the results before you decide whether to save the updated results, or discard the changes.

If you save the filter chain to a result, the variants that are included reflect the filtered results when the results are later opened.

For information about system-installed filter chains, and how manager- and administrator-level users can create custom filter chains, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, select a sample of interest.
3. In the **Results** screen, click the **Variants** tab.
4. To refine the list of variants shown in the table, select a variant type.
   - **SNVs/Indels**
   - **Fusions**
   - **CNVs**
5. Above the variant table in the **Results** screen, click **Edit Filters**, then select a filter chain.
   - Click **Edit** to make changes to a draft filter chain, if needed.
   - Click **Copy** to copy, then edit the selected filter chain, if needed.

   The list of results changes to reflect the selected filter chain. If you made edits to the filter chain, all sample results to which the edited filter chain is applied are also updated with the changes.
6. Click **Save** if you want the filter chain to be applied to the results when the results are later opened. The filter chain is selected and applied to the results when the results are reopened.

Variant report

The variant report is a PDF report of the results for each sample in a sequencing run. You can use a system-installed report template. For system-installed report templates, the assay used in the run determines the data that are included in the report.

Alternatively, you can customize the layout and contents of a variant report.

To automatically generate a variant report for each sample during data analysis of a run, enable **Generate Report** in the **Setup** step when you plan the run (for more information, see Chapter 6, "Plan a run"). To generate a variant report for each sample after a run is complete, see "Generate a variant report" on page 170.

When a variant report has been generated for a sample, it is available for download in two places:

- A link is available in the **Results / Sample Results** screen when you place the pointer over the row for that sample. Click the link to download the PDF.
- A **Download Report** button is available in the **Variant Report** pane in the **Reports** tab.

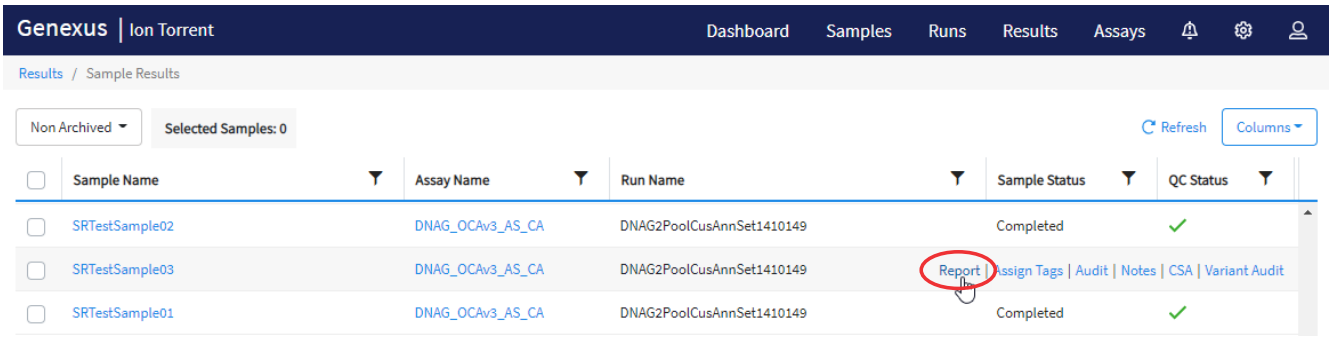
Variant reports can be electronically or manually signed by users. Electronically signed reports have **(Sign off)** after the sample name in the **Sample Results** screen. The electronic signature is included in the footer of the report. If included, electronic signatures also appear in the Electronic Signature section of the report. A sample name followed by indicates that the variant report is locked. For more information, see "Sign off on the run results" on page 169.

Download a variant report

You can download a variant report for a sample result of interest from the **Results / Sample Results** screen.

Note: The variant report is also available for download as part of the results files in the **Results** screen for a specific sample. For more information, see "Results files" on page 172.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, place the pointer over the row of the sample of interest, then click **Report**.



A ZIP file that contains the PDF report downloads automatically.

3. Extract the downloaded files, then open the PDF file in an appropriate viewer.

Reanalyze a run

If a sequencing run fails to meet one or more QC parameters defined by the assay, you can adjust the assay parameters and reanalyze a run. For more information, see “Copy an assay (manager/administrator)” on page 58.

Reanalysis of runs can start from the alignment, basecalling, or signal processing steps. When you reanalyze a run, the reanalysis is applied to all samples in the assay.

Reanalysis from the	Description
Signal processing step	The option to reanalyze a run from the signal processing step is available only when the following conditions are met. • When a run fails during the basecalling step or earlier. • When the run has not already been successfully reanalyzed. Reanalysis at the signal processing step uses DAT files.
Basecalling step	The option to reanalyze a run from the basecalling step is available only when a run or reanalysis successfully completes the signal processing step. Reanalysis at the basecalling step uses .wells files.
Alignment step	The option to reanalyze a run from the alignment step is available only when a run or reanalysis successfully completes the basecalling step. Reanalysis at the alignment step uses BAM files.

Note:

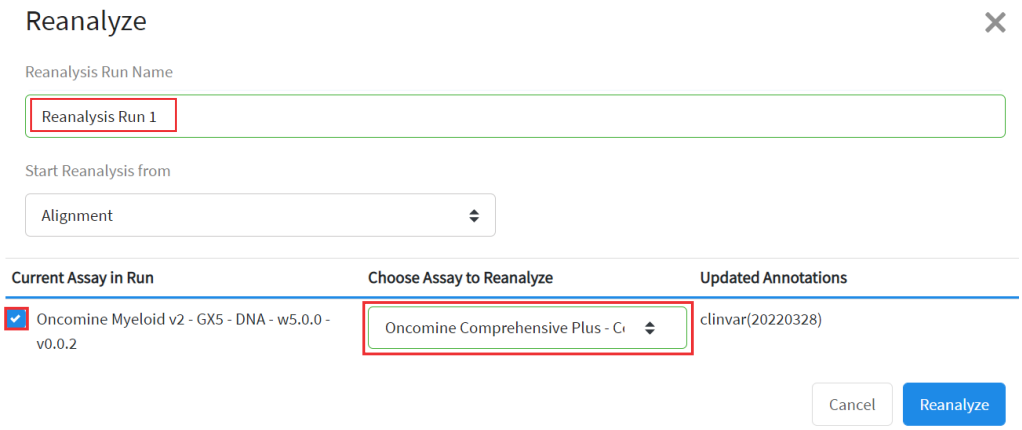
- Manager- and administrator-level users can reanalyze a sequencing run only if the run completed without any critical alarms or errors. If the run aborted or produced major alarms or errors, the run cannot be reanalyzed.
- QC parameters at the limits of stringency cannot be adjusted further. If samples fail QC and you cannot adjust the QC parameters further, you can sequence the sample library again. Alternatively, you can prepare a new library for sequencing.

The files needed for the stage of reanalysis that you select must be present in the software. For example, if the .wells files for the run have been removed from the software, you cannot reanalyze from basecalling. Instead, reanalyze the BAM files at alignment. Administrator-level users can manage the settings and schedule to backup and delete files and data. For more information, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

You can reanalyze a run with any compatible assay that exists in the software. Alternatively, you can create a new assay or copy the original assay that was used in a run and modify assay parameters if needed. For more information, see Chapter 4, “Create and manage assays (manager/administrator)”.

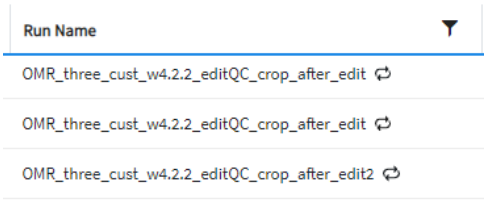
1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Results / Run Results** screen, in the **Run Name** column, click the run name of interest.
3. In the upper right corner of the screen, click ··· **(More Options)** ▸ **Reanalyze**.

4. In the **Reanalyze** dialog box, enter or select the following information.
   a. In **Reanalysis Run Name** field, enter a reanalysis run name.
   b. In **Start Reanalysis from** dropdown list, select **Alignment**, **Basecalling** or **Signal Processing**.
   c. In the **Current Assay in Run** column, select the checkbox in the row of each assay that you want to reanalyze, then in the **Choose Assay to Reanalyze** column, select an assay that you want to use for each reanalysis from the dropdown list.



5. Click **Reanalyze**.

Follow the progress of the reanalysis in the **Results / Run Results** screen in the **Run Status** column, and in the **Results / Sample Results** screen in the **Sample Status** column. When reanalysis is complete, the new results can be viewed by clicking the run name corresponding to the reanalysis assay in the **Results / Run Results** screen. Runs that have been reanalyzed are appended with ⟲ after the run name.



Reanalyze a sample

If a sample fails to meet one or more QC parameters defined by the assay, you can adjust the assay parameters and reanalyze a sample. A sample can be reanalyzed starting only from the alignment step. You can reanalyze all samples in a run from the basecalling or signal processing steps. For more information, see “Reanalyze a run” on page 166.

Reanalysis at the alignment step uses BAM files.

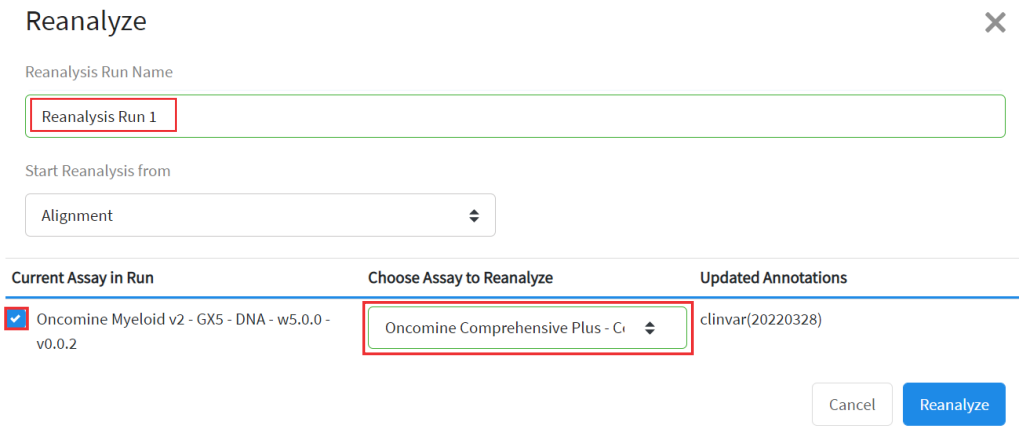
Note: Manager- and administrator-level users can reanalyze a sample only if the run completed without any critical alarms or errors. If the run aborted or produced major alarms or errors, the sample cannot be reanalyzed.

The option to reanalyze a sample from the alignment step is available only when a sample or reanalysis successfully completes the basecalling step.

You can reanalyze a sample with any compatible assay that exists in the software. Alternatively, you can create a new assay or copy the original assay that was used in a run and modify assay parameters if needed. For more information, see Chapter 4, “Create and manage assays (manager/administrator)”.

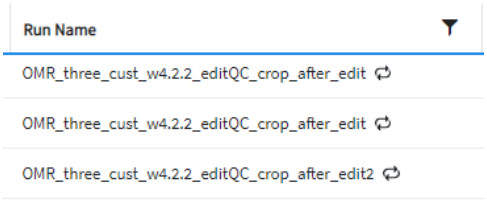
1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, place the pointer over the row of a sample of interest, then click **Reanalyze**.

   Alternatively, you can reanalyze a sample when you click a sample name, then click ··· **(More Options)** ▸ **Reanalyze**.
3. In the **Reanalyze** dialog box, enter or select the following information.
   a. In **Reanalysis Run Name** field, enter a reanalysis run name.
   b. In the **Current Assay in Run** column, select the checkbox in the row of each assay that you want to reanalyze, then in the **Choose Assay to Reanalyze** column, select an assay that you want to use for each reanalysis from the dropdown list.



4. Click **Reanalyze**.

Follow the progress of the reanalysis in the **Results / Sample Results** screen in the **Sample Status** column. When reanalysis is complete, the new results can be viewed by clicking the sample name corresponding to the reanalysis assay in the **Results / Sample Results** screen. Samples that have been reanalyzed are listed in the table of sample results with the run name appended with ↻.



Sign off on the run results

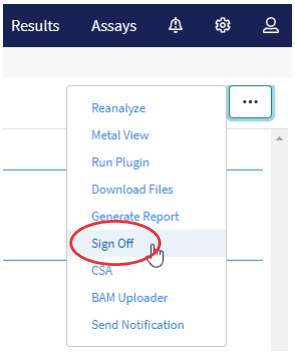
Manager- and administrator-level users can provide their electronic signature on sample results for completed runs. In the **Results / Sample Results** screen, a sample name followed by ✎ indicates that a manager- or administrator-level user has approved the sample results. The signature information appears in the variant report PDF file or a user-created report, if selected. For more information, see “Variant report” on page 165. For information on how to create a report template, see the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953), or the software help system.

Multilanguage support for PDF report generation is provided.

This feature allows you to meet Title 21 CFR Part 11 of Federal Regulations that establishes the United States Food and Drug Administration regulations on electronic records and signatures, password policies, and user activity auditing.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, click the sample result of interest.
3. In the upper right corner of the screen, click ··· **(More Options)** ▸ **Sign Off**.

   The **Sign Off Report** dialog box opens.



4. In **Password**, enter the password.
5. In the **Electronic Signature** dropdown list, select the meaning of the signature.
6. In the **Report Template** dropdown list, select the report template that you want to use.

   The option to select the report template is available only before the variant report has been signed by any user.
7. In **Sign Off Comments**, enter a comment.

8. In **Report Name**, change the name of the report if needed.

   The option to change the report name is available only before the variant report has been signed by any user.

9. In **Select Your Language**, change the language of the report if needed.

   The option to change the language is available only before the variant report has been signed by any user.

10. In **Footer Field**, enter any text that you want to appear in the footer of the PDF report pages.

    If you entered footer information in the **Footer Field** when you created a report template, the same footer information appears in the **Electronic Signature** dialog box. You can enter new footer information to override the report template.

11. Click **Sign Off** to confirm the electronic signature.

The report is signed. If the signature is designated to lock the report, the variant report is signed and locked.

For information to download the signed report, see “Download a variant report” on page 165.

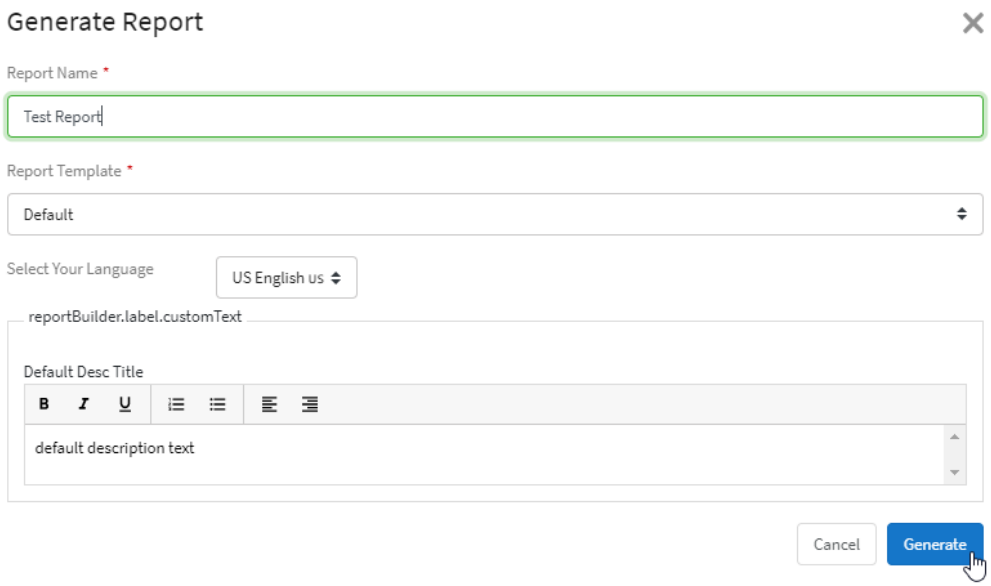
Generate a variant report

When generating a customized report, you can update any report template selections.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Results / Sample Results** screen, click the sample of interest in the **Sample Name** column.
3. Click the **Reports** tab.
4. In the **Variant Report** pane, click **Generate Report**.
5. In the **Generate Report** dialog box, change the name of the report that is generated by the software, if needed, enter an optional description, and select the report template and language of the report.
   a. If the report template includes an image from the results, select the images to include in the report.
   b. If the custom text that was designated as **Editable on Report Generation** when the report template was created, enter text if needed.

   The **Report Template** list includes the report templates that are associated with the assay that was used in the run. For information on creating report templates, see the software help system, or the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).

If you have previously generated a variant report that has not been locked and then you select the same report template that was used to generate the variant report, the new report and any new selections you make override the previous variant report.



6. Click **Generate**.
   A ZIP file that contains all the selected reports and other files is downloaded.

Reports that have been generated are available for download in the **Reports** tab and in the **Sample Results** screen.

Download results files

You can download results files in Genexus™ Software.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Name** column, click the sample name of interest.
3. Click ··· **(More Options)** ▸ **Download Files**.
4. In the **Download Files** dialog box, select the files to download, then click **Download**.

The selected results files are downloaded in one ZIP folder. For information about the files, see “Results files” on page 172.

Results files

The following files can be downloaded from the **View Results** screen for each sample. The files that are available for download vary depending on the assay used. Results files include the sequencing data, results from the analyses, such as variant files, and audit and log files.

For a list and descriptions of plugin output files, see "Output files generated by the coverageAnalysis plugin" on page 175.

For instructions to download results files, see "Download results files" on page 171.

Option	File name	Description
Variants		
Filtered Variants (.vcf)[1]	`<Filter name>_filtered.vcf`	Summary of filtered variant results in variant call format (VCF).
All Variants (.vcf)[1]	`Oncomine_<LibPrepID>_ <analysisID>.vcf`	Summary of variant results in variant call format (VCF).
Variant Summary (.tsv)	`Summary.tsv`	File that lists SNV/INDEL, copy number, and fusion results in tab-separated value format (TSV).
Snvindel (.tsv)	`Snvindel.tsv`	File that lists SNV/INDEL variant results in tab-separated value format (TSV).
Fusion (.tsv)	`Fusion.tsv`	File that lists fusion results in tab-separated value format (TSV).
CNV (.tsv)	`Cnv.tsv`	File that lists copy number variant results in tab-separated value format (TSV).
Sequencing Results		
DNA Unmapped Bam File (.bam)	`<barcode> _rawlib.basecaller.bam`	Unmapped DNA barcode BAM file; output after mapping reads to reference.
DNA mapped bam file (.bam)	`merged.bam`	Mapped BAM file of combined barcode reads.
DNA Mapped Bam Index File (.bai)	`merged.bam.bai`	Mapped BAM Index file.
DNA Basecaller FASTAQ File (.fastq)	`<barcode>_rawlib. basecaller.fastq`	FASTQ file of the DNA barcodes used.
DNA Processed Bam File	`merged.bam.ptrim.bam`	Mapped BAM file of combined barcode reads.
DNA Processed Bam Index	`merged.bam.ptrim.bam.bai`	Mapped BAM index file.
RNA Unmapped Bam File (.bam)	`<barcode>_rawlib. basecaller.bam`	Unmapped RNA BAM file; output of base calling, contains unmapped reads.

(continued)

Option	File name	Description
RNA Mapped Bam File (.bam)	`<barcode>_rawlib.basecaller_alignments.bam`	Mapped BAM file of combined barcode reads.
RNA Mapped Bam Index File (.bai)	`<barcode>_rawlib.basecaller_alignments.bam.bai`	Mapped BAM index file.
RNA Basecaller FASTAQ File (.fastq)	`<barcode>_rawlib.basecaller.fastq`	FASTQ file generated from the unmapped BAM file of the RNA barcodes used.
Test Fragment Basecaller FASTAQ File (.fastq)	`rawtf.basecaller.fastq`	FASTQ file for the test fragment.
Audit and Log		
Analysis Log File	`analysis.log`	Analysis log file.
Run Summary[2]	`Info.csv`	Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary.
Run Audit	`PlannedRun-AuditTrail.pdf`	Contains all audit records pertaining to the run.
Reports		
Report	`<language>_<sampleName>_<mode>_<templateName>_<assayName>_<date>.pdf`	A PDF report that contains sample-specific results. For more information, see “Variant report” on page 165.
Sample Summary[3]	`Info.csv`	Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary.
Troubleshooting Files		
Log Files[4]	`analysis.log`	Analysis log file.
	`summary.<timestamp>.log`	Start and end time for each time an assay module is executed for the analysis.
	`various.err` `various.out`	Analysis pipeline logs used by field service engineers for troubleshooting.

(continued)

Option	File name	Description
Log Files[4]	`Info.csv`	Contains information about the run and analysis, including software version details, sample details, library details, run details, assay details, reagent and consumable information, run and sample QC metrics, and instrument summary.
	`PlannedRun-AuditTrail.pdf`	Contains the audit trail of the run plan in PDF format.
Other[4]	`analysis.ini` `analysisSamples.json`	Analysis configuration files, including secondary and tertiary INI files.
VCF Files	`analysis.vcf`	Summary of variant results in variant call format (VCF).

[1] You can view the extracted files individually, or upload a VCF file to a software application that accepts VCF files, such as Ion Torrent™ Oncomine™ Reporter software.
[2] Files are available only for assays from Genexus™ Software version 6.2 and earlier.
[3] Files are available only for custom and system installed assays in Genexus™ Software version 6.6.
[4] Separate folders are generated for each sample. If included in the run, separate folders are also generated for an NTC and positive control.

Review coverageAnalysis plugin results

The coverageAnalysis plugin generates a Coverage Analysis Report. This report includes read statistics and several charts. The statistics and charts that are presented depend on the library type for the analysis.

The report summary lists the barcode, the sample, the number of mapped reads, the percentage of on target reads, mean base coverage depth, and base coverage uniformity. Microsoft™ Excel™-compatible reports are also generated, including differential expression tables. Additional details regarding read coverage are also provided on a per-barcode basis, along with a list of gene annotations for each sequenced region.

You can download coverageAnalysis plugin output files from the **Results** screen for a sample. For more information, see “Results files” on page 172 and “Output files generated by the coverageAnalysis plugin” on page 175.

1. In the menu bar, click **Results** ▸ **Sample Results**.
2. In the **Sample Results** screen, in the **Sample Name** column, click the sample of interest.
3. Click the **Plugins** tab.
   A summary table of the coverage analysis, by barcode, is included in the **coverageAnalysis** summary pane.
4. *(Optional)* From the **Executed At** dropdown list, select an alternate timestamp, if available, to view additional reports.

5. *(Optional)* Click **View Log** to view the coverageAnalysis log.
6. *(Optional)* Click **Delete** to delete the coverageAnalysis plugin output for the selected timestamp.

   IMPORTANT! If you click **Delete**, the report is deleted without the appearance of confirmation dialog window. Ensure that you intend to delete the report before clicking **Delete**.

7. In the **coverageAnalysis** summary pane, in the **Barcode Name** column, click the link in the row of the barcode of interest.
   The detailed **Coverage Analysis Report** for the barcode opens in a separate window.

Output files generated by the coverageAnalysis plugin

You can download coverageAnalysis plugin results files from links that are contained in the **File Links** section.

Sometimes the file name can be too long to open in applications such as Microsoft™ Excel™. To resolve this problem, right-click the file and click **Save As** to rename the downloaded file.

Click **(Help)** next to the file to open a description of the file.

The following is an example of the content of a results file that is generated by the coverageAnalysis plugin.

The list of files depends on the application type selected.

File	Description
Coverage statistics summary	A summary of the statistics presented in the tables at the top of the plugin report. The first line is the title. Each subsequent line is either blank or contains a statistic title followed by a colon (:) and its value.
Base depth of coverage	Coverage summary data used to create the Depth of Coverage Chart. This file contains the following fields: • `read_depth`: the depth at which a (targeted) reference base has been read. • `base_cov`: the number of times any base was read (covered) at this depth. • `base_cum_cov`: the cumulative number of reads (coverage) at this read depth or greater. • `norm_read_depth`: the normalized read depth (depth divided by average base read depth). • `pc_base_cum_cov`: same as `base_cum_cov` but represented as a percentage of the total base reads.

(continued)

File	Description
Amplicon coverage summary	Coverage summary data used to create the Amplicon Coverage Chart. This file contains these fields: • `contig_id`: the name of the chromosome or contig of the reference for this amplicon. • `contig_srt`: the start location of the amplicon target region. This coordinate is 1-based, unlike the corresponding 0-based coordinate in the original targets BED file. • `contig_end`: the last base coordinate of this amplicon target region. **Note:** The length of the amplicon target is given as tlen = (contig_end - contig_srt + 1). • `region_id`: the ID for this amplicon as given as the 4th column of the targets BED file. • `gene_id`: the gene symbol as given as the last field of the targets BED file. • `gc_count`: the number of G and C bases in the target region. %GC = 100% * gc / tlen. • `overlaps`: the number of times this target was overlapped by any read by at least one base. Individual reads might overlap multiple amplicons where the amplicon regions themselves overlap. • `fwd_e2e`: the number of assigned forward strand reads that read from one end of the amplicon region to the other end. • `rev_e2e`: the number of assigned reverse strand reads that read from one end of the amplicon region to the other end. • `total_reads`: the total number of reads assigned to this amplicon. This value is the sum of `fwd_reads` and `rev_reads` and is the field that rows of this file are ordered by (then by `contig id`, `srt` and `end`). • `fwd_reads`: the number of forward strand reads assigned to this amplicon. • `rev_reads`: the number of reverse strand reads assigned to this amplicon. • `cov20x`: the number of bases of the amplicon target that had at least 20 reads. • `cov100x`: the number of bases of the amplicon target that had at least 100 reads. • `cov500x`: the number of bases of the amplicon target that had at least 500 reads.
Chromosome base coverage summary	Base reads per chromosome summary data used to create the default view of the Reference Coverage Chart. This file contains these fields: • `chrom`: the name of the chromosome or contig of the reference. • `start`: the coordinate of the first base in this chromosome. This is always 1. • `end`: the coordinate of the last base of this chromosome. Also its length in bases. • `fwd_reads`: the total number of forward strand base reads for the chromosome. • `rev_reads`: the total number reverse strand base reads for the chromosome. • `fwd_ontrg` (if present): the total number of forward strand base reads that were in at least one target region. • `seq_reads`: the total sequencing (whole) reads that are mapped to individual contigs.

(continued)

File	Description
Aligned reads BAM file	Contains all aligned reads that are used to generate this report, in BAM format. This is the same file that can be downloaded from the main report (for the specific barcode). See the current SAM tools documentation for more file format information.
Aligned reads BAI file	Binary BAM index file as required by some analysis tools and alignment viewers such as IGV. This is the same file that can be downloaded from the main report (for the specific barcode).

Upload results files to another Genexus™ Integrated Sequencer

When a run completes successfully in Genexus™ Software, you can upload the results (BAM files) to another Genexus™ Integrated Sequencer.

Tip: To automatically upload BAM files when a run is complete, select the **Upload BAM files to Server** checkbox in the **Setup** step of planning a run.

Before you can upload results files to another Genexus™ Integrated Sequencer, you must link the accounts that you can use for data uploads. For more information, see “Register Genexus™ Software accounts (manager/administrator)” on page 232.

1. In the menu bar, click **Results** ▸ **Run Results**.
2. In the **Run Results** screen, place the pointer over the row of a run of interest, then click **BAM Uploader**.
3. In the **Upload Samples to Server** dialog box, make the following selections.
   a. From the **Configured Account** list, select the Genexus™ Software account and the software version.
   b. In the **Select** column, select one or more assays from the list to upload the results files from the selected assays.
   c. In the **Genexus Workflow** column, select whether you want analysis to start on the selected Genexus™ Integrated Sequencer immediately.
      - Select **Upload Only** to upload the sample results (BAM files) to the software.
      - Select an assay of interest to upload the sample results (BAM files) and automatically start analysis with the selected assay on the Genexus™ Integrated Sequencer.
   d. Click **Upload**.

The results are uploaded to another Genexus™ Integrated Sequencer.

View verification run results

Manager- and administrator-level users can view verification run details, including the run summary, QC summary, and the reagents used in the run. Verification runs are performed during installation to validate the performance of the instrument or instruments. Sequencing verification runs are performed to validate the Genexus™ Integrated Sequencer.

If a Genexus™ Purification Instrument is also connected, a verification run that validates both the purification instrument and sequencer is performed.

Note: Field Service Engineers use system-installed verification templates to validate the installation of the Genexus™ Integrated Sequencer. Field Service Engineers can view the template information but cannot modify it.

1. In the menu bar, click **Results** ▸ **Verification Results**.
2. In the **Verification Results** screen, view or download information for the verification run.

Option	Description
View verification results details	In the **Run Name** column, click the verification run name of interest to open the **Verification Results Details** screen. In the **QC** section of the **Verification Results Details** screen, click the **>** symbol to the left of the **Lane** column in the lane of interest to expand the results details pane.
Download a verification report that has been signed	Place the pointer over the row of the verification run of interest, then click **Report**.

3. You can perform the following actions for **Verification Results**. Action links are available when you place the pointer over the row of a verification result.

Option	Description
View Plan	If the run has not started on an instrument, you can view the run plan for the verification run.
Audit	Displays the list of users who created and edited the run. You can export and print information from the list from the **Audit Trail** dialog box.
CSA	**CSA**—Customer Support Archive. Click this link to download all of the sequencer log files. Log files contained within the CSA can be useful when troubleshooting issues with the sequencer. For more information, see the *Genexus™ Software 6.6 User Guide* (Pub. No. MAN0024953).
Report	Download a verification report that has been signed.

Verification runs

The following information is available in the **Results / Verification Results** screen.

Column	Description
Run Name	The name of the run, created when the run was planned. Click the name to open the **Verification Results Details** screen.
Assay Full Name	The name of the assay used in the run.
Field Engineer Name	The name of the support specialist who performed the run.
Instrument Name	The name of the sequencer that was verified.
Run Status	The current status of the full sequencing run, including analysis.
QC Status	Indicates whether a sequencing run passed or failed, based on the sequencing QC metrics selected for the assay.
Started On	The date and time that the run was started.
Updated On	The date and time that the run was last updated.

Sign verification run reports (manager/administrator)

Manager- and administrator-level users can sign results reports for verification runs. However, we recommend that only qualified support specialists sign performance qualification (PQ) reports.

Verification reports can be electronically signed only when all samples pass QC analyses.

1. Click **Results** ▸ **Verification Results**.
2. In the **Verification Results** screen, click the name of the verification run of interest.
3. In the **Verification Results Details** screen, click **Sign Off**.
4. In the **Sign Off Report** dialog box, enter or make the following selections.
   a. Enter the password.
   b. In **Electronic Signature**, select **Approval**.
   c. In **Sign off Comments**, enter a comment.
   d. In **Laboratory Comments** and **Footer Field**, enter optional information.
5. Click **Sign Off.**

The verification run report is electronically signed.

You can view, print, or download the report in a PDF file. For more information, see “Verification runs” on page 179.

A Troubleshooting

Customer Support Archive (CSA)

You can download an archive that a Technical Support representative can use to diagnose Genexus™ Software issues. The Customer Support Archive (CSA) contains log files and other technical data about your Genexus™ Software and other files from sequencing runs on a Genexus™ Integrated Sequencer.

You can access customer support archive files from the **Results / Sample Results** and the **Results / Run Results** screens.

Download a customer support archive

When you download a customer support archive (CSA) in Genexus™ Software, files are included for all samples for a specific assay. If the run includes only one assay, files for all samples are included. If the run includes more than one assay, you can specify the assay of interest when you download the CSA.

1. In the menu bar, click **Results** ▸ **Sample Results**.

2. In **Sample Results** screen, place the pointer over the row of the sample result of interest, then click **CSA**.

   Alternatively, you can download CSA files three other ways.

   - Click **Results** ▸ **Sample Results**. Click the sample name of interest. Then, click ··· **(More Options)** ▸ **CSA.**
   - Click **Results** ▸ **Run Results**. Click the run name of interest. Place the pointer over the run of interest, then click **CSA**. In the **Download CSA** dialog box that appears, select the assay of interest, then click **Download**.
   - Click **Results** ▸ **Run Results**. Click the run name of interest. Then, click ··· **(More Options)** ▸ **CSA**. In the **Download CSA** dialog box that appears, select the assay of interest, then click **Download**.

An XZ compressed TAR archive (TXZ) file is downloaded to the folder that you specified to download files from the browser. This location depends on the browser settings. You can attach the archive to an email to send to Customer Support.

Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls

You can use quality control results to troubleshoot Genexus™ Integrated Sequencer runs to help identify the cause of performance problems. If you select the **Include Inline Controls** checkboxes for DNA and RNA in the **Reagent** step when you create an assay (see "Create a new assay (manager/administrator)" on page 45), you include the inline control analysis in the post-run results analysis. Inclusion of a set of six control amplicons (covering a range of amplicon length) and spike-in nucleic acid into sample library preparation reactions helps determine whether poor performance is due to insufficient sample input and/or poor sample quality, or is unrelated to sample input and quality. With 10 ng sample input, the read ratio of endogenous sample reads to spike-in control reads is expected to be ~3. Using more than 10 ng sample input results in a proportionally higher read ratio. For example, if you load 20 ng of sample, the read ratio should be ~6.

The CF-1 templating control serves as a check on templating and sequencing performance that is independent of library preparation.

Use the following table as a guide to help identify the source of performance problems. For recommended actions, see the troubleshooting topics under "Genexus™ Integrated Sequencer—general and QC troubleshooting" on page 182.

QC category	Run diagnostic			
	Successful run	Sample input and/or quality problem	Library preparation problem unrelated to sample	Templating or Sequencing problem
Sample QC (endogenous sample reads)	Passed	Failed	Failed	Failed
Read ratio for inline controls (endogenous to spike-in reads)	Normal Read ratio ~3	Low Read ratio <<3	Normal or variable	—
Templating Control QC - CF-1	Passed	Passed	Passed	Failed

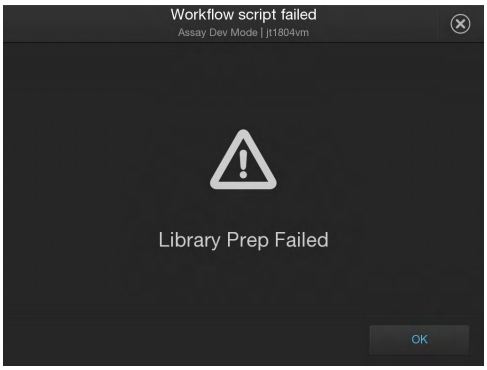
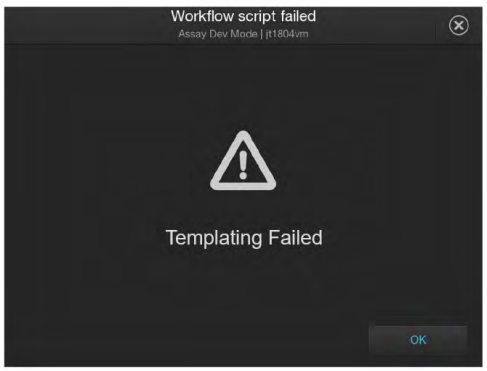
Genexus™ Integrated Sequencer—general and QC troubleshooting

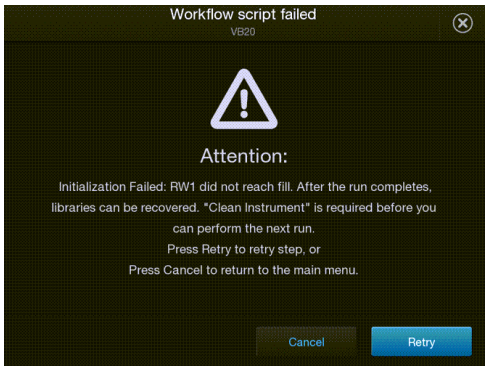
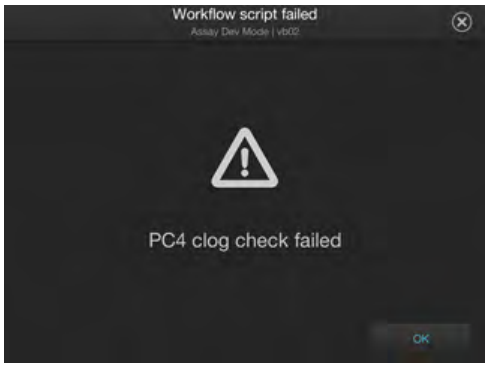
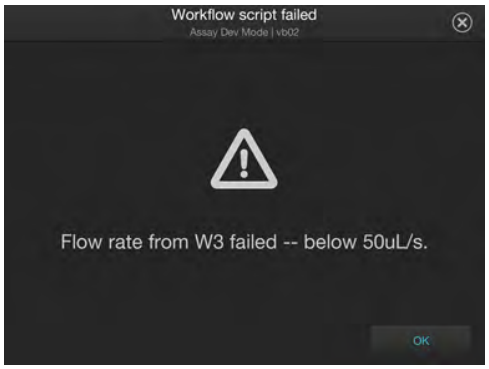
Observation	Possible cause	Recommended action
A consumable is not recognized by the sequencer after loading on the deck	The consumable (for example, a strip, barcode plate, pipette tip box) is correctly placed but is not completely inserted into its position, causing it to be misaligned with its expected position.	Ensure that the consumable is pressed completely into place. Apply firm pressure on the item so that it fits snugly into its deck position.
	The barcode of the consumable is not readable by the instrument.	Tap **Help** in the lower left corner of the **Load Instrument** screen and follow on-screen instructions to override the block manually. Note that the name of the consumable does not appear in the list of consumables in the run summary.
		If the behavior continues in subsequent runs, contact Technical Support.
	Consumable version does not match the Genexus™ Software version. For example, a consumable compatible with Genexus™ Software 6.2 is installed in a sequencer updated for Genexus™ Software 6.6.2.1.	Ensure that you are using consumables compatible with the software installed on the sequencer.
Run Status = **Failed** **Details:** In the Genexus™ Software **Run Result** screen, the **Run Status** for a completed run is listed as "**Failed**". In the **Sample Results** screen, the **Sample Status** is listed as "**BaseCallingActor FAILED**".	Chip calibration failed due to a chip problem, or an instrument problem.	Repeat the run with a new chip. If the problem persists, contact Technical Support.
A lane that has been used is not crossed out in the sequencer screen **Details:** After completion of a run, the lane used in the run was not crossed out, so that the next run could reuse the lane.	A chip problem caused a datacollect failure to read efuse.	In the sequencer screen, tap **Settings** ▸ **Clean instrument** to perform a clean instrument. For details, see “Perform a Clean instrument procedure” on page 206. After cleaning, start a new run.

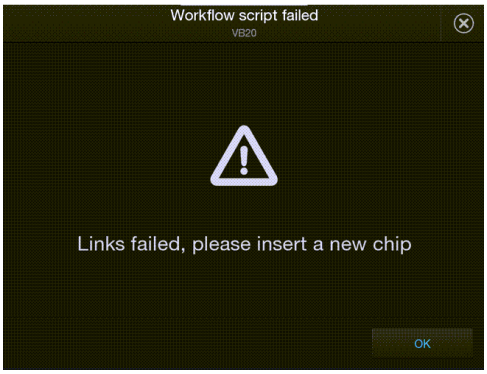
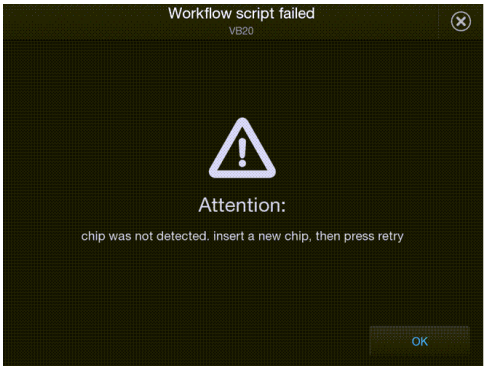
Observation	Possible cause	Recommended action
The number of sample reads is low, CF-1 metrics pass QC, but read ratio of inline controls is low. **Details:** If CF-1 reads per lane, accuracy, and mean AQ20 read length are good, and read ratio of inline controls (endogenous vs. spike-in) is low (<< 3), a problem with sample input is indicated. For more information, see "Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls" on page 181.	Nucleic acid input may have been insufficient, and/or the nucleic acid was degraded.	For a sample run, re-quantify nucleic acid samples and/or perform sample QC to ensure that the expected nucleic acid input and size was loaded.
		If needed, re-isolate and purify nucleic acid samples.
The number of sample reads is low, but CF-1 metrics pass QC, and read ratio of inline controls is normal **Details:** If CF-1 metrics passed QC, and read ratio of inline controls is normal (~ 3), a problem in library preparation unrelated to sample input or quality may be indicated. For more information, see "Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls" on page 181.	One or more of the Genexus™ Strip 1 strips used in the run had magnetic beads trapped in the tube 5 keyhole.	Repeat the run with strips that you have verified have no trapped beads. For more information, see "Before you begin" on page 95.
	An incorrect assay was selected for the run, or library amplification parameters were not optimal.	Ensure that you have selected the correct assay and reviewed assay parameters.
	Library strips were inadequately equilibrated to room temperature (Genexus™ Strip 1), or incompletely thawed (Genexus™ Strip 2-AS or Genexus™ Strip 2-HD) before loading in the sequencer.	Ensure that Genexus™ Strip 1 strips are fully equilibrated to room temperature, and Genexus™ Strip 2-AS strips are completely thawed before loading in the sequencer.
The number of sample reads is low, and CF-1 metrics fail QC **Details:** If CF-1 metrics failed QC, a problem in templating or sequencing is indicated. For more information, see "Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls" on page 181.	One or more of the Genexus™ Strip 3-GX5™ strips used in the run may have had an excessive amount of magnetic beads trapped in the tube 6 or 7 keyhole.	Repeat the run with strips that you have verified have no trapped beads. For more information, see "Before you begin" on page 95.
	Template strips were inadequately equilibrated to room temperature (Genexus™ Strip 3-GX5™), or incompletely thawed (Genexus™ Strip 4) before loading in the sequencer.	Ensure that Genexus™ Strip 3-GX5™ strips are fully equilibrated to room temperature, and Genexus™ Strip 4 strips are completely thawed before loading in the sequencer.
	The sequencing chip or coupler was faulty or leaky.	Repeat the run with new chip and coupler. If low performance continues, contact Technical Support.

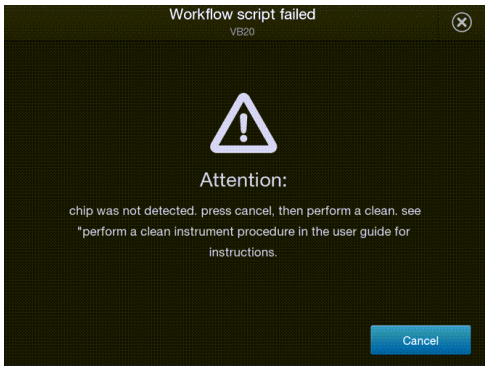
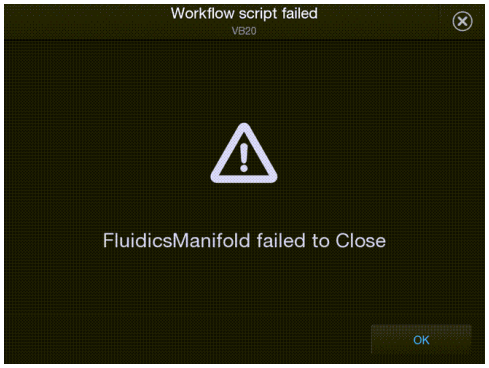
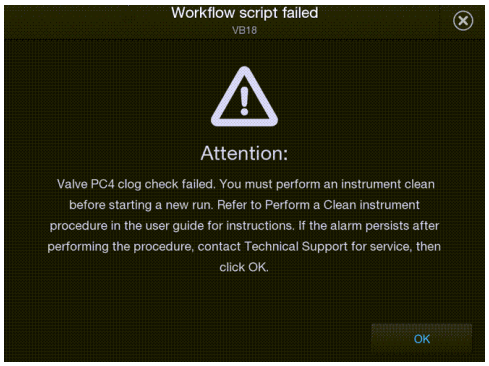
Observation	Possible cause	Recommended action
The number of sample reads is low, and CF-1 metrics fail QC **Details:** If CF-1 metrics failed QC, a problem in templating or sequencing is indicated. For more information, see "Troubleshoot Genexus™ Integrated Sequencer performance with CF-1 and inline controls" on page 181. *(continued)*	The run was started >14 days after the last initialization was performed, or on an expired initialization.	Perform a Clean instrument procedure (**Settings ▸ Clean instrument**), install new a chip, and new sequencing reagent bottles and cartridge in the sequencing reagents bay, then repeat the run. **Note:** Reagents are stable on the sequencer for 14 days, after which you may experience reduced performance. For further information, see "Options for an expired sequencer initialization" on page 111.
Pipette tips remain in the used tip rack	Tips were unable to be picked up by the pipettor.	In Genexus™ Software 6.6 and later, if the pipettor cannot pick up a tip, it leaves the problematic tip in its place and moves to the next available tip. No action is needed.

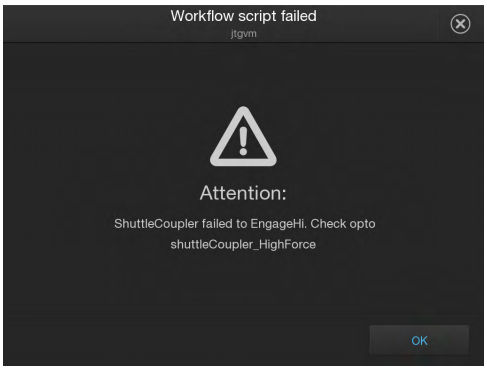
Genexus™ Integrated Sequencer error and warning messages

Observation	Possible cause	Recommended action
Error message: **Library Prep Failed** 	• The sequencer failed xy homing, *or* • The sequencing chip failed to engage at pipette position.	Confirm that no analyses are in progress, then reboot the instrument. Repeat the run. If the failure continues, contact Technical Support.
Error message: **Templating Failed** 	• The sequencer failed xy homing, *or* • The Genexus™ Coupler was misaligned.	Confirm that no analyses are in progress, then reboot the instrument. Repeat the run, and ensure that the coupler is installed with proper alignment. If the failure continues, contact Technical Support.

Observation	Possible cause	Recommended action
Error message: **Initialization Failed: XXX did not reach fill** 	Genexus™ Bottle 2 flow was restricted, or a flow sensor malfunctioned. OR During a post-chip clean, the Genexus™ Bottle 3 was empty, or Genexus™ Bottle 3 flow was restricted. **Note:** XXX is the name of the conical tube that did not reach fill.	Allow the run to complete, then select **Settings ▸ Clean instrument** to do a clean. After cleaning completes, start a new run. If the error message appears again, contact Technical Support for service. **Note:** After the run completes, you can recover libraries from the amplification plate, if desired. For more information, see Appendix E, "Library QC Archive: recover library preparations from the Genexus™ Integrated Sequencer for reuse".
Error message: **XXX clog check failed** 	Clog check following Clean Instrument failed. Incomplete fluid flush or debris has caused a clog. **Note:** XXX refers to the failed waste lines, chip, or main 1-4.	Contact Technical Support for recommended action.
Error message: **Flow rate from W3 failed – below 50 µL/s** 	During the post-chip clean the Genexus™ Bottle 3 was empty, or Genexus™ Bottle 3 flow was restricted.	Select **Settings ▸ Clean instrument** to do a clean. Ensure that the Genexus™ Bottle 3 is installed correctly, and the bottle contains sufficient volume. Replace as needed. For details, see "Perform a Clean instrument procedure" on page 206. If the failure persists, contact Technical Support.

Observation	Possible cause	Recommended action
Error message: **PostChipClean failed**	The post-chip clean aborted due to a chip error.	In the sequencer screen, tap **Settings ▸ Clean instrument** to re-do a Clean instrument procedure. For details, see “Perform a Clean instrument procedure” on page 206. You may or may not need to replace the chip during deck set up.
Error message: **Links failed, please insert a new chip** 	A chip failed to come up when shuttled to the sequencing position during run setup.	Replace the chip, then try to proceed again. If the error message appears again, contact Technical Support.
Error message: **Chip was not detected. Insert a new chip, then press retry** 	Chip connection was lost and sequencing failed.	1. Insert a new chip, then press **Retry**. 2. If this does not resolve the problem, remove the chip from the instrument. An instrument clean is required before a new run can proceed. 3. Force an instrument clean by navigating to **Settings ▸ Clean instrument**. 4. After the clean, repeat the run with a new chip and reagents.

Observation	Possible cause	Recommended action
Error message: **Chip was not detected. Press cancel, then perform a clean** 	Chip connection was lost and sequencing failed.	An instrument clean will be required before a new run can proceed. 1. Press **Cancel**. 2. Force an instrument clean by navigating to **Settings ▸ Clean instrument**. 3. After the clean, repeat the run with a new chip and reagents. **Note:** For more information, see "Perform a Clean instrument procedure" on page 206.
Error Message: **FluidicsManifold failed to close** 	The sequencing clamp hardware did not function properly.	Instrument service is required to proceed. Libraries can be recovered if the error occurred after library preparation was completed. For more information, see Appendix E, "Library QC Archive: recover library preparations from the Genexus™ Integrated Sequencer for reuse".
Error message: **Valve PC4 clog check failed** 	A clog was detected during the post-chip clean.	1. Perform an instrument clean by navigating to **Settings ▸ Clean instrument**. 2. After cleaning completes, begin a new run. 3. If the error message appears again, contact Technical Support for service.

Observation	Possible cause	Recommended action
Error message: **ShuttleCoupler failed to EngageHi** 	Chip shuttle hardware did not function properly.	Instrument service is required to proceed. **Note:** Libraries can be recovered if the error occurred after library preparation was completed. For more information, see Appendix E, “Library QC Archive: recover library preparations from the Genexus™ Integrated Sequencer for reuse”.
Error message: **Vacuum Clean Failed: Instrument Service might be required**	XY motion failed, OR a leak was detected in the vacuum system.	Contact Technical Support for recommended action.
Warning message: **Pressure Leak Detected in vac pressure drop leakrate** **Press Ok to open doors and fix the leak.**	Genexus™ Bottle 1 leak was detected.	Remove the Genexus™ Bottle 1 and ensure that the cap is tight, then replace the bottle securely on the instrument. Continue with the run. If the pressure leak message appears again, replace the Genexus™ Bottle 1 with a new bottle, then continue with the run. If the failure persists, contact Technical Support.
Warning message: **Pressure Leak Detected in vac time to pressure** **Press Ok to open doors and fix the leak.**	A Genexus™ Bottle 1 leak or over-fill was detected.	Ensure that a new Genexus™ Bottle 1 is installed, and the cap is tight and the bottle is secure on the instrument. Continue with the run. If the pressure leak message appears again, replace the Genexus™ Bottle 1 with a new bottle, then continue with the run. If the failure persists, contact Technical Support.

Observation	Possible cause	Recommended action
Warning message: **Pressure Leak Detected in vac pressure baseline** **Press Ok to open doors and fix the leak.**	Genexus™ Bottle 1 leak was detected.	Remove the Genexus™ Bottle 1 and ensure that the cap is tight, then replace the bottle securely on the instrument. Continue with the run. If the pressure leak message appears again, replace Genexus™ Bottle 1 with a new bottle, then continue with the run. If the failure persists, contact Technical Support.
Warning message: **Pressure Leak Detected in W2P** **Press Ok to open doors and fix the leak.**	A leak was detected in the Genexus™ Bottle 2 at the left position.	Remove Genexus™ Bottle 2 installed at the left position, ensure that the cap is tight, then reinstall the bottle securely. Continue with the run. If the pressure leak message is seen again, replace the left Genexus™ Bottle 2 with a new bottle, then continue with the run. If the failure persists, contact Technical Support.
Warning message: **Pressure Leak Detected in W22P** **Press Ok to open doors and fix the leak.**	A leak was detected in the Genexus™ Bottle 2 installed at the right position.	Remove the Genexus™ Bottle 2 installed at the right position, ensure that the cap is tight, then reinstall the bottle securely. Continue with the run. If the pressure leak message appears again, replace the right Genexus™ Bottle 2 with a new bottle, then continue with the run. If the failure persists, contact Technical Support.

Observation	Possible cause	Recommended action
Warning message: **Pressure Leak Detected in W3P** **Press Ok to open doors and fix the leak.**	A leak was detected in the Genexus™ Bottle 3.	Remove the Genexus™ Bottle 3, ensure that the cap is tight, then reinstall the bottle securely. Continue with the run, or repeat the clean if this message appears in a post-chip clean. If the pressure leak message appears again, replace the Genexus™ Bottle 3 with a new bottle. Continue with the run, or repeat the clean if this message appears in a post-chip clean. If the failure persists, contact Technical Support.
Warning message: **Pressure Leak Detected in Conicals & Reagent Cartridge** **Press Ok to open doors and fix the leak.**	A leak was detected in one or more of the Genexus™ Conical Bottles or the Genexus™ Cartridge.	Tighten all Genexus™ Conical Bottles one bottle at a time to prevent accidental position changes. Inspect the Genexus™ Cartridge for defects. Continue with the run. If the failure persists, contact Technical Support.
Warning message: **Liquid detected in conicals** **Press Ok to open doors and fix the issue.**	The previous post-chip clean did not remove all of the liquid in the Genexus™ Conical Bottles, causing failure of the Conicals empty test and Conical volume test.	Replace the Genexus™ Conical Bottles with new bottles, then tap **Settings ▸ Clean instrument** to clean the instrument. After cleaning, start a new run.

Genexus™ Software

Observation	Possible cause	Recommended action
Cannot sign in to Genexus™ Software	You either entered an incorrect password or you are signed out due to several failed login attempts.	Contact the Genexus™ Software system administrator.
Batch sample import fails	One or more entries in the sample-import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the sample-import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean sample-import template file, and be careful to copy only those rows that contain actual data.
	The sample import spreadsheet contains a nonunique sample name.	Every sample name in the software must be unique. Ensure that the spreadsheet does not contain any duplicate sample names, then repeat the import. Note that the system check is not case-sensitive, so a sample name of ABC1 conflicts with abc1.
	The headings in the sample import spreadsheet do not match the sample attributes in the software.	The headings must match the sample attributes in the software exactly. Check the headings for spelling or other errors. Map a sample attribute if needed.
Library batch import fails	One or more entries in the library batch import spreadsheet contains special characters, lines breaks, unexpected spaces, incorrect entry length, incorrect date formatting, or other formatting errors.	Check each entry for correct formatting, correct any errors, and repeat the import.
	Blank rows were copied into the library batch import template file from a different source.	Rows that appear empty can contain hidden formatting that conflicts with the import function. Start with a clean library batch import template file, and be careful to copy only those rows that contain actual data.
	The library batch import spreadsheet contains a nonunique **Library Batch ID**.	Each **Library Batch ID** in the software must be unique. Ensure that the spreadsheet does not contain any duplicate IDs, then repeat the import. The system check is not case-sensitive. For example, a **Library Batch ID** of ABC1 conflicts with abc1.

Observation	Possible cause	Recommended action
Library batch import fails *(continued)*	A sample name entered in the library batch import spreadsheet does not match a sample name listed in the **Manage Samples** screen.	Ensure that the sample names entered into the spreadsheet are correct and match an existing sample name added to the software.
	The Barcode ID name format does not exactly match the format that is used in the **Prepare Library Batch** dialog box.	Use the name format following the Barcode ID name format found in the Barcode Set reference lists (**Settings ▸ References ▸ Barcode Set**), for example: IonDual_0101 through IonDual_0196, or IonHDdual_0101 to IonHDdual_0132.
	An invalid library, control, or panel kit barcode has been entered in the spreadsheet.	Ensure that you have correctly entered a valid kit barcode in the appropriate cell of the spreadsheet.
	The spreadsheet template that you used is from a previous software version.	New fields in the template file can be added with new software versions. Ensure that you download the template file from the current software version.
Cannot upload my panel or hotspots	Issues with BED file format or files do not end in ***.bed***.	Ensure your file is in the correct BED format and has a ***.bed*** extension.
Variants tab is missing hotspot entries **Details:** The remaining entries are present.	Hotspot BED file contains entries that are incorrectly formatted.	Check that BED file entry is correctly formatted. See the following examples: SNP entry: chr1 2337276 2337277 SVA_322 0 + REF=C;OBS=T;ANCHOR=G AMPL Deletion entry: chr1 201341175 201341180 SVA_497 0 + REF=AGAAG;OBS=;ANCHOR=C AMPL Insertion entry: chr1 236978992 236978992 SVA_621 0 + REF=;OBS=TCTG;ANCHOR=T AMPL
		Ensure that the REF values match the actual reference coordinate of hg19.
The results of the run do not appear in the **Results / Run Results** screen	The instrument disk space is full.	Clear disk space on the sequencer. For more information, see “Manually delete run data” on page 213.
Cannot download run result files	The run failed.	Create an assay with the correct configuration for the samples, then reanalyze the samples.

B

Maintain the sequencer

Materials required

Cleaning

- Lint-free wipes
- Deionized water
- 70% isopropanol

Liquid waste filter replacement

- Genexus™ Filter (ordered separately [Cat. No. A40302])

Conical bottle replacement

- Genexus™ Conical Bottles (from the Genexus™ Installation and Training Kit, or ordered separately [Cat. No. A40275])

Clean or decontaminate the sequencer

If a spill or leak occurs inside the instrument, perform the following steps to clean or decontaminate the sequencer.

Note: Dispose of all waste in appropriate liquid or solid waste containers.

1. Remove each Genexus™ Bottle 2, then remove and empty the Genexus™ Bottle 1 and Waste carboy.
2. Remove the Genexus™ Cartridge.
3. Inspect the floor of the sequencing reagents bay and Genexus™ Cartridge port for liquid.
4. Using lint-free wipes, soak up as much liquid as possible, then clean the affected area with wipes moistened with deionized water.
5. Wipe the affected surfaces with 70% isopropanol, then allow to air-dry.

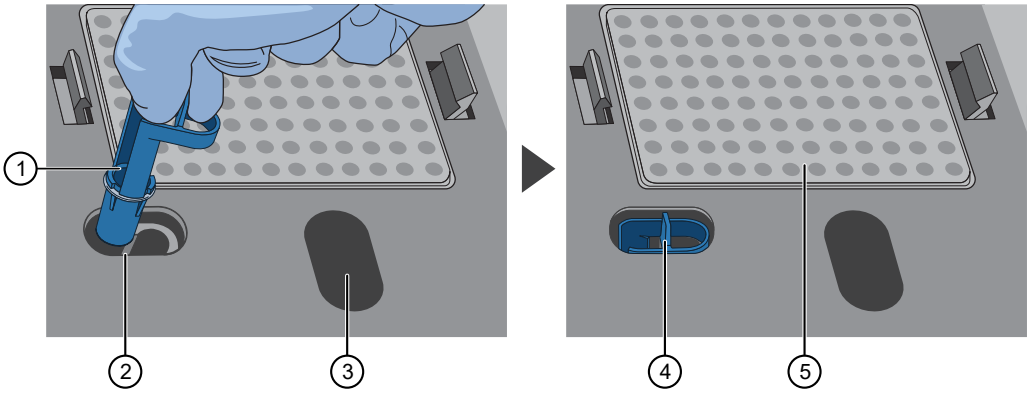
Replace the Genexus™ Filter

The Genexus™ Filter captures particulate matter in the liquid waste to prevent blockage of the waste line over time. The filter needs to be replaced with a new filter after one year of regular instrument use.

1. Remove the old filter from the liquid waste disposal port on the instrument deck by grasping the filter firmly and pulling up. Dispose of the filter as regular waste.

   Note: If an interim filter is installed in the port, use a straight needle probe to pierce the filter, then lift out the filter using the probe.

2. Insert a new Genexus™ Filter into the liquid waste disposal port, then press firmly to seat the filter O-ring securely.



① Genexus™ Filter
② Liquid waste disposal port
③ Used pipette tip disposal port
④ Installed filter
⑤ Sample plate station

Replace the Genexus™ Conical Bottles

Genexus™ Conical Bottles need to be replaced when filters in the bottles become partially clogged, resulting in reduced flow rate. The Genexus™ Integrated Sequencer measures flow rate in a Conical Flow Rate test that is performed during every post-chip clean. If the conical flow rate test fails, the sequencer automatically redirects to the conical bottle replacement procedure. During a normal run, a post-chip clean is performed in three situations:

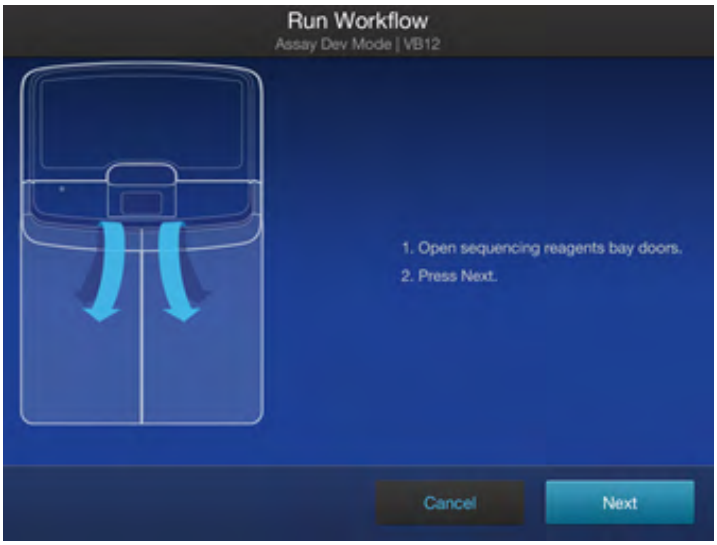
- At the end of the run if all four lanes have been used.
- At the end of the run if you selected **Do Force Clean** at run setup.
- At run setup when a run is selected but insufficient unused lanes in the installed chip are available for the run. If you continue with the run, a post-chip clean is performed before the run can proceed.

Follow these steps to replace the Genexus™ Conical Bottles.

1. After a run, in the **Run Complete** screen, tap **Next**.

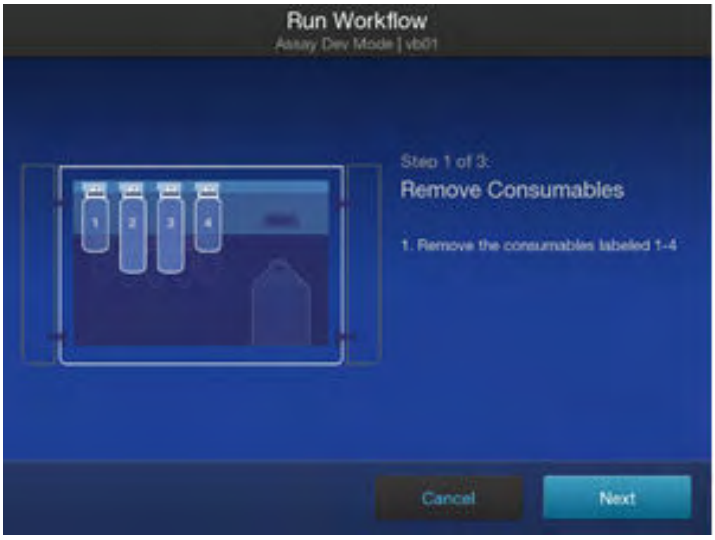
If the Conical Flow Rate test failed, a screen appears directing you to open the sequencing reagents bay doors.

2. Open the sequencing reagents bay doors, then tap **Next**.

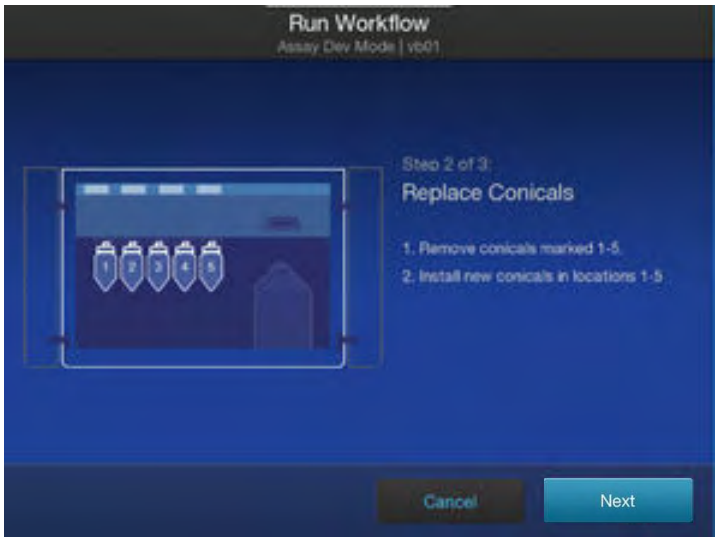


The **Remove Consumables** screen appears.

3. Remove, but do not discard, the Genexus™ Bottle 1 (position 1), the two Genexus™ Bottle 2 bottles (positions 2 and 3), and the Genexus™ Bottle 3 (position 4), then tap **Next**.



4. Remove the five used Genexus™ Conical Bottles, install five new conical bottles, then tap **Next**.



Note: Discard the used conical bottles appropriately.

5. Replace the four reagent bottles that you removed in step 3 in their original positions.

6. After replacing the bottles, close the sequencing reagents bay doors, then tap **Next** in the **Insert Consumables** screen.

   After you tap **Next**, the sequencer starts a **Leak Test**.

Note: If you tap **Next** while the sequencing reagents bay doors are open, an alert appears asking you to close the doors before proceeding.

- If the sequencer passes the **Leak Test**, no action is needed. The sequencer proceeds to a cleaning cycle.

- If the sequencer fails the **Leak Test**, a notification appears. Tap **OK** to return to step 2 to fix the leak. Open the sequencing reagents bay doors, check the tightness of each conical bottle that you installed, then ensure that the four reagent bottles are installed correctly. Close the sequencing reagents bay doors, then tap **Next** to repeat the **Leak Test**.

After completion of a successful **Leak Test** and cleaning, the sequencer moves to a normal workflow.

- If the sequencer is at the end of the run, the next screen is a **Clear Deck** screen, followed by a **Clear Sequencing Reagents** screen.
- If the sequencer is at the start of the run, the next screen is a **Clear Deck** screen, followed by a **Load Deck** screen.

Genexus™ Integrated Sequencer power off and power on before and after a long-term shutdown

Follow these procedures to power off the sequencer if it will not be used for more than 28 days, and power on after a long-term shutdown.

Power off before a long-term shutdown

- If all four lanes of a chip have been used, and a post-chip clean has completed successfully, follow these steps to power off the sequencer, as described in “Power off” on page 38.
  a. In the home screen, tap **Settings** ▸ **System Tools** ▸ **Shut down**.
  b. Select **Shutdown**.
  A confirmation message appears. Select **Yes** to power off the instrument.
  c. Turn the power switch located at the back of the instrument to the off (O) position.
- If a partially used chip is installed on the instrument, follow these steps to perform a Clean instrument procedure as described in page 206, then power off the instrument.
  a. In the home screen, tap **Settings** ▸ **Clean Instrument**.
  b. Follow the on-screen instructions to start the cleaning.

  Note: If an alarm appears, follow the on-screen prompts, or contact technical support for further help. Do not continue until the problem is resolved.

  c. After the cleaning completes, power off the system in the home screen by tapping **Settings** ▸ **System Tools** ▸ **Shut down**.
  d. Select **Shutdown**.
  A confirmation message appears. Select **Yes** to power off the instrument.
  e. Turn the power switch located at the back of the instrument to the off (O) position.

Power on after a long-term shutdown

After a long-term shutdown (>28 days), follow these steps to power on the sequencer, as described in “Power on” on page 37.

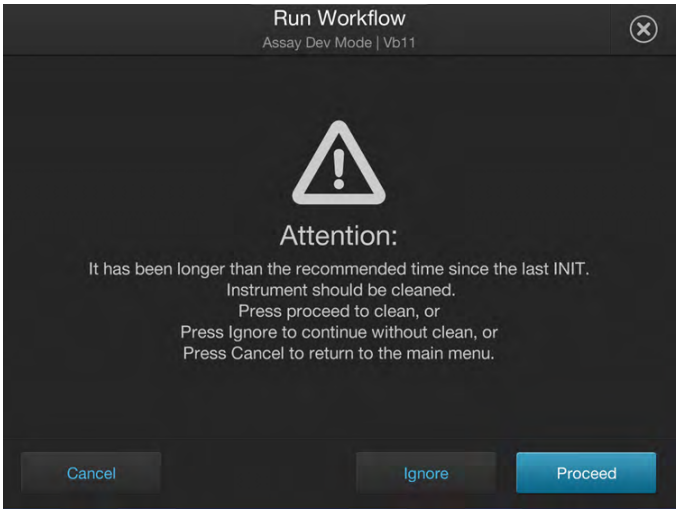
1. Turn the power switch on the back of the sequencer to the on (|) position.
2. Press the power button on the front of the instrument.
The button illuminates.
3. Sign in with your user name and password.
After the home screen appears, the sequencer is ready for use. Check for instrument alarms, if any. If a post-chip clean was performed before the system was powered off, the instrument is ready to use.

4. If a post-chip clean was not performed before the sequencer was powered off, perform a Clean instrument procedure as described in "Perform a Clean instrument procedure" on page 206.
   a. In the home screen, tap **Settings** ▸ **Clean Instrument**.
   b. Follow the on-screen instructions to start the cleaning.

      Note: If an alarm appears, follow the on-screen prompts, or contact technical support for further help. Do not continue until the problem is resolved.

   c. After the cleaning is complete, the instrument is ready to use.

5. If the instrument was in an initialized state before the system was powered off, and shows the following alarm for an expired initialization, perform the following steps to clean the instrument as described in "Options for an expired sequencer initialization" on page 111.



   a. Select **Cancel** to return to the home screen, then tap **Settings** ▸ **Clean Instrument**.
   b. Follow the on-screen instructions to start the cleaning.

      Note: If an alarm appears, follow the on-screen prompts, or contact technical support for further help. Do not continue until the problem is resolved.

   c. After the cleaning is complete, the instrument is ready to use.

Instrument planned maintenance

The Genexus™ Integrated Sequencer needs annual planned maintenance to keep it operating in peak condition. A notification appears when maintenance is due. To schedule a planned maintenance visit, contact your Thermo Fisher Scientific Field Service Engineer.

C

Touchscreen reference

Sequencer touchscreen icons

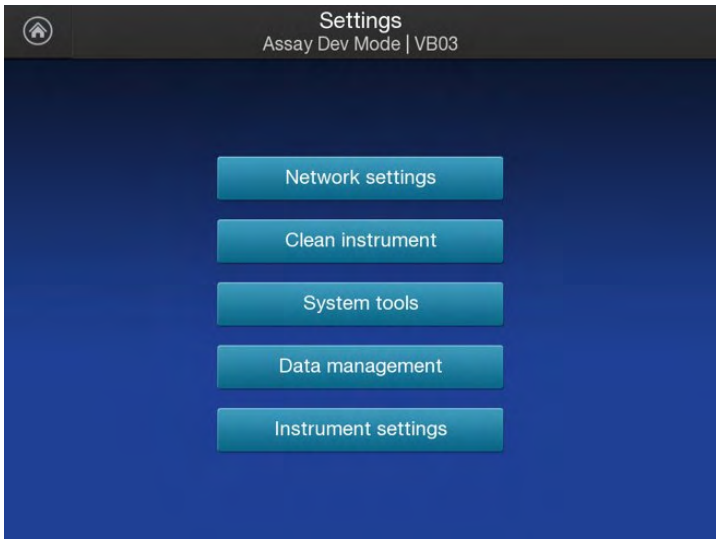
Number	Icon	Description
1		Network connectivity – connected
		Network connectivity – not connected
2		Instrument idle
		Sequencing in progress
		Instrument ready
		Error
3		Chip status – Absent

(continued)

Number	Icon	Description
3		Chip status – Standby
		Chip status – Connecting
		Chip status – Ready
		Chip status – Imaging
		Chip status – Error
4		Chip lane status – 4 lanes available
		Chip lane status – 1 lane in use or used, 3 lanes available
		Chip lane status – 2 lanes in use or used, 2 lanes available
		Chip lane status – 3 lanes in use or used, 1 lane available
		Chip lane status – 4 lanes in use or used, 0 lanes available
5		Instrument File System Space – the percent of file space used is indicated. The instrument checks for sufficient disk space before each run, and notifies the user if there is not enough disc space for a run. The indicator turns yellow when disk space is ≥67% full. The indicator turns red when ≥90% full.
6		Instrument Server File System Space – the percent of file space used is indicated. If the indicator turns red, archive data from the server to free up disk space. See the software help system for information on archiving data.

Settings

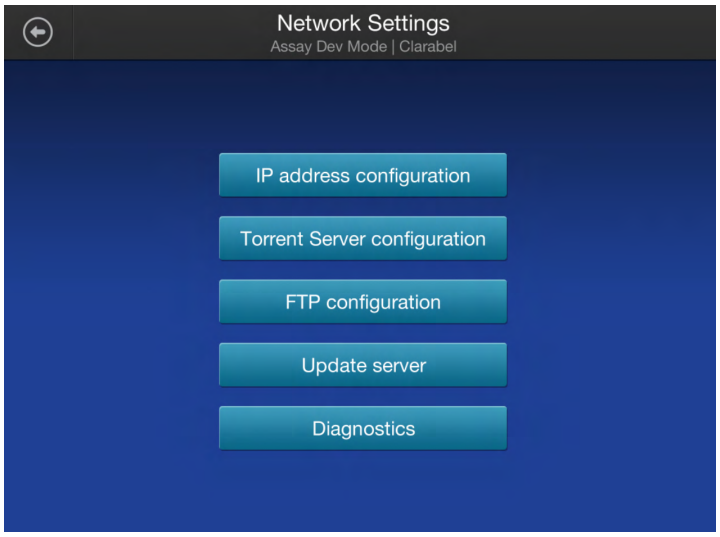
Use the **Settings** menu to view and/or change instrument settings, manage data and network configurations, and clean the instrument.

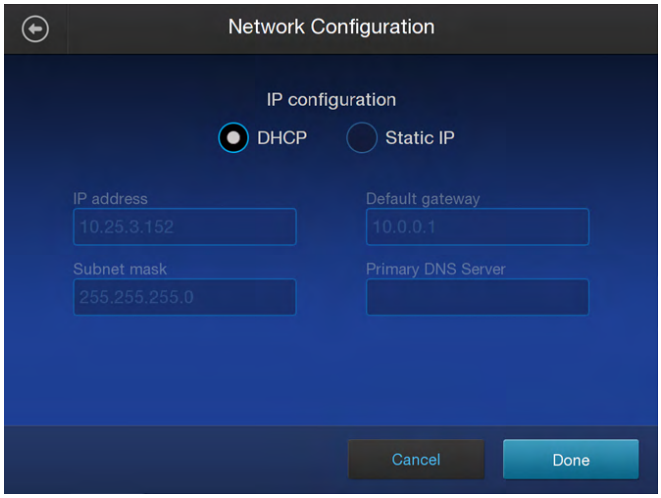
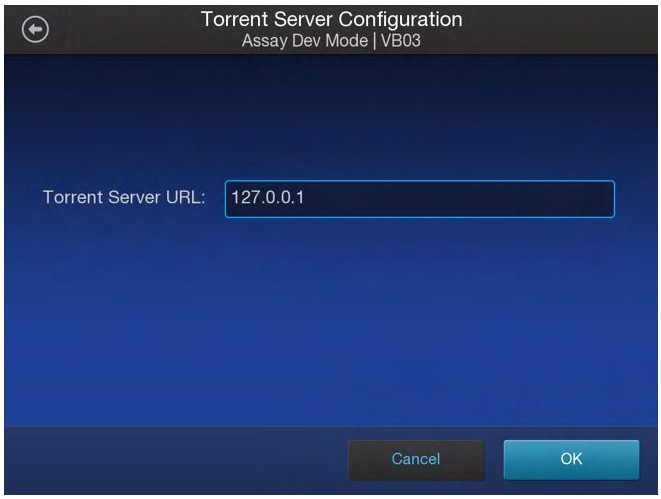


Note: The **System tools** option is for use only by trained service personnel.

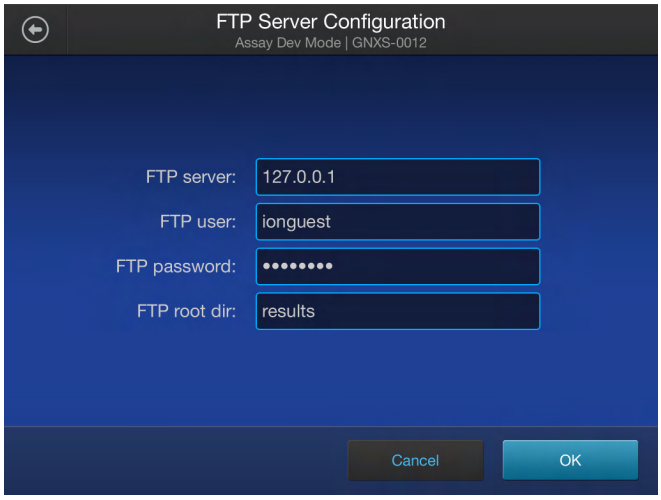
Network Settings

Use the **Network Settings** menu to configure IP address, Ion Torrent™ Server, and FTP settings.



Screen	Description	When/How to use
IP Address Configuration	Set or change the IP configuration (DHCP or Static IP). 	1. Select either the **DHCP** or the **Static IP** option. 2. Touch the screen in the field you want to edit to activate the field. A virtual keyboard appears. 3. Enter the new information, then press **Done**.
Ion Torrent™ Server Configuration	Change the Ion Torrent™ Server IP address and user information.  Enter the following IP address in the **Torrent Server URL** field for your instrument: 127.0.0.1	Use this screen when a change to the Ion Torrent™ Server IP address or user information is required. **IMPORTANT!** Do not change the address unless directed to do so by a Thermo Fisher Scientific field service engineer.

(continued)

Screen	Description	When/How to use
FTP Server Configuration	Change the FTP Server IP address and user information.  Enter the following IP address in the **FTP server** field for your instrument: 127.0.0.1	USe the screen when a change to the FTP Server IP address or user information is required. **IMPORTANT!** Do not change the address unless directed to do so by a Thermo Fisher Scientific field service engineer.

Perform a Clean instrument procedure

Cleaning is normally performed automatically at the completion of the previous sequencing run. Perform a **Clean instrument** procedure if:

- the sequencing run was aborted or had a power failure during a run, or
- the normal post-sequencing run cleaning was not completed.

The **Clean instrument** command initiates the following cleaning procedures:

- UV clean—irradiation of the deck surface with UV light. For more information see “Clear the instrument deck and perform a UV Clean” on page 108.
- Vacuum clean—cleaning of the four chip vacuum lines and robotic waste line.
- Post-chip clean—cleaning of sequencer fluidic lines with conical flow rate test.

IMPORTANT! The **Clean instrument** procedure renders remaining sequencing reagents and unused lanes on the installed chip unusable in a sequencing run after the cleaning.

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1. In the **Settings** menu, tap **Clean instrument**.

2. Follow the on-screen instructions, then tap **Proceed** to go to the following sequence of screens: **Clear Deck** if there are items to be cleared, **UV Clean**, **Load Deck**, **Clear Sequencing Reagents**, and **Load Sequencing Reagents**. For more information, see “Clear the instrument deck and perform a UV Clean” on page 108.

   Note: To save on reagents and expense, you can perform a clean procedure with used sequencing reagent bottles, if available, instead of using new bottles. Tap **Help** in the lower left corner of the screen to bypass warnings for installing used bottles.

   ---

3. Follow the on-screen instructions in the **Clear Deck** and **Clear Sequencing Reagents** screens. After sequencing reagents are cleared, tap **Next** to return to the home screen.

Replace Genexus™ Conical Bottles during a Clean instrument

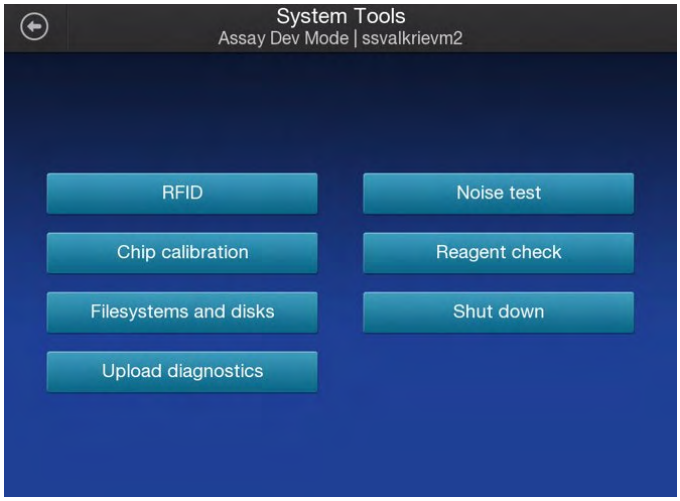
If the sequencer detects restricted flow from the Genexus™ Conical Bottles during a **Clean instrument** procedure, the following alert is displayed.

If you see this alert, follow these steps to replace the five Genexus™ Conical Bottles.

1. Tap **OK**. The screen returns to the run screen.
2. Tap **Settings** ▸ **Clean instrument** again.
3. Follow the screen instructions to clear the deck, then load the deck.
4. When the sequencing reagent bay doors open, do the following.
   a. Remove, but do not discard, the Genexus™ Bottle 1, the two Genexus™ Bottle 2 bottles, and the Genexus™ Bottle 3 to gain easier access to the Genexus™ Conical Bottles.
   b. Remove the five used Genexus™ Conical Bottles, then install five new conical bottles.
   c. Replace the four reagent bottles that you removed in their original positions.
   d. Tap **Help** in the lower left corner of the screen to ignore the reagent bottles and Genexus™ Cartridge as used so they can be reused in the next clean.
5. Close the sequencing reagent bay doors.
   The **Clean instrument** procedure begins automatically.
6. After the cleaning finishes, follow the screen instructions to clear the deck and remove the sequencing reagents.
   For further information, see “Replace the Genexus™ Conical Bottles” on page 195.

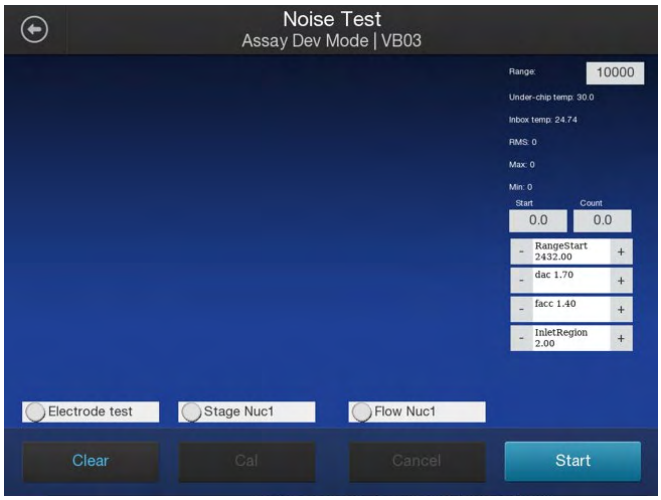
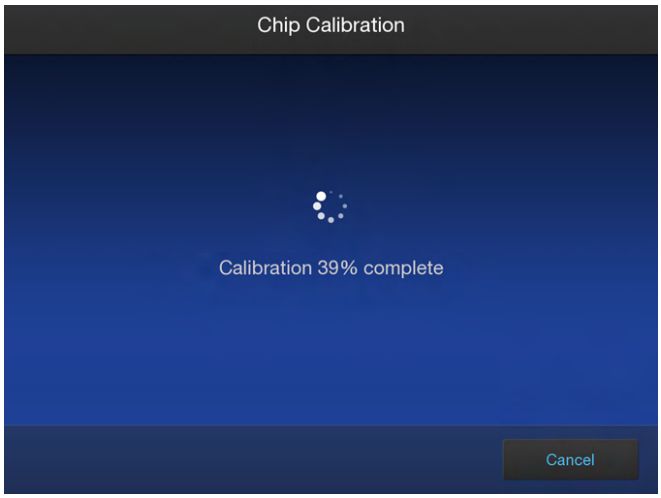
System Tools

The **System Tools** menu enables you to upload instrument diagnostics, manage data, and shut down or reboot the instrument.

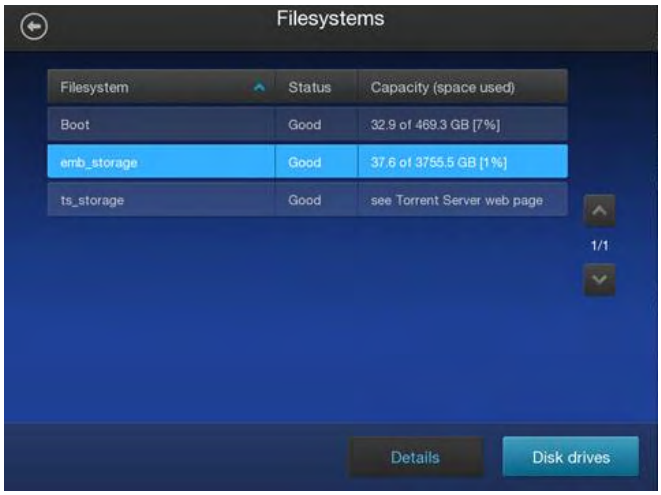
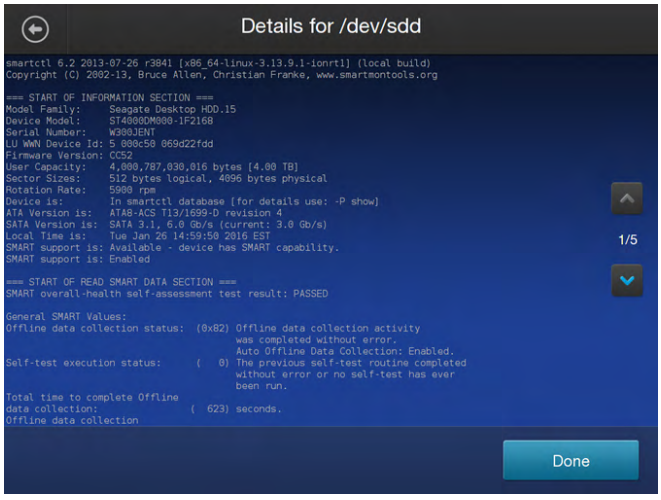


Tool	Description	When/How to use
RFID	Lists the product, product expiration date, and remaining uses of the product. 	To determine if reagents that were left on an instrument during a period of instrument shutdown have expired. 1. Tap the screen within the row of the product to view an **RFID Tag Detail** page. 2. Tap **Done** to return. **Note:** You can also use the RFID Tags screen to track remaining days of reagent use before a sequencer initialization expires. We recommend that you use reagents within 14 days of initialization. After 28 days, the sequencer displays a warning at run start with options to proceed to a clean and new initialization, ignore to continue without clean, or cancel the run. For further information, see “Options for an expired sequencer initialization” on page 111.

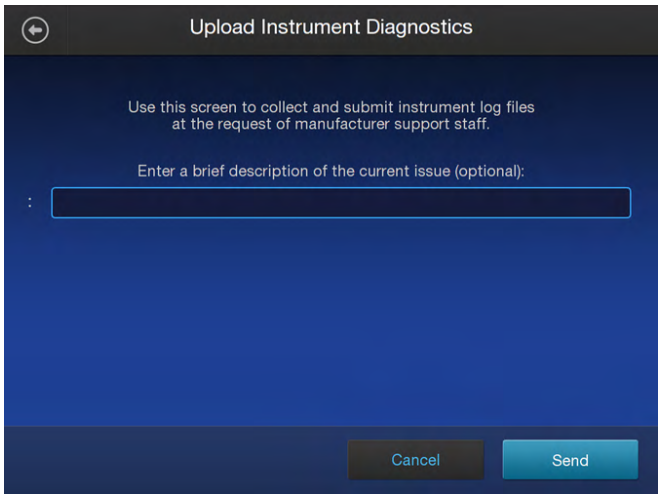
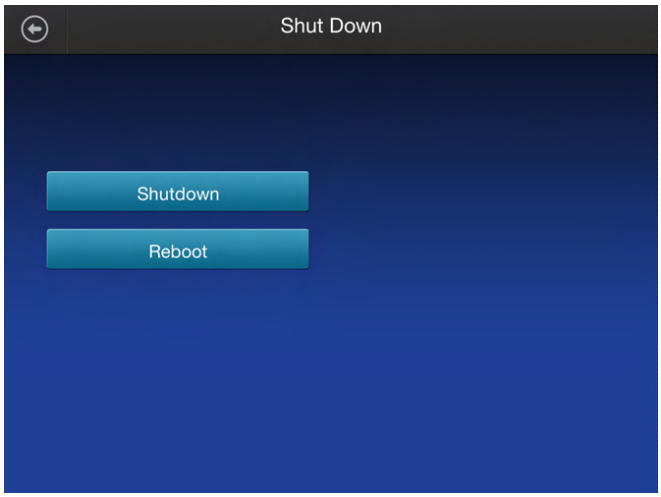
(continued)

Tool	Description	When/How to use
Noise test	Provides real-time measurement of electrical noise readings on the chip. 	For troubleshooting if directed to do so by Technical Support.
Chip calibration	Checks the status of a chip. **IMPORTANT!** Do not perform a chip calibration unless instructed to do so by a field service engineer. 	1. Insert a chip into the chip installation slot. 2. In the System Tools menu, tap **Chip Calibration** to start the chip calibration. 3. In the dropdown lists, select the **Image** to display and the type of **Calibration** to perform. 4. After a chip calibration, perform a **Clean instrument** before removing the chip. **Note:** Tap **Cancel** to quit the chip calibration.

(continued)

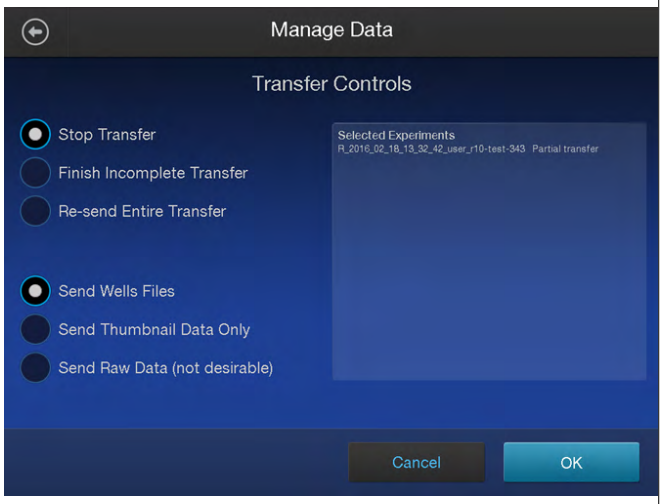
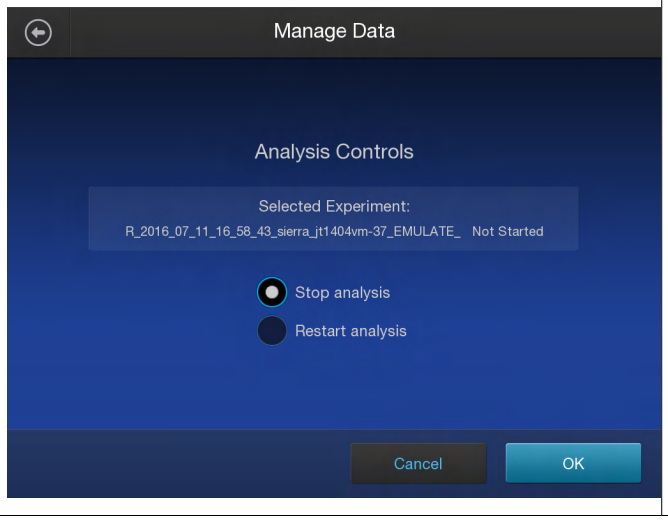
Tool	Description	When/How to use
Filesystems and disks	Provides real-time drive activity status and disk space usage.   	Use if directed to do so as part of a troubleshooting procedure: 1. In the System Tools menu, press **Filesystems and disks**. The **Filesystems** screen opens. 2. Tap the screen within the row of the **Filesystem** that you want to view, then press **Details**. The **Disk Drives in <Filesystem>** screen opens. 3. Tap the screen within the row of the **Drive** to view details of the disk drive. The **Details for <Drive>** screen opens. 4. Tap **Done** to return to the previous screen.

(continued)

Tool	Description	When/How to use
Upload diagnostics	Upload instrument diagnostics files for review by support personnel. 	Upload an instrument diagnostics file after an aborted run, or if otherwise directed to do so by Technical Support. **Note:** The file is pushed to Genexus™ Software. To retrieve the file using Genexus™ Software, navigate to ⚙ **(Settings) ▸ About**, click **Instrument Diagnostics**, then follow the on-screen instructions.
Shut down	Provides access to "Shutdown" and "Reboot" commands. 	Use these commands if directed to do so by Technical Support as part of a troubleshooting procedure, or if the instrument will not be used for more than three days. **Note:** It is not necessary or recommended to shut down the instrument overnight or over the weekend. To power on the instrument after a shut down, see “Power on” on page 37.

Data Management

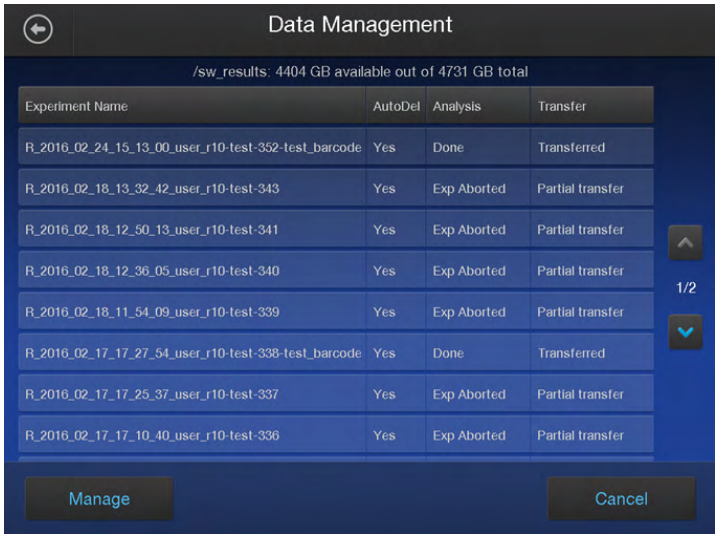
The **Data Management** function allows you to delete run data manually, or transfer data in the event of a failure of automatic transfer. Under normal conditions, run data are automatically transferred to the analysis server, then deleted from the instrument hard drive.

Item	Description	When/How to use
Delete data	Manually delete run data from the instrument.	If the instrument hard drive becomes full, see “Manually delete run data” on page 213.
Transfer Controls	Transfer run data from the instrument hard drive to the Ion Torrent™ Server. 	1. Tap the screen in the **Experiment Name** row of the individual experiment to be transferred. 2. Tap **Transfer**. 3. Select the transfer action to be performed. 4. Select the file transfer option. 5. Tap **OK**.
Analysis Controls	Stop the analysis of the data of individual runs. 	1. Tap the screen in the **Experiment Name** row of the individual experiment to be analyzed, then tap **Analysis**. 2. Select **Stop Analysis**, then tap **OK**. **Note: Restart analysis** is currently not supported.

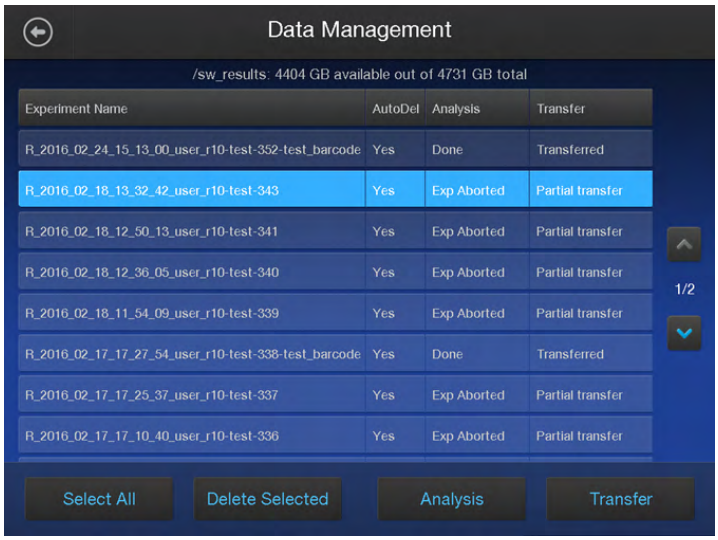
Manually delete run data

To troubleshoot data management problems, use the **Data Management** function to delete run data manually, or transfer the data to an external server.

1. In the **Settings** menu, tap **Data Management** to access the **Data Management** screen, then tap **Manage**.



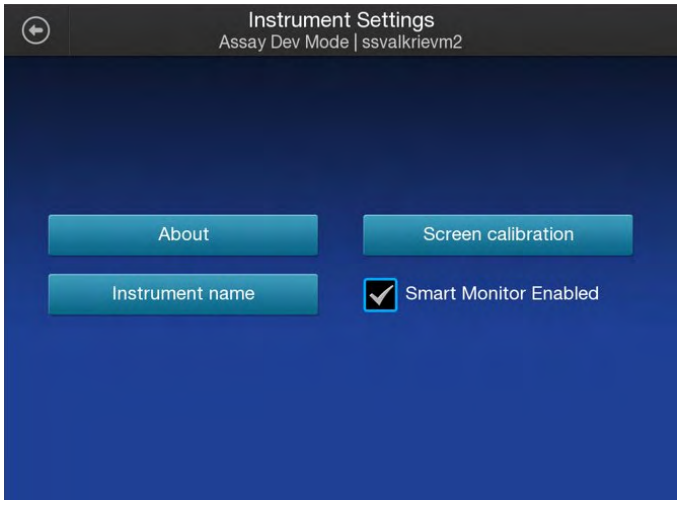
2. Tap **Select All** to select all the available experiments, or tap the screen in the **Experiment Name** row of the individual experiment to be managed.

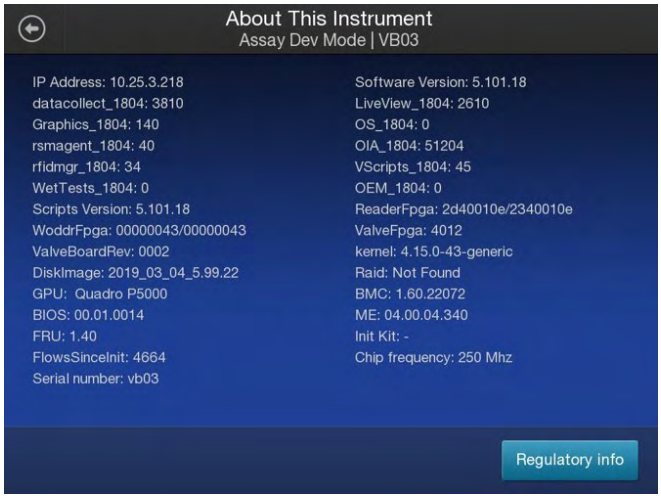


3. Tap **Delete Selected**.

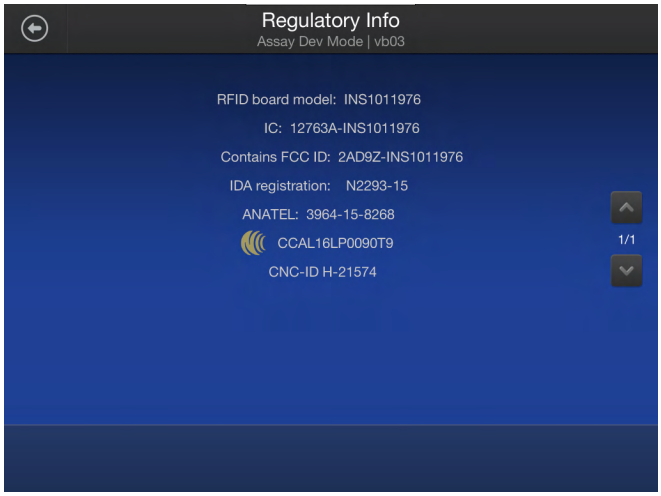
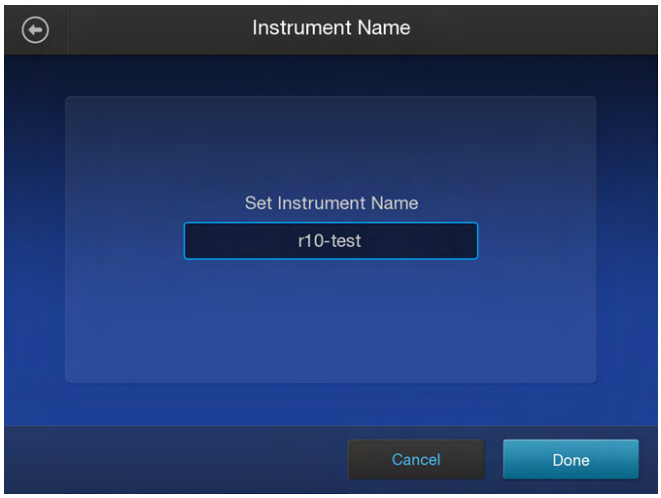
Instrument settings

The **Instrument Settings** buttons access information about the instrument and allow you to set the instrument name and calibrate the touchscreen.

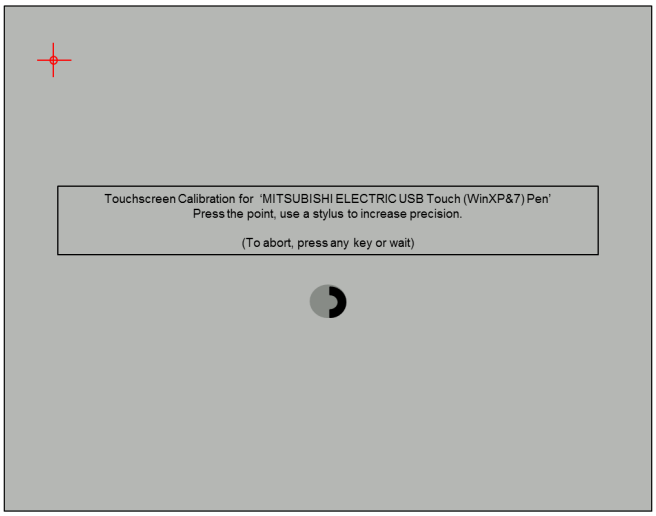


Button	Description	When/How to use
About	Provides instrument details. 	Use the screen to view instrument reference information or access regulatory information. Click **Regulatory info** to view the following screen.

(continued)

Button	Description	When/How to use
Regulatory info	Lists instrument-specific regulatory information. 	To view instrument RFID regulatory information. See “Radio compliance standards” on page 241 for compliance information.
Instrument name		To change the instrument name.

(continued)

Button	Description	When/How to use
Screen calibration		For troubleshooting if directed to do so by Technical Support. Touch the red cross with your finger or a stylus each time it appears. In total, you touch the screen 4 times, one time in each corner.
Smart Monitor Enabled	—	Select the checkbox to enable remote monitoring of instrument runs by Thermo Fisher Scientific for troubleshooting.

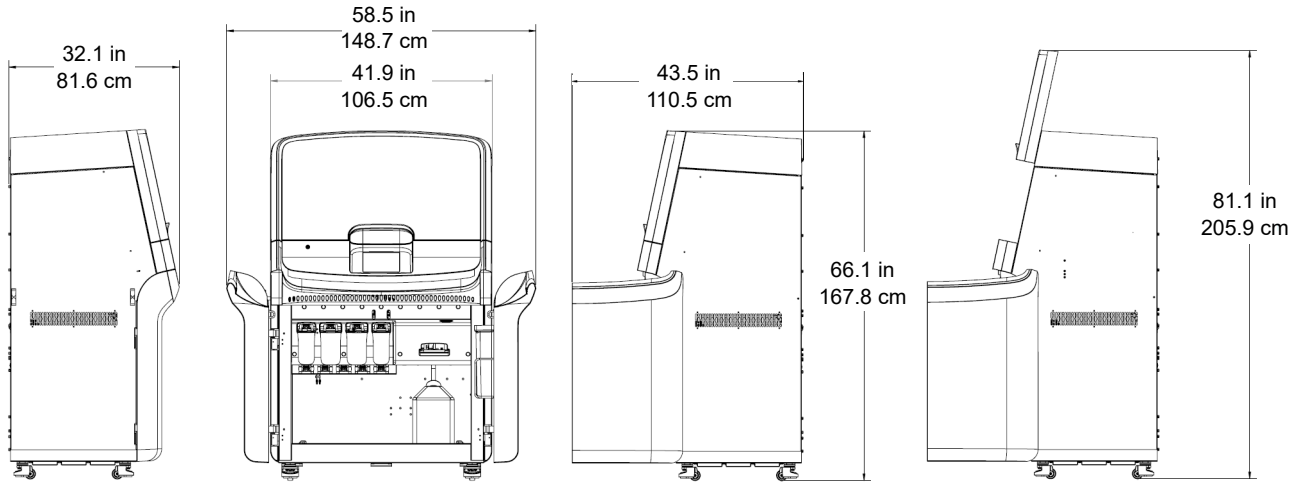
D

Instrument specifications

Instrument dimensions, weight, and clearances

Instrument dimensions and weight

Component	Height		Length (depth)		Width		Weight
	Open	Closed	Open	Closed	Open	Closed	
Genexus™ Integrated Sequencer	81.1 in (205.9 cm)	66.1 in (167.8 cm)	43.5 in (110.5 cm)	32.1 in (81.6 cm)	58.5 in (148.7 cm)	41.9 in (106.5 cm)	450 lb (204.1 kg)



Instrument clearances

During instrument setup and maintenance, it is necessary to access the back and sides of the instrument. If the back of an instrument component faces a wall, it is necessary to have enough space to roll the instrument out from the wall to enable access to the back of the instrument.

IMPORTANT! For safety, the power outlet that is used for powering the instrument must be accessible at all times.

Component	Top	Front	Left/Right	Back
Genexus™ Integrated Sequencer	20 in (50.8 cm)	18 in (45.7 cm)[1]	6 in/12 in[2] (15.2 cm/30.5 cm)	12 in (30.5 cm)

[1] The instrument requires 36.0 in (90.0 cm) aisle in front of bench for operator access.

[2] To allow sufficient side clearance for the instrument doors to open.

Environmental requirements

Ensure that the installation room is maintained under correct environmental conditions. Avoid placing the sequencer next to heaters, cooling ducts, or in direct sunlight. Place the sequencer at least 1 meter away from major sources of electronic noise such as refrigerators or microwaves. Fluctuations between day and night temperatures can cause system instability.

Component	Acceptable range
Altitude	Located between sea level and 2,500 m (8,200 ft) above sea level
Humidity	20%–70%, non-condensing
Operating temperature	15°C to 30°C (59°F to 86°F) **IMPORTANT!** • The recommended operating temperature for the Genexus™ Integrated Sequencer during install qualification and performance qualification runs is 23°C (73°F). • If the sequencer is operated at an elevation of 1,800 m (5,900 ft) or greater above sea level, the maximum operating temperature is 23°C (73°F). • The room temperature must not fluctuate more than 2°C (3.6°F) over a 2-hour period.
Vibration	Do not install the instrument(s) near equipment that causes vibration (freezers, pumps, and similar equipment). Significant vibration during sequencing can add noise and reduce the quality of the sequencing measurements.
Electromagnetic interference	The electromagnetic environment should be evaluated before placement and operation of the instrument. Do not use the instrument in close proximity to sources of strong electromagnetic radiation (for example, unshielded intentional RF sources), as these sources can interfere with proper operation.

(continued)

Component	Acceptable range
Pollution	The Genexus™ Integrated Sequencer is intended to be used in Office or Laboratory controlled environments.
Other conditions	For indoor use only. Install the sequencer on a level surface. The installation location must be away from any vents that could expel particulate material on the system components.

Thermal specifications for the instrument

During operation, the thermal output based on the typical current draw of the instrument is:

Component	Typical draw[1]	Thermal output
Genexus™ Integrated Sequencer	1000W	3412 BTU/h

[1] Maximum draw: 1200W

Ventilation requirements

Allow at least 12 in (30 cm) of clearance around the Genexus™ Integrated Sequencer for ventilation. Do not block air inlets or outlets that allow proper ventilation.

Electrical requirements

WARNING! For safety, the power outlet used for powering the instrument must be accessible at all times. See “Instrument clearances” on page 218 for information about the space needed between the wall and the instrument. In case of emergency, you must be able to immediately disconnect the main power supply to the instrument. Allow adequate space between the wall and the equipment so that the power cord can be disconnected in case of emergency.

WARNING! Par souci de sécurité, la prise de courant alimentant l’instrument doit être accessible à tout moment. En cas d’urgence, il doit être possible de débrancher immédiatement l’alimentation principale de l’ensemble des équipements. Laisser suffisamment d’espace entre le mur et les équipements afin de pouvoir débrancher les câbles d’alimentation sans encombre, en cas d’urgence.

- Electric receptacle required: 2-prong with ground pin
- Mains AC line voltage tolerances can be up to ± 10% percent of nominal voltage
- Use only the specified power cable supplied with the instrument to connect the sequencer to a wall receptacle. Route the power cord away from the workspace to avoid accidental disconnection.

Component	Rated input voltage	Rated current[1]	Rated frequency	Rated power[2]
Genexus™ Integrated Sequencer	100–240 VAC	12–5A	50/60 Hz	1200W

[1] Based on rated current at minimum input voltage.
[2] Average power is 1000W.

A power cord is provided with the instrument. If not suitable for installation in your region, ensure any power cord you do use is:

- Maximum 10 ft (3 m) in length
- Grounding type
- Compatible with the power supply receptacles used to connect to main power
- Suitable for the rating of the instrument and mains power supply
- Compliant with local safety requirements (for example, UL Listed for North America, JIS approved for Japan, HAR or agency certified for Europe)

Library QC Archive: recover library preparations from the Genexus™ Integrated Sequencer for reuse

After a run completes on the Genexus™ Integrated Sequencer, a variable volume of library prepared during the run is left over, and can be manually recovered from Zone 2 wells (columns 5–8) of the PCR amplification plate. The leftover volume depends on the number of samples used per library strip. If one sample is used per strip, 7 µL is present; if four samples are used per strip, 55 µL is present. The recovered pre-pooled libraries can be purified, quantified, pooled, and reused in a Library to Result run on the Genexus™ Integrated Sequencer. The recovered and purified libraries can also be archived for later use.

Materials and equipment required

Unless otherwise indicated, all materials are available through **thermofisher.com**.

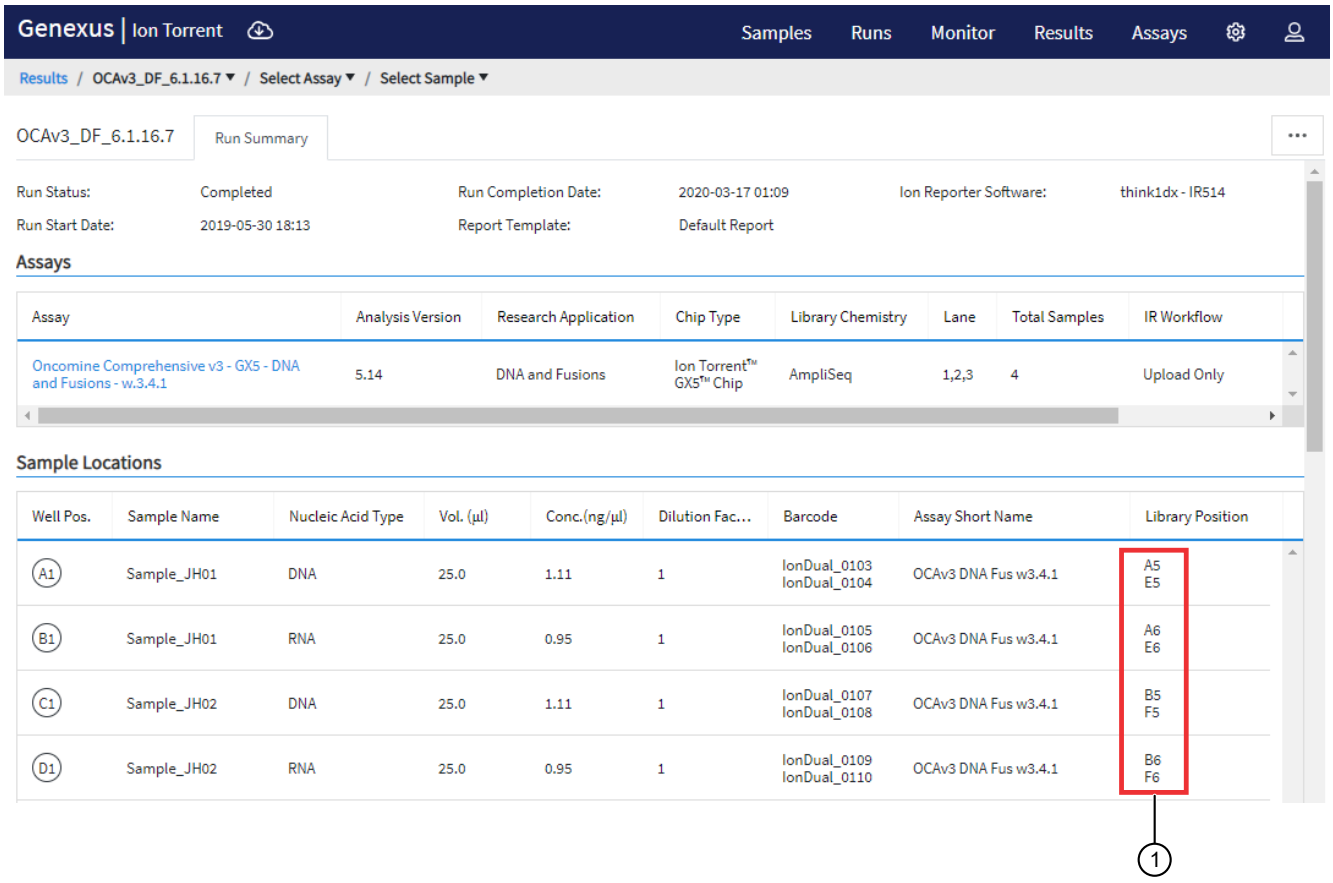
Item	Source
Agencourt™ AMPure™ XP Reagent	NC9959336, NC9933872 (**fisherscientific.com**)
DynaMag™–96 Side Magnet, or equivalent	12331D
Ethanol, Absolute, Molecular Biology Grade	BP2818500 (**fisherscientific.com**)
Nuclease-free water	AM9932
Low TE buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0)	—
Eppendorf™ DNA LoBind™ Microcentrifuge Tubes, 1.5-mL	13-698-791 (**fisherscientific.com**)
MicroAmp™ Optical 96-Well Reaction Plate with Barcode	4306737
MicroAmp™ Clear Adhesive Film	4306311

(continued)

Item	Source
Ion Library TaqMan™ Quantitation Kit	4468802
Applied Biosystems™ 7500 Fast Real-Time PCR Instrument, or equivalent	4351106

Recover libraries from the sequencer and purify

After a sequencing run, leftover volume from each library prepared from a sample and primer pool (7–55 µL, depending on the number of samples used per library strip) is present in the plate loaded in the PCR amplification station. The library order in the plate is provided in the **Run Summary** tab (**Results** ▸ **Run Results** ▸ **Run name**). The following figure shows the library positions for 2 samples in a run using Oncomine™ Comprehensive Assay v3 GX with two primer pools each for DNA and RNA.



① Library position in PCR plate

The aqueous library layer is overlayed with approximately 25 µL of mineral oil. Use the following procedure to recover and purify the libraries.

Before you start the procedure, do the following.

- Prepare sufficient 70% ethanol to have 400 µL for each library to be recovered and purified.
- Warm the Agencourt™ AMPure™ XP Reagent to room temperature and vortex thoroughly to disperse the beads before use. Pipet the suspension slowly.

IMPORTANT! Do not substitute a Dynabeads™-based purification reagent for the Agencourt™ AMPure™ XP Reagent.

1. Remove the PCR amplification plate from the sequencer deck, then transfer 30 µL of the lower aqueous layer to a new PCR plate.

   Note:
   - If a well contains less than 30 µL, transfer the entire aqueous volume.
   - It is not critical if you transfer some of the oil overlay. The oil does not interfere in the purification procedure.

2. Add 30 µL of nuclease-free water to each well with library.
3. Add 60 µL (1X sample volume) of Agencourt™ AMPure™ XP Reagent to each library, then pipet up and down 5 times (with the pipettor set to 60 µL) to mix the bead suspension with the DNA thoroughly. Visually inspect each well to ensure that the mixture is homogeneous. Use a new pipette tip for each library.
4. Incubate for 5 minutes at room temperature.
5. Place the plate in a magnetic rack such as the DynaMag™-96 Side Magnet, then incubate for 2 minutes, or until the suspension clears.
6. Carefully remove the supernatant without disturbing the pellet, then discard the supernatant.
7. Add 150 µL of freshly prepared 70% ethanol, then move the plate side-to-side in the two positions of the magnet for 30 seconds to wash the beads.

   Note: If your magnet does not have two positions for shifting the beads, remove the plate from the magnet and gently pipet up and down 5 times (with the pipettor set at 100 µL), then return the plate to the magnet and incubate for 2 minutes or until the solution clears.

8. Carefully remove the supernatant without disturbing the pellet, then discard.
9. Repeat step 7 and step 8 for a total of two 70% ethanol washes.
10. Ensure that you remove all ethanol droplets from the wells. Keeping the plate in the magnet, air-dry the beads for 5 minutes at room temperature.

    IMPORTANT! Residual ethanol can inhibit later reactions. If needed, centrifuge the plate and remove remaining ethanol before air-drying the beads. Under conditions of low relative humidity the beads air-dry rapidly. Do not overdry.

11. Remove the plate from the magnet, then add 50 µL of Low TE to each pellet to disperse the beads.

12. Seal the plate with MicroAmp™ Clear Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.
13. Incubate the plate for at least 2 minutes at room temperature.
14. Place the plate on the magnet for at least 2 minutes.
15. Remove the supernatant, which contains the purified library, and transfer it to a new labeled Eppendorf LoBind™ tube.

Proceed to “Quantify the purified libraries”.

Quantify the purified libraries

1. Dilute 2 µL of each purified library with 198 µL Nuclease-free water for a 100-fold dilution.
2. Use the dilution to quantify your libraries with the Ion Library TaqMan™ Quantitation Kit. For detailed procedures, see the *Ion Library TaqMan™ Quantitation Kit User Guide* (Pub. No. MAN0015802), available at **thermofisher.com**.

Quantified libraries can be combined with sample libraries of similar panel and barcode type (Ion Torrent™ Dual Barcode) and used in library runs on the Genexus™ Integrated Sequencer, or in templating reactions for sequencing on other Ion Torrent™ platforms. For details, see “Combine libraries”.

Combine libraries

After quantification, combine libraries that were prepared with different barcodes according to the assay used.

IMPORTANT! Be careful not to combine libraries barcoded with the same barcode adapter.

- For single primer pool assays, and for multiple primer pool DNA *or* fusion assays, adjust sample library concentration to 200 pM, then combine an equal volume of each library so that the total volume is ≥125 µL, or the volume specified in the setup guide for the library run that you plan.
- For DNA and fusion assays, adjust sample library concentration to 200 pM, then follow the recommendations in the table below to combine the DNA and RNA libraries prepared for a given sample in the appropriate ratio. Prepare sufficient volume so that the total volume of combined libraries is ≥125 µL.

Assay	DNA:RNA library ratio[1]	
	FFPE sample	High-molecular weight sample
Oncomine™ Comprehensive v3 - GX5 - DNA and Fusions	70:30	80:20
Oncomine™ Myeloid v2 - GX5 - DNA and Fusions	—	90:10

[1] DNA:RNA library pooling ratio in system-installed assays can be found in the Library Pooling Percent DNA and Library Pooling Percent RNA assay parameters.

Note: For DNA or RNA panels with two primer pools, the fraction of each pool should be equal.

Proceed to "Plan a Library to Result run" on page 84 to use the purified and combined libraries in a library run on the Genexus™ Integrated Sequencer. To achieve the required number of reads per sample specified in the assay that is used, follow guidance during library run planning on how many samples you can combine in a single run with the sequencing chip that is loaded.

Store libraries

You can store libraries at 4–8°C for up to 1 month. For longer lengths of time, store at –30°C to –10°C.

Supplemental information

Quantify FFPE DNA with the Qubit™ Fluorometer

When using the Ion AmpliSeq™ Direct FFPE DNA Kit, the DNA concentration can be estimated using a Qubit™ Fluorometer and the Qubit™ dsDNA HS Assay Kit (Cat. No. Q32851). See the *Qubit™ dsDNA HS Assay Kits User Guide* (Pub. No. MAN0002326) for more information.

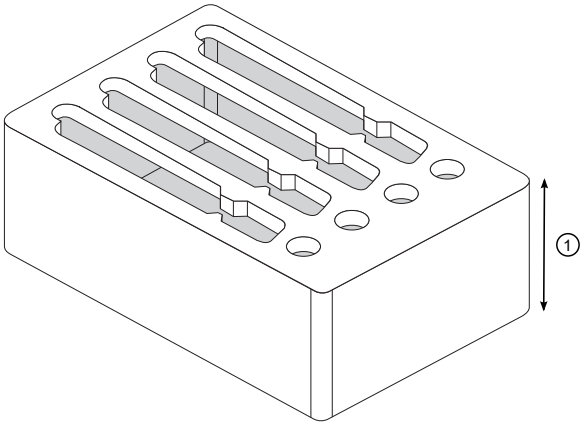
1. Set up the required number of 0.5-mL Qubit™ Assay tubes for standards and samples. The Qubit™ dsDNA HS Assay requires 2 standards.
2. Prepare sufficient Qubit™ working solution for all samples and standards by diluting Qubit™ dsDNA HS Reagent 1:200 in Qubit™ dsDNA HS Buffer.
3. Combine 2 µL of the FFPE DNA sample with 198 µL (200-µL final volume) of working solution, mix well, then incubate for at least 2 minutes.
4. Prepare each Qubit™ standard as directed in the user guide.
5. Measure the concentration of each sample and standard on the Qubit™ Fluorometer.
6. *(Qubit™ 2.0 Fluorometer only.)* Calculate the concentration of the undiluted sample by multiplying by the dilution factor. Alternatively, use the **Calculate Stock Conc.** feature on your instrument.

Proceed to “Dilute or concentrate the samples, if needed, then load the sample plate—Nucleic Acid to Result run” on page 92.

Centrifuge library and templating reagent strips using the Genexus™ Strip Centrifuge Adapter

You can use the Genexus™ Strip Centrifuge Adapter as a holder for centrifuging library and templating strips to collect contents in the bottom of strip wells after vortexing the strips. Vortexing and centrifuging strips is recommended to reduce errors in the workflow due to air bubbles in strip wells or beads trapped near the foil seal. Users can request this part from a Thermo Fisher Scientific Field Service Engineer.

Use of the adapter requires a centrifuge with buckets that support the height of the adapter at 44 mm so that buckets swing freely in the centrifuge rotor when loaded with strips. For more information, see “Required materials—general laboratory equipment and supplies” on page 30.



Genexus™ Strip Centrifuge Adapter

① 44 mm height

To use the Genexus™ Strip Centrifuge Adapter, follow these steps.

1. After thawing and vortexing, load the strips in the adapters in a balanced orientation. The centrifuge buckets must support the height of the adapters loaded with strips.
2. Place each adapter loaded with strips in the centrifuge.
3. Centrifuge the strips at 300 × *g* for 15 seconds.
4. Remove strips from the adapters, then inspect the strips to ensure that contents have been collected and air bubbles are not present.
5. If brown magnetic beads are still visible in the tube keyhole near the foil seal, invert the strip to resuspend the contents, then repeat step 3.

   Note: It is not necessary to dislodge all of the beads trapped in a keyhole—dislodging most of the beads is sufficient.
6. Repeat step 1 through step 4, if needed, for the remaining library and templating strips to be loaded in the sequencer.
7. After centrifugation, return Genexus™ Strip 2-AS or Genexus™ Strip 2-HD, and Genexus™ Strip 4 to ice or 4°C until you are ready to load the strips in the sequencer.

Guidelines for using custom assays with the Genexus™ Integrated Sequencer

Follow these guidelines for setting up assays on the Genexus™ Integrated Sequencer if you are using a custom Ion AmpliSeq™ or Ion AmpliSeq™ HD assay.

Ion AmpliSeq™ library chemistry:

- For germline Ion AmpliSeq™ assays, start with a coverage depth of 150 reads per amplicon for calculating the **Minimum Read Count Per Sample** that you enter in the **Panel** step of assay setup.

  Example: Your panel has 500 amplicons in each of two primer pools.

  Minimum Read Counts Per Sample =

  (500 amplicons × 2 pools) × 150 reads/amplicon/sample = 150,000
- For somatic Ion AmpliSeq™ assays, start with a coverage depth of 2,500 reads per amplicon for calculating the **Minimum Read Count Per Sample** when you set up your assay.

  Example: Your panel has 500 amplicons in each of two primer pools.

  Minimum Read Counts Per Sample =

  (500 amplicons × 2 pools) × 2,500 reads/amplicon/sample = 2,500,000
- Refer to the information in the following table for entering the number of target amplification cycles and anneal/extend time parameters for a custom Ion AmpliSeq™ panel.

Primer pairs per pool	Recommended number of amplification cycles (10 ng high-quality DNA/RNA)[1]	Anneal/Extend time[2]
RNA fusion panels	28	4 minutes
12–24	22	4 minutes
25–48	21	4 minutes
49–96	20	4 minutes
97–192	19	4 minutes
193–384	18	4 minutes
385–768	17	4 minutes
769–1,536	16	8 minutes
1,537–3,072	15	8 minutes
3,073–6,144	14	16 minutes
6,145–24,576	13	16 minutes

[1] Add 3 cycles for low quality (FFPE) samples.

[2] For Ion AmpliSeq™ panels using a 375-bp amplicon design, add 4 minutes to the anneal/extend time recommended in the table.

Ion AmpliSeq™ HD library chemistry:

- To calculate the **Minimum Read Count Per Sample** parameter that you enter during assay setup, estimate the coverage depth that you require to achieve the limit of detection (LoD) needed for your samples. The limit of detection depends on coverage depth and amount of input material.

  For more information, see the *Ion AmpliSeq™ HD Library Kit User Guide* (Pub. No. MAN0017392).

LoD	Minimum amplicon coverage
5%	1,400
1%	7,000
0.5%	14,000
0.1%	50,000

  Examples:

  - Your panel has 200 amplicons in each of two primer pools, and you require 5% LoD and 1,400 reads/amplicon for a solid tumor sample.

    Minimum Read Counts Per Sample =

    (200 amplicons × 2 pools) × 1,400 reads/amplicon/sample = 560,000

  - Your panel has 100 amplicons in each of two primer pools, and you require 0.1% LoD and 50,000 reads/amplicon for a cfTNA sample.

    Minimum Read Counts Per Sample =

    (100 amplicons × 2 pools) × 50,000 reads/amplicon/sample = 10,000,000

  Note: These calculations are based on 100% uniformity and 100% on target reads. Depending on the actual uniformity of the panel, the quality of input sample, and panel on-target percentage, minimum read counts per sample may need adjustment.

- Refer to the information in the following table for entering the number of library amplification cycles for a custom Ion AmpliSeq™ HD panel.

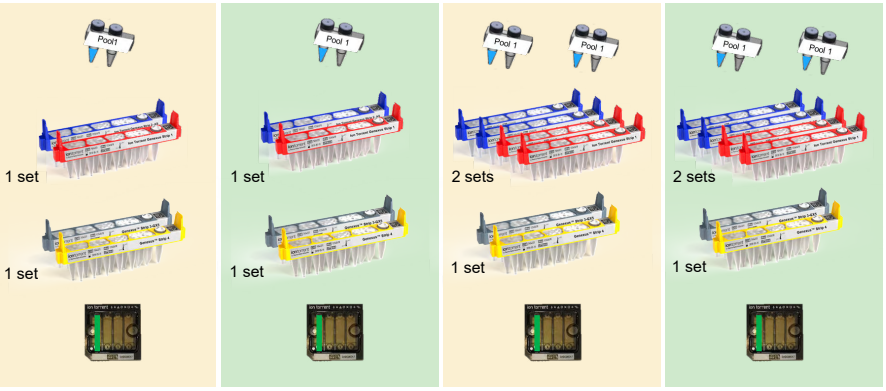
Primer pairs per pool	Recommended number of cycles
12–500	20
501–1,000	19
1,001–2,000	18
2,001–5,000	17

Planning sequencing runs for efficient use of consumables

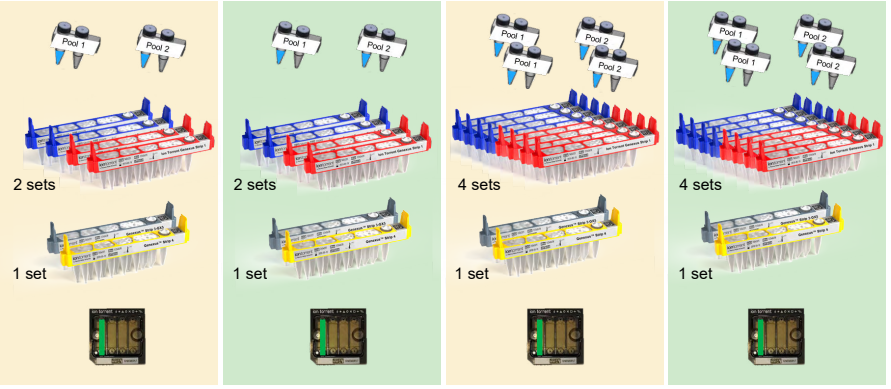
Genexus™ Integrated Sequencer consumables are designed to be used most efficiently when samples are grouped in multiples of four. For example, running one sample uses the same number of primer pool tubes, library and template strips, and chip lanes as four samples. Running five or six samples requires the same consumables as eight samples. For the most economical use of reagents, therefore, you should try to group samples in your runs in multiples of four, if possible.

The following tables show the relationship between sample number, the number of pools in an assay, the required number of reads per sample, and the amount of deck consumables required for a sequencing run.

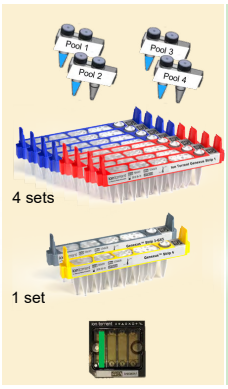
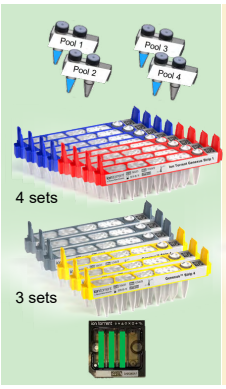
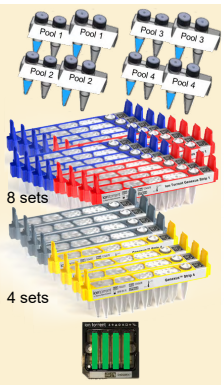
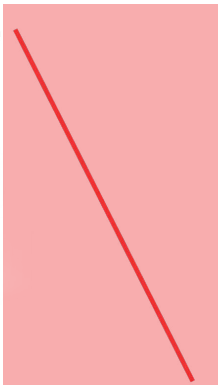
One-pool assay, requiring 500K reads per sample

Consumable	1 Sample	4 Samples	6 Samples	8 Samples
Primer pools				
Library strips				
Template strips				
GX5™ Chip (12–15M reads per lane)				

Two-pool assay, requiring 1M reads per sample

Consumable	1 Sample	4 Samples	6 Samples	8 Samples
Primer pools				
Library strips				
Template strips				
GX5™ Chip (12–15M reads per lane)				

2 × two-pool assay, requiring 8M reads per sample

Consumable	1 Sample	4 Samples	6 Samples	8 Samples
Primer pools Library strips Template strips GX5™ Chip (12–15M reads per lane)				

Note: Eight samples at 8M reads per sample exceed the capacity of the GX5™ Chip. You can proceed with the run using eight pairs of library strips and four pairs of template strips, but you may not achieve the minimum reads per sample set for the assay.

Register Genexus™ Software accounts (manager/administrator)

Manager- and administrator-level users can register Genexus™ Software accounts. After the accounts are registered, you can transfer results from one Genexus™ Integrated Sequencer to another.

1. In Genexus™ Software, click **(Settings)** ▸ **Thermo Fisher Account**.
2. In the **Thermo Fisher Account Settings** screen, click **Register Account**.
3. In the **Register Thermo Fisher Account** dialog box, enter the information that is required to create the account.

Item	Description
Account Type	Select **Genexus**.
Name	Enter a name to identify the account in the **Thermo Fisher Account Settings** screen in Genexus™ Software. The name can contain only alphanumeric characters (0-9, Aa-Zz), periods (.), underscores (_), or hyphens (-). For example, enter ***Lab_Admin***.
User Name	Enter the name that is used to sign in to the Genexus™ Software account that you want to link.
Password	Enter the password for the Genexus™ Software account that you want to link.
Server	Enter the name of the Genexus™ Integrated Sequencer account that will be used for file uploads.
Port	Enter the port of the Genexus™ Integrated Sequencer.
Version	Click **Get Ion Reporter Software Versions**, then select the software version.
Set as Default Account	Click the toggle switch to make the account the default account that is used to upload results files.

The configured account is listed in the **(Settings) / Thermo Fisher Account** screen. The **Account Type** is **Genexus**. A successfully authenticated account has **Active** listed in the **Status** column.

G Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit **thermofisher.com/support**.

Symbols on this instrument

Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.

- **CAUTION!**—Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.
- **WARNING!**—Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.
- **DANGER!**—Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury.

Standard safety symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.
CAUTION! Risk of electrical shock.
CAUTION! Hot surface.

(continued)

Symbol and description
CAUTION! Potential biohazard.
CAUTION! Ultraviolet light.

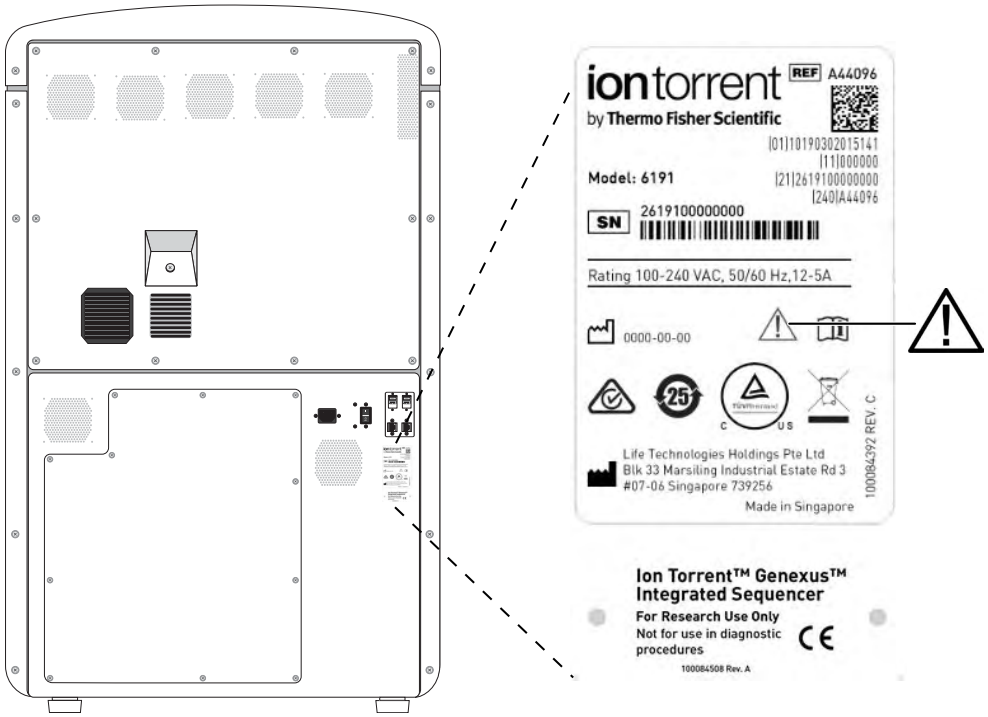
Symbole et description
MISE EN GARDE ! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
MISE EN GARDE ! Risque de choc électrique.
MISE EN GARDE ! Surface chaude.
MISE EN GARDE ! Danger biologique potentiel.
MISE EN GARDE ! Rayonnement ultraviolet.

Additional safety symbols

Symbol and description
CAUTION! Moving parts.
CAUTION! Piercing hazard.

Symbole et description
MISE EN GARDE ! Parties mobiles.
MISE EN GARDE ! Danger de perforation.

Location of safety labels

Label and location
blank Purification instrument rear panel
 Sequencer rear panel

Control and connection symbols

Symbol	Description
	On (Power)
	Off (Power)
	Protective conductor terminal (main ground)
	Alternating current

Conformity symbols

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and U.S.A.
	Indicates conformity with China RoHS requirements.
	Indicates conformity with European Union requirements.
	Indicates conformity with Australian standards for electromagnetic compatibility.
	Indicates conformity with the WEEE Directive 2012/19/EU. **CAUTION!** To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Instrument safety

General

CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

Physical injury

CAUTION! Moving and Lifting Injury. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. Improper lifting can cause painful and permanent back injury.

Things to consider before moving the instrument:

- Depending on the weight, moving may require two or more persons.
- If you decide to move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.

CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety

WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.

AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.

WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility.

AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.

WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.

AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Cleaning and decontamination

CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.

MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety standards

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-010 EN 61010-2-010	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-010: Particular requirements for laboratory equipment for the heating of materials*
IEC 61010-2-020 EN 61010-2-020	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-020: Particular requirements for laboratory centrifuges*
IEC 61010-2-081 EN 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*
IEC 61010-2-101 EN 61010-2-101	*Safety requirements for electrical equipment for measurement, control and laboratory use - Part 2-101: Particular requirements for in vitro diagnostic (IVD) medical equipment*

EMC standards

Reference	Description
EU Directive 2014/30/EU	European Union “EMC Directive”
EN 61326-1 IEC 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
EN 61326-2-6 IEC 61326-2-6	*Electrical equipment for measurement, control and laboratory use. EMC requirements. Particular requirements. In vitro diagnostic (IVD) medical equipment*
FCC Part 18 (47 CFR)	U.S. Standard “Industrial, Scientific, and Medical Equipment”
AS/NZS CISPR 11	*Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment*

(continued)

Reference	Description
ICES-001, Issue 4	*Industrial, Scientific and Medical (ISM) Radio Frequency Generators*
FCC Part 15 Subpart B (47 CFR)	*U.S. Standard Radio Frequency Devices* This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference, in which case, you may need to take measures to mitigate the interference. Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these can interfere with proper operation.

Environmental design standards

Reference	Description
Directive 2012/19/EU	European Union WEEE Directive on waste electrical and electronic equipment.
Directive 2011/65/EU	European Union RoHS Directive on restriction of hazardous substances in electrical and electronic equipment.
SJ/T 11364-2014	China RoHS Standard—Marking for the restriction of the use of hazardous substances in electronic and electrical products For instrument specific certificates, visit our customer resource page at **www.thermofisher.com/us/en/home/technical-resources/rohs-certificates.html**.

Radio compliance standards

Reference	Description
Directive 2014/53/EU	European Union: *RE Directive*—Radio equipment
RFID	FCC Notice (for U.S. Customers): This device complies with Part 15 of the FCC Rules. Operation is subject to the following 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. Changes and modifications not expressly approved byThermo Fisher Scientific can void your authority to operate this equipment under Federal Communications Commissions rules.

(continued)

Reference	Description
RFID	Canada: This device complies with Industry Canada license-exempt RSS standard(s). Operation is subject to the following two conditions: (1) this device may not cause interference, and (2) this device must accept any interference, including interference that may cause undesired operation of the device.
RFID	Canada (Français québécois): Le présent appareil est conforme aux CNR d'Industrie Canada applicables aux appareils radio exempts de licence. L'exploitation est autorisée aux deux conditions suivantes : (1) l'appareil ne doit pas produire de brouillage, et (2) l'utilisateur de l'appareil doit accepter tout brouillage adioélectrique subi, même si le brouillage est susceptible d'en compromettre le fonctionnement.
Notice 423 [2005] Industrial and Information Department Radio, BRR[1]	China: Notice on issuing the technical requirements for micropower (short range) radio equipment.
GB/T 12572-2008	China: *Universal requirements and measurement methods of parameters for radio transmitting equipment*
ETSI EN 300 330-1 (2015-03)	China: *Electromagnetic compatibility and Radio spectrum Matters (ERM); Short Range Devices (SRD); Radio equipment in the frequency range 9 kHz to 25 MHz and inductive loop systems in the frequency range 9 kHz to 30 MHz; Part 1 Technical characteristics and test methods*
No 1 [2014]1 of Industrial and Information Department Radio, BRR[1]	China: *Methods for the administration of type approval of radio transmitting modules used in independent operation*
China: The Ministry of Industry and Information Technology (MIIT) issued a notice on micro-power short-range radio transmitting equipment.	

[1] Bureau of Radio Regulation of the Ministry of Industry and Information Technology, State Radio Office

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020 **https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf**
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs) **www.who.int/publications/i/item/9789240011311**

Documentation and support

Related documentation

Document	Publication number
Genexus™ Software 6.6 User Guide	MAN0024953
Genexus™ Integrated Sequencer Quick Reference	MAN0017912
Genexus™ Purification System User Guide	MAN0018475
Ion AmpliSeq™ SARS-CoV-2 Insight Research Assay – GX User Guide	MAN0024933
Oncomine™ Precision Assay GX User Guide	MAN0018508
Oncomine™ Comprehensive Assay v3 GX User Guide	MAN0018512
Oncomine™ TCR Beta-LR Assay GX User Guide	MAN0018513
Oncomine™ Myeloid Assay GX v2 User Guide	MAN0025830
Oncomine™ BRCA Assay GX User Guide	MAN0018514
Genexus™ Integrated Sequencer Site Preparation Guide	MAN0017918
Genexus™ Integrated Sequencer IT Checklist	MAN0018466
Ion Library TaqMan™ Quantitation Kit User Guide	MAN0015802

Customer and technical support

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- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

    Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.