

Biomark/EP1 Data Collection User Guide

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About this User Guide

IMPORTANT: Before using the Biomark™ or EP1™ system, read and understand the safety guidelines in this document. Failure to follow these guidelines may result in undesirable effects, injury to personnel, and/or damage to the instrument or to property.

How To Use This Guide

The following chapters provide information about the data collection software on the Biomark or EP1 system.

Safety Alert Conventions




CAUTION! ABBREVIATED SAFETY ALERTS. Hazard symbols and hazard types specified in procedures may be abbreviated in this document. For the complete safety information, see [Appendix C, Safety](#), on [page 105](#).

Fluidigm documentation uses specific conventions for presenting information that may require your attention. Refer to the following safety alert conventions.


Safety Alerts for Chemicals

For hazards associated with chemicals, this document follows the United Nations Globally Harmonized System of Classification and Labelling of Chemicals (GHS) and uses indicators that include a pictogram and a signal word that indicates the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a red diamond-shaped frame. Refer to the individual safety data sheet (SDS) for the applicable pictograms and hazards pertaining to the chemicals being used.
DANGER	Signal word that indicates more severe hazards.
WARNING	Signal word that indicates less severe hazards.

Safety Alerts for Instruments

For hazards associated with instruments, this document uses indicators that include a pictogram and signal words that indicate the severity level:

Indicator	Description
	Pictogram (see example) consisting of a symbol on a white background within a black triangle-shaped frame. Refer to the instrument user guide for the applicable pictograms and hazards pertaining to instrument usage.
DANGER	Signal word that indicates an imminent hazard that will result in severe injury or death if not avoided.
WARNING	Signal word that indicates a potentially hazardous situation that could result in serious injury or death if not avoided.
CAUTION	Signal word that indicates a potentially hazardous situation that could result in minor or moderate personal injury if not avoided.
IMPORTANT	Signal word that indicates information necessary for proper use of products or successful outcome of experiments.

Safety Data Sheets

Read and understand the SDSs before handling chemicals. To obtain SDSs for chemicals ordered from Fluidigm, either alone or as part of this system, go to fluidigm.com/sds and search for the SDS using either the product name or the part number.

Some chemicals referred to in this user guide may not have been provided with your system. Obtain the SDSs for chemicals provided by other manufacturers from those manufacturers.

The Biomark™ System and EP1™ Reader

1

Biomark System Overview



The Biomark™ System includes the optical, thermal cycling, and software components necessary to perform real-time qPCR gene expression, genotyping and digital analysis, as well as end-point analysis for genotyping and digital applications.

The Biomark™ System provides orders of magnitude higher throughput for real-time qPCR compared to conventional platforms due to its integrated fluidic circuits (IFCs)—nanofluidic IFCs that contain fluidic networks that automatically combine sets of samples with sets of assays. This innovative solution for real-time qPCR provides experiment densities far beyond what is possible with microplate platforms, and significantly reduces the number of liquid-handling steps and volumes per reaction.

Components of the Biomark System

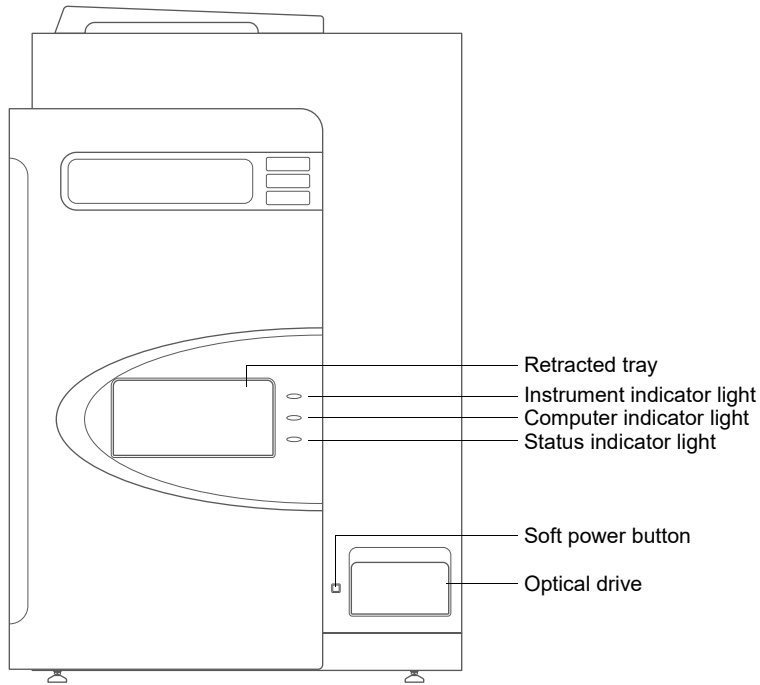


Figure 2. Biomark front view

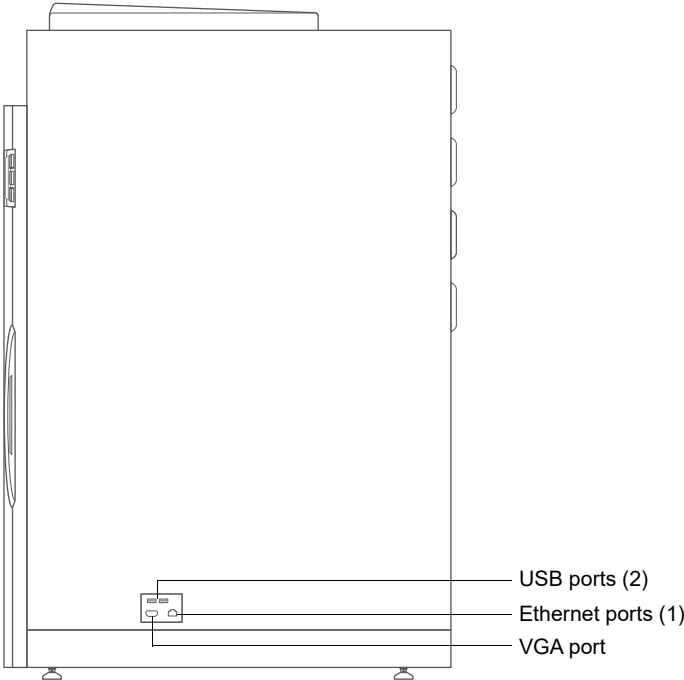


Figure 3. Biomark right side view

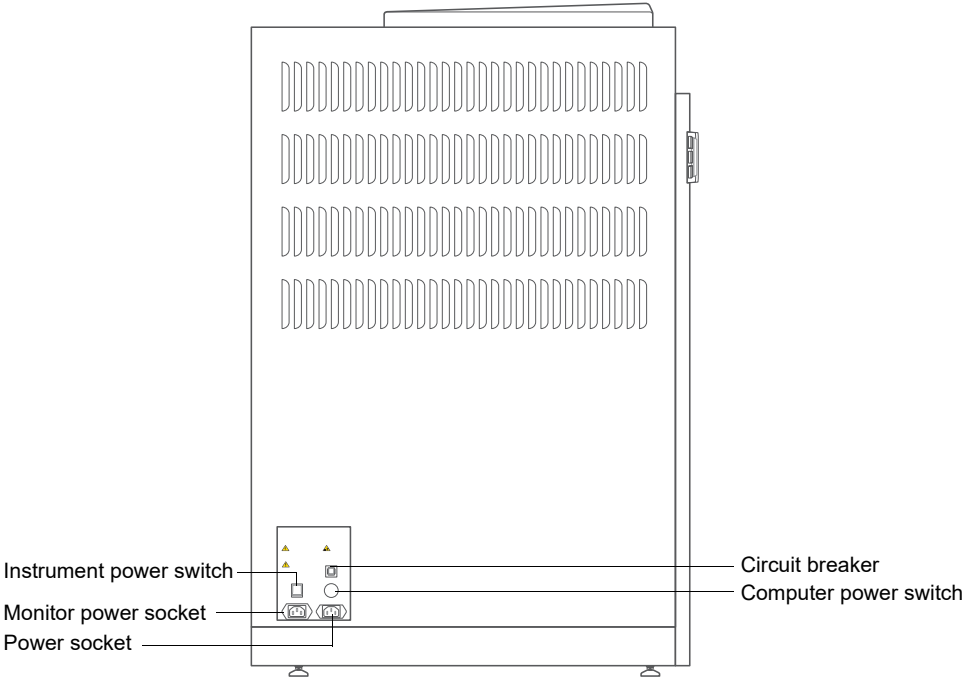



Figure 4. Biomark rear view

Components Included in the Biomark Shipping Box

	Component	Purpose	Qty
1.	Biomark instrument	Thermal-cycles the IFC and collects real-time and endpoint data.	1
2.	Power cable	Country specific power cable to connect the Biomark to the wall socket.  The instrument has a connection to protective earth through the power cord provided by Fluidigm. Ensure that the electrical receptacle provides an earth ground before connecting the power cord. Use only power cords provided by Fluidigm or power cords that meet the minimum ratings of 250V/10A, 16AWG and a length not exceed 2 meters (6 feet).	1
3.	Flat screen monitor	Displays the status of the Biomark system and Data Collection software.	1
4.	Keyboard and mouse	Input devices for using the Biomark software.	1
5.	IFC Controller	Controls filling of reagents and valve actuation in IFCs	1

Biomark Operating Environment

The Biomark system should be used in an environment that meets the requirements.

IMPORTANT: The Biomark system is for indoor use only.

Temperature	Ambient between 15 °C and 30 °C (59-86 °F)
Humidity	20%-80%, non-condensing
Pollution	Degree 2
Electrical installation	Category II
Altitude	Up to 2,000 m (6,562 ft)

Biomark Power Options

Biomark operates through 100-240 VAC power at 50/60 Hz, 9.0 A, 100V; 3.7A, 240V.

IMPORTANT: Fluidigm recommends a UPS with voltage regulating capabilities. See the Uninterruptible Power Supply Recommendation in the Biomark Site Requirements Guide (PN 68000082).

Biomark Power Cord Requirements

Fluidigm will provide a country-specific power cord.

Location	Minimum Wire Gauge (AWG)	Maximum Length (Meters)	Plug End Instrument	Plug End Receptacle
Japan, USA, Canada	14	2	IEC C13	Country Specific
Europe, Australia	16	2	IEC C13	Country Specific

Biomark Regulatory Compliance

The following directives and harmonized standards were used to evaluate the safety and performance of the Biomark system.



General Regulations and Requirements

- 73/23/EEC European Parliament Low Voltage Directive
- 89/336/EEC European Parliament Directive: Electromagnetic Compatibility

Harmonized Standards

- IEC/EN 61326: 2003
- IEC/EN 61010: 2001

Conformity Symbols on the Instrument

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and the United States.
	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.

Refer to the Biomark System Site Requirements Guide (PN 68000082) for more detailed information on the recommended environmental conditions.

EP1™ System Overview



The Fluidigm EP1™ Reader enables high-throughput SNP (single nucleotide polymorphism) genotyping and digital PCR. The Reader is a cost-effective end-point detection system. Add the Stand-Alone Thermal Cycler and an IFC Controller to scale up the number of daily experiments performed on a single EP1™ Reader.

Components of the EP1 System

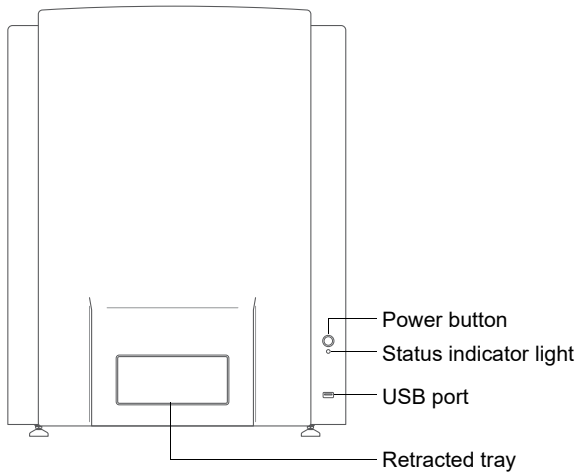


Figure 5. EP1 front view

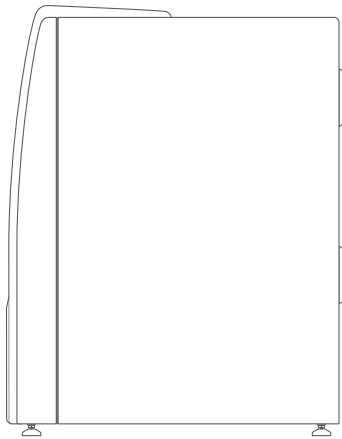


Figure 6. EP1 side view

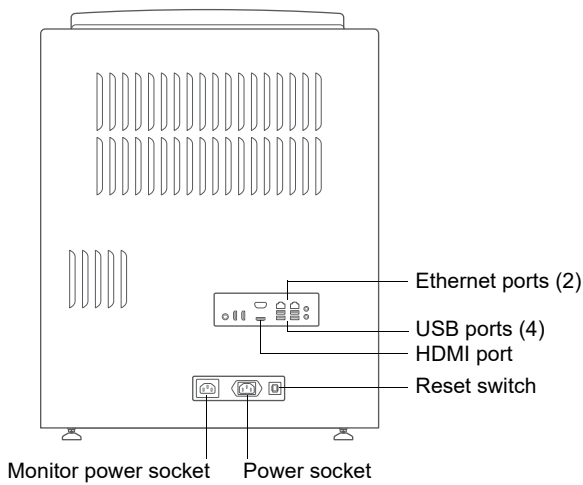



Figure 7. EP1 rear view

Components Included in the EP1 Shipping Box

	Component	Purpose	Qty
1.	EP1 instrument	Collects endpoint data.	1
2.	Power cable	<p>Country specific power cable to connect the EP1 to the wall socket.</p>  <p>The instrument has a connection to protective earth through the power cord provided by Fluidigm. Ensure that the electrical receptacle provides an earth ground before connecting the power cord.</p> <p>Use only power cords provided by Fluidigm or power cords that meet the minimum ratings of 250V/10A, 16AWG and a length not exceed 2 meters (6 feet).</p>	1
3.	Flat screen monitor	Displays the status of the EP1 system and Data Collection software.	1
4.	Keyboard and mouse	Input devices for using the EP1 software.	1

EP1 Operating Environment

The EP1 system should be used in an environment that meets the requirements.

IMPORTANT: The EP1 system is for indoor use only.

Temperature	Ambient between 15 °C and 30 °C (59-86 °F)
Humidity	20%-80%, non-condensing
Pollution	Degree 2
Electrical installation	Category II
Altitude	Up to 2,000 m (6,562 ft)

EP1 Power Options

EP1 operates through 100-240 VAC power at 50/60 Hz, 5 A.

IMPORTANT: Fluidigm recommends a UPS with voltage regulating capabilities. See the Uninterruptible Power Supply Recommendation in the EP1 Site Requirements Guide (PN 100-2195).

EP1 Power Cord Requirements

Fluidigm will provide a country-specific power cord.

Location	Minimum Wire Gauge (AWG)	Maximum Length (Meters)	Plug End Instrument	Plug End Receptacle
USA, Canada, Japan, Europe, Australia	16	2	IEC C13	Country Specific

EP1 Regulatory Compliance

The following directives and harmonized standards were used to evaluate the safety and performance of the EP1 system.



General Regulations and Requirements

- 2014/35/EU European Parliament Low Voltage Directive
- 2014/30/EU European Parliament Directive: Electromagnetic Compatibility

Harmonized Standards

- IEC/EN 61326-1
- IEC/EN 61010-1
- IEC/EN 61010-2-081

Conformity Symbols on the Instrument

Conformity mark	Description
	Indicates conformity with safety requirements for Canada and the United States.
	Indicates conformity with European Union requirements for safety and electromagnetic compatibility.

Refer to the EP1 System Site Requirements Guide (PN 100-2195) for more detailed information on the recommended environmental conditions.

Integrated Fluidic Circuits (IFCs)

The IFC (integrated fluidic circuit) has various features and benefits.

IFC Features	IFC Benefits
Lower running costs Nanoliter reaction volumes	Saves on reagents and pipette tips, and on the upkeep of liquid-handling robots.
Higher throughput High-density reaction chambers	Provides far more data per run.
More Informative An N x M architecture coupled with nanoliter reaction volumes	Generates multiple readouts per sample, without the spectral overlap and cross-amplicon influence of multiplexed PCRs
Highly Flexible Input frame with microwells together with an N x M architecture	Facilitates the input of any set of samples and any set of primers/probes, delivering the throughput of a fixed array.

IFC Barcodes Related to Applications

Each IFC has a unique barcode. The first three digits of the barcode determine which applications are listed in the Applications menu in the Data Collection Software.

Gene Expression	Genotyping	Digital PCR
113x 48.48 Dynamic Array™ IFC	124x 48.48 Dynamic Array™ IFC	115x 12.765 Digital Array™ IFC
136x 96.96 Dynamic Array™ IFC	138x 96.96 Dynamic Array™ IFC	148x 48.770 Digital Array™ IFC
153x Flex Six™ Gene Expression IFC	154x Flex Six™ Genotyping IFC	167x qdPCR 37K™ IFC
158x 24.192 Dynamic Array™ IFC	166x 192.24 Genotyping IFC	
169x 192.24 Gene Expression IFC		

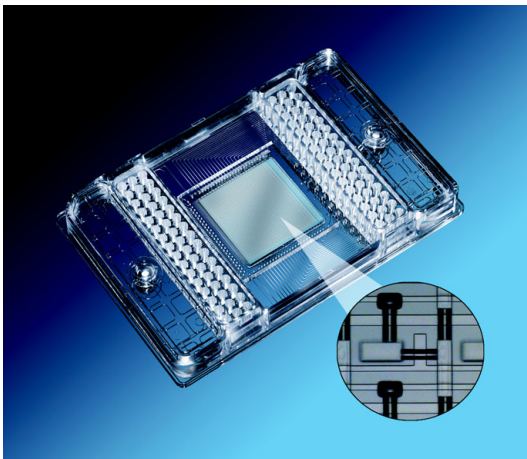
Flex Six™ Gene Expression IFC (Biomark™ only) and Flex Six™ Genotyping IFC (Biomark™ and EP1™)



The Fluidigm Flex Six™ Gene Expression IFC and Flex Six™ Genotyping IFC address the requirement for substantial variation in sample and assay numbers during target selection while allowing complete use of the IFC. It utilizes a completely new architecture which incorporates six 12 X 12 partitions that can be organized in any configuration, in up to six separate experimental runs. This new IFC adjusts to

customers' experimental needs during target selection and largely eliminates the need for microplate-based experiments.

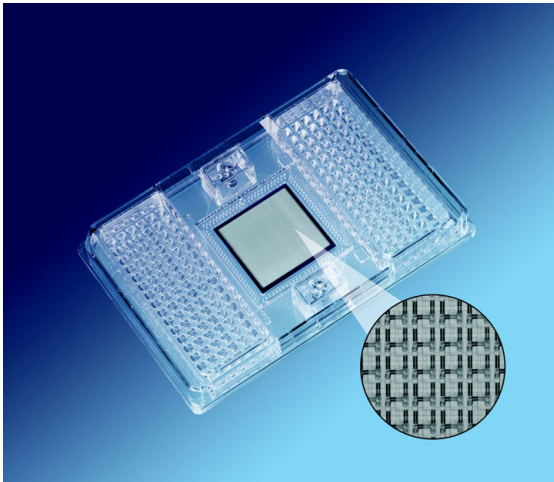
48.48 Dynamic Array™ IFC



The Fluidigm 48.48 Dynamic Array™ IFC is an efficient solution for large-scale, real-time qPCR. The key to this efficiency is the matrix of channels, chambers, and integrated valves finely patterned into layers of silicone. This material is gas permeable, allowing the blindfill of fluids into valve-delimited chambers. The valves partition samples and reagents and allow them to be systematically combined into 2,304 assays.

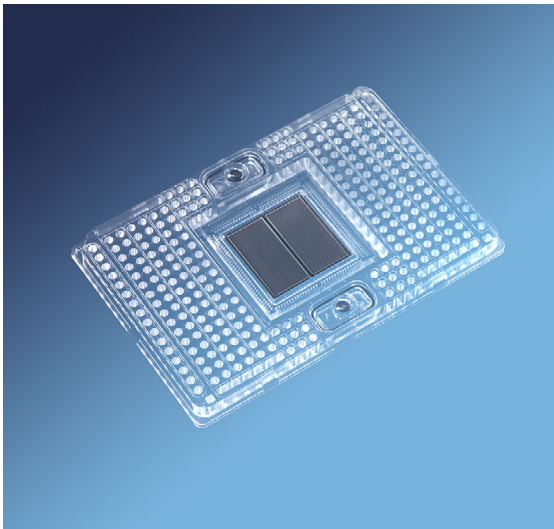
The significance of this approach to operational efficiency is immense. Managing a gene expression study involving 2,000 samples against a set of 48 genes would require 1,000 96-well plates as compared to 42 Dynamic Array™ IFCs. Managing the same study would require 192,000 steps on microplates but only 4,032 liquid-transfer steps on the IFCs. Comparative time required to complete such a study would typically involve 100 days on plates but just 4 1/2 days on IFCs. In addition, the running cost is reduced by half or more.

96.96 Dynamic Array™ IFC



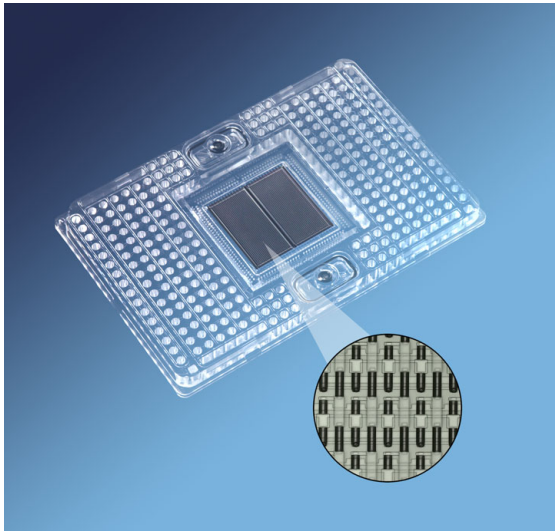
The Fluidigm 96.96 Dynamic Array™ IFC is similar to the 48.48 Dynamic Array™ IFC but with high throughput. On one side of the frame are 96 wells to accept the samples and, on the other, 96 wells to accept the probe and primer pairs. Once in the wells, the components are pressurized into the IFC using an IFC controller. The components are then systematically combined into 9,216 parallel reactions.

24.192 Dynamic Array™ IFC for Gene Expression (Biomark system only)



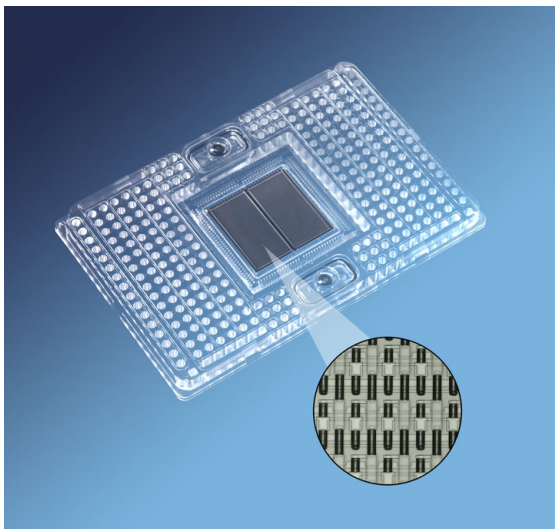
The Fluidigm 24.192 Dynamic Array IFC for gene expression supports the analysis of up to 192 markers per sample, the largest panel size of any Fluidigm gene expression IFC. The GE 24.192 IFC is well suited for customers who want to maximize data collection from rare samples. It is also a valuable tool in custom panel development, where it can be used to validate performance of potential gene expression assays prior to final panel selection and testing on a larger number of samples (for example, by using the 96.96 Dynamic Array IFC).

192.24 Genotyping IFC



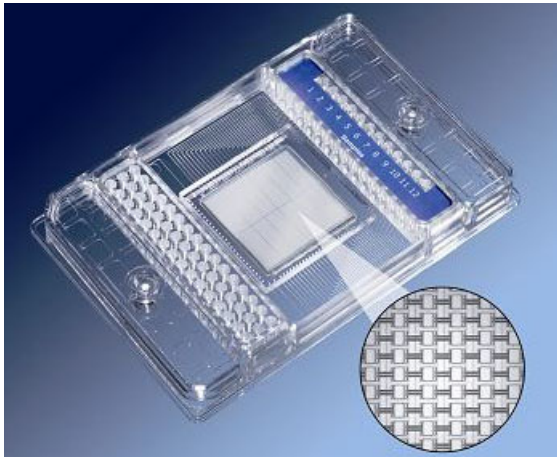
The Fluidigm 192.24 Genotyping IFC provides a solution for targeted high sample throughput SNP genotyping. It is designed to genotype 192 samples against 24 assays in a single run and incorporates innovative design features to greatly increase sample throughput. Once in the wells, the components are pressurized into the IFC using an IFC Controller. The components are then systematically combined into 4,608 parallel reactions.

192.24 Gene Expression IFC (Biomark™ System only)



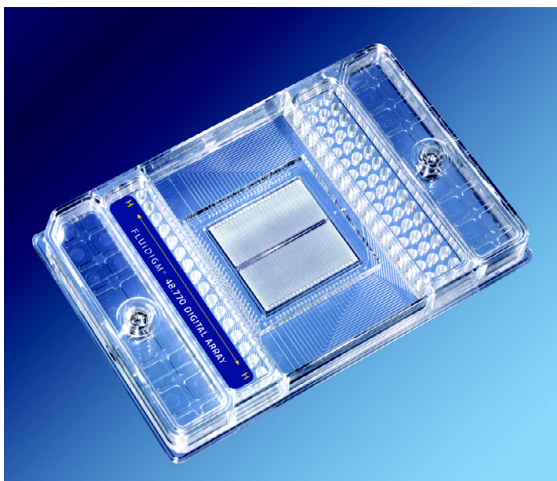
The Fluidigm 192.24 Gene Expression IFC meets the needs of production users who have narrowed their gene panel and require high sample throughput. Together with the Biomark™ System, this IFC enables maximum throughput, with minimal hands-on time. The 192.24 Gene Expression IFC can be particularly useful in clinical research and production environments, where users will benefit from the low-cost per sample, high reliability, and simple workflow of the IFC.

12.765 Digital Array™ IFC



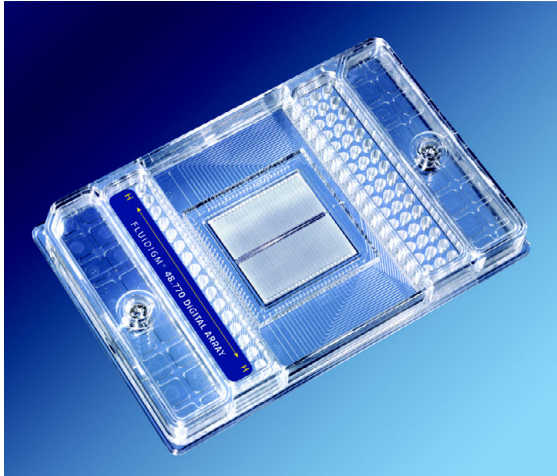
The Fluidigm 12.765 Digital Array™ IFC is an efficient solution for absolute qPCR. The key to this efficiency is the matrix of channels, chambers, and integrated valves finely patterned into layers of silicone. This material is gas permeable, allowing the blindfill of fluids into valve-delimited chambers. The valves partition samples into 765 chambers in each of the 12 panels (765 chambers x 12 panels = 9,180 reactions per IFC).

48.770 Digital Array™ IFC



The Fluidigm 48.770 Digital Array™ IFC delivers high-throughput digital PCR enabling 48 samples to be tested at the same time. This IFC is ideal for copy number variation studies, absolute quantification, and mutation detection. The 48.770 Digital Array™ IFC is capable of producing 36,960 individual PCR reactions simultaneously.

qdPCR 37K™ IFC



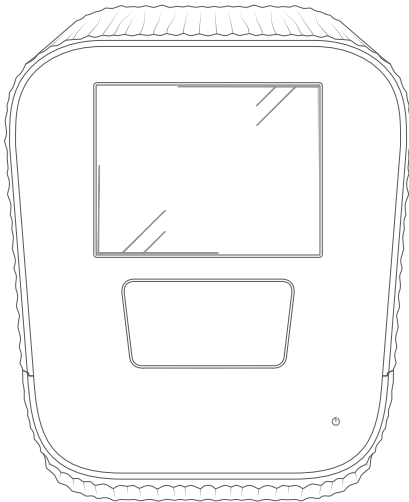
The qdPCR 37K™ IFC combines both digital and quantitative real-time PCR. Real-time analysis of digital PCR reactions allows removal of false positives. The qdPCR 37K™ IFC also provides high amplification success rate with the lowest cost-per-sample available in the market today.

The qdPCR 37K™ IFC is capable of producing 36,960 individual PCR reactions simultaneously.

Controllers for IFCs

Juno™

Juno is a universal controller that primes, loads, and thermal cycles assay-sample mixes.



IFC Controllers: MX, HX, and RX

These are the controllers for use with the Biomark™ System:

- IFC Controller MX
- IFC Controller HX (pictured)
- IFC Controller RX



The controllers were designed specifically to work with specific IFCs:

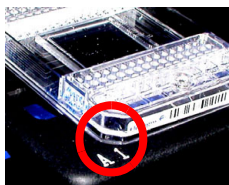
- IFC Controller MX -- for priming and loading the 48.48 Dynamic Array™ IFC, the 12.765 Digital Array™ IFC, the 48.770 Digital Array™ IFC, and the qdPCR 37K™ IFC.
- IFC Controller HX -- for priming and loading the Flex Six™ Gene Expression IFC and Flex Six™ Genotyping IFC, 96.96 Dynamic Array™ IFC.
- IFC Controller RX -- for loading the 192.24 Gene Expression IFC, 192.24 Genotyping IFC, and the 24.192 Dynamic Array IFC for gene expression.

System Process Overview

IMPORTANT: Many materials, including many well-known inhibitors, can have profound impact on PCR performance. Such contaminants can have a more significant impact on PCR performance in microfluidic devices than in microtiter plates. Prior to working with the IFCs, clear the workstation of all nonessential materials and wipe it clean of all debris. Avoid using any source of fluorescent dyes, especially cloth- or felt-tipped pens and highlighters, in the vicinity of an IFC.

The workflow for running an experiment on Biomark or EP1 consists of the following steps:

- 1 Inject Control Line Fluid into each accumulator on the IFC.
- 2 Remove and discard the protective film from the bottom of the IFC.
- 3 Place the IFC into the appropriate controller, then run the appropriate *Prime* script to prime the Control Line Fluid into the IFC. Each IFC type corresponds to a script number. For example, you would choose *Prime (148x)* for a 48.770 IFC.
- 4 Remove the IFC from the controller, and pipet samples and assay solutions into the inlets on the IFC.
- 5 Return the IFC to the controller.
- 6 Run the appropriate *Load Mix* script. Air pressure forces samples and assays into the IFC where they mix.
- 7 Remove the loaded IFC from the controller.
- 8 Place the IFC into the Biomark or EP1 instrument, making sure the A1 corner on the IFC aligns with the A1 on the instrument tray.



- 9 Run your experiments.

Supported Detection Reagents

We support the following detection reagents on the Biomark™ System and the EP1 Reader.

Probe Types

- FAM-MGB
- VIC-MGB
- FAM-TAMRA
- FAM-non fluorescent quencher
- SNP Type™-HEX
- SNP Type-FAM

Additional Probe Types

Fluidigm does not support other probe types at this time, however, you may run additional probe types using the following guidelines:

Fluorophores With...		
Excitation Wavelengths		Emission Wavelengths
between 465 and 505 nm	and	between 500 and 550 nm
between 510 and 550 nm	and	between 540 and 600 nm

Turning On the Biomark™ System

Turning On the Personal Computer (PC)

- 1 Push in the round button (hard power) on the side panel of the Biomark.



Hard power to PC

- 2 Turn on the PC monitor. The computer may take up to 10 seconds to power on.
- 3 If the computer does not power on, push the green button (soft power) on the front panel of the Biomark.



Soft power

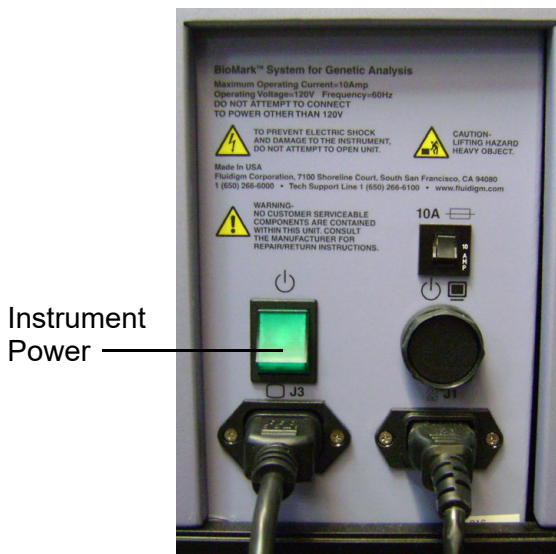
NOTE: The green button on the front of the system does not open the DVD drive cover. Manually open the door and use the small black button to load/unload DVDs or CDs.



Button to load/
unload DVDs/CDs

Turning On the Instrument

- 1 Toggle the green switch on the side panel of the Biomark™ System to turn the instrument on.



You are now ready to use the system.

Turning Off the Biomark™ System

Turning Off the PC

- 1 Click the Start menu.



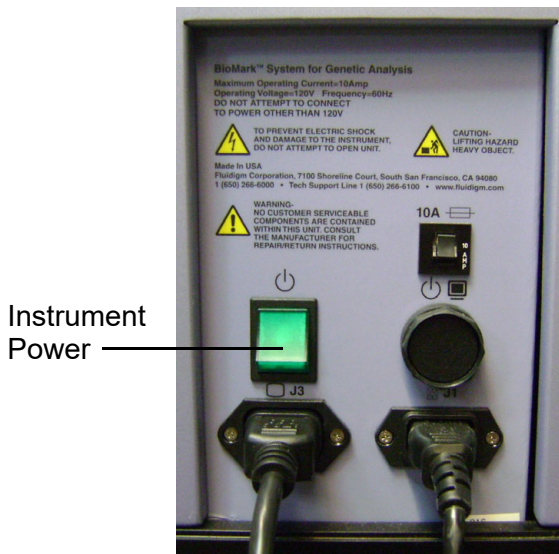
- 2 Select Shut down.



NOTE: Do not use the round button (hard power) at the side of the system to turn off the computer.

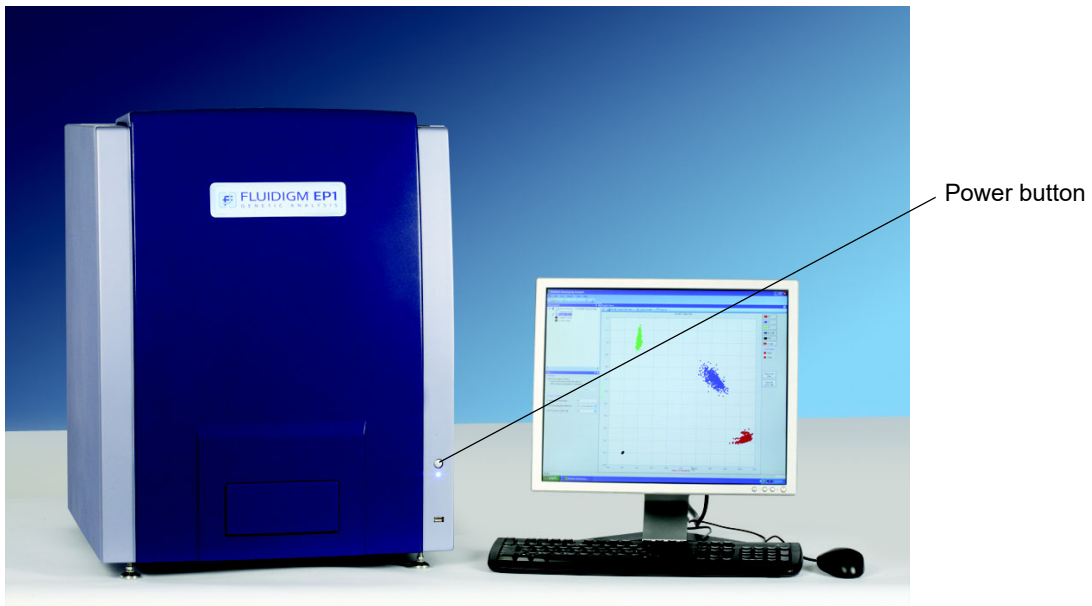
Turning Off the System

- 1 Toggle the green switch on the side of the Biomark™ System to turn the system off.



Turning On the EP1™ Reader

- 1 Press the power button on the front of EP1 to turn on both the computer and the instrument.



Turning Off the EP1™ Reader

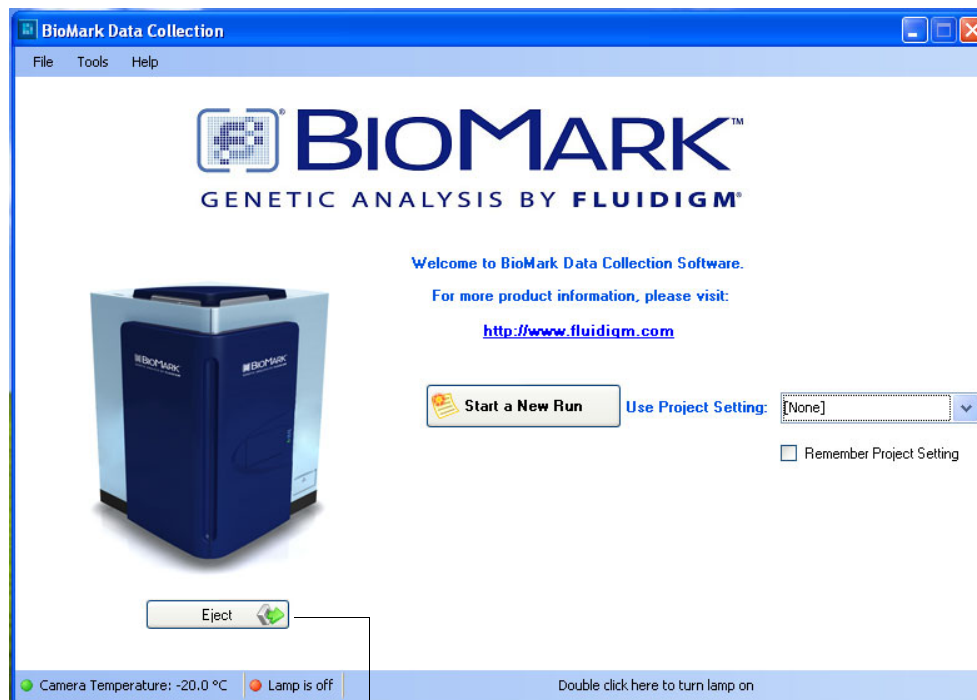
- 1 Press the power button to turn off both the computer and the instrument.

Launching Fluidigm Data Collection Software

IMPORTANT: Start the Fluidigm Data Collection software 20 minutes before starting the run to allow the lamp to warm to operating temperature. If the lamp has automatically turned off due to inactivity, double-click the status bar to reset.

- Double-click the software icon on the desktop to open the Start screen.

NOTE: Once past the start screen, you cannot access the lamp control (See [“Using the Data Collection Tools Menu”](#) on page 32).

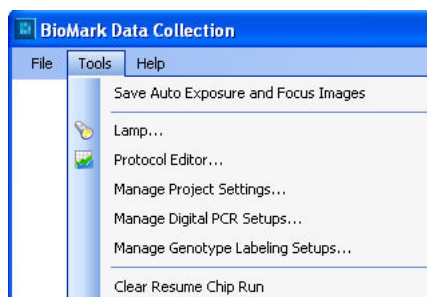


Camera and lamp status

Click to open and close the system tray.

Using the Data Collection Tools Menu

Each Tool is described in this manual.



“Saving Auto Exposure and Focus Images” on page 52

“Lamp Control” on page 33

“Using the Protocol Editor” on page 81

Project Settings Editor on page 56

“Setting Up a Digital PCR Project” on page 56

“Genotyping IFC Runs” on page 59

“Resuming an IFC Run” on page 77

“Clearing an IFC Run” on page 79

Lamp Control

Click **Tools > Lamp** to access the Lamp Control dialog box.

NOTE: Once past the start screen, you cannot access the lamp control.

Use the Lamp Control dialog box to check bulb status, and to manage lamp parameters that help extend the life of the lamp. For example, setting a low idle time shuts off the lamp when not in use.

Lamp On/Off Status

Turn lamp On or Off, and check how long before lamp shuts down.

Lamp Usage Status

- Expected lamp life is shown in hours.
- Click *Reset* after changing the lamp to begin tracking lamp life from 0.
- Remaining lamp life is shown in hours.



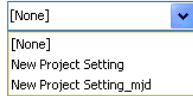

Lamp Idle Times

Shows lamp idle times and when to power off for the day.

Click OK after changing any parameter to accept the changes.

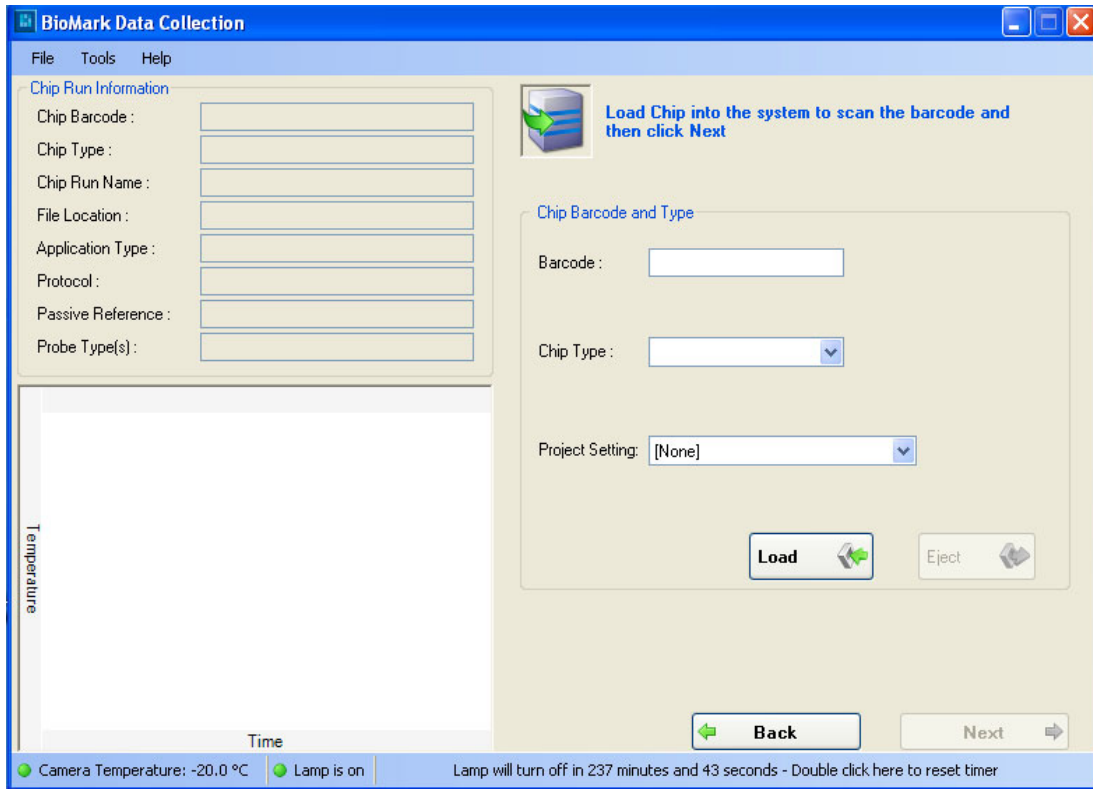
Starting a Run

You have some options to consider before starting a run. Use the table below as a guide.

If you want to...	Then...	and then...
Start a new run (See “Starting a New Run With No Parameters Pre-defined” on page 35)	Click 	Follow the software wizard
Start a new run and save the run settings	Click <input checked="" type="checkbox"/> Remember Project Setting	Click 
Start a new run using settings from a previous run (See “Starting a Run Using Previously Created Project Settings” on page 46)	Choose the run from the drop-down menu Use Project Setting: 	Click 

Starting a New Run With No Parameters Pre-defined

- 1 Launch the Fluidigm Data Collection Software.
- 2 Click **Start a New Run**. The Chip Run Information window opens.



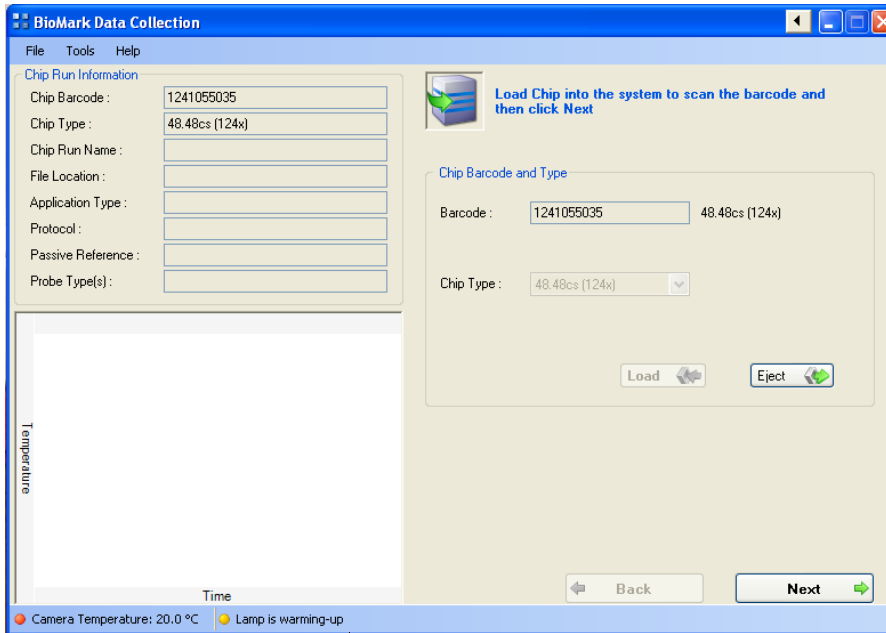
The lamp is heating and the camera is cooling.

- 3 Place the IFC on the Biomark™ System and/or EP1™ Reader tray, ensuring that the notched A1 corner on the IFC is aligned with the A1 on the system tray.

IMPORTANT: Be sure to remove:

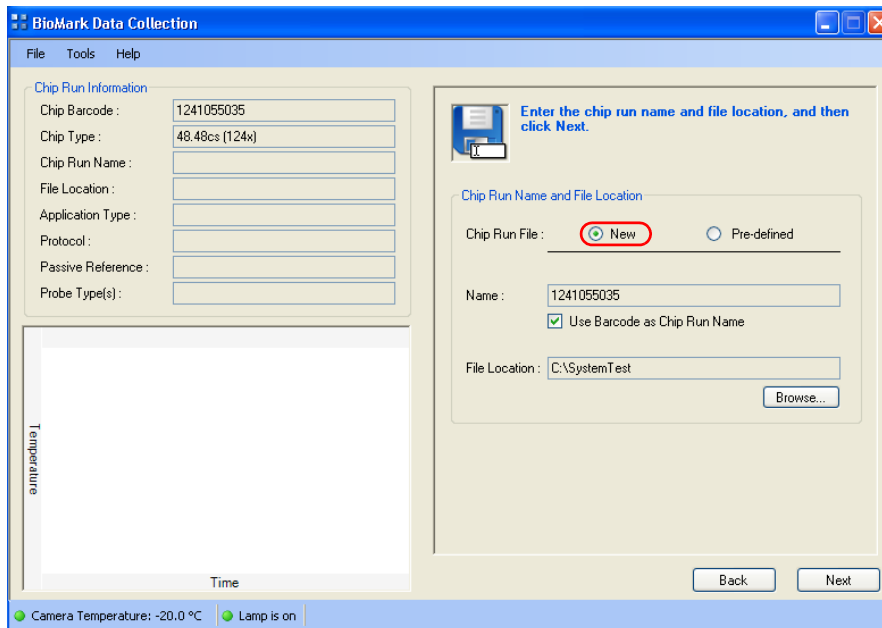
- a. The protective film from beneath the IFC.
- b. Dust particles from the top of the IFC with tape.

- 1 Click **Load**. The IFC retracts into the system, the barcode is scanned, and the IFC type identified. The barcode and IFC type display, as shown below.



Camera and lamp status display

- 2 Click **Next**. The Chip Run Name and Location dialog box opens.



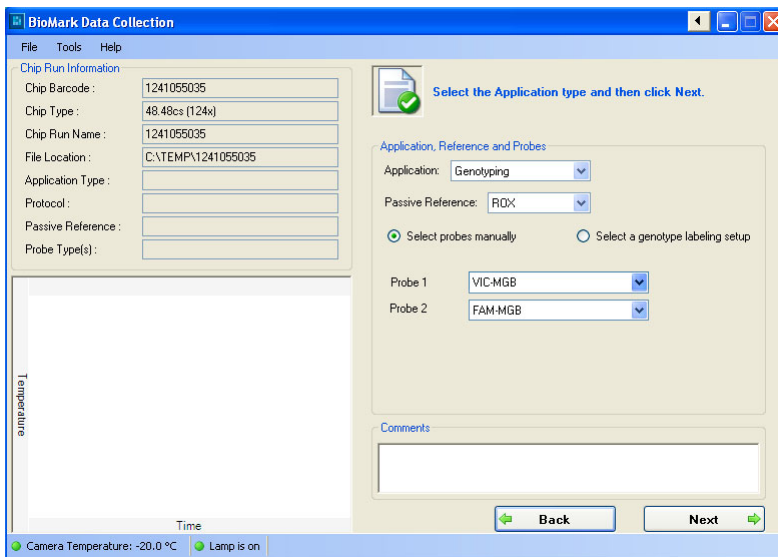
- 3 In the Chip Run File panel, click **New**.
If you click **Pre-defined**, click **Browse** to locate the .bml file you want to use.
- 4 Click **Use Barcode as Chip Run Name** or, uncheck and then type a unique

name in the New Chip Run Name text box.

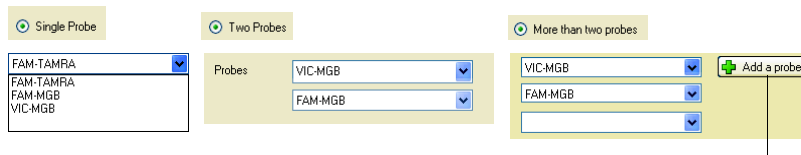
When you de-select 'Use Barcode as Chip Run Name,' the barcode remains in the New Chip Run Name text box but it is now editable.

- 5 Click **Browse** to specify a desired file location for your IFC run. You need to do this only once per IFC.

- 6 Click **Next**. The Application, Reference and Probes window opens.

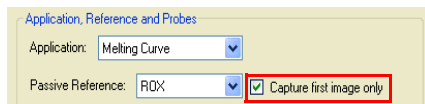


- 7 Select an application from the Application drop-down menu (see descriptions below for more information).
- *Gene Expression* (on the Biomark™ System only). If you select Gene Expression from the Application drop-down menu, a separate drop-down menu appears for each probe you select, as shown below.

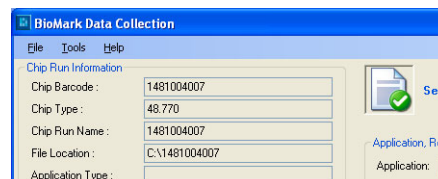
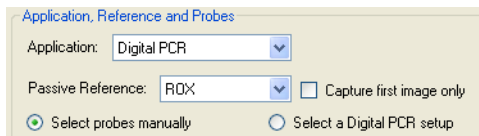


If you are using more than 3 probes, click Add a probe for each additional probe set.

- *Melting Curve* (on the Biomark™ System only). This application has the same options as Gene Expression, shown above.

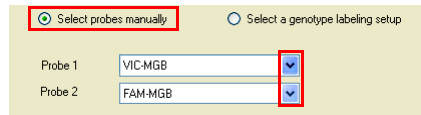


- *Digital PCR*. This application has the same options as Genotyping, described below except there is no labeling. Click **Select a Digital PCR Setup** and then choose a setup.

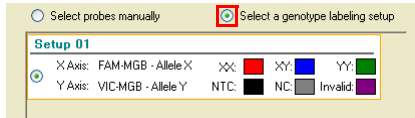


- *Genotyping*. This application has the same options as Digital PCR above except that you have three labeling options:

- Select probes manually. Click Probe 1 and Probe 2 drop-down menus to select probes.



- Select a pre-defined setup. Click **Select a genotype labeling setup** to open the default setup or any that you have created. You can also modify setups from this pane.



Click the setup to select it.

- Create a new setup. To create a new setup or edit an existing setup, click **Tools > Manage Genotype Labeling Setups**. Follow the sequence shown in the illustration below.

1

2

3

Click to create a new labeling setup

Click to edit an existing labeling setup

When adding a new labelling setup:

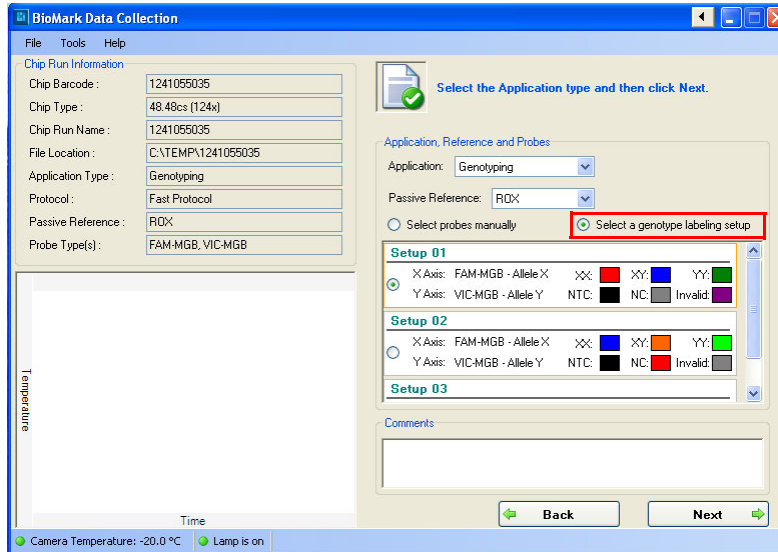
- Use the Probe Type drop-down menu to select your probe sets.
- Click on colors to change them.
- Type a name for the new setup.
- Click **OK**.

When editing an existing labelling setup:

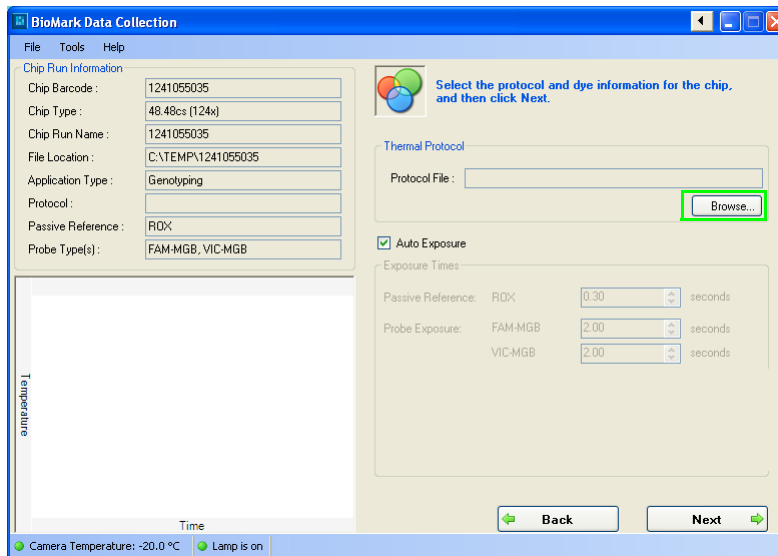
- Use the Probe Type drop-down menu to change your probe sets.
- Click on colors to change them.
- Type a name for the edited setup.
- Click **OK**.

NOTE: Set up a new genotype labeling and color scheme at any time by going to **Tools > Manage Genotype Label Setups > Add New Genotype Labeling Setup**.

Any new labeling setups you create are shown when you click **Select a genotype labeling setup**, as shown below.



11 After you have chosen the application and the probe-sets, click **Next**. The Thermal Protocol window opens.

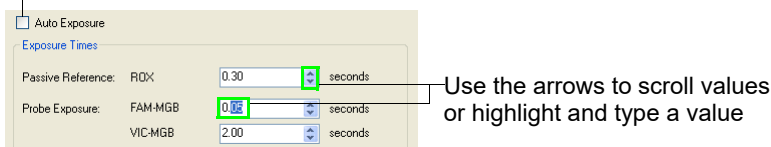


12 Click **Browse** in the Thermal Protocol pane to navigate to the thermal protocol you want to use (.pcl file extension).

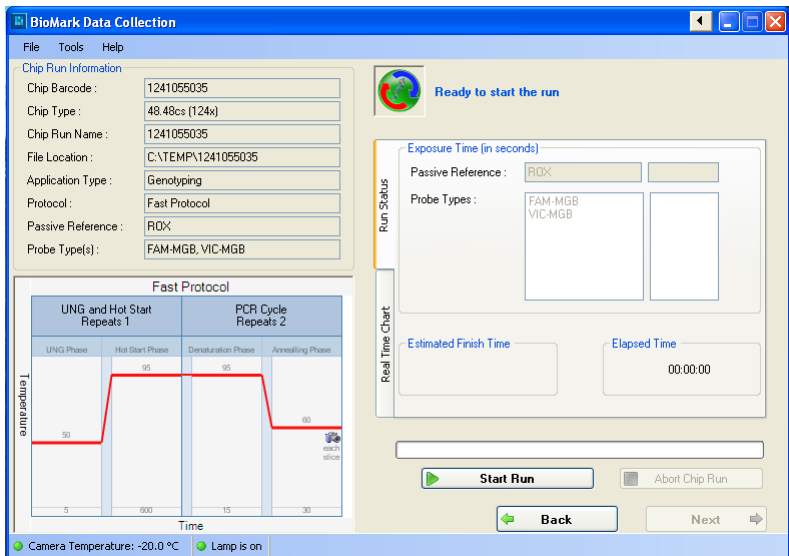
NOTE: If you are unsure which thermal protocol to use, contact Technical Support.

- 1 (Optional) Uncheck **Auto Exposure** and enter your custom exposure times. For most, Auto Exposure works well and they find no need to change the default setting. However, if you find, for example, that your images are too bright, you may want to uncheck Auto Exposure and adjust values manually until you have images with which you are satisfied.

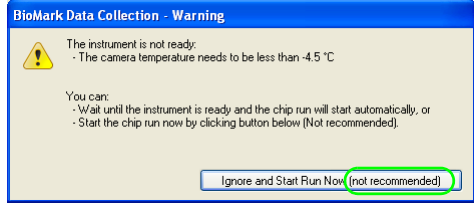
Click to uncheck and check



- 2 Click **Next**. Check experiment parameters in the Chip Run Information pane. If the information is not correct, click **Back** until you reach the screen that you want to change.



- 3 If the experiment information is correct, click **Start Run**. If the camera or the lamp is not ready and you click Start Run, a warning opens, similar to the one below:

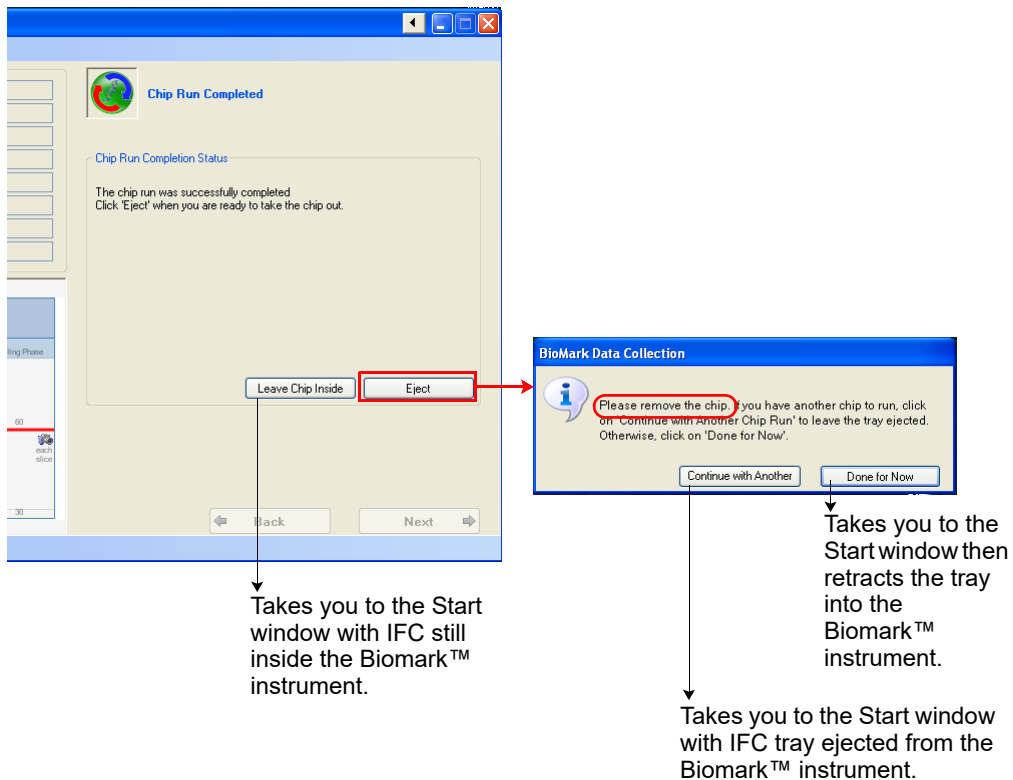


- 4 The run starts automatically when the camera and the lamp achieve optimum temperature.

IMPORTANT: We do not recommend that you ignore instrument warnings. Your data may be compromised if you do.

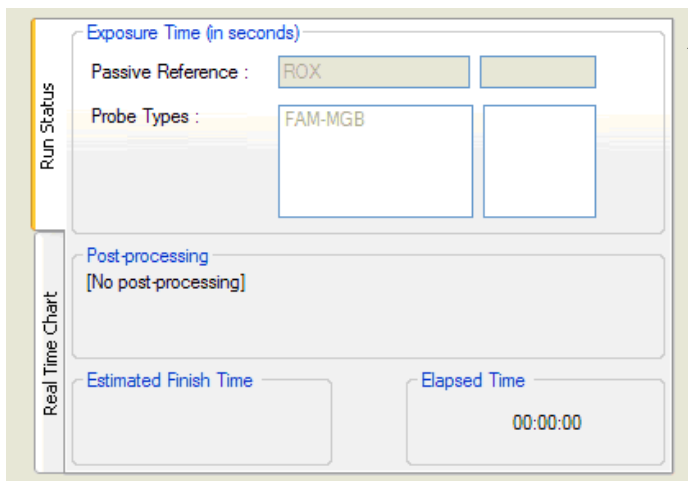
NOTE: At this point, no other interaction by the user is required.

- 1 If you click **Eject** when the IFC run is completed, a dialog box opens.

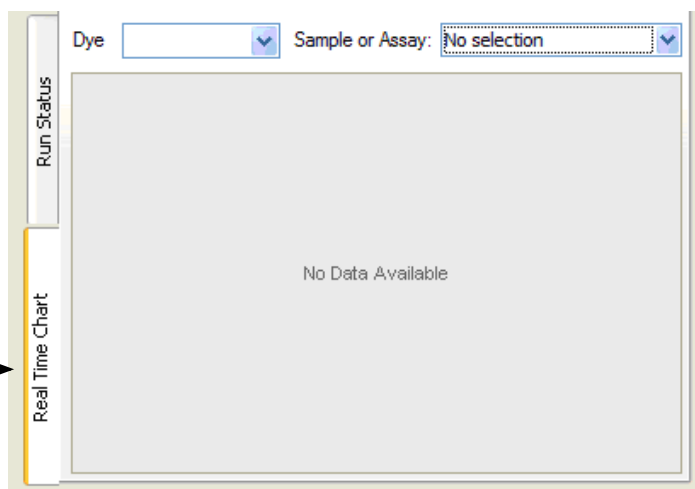


Viewing Real-Time Curves During an IFC Run

When the IFC run begins, you can track progress in two panes, *Run Status* and *Real Time Chart*. The software processes the images as they are taken, enabling display of real-time intensity curves (on the Biomark™ System only).



← **Run Status.** The Run Status pane shows the elapsed time, the estimated finish time, and the exposure time for each probe-set and for the passive reference.



→ **Real Time Chart.** Displays the raw data as the software collects the images. You must choose a dye and sample/assay to see curves in the Real-Time Chart.

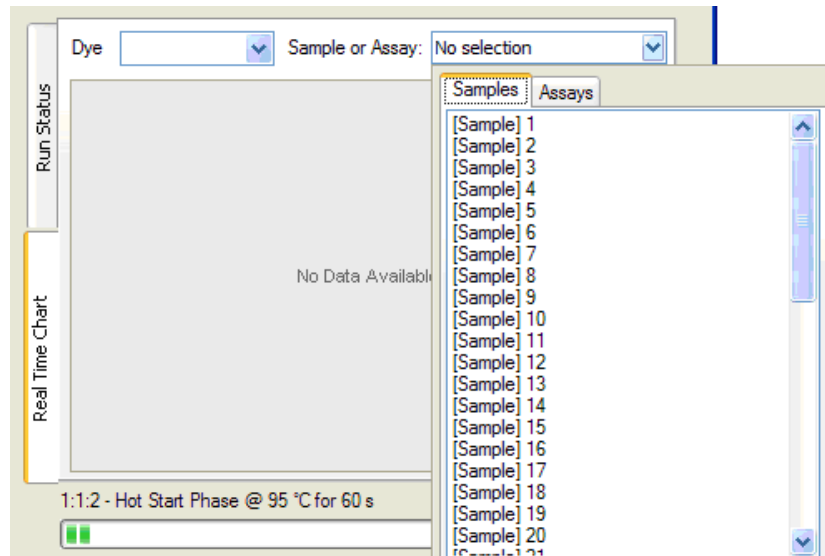
Using the Normalized Intensity Curves Feature

For digital IFC runs, when you select a panel, all intensity curves for the wells in the selected panel are displayed.

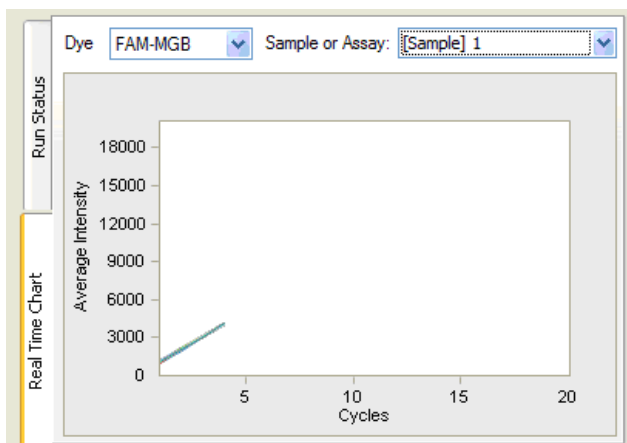
For all other IFC runs, when you select a sample or assay, intensity curves for wells that contain them are displayed.

Choose the sample or the assay from the drop-down menu, as shown below.

Additionally, you may select which probe or passive reference to display.



Once you select your probes/passive reference, or samples/assays/panel to view, the intensity curves are displayed in real time, as shown below.



NOTE: This feature works only if wells can be properly located. If well locations cannot be found, the Real-Time chart is disabled, and an error message displays.

Starting a Run Using Previously Created Project Settings

The Use Project Settings feature is visible on the Start screen only if at least one set of IFC run parameters has been created in the Manage Project Settings feature. To learn how to use Manage Project Settings see, [“Creating Run Parameters in the Manage Project Settings Feature”](#) on page 47.

To Start a Run using run parameters previously created:

- 1 Select in the Use Project Settings drop-down menu.



- 2 Click **Start a New Run**.
- 3 Edit parameters, if necessary.
- 4 Continue through the screens to complete the run.

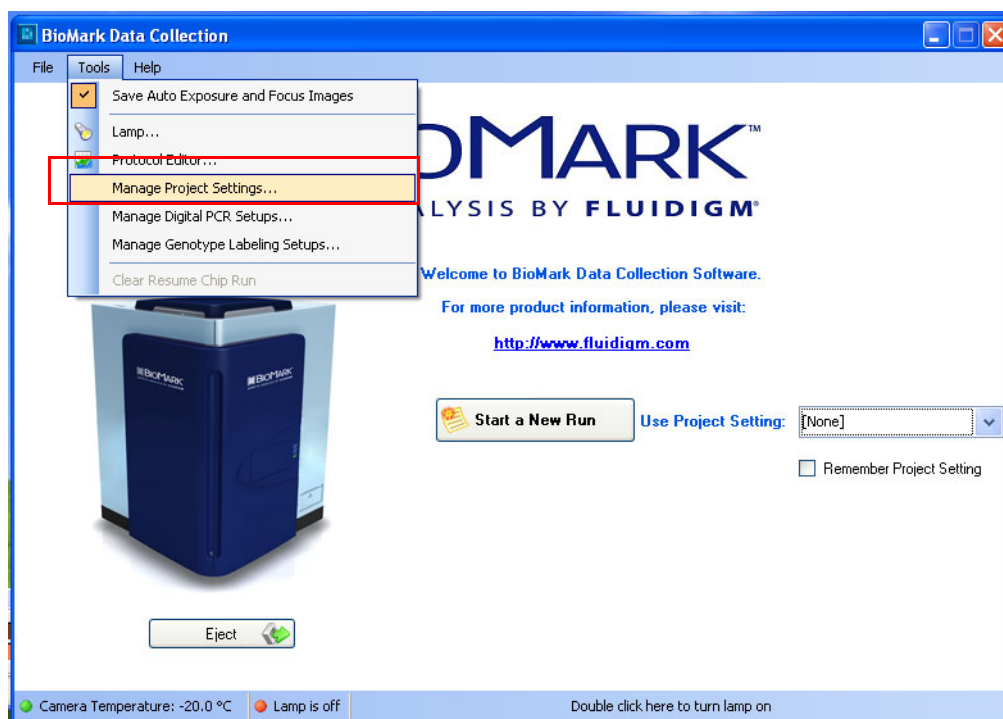
Creating Run Parameters in the Manage Project Settings Feature

The Manage Project Settings feature provides you with a way to store a set of IFC run parameters that can be reused for subsequent IFC runs, accessed through the Use Project Settings drop-down menu.

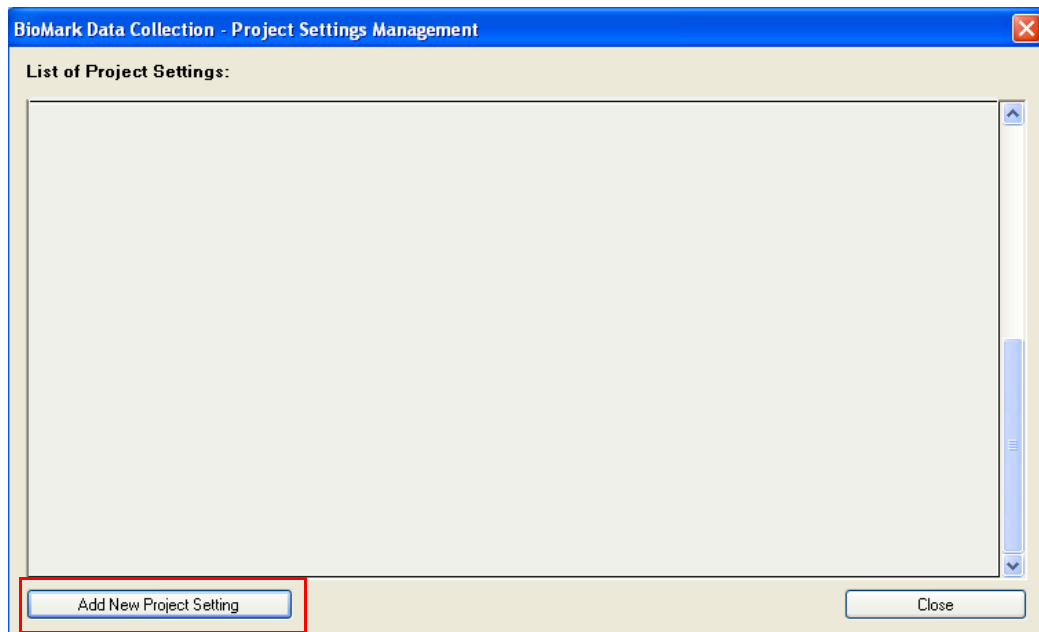
The Use Projects Setting drop-down menu is visible at startup and on the Chip Load page, only if at least one project setting has been saved or created in Manage Project Settings.

To create a new IFC run in Manage Project Setting:

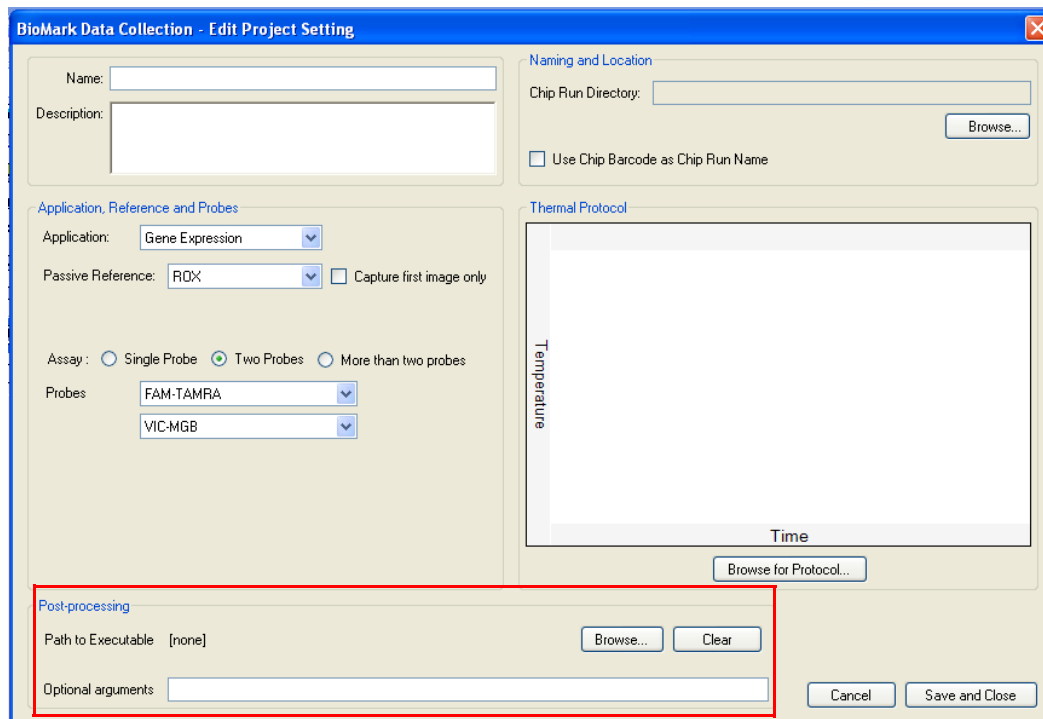
- 1 Launch Biomark™ Data Collection software.
- 2 Click **Tools > Manage Project Settings**.



3 Click Add New Project Setting.



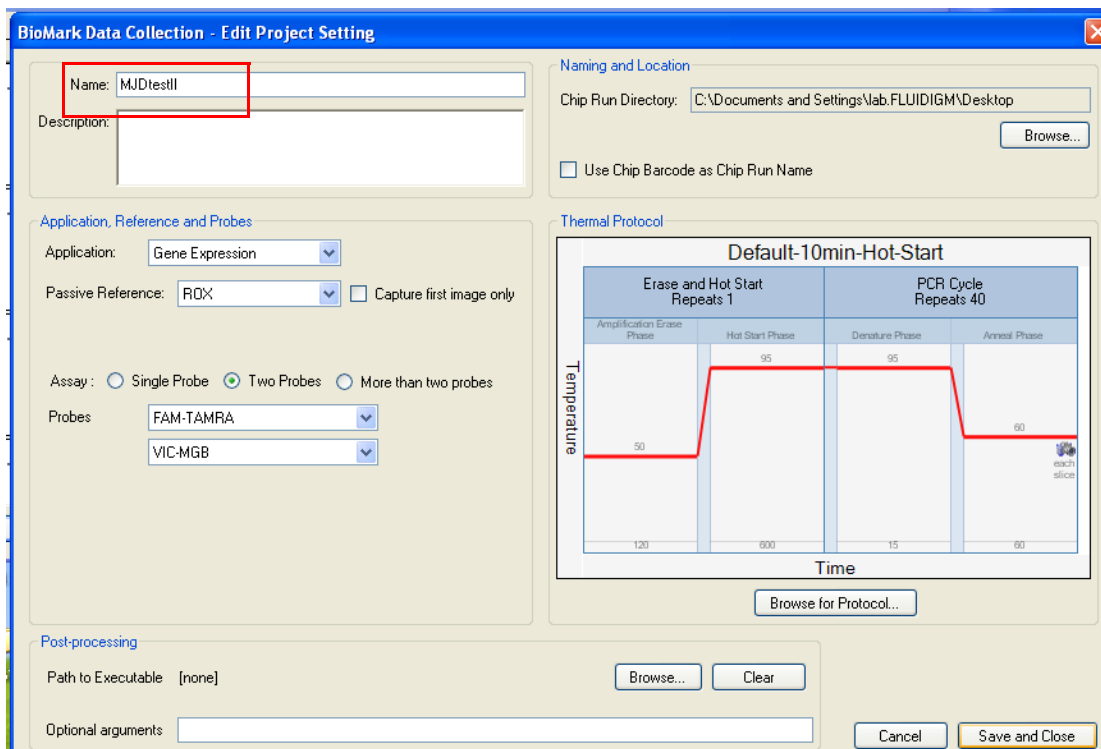
The Edit Project Settings window opens.



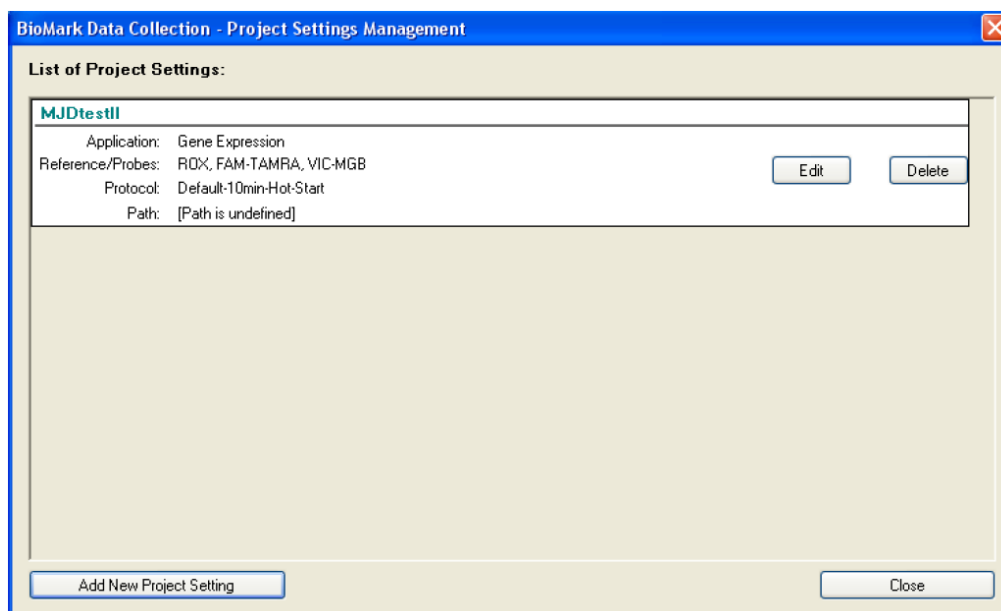
All parameters required to define an IFC run are located in this window. These are the same options found in the IFC run wizard for a run with no parameters chosen, except for the Postprocessing feature (see graphic above).

Use this feature if, after a run is completed, you want to batch process, or update a LIMS database with the current IFC run data. To run a batch file, browse to the executable, enter additional command-line instructions in the Optional arguments text box, and click Save and Close.

- 4 Click **Save and Close** when you complete the parameters, which may look like the graphic below.



Your newly defined IFC run is now visible in the Project Settings Management window as shown below.



The IFC run parameters can now be selected from the Use Project Settings drop-down menu on the Start window, as shown below.



All parameters populate the screen and user can start a new IFC run.

Digital PCR Runs

Setting Up a New Digital PCR Run

- 1 Click **Start a New Run**.
- 2 Click **Load**. The IFC retracts into the system, the barcode is scanned, and the IFC type identified, as shown below.

BioMark Data Collection

File Tools Help

Chip Run Information

Chip Barcode : 1481011103

Chip Type : 48.770

Chip Run Name :

File Location :

Application Type : Digital PCR

Protocol : M96 Protocol Candidate 5

Passive Reference : ROX

Probe Type(s) : FAM-TAMRA, VIC-MGB

Load Chip into the system to scan the barcode and then click Next

Chip Barcode and Type

Barcode : 1481011103 48.770

Chip Type : 48.770

Project Setting: HD-DID

Load Eject

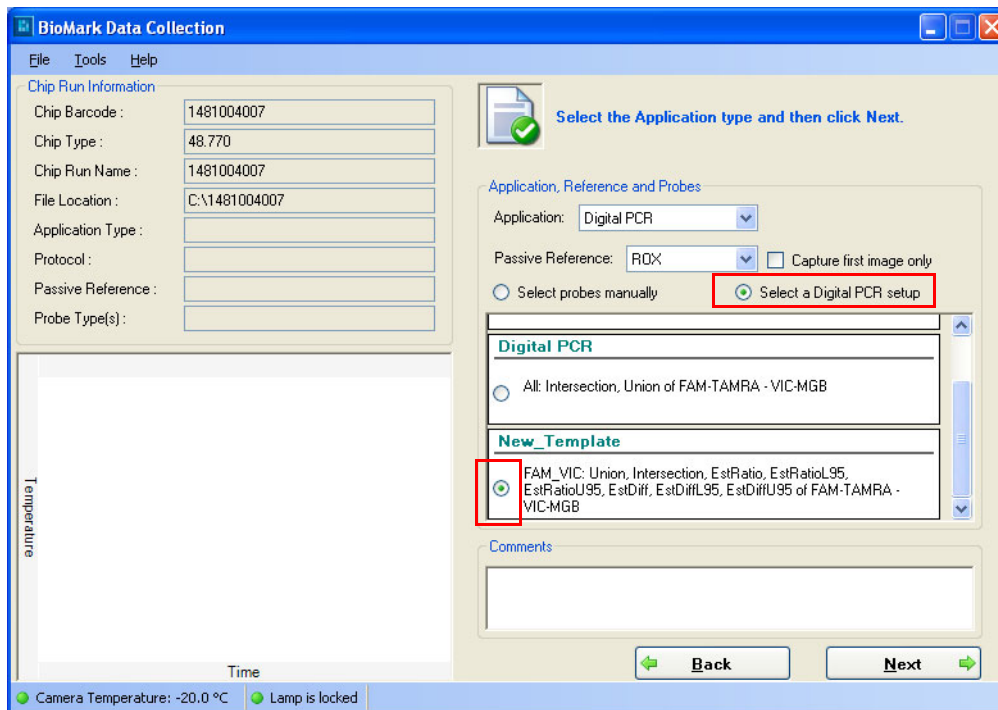
Back Next

Camera Temperature: -20.0 °C Lamp is locked but still warming-up Warm-up will be completed in 0 minutes 20 seconds

M96 Protocol Candidate 5

Thermal Mix Repeats 1			UNG and Hot Start Repeats 1		PCR Cycle Repeats 10		PCR Cycle Repeats 30	
50C	70C	25C	UNG Phase	Hot Start Ph	Denatur ation Ph	Annealation Ph	Denatur ation Ph	Annealation Ph
120	1800	600	120	600	15	60	15	60
50	70	25	50	95	95	70	95	52

- 3 Click **Next**.
 - 4 Click **Browse** to locate the .bml file you want to use.
 - 5 In the Chip Run Name and File Location panel, select **New**.
 - 6 Click **Next**.
 - 7 Select **Digital PCR** from the drop-down menu.
 - 8 (Optional) Click **Capture First Image Only**. For more information, see [page 42](#).
- Select probes manually Select a **Digital PCR setup** if one has previously been set up.
- In the following example Select Digital PCR Setup has been selected and labeled “New Template”.



9 Click Next.

10 Click Start Run.

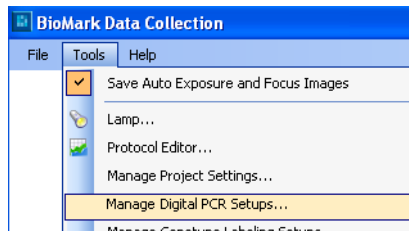
Saving Auto Exposure and Focus Images

When this option is checked—It is on by default—all images from auto exposure and image focusing are saved as part of your IFC run. If you do not want to save them, uncheck this option.

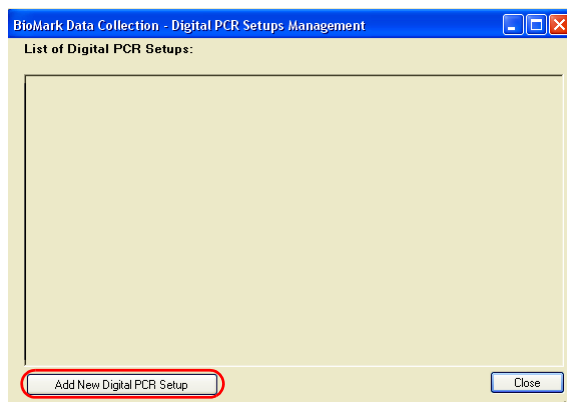
Managing Digital PCR Setups

Before performing a new Digital PCR run, you may choose to set up all the essential parameters first. This prepares the IFC run for being viewed in the Analysis software application. The following procedure explains how.

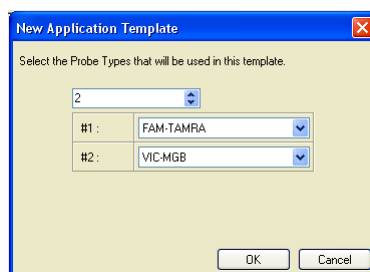
- 1 Click **Tools > Manage Digital PCR Setups**.



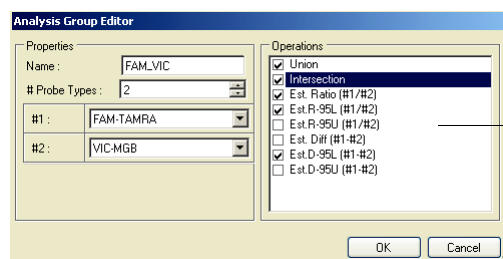
- 2 Click **Add New Digital PCR Setup**.



- 3 Select the number and the type of probes.

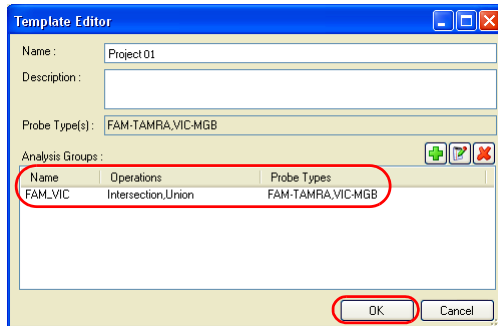


- 4 Click **OK**.
- 5 Click the **Analysis Group Editor** icon. The Analysis Group Editor opens.

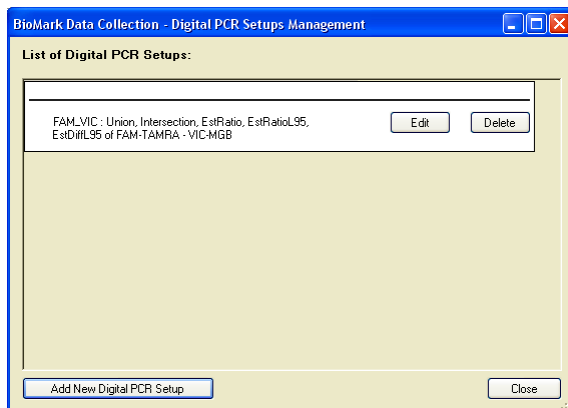


See [pages 55](#) below for a description of these options

- 6 Type a name for the analysis group you are about to create.
- 7 Type the amount of probe types or use the spin box arrows.
- 8 From the drop-down menus, choose the appropriate probes.
- 9 Click the **operations** that you want associated with this analysis group
- 10 Click **OK**. The new analysis group is now listed in the Template Editor, Analysis Groups pane.



- 11 Click **OK**. The new analysis group and all the parameters associated with it, are displayed in the List of Digital PCR Setups.

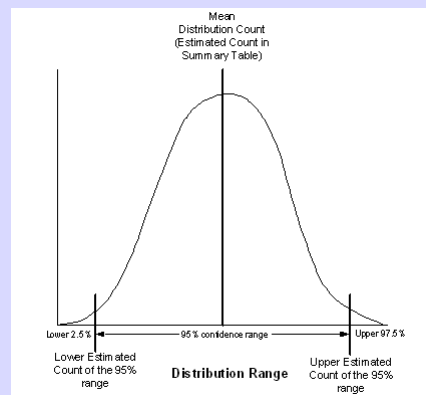


- 12 Click **Close** to finish the setup.
- 13 (Optional) Click **Add New Digital PCR Setup** to add another. Or, Click **Edit** to change elements in the analysis group.
- 14 Set up your Digital PCR project (go to [“Setting Up a Digital PCR Project”](#) on page 56)

Description of the Analysis Group Editor and its Operations

The Analysis Group Editor allows you to specify the relationship between two or more probe types. This relationship defines the operations the application is to perform on the hit data for each panel. Each operation selected in the Operations checklist adds the appropriate column to the Summary tables and exported data in the Biomark™ Digital PCR Analysis Software v2.1 (for more information see, *Biomark™ Digital PCR Analysis Software v2.1 User Guide*, PN 68000100). Many operations are limited to two-probe type analysis groups. The operations are:

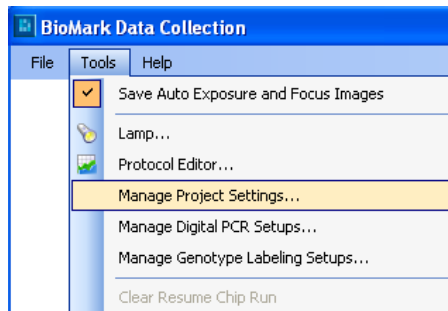
- **Union** - Selecting this option allows the total number of counts in both probe types to be displayed in a “Union” column in the summary table. Intersection counts are subtracted from the total number.
- **Intersection** - Selecting this option allows the number of counts common to both probe types to be displayed in an “Intersection” column in the summary table.
- **Est. Ratio (#1/#2)** - Selecting this option allows the estimated ratio between the distribution estimated targets of each probe type to be displayed in an “Est. Ratio” column in the summary table in the Biomark™ Digital PCR Analysis Software v2.1.
- **Est. R-95L (#1/#2)** - Selecting this option allows the lower estimated target of the 95% confidence range to be displayed in an “Est. R-95L” column in the summary table in the Biomark™ Digital PCR Analysis Software v2.1 (graphic at right).
- **Est. R-95U (#1/#2)** - Selecting this option allows the upper estimated target of the 95% confidence range to be displayed in an “Est. R-95U” column in the summary table in the Biomark™ Digital PCR Analysis Software v2.1 (graphic at right).
- **Est. Diff (#1/#2)** - Selecting this option allows the estimated difference in the targets for each probe type to be displayed in an “Est. Diff” column in the summary table in the Biomark™ Digital PCR Analysis Software v2.1.
- **Est. D-95L (#1/#2)** - Selecting this option allows the lower estimated target of the 95% confidence range to be displayed in an “Est. D-95L” column in the summary table in the Biomark™ Digital PCR Analysis Software v2.1 (graphic at right).
- **Est. D-95U (#1/#2)** - Selecting this option allows the upper estimated target of the 95% confidence range to be displayed in an “Est. D-95U” column in the summary table in the Biomark™ Digital PCR Analysis Software v2.1 (graphic at right).



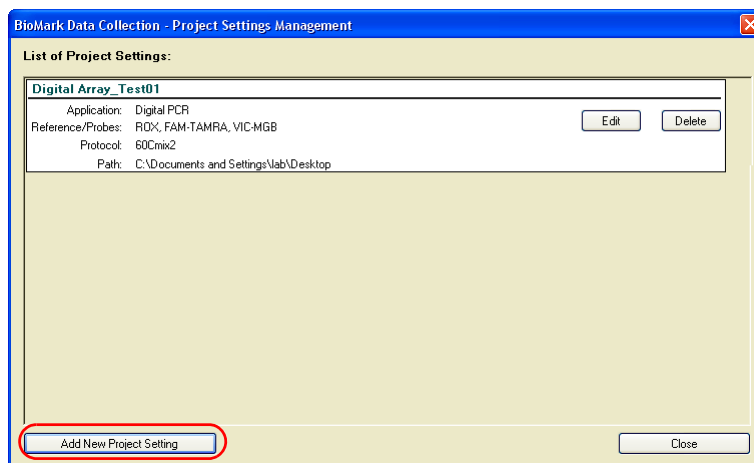
Setting Up a Digital PCR Project

Now that you have the digital PCR setup complete, set up a digital PCR project.

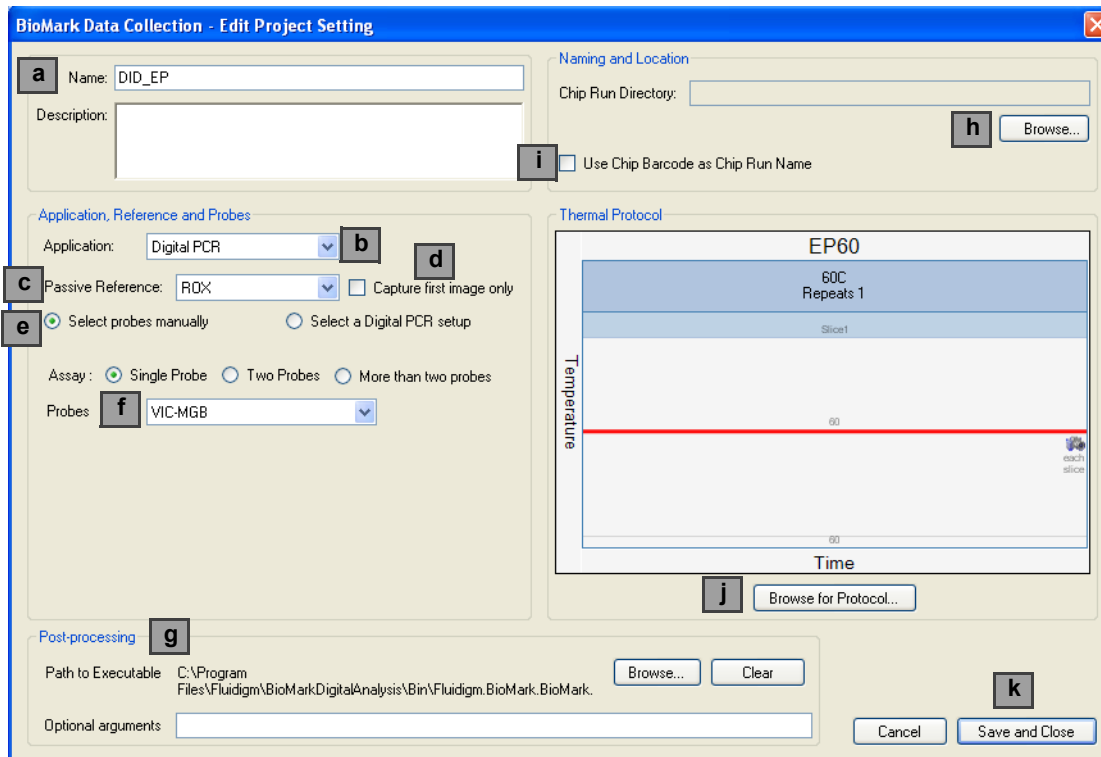
- 1 Click **Tools > Manage Project Settings**.



- 2 Click **Add New Project Settings**.



The Project Settings Editor opens.



- 3 Complete the settings using the following steps as a guide:
- Type a name for the new project and, optionally, type a description.
 - Click the appropriate application from the Application drop-down menu.
 - ROX is chosen by default.
 - Click **Capture first image only**, if appropriate (see [pages 57](#) below for a description of this option).
 - Click **Select a Digital PCR setup** to display the analysis group you created in the Managing Digital PCR Setups section. If you click **Select Digital manually**, choose the number and the type.

When to Check Capture First Image Only

All passive reference and probes must be distinct, that is, they must use a different pair of excitation/emission filters.

There is an exception if the “Capture first image only” option is used for the passive reference. In this case, a probe can use the same filter pair as the passive reference.

This option is unavailable for genotype application. As a consequence the software shows the “more than 2 probes” option in the probe selection page only if there are more than 3 different filter pairs installed in the instrument, and the maximum number of select able probes is the number of distinct

- Click the setup you created to activate it.

- g (Optional) Click **Post Processing** and add command line comments as appropriate.

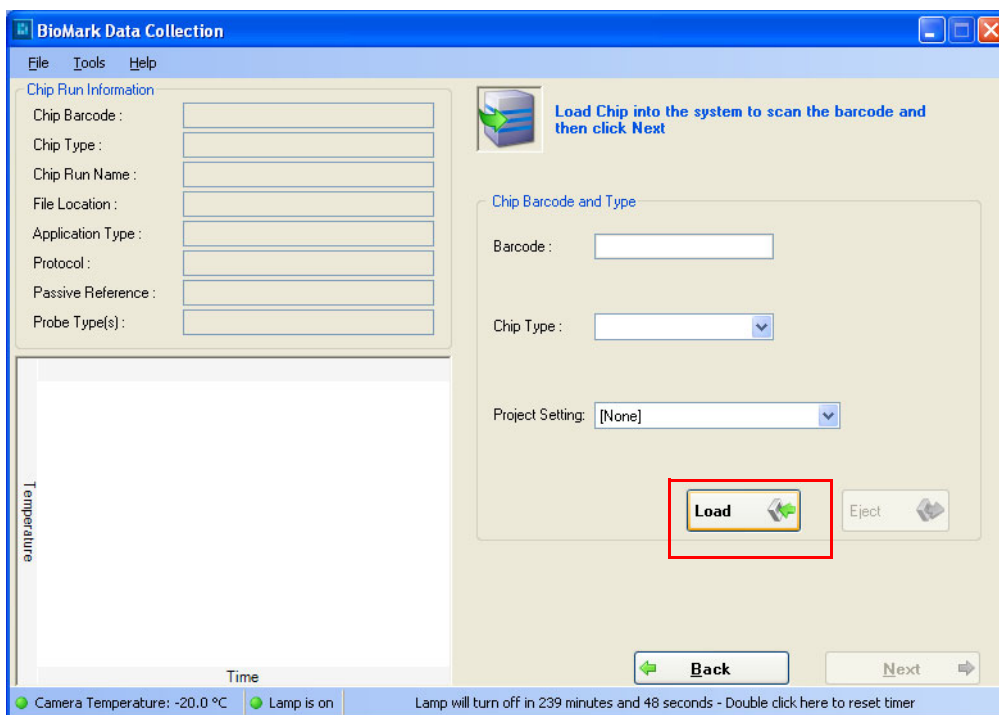
When to use Post Processing

Check this option if, after a run is completed, you want to batch process, or update a LIMS database. To run a batch file, input the path, add IFC information, and then continue to step h.

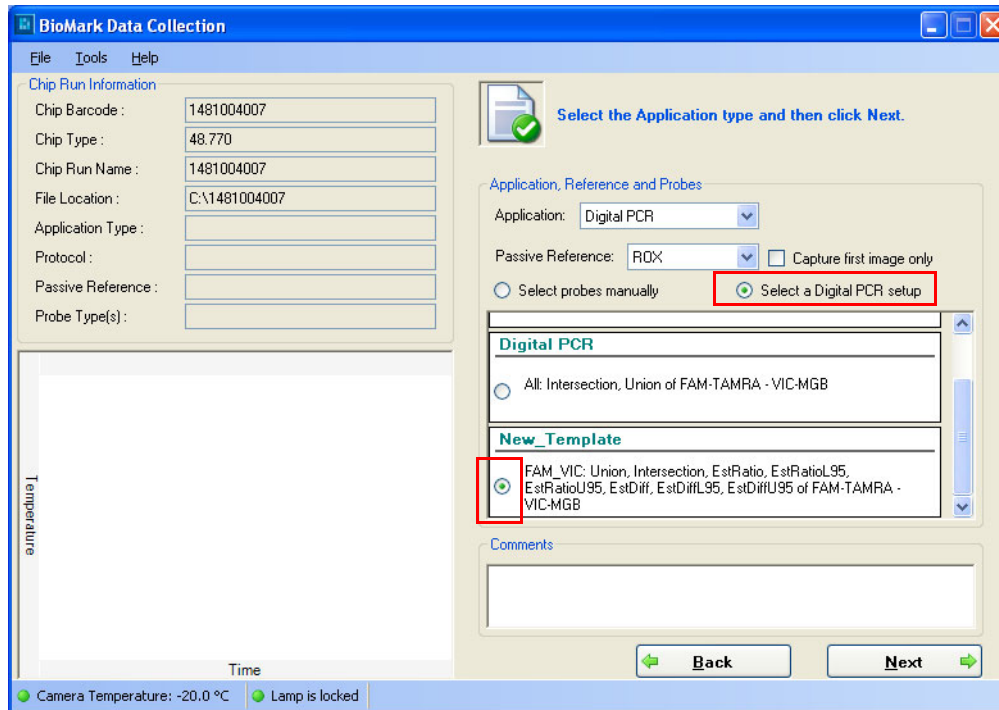
- h Click **Browse** to navigate to a save location.
 - i Click **Use Chip Barcode as Chip Run Name**.
 - j Click **Browse for Protocol**. Double-click it to open.
 - k Click **Save and Close**.
- 4 Start the run.
At the Start Window, Select the project from the Use Project Setting drop-down menu.
- 5 Click **Start a New Run**.
Optionally, follow the steps below to use a Digital PCR Setup.

Using a Digital PCR Setup

- 1 Click **Start a New Run**.
- 2 Click **Load**, as shown below



- 3 Click **Select a Digital PCR setup**.
A list of all previously created Digital PCR Setups display, as shown below.



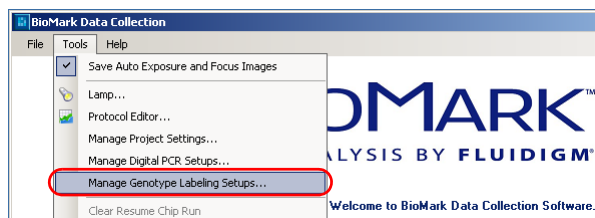
- 4 Click the setup you want to use (in the example, *New Template* is selected).
- 5 Click **Next** and complete the sequential windows that follow.

Genotyping IFC Runs

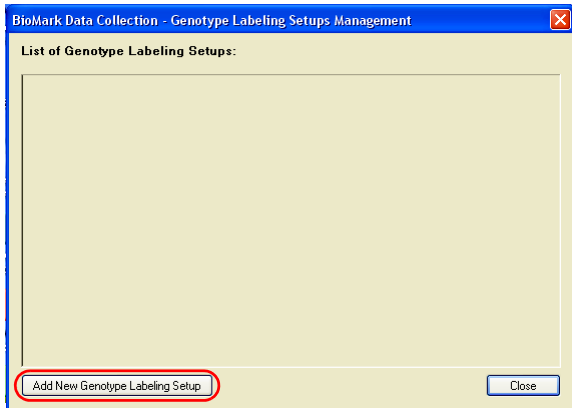
Managing Genotyping Labeling Setups

Before you start a new genotyping run, set up the new labeling scheme.

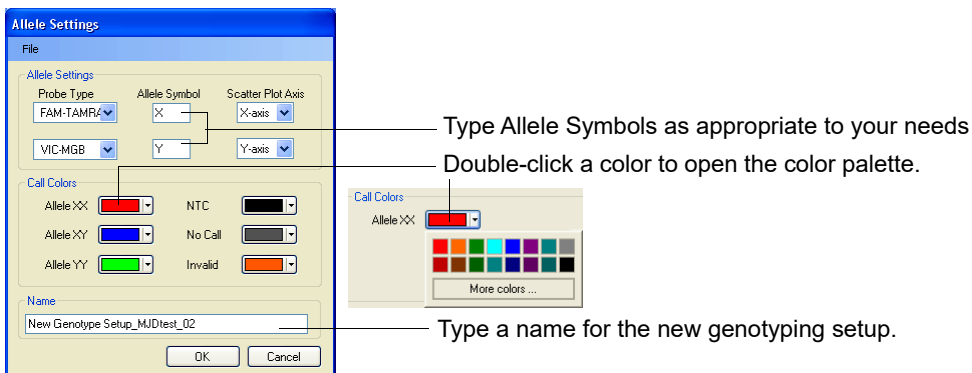
- 1 Click **Tools > Manage Genotyping Labeling Setups**.



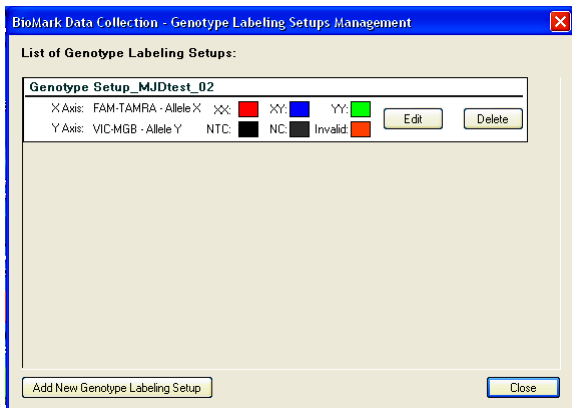
- 2 Click **Add New Genotyping Labeling Setup**.



- 3 Configure allele settings as appropriate. Use the drop-down menus to change values.



- 4 Click **OK** to accept the changes. The new genotyping labeling setup opens in the List of Genotype Labeling Setups dialog.



- 5 Click **Close** to finish the setup.
- 6 (Optional) Click **Add New Genotype Labeling Setup** to add another. Or, Click **Edit** to change elements in any genotype setup listed.
- 7 Set up your genotyping project (go to [“Setting Up a New Genotyping Run”](#) on page 61).

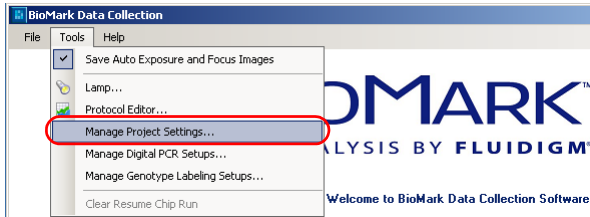
Setting Up a New Genotyping Run

- 1 Double-click the Data Collection Software icon on the desktop.
- 2 Click **Start a New Run**.
- 3 Check the status bar to verify that the lamp and camera are ready. Make sure both are green before proceeding.
- 4 Place the IFC into the reader.
- 5 Click **Load**.
- 6 Verify IFC barcode and IFC type.
 - a Choose project settings (if applicable).
 - b Click **Next**.
- 7 Chip Run file:
 - a Select **New** or **Predefined**.
 - b Browse to a file location for data storage.
 - c Click **Next**.
- 8 Application, Reference and Probes:
 - a Select Application Type—**Genotyping**.
 - b Select Passive Reference (ROX).
 - c Select probe types.
 - d Click **Next**.
- 9 Click Browse to find the appropriate thermal protocol file.
- 10 Confirm **Auto Exposure** is selected.
- 11 Click **Next**.
- 12 Verify that the IFC run information is correct.
- 13 Click **Start Run**.

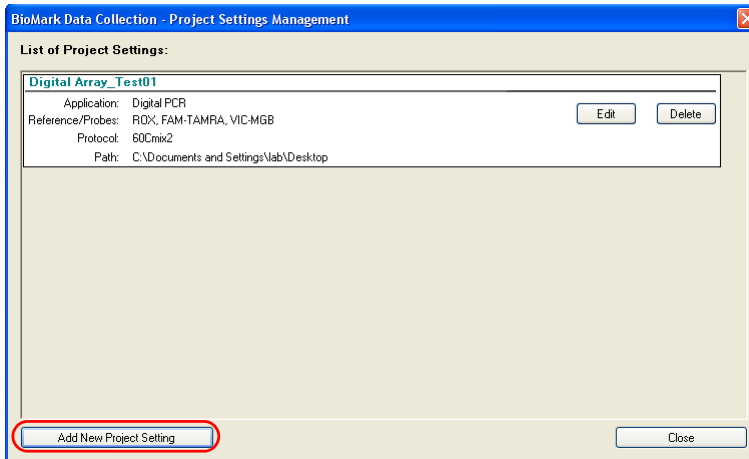
Setting Up a Genotyping Project

Now that you have the labeling set up, create a genotyping project.

- 1 Click **Tools > Manage Project Settings**.

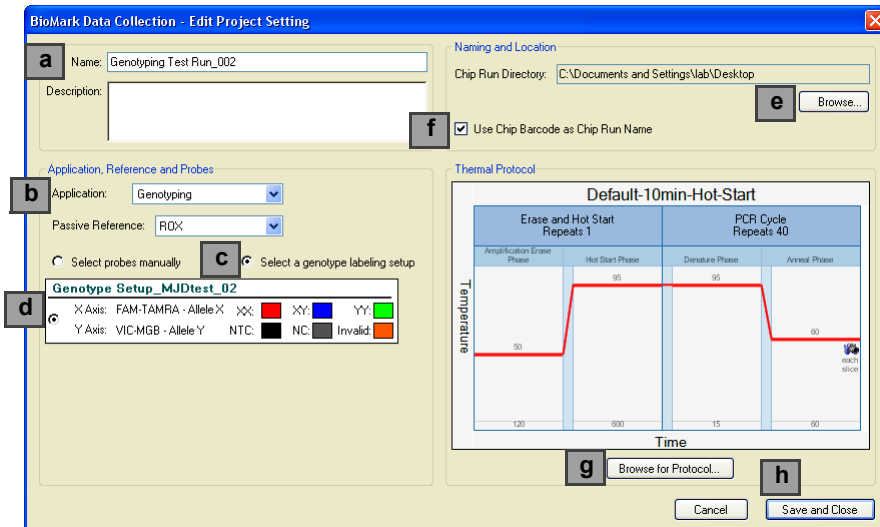


- 2 Click **Add New Project Settings**.



The Project Settings Editor opens.

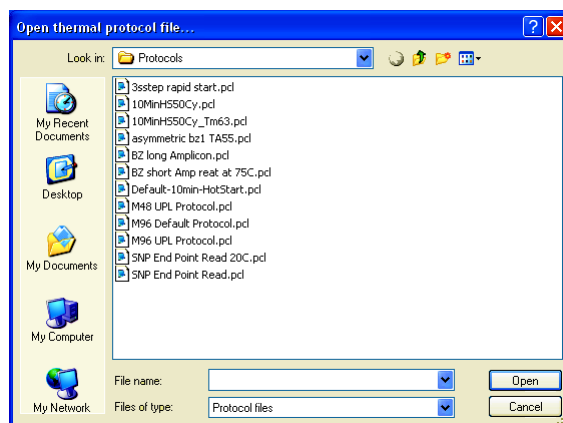
Project Settings Editor



- 3 Complete the settings using the following steps as a guide:
 - a Type a name for the new project and, optionally, type a description.
 - b Click **Genotyping** from the Application drop-down menu.

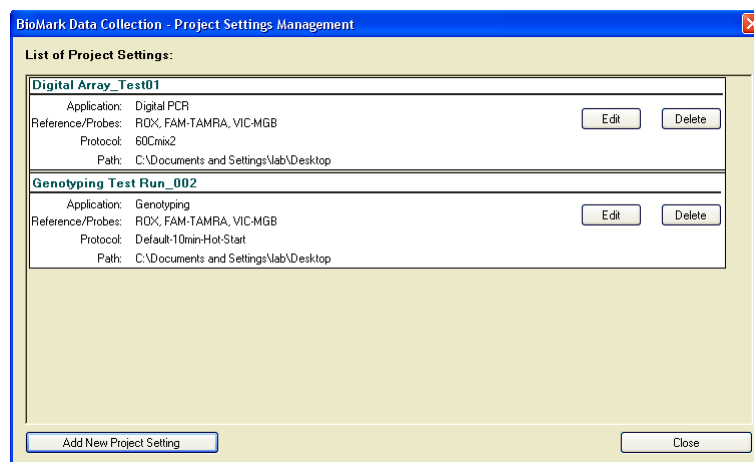
NOTE: ROX is the default passive reference.

- a Click **Select a genotype labeling setup**. The setup you created is now visible.
- b Click the setup you created to activate it.
- c Click **Browse** to navigate to a save location.
- d Click **Use Chip Barcode as Chip Run Name**.
- e Click **Browse for Protocol**. When you get the thermal protocol, double-click it to open it.



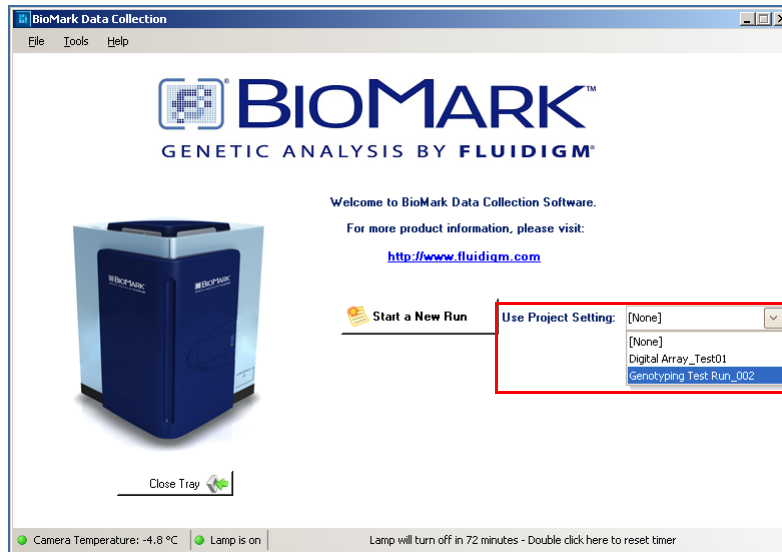
These are example files only and may not apply to your situation

- f Click **Save and Close**. The new project settings—with all the parameters you set—are listed in the Project Settings list.



Now, when you start a new run, click the Use Project Settings drop-down menu on the Start screen, and choose your custom project. As an example, you would:

- 1 Select a project setting from the drop-down menu.
- 2 Click **Start a New Run** and follow the software prompts.

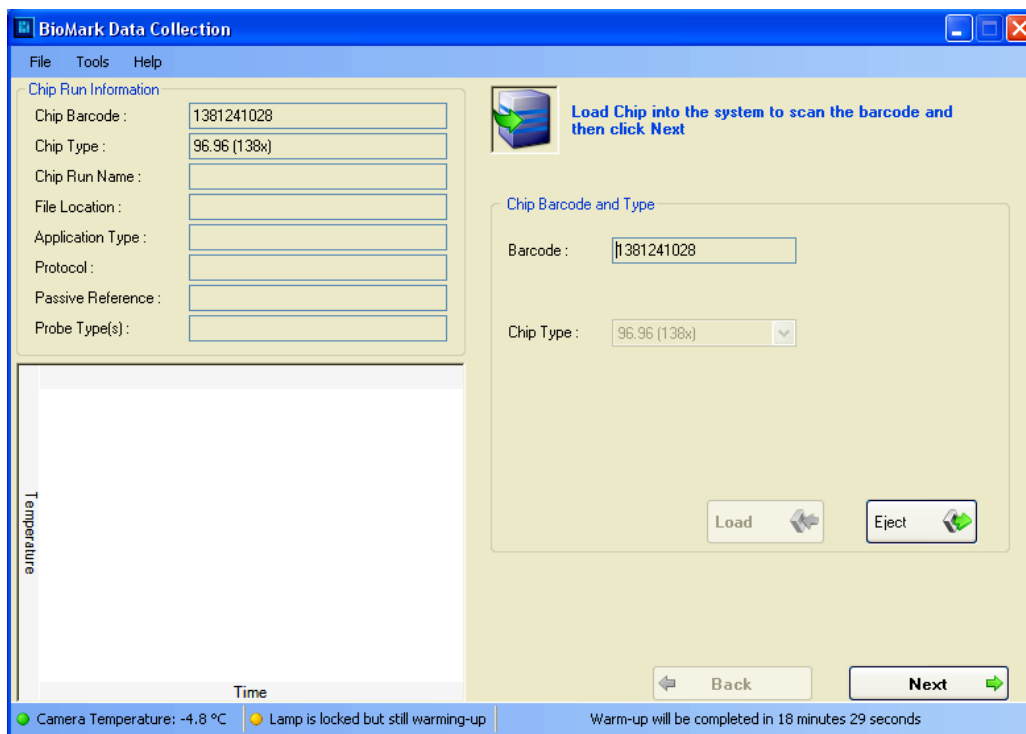


Gene Expression IFC Runs

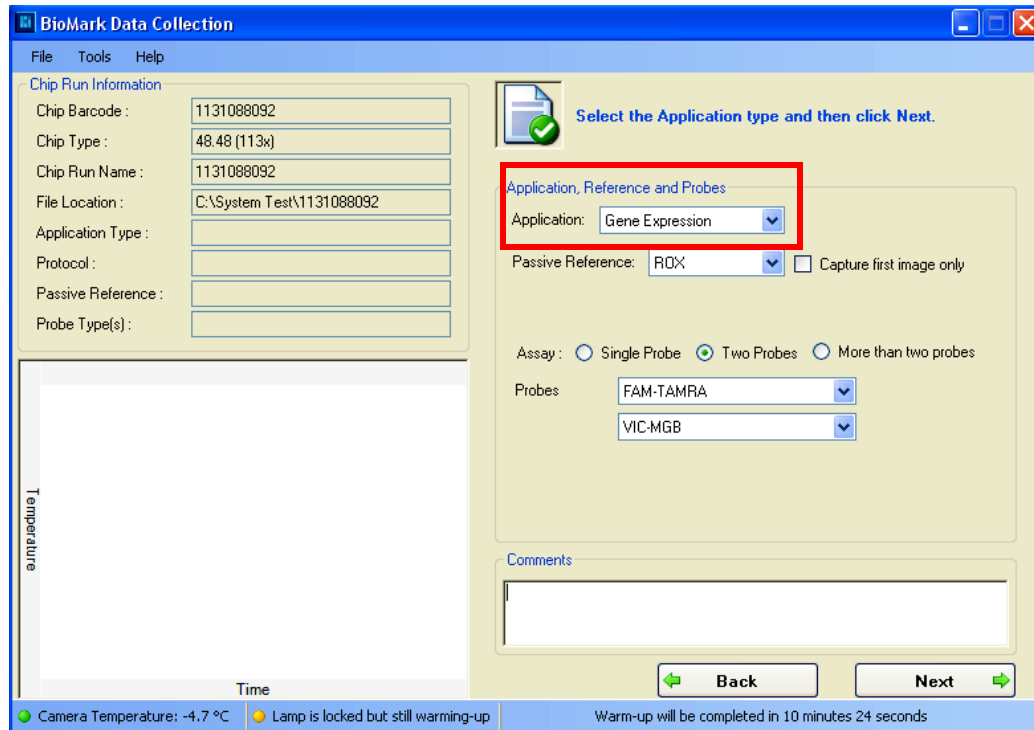
Setting Up a New Gene Expression Run

A gene expression run can be set up with or without creating a new project. In the following example, a new run is created without setting up a new project first. To Perform a Gene Expression Run:

- 1 Place the IFC onto the Biomark™ System tray.
- 2 Click **Load**. The IFC goes into the Biomark™ instrument where the barcode is read, and then displayed, as shown below.



- 3 Click **Next**.
- 4 Click **This is a new chip run** to open the following window.



- 5 From the Application drop-down menu, select **Gene Expression** or, **Melting Curve**.
- 6 Choose the number of probes you are using.
- 7 Select the name of the probes from the Probes drop-down menus.
- 8 Click **Next**.
- 9 Click **Browse** to go to thermal protocols (.pct).
- 10 Double-click the appropriate protocol to open it, as shown below.

BioMark Data Collection

File Tools Help

Chip Run Information

Chip Barcode : 1131088092
 Chip Type : 48.48 (113x)
 Chip Run Name : 1131088092
 File Location : C:\System Test\1131088092
 Application Type : Gene Expression
 Protocol : Default-10min-Hot-Start
 Passive Reference : RDX
 Probe Type(s) : FAM-TAMRA, VIC-MGB

Select the protocol and dye information for the chip, and then click Next.

Thermal Protocol

Protocol File : C:\Program Files\Fliudigm\BioMarkDataCollection\Application
 Browse...

Auto Exposure

Exposure Times

Passive Reference: RDX 0.30 seconds
 Probe Exposure: FAM-TAMRA 2.00 seconds
 VIC-MGB 2.00 seconds

Default-10min-Hot-Start

Erase and Hot Start Repeats 1		PCR Cycle Repeats 40	
Amplification Erase Phase	Hot Start Phase	Denaturation Phase	Anneal Phase
50	95	95	60
120	600	15	60

Temperature

Time

each slice

Back Next

Camera Temperature: -4.9 °C Lamp is locked but still warming-up Warm-up will be completed in 6 minutes 20 seconds

11 Click Next.

12 Click Start Run and follow the prompts.

BioMark Data Collection

File Tools Help

Chip Run Information

Chip Barcode : 1131088092
 Chip Type : 48.48 (113x)
 Chip Run Name : 1131088092
 File Location : C:\System Test\1131088092
 Application Type : Gene Expression
 Protocol : Default-10min-Hot-Start
 Passive Reference : RDX
 Probe Type(s) : FAM-TAMRA, VIC-MGB

Ready to start the run

Exposure Time (in seconds)

Passive Reference : RDX
 Probe Types : FAM-TAMRA, VIC-MGB

Run Status

Estimated Finish Time
 Elapsed Time 00:00:00

Real Time Chart

Start Run Abort Chip Run

Back Next

Camera Temperature: -4.8 °C Lamp is locked but still warming-up Warm-up will be completed in 5 minutes 22 seconds

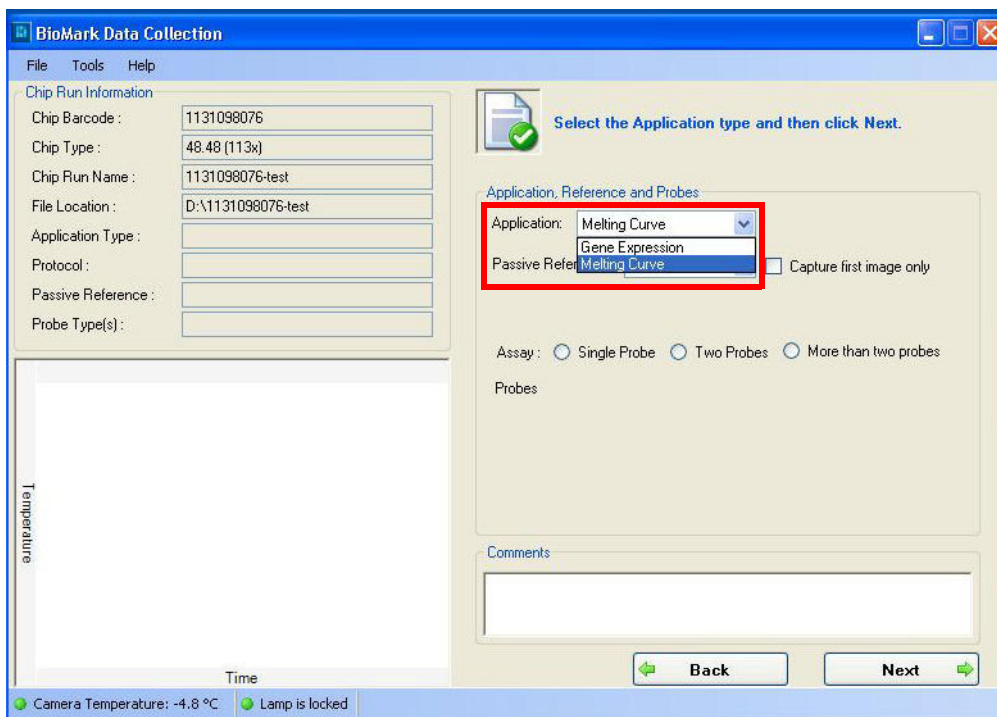
Setting up a Gene Expression Project

To set up a gene expression project (on the Biomark™ System only) go to [“Setting Up a New Genotyping Run” on page 61](#). Setting up a gene expression project is identical to setting up a genotyping project except that you do not need to set up labeling.

Melting Curve IFC Runs

Setting up a Melting Curve IFC Run

Setting up a melting curve IFC run (on the Biomark™ System only) is identical to setting up a gene expression IFC run except that you choose Melting Curve from the Application drop-down menu, as shown below. Then, follow the screen prompts.



- 1 Double-click the Data Collection Software icon on the desktop.
- 2 Click **Start a New Run**.
- 3 Check the status bar to verify that the lamp and camera are ready. Make sure both are green before proceeding.
- 4 Place the IFC into the reader.
- 5 Click **Load**.
- 6 Verify IFC barcode and IFC type.
 - a Choose project settings (if applicable).

- b Click **Next**.
- 7 Chip Run file:
 - a Select **New** or **Predefined**.
 - b Browse to a file location for data storage.
 - c Click **Next**.
- 8 Application, Reference and Probes:
 - a Select Application Type—**Melting Curve**.
 - b Select Passive Reference (ROX).
 - c Select Assay—**Single probe**, **Two probes** or **More than two probes**.
 - d Click **Next**.
- 9 Click Browse to find the appropriate thermal protocol file.
- 10 Confirm **Auto Exposure** is selected.
- 11 Click **Next**.
- 12 Verify that the IFC run information is correct.
- 13 Click **Start Run**.

Setting Up a Melting Curve Project

To set up a melting curve project (on the Biomark™ System only) go to [“Setting Up a New Genotyping Run” on page 61](#). Setting up a melting curve project is identical to setting up a genotyping project except that you do not need to set up labeling.

Starting and Tracking Flex Six™ IFC Runs

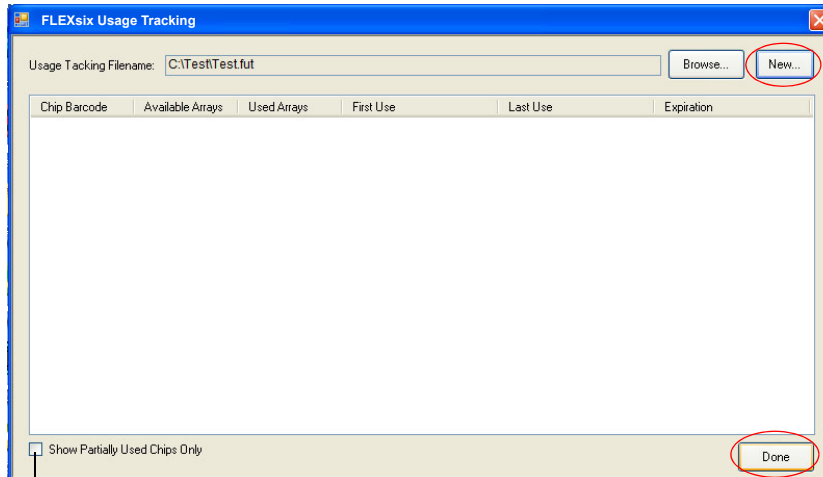
Setting Up a Tracking File

If this is the first time you are running a Flex Six™ IFC after installing the Data Collection software or after restarting the computer, you will need to set up a tracking file. After the tracking file is set up initially, the software creates a directory and stores all Flex Six™ data in this location.

NOTE: If the directory containing tracking files changes, the Data Collection software will no longer be able to track IFC usage.

NOTE: If you have multiple Biomark™ Systems, you should create the tracking file in a location that can be accessed by all the Biomark™ Systems. Only usage tracking files should be stored this way; we do not recommend storing IFC run data on a network.

- 1 On the Home page, select the **Tools > FLEXsix Usage Tracking**.
- 2 Click **New** to create a new tracking file, or you can select an existing tracking file by using the Browse button.



Allows you to search for partially used IFCs

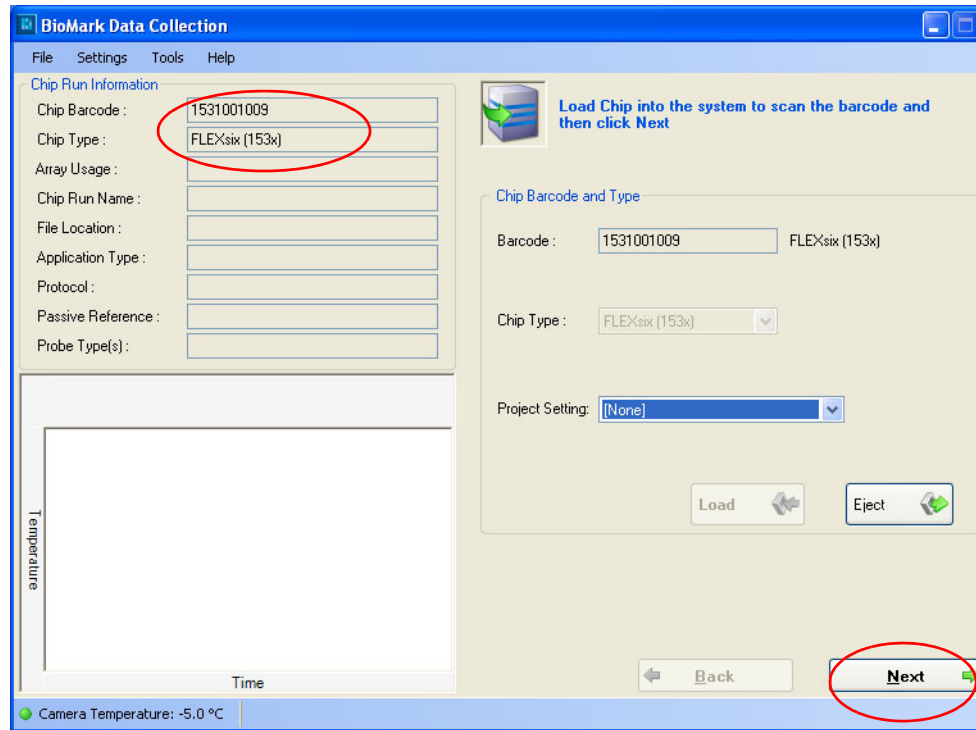
- 3 Click **Done**.

Starting a Flex Six™ IFC Run

Multiple gene expression and genotyping experiments can be run on a Flex Six™ IFC. You can run one experiment at a time or several of the same application at a time.

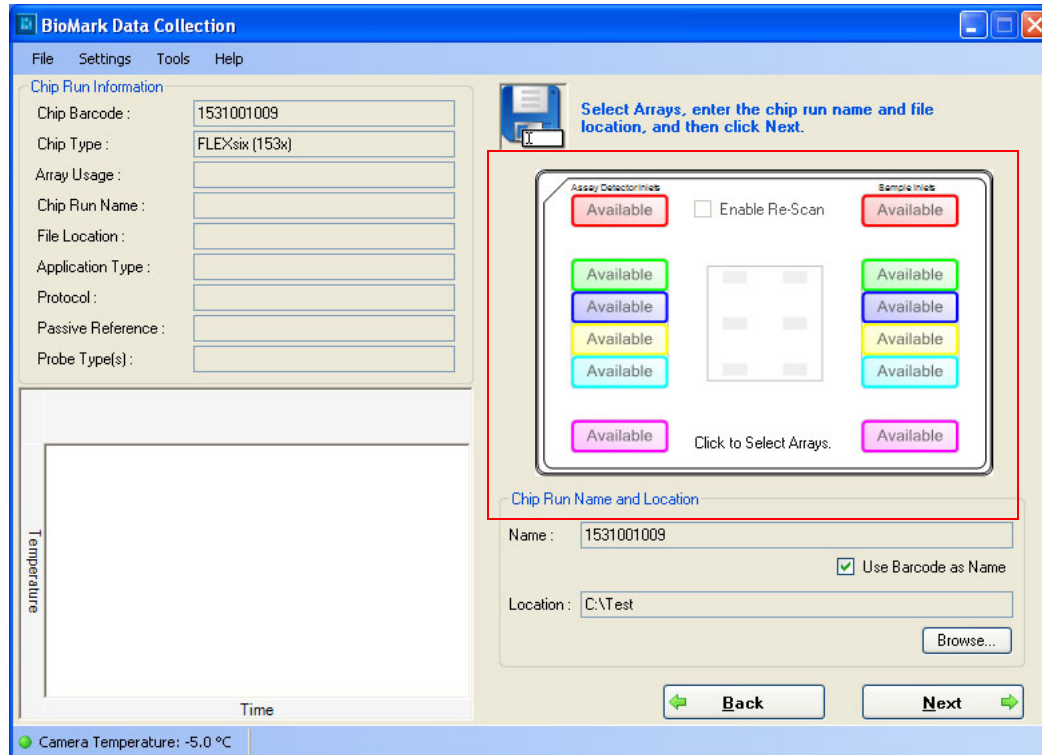
- 1 From the **Start** screen, click **Start a New Run**. The Data Collection screen appears.
- 2 Load the IFC on the Biomark™ tray.
 - a Remove the protective film from the bottom of the IFC and place the IFC on the Biomark™ tray. Make sure the A1 corner on the IFC aligns with the A1 on the tray.

- b Click **Load**. The tray retracts, and the system scans the barcode and identifies the IFC.



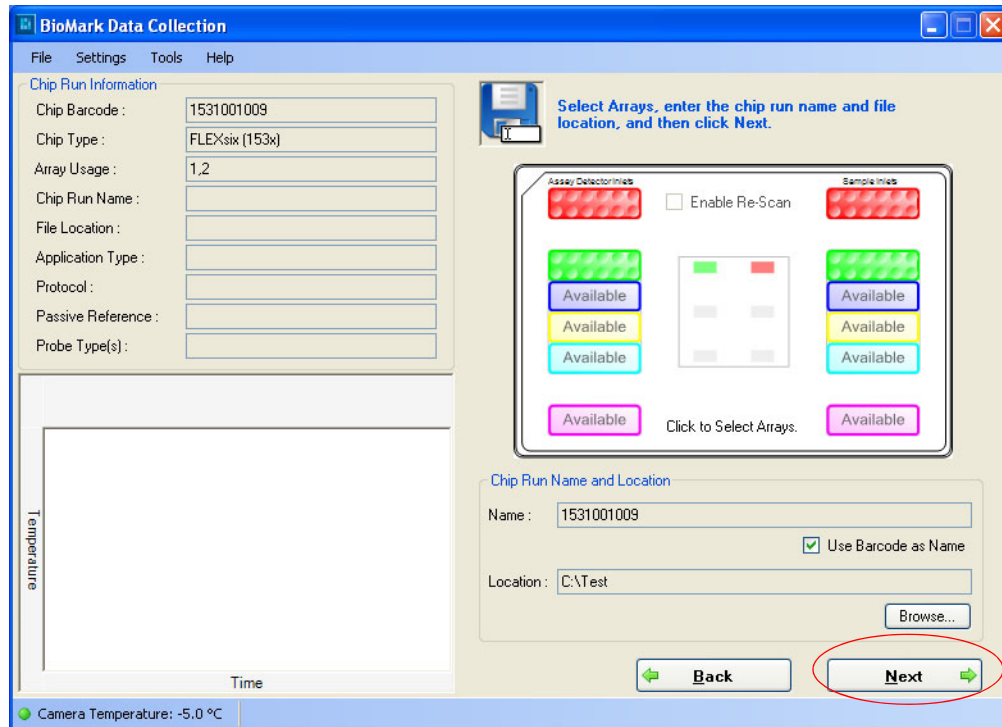
c Click **Next**.

Now you will see the available arrays.



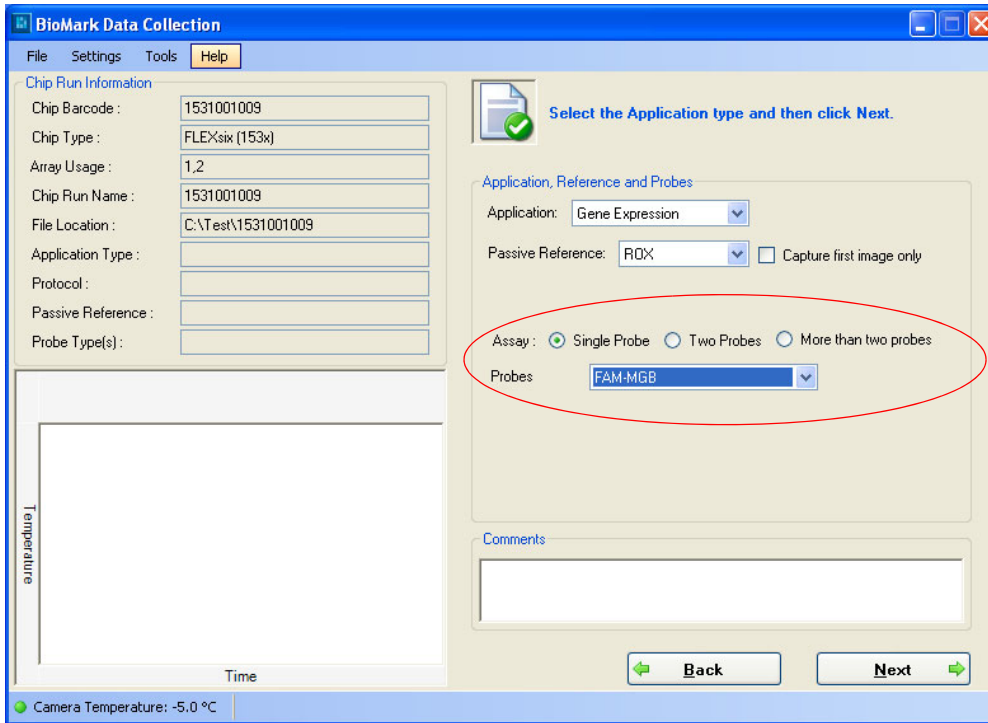
- 3 Select the arrays you wish to run. (Selected arrays should be contiguous.)
- 4 Determine the IFC run name and file location for the new IFC run.

- a Click the **Use Barcode as Chip Run Name** checkbox to use the barcode as the name or uncheck the box and enter a unique name. Browse to a different location for saving the file, if needed.



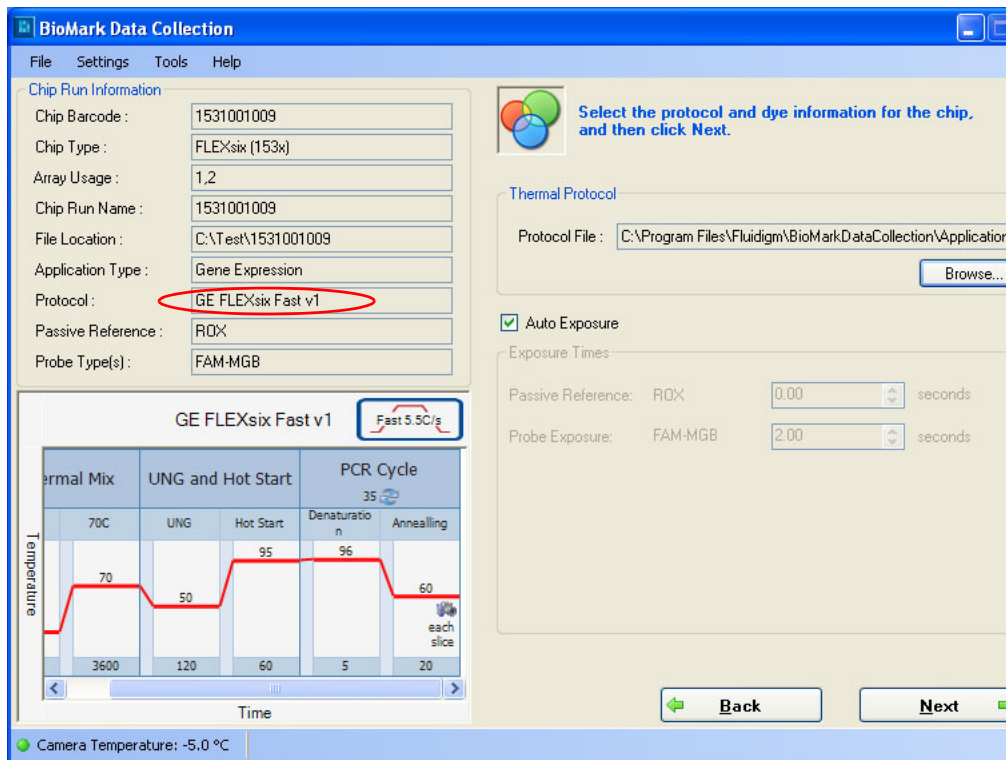
- b Click **Next**.
- 5 Select the application and select probes.
 - a For the Assay, select **Single Probe**, **Two Probes**, or **More than two probes**.

b In the Probes drop-down box, select the probes.



c Click **Next**.

6 Select a protocol file.



a Click **Next**.

You are now ready to start your run.

7 Click Start Run.

BioMark Data Collection

File Settings Tools Help

Chip Run Information

Chip Barcode : 1531001009

Chip Type : FLEXsix (153x)

Array Usage : 1,2

Chip Run Name : 1531001009

File Location : C:\Test\1531001009

Application Type : Gene Expression

Protocol : GE FLEXsix Fast v1

Passive Reference : ROX

Probe Type(s) : FAM-MGB

Ready to start the run

Exposure Time (in seconds)

Passive Reference : ROX

Probe Types : FAM-MGB

Estimate the Exposure Times at 70.0 C

Post-processing
[No post-processing]

Estimated Finish Time

Elapsed Time
00:00:00

Start Run Abort Chip Run

Back Next

GE FLEXsix Fast v1 Fast 5.3C/s

Thermal Mix	UNG and Hot Start		PCR Cycle	
70C	UNG	Hot Start	Denaturation	Annealing
3600	120	60	5	20

Temperature

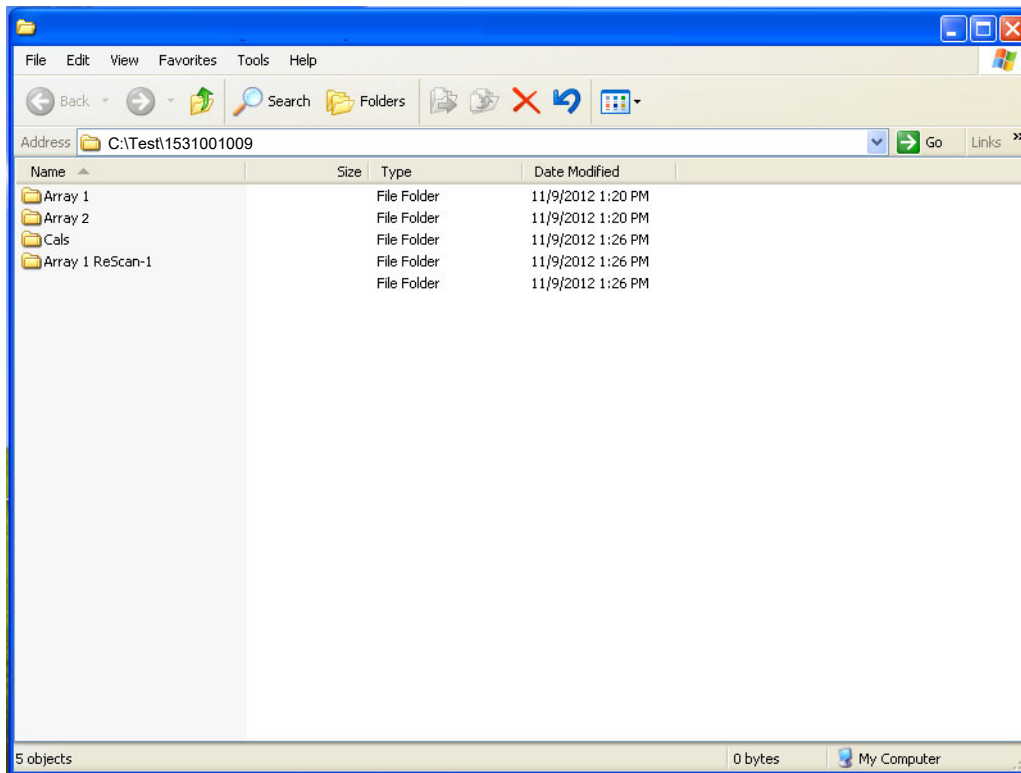
Time

Camera Temperature: -5.0 °C

In subsequent use of the same IFC, dates will appear on the used arrays.

Directory Structure

The array IFC runs will be stored in a directory in which each array has its own folder.



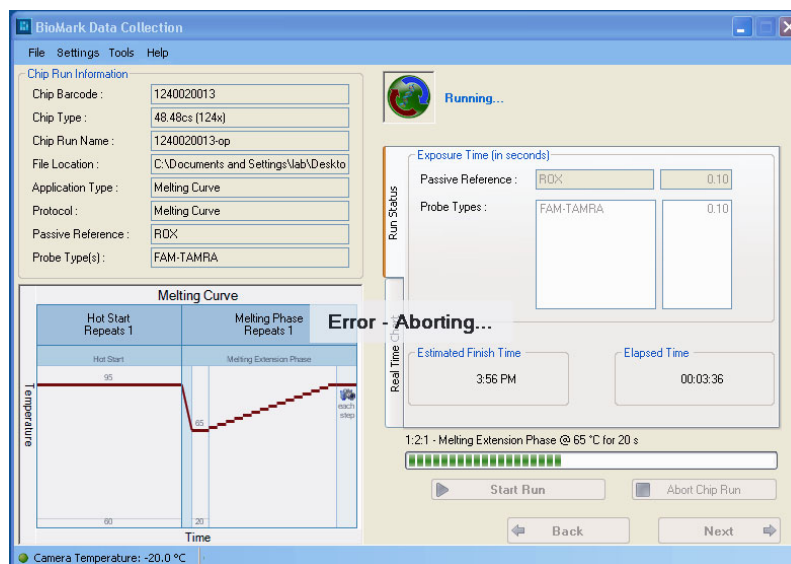
NOTE: If you want to re-scan the IFC for any reason, select the array and check mark **Enable Re-scan**. The software creates a Re-Scan folder for these re-scanned IFC runs.

Troubleshooting

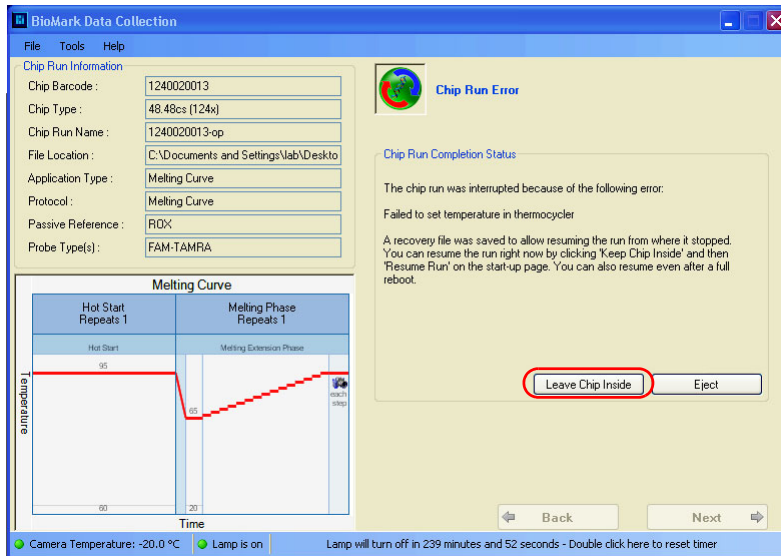
Resuming an IFC Run

In the rare event of a system failure during a run, you may be able to resume your run when the system is restored (on the Biomark™ System only).

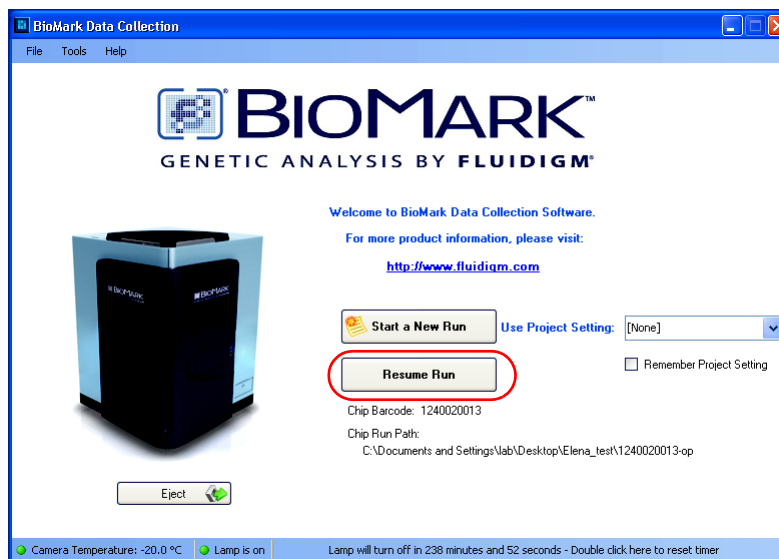
If such an event occurs—a sudden powering down of the thermal cycler, for example—Data Collection software issues a warning, as shown below.



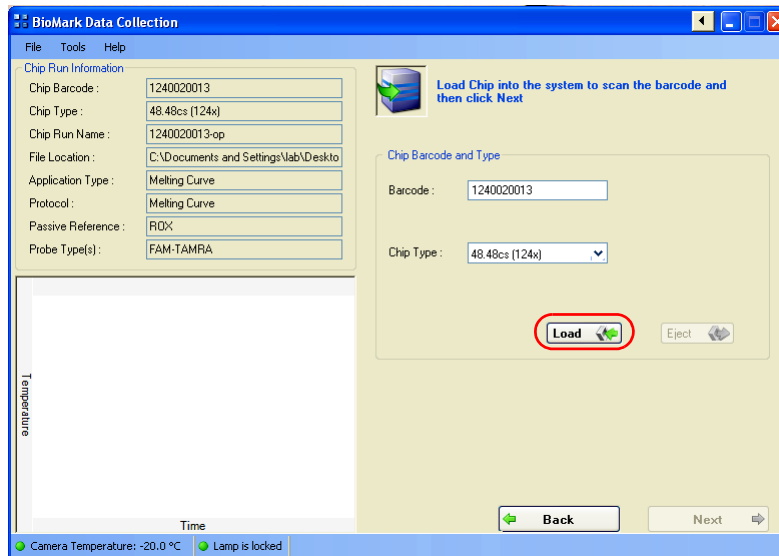
After the problem is fixed and the system is up, the following Chip Run Error screen displays.



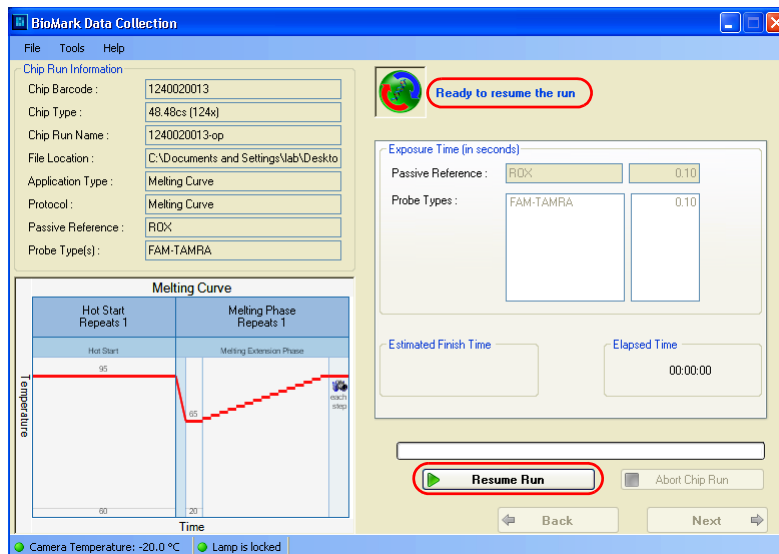
1 Click **Leave Chip Inside** to display the following screen.



2 Click **Resume Run** to display the Load screen, as shown below.



- 3 Click **Load** to return ejected IFC tray back into the Biomark™ instrument. After the IFC is back in the instrument, the window where the IFC run was interrupted is displayed, as shown below.

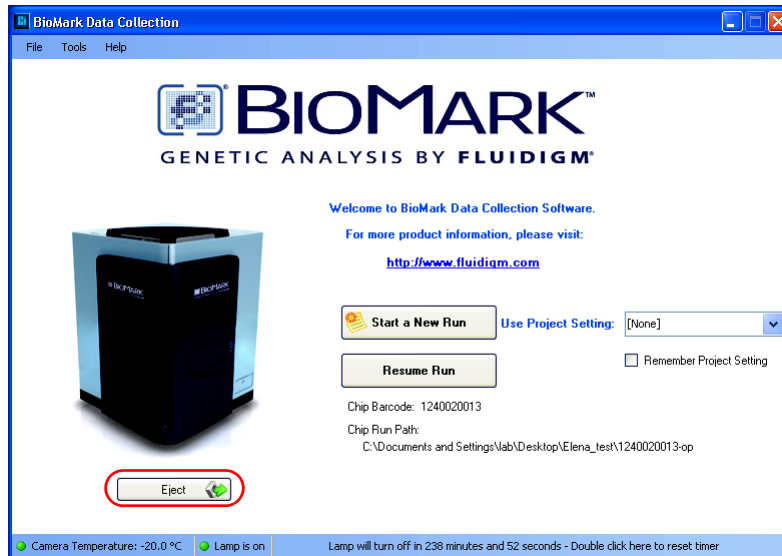


- 4 Click **Resume Run** to continue your IFC run.

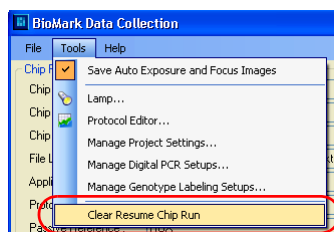
Clearing an IFC Run

If, after a system error you do not want to continue the interrupted IFC run:

- 1 Click **Eject** to eject the IFC from the instrument.



2 Click Tools > Clear Resume Chip Run to clear the interrupted run.



Using the Protocol Editor

Through the Protocol Editor you can:

- Create a new protocol using the template wizard
- Change protocol parameters
- Create a customized protocol

Accessing the Protocol Editor

1 To open the Protocol Editor:

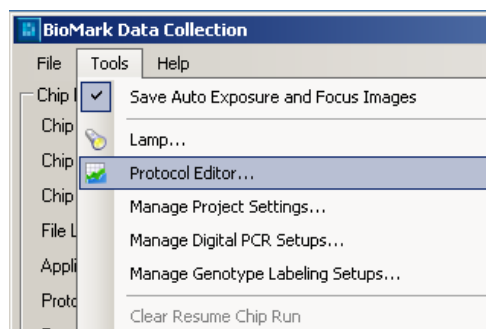
- Without opening Data Collection software:

Click **Start > All Programs > Fluidigm > BioMark > Protocol Editor**.



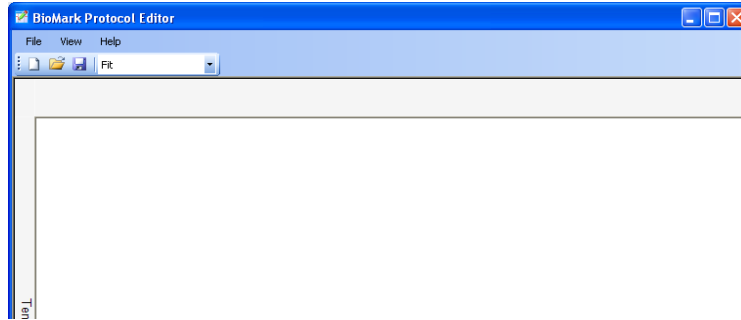
- Within Data Collection software:

Click **Tools > Protocol Editor**.



Protocol Editor

First screen.

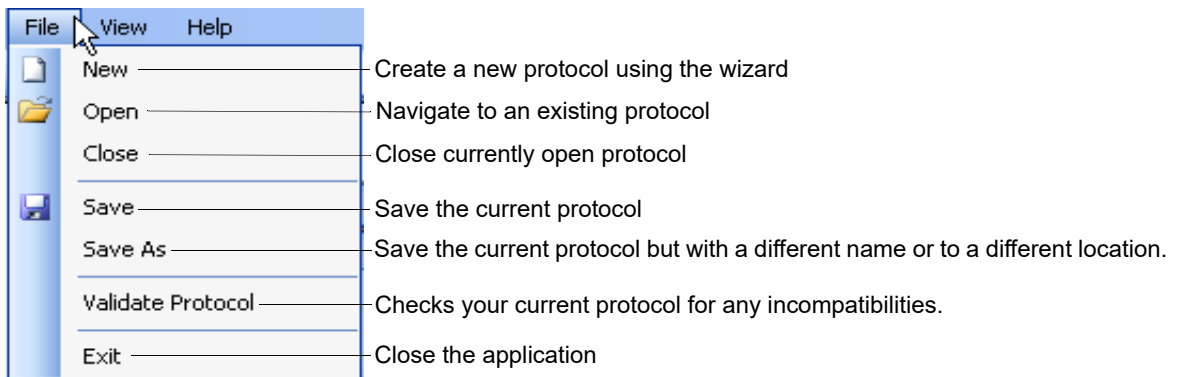


Protocol Editor Menu Bar

A brief description of each option follows.

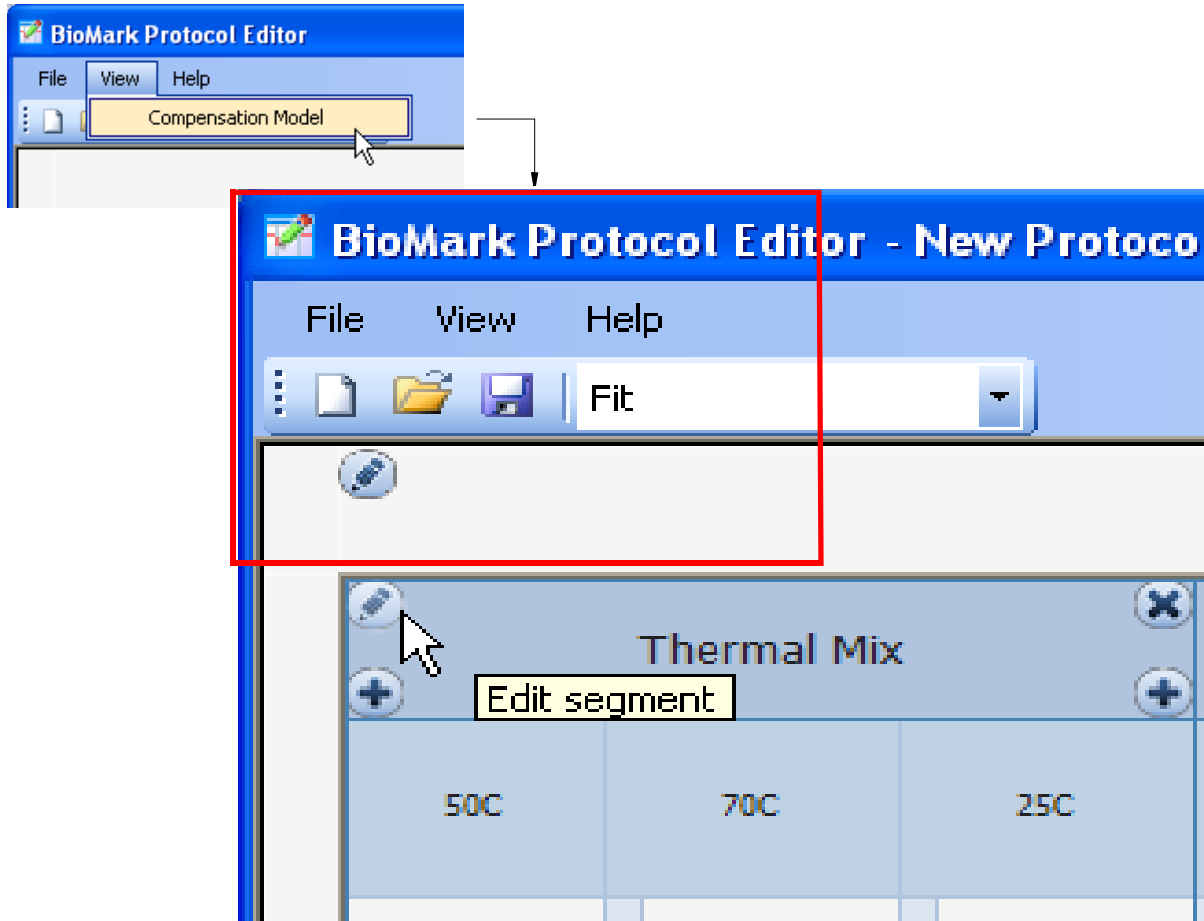
File

Click **File** to access the following options.



View

Click **View > Compensation Model** to open the Protocol Viewer which contains a view of the expanded and compensated protocol in one scrollable window.



Creating a New Protocol

Overview

As you follow the New Protocol Wizard, you are offered options from which you must choose in order to complete each window. Choices are dependent on the protocol type that you choose from the drop-down menu in the Wizard Properties panel (graphic below). The protocol types are self-evident—just follow the wizard—except for Manual, which is described beginning on page 98.

Following the Wizard

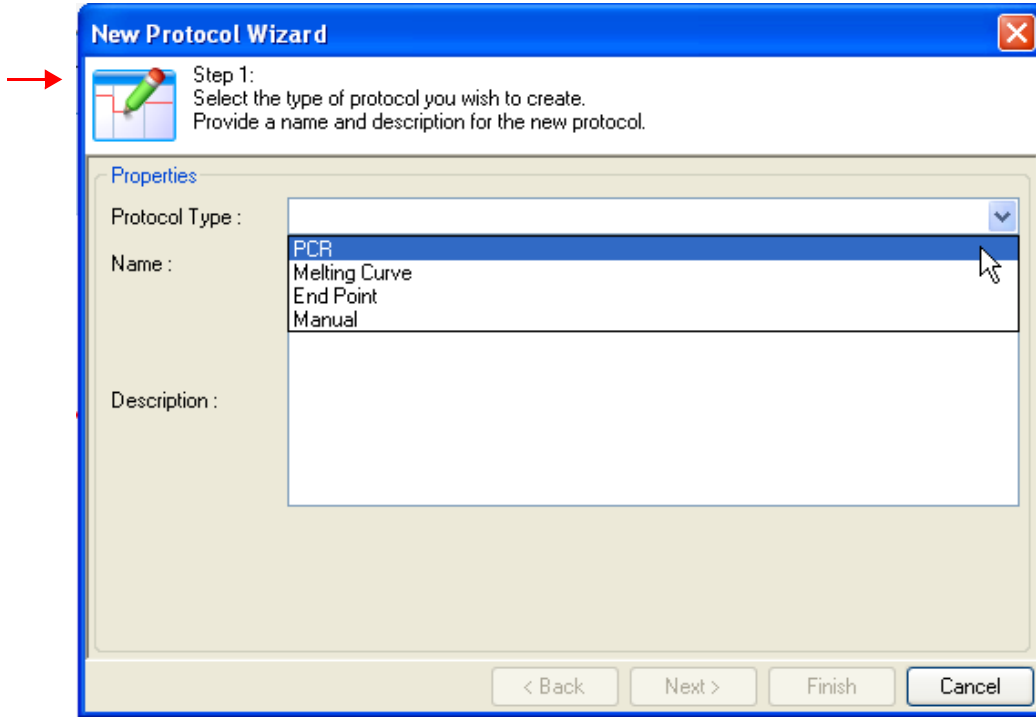
In the example below, screens and options are shown as the user follows the wizard for the first choice, PCR.

To create a new protocol:

- 1 In the Protocol Editor, click **Protocol > New**.
- 2 Follow the **New Protocol Wizard**.

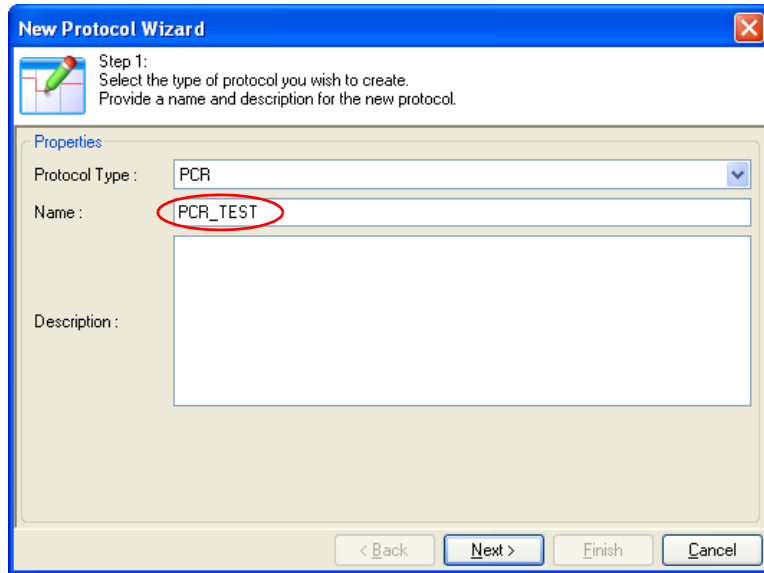
NOTE: For the other protocol types, follow the wizard and choose appropriate options.

- 1 Select a **Protocol Type** (PCR, for this example).



Protocol types in the Properties panel

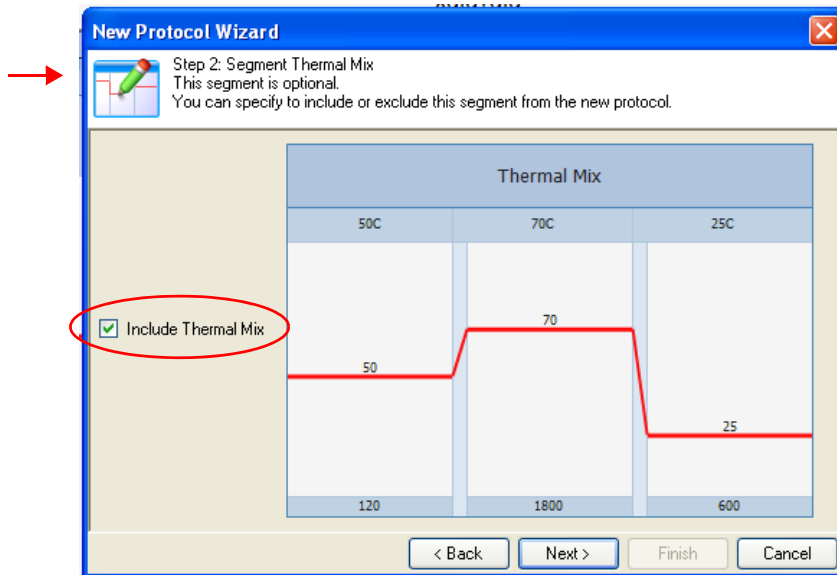
2 Name the new protocol.



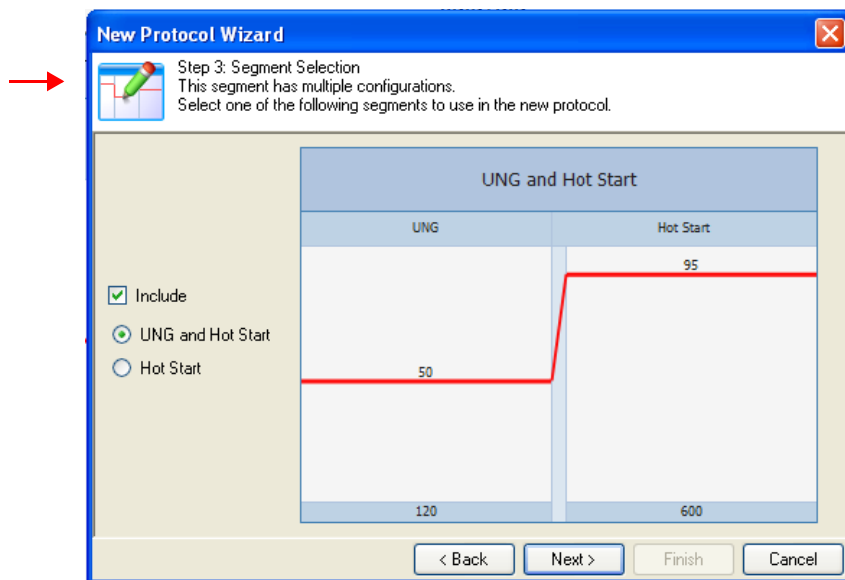
3 *Optional:* Add a description.

4 Click **Next**.

5 Include **Thermal Mix** segment by checking the box or, uncheck to exclude.



- 6 Click **Next**.
- 7 Click **Include** to activate, and then click either **UNG and Hot Start** or, **Hot Start**.
Or,
Deactivate **Include** to omit both segments.



NOTE: UNG = uracil-DNA glycosylase. The Uracil-DNA Glycosylase catalyzes the hydrolysis of the N-glycosylic bond between the uracil and sugar, leaving an apyrimidinic site in uracil-containing single or double-stranded DNA. Shows no activity on RNA.

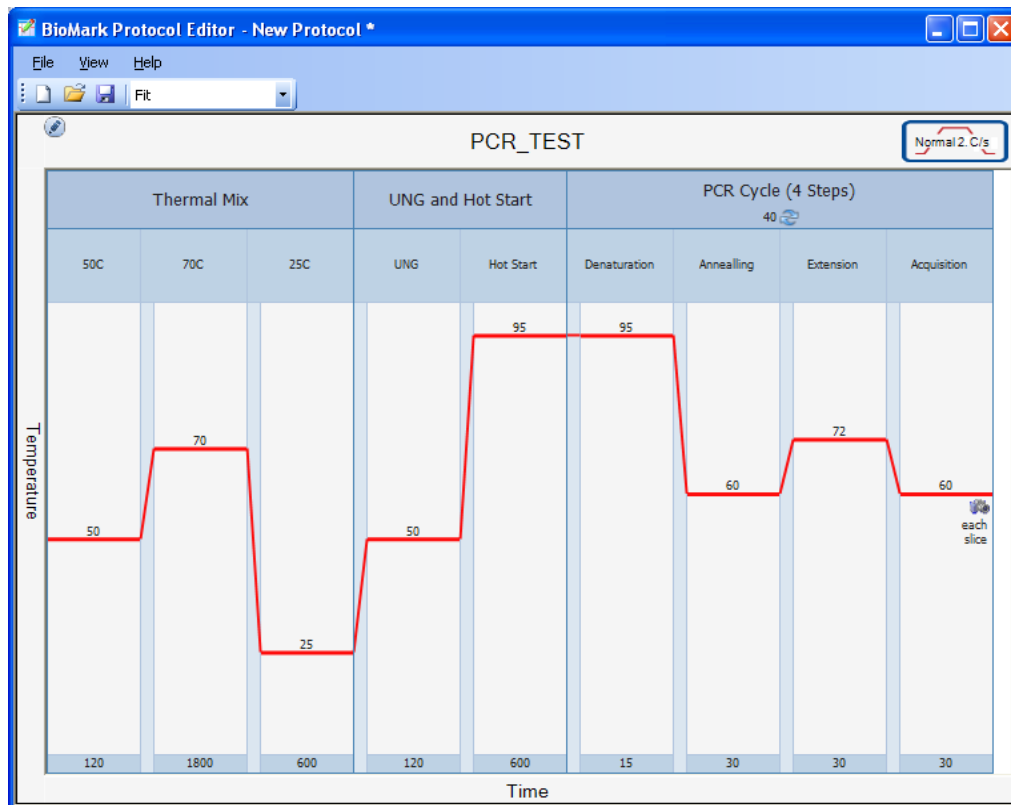
- 8 Click **Next**.

9 Choose a PCR Cycle using the following screens as a guide.

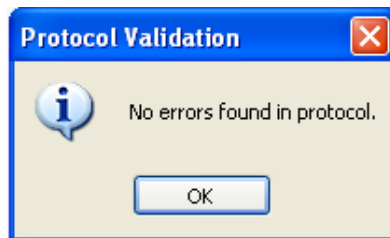
The figure shows three sequential screenshots of the 'New Protocol Wizard' software interface, illustrating the selection of different PCR cycle configurations. Each screenshot is titled 'New Protocol Wizard' and shows 'Step 4: Segment Selection' with the instruction: 'This segment has multiple configurations. Select one of the following segments to use in the new protocol.'

- Top Screenshot: PCR Cycle (2 Steps)**
 - Selected option: PCR Cycle
 - Temperature profile: Denaturation at 95°C (15s), Annealing at 60°C (60s).
 - Buttons: < Back, Next >, Finish, Cancel.
- Middle Screenshot: PCR Cycle (3 Steps)**
 - Selected option: PCR Cycle (3 Steps)
 - Temperature profile: Denaturation at 95°C (15s), Annealing at 60°C (30s), Extension at 72°C (30s).
 - Buttons: < Back, Next >, Finish, Cancel.
- Bottom Screenshot: PCR Cycle (4 Steps)**
 - Selected option: PCR Cycle (4 Steps)
 - Temperature profile: Denaturation at 95°C (15s), Annealing at 60°C (30s), Extension at 72°C (30s), Acquisition at 60°C (30s).
 - Buttons: < Back, Next >, Finish, Cancel.

10 Click **Finish** to open the new protocol template.



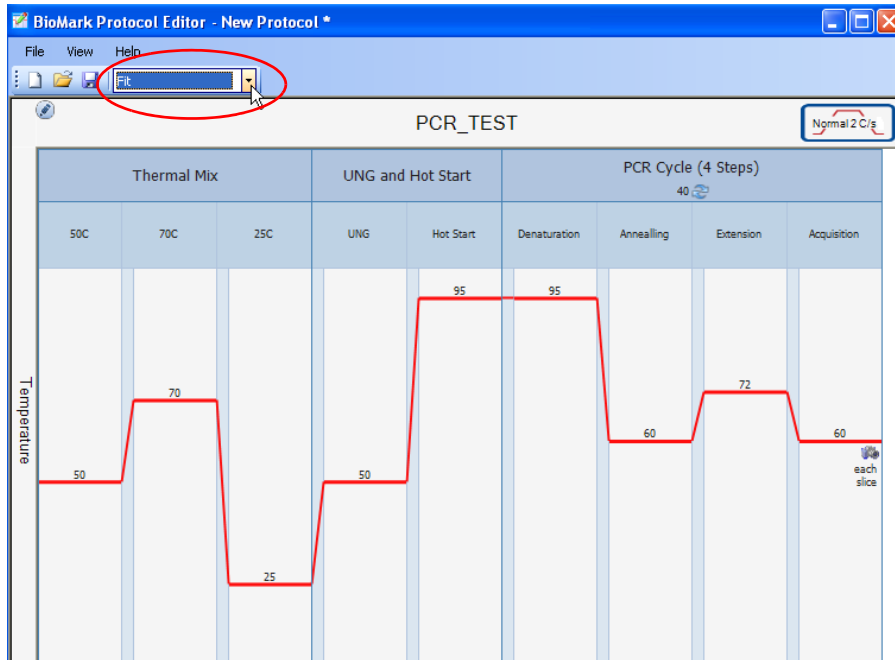
11 *Optional*: Click **File > Validate Protocol** to ensure that your protocol is ready to use.



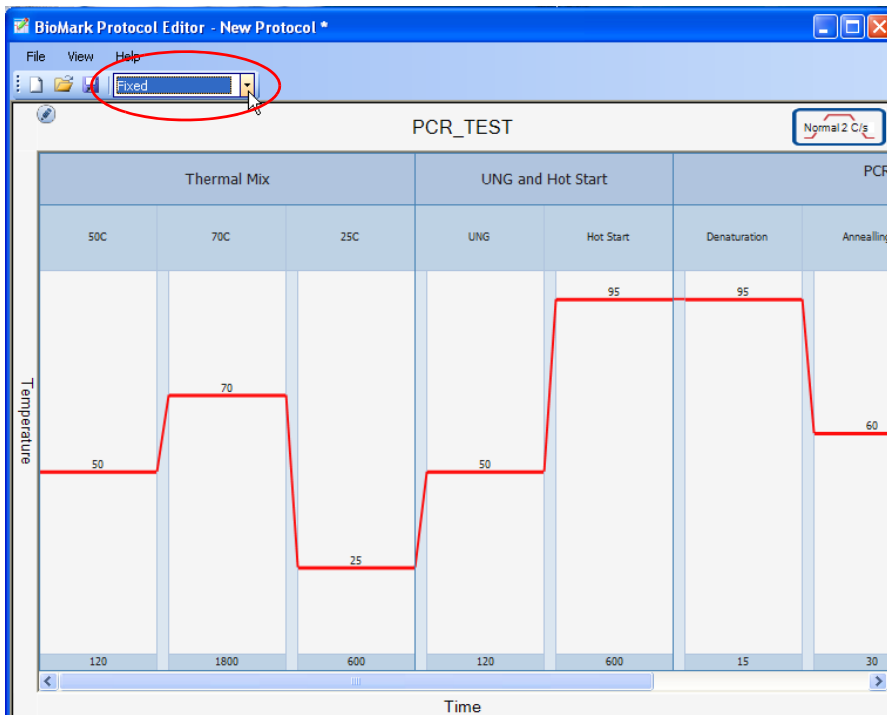
Fit/Fixed Drop-Down Menu

Toggle between the Fit or Fixed view. Fit reduces the size of the protocol so that it fits in the active window. Fixed increases the size of the protocol so that the segments and slices are easier to see but the entire protocol is not visible without horizontal scrolling

Fit view example:



Fixed view example:

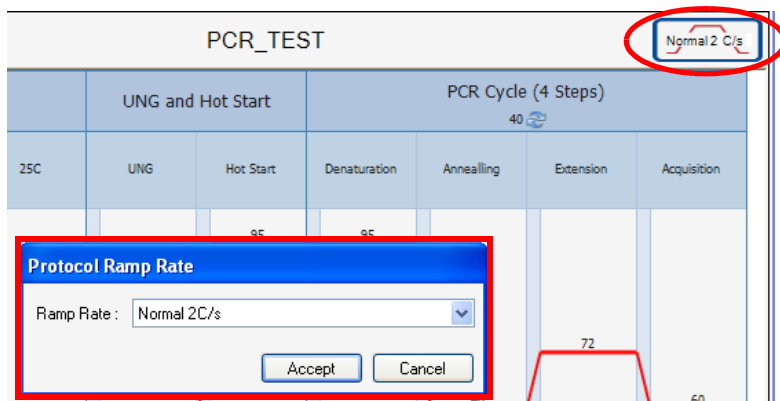


Changing the Global Ramp Rate

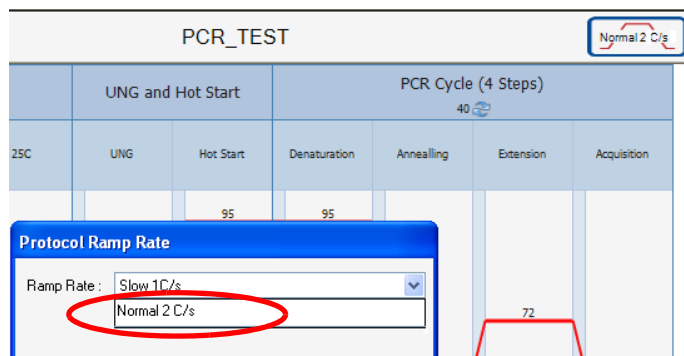
The ramp rates available for selection are specific to the hardware configuration of the system running the editor. The system configuration files define which thermal cycler is connected to the machine. Each thermo cycler type has a different set of ramp rates.

To change the ramp rate for all segments simultaneously:

- 1 Click the **Ramp Rate** icon to open the Ramp Rate dialog.



- 2 Click the **drop-down menu** to display ramp rate choices.



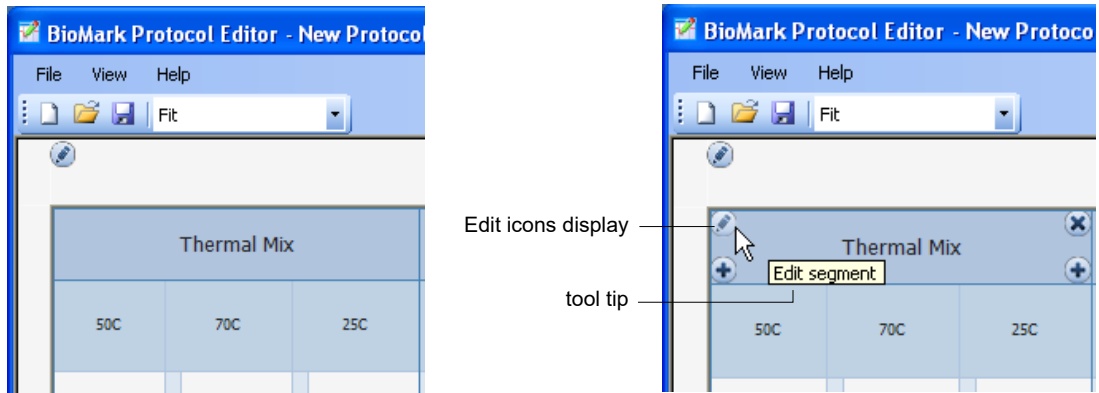
- 3 Click a new ramp rate.
- 4 Click **Accept**. Now, the ramp rate is the same in all segments and slices.

The image shows a software interface for editing a PCR protocol. At the top, a table titled "PCR_TEST" lists the steps: UNG and Hot Start, PCR Cycle (4 Steps), and Acquisition. The "PCR Cycle (4 Steps)" step is expanded to show sub-steps: UNG, Hot Start, Denaturation, Annealing, Extension, and Acquisition. A red circle highlights the "Normal 2 C/s" ramp rate setting in the top right corner of the protocol editor. Below this, two dialog boxes are open. The "Segment Properties" dialog box shows the "PCR Cycle (4 Steps)" segment with a "Repeat" of 40 and a "Ramp Rate" of "Normal 2 C/s", which is circled in red. The "Slice Properties" dialog box shows the "Acquisition" slice with a "Temperature (C)" of 60, a "Duration (s)" of 30, and a "Ramp Rate" of "Normal 2 C/s", also circled in red. Both dialog boxes have "Accept" and "Cancel" buttons. A temperature profile graph is partially visible at the bottom left, showing a ramp from 50 to 60 degrees Celsius.

Context Sensitive Edit Tools

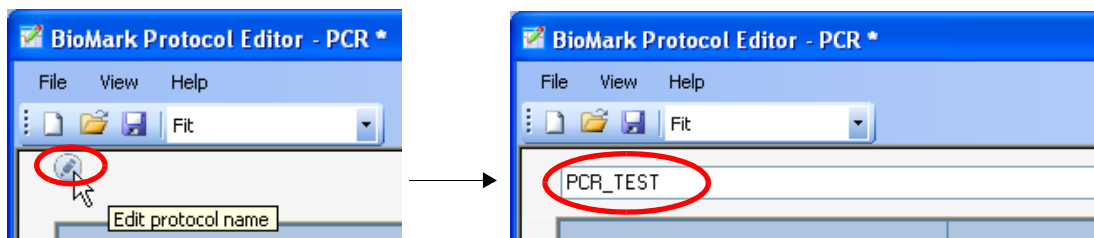
The Protocol Editor includes context-sensitive tool tips. They work in two ways:

- Hover your cursor anywhere within a area to reveal Edit icons.
- Hover your cursor over an Edit icon to reveal its function in a tool tip.

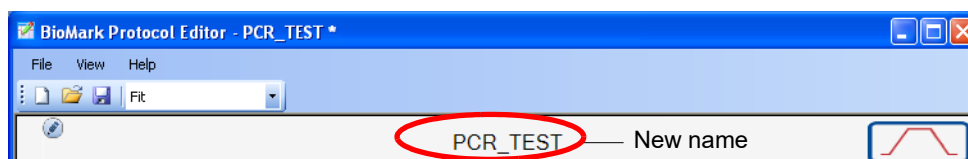


Changing the Protocol Name

- 1 Click the **Edit protocol name** icon or, double-click within the title area.



- 2 Type a new name for the protocol.
- 3 Hit **Enter** to commit, or tap **Esc** to return to original name.



Changing Segment Parameters

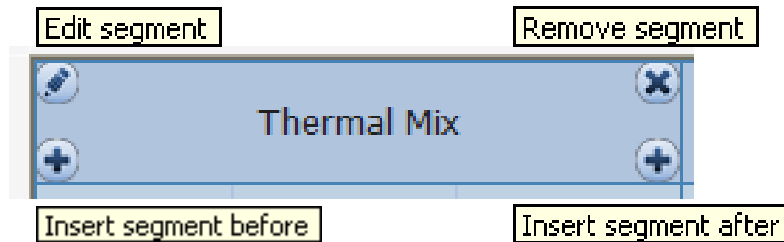
To change parameters of individual segments, hover your cursor on the segment you want to change, to reveal editing tool icons.

Some general rules for editing protocols:

- A slice defines specific parameters for the controlling the thermocycler to perform one step of the containing segment's function.
- A segment is a logical grouping of slices to perform a specific function. A segment always contains at least one slice.
- A protocol always contains at least one segment.
- All non-manual segments are constrained.
- Constrained segments.
 - All segments except manual ones are constrained, specifically meaning that the user cannot add or remove a slice from a constrained segment.
 - The user can add or remove segments from a non-manual protocol but cannot modify the slice composition of a non-manual segment. Specifically, a constrained segment performs a defined task and modification of the slice composition will interfere or prevent the segments function from completing. If the user wishes to create a custom segment they can add the Manual segment and modify the slice composition themselves.

Segment Editing Tools

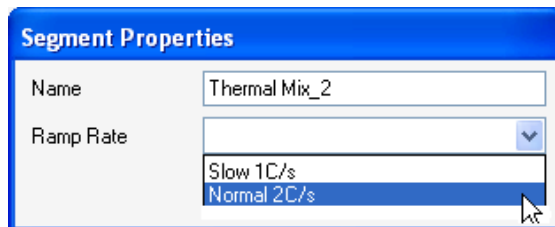
The following graphic shows the tip tools that open when you hover your cursor over the icons.



What the Editing Tools Do

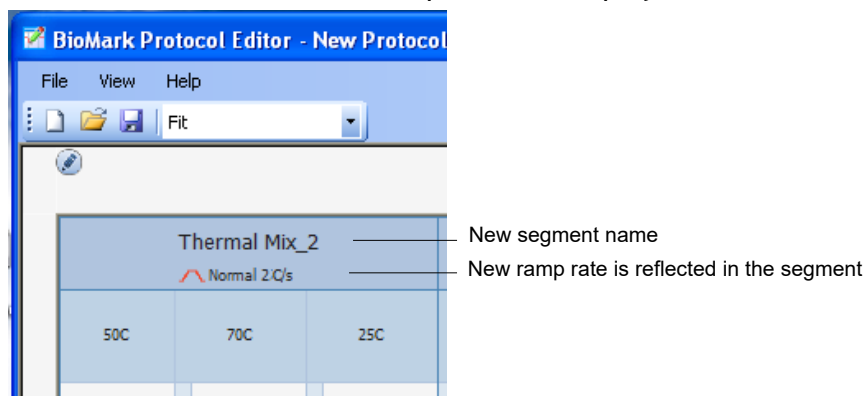
Edit segment

- 1 Click the **Edit segment icon** or, **double-click** inside the segment, to open the Segment Properties dialog.
- 2 Rename the segment.



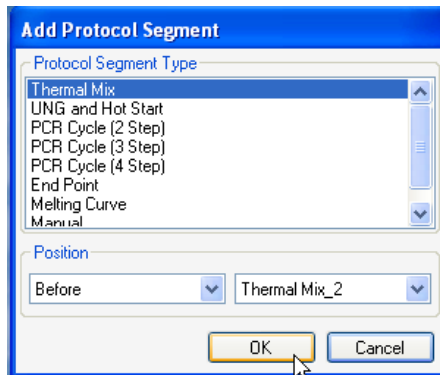
- 3 Select an appropriate ramp rate for the segment.
- 4 Click **Accept**.

The custom name and new ramp rate are displayed in the altered segment.

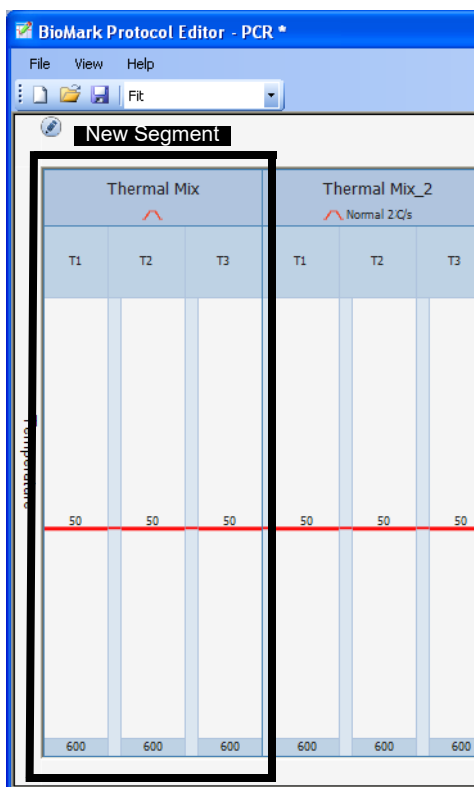


Insert segment before

- 1 Click the **Insert segment before icon** to open the Add Protocol Segment dialog.



- 2 Click **OK** accept the default positions. The new segment is added to the protocol, before (in front of) the segment in which you started.



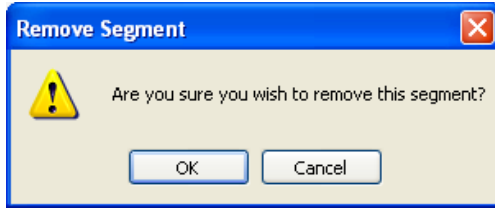
Any parameters that you change are reflected in the title of the segment or the slice that you alter.

Insert segment after

Follow the procedure for inserting a segment before, described above. The result will place a new segment after (behind) the segment in which you started.

Remove segment

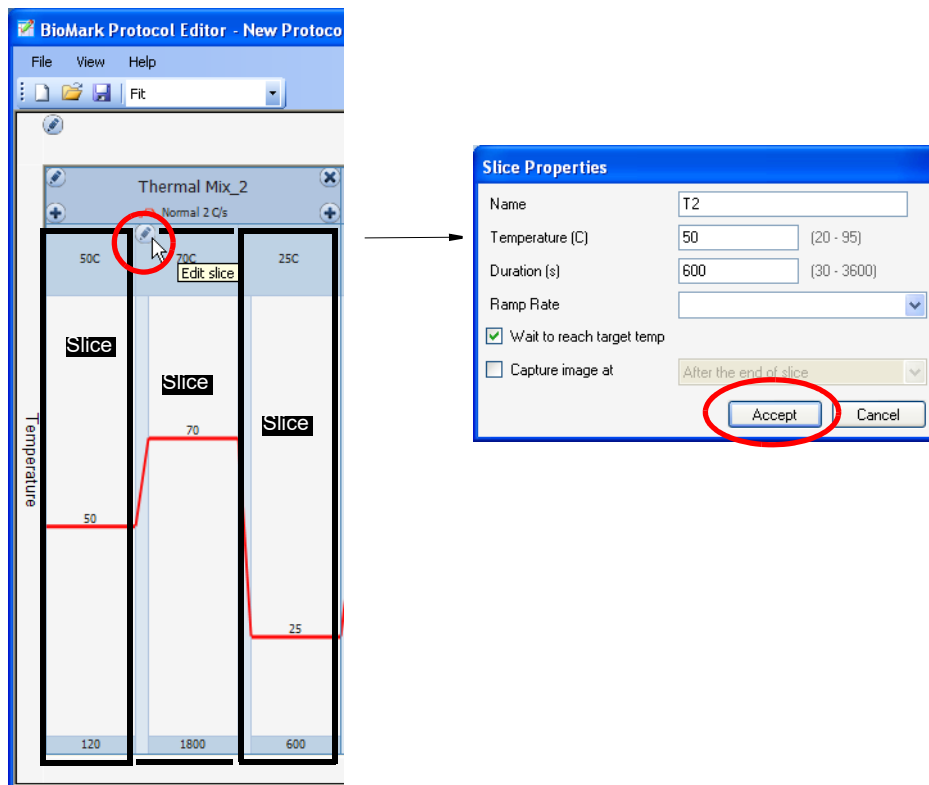
When you click **Remove segment**, a warning displays. Click **OK** to remove the segment or, **Cancel** to keep the segment.



IMPORTANT: Removed segment cannot be recovered.

Changing Slice Parameters

- 1 Hover your cursor inside the segment slice you want to alter.
- 2 Click the **Edit slice** icon or, **double-click** inside the slice, to open the Slice Properties dialog.



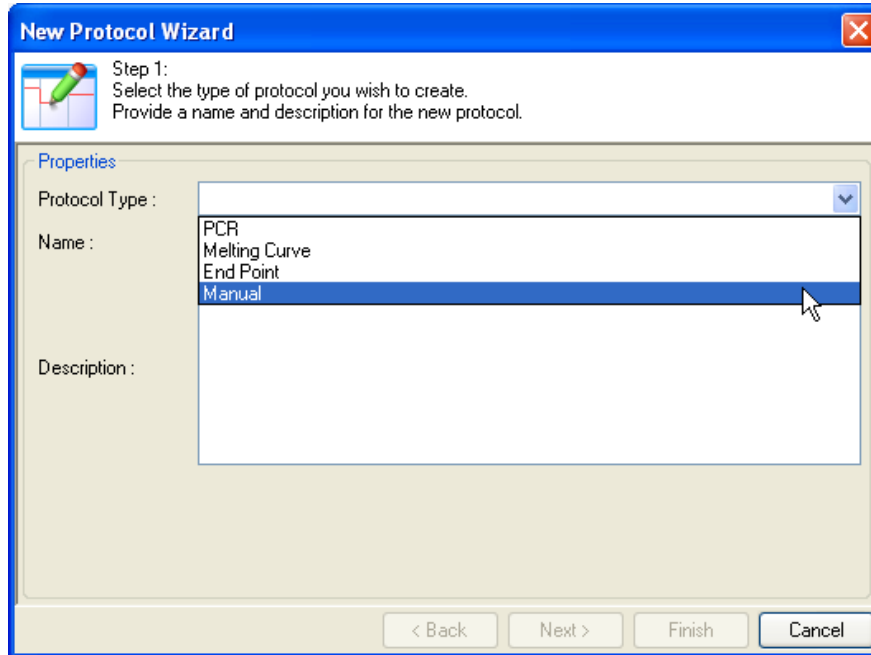
- 3 Adjust parameters appropriately.
- 4 Click **Accept**.

Using the Manual Type Protocol

Choosing Manual enables you to create a protocol by adding elements from the template like, segments, slices, and ramp rates as needed.

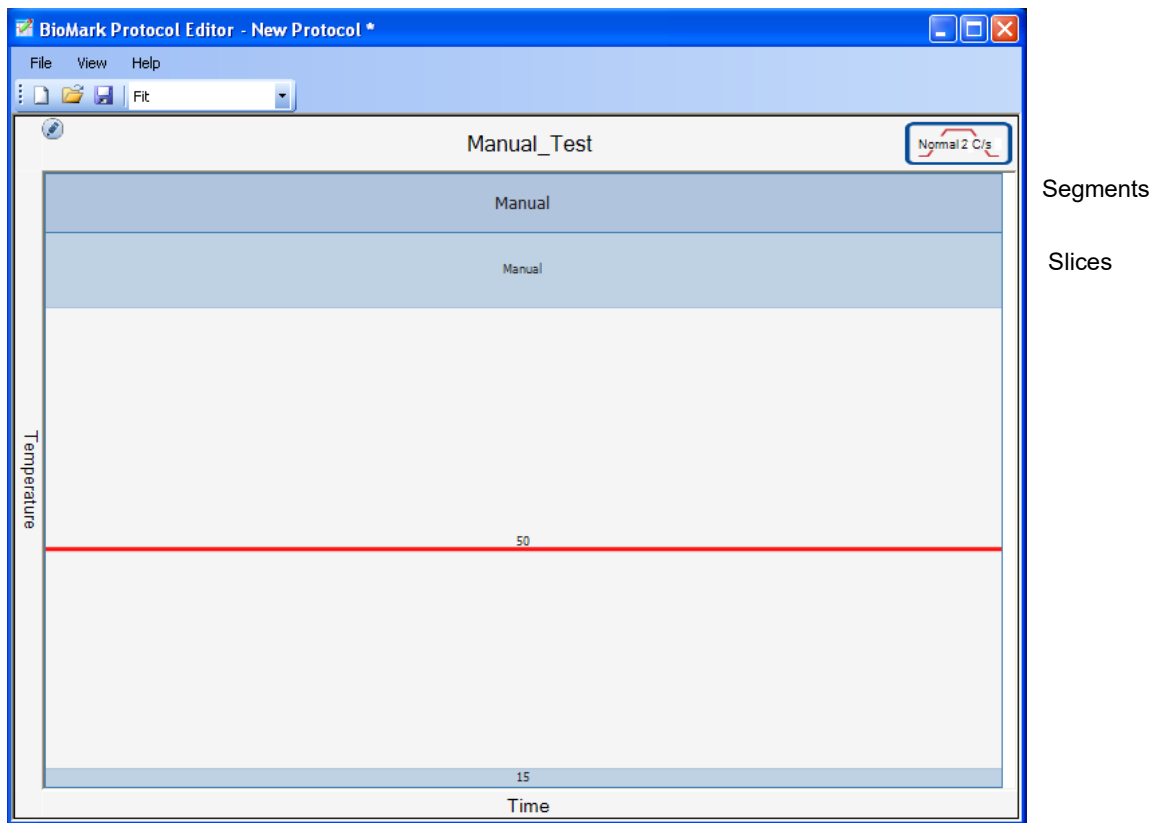
To use the Manual protocol type:

- 1 In the Protocol Editor, click **Protocol > New**.
- 2 Click **Manual** from the Protocol Type drop-down menu.



- 3 Name the new protocol.

- 4 Click **Finish** to open the blank protocol.



IMPORTANT: All protocols must have at least one segment and one slice.

- 1 Add segments by hovering your cursor in the segment to display edit tools.

Maintenance and Decontamination

A

This section describes how to clean and maintain your Biomark or EP1 system for optimal performance.

IMPORTANT: Before using a cleaning or decontamination method other than those recommended by Fluidigm, verify with Fluidigm technical support that the proposed method will not damage the equipment

Preventive Maintenance

This section describes how to clean and maintain your Biomark or EP1 system for optimal performance.

Preventive Maintenance Recommendations

For optimal performance of your Biomark or EP1 system, we recommend:

- Performing annual maintenance by a certified Fluidigm service technician.
- Only using replacement parts supplied by Fluidigm Corporation.

Clean the Biomark or EP1 System

Use a lint-free cloth and 70% ethanol or 70% isopropyl alcohol to gently wipe the top of the instrument tray and the instrument covers as needed.

IMPORTANT:

- Do not spray cleaning solution directly on the instrument, as it may penetrate the seams and cause damage. Spray cleaning solution on a lint-free cloth and then gently wipe instrument surface.
- Do not use bleach to clean the instrument as it is corrosive to metal.
- Before using a cleaning or decontamination method other than those recommended by Fluidigm, verify with Fluidigm technical support that the proposed method will not damage the equipment.

Decontamination of the Biomark or EP1 System

Ensure that the Biomark or EP1 system is cleaned and/or decontaminated prior to servicing the equipment, removing it from use, or transporting it for disposal. Refer to the instructions contained in this document and use only those materials specified.

Biological Agents

- 1 Using a soft cloth, apply 70% ethyl alcohol or 70% isopropyl alcohol to all accessible surfaces.
- 2 Keep surfaces wet for at least 5 minutes, then wipe dry.
- 3 Repeat steps 1 and 2 once.
- 4 Clean all decontaminated surfaces with a wet cloth to remove residual alcohol and wipe dry.

Hazardous Chemicals

- 1 Using a soft cloth, apply 70% ethyl alcohol or 70% isopropyl alcohol to all accessible surfaces.
- 2 Before use, ensure that alcohol is compatible with the chemicals used.
- 3 Keep surfaces wet for at least 5 minutes, then wipe dry.
- 4 Repeat steps 1 and 2 once.
- 5 Clean all decontaminated surfaces with a wet cloth to remove residual alcohol and wipe dry.

Radioactive Materials

- 1 Using a soft cloth, apply an industry standard radioactivity decontaminant to all accessible surfaces.
- 2 Wipe the surfaces as directed by the decontaminant manufacturer.
- 3 Survey the instrument with an appropriate radioactivity measuring device.
- 4 Ensure that the survey results are at or below background level.

Disposal of Products

Used IFCs should be handled and disposed of in accordance with federal, state, regional, and local laws for hazardous waste management and disposal.

Do not dispose of this product in unsorted municipal waste. This equipment may contain hazardous substances that could affect health and the environment. Use appropriate take-back systems when disposing of materials and equipment.

Learn more at fluidigm.com/compliance



Related Documents

B

This document is intended to be used in conjunction with these related documents:

- Biomark Site Requirements Guide (PN 69000082)
- EP1 Site Requirements Guide (PN 100-2195)
- *Fluidigm® Real-Time PCR User Guide* (PN 68000088)
- *Fluidigm® SNP Genotyping Analysis Software v3.0 User Guide* (PN 68000098)
- *Fluidigm® Digital PCR Analysis Software v3.0 User Guide* (PN 68000100)
- *Fluidigm® Melting Curve Analysis Software v3.0 User Guide* (PN 68000118)
- *Fluidigm IFC Controller Setup Quick Reference* (PN 68000117)
- *Fluidigm IFC Controller Usage Quick Reference* (PN 68000126)
- *Fluidigm Control Line Fluid Loading Procedure Quick Reference* (PN 68000132)
- *Gene Expression Analysis with the GE 24.192 IFC Using TaqMan Assays Protocol* (PN 101-7571)
- *Gene Expression Analysis with the GE 24.192 IFC Using TaqMan Assays Quick Reference* (PN 101-7916)
- *Fluidigm Flex Six™ IFC TaqMan Fast/Standard Gene Expression Workflow Quick Reference* (PN 100-7251)
- *Fluidigm 48.48 Real-Time PCR Workflow Quick Reference* (PN 68000089)
- *Fluidigm 48.48 Genotyping Workflow Quick Reference* (PN 68000099)
- *Fluidigm 48.48 SNPtype Genotyping Workflow Quick Reference* (PN 100-3910)
- *Fluidigm 96.96 Real-Time PCR Workflow Quick Reference* (PN 68000130)
- *Fluidigm 96.96 Genotyping Workflow Quick Reference* (PN 68000129)
- *Fluidigm 96.96 SNPtype Genotyping Workflow Quick Reference* (PN 100-3912)
- *Fluidigm 192.24 Genotyping Workflow Quick Reference* (PN 100-3184)
- *Fluidigm 192.24 SNPtype Genotyping Workflow Quick Reference* (PN 100-3913)
- *Fluidigm 192.24 Real-Time PCR Workflow Quick Reference* (PN 100-6170)
- *Fluidigm 192.24 Fast/Standard Delta Gene™ Gene Expression Workflow* (PN 100-7222)
- *Fluidigm 12.765 Digital PCR Workflow Quick Reference* (PN 68000096)
- *Fluidigm 48.770 Digital PCR Workflow Quick Reference* (PN 68000147)
- *Fluidigm qdPCR 37K™ IFC Workflow Quick Reference* (PN 100-6896)

IMPORTANT: For translations of the instrument safety information, see Safety Information for Genomics Instruments (PN 101-6810).

General Safety

In addition to your site-specific safety requirements, Fluidigm recommends the following general safety guidelines in all laboratory and manufacturing areas:

- Use personal protective equipment (PPE): safety glasses, fully enclosed shoes, lab coats, and gloves.
- Know the locations of all safety equipment (fire extinguishers, spill kits, eyewashes/showers, first-aid kits, safety data sheets, etc.), emergency exit locations, and emergency/injury reporting procedures.
- Do not eat, drink, or smoke in lab areas.
- Maintain clean work areas.
- Wash hands before leaving the lab.

Instrument Safety

The instrument should be serviced by authorized personnel only.



WARNING! Do not modify this instrument. Unauthorized modifications may create a safety hazard.



WARNING! BIOHAZARD. If you are putting biohazardous material on the instrument, use appropriate personal protective equipment and adhere to Biosafety in Microbiological and Biomedical Laboratories (BMBL), a publication from the Centers for Disease Control and Prevention, and to your lab's safety protocol to limit biohazard risks. If biohazardous materials are used, properly label the equipment as a biohazard. For more information, see the BMBL guidelines online at [cdc.gov/biosafety/publications/index.htm](https://www.cdc.gov/biosafety/publications/index.htm)



WARNING! PHYSICAL INJURY HAZARD. Do not attempt to lift or move any boxed or crated items unless you use proper lifting techniques. The crated Biomark System weighs approximately 249 kg (550 lb). The crated EP1 System weighs approximately 90 kg (198 lb). If you choose to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others. Use appropriate moving equipment and proper lifting techniques to minimize the chance of physical injury.

Symbols on the Instrument

The following table describes the hazard symbols that may be used in this document or on labels on the instrument.

Symbol	Description
	Hazard. Consult the user guide for further information.
	Biohazard.
	Electricity hazard. Indicates high electricity levels and a threat of electric shock from machines and/or equipment in the vicinity. You may suffer severe injuries or death.
	Lifting hazard.
	Power and standby symbol.
	Power switch is in the Off position.
	Power switch is in the On position.
	Protective conductor terminal (main ground). It must be connected to earth ground before any other electrical connections are made to the instrument.
	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. Contact customer service for information about responsible disposal options.

Electrical Safety

NOTE: The main power disconnect is on the rear panel of the instrument.



WARNING! ELECTRICAL HAZARD. DO NOT REMOVE THE COVERS. Electrical shock can result if the instrument is operated without its protective covers. No internal components are serviceable by the user.



WARNING! ELECTRICAL HAZARD. Plug the instrument into a properly grounded receptacle with adequate current capacity.

Chemical Safety

The responsible individuals must take the necessary precautions to ensure that the surrounding workplace is safe and that instrument operators are not exposed to hazardous levels of toxic substances. When working with any chemicals, refer to the applicable safety data sheets (SDSs) provided by the manufacturer or supplier.



For technical support visit
fluidigm.com/support.