MiniOpticon™ System

Instruction Manual

For MiniOpticon real-time PCR detection system with CFX Manager™ software

Catalog #CFB-3120

BIO-RAD

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Bio-Rad Resources

Bio-Rad provides many resources for scientists. Table 1 lists Bio-Rad resources and how to locate what you need.

Table 1. Bio-Rad resources

Resource	How to Contact
Local Bio-Rad Laboratories representatives	Find local information and contacts on the Bio-Rad website by selecting your country on the home page (**www.bio-rad.com**). Find the nearest international office listed on the back of this manual
Technical notes and literature	Go to the Bio-Rad website (**www.bio-rad.com**) or Gene Expression Gateway (**www.bio-rad.com/genomics/**). Type a search term in the Search box and select **Literature** to find links to technical notes, manuals, and other literature
Technical specialists	Bio-Rad's Technical Support department is staffed with experienced scientists to provide customers with practical and expert solutions To find local technical support on the phone, contact your nearest Bio-Rad office. For technical support in the United States and Canada, call 1-800-424-6723 (toll-free phone), and select the technical support option

Writing Conventions Used in this Manual

This manual explains how to safely set up and operate the MiniOpticon system. The manual uses the writing conventions listed in Table 2.

Table 2. Conventions used in this manual

Convention	Meaning
TIP:	Provides helpful information and instructions, including information explained in further detail elsewhere in this manual
NOTE:	Provides important information, including information explained in further detail elsewhere in this manual
WARNING!	Explains very important information about something that might damage the researcher, damage an instrument, or cause data loss
X > Y	Select X and then select Y from a toolbar, menu or software window

For information about safety labels used in this manual and on the MiniOpticon system, see, "Safety and Regulatory Compliance" on page iii.

Safety and Regulatory Compliance

For safe operation of the MiniOpticon system, we strongly recommend that you follow the safety specifications listed in this section and throughout this manual.

Safety Warning Labels

Warning labels posted on the instrument and in this manual warn you about sources of injury or harm. Refer to Table 3 to review the meaning of each safety warning label.

Table 3. Meaning of safety warning labels

	**CAUTION: Risk of danger!** This symbol identifies components that pose a risk of personal injury or damage to the instrument if improperly handled. Wherever this symbol appears, consult the manual for further information before proceeding
	**CAUTION: Hot surface!** This symbol identifies components that pose a risk of personal injury due to excessive heat if improperly handled

Instrument Safety Warnings

The warning labels shown in Table 4 also display on the instrument, and refer directly to the safe use of the MiniOpticon real-time PCR detection system.

Table 4. Instrument Safety Warning Labels

Icon	Meaning
	**Warning about risk of harm to body or equipment**. Operating the MiniOpticon real-time PCR detection system before reading this manual can constitute a personal injury hazard. For safe use, do not operate this instrument in any manner unspecified in this manual. Only qualified laboratory personnel trained in the safe use of electrical equipment should operate this instrument. Always handle all components of the system with care, and with clean, dry hands
	**Warning about risk of burning.** A thermal cycler generates enough heat to cause serious burns. Wear safety goggles or other eye protection at all times during operation. Always allow the sample block to return to idle temperature before opening the lid and removing samples. Always allow maximum clearance to avoid accidental skin burns
	**Warning about risk of explosion**. The sample blocks can become hot enough during the course of normal operation to cause liquids to boil and explode

Safe Use Specifications and Compliance

Table 5 lists the safe use specifications for the MiniOpticon system. Shielded cables (supplied) must be used with this unit to ensure compliance with the Class A FCC limits.

Table 5. Safe Use Specifications

Safe Use Requirements		Specifications
Temperature	Indoor use	Ambient temperature of 15—31°C. Relative humidity maximum of 80% noncondensing
Altitude		Up to 2,000 meters above sea level

Regulatory Compliance

This device complies with Part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) this device may not cause harmful interference, and (2) this device must accept any interference received, including interference that may cause undesirable operation.

This device has been tested and found to comply with the EMC standards for emissions and susceptibility established by the European Union at time of manufacture.

This digital apparatus does not exceed the Class A limits for radio noise emissions from digital apparatus set out in the Radio Interference Regulations of the Canadian Department of Communications.

LE PRESENT APPAREIL NUMERIQUE N'EMET PAS DE BRUITS RADIOELEC¬TRIQUES DEPASSANT LES LIMITES APPLICABLES AUX APPAREILS NUMERIQUES DE CLASS A PRESCRITES DANS LE REGLEMENT SUR LE BROUILLAGE RADIOELECTRIQUE EDICTE PAR LE MINISTERE DES COMMUNICATIONS DU CANADA.

This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

FCC Warning

> NOTE: Changes or modifications to this unit not expressly approved by the party responsible for compliance could void the user's authority to operate the equipment.

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.

Although this design of instrument has been tested and found to comply with Part 15, Subpart B of the FCC Rules for a Class A digital device, please note that this compliance is voluntary, for the instrument qualifies as an "Exempted device" under 47 CFR § 15.103(c), in regard to the cited FCC regulations in effect at the time of manufacture.

Table of Contents

1 System Installation

Read this chapter for information about setting up the MiniOpticon™ real-time PCR detection system:

System Overview

The MiniOpticon system uses an array of 48 light-emitting diodes (LEDs) to sequentially illuminate each of the 48 wells in the cycler block. The LEDs efficiently excite fluorescent dyes with absorption spectra in the 470–505 nm range. The MiniOpticon system uses two filtered photodiodes for fluorescence detection. The first channel is optimized to detect dyes with emission spectra in the 523–543 nm range, such as SYBR® Green I and FAM. The second channel is optimized for dyes with emission spectra of 540–700 nm. The MiniOpticon detector is calibrated at the factory and requires no further calibration before use.

The MiniOpticon system includes:

- **Optical tower.** This tower includes an optical system to collect fluorescent data

  NOTE: The serial number of the MiniOpticon system is located on a sticker on the back of the optical tower.

- **MJ Mini thermal cycler base.** The MiniOpticon system includes a thermal cycler block that rapidly heats and cools samples.

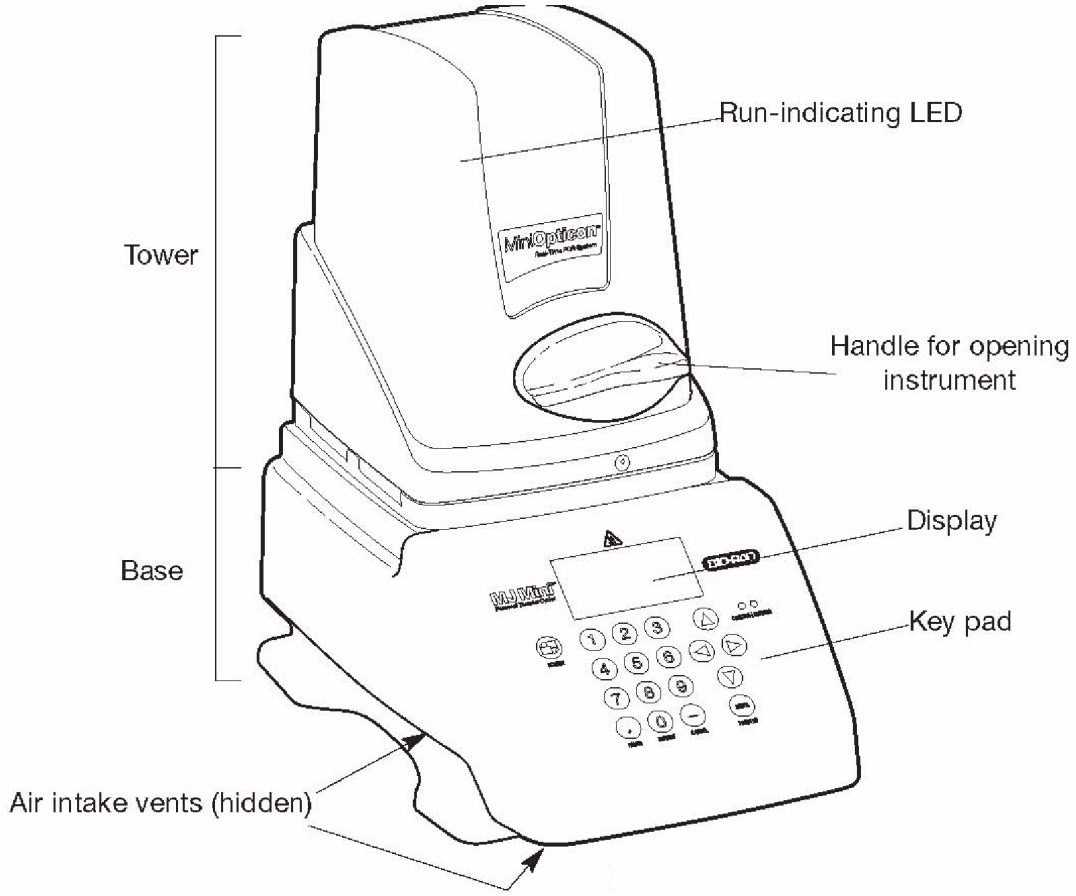


Figure 1. Front view of the MiniOpticon system.

When open, the MiniOpticon system includes these features:

- **Inner lid with heater plate.** The heater lid maintains temperature on the top of the reaction vessel to prevent sample evaporation. Avoid touching or otherwise contaminating the heater plate. Never poke anything through the holes, the apical system can be damaged.
- **Block.** Load samples in this block before the run

  WARNING! Prevent contamination of the instrument by spills, and never run a reaction with an open or leaking sample lid. For information about general cleaning and maintenance of the instrument, see “Instrument Maintenance” (page 112).

  WARNING! Avoid touching the inner lid or block: These surfaces can be hot.

The back panel of the MiniOpticon system includes these features (Figure 2):

- **Power switch.** Press the power switch to turn the power on
- **Power input.** Plug in the power cord here

- **USB connections.** Use these ports to connect the MiniOpticon system to a computer

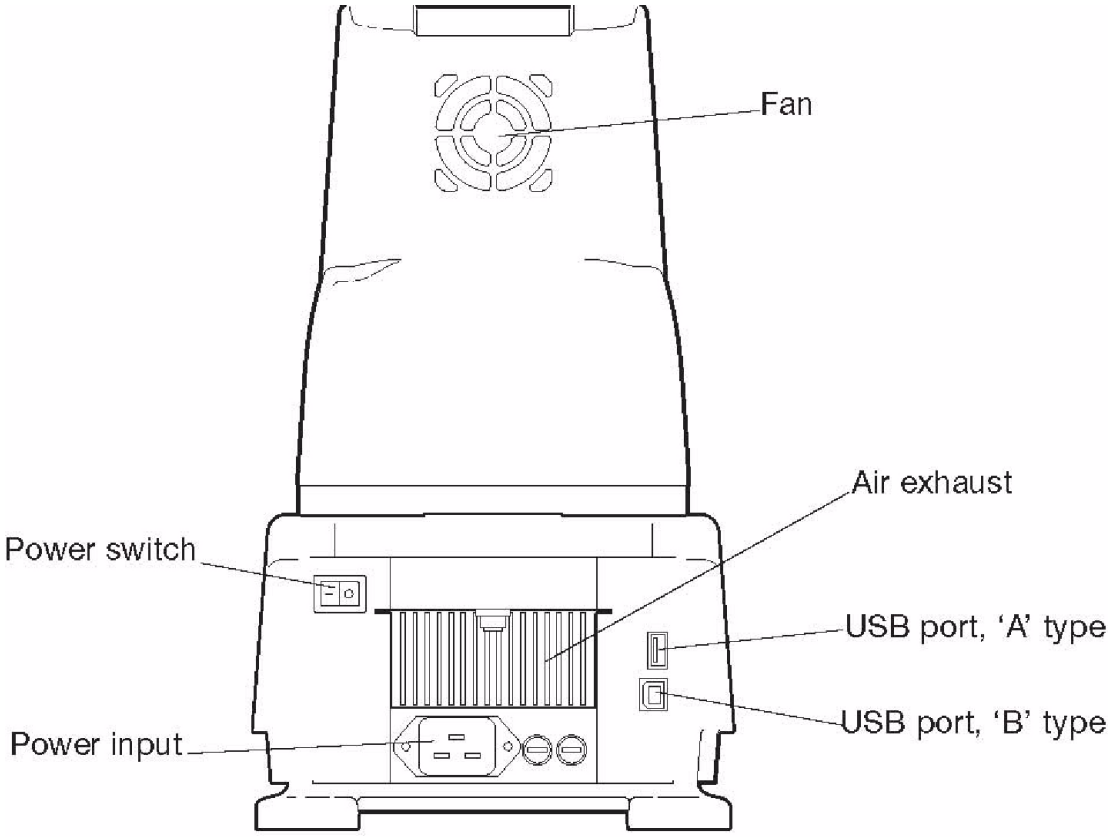


Figure 2. Back panel of MiniOpticon System.

WARNING! Avoid contact with the back panel during operation.

System Requirements

To operate the MiniOpticon system, use the following power sources and cables:

- **Input power.** 100—240 VAC, 50—60 Hz
- **Indoor use.** Ambient temperature of 15—31°C. Relative humidity maximum of 80% (non-condensing)
- **Air Supply.** The MiniOpticon system requires a constant supply of air that is 31°C or cooler in order to remove heat from the heat sink. Air is taken in from the lower vents located on the sides and front of the instrument and exhausted from the fan in the back. If the air supply is inadequate or too hot, the instrument can overheat, causing performance problems and even automatic shutdowns

  WARNING! Do not place the MiniOpticon system on a lab bench covered by bench paper. The bench paper can prohibit sufficient air circulation.
- **USB cable.** Control the MiniOpticon system using only the USB cable provided from Bio-Rad. This cable is sufficiently shielded to help prevent data loss

Setting Up the system

The MiniOpticon system should be installed on a clean, dry, level surface with sufficient cool airflow to provide adequate air supply to run properly. The MiniOpticon system requires a location with power outlets to accommodate the MiniOpticon system and the computer.

NOTE: Only one MiniOpticon system should be connected to a computer at one time.

Installing the MiniOpticon System

To install the MiniOpticon system:

1. Your MiniOpticon system shipment includes the components listed below. Remove all packing materials and store them for future use. If any items are missing or damaged, contact your local Bio-Rad office.
   - MiniOpticon system
   - USB cable
   - CFX Manager software installation CD
   - Instruction manual
   - CFX Manager™ software quick guides for protocol, plate, data analysis, and gene expression analysis
2. Firmly grasp the instrument from beneath to support the weight of the cycler and the optical tower. Carefully lift the instrument out of the shipping box.

   WARNING! Do not lift the instrument by the green handle.
3. Insert the power cord plug into its jack at the back of the instrument.
4. Plug the power cord into a standard 110 V or 220 V electrical outlet. The MiniOpticon system will accept 220 V automatically. Avoid plugging the MiniOpticon system into a power outlet that is already being used for other laboratory equipment

   NOTE: Turn the system on only after installing CFX Manager software. The power switch is on the back right-hand side of the MiniOpticon system.

Installing the CFX Manager Software

CFX Manager software is run on a PC computer with either the Windows XP or Windows Vista operating system and is required to run and analyze real-time PCR data from the MiniOpticon system. Table 6 lists the computer system requirements for the software on Windows XP and Windows Vista.

Table 6. Computer requirements for CFX Manager software

System	Minimum	Recommended
Operating system	Windows XP Professional Service Pack 2 and above or Windows Vista Home Premium and above	Windows XP Professional SP2 and above or Windows Vista Home Premium and above
Drive	CD-ROM drive	CD-RW drive
Hard drive	10 GB	20 GB
Processor speed	2.0 GHz	2.0 GHz
RAM	1 GB RAM (2 GB for Windows Vista)	2 GB RAM
Screen resolution	1024 x 768 with true-color mode	1280 x 1024 with true-color mode

Table 6. Computer requirements for CFX Manager software (continued)

System	Minimum	Recommended
USB	USB 2.0 Hi-Speed port	USB 2.0 Hi-Speed port
Internet browser	Internet Explorer	Internet Explorer
Software		Microsoft Office Suite

WARNING! Running a MiniOpticon system with CFX Manager software on a PC computer with a Windows 64-bit operating system is not supported due to incompatible USB drivers. A PC computer with a 64-bit processor (like Intel) on a 32-bit Windows operating system is supported.

WARNING! CFX Manager software can be installed on the same computer that already has Opticon Monitor version 3.1 installed. There may be conflicts controlling the instrument if both software packages are opened at the same time with the MiniOpticon turned on.

WARNING! If the computer with the CFX Manager software is running a virus scan program, make sure scans are performed when the MiniOpticon system is idle.

To install the CFX Manager software:

1. Log in to the computer with administrative privileges, the software must be installed on the computer by a user with administrative privileges.
2. Place the CFX Manager software CD in the computer's CD drive.
3. The software launch page should appear automatically. Double-click **Install Software** on the software launch page (Figure 3).

Figure 3. Software installation screen.

TIP: Click the **Documentation** button to find searchable PDF copies of instrument manuals and other documentation.

4. Accept the terms in the license agreement to continue.
5. Follow the instructions on the screen to complete the installation. When completed, the Bio-Rad CFX manager software icon will appear on the desktop of the computer.

6. If the launch page does not appear automatically, double-click on (**CD drive):\Bio-Rad CFX**, then open and follow instructions in the **Readme.txt** file.

   NOTE: For Windows Vista operating system, you will be prompted to install device software for **Jungo** during the CFX Manager software installation. Click **Install** to proceed. If prompted with the warning "Windows can't verify the published of this driver software," Click **Install this driver software anyway** to proceed.

Installing MiniOpticon System Drivers

The MiniOpticon system drivers must be installed on the computer in order to properly communicate with the device and perform real-time PCR experiments. The drivers are installed automatically during CFX Manager software installation for computers running Windows Vista operating system. Drivers must be installed manually for computers running Windows XP operating system.

NOTE: For Windows XP operating system, three drivers must be installed: Bio-Rad Thermal Cycler (EEPROM Empty), Bio-Rad Mini Optical Module and Bio-Rad Mini Cycler. The driver installation package provides instructions on how to install the drivers correctly.

To install the system drivers for Windows XP:

1. Connect the MiniOpticon system to the computer by plugging a USB cable (square end) into the USB 2.0 port located on the back of the MiniOpticon system, and then connecting the cable (flat end) into the USB 2.0 port located on the computer.
2. Turn the MiniOpticon system on by pressing the switch on the back of the system so that the side marked "I" is depressed.
3. Follow the instructions in the **Found New Hardware Wizard** that launches after the instrument is first detected by the computer.
4. On the first screen, select **Yes, this time only** to instruct the Windows operating system to connect to Windows Update to search for software (Figure 4). Click **Next**.

Figure 4. Found New Hardware Wizard.

5. Select **Install the software automatically** to install the Bio-Rad Thermal Cycler (EEPROM Empty) driver. Click **Next** (Figure 5).

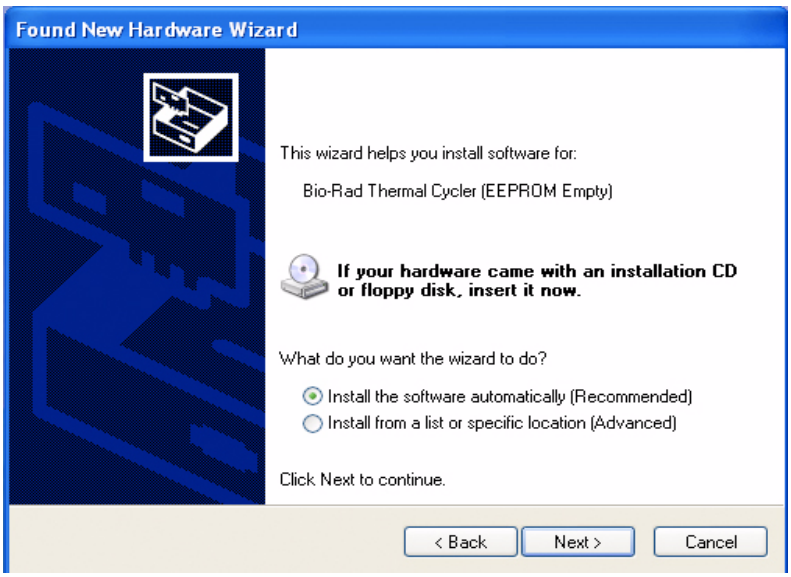


Figure 5. Software (Driver) installation screen.

6. A window will appear indicating the driver being installed has not passed Windows Logo testing to verify its compatibility with Windows XP. Click **Continue Anyway** to proceed.
7. Click **Finish** at the software installation completion screen when the driver is installed.

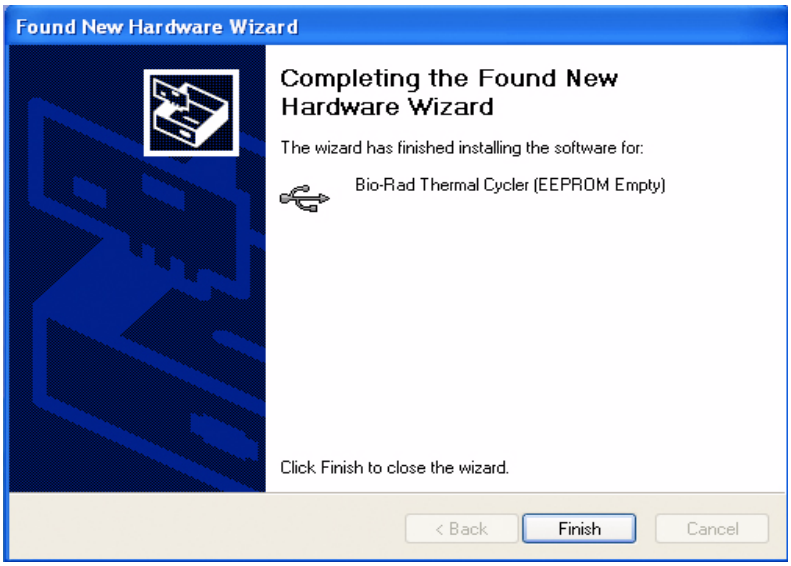


Figure 6. Finished Driver installation screen.

8. Repeat the driver installation for the Bio-Rad Mini Optical Module and the Bio-Rad MiniCycler drivers.

Running Experiments

Be sure that the MiniOpticon system is connected to the computer and turned on before launching the CFX Manager software. The green protocol-indicator light on the front of the MiniOpticon detector is illuminated only during a protocol run.

WARNING! Remove the shipping plate from the thermal cycler block to operate.

Loading the Block

1. To access the MiniOpticon system's block, turn the front green handle counter-clockwise until it snaps into the open position. Rotate the entire tower outward, to the left.
2. Place the 48-well, 0.2 ml microplate, or tube strips with sealed lids in the block. Check that the tubes are completely sealed to prevent leakage. For optimal results, load sample volumes of 15–30 µl.
3. To ensure uniform heating and cooling of samples, sample vessels must be in complete contact with the sample holder. Adequate contact is ensured by:
   - Verifying the sample holder is clean before loading samples
   - Firmly pressing tubes, or a 48-well microplate into the sample holder

   TIP: Spin down reactions in tubes or microplates before loading into the thermal cycler block. Air bubbles in samples, or liquid on the plate deck, can affect results.

- Bio-Rad strongly recommends that oil not be used to thermally couple sample vessels to the block

  NOTE: Do not open the MiniOpticon detector while the green protocol-indicator light is illuminated. Opening the door, particularly during a scan of the plate, may interrupt the software's control of the protocol.

4. To close the instrument, rotate the tower back into the closed position and then turn the green handle clockwise (Figure 1). Both the tower and the handle have spring mechanisms that facilitate closure.

   NOTE: For accurate data analysis, check that the orientation of reactions in the block is exactly the same as the orientation of the well contents in the software Plate tab (see "Plate Tab" on page 19). If needed, edit the well contents before, during, or after the run.

   WARNING! When running the MiniOpticon system, always balance the tube strips or cut microplates in the wells. For example, if you run one tube strip on the left side of the block, run an empty tube strip (with caps) on the right side of the block to balance the pressure applied by the heated lid.

   WARNING! Be sure that nothing is blocking the lid when it closes. Although there is a safety mechanism to prevent the lid from closing if it senses an obstruction, do not place anything in the way of the closing lid.

Recommended Plastic Consumables

Run only white-welled 48-well plates or white-welled strip tubes in the MiniOpticon system. For optimal results, Bio-Rad provides the following consumables for the MiniOpticon system (catalog numbers are provided in bold):

- **MLL-4851.** Multiplate low-profile 48-well unskirted PCR plates, white color wells
- **TLS-0851.** Low-profile 8-tube strips, 0.2 ml, without caps, white color wells
- **TCS-0803.** Optical flat 8-cap strips, for 0.2 ml tubes and plates, ultraclear

2 Introduction to CFX Manager Software

Read this chapter for information about getting started with CFX Manager software.

Main Software Window

For instructions about running the system, refer to one of the quick guides that ship with the system:

- Protocol quick guide
- Plate quick guide
- Data Analysis quick guide
- Gene Expression Analysis quick guide

  TIP: See the software Help for more guides about running experiments.

Get started in the main software window by using these features (Figure 7):

- **Status bar.** View the current software and instrument status (page 10)
- **Menu bar.** Select software commands to create or open files(page 10)
- **Toolbar buttons.** Open software files (page 12), the Startup Wizard (page 15), and the Experiment Setup window (page 17)
- **Detected Instruments pane.** View a list of attached instruments (page 13)

- **Startup Wizard window.** Access common software commands (page 14)

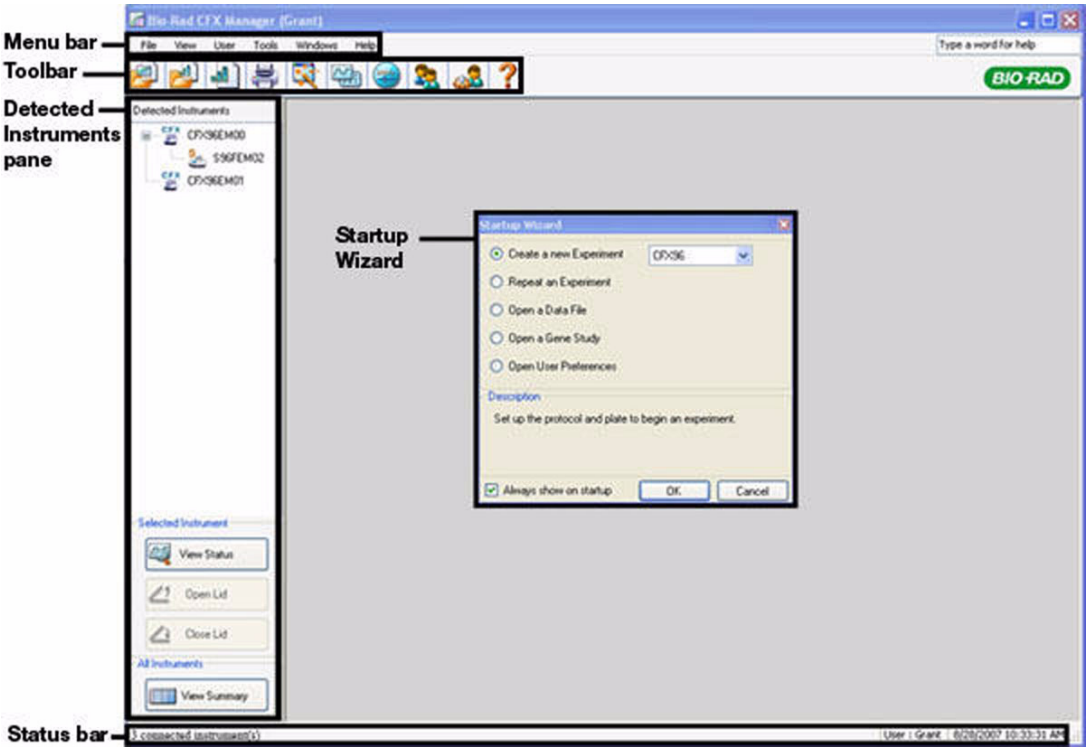


Figure 7. The main software window.

Status Bar

The status bar at the bottom of the main software window shows the status of the software. View the left side of the status bar (Figure 8) to see the current status of instruments.

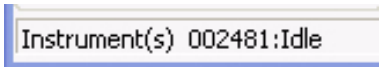


Figure 8. Left side of status bar in main software window.

View the right side of the status bar (Figure 9) to see the current user name, date, and time.

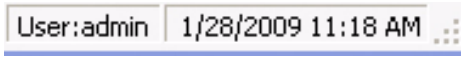


Figure 9. Right side of status bar in the main software window.

Click and drag the right corner of the status bar to resize the main window.

Menu Bar

The menu bar of the main software window provides the items listed in Figure 10.

Figure 10. Menu bar in the main software window.

Select the commands shown in the menu bar (Table 7).

Table 7. Menu bar items in the main software window

Menu Item	Command	Function
File	New	Create a new protocol, plate, experiment, or Gene Study
	Open	Open existing files, including protocol (.prcl), plate (.pltd), data (.pcrd), and Gene Study (.mgxd) files, and stand-alone run files (.zpcr)
	Recent Data Files	View a list of the ten most recently viewed data files, and select one to open in Data Analysis
	Repeat an Experiment	Open the Experiment Setup window with the protocol and plate from a completed run to quickly repeat the run
	Exit	Exit the software program
View	Application Log	Display the application log for the software
	Run Reports	Select a run report to review from a list
	Startup Wizard	Open the Startup Wizard
	Experiment Setup	Open the Experiment Setup window
	Instrument Summary	Open the Instrument Summary window
	Detected Instruments	Show or hide the Detected Instruments pane
	Toolbar	Show or hide the main software window toolbar
	Status Bar	Show or hide the main software window status bar
User	Select User	Open the Select User window to change software users
	Change Password	Change your user password
	User Preferences	Open the User Preferences window
	User Administration	Manage users in the User Administration window
Tools	Dye Calibration Wizard	Open the Dye Calibration window to calibrate an instrument for a new fluorophore
	Protocol AutoWriter	Open the Protocol AutoWriter window to create a new protocol
	Ta Calculator	Open the Ta Calculator window to calculate the annealing temperature of primers
	View Block Status Log	View a log of the thermal cycler block
	Application Data Folder	Open the Application Data folder to view software files
	User Data Folder	Open the Data folder to view protocol, plate, and data files
	Properties All Instruments	View properties of all detected instruments, including serial numbers
	Zip Data and Log Files	Choose and condense selected files in a zipped file for storage or to email
	Options	Configure software email settings

Table 7. Menu bar items in the main software window (continued)

Menu Item	Command	Function
Windows	Cascade	Arrange software windows on top of each other
	Tile Vertical	Arrange software windows from top to bottom
	Tile Horizontal	Arrange software windows from right to left
	Close All	Close all open software windows
Help	Contents	Open the software Help for more information about running PCR and real-time PCR
	Index	View the index in the software Help
	Search	Search the software Help
	Gene Expression Gateway Web site	Open a web site to find information about running PCR and real-time PCR experiments
	PCR Reagents Website	View a website that lists Bio-Rad consumables for PCR and real-time PCR reagents
	PCR Plastic Consumables Website	View a website that lists Bio-Rad consumables for PCR and real-time PCR experiments
	Software Updates	Check for software updates from Bio-Rad
	About	Open a window to see the software version

Toolbar Buttons

Click a button in the toolbar of the main software window (Table 8) for quick access to common software commands.

NOTE: To show or hide the toolbar, select **View > Toolbar** in the menu bar.

Table 8. Toolbar buttons in the main software window

Button	Button Name	Function
	Open a Data File	Open a browser window to locate a data file (*.pcrd extension) and open it in the Data Analysis window (page 49)
	Open a Gene Study	Open a browser window to locate a Gene Study file (.mgxd extension) and open it in the Gene Study window (page 79)
	Create a New Gene Study	Open the Gene Study window (page 79) to add files and create a new study
	Print	Print the current software window
	Startup Wizard	Open the Startup Wizard that links you to common software functions (page 14)

Table 8. Toolbar buttons in the main software window (continued)

Button	Button Name	Function
	Experiment Setup	Open the Experiment Setup window to run an experiment (page 17)
	Protocol AutoWriter	Open the Protocol AutoWriter window to create a new protocol (page 33)
	Select User	Open the Select User window to change software users (see “Logging in and Selecting a User” on page 101)
	User Preferences	Open the User Preferences window (page 101)
	Help	Open the software Help window for more information about running PCR and real-time PCR

Startup Wizard

The Startup Wizard automatically appears when CFX Manager software is first opened (Figure 11). If it is not shown, click the **Startup Wizard** button on the main window toolbar.

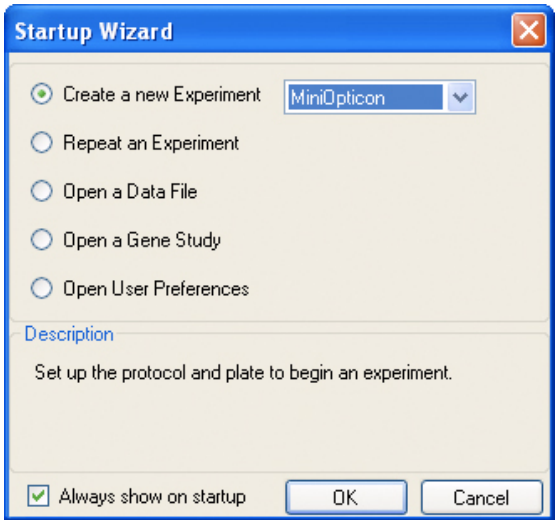


Figure 11. Startup Wizard window.

NOTE: Make sure you select MiniOpticon from the instrument pull-down menu.

Options in the Startup Wizard include the following:

- **Create a new Experiment (page 17).** Set up the protocol and plate to begin a new experiment.

  NOTE: Select the appropriate instrument in the pull down list to make sure the default plate settings match the instrument to be used for the experiment.
- **Repeat an Experiment.** Set up an experiment with the protocol and plate from a completed run. If needed, you can edit the experiment before the run
- **Open a Data File (page 49).** Open a data file to analyze results
- **Open a Gene Study (page 79).** Open a multi-file gene expression study to analyze results from multiple gene expression experiments
- **Open User Preferences (page 101).** Open the User Preferences window to customize software settings

Detected Instruments Pane

A connected MiniOpticon system will appear in the Detected Instruments pane, which shows each instrument as an icon named with the MiniOpticon serial number.

Figure 12 shows detected MiniOpticon system with serial number 002481.

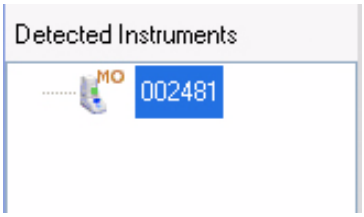


Figure 12. Instruments listed at the top of the Detected Instruments pane.

Right-click on the instrument icon to select one of these options:

- **View Status.** Open the Run Details window to check the status of the selected instrument block
- **Properties.** Open the Instrument Properties window
- **Collapse All.** Collapse the list of instruments in the Detected Instruments pane
- **Expand All.** Expand the list of instruments in the Detected Instruments pane

You can also control a block by clicking the **View Status** button in the Selected Instrument pane to launch the Run Details window (Figure 13).

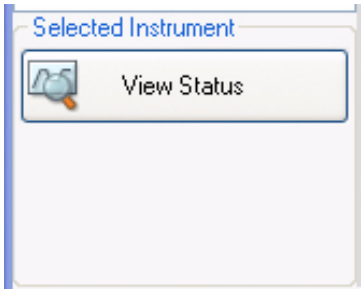


Figure 13. View Status button at the bottom of the Detected Instruments pane.

Instrument Properties Window

To open the Instrument Properties window to view information about an instrument, right-click on the instrument icon in the Detected Instruments pane (Figure 12). The window includes two tabs (Figure 14):

- **Properties.** View serial numbers of the MiniOpticon system
- **Calibrated Dyes.** View the list of calibrated fluorophores

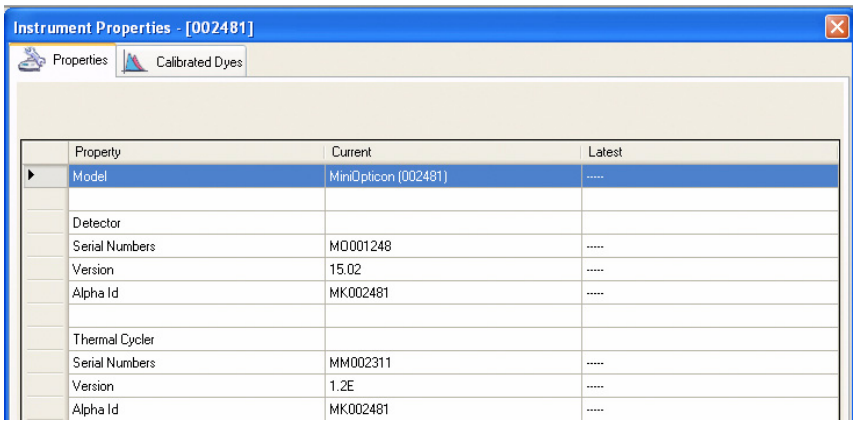


Figure 14. Instrument Properties window.

Properties Tab

The Properties tab displays important serial numbers for the connected instrument. The firmware versions are also displayed. The default name for an instrument is the MiniOpticon serial number, which appears in many locations in the software.

Calibrated Dyes Tab

Open the Calibrated Dyes tab to view the list of calibrated fluorophores and plates for the selected instrument. Click an **Info** button to see detailed information about a calibration.

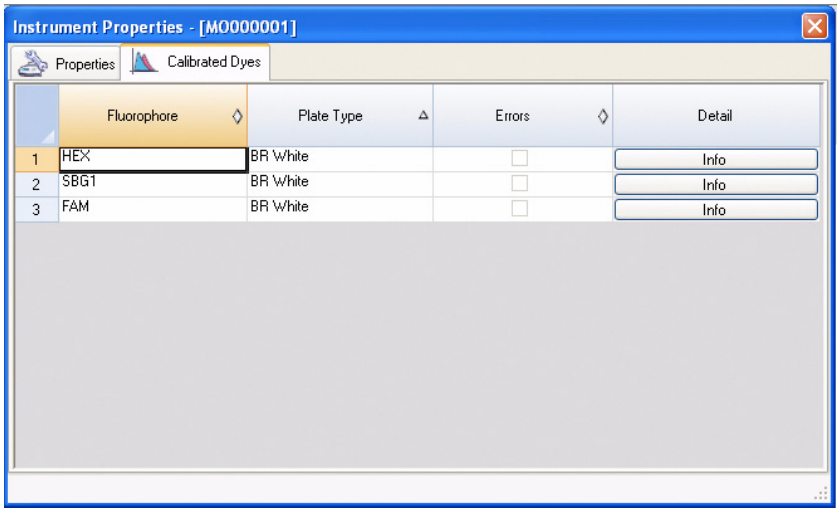


Figure 15. Calibrated Dyes tab in the Instrument Properties window.

Software Files

CFX Manager software stores information about experiments in specific files (Table 9):

Table 9. File types used with CFX Manager software

File Type	Extension	How to View and Edit File
Protocol	.prcl	Select in Experiment Setup and edit in Protocol Editor
Plate	.pltd	Select in Experiment Setup and edit in Plate Editor
Data	.pcrd	View and analyze in Data Analysis window
Gene Study	.mgxd	View and analyze in Gene Study window

Software Help Tools

The Help option of the CFX Manager software provides the following tools:

- Select the **Search** or **Index** tabs in this Help site to search for more information
- Open the **Glossary** to look up words that are specifically used in this software. For widely used words, consult a PCR dictionary or glossary
- Press **F1** on your keyboard to open software help about topics in many of the software windows
- Print any Help page by right-clicking on it and selecting **Print**

Tips and Tricks

Tips and tricks for using CFX Manager software are listed below.

- Open any Protocol, Plate, Data, or Gene Study file by dragging it from a folder to an open software window
- Print or export the information shown in many windows by right-clicking a chart, spreadsheet, or well selector
- Change the size of any window by clicking and dragging the edges
- Open the User Preferences window to choose default settings that activate every time you log in to the software
- Add data files to a Gene Study by dragging from a folder to an open Gene Study window
- Open multiple Data Analysis and Gene Study files at the same time
- Click the **Settings** or **Tools** menu to find advanced functions
- To add or delete files from the Express Load menu, add or delete the files (.prcl and .pltd extensions) in the ExpressLoad folder, select **Tools > User Data Folder** in the menu bar of the main software window
- To view all the information loaded into one well in a plate, double-click the well to open the Well Info window
- Right-click any graph or chart to change viewing and data analysis options
- Edit well contents before, during, or after the run
- Select a well group to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group pull-down menu in the toolbar

3 Running Experiments

Read this chapter for information about running experiments using CFX Manager software:

- Experiment Setup window (below)
- Protocol tab (page 18)
- Plate tab (page 19)
- Start Run tab (page 20)
- Run Details window (page 22)
- Instrument Summary Window (page 24)

Experiment Setup Window

The Experiment Setup window provides quick access to the files and settings needed to set up and run an experiment. To open the Experiment Setup window, follow one of these options:

- Click the **Create a New Experiment** option in the Startup Wizard (page 14)

  NOTE: Make sure you have MiniOpticon selected in the instrument pull down menu
- Click the **Experiment Setup** button in the main software toolbar (page 12)
- Select **File > New > Experiment** in the main software menu bar (page 10)

The Experiment Setup window includes three tabs:

- **Protocol.** Click the Protocol tab to select an existing protocol to run or edit, or to create a new protocol in the Protocol Editor window (page 27)
- **Plate.** Click the Plate tab to select an existing plate to run or edit, or to create a new plate in the Plate Editor window (page 37)
- **Start Run.** Click the Start Run tab (page 20) to check the run settings, select one or more instrument blocks, and begin the run

  NOTE: If the protocol currently selected in the Protocol tab does not include a step with a plate read for real-time PCR analysis, then the Plate tab is hidden. To view the Plate tab, add a “Plate Read” (page 30) in at least one step in the protocol.

  NOTE: Start a new experiment from a previous run by selecting **File > Repeat an Experiment** in the main software menu bar. Then select the data file (.pcrd) for the experiment you want to repeat.

The Experiment Setup window opens with the Protocol tab in front (Figure 16). To open another tab, click that tab or click **Prev** and **Next** at the bottom of the window.

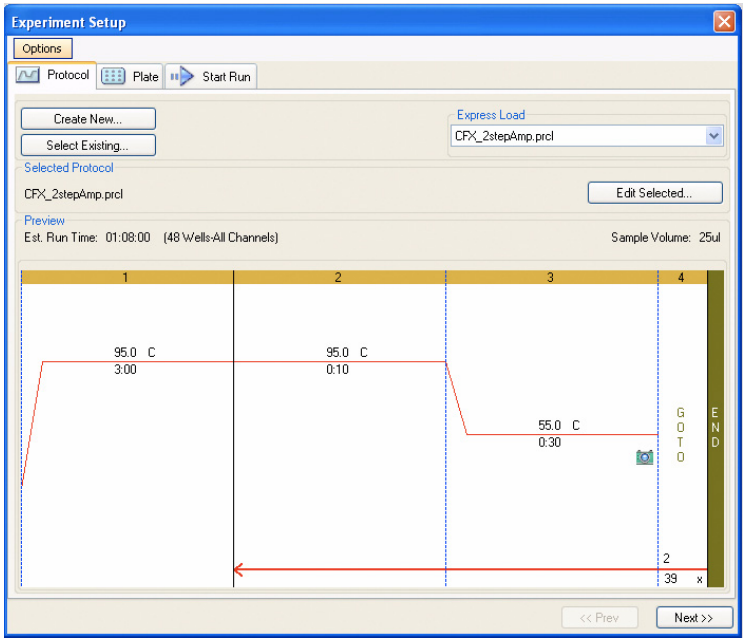


Figure 16. Experiment Setup window, including the Protocol, Plate, and Start Run tabs.

Protocol Tab

The Protocol tab shows a preview of the selected protocol file loaded in the Experiment Setup (Figure 16). A protocol file contains the instructions for the instrument temperature steps, as well as instrument options that control the ramp rate and lid temperature.

Select one of the following options to select an existing protocol, create a new protocol, or edit the currently selected protocol:

- **Create New.** Open the Protocol Editor to create a new protocol
- **Select Existing.** Open a browser window to select and load an existing protocol file (.prcl extension) into the Protocol tab
- **Express Load pull-down menu.** Quickly select a protocol to load it into the Protocol tab

  TIP: To add or delete protocols in the **Express Load** menu, add or delete files (.prcl extension) in the **ExpressLoad** folder. To locate this folder, select **Tools > User Data Folder** in the menu bar of the main software window
- **Edit Selected.** Open the currently selected protocol in the Protocol Editor

End Point Only Runs

To run a protocol that contains only an end point data acquisition step, select **Options > End Point Only Run** from Options in the menu bar of the Experiment Setup window. The default end point protocol, which includes two cycles of 60.0°C for 30 seconds, is loaded into the Protocol tab.

Figure 17 shows the default end point protocol.

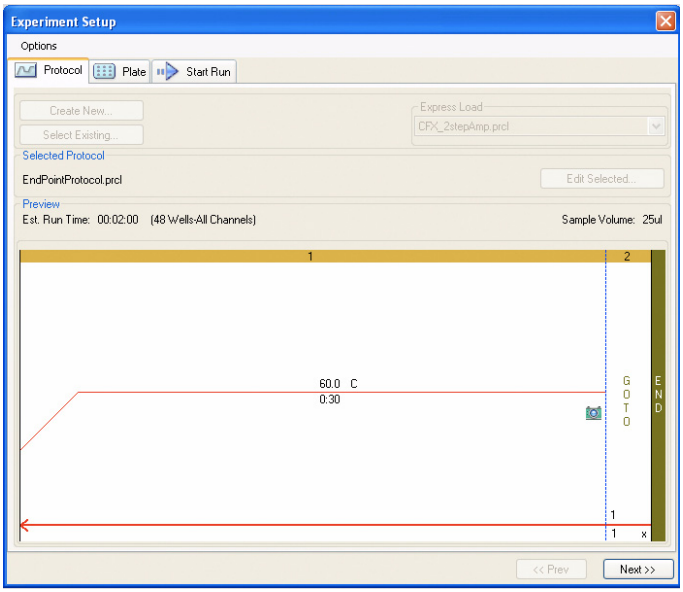


Figure 17. End Point Only protocol.

To change the step temperature or sample volume for the end point only run, click the **Start Run** tab and edit the **Step Temperature** or **Sample Volume**. Figure 18 shows the step temperature is changed to 55.0°C.

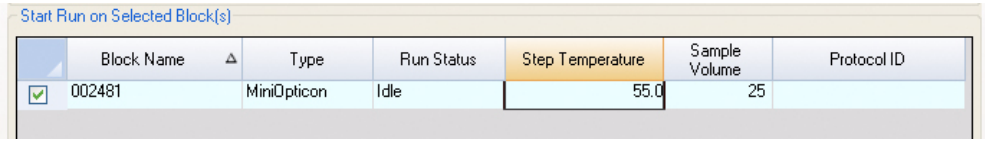


Figure 18. Change the sample volume on the Start Run tab.

Plate Tab

The Plate tab shows a preview of the selected plate file loaded in the Experiment Setup window (Figure 19). In a real-time PCR experiment, the plate file contains a description of the contents of each well, the scan mode, and the plate type. CFX Manager software uses these descriptions for data collection and analysis.

Select one of the following options to select an existing plate, create a new plate, or edit the currently selected plate:

- **Create New button.** Open the Plate Editor to create a new plate
- **Select Existing button.** Open a browser window to select and load an existing plate file (.pltd extension) into the Plate tab
- **Express Load pull-down menu.** Quickly select a plate to load it into the Plate tab

  TIP: To add or delete plates in the **Express Load** menu, add or delete files (.pltd extension) in the **ExpressLoad** folder. To locate this folder, select **Tools > User Data Folder** in the menu bar of the main software window.

- **Edit Selected button.** Open the currently selected plate in the Plate Editor

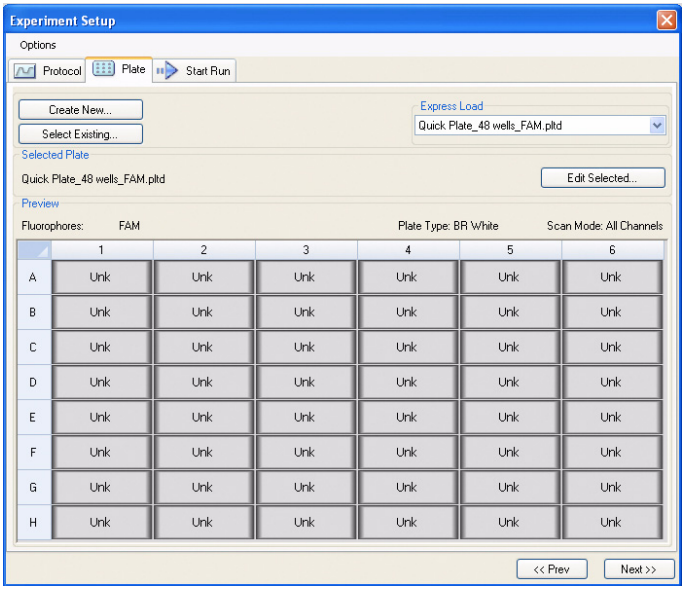


Figure 19. Plate tab window.

Start Run Tab

The Start Run tab (Figure 20) includes a section for checking information about a run that is going to be started, including the selected protocol and plate files, and a section for selecting the instrument block.

- **Run Information pane.** View the selected Protocol file, Plate file, and data acquisition Scan Mode setting. Enter optional notes about the experiment in the **Notes** box
- **Start Run on Selected Block(s) pane.** Edit run parameters (if necessary), and then click the **Start Run** button to begin the experiment

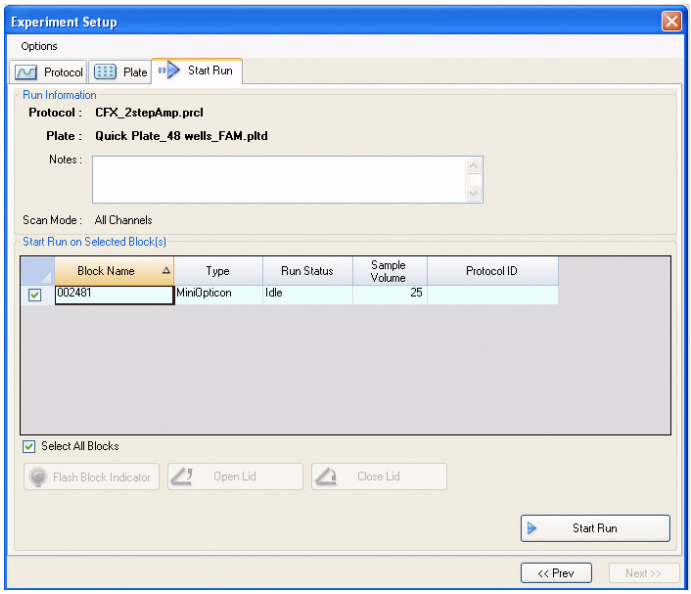


Figure 20. The Start Run tab.

By default, the **Start Run on Selected Block(s) pane** shows the block name, block type, and status of the instrument detected by the software. These descriptions cannot be edited.

NOTE: You can override the Sample Volume loaded in the Protocol file by selecting the volume in the spreadsheet cell and typing a new volume.

NOTE: A Protocol ID can be entered for each block by selecting the cell and typing an ID or by selecting the cell and scanning with a bar code reader.

To add or remove run parameters from the spreadsheet in the **Start Run on Selected Block(s)** pane, right-click on the list and select an option in the menu to display. Choose the value to change by clicking the text inside the cell to select it and then typing in the cell, or by selecting a new parameter from the pull-down menu. Editable parameters include:

- **Lid Temperature.** View the temperature of the lid. Override the lid temperature by selecting the text and typing a new temperature

Table 10 shows additional options on the right-click menu in the Start Run on Selected Block(s) pane:

Table 10. Start Run on Selected Block(s) right-click menu options

Right-Click Option	Function
Copy	Copy selected text
Copy as Image	Copy an image of the Start Run on Selected Block(s) pane
Print...	Print the current view of the list of selected blocks
Print Selection...	Print the column that is currently selected
Export to Excel...	Export the list of blocks to an Excel spreadsheet file
Export to Text...	Export the list of blocks to a text file
Find...	Find text in the list of blocks
Sort...	Sort up to three columns in the list of blocks

Run Details Window

When you click **Start Run**, CFX Manager software prompts you to save the name of the data file and then opens the Run Details window (Figure 21). Review the information in this window to monitor the progress of a run.

- **Run Status tab.** Check the current status of the protocol, open the lid, pause a run, add repeats, skip steps, or stop the run
- **Real-Time Status tab.** View the real-time PCR fluorescence data as they are collected

- **Time Status tab.** View a full-screen countdown timer for the protocol

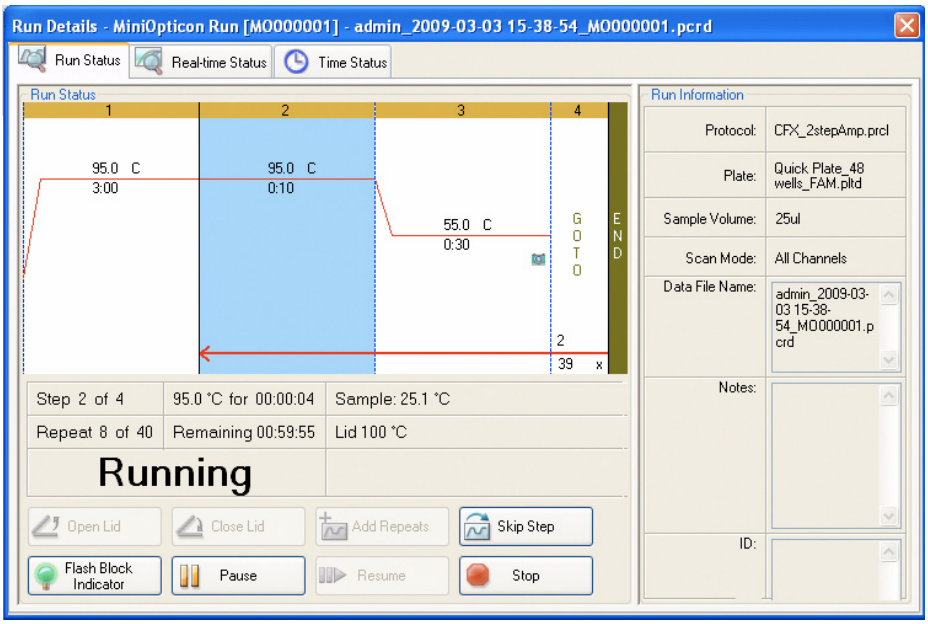


Figure 21. Run Details window.

Run Status Tab

The Run Status tab (Figure 21) shows the current status of a run in progress in the Run Details window and provides buttons to control the lid and change the run in progress.

- **Run Status pane.** Displays the current progress of the protocol, including the current step, current GOTO repeat, block temperature, remaining hold time for the current step, sample temperature, lid and shuttle temperature
- **Run Status buttons.** Click one of the buttons to remotely operate the instrument or to interrupt the current protocol
- **Run Information pane.** Displays experiment details

Run Status Tab Buttons

Click one of the buttons listed in Table 11 to operate the instrument from the software, or to change the run that is in progress.
NOTE: Changing the protocol during the run, such as adding repeats, does not change the protocol file associated with the run. These actions are recorded in the Run Log.

Table 11. Run Status buttons and their functions

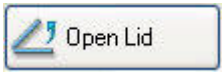
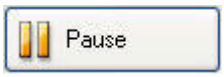
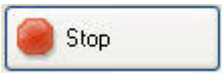
Button	Function
	This button is disabled for the MiniOpticon System
	This button is disabled for the MiniOpticon system

Table 11. Run Status buttons and their functions (continued)

Button	Function
	Add more repeats to the current GOTO step in the protocol. This button is only available when a GOTO step is running
	Skip the current step in the protocol. If you skip a GOTO step, the software verifies that you want to skip the entire GOTO loop and proceed to the next step in the protocol
	Flash the run indicating LED on the MiniOpticon
	Pause the protocol NOTE: This action is recorded in the Run Log.
	Resume a protocol that was paused
	Stop the run before the protocols ends, which may alter your data

Real-Time Status Tab

The Real-time Status tab (Figure 22) shows real-time PCR data collected at each cycle during the protocol after the first two plate reads. This tab also shows the well selector and text describing the protocol status at the bottom of the window.

TIP: Click **View/Edit Plate** to open the Plate Editor window. During the run, you can enter more information about the contents of each well in the plate.

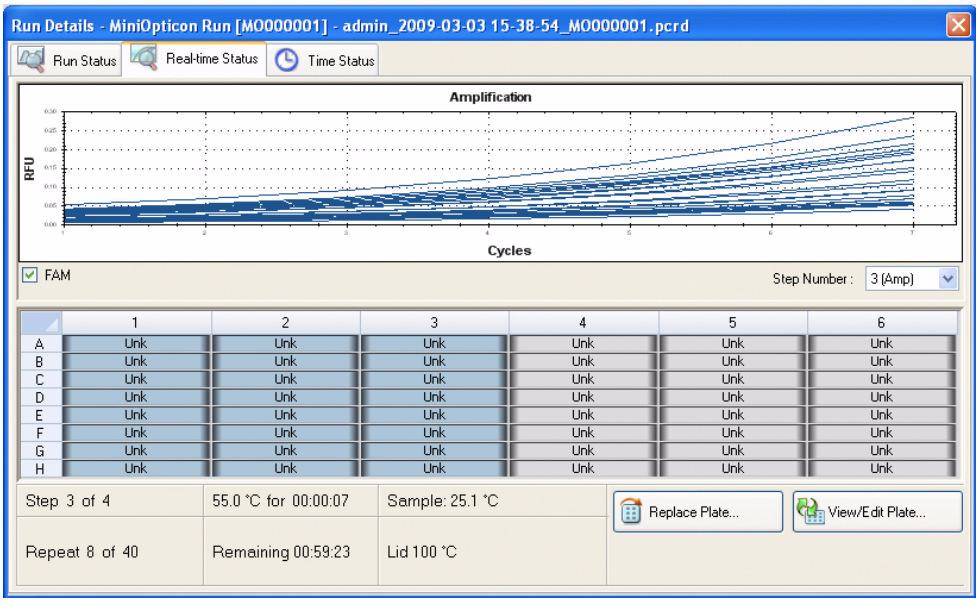


Figure 22. The Real-time Status tab displays the data during a run.

Replacing a Plate File

During a run, replace the plate file by clicking **Replace Plate** (Figure 22) in the Real-time Status tab. Select the new plate file (.pltd) from the list in the windows browser.

TIP: Replacing a plate file is especially useful if you start a run with a Quick Plate file in the Express Load folder.

Time Status Tab

The Time Status tab shows a countdown timer for the current run (Figure 23).

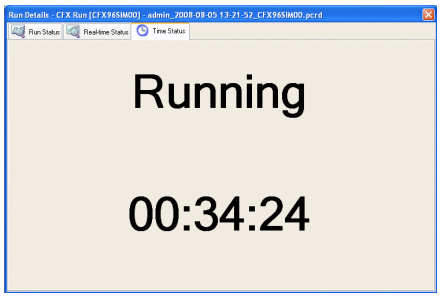


Figure 23. The Time Status tab displays a count-down timer for the current run.

Instrument Summary Window

The Instrument Summary window shows the status of a detected instrument. Open this window by selecting **View >Instrument Summary** on the menu bar. Click the buttons in the tool bar to change the status of each instrument.

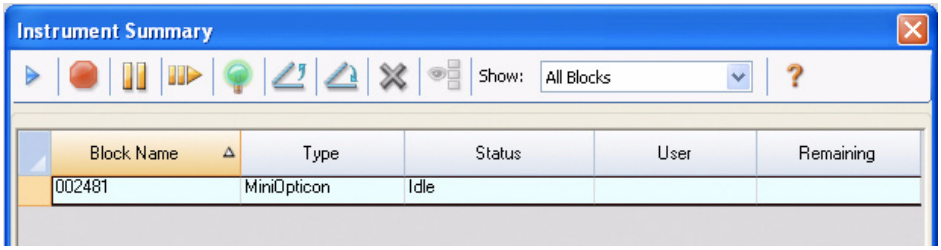


Figure 24. Instrument Summary window.

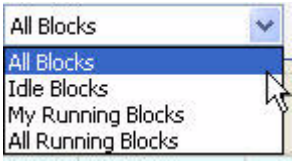
Instrument Summary Toolbar

The Instrument Summary toolbar includes buttons and functions listed in Table 12.

Table 12. Toolbar buttons in the Instrument Summary window

Button	Button Name	Function
	Set Up Experiment	Set up an experiment on the selected block by opening the Experiment Setup window
	Stop	Stop the current run on selected blocks

Table 12. Toolbar buttons in the Instrument Summary window (continued)

Button	Button Name	Function
	Pause	Pause the current run on selected blocks
	Resume	Resume the run on selected blocks
	Flash Block Indicator	Flash the run indicating LED on the MiniOpticon system
	Open Lid	This button is disabled for the MiniOpticon system
	Close Lid	This button is disabled for the MiniOpticon system
	Hide Selected Blocks	Hide the selected blocks in the Instrument Summary list
	Show All Blocks	Show the selected blocks in the Instrument Summary list
	Show	Select which blocks to show in the list. Select one of the options to show all detected blocks, all idle blocks, all the blocks that are running with the current user, or all running blocks

Right-Click Menu

Right-click in the Instrument Summary window to change the list of options that appear:

- **Block Name.** View the name of the block
- **Type.** View the size and type of the block
- **Status.** View the current status of the block
- **User.** View the current user who is logged in to the software
- **Remaining.** View the time remaining in the current run
- **Copy.** Copy the entire list
- **Copy as Image.** Copy the list as an image file
- **Print.** Print the list
- **Print Selection.** Print only the selected cells in the list
- **Export to Excel.** Export the list as an Excel formatted file
- **Export to Text.** Export the list as a text file
- **Find.** Find text in the list
- **Sort.** Sort the list by selecting up to three columns of data in the Sort window

4 Protocols

Read the following chapter for information about creating and editing protocol files:

- Protocol Editor window (below)
- Protocol Editor controls (page 29)
- Temperature control modes (page 33)
- Protocol AutoWriter (page 33)

Protocol Editor Window

A protocol instructs the instrument to control the temperature steps, lid temperature, and other instrument options. Open the Protocol Editor window to create a new protocol or to edit the protocol currently selected in the Protocol tab. Once a Protocol is created or edited in the Protocol Editor, click **OK** to load the protocol file into the Experiment Setup window and run it.

Opening the Protocol Editor

To open the Protocol Editor, follow one of these options:

- To create a new protocol, select **File > New > Protocol** or click **Create New** in the Protocol tab (page 18)
- To open an existing protocol, select **File > Open > Protocol**, or click **Open Existing** in the Protocol tab (page 18)
- To edit the current protocol in the Protocol tab, click **Edit Selected** in the Protocol tab (page 18)

  TIP: To change the default settings in the Protocol Editor window, enter the changes in the Protocol tab in the user Preferences window (page 104)

Protocol Editor Window Features

The Protocol Editor window (Figure 25) includes the following features:

- **Menu bar.** Select settings for the protocol
- **Toolbar.** Select options for editing the protocol
- **Protocol.** View the selected protocol in a graphic (top) and text (bottom) view. Click the temperature or dwell time in the graphic or text view of any step to enter a new value

- **Protocol Editor buttons.** Edit the protocol by clicking one of the buttons to the left of the text view

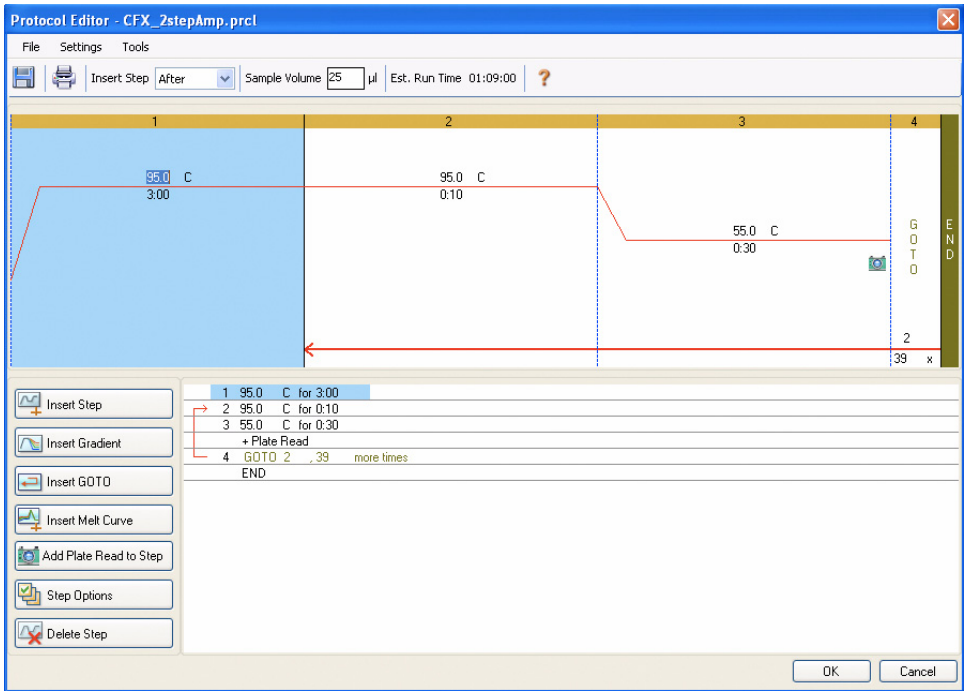


Figure 25. Protocol Editor window with buttons for editing protocols.

Protocol Editor Menu Bar

The menu bar in the Protocol Editor window provides the menu items listed in Table 13.

Table 13. Protocol Editor menu bar

Menu Item	Command	Function
File	Save	Save the current protocol
	Save As	Save the current protocol with a new name or in a new location
	Close	Close the Protocol Editor
Settings	Lid Settings	Open the Lid Settings window to change or set the Lid Temperature
Tools	Gradient Calculator	Select the block type for a gradient step. Choose 48 Wells for the MiniOpticon system
	Run-time Calculator	Select the instrument and scan mode to be used for calculating the estimated run time in the Experiment Setup window

The toolbar (Figure 26) in the Protocol Editor window provides quick access for important functions.

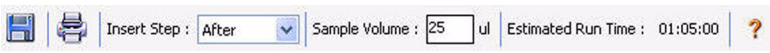


Figure 26. Protocol Editor toolbar.

Table 14 lists the function of the Protocol Editor toolbar buttons.

Table 14. Protocol Editor toolbar buttons

Toolbar Button and Menus	Name	Function
	Save	Save the current protocol file
	Print	Print the selected window
	Insert Step	Select **After** or **Before** to insert steps in a position relative to the currently highlighted step
	Sample Volume	Enter a sample volume in µl between 0 and 50. If you are using higher than 50 µl reactions, select 50 µl. Sample volume determines the Temperature Control mode (page 33). Enter zero (0) to select Block mode
	Run Time	View an estimated run time based on the protocol steps and ramp rate
	Help	Open the software Help for more information about protocols

Protocol Editor Controls

The Protocol Editor window includes buttons for editing the protocol. First, select and highlight a step in the protocol by left clicking it with the mouse pointer. Then click one of the Protocol Editor buttons at the bottom left side of the Protocol Editor window to change the protocol. The location for inserting a new step is determined by the status of the Insert Step box located in the toolbar.

Inserting a Step

To insert a temperature step before or after the currently selected step:

1. Click **Insert Step**.
2. Edit the temperature or hold time by clicking the default value in the graphic or text view, and entering a new value.

3. (Optional) Click **Step Options** to enter an increment or extend option to the step (page 32). Figure 27 shows the new step that was inserted after step 2.

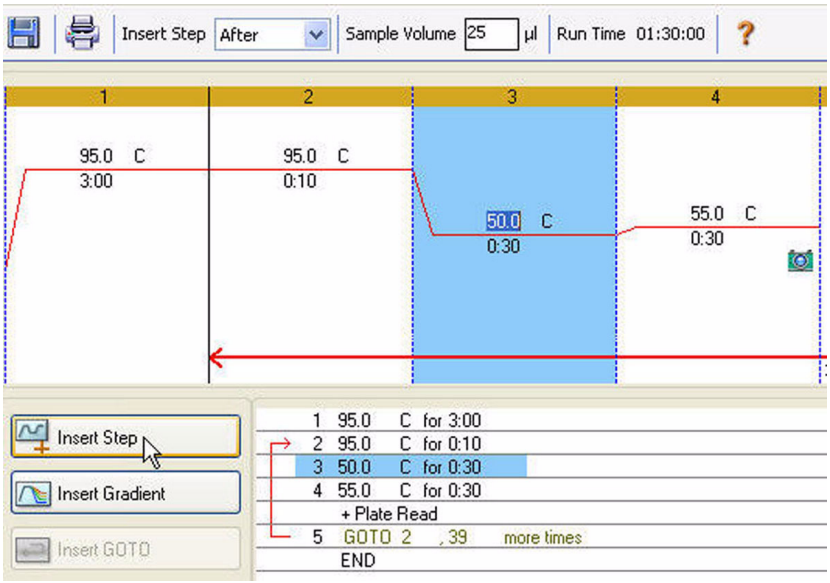


Figure 27. Protocol with inserted step.

Adding or Removing a Plate Read

To add a plate read to a step or to remove a plate read from a step:

1. Select the step by clicking the step in either the graphical or text view.
2. Click **Add Plate Read to Step** to add a plate read to the selected step. If the step already contains a plate read, the text on the button changes, so now the same button reads **Remove Plate Read**. Click to remove a plate read from the selected step.

In Figure 27, notice that the camera icon in the graphic view (top) shows that step 4 includes a plate read.

Inserting a Gradient

To insert a gradient step before or after the currently selected step:

1. Insert a temperature gradient step by clicking **Insert Gradient**.
2. Make sure the plate size for the gradient matches the block type of the instrument. Select the plate size for the gradient by selecting **Tools > Gradient Calculator** in the Protocol Editor menu bar.
3. Edit the gradient temperature range by clicking the default temperature in the graphic or text view, and entering a new temperature. Alternatively, click the **Step Options** button to enter the gradient range in the Step Options window (page 32).
4. Edit the hold time by clicking the default time in the graphic or text view, and entering a new time.

Figure 28 shows the inserted gradient step. The temperature of each row in the gradient is charted on the right side of the window.

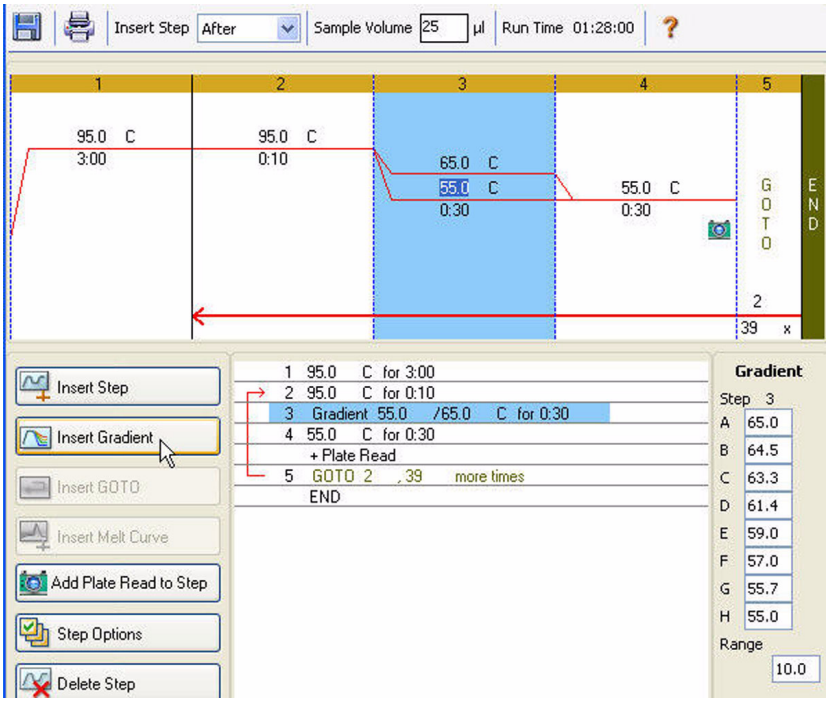


Figure 28. Protocol with inserted gradient step.

Inserting a GOTO Step

To insert a GOTO step before or after the selected step:

1. Click **Insert GOTO**.
2. Edit the GOTO step or number of GOTO repeats by clicking the default number in the graphic or text view, and entering a new value.

Figure 28 shows an inserted GOTO step at the end of the protocol. Notice that the GOTO loop includes steps 2 through 4.

Inserting a Melt Curve

To insert a melt curve step before or after the selected step:

1. Click **Insert Melt Curve**.
2. Edit the melt temperature range or increment time by clicking the default number in the graphic or text view, and entering a new value. Alternatively, click **Step Options** to enter the gradient range in the Step Options window (page 32).

   NOTE: You cannot insert a melt curve step inside a GOTO loop.

   NOTE: The melt curve step includes a 30-second hold at the beginning of the step that is not shown in the protocol.

Figure 29 shows a melt curve step added after step 6.

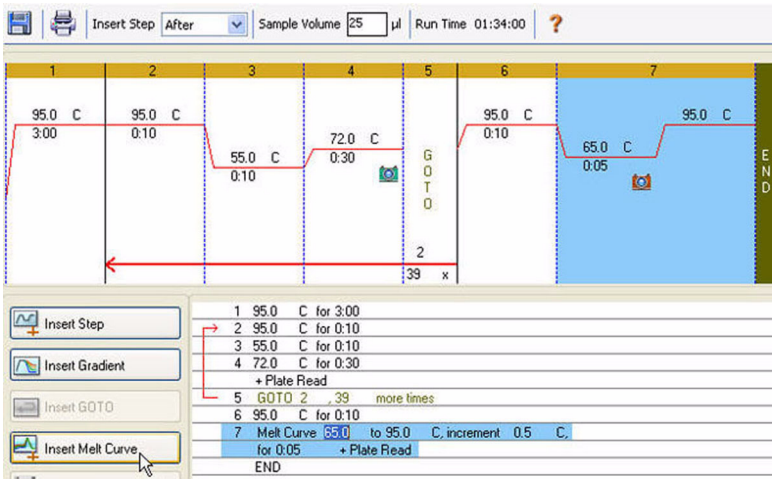


Figure 29. Protocol with inserted melt curve step.

Changing Step Options

To change a step option for the selected step:

1. Click on a step in the graphic or text view.
2. Click **Step Options** to open the Step Options window.
3. Add or remove options by entering a number, editing a number, or clicking a check box.
   TIP: To hold a step forever (an infinite hold), enter zero (0.00) for the time.

Figure 30 shows the selected step with a gradient of 10°C. Notice that some options are not available in a gradient step. A gradient step cannot include an increment or ramp rate change.

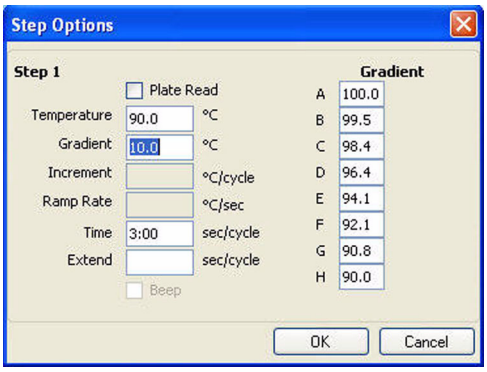


Figure 30. Step option for a gradient.

NOTE: A gradient runs with the lowest temperature in the front of the block (row H) and the highest temperature in the back of the block (row A).

The **Step Options** window lists the following options you can add or remove from steps:

- **Plate Read**. Check the box to include a plate read
- **Temperature**. Enter a target temperature for the selected step
- **Gradient.** Enter a gradient range for the step

- **Increment.** Enter a temperature to increment the selected step; the increment amount is added to the target temperature with each cycle
- **Ramp Rate.** Enter a rate for the selected step; the range depends on the block size
- **Time.** Enter a hold time for the selected step
- **Extend.** Enter a time to extend the selected step. The extend amount is added to the hold time with each cycle
- **Beep.** Check the box to include a beep at the end of the step

  TIP: When you enter a number that is outside the option range, the software changes the number to the closest entry within the range.

Deleting a Step

To delete a step in the protocol:

1. Select a step in the graphic or text view.
2. Click **Delete Step** to delete the selected step.

   WARNING! You cannot undo this function.

Temperature Control Modes

The instrument uses one of two temperature control modes to determine when the sample reaches the target temperature in a protocol.

TIP: The sample volume can be changed before a run by editing the Sample Volume parameter in the Start Run tab (see “Start Run Tab” on page 20).

Enter a sample volume in the protocol editor to select a temperature control mode:

- **Calculated mode.** When you enter a sample volume between 1 and 50 µl the thermal cycler calculates the sample temperature based on the sample volume. This is the standard mode
- **Block mode.** When you enter a sample volume of zero (0) µl, the thermal cycler records the sample temperature as the same as the measured block temperature

Protocol AutoWriter

Open the Protocol AutoWriter to quickly write protocols for PCR and real-time PCR experiments. To open the Protocol AutoWriter, select one of these options:

- Click the **Protocol AutoWriter** button in the main software window toolbar
- Select **Tools > Protocol AutoWriter** from the menu bar in the main software window

Figure 31 shows a protocol (bottom of window) written by the Protocol AutoWriter.

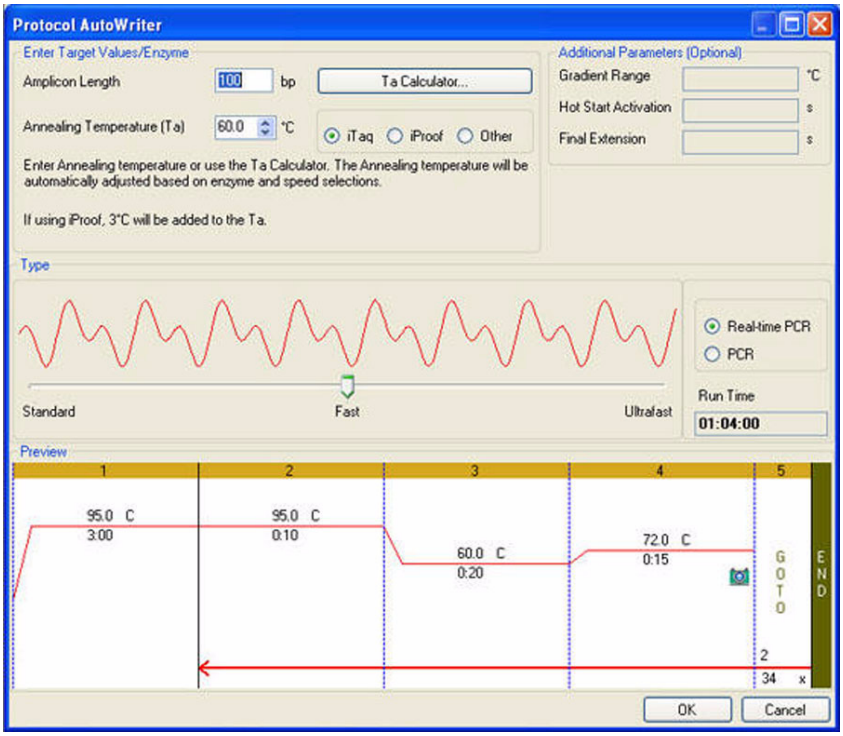


Figure 31. Protocol AutoWriter window with a new protocol.

Overview of the Protocol AutoWriter

The Protocol AutoWriter window uses information about your reaction to automatically generate a protocol file. Enter the following information about your PCR experiment:

- **Annealing Temperature (Ta) or primer sequence.** Enter the annealing temperature for the primers. If the annealing temperature is unknown, click **Ta Calculator** to enter the primer sequence so the Protocol AutoWriter calculates this value
- **Amplicon Length (bp).** Enter the expected length of the PCR product
- **Enzyme.** Select the DNA polymerase enzyme (**iTaq**, **iProof**, or **Other**) and enter additional information including hot start activation time, and final extension time
- **Run time and type.** Enter a speed (**Standard, Fast, Ultrafast**) to adjust the total run time, and select the type of PCR (Real-time PCR or PCR)

The run time for any protocol is influenced by the number of steps and cycles, the incubation time at each step, and the time it takes to reach uniformity at the target temperature. To reduce the overall run time, the Protocol AutoWriter makes one or more of the following changes:

- Reduces the total number of protocol steps
- Reduces the number of GOTO repeats
- Minimizes the hold time in each temperature step
- Minimizes the ramp time between steps by reducing the temperature change from one step to the next

For example, a typical PCR protocol includes the following three sets of steps with a total run time of 1.5 to 2.0 hours:

1. Initial template denaturation and enzyme activation (95°C for 3-10 minutes).

2. Cycles of three temperature steps (30 to 40 cycles): Denaturation of template (94-95°C for 15-30 seconds), annealing of primers (anneal for 15-30 seconds), and extension of product (72°C for 15-60 seconds).
3. Final extension (72°C for 10 minutes).

The Protocol AutoWriter might make these modifications to shorten a typical protocol:

- Change the initial template denaturation and enzyme activation step from 95°C for 3 minutes to 98°C for 30 seconds
- Change the denaturation step in each cycle from 95°C for 30 seconds to 92°C for 1 second
- Combine the annealing and extension steps into a single step at 70°C for 20 seconds

  NOTE: Combining the annealing and extension steps imposes limits on the melting temperature of the primers. If the melting temperatures of the primers do not fall within the specified range, adjust the primers. For example, shorten the primers by 2 to 3 bases (bp), or redesign them to adjust the melting temperature.

Creating a Protocol With the Protocol AutoWriter

Follow these steps to create a new protocol using the Protocol AutoWriter:

1. Click **Protocol AutoWriter** on the toolbar to open the Protocol AutoWriter window.
2. Enter the **Annealing Temperature (Ta)** and **Amplicon Length** in the boxes within the **Enter Target Values/Enzymes** pane. If you do not know the annealing temperature for primers, click **Ta Calculator** to enter the primer sequences and calculate the annealing temperature. For information about the calculations used in the Ta Calculator see Breslauer et al. 1986.
3. Select an enzyme type from the list of options (**iTaq, iProof, or Other**).
4. Add parameters in the **Additional Parameters (Optional)** pane if you want to add a Gradient Range, Hot Start Activation temperature, or Final Extension time in the protocol.
5. Select a protocol speed (**Standard, Fast, or Ultrafast**) by moving the sliding bar in the **Type** pane. When you move the sliding bar, the software adjusts the total run time. Select **Real-time PCR** to tell the software to collect fluorescence data.
6. Review the protocol and total run time in the Preview pane. Make changes as needed.

   TIP: Enter the lid temperature and sample volume before each run by editing the parameters in the Start Run tab (see “Start Run Tab” on page 20).
7. Click **OK** to save the new protocol, or click **Cancel** to close the window without saving the protocol.

   TIP: To edit a protocol written with the Protocol AutoWriter, open the protocol file (.prcl extension) in the Protocol Editor window (page 27).

   NOTE: Bio-Rad Laboratories does not guarantee that running a protocol written in the Protocol AutoWriter window will always result in a PCR product.

5 Plates

Read this chapter for information about creating and editing plate files:

- Plate Editor window (next section)
- Plate size and type (page 40)
- Select Fluorophores window (page 41)
- Well loading controls (page 42)
- Well Groups Manager window (page 46)

Plate Editor Window

A plate file contains run parameters such fluorophores and well contents, and instructs the instrument about how to analyze the data. Open the Plate Editor window to create a new plate or to edit the plate currently selected in the Plate tab. Once a plate file is created or edited in the Plate Editor, click **OK** to load the plate file into the Experiment Setup window and run it.

To run a real-time PCR experiment, you must load the minimal required information in the Plate Editor: at least one well must contain a loaded sample type and fluorophore.

> TIP: You can change well contents before, during, and after running the experiment. However, the scan mode and plate size cannot be changed during or after the run.

Opening the Plate Editor

To open the Plate Editor window (Figure 32), follow one of these options:

- To create a new plate, select **File > New > Plate** or click the **Create New** button in the Plate tab (page 19)
- To open an existing plate, select **File > Open > Plate**, or click the **Open Existing** button in the Plate tab (page 19)
- To edit the current plate in the Plate tab, click the **Edit Selected** button in the Plate tab (page 19)
- To open the plate associated with a data file, in the Data Analysis window (page 49), click **View/Edit Plate** on the toolbar

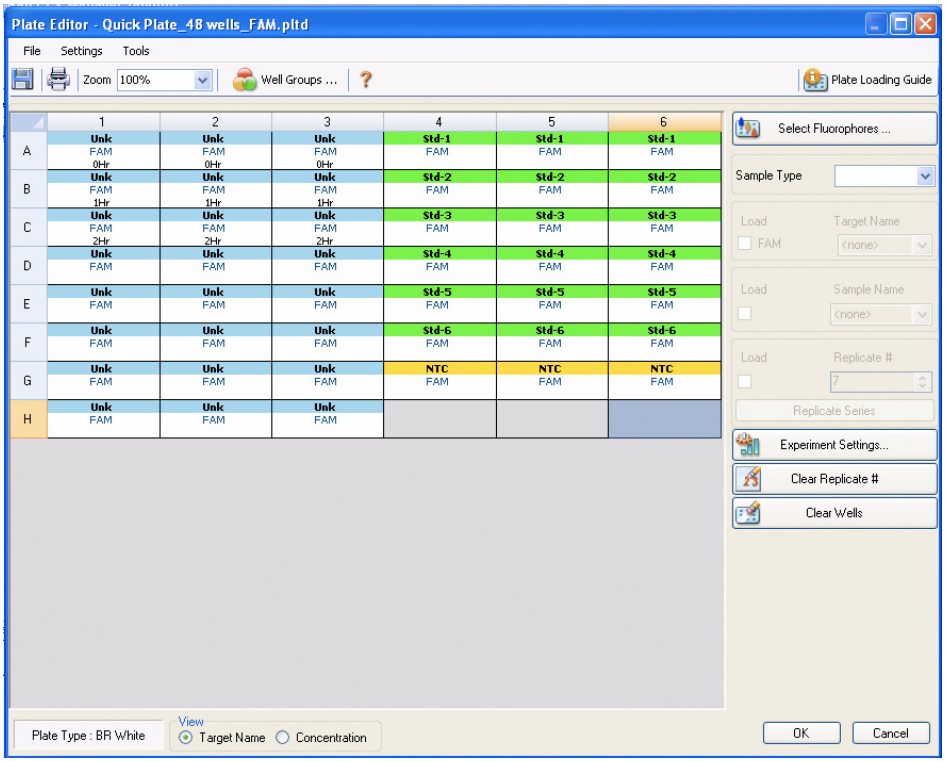


Figure 32. Plate Editor window.

Plate Editor Window Features

The Plate Editor window includes the following features:

- **Menu bar.** Select settings for the plate size, plate type, number conventions, and units (page 38)
- **Toolbar.** Select settings for Well Groups (page 46) or open the Plate Loading Guide window for a quick overview of instructions to load a plate.
- **Plate view.** View the current well contents. Load wells by using the plate loading options on the right side of the plate view (Figure 32)
- **Well loading controls.** Choose the contents to load in the wells (page 42) from the controls on the right side of the plate view

The Plate Editor example shows some loaded wells with unknowns (**Unk**) and standards (**Std**) (Figure 32). The bottom of the plate lists the plate type (**BR White**) and a selector to view **Target Name** or **Concentration** when wells are loaded with a standard sample type.

Plate Editor Menu Bar

The menu bar in the Plate Editor window provides the menu items shown in Table 15.

Table 15. Menu bar items in the Plate Editor

Menu Item	Command	Function
File	Save	Save the plate files

Table 15. Menu bar items in the Plate Editor (continued)

Menu Item	Command	Function
	Save As	Save the plate file with a new file name
	Exit	Exit the Plate Editor
Settings	Plate Size	Select a plate size that reflects the number of wells in the instrument block. Choose **48 Wells** for the MiniOpticon system NOTE: Plate Size must be the same as the block size in the instrument on which the experiment will be run
	Plate Type	Choose the type of wells in the plate that holds your samples. The MiniOpticon system is only factory calibrated for BR White plates. For accurate data analysis, the plate type must be the same as the plate well type used in the experiment NOTE: You must calibrate new plate types (page 111).
	Number Convention	Select or cancel the selection for Scientific Notation
	Units	Select the units to show in the spreadsheets when performing quantitation of unknowns versus a standard curve. Select copy number, fold dilution, micromoles, nanomoles, picomoles, femtomoles, attomoles, milligrams, micrograms, nanograms, picograms, femtomoles, attograms, or percent
Tools	Show Spreadsheet View	Show the plate information in a spreadsheet view for export or printing
	Plate Loading Guide	Show a quick guide about how to set up a plate and load the wells
	Show Well Notes	Select to show this pane in the well loading controls. Enter notes about one or more wells
	Show Collection Name	Select to show this pane in the well loading controls. Select to enter collection names for one or more wells
Help	Help Contents	Open the Help for more information about plates

Plate Editor Toolbar

The toolbar in the Plate Editor provides quick access to important plate loading functions.

Figure 33. Plate Editor toolbar.

TIP: To change the default settings in the Plate Editor window, open the User Preferences window and enter the changes in the Plate tab.

Table 16 lists the functions available in the Plate Editor toolbar.

Table 16. Toolbar items in the Plate Editor

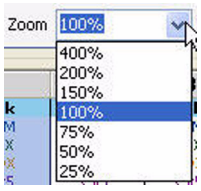
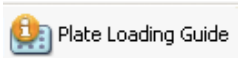
Toolbar Item	Name	Function
	Save	Save the current plate file
	Print	Print the selected window
	Zoom	Increase or decrease magnification in plate view
	Well Groups	Open the Well Groups Manager window and set up well groups for the current plate
	Help	Open the software Help for information about plates
	Plate Loading Guide	Show a quick guide about how to set up a plate and load the wells

Plate Loading Guide

Open the Plate Loading Guide window for information on how to load wells (Figure 34).

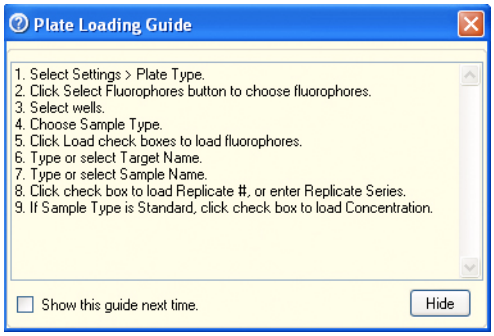


Figure 34. Plate Loading Guide window.

Plate Size and Type

The software applies these plate settings to all the wells during the experiment:

- **Plate Size.** Select a plate size that represents the size of the reaction module block of your instrument. Choose **MiniOpticon** from the pull-down menu option on the Startup Wizard to change the default plate size loaded in the Plate tab of the Experiment Settings

window. In the Plate Editor, select the plate size from the Settings menu (see Table 15). Plate size cannot be changed during or after the experiment

- **Plate Type.** For the MiniOpticon system, select the appropriate white wells designation from the Settings menu. For older systems, the plate type **MJ White** may need to be selected. For new systems, select the plate type **BR White**. Make sure the fluorophore being used in the experiment is calibrated for the selected plate type

  NOTE: The MiniOpticon system is factory calibrated for plates with white wells. Calibration is specific to the instrument, dye, and plate type. To calibrate a new combination of dye and plate type on an instrument, select **Tools > Calibration Wizard** (see “Calibration Wizard” on page 111).

Select Fluorophores Window

The Select Fluorophores window lists fluorophores that can be selected to load into the Plate Editor well loading controls. To open the Select Fluorophores window, click **Select Fluorophores** on the right side of the Plate Editor.

NOTE: You cannot add or remove fluorophores in this list; you must calibrate the new fluorophores on an instrument in the Calibration Wizard (page 111). After calibration, the new fluorophore is added to the Select Fluorophore window.

NOTE: The MiniOpticon system is only factory calibrated for FAM, SYBR® and HEX. If you intend to use another dye, you must perform a dye calibration. To calibrate a new combination of dye and plate type on an instrument, select **Tools > Calibration Wizard** (see “Calibration Wizard” on page 111)

- Click the **Selected** check box next to the fluorophore name to add or remove a fluorophore that appears in the list on the right side of the Plate Editor window (Figure 35).

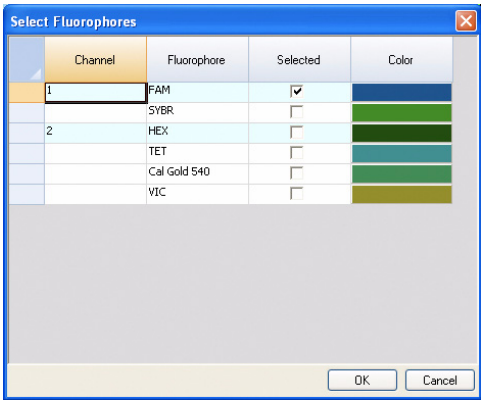


Figure 35. Select Fluorophores window.

- Click the **Color** box next to the fluorophore name and select a new color to represent each fluorophore in the Plate Editor window and Data Analysis charts

  NOTE: Before beginning the run, the software verifies that the fluorophores you specified in the plate are calibrated on that instrument. You cannot run a plate if it includes fluorophores that have not been calibrated on that instrument.

Well Loading Controls

A plate file contains information about the contents of each well loaded with sample for an experiment. After the run, the software links the well contents to the fluorescence data collected during the protocol and applies the appropriate analysis in the Data Analysis window. For example, wells loaded with a standard sample type are used to generate a standard curve.

When setting up a gene expression experiment, consider the following guidelines for well contents:

- **Target Name.** One or more targets of interest (genes or sequences) in each loaded well. Each target is assigned to one fluorophore
- **Sample Name.** One identifier or condition that corresponds to the sample in each loaded well, such as “0 hr”, “1 hr”, or “2 hr”

  TIP: Target names and sample names must match between wells to compare data in the Gene Expression tab in the Data Analysis window. Each name must contain the same punctuation and spacing. For example, “Actin” is not the same as “actin”, and “2hr” is not the same as “2 hr”. To facilitate consistency in names, enter them in the Target and Sample Names Libraries in the Plate tab in the User Preferences window (page 105).

Select a well to load contents into by left clicking with the mouse pointer in the plate view. Hold down the mouse button and drag to select multiple wells. The buttons and lists on the right side of the plate view include all the options needed to load the wells (Table 17).

Table 17. Options for loading the plate and wells in the Plate Editor

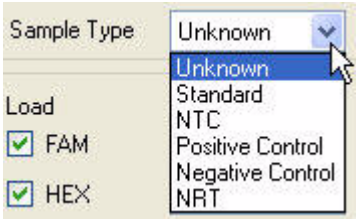
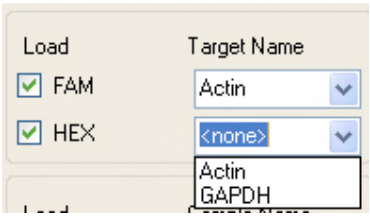
Option	Function
	After selecting wells, the Sample Type must be loaded first. Select a **Sample Type** from the pull-down menu to load it in the selected wells. Sample Types include Unknown, Standard, NTC (no template control), Positive Control, Negative Control, and NRT (no reverse transcriptase)
	Click a **Load** box to add a fluorophore to the selected wells; each fluorophore corresponds to a target name. To add fluorophores to the Load list, select them in the **Select Fluorophores** window For gene expression analysis or to distinguish between multiple targets, select a name in the **Target Name** pull-down menu and press **Enter** to load the target name in the well. To delete a target name, select it, press **Delete**, and then press **Enter** TIP: To add a new target name to the pull-down menu in the current plate only, type a name in the pull-down box and press **Enter**

Table 17. Options for loading the plate and wells in the Plate Editor (continued)

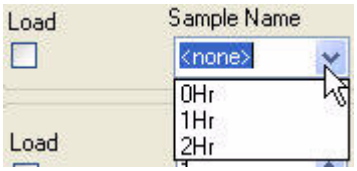
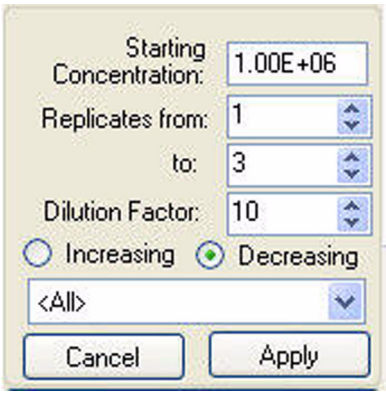
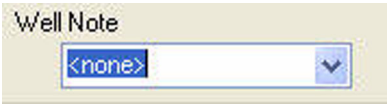
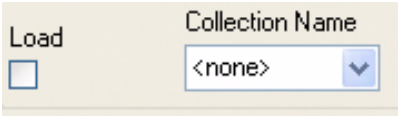
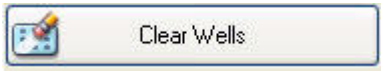
Option	Function
	For gene expression analysis or to distinguish between multiple samples, select a **Sample Name** from the pull-down menu to load that sample name in the selected wells. To delete a sample name, select it in the menu, press the **Delete** key on your keyboard, and then press **Enter**. TIP: To add a new sample name to the pull-down menu in the current plate, type a new name in the pull-down box and press the **Enter** key
	To load replicate numbers, selected wells must contain identical well contents. If they do not, the software disables this loading control Click the **Load** box to add a Replicate # to the selected wells. Click **Clear Replicate #** to clear the replicate number from selected cells. TIP: To load multiple replicate numbers across a series of wells, click the **Replicate Series** button.
	In the **Replicate Series** pane you can apply a replicate series to a set of selected wells. Enter the **Replicate Group Size** by selecting a number from the pull-down menu that represents the number of samples (wells) in each group of replicates. Select a **Starting Replicate #** from the pull-down menu to add replicates. NOTE: You can load replicate groups with replicate numbers progressing from left to right (**Horizontal**), or progressing from top to bottom (**Vertical**).
	Enter a concentration to the selected wells with standard sample type by editing or typing a number in the **Concentration** box. To apply the concentration to one fluorophore in the well, select a single fluorophore from the pull-down menu (**<All>**) under the concentration box.To delete a concentration, select it, press **Backspace** on your keyboard and then press **Enter** Select multiple wells with a Standard sample type to activate the **Dilution Series** button

Table 17. Options for loading the plate and wells in the Plate Editor (continued)

Option	Function
	Click **Dilution Series** to enter a dilution series for the concentration of Standard samples, and load a standard curve Enter the **Starting Concentration** for the dilution series, the **Replicates from** (starting replicate number) and **to** (ending replicate number), and the **Dilution Factor** (amount to change the concentration with each replicate group). Select **Increasing** for a dilution series that increases, or select **Decreasing** for a dilution series that decreases. Finally, select the fluorophore used for the dilution series from the pull-down menu and click **Apply**
	Select **Tools > Show Well Notes** to show this pane. Enter notes about one or more wells by selecting the wells and typing the notes in the pull-down menu. Any notes you add appears in the spreadsheet on the Quantitation Data tab (page 58)
	Select **Tools > Show Collection Name** to show this pane. Enter sample collection information about one or more wells by selecting the wells and typing a collection name in the pull-down menu. Any collection name you add to wells appear in the Gene Expression Analysis window and enables sample grouping options
	Click **Experiment Settings** to open the Experiment Settings window to manage the lists of Targets and Samples, and to set up a gene expression experiment
	Click **Clear Replicate #** to clear the replicates numbers in the selected wells
	Click **Clear Wells** to clear all content in the selected wells NOTE: Clicking **Clear Wells** permanently removes the content of the wells.

Experiment Settings Window

To open the Experiment Settings window, follow one of these options:

- In the Plate Editor, click **Experiment Settings**
- While analyzing data in the Data Analysis window, click **Experiment Settings** in the Gene Expression tab

Open the Experiment Settings window to view or change the list of Targets and Samples (Figure 36 and Figure 37) or to set the gene expression analysis sample group to be analyzed if **Collection Names** have been added to the wells.

- **Targets.** A list of target names for each PCR reaction, such as a genes or sequences of interest. Click the Reference column to assign reference genes in an experiment

- **Samples.** A list of sample names that indicate the source of the target, such as a sample taken at 1 hour (1 hr), or taken from a specific individual (“mouse1”). Click the **Control** column to assign the control condition for an experiment

Figure 36 shows the Targets tab with the analysis settings shown.

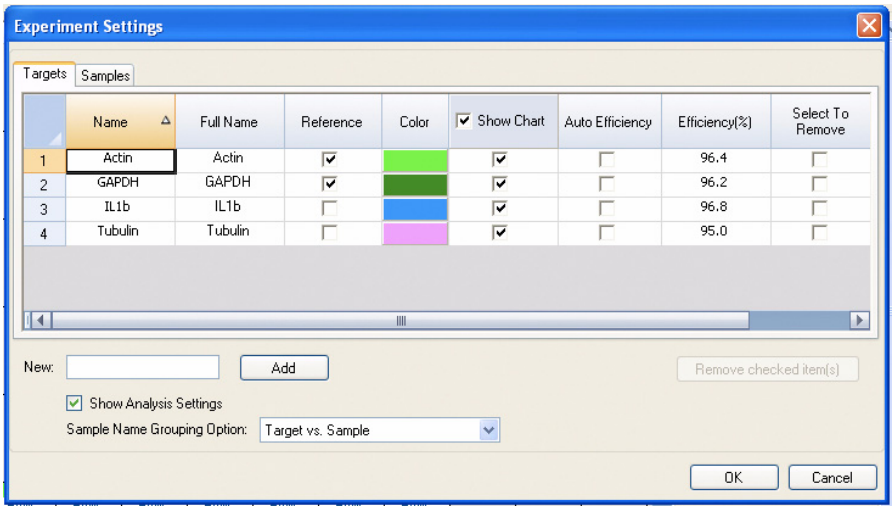


Figure 36. Targets tab in Experiment Settings window.

Figure 37 shows the Samples tab with the Analysis Settings shown.

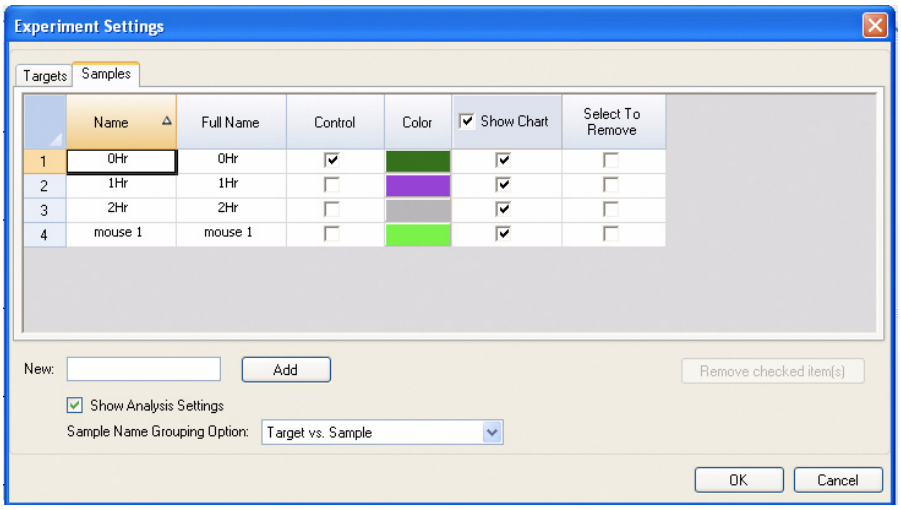


Figure 37. Samples tab in Experiment Settings window.

To adjust the Targets and Samples lists, use the following functions:

- Add a target or sample name by typing a name in the **New** box, and clicking **Add**
- Remove a target or sample name from the list by clicking the **Select to Remove** box for that row, and then clicking **Remove checked items(s)**
- Select the target as a reference for gene expression data analysis by clicking the box in the **Reference** column next to the name for that target
- Select a control sample for gene expression data analysis by clicking the box in the **Control** column next to the name for that sample

Click the **Show Analysis Settings** box in the Experiment Settings window to view or change analysis parameters applied in the Gene Expression tab.

To adjust target parameters:

- Click a cell in the **Color** column to change the color of the targets graphed in the Gene Expression chart
- Enter a number for the efficiency of a target. The software calculates the relative efficiency for a target using **Auto Efficiency** if the data for a target includes a standard curve. Alternatively, type a previously determined efficiency

To adjust the settings for a sample in the Samples tab:

- Click a color in the **Color** column to change the color of the samples graphed in the Gene Expression chart
- Click a box in the **Show Chart** column to show the sample in the Gene Expression chart using a color that is selected in the **Color** column

Sample Name Grouping Option

Loading **Collection Names** in the wells enables samples to be analyzed in the Gene Expression data analysis tab using one of four configurations defined by the Sample Name Grouping Option. These options are available from the pull-down menu in the Experiment Settings tab.

- Target vs. Sample
- Target vs. Collection
- Target vs. Sample_Collection
- Target vs. Collection_Sample

Well Groups Manager Window

Well groups divide a single plate into subsets of wells that can be analyzed independently in the Data Analysis window. Once well groups are set up, select a well group in the Data Analysis window to analyze the data in an independent group. For example, set up well groups to analyze multiple experiments run in one plate, or to analyze each well group with a different standard curve.

NOTE: The default well group is **All Wells**.

Create Well Groups

To create well groups in the Well Groups Manager window:

1. Click **Well Groups** in the toolbar of the Plate Editor.
2. Click **Add** to create a new group. The pull-down menu shows the group name as **Group 1** for the first group.
3. Select the wells that will compose the well group in the plate view by clicking and dragging across the group of wells. Selected wells turn blue in color (Figure 38).
4. (Optional) Change the name of the group by selecting the group name in the pull-down menu and typing a new name.
5. (Optional) Create more well groups by repeating steps 1 and 2.

6. (Optional) Delete well groups by selecting the group name in the pull-down list, and clicking **Delete**.
7. Click **OK** to finish and close the window, or click **Cancel** to close the window without making changes.

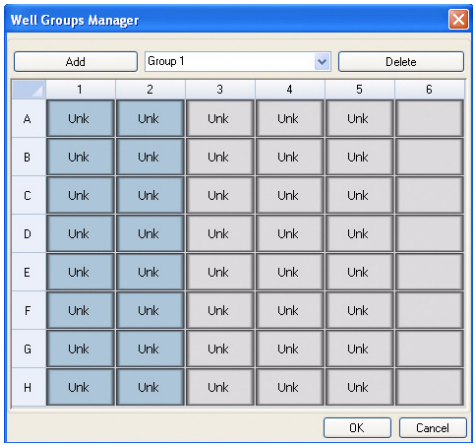


Figure 38. Color of wells in the Well Group Manager window.

Plate Spreadsheet View Window

The Plate Spreadsheet View window shows the contents of a plate in the Plate Editor. Open the Plate Spreadsheet View window (Figure 39) by selecting **Tools > Show Spreadsheet View** in the Plate Editor menu bar.

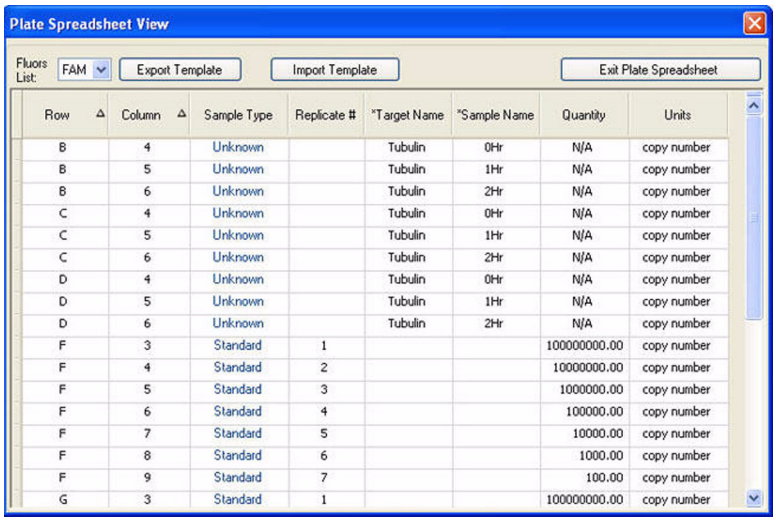


Figure 39. Plate Spreadsheet View window.

Open the spreadsheet view to import or export the well contents to Excel or to another tab-delimited format:

- Click **Import Template** to import well contents from a comma delimited file
- Click **Export Template** to export well contents in Excel file (.csv format)

To sort or edit a column, select it and use these methods:

- Sort the spreadsheet according to the data in one column by clicking the diamond next to a column name
- Edit the contents of a column that has an asterisk (*) at the top by clicking and typing in each well

  NOTE: Select the units for the standard curve data in the Quantity column by opening the Plate Editor and selecting **Settings > Units** in the menu bar. After the plate runs, the data from these standards appear in the Standard Curve chart of the Quantitation tab (Data Analysis window) with the units you select. Open the spreadsheet view to import or export the plate contents to Excel or another tab-delimited format.

Right-click on the spreadsheet to select one of these options from the right-click menu:

- **Copy.** Copy the entire spreadsheet
- **Copy as Image.** Copy the spreadsheet as an image file
- **Print.** Print the spreadsheet
- **Print Selection.** Print only the selected cells
- **Export to Excel.** Export the file as an Excel formatted file
- **Export to Text.** Export the file as a text file
- **Find.** Find text in the spreadsheet
- **Sort.** Sort the spreadsheet by selecting up to three columns of data in the Sort window

6 Data Analysis Overview

Read this chapter for information about data analysis:

Data Analysis Window

During data analysis, changing the way the data are displayed by changing the contents of wells in the Plate Editor never changes the fluorescence data that were collected from each well during the run. Once the module collects fluorescence data, you cannot delete those data, but you can choose to remove data from view and analysis.

To change the content of wells after a run, open the Plate Editor by clicking **Edit/View Plate** at the top of the Data Analysis window.

> TIP: You can add or edit information about the contents of the well before, during, or after you run the real-time PCR experiment. You must assign the scan mode and plate size before the run, and these parameters cannot be changed after the run.

CFX Manager software processes real-time PCR data automatically at the end of each run, and opens the Data Analysis window to display these data. Choose one of these methods to open existing data files in the Data Analysis window:

- Drag a data file (.pcrd extension) over the main software window and release it
- Select **File > Open > Data File** in the main software window to select a file in the Windows browser
- Click **Data Analysis** in the main software window toolbar to select a file in the Windows browser
- Select **File > Recent Data Files** to select from a list of the ten most recently opened data files

The Data Analysis window displays up to nine tabs (Figure 40). Each tab shows the analyzed data for a specific analysis method.

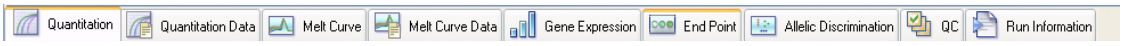


Figure 40. All the tabs that can be displayed in Data Analysis.

The software only displays a tab in the Data Analysis window if the data are collected in the run and data are available for that type of analysis. For example, the Melt Curve and Melt Curve Data tabs do not appear if the experiment does not include a melt curve step.

Data Analysis Toolbar

The toolbar in the Data Analysis window (Figure 41) provides quick access to important data analysis functions.

Figure 41. Toolbar in the Data Analysis window.

Table 18 lists the functions of buttons in the toolbar.

Table 18. Toolbar in the Data Analysis window

Toolbar button	Name	Function
	Save	Save the current data file
	Print	Print the selected window
	Trace Style	Open the Trace Style window
	Report	Open a Report for the current data file
	View/Edit Plate	Open the Plate Editor to view and edit the contents of the wells
	Well Groups...	Select a well group name from the pull-down menu. The default selection is All Wells
	Help	Open the software Help site for more information about data analysis

Data Analysis Menu Bar

The menu bar in the Data Analysis window (Figure 42) provides these menu items:

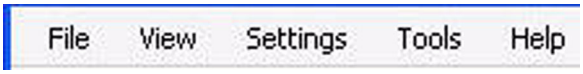


Figure 42. Menu bar in the Data Analysis window.

Table 19 lists the functions of items in the menu bar.

Table 19. Menu bar items in Data Analysis window

Menu Item	Command	Function
File	Save	Save the file
	Save As	Save the file with a new name
	Repeat Experiment	Extract the protocol and plate file from the current experiment to rerun it
	Exit	Exit the Data Analysis window
View	Run Log	Open a Run Log window to view the run log of those data file
Settings	Analysis Mode	Select Baseline Subtraction method for the selected well groups in the data
	C(t) Determination Mode	Select Regression or Single-Threshold mode to determine how threshold-cycle (C(t)) values are calculated for each trace
	Baseline Thresholds	Open the Baseline Thresholds window to adjust the baseline or the threshold
	Trace Styles	Open the Trace Styles window
	View/Edit Plate	Open the Plate Editor to view and edit the plate
	Mouse Highlighting	Turn on or off the simultaneous highlighting of data with the mouse pointer TIP: If Mouse Highlighting is turned off, then hold down the Control key to temporarily turn on the highlighting
	Display Threshold Values	Display the value of the threshold line in the chart
Tools	Reports	Open the Report for this data file
	Import Fluorophore Calibration	Select a calibration file to apply to the current data file
	Replace Plate	Replace the current plate file in the data analysis
	Export All Data Sheets to Excel	Export all the spreadsheet views from every tab to a separate Excel formatted file
Help		Open software Help for more information about data analysis

Quantitation Tab

Each tab in the Data Analysis window displays data in charts and spreadsheets for a specific analysis method, with a well selector to select the data you want to show. The Data Analysis window opens with the Quantitation tab (Figure 43) in front. The **Amplification** chart data in this tab should be used to determine the appropriate analysis settings for the experiment.

NOTE: The **Amplification** chart shows the relative fluorescence units (RFU) for each well at every cycle. Each trace in the chart represents data from a single fluorophore in one well.

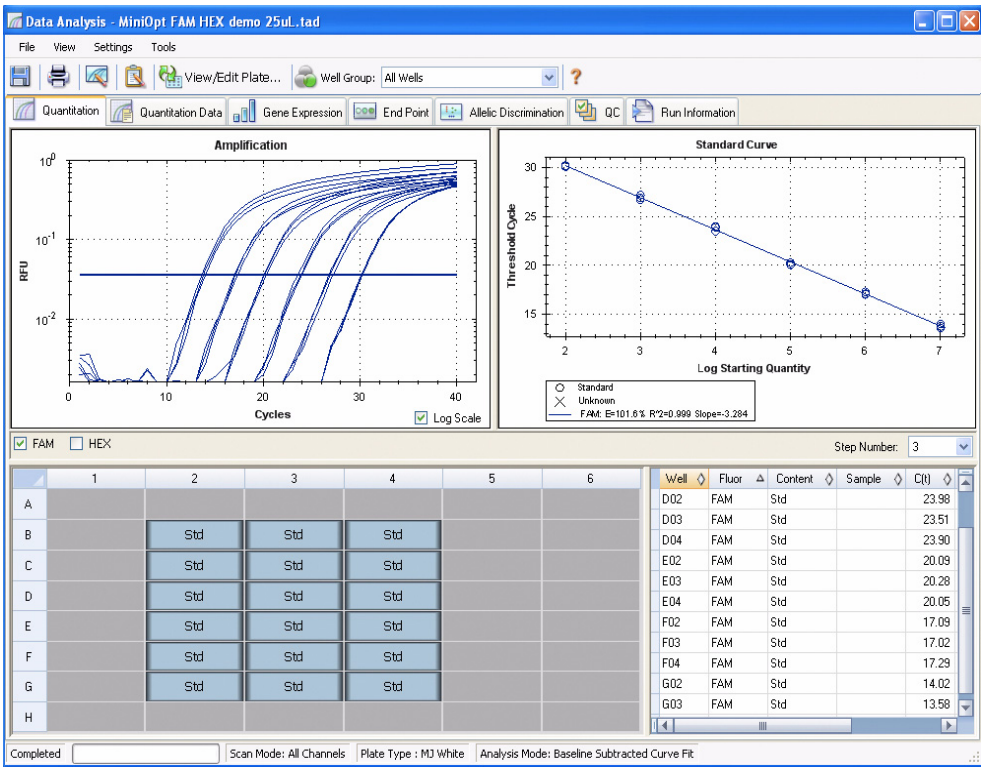


Figure 43. Layout of the Quantitation tab in the Data Analysis window.

NOTE: The software links the data in the panes of each data analysis tab. For example, highlighting a well by placing the mouse pointer over the well in the well selector view highlights the data in all the other panes.

Step Number Selector

The MiniOpticon system can acquire fluorescence data at multiple protocol steps; the software maintains the data acquired at each step independent. The software displays the **Step Number** selector below the Standard Curve chart on the Quantitation tab whenever a protocol contains more than one data collection step. When you select a step, the software applies that selection to all the data that are shown in the Data Analysis window. In Figure 44 the data collection step number is **3** for all the data.

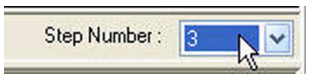


Figure 44. Step Number selection in the Data Analysis window.

Viewing Well Groups the in Data Analysis Window

Wells in the plate can be grouped into subsets for independent analysis using well groups. When you create well groups in the **Well Groups Manager** window in the Plate Editor (page 46), group names appear in the Data Analysis window within the Well Groups list on the toolbar.

> TIP: To open the Plate Editor, click **View/Edit Plate** in the Data Analysis window toolbar.

By default, **All Wells** is selected as the well group when the Data Analysis Window is first opened, with the data for all wells shown in the charts and spreadsheets.

Figure 45 shows Group 2 selected in the Well Groups menu. Only the wells in that well group appear loaded with content in the well selector and data only for these wells are included in the data analysis calculations.

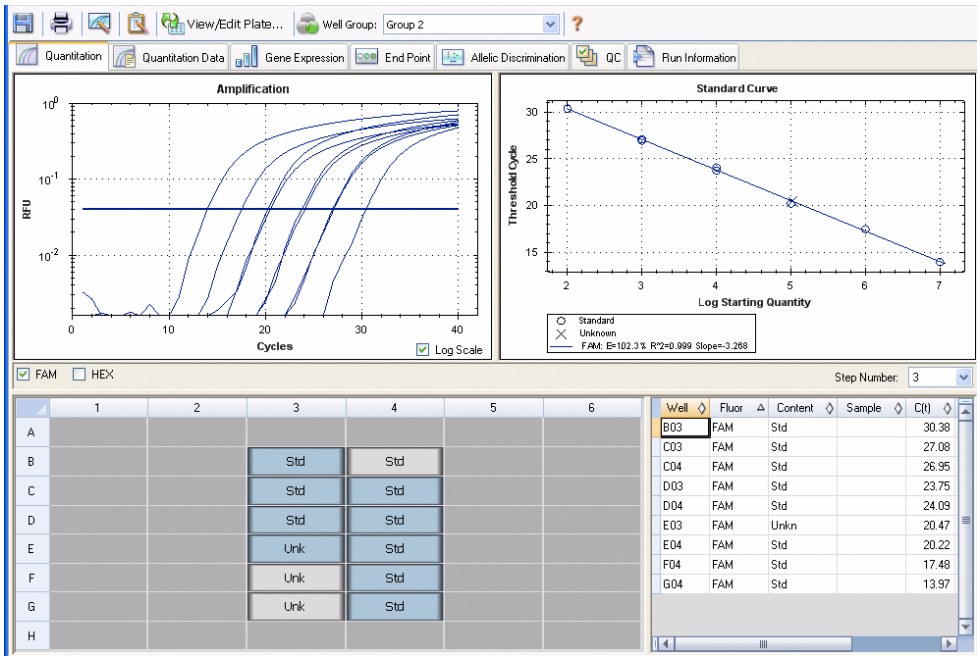


Figure 45. Data Analysis window with Group 2 selected.

Data Analysis Settings

The **Amplification** chart data in the Quantitation tab shows the RFU for each well at every cycle. Each trace in the chart represents data from a single fluorophore in one well. These data are used to determine C(t) values for each well on a per fluorophore basis. The software uses one of two modes to determine C(t) values:

- **Regression.** This mode applies a multivariable, nonlinear regression model to individual well traces and then uses this model to compute an optimal C(t) value
- **Single Threshold.** This mode uses a single threshold value to calculate the C(t) value based on the threshold crossing point of individual fluorescence traces

Adjusting the Threshold

In Single-Threshold mode, adjust the threshold for a fluorophore by clicking on the threshold line in the Amplification chart and moving the mouse pointer vertically. Alternatively, specify an exact crossing threshold for the selected fluorophore by following these instructions:

1. Select one fluorophore in the fluorophore selector in the Quantitation tab (Figure 43) by clicking the boxes next to the fluorophore name located under the Amplification chart.
2. Select **Settings > Baseline Thresholds** in the menu bar to open the Baseline Thresholds window.
3. Adjust the crossing threshold (Figure 46) for the fluorophore by clicking **User Defined** and entering a threshold number.

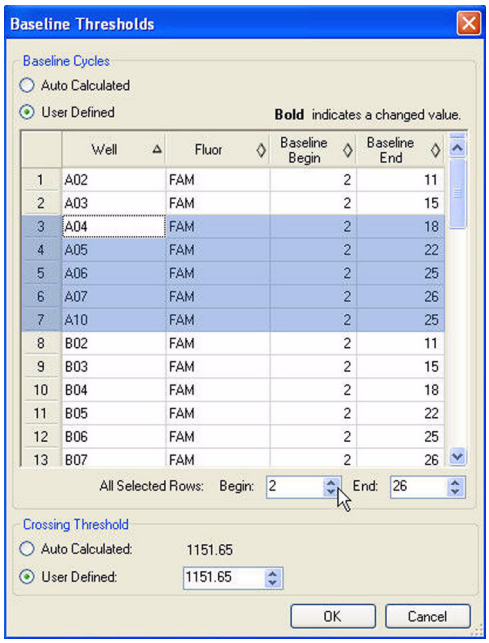


Figure 46. Baseline Thresholds window.

4. Click **OK** to confirm the change and close the window.

Baseline Settings

The software automatically sets the baseline individually for each well. Once you select the wells for analysis, check the baseline settings in these wells. Open the Baseline Thresholds window (Figure 46) to change the default baseline for selected wells. To open this window:

1. Select one fluorophore in the fluorophore selector in the Quantitation tab (Figure 43) by clicking the boxes next to the fluorophore name located under the Amplification chart.
2. Select **Settings > Baseline Thresholds** to open the Baseline Thresholds window.

To adjust the baseline begin and end cycle for each well:

1. In the Baseline Cycles pane, select one or more wells by clicking the row number, clicking the top left corner to select all wells, holding down the Control key to select multiple individual wells, or holding down the shift key to select multiple wells in a row.
2. Adjust the **Baseline Begin** cycle and **Baseline End** cycle for all selected wells or change the **Begin** and **End** cycle number at the bottom of the spreadsheet (Figure 46).

3. Click **OK** to confirm the change and close the window.

Selecting the Analysis Mode

Select the Analysis Mode to determine the method of baseline subtraction for all fluorescence traces. Select **Settings > Analysis Mode** to choose one of these three options:

- **No Baseline Subtraction.** The software displays the data as relative fluorescence traces. Some analysis is not possible in this analysis mode, and therefore the software does not display the Gene Expression, End Point, and Allelic Discrimination tabs
- **Baseline Subtracted.** The software displays the data as baseline subtracted traces for each fluorophore in a well. The software must baseline subtract the data to determine threshold cycles, construct standard curves, and determine the concentration of unknown samples. To generate a baseline subtracted trace, the software fits the best straight line through the recorded fluorescence of each well during the baseline cycles, and then subtracts the best fit data from the background subtracted data at each cycle
- **Baseline Subtracted Curve Fit.** The software displays the data as baseline subtracted traces, and the software smoothes the baseline subtracted curve using a centered mean filter. This process is performed so that each C(t) is left invariant

Well Selectors

Click the wells in the well selector to show or to hide the data in the charts or spreadsheets throughout the Data Analysis window:

- To hide one well, highlight and click the individual well. To show that well, highlight and click the well again
- To hide multiple wells, click and drag across the wells you want to select. To show those wells, click and drag across the wells again
- Click the top left corner of the plate to hide all the wells. Click the top left corner again to show all wells
- Click the start of a column or row to hide those wells. Click the column or row again to show the wells

Only wells loaded with content (entered in the Plate Editor) can be selected in the well selector, and their color shows if they are selected. As shown in Figure 47, the well selector shows these three types of wells:

- **Selected, loaded wells (blue).** These wells contain a loaded **Unk** (unknown) sample type. The data from these wells appear in the Data Analysis window
- **Unselected, loaded wells (light gray).** These wells contain loaded **Std** and **Pos** sample types. The data from unselected wells do not appear in the Data Analysis window

- **Empty wells (dark gray).** These wells were not loaded in the Plate Editor window

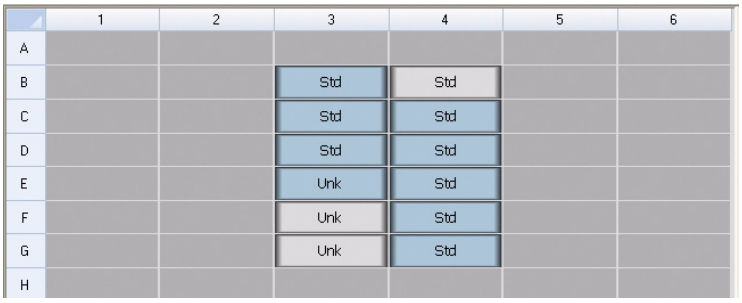


Figure 47. Three well colors appear in a well selector.

Well Selector Right-Click Menu Items

Right-click any well selector view to select the items listed in Table 20.

Table 20. Right-click menu items in the well selectors

Item	Function
Copy	Copy the content of the well to a clipboard, including Sample Type and optional Replicate #
Copy as Image	Copy the well selector view as an image
Print...	Print the well selector view
Print Selection...	Print the current selection
Export to Excel...	Export the data to an Excel spreadsheet

Excluding Wells From Analysis

To exclude any wells from data analysis temporarily, follow these instructions:

RIGHT-CLICK OPTION

1. Right-click on the well in the well selector, on a fluorescence trace, or on a point plotted on the standard curve.
2. Choose **Exclude Well XX from Analysis** from the menu options.

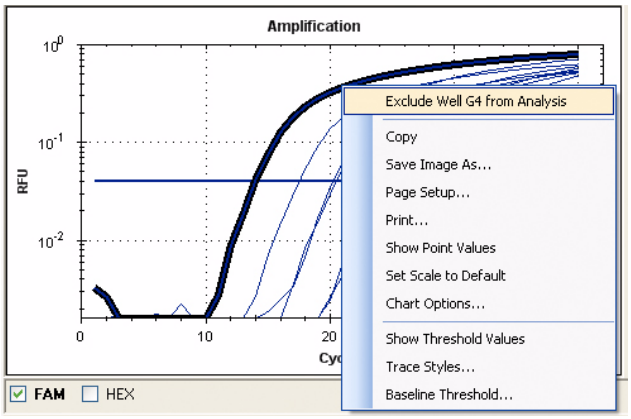


Figure 48. Exclude well XX from analysis.

NOTE: Unselect the **Exclude Well from Analysis** from the right-click menu to re-include the well.

Plate Editor Options

1. Click **View/Edit Plate** on the toolbar in the Data Analysis window.
2. Select one or more wells in the well selector view.
3. Click **Exclude Wells in Analysis** (Figure 49) to exclude the selected wells. This checkbox is at the bottom of the Plate Editor controls on the right side of the window.

Figure 49. Click exclude Wells in Analysis checkbox at bottom of the pane.

In Figure 50, one well (under the pointer) was excluded from data analysis in the Plate Editor. Notice that the excluded well is grey and marked with an asterisk (*).

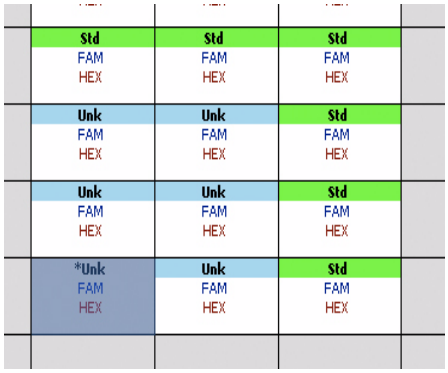


Figure 50. The excluded well is marked with * in the Plate Editor.

Alternatively, to permanently remove wells from analysis, clear the contents from wells in the Plate Editor by clicking **Clear Wells**.

WARNING! You will have to reenter any well content that is cleared.

Charts

Each chart in the Data Analysis window displays the data in a different graph and includes options for adjusting the data. To magnify an area of the chart, select the area by clicking and dragging the mouse. The software resizes the chart and centers it on the selected area.

TIP: Return the chart to a full view by right-clicking on the chart and selecting **Set Scale to Default** from the right-click menu.

Common Right-Click Menu Items for Charts

Right-click menu items are available on all charts. Some of the available items are present for all charts. These items can be used to change how the data are displayed or they can also be used to easily export the data from a chart (Table 21).

Table 21. Right-click menu items for charts

Item	Function
Copy	Copy the chart into the clipboard
Save Image As...	Save the chart image in the selected image file type. Select from these formats: **PNG** (default), **GIF**, **JPG**, **TIF**, or **BMP**
Page Setup...	Preview and select page setup for printing
Print...	Print the chart
Show Point Values	Show the point values when the mouse moves over a point on the chart
Set Scale to Default	Return to default chart view after magnifying the chart
Chart Options...	Open the Chart Options window to change the chart title, select limits for the x- and y-axes, choose to show or hide showing grid lines, and choose to show or hide minor ticks in the axes

NOTE: Menu items that apply to specific charts are described in the next chapter “Data Analysis Windows” (page 61).

Spreadsheets

The spreadsheets shown in Data Analysis include options for sorting and transferring the data. Sort the columns by one of these methods:

- Click and drag a column to a new location in the selected table
- Click the column header to sort the data in ascending or descending order

To sort up to three columns of data in the Sort window, follow these steps:

1. Right-click on the spreadsheet to open the menu and select **Sort**.
2. In the Sort window, select the first column title to sort. Sort the data in ascending or descending order.
3. Select more than one column title by selecting the title in the pull-down menu. Select **Ascending** or **Descending** to sort the column in that order.
4. Click **OK** to sort the data, or click **Cancel** to stop sorting.

Highlight the data on the associated charts and well selector by holding the mouse pointer over a cell. If you click in the cell, you can copy and paste the contents into another software program.

Common Right-Click Menu Items for Spreadsheets

Right-click any spreadsheet view to select the items shown in Table 22.

Table 22. Right-click menu items for spreadsheets

Item	Function
Copy	Copy the contents of the selected wells to a clipboard. Then, paste the contents into a spreadsheet such as Excel
Copy as Image	Copy the spreadsheet view as an image file and paste it into a file that accepts an image file such as text, image, or spreadsheet files
Print...	Print the current view
Print Selection...	Print the current selection
Export to Excel...	Export the data to an Excel spreadsheet
Export to Text...	Export the data to a text editor
Export to Xml	Export the data to an XML file
Export to Html	Export the data to an HRML file
Find...	Search for text
Sort...	Sort the data in up to three columns

7 Data Analysis Windows

Read this chapter for more information about the tabs in the Data Analysis window:

Quantitation Tab

Use the data in the Quantitation tab (Figure 51) to set the data analysis conditions, including the baseline settings for individual wells and the threshold settings. The Quantitation tab shows data in these four views:

- **Amplification chart.** Shows the relative fluorescence units (RFUs) for each well at every cycle. Each trace in the chart represents data from a single fluorophore in one well

  NOTE: To select the fluorophore data to display or hide in the Quantitation tab charts and spreadsheets, click the fluorophore selector below the Amplification chart (Figure 51).

  NOTE: Click the **Log Scale** box at the bottom of the Amplification chart to view the fluorescence traces in a semi-log scale

  TIP: To magnify any area of the chart, click and drag the mouse across an area. To return to a full view, right-click and select **Set Scale to Default** from the menu.
- **Standard curve.** This graph is only shown if the experiment includes wells designated as a standard sample type. The standard curve is displayed with the threshold cycle plotted against the log of the starting quantity. The legend shows the Reaction Efficiency (E) for each fluorophore in the wells with a standard sample type
- **Well selector.** Selects the wells with the fluorescence data you want to show

- **Spreadsheet.** Shows a spreadsheet of the data collected in the selected wells

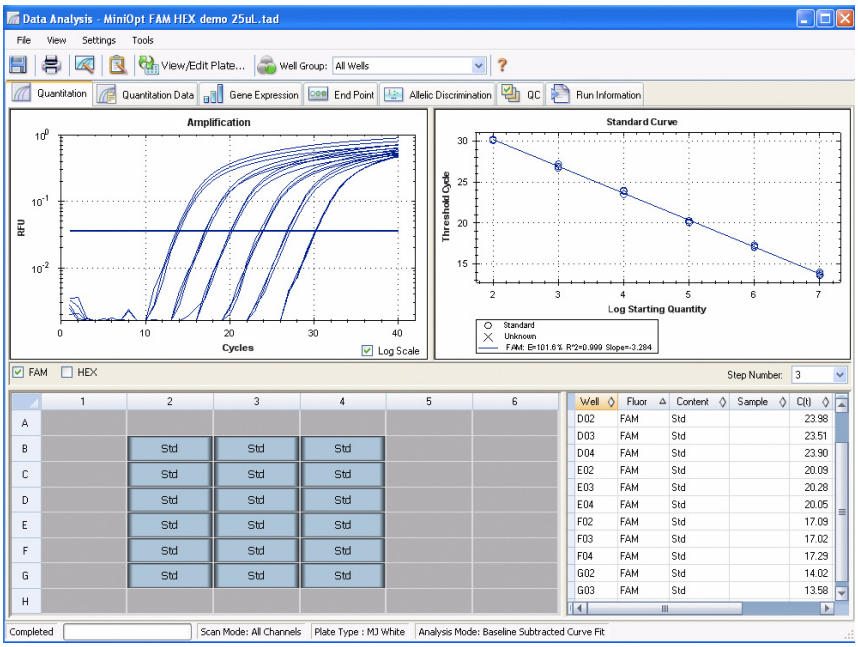


Figure 51. Layout for the Quantitation tab in Data Analysis window.

Trace Styles Window

Open the Trace Styles window (Figure 52) to adjust the appearance of traces in the amplification and melt curve charts in the Quantitation and Melt Curve tabs.

To open this window, follow these steps:

1. Select only one fluorophore under the Amplification chart.
2. Click **Trace Styles** in the Data Analysis toolbar, or select **Settings > Trace Styles** in the Data Analysis menu bar.

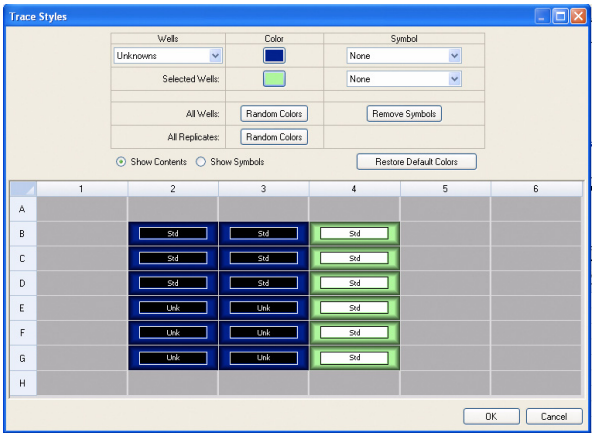


Figure 52. Trace Styles window.

Use the tools in the Trace Styles window to adjust appearance of traces, and preview the changes in the well selector at the bottom of the window.

- Select a specific set of wells by using the well selector at the bottom of the window. Alternatively, select wells that contain one sample type in the pull-down menu in the **Wells** column, including **Unknown**, **Standard**, **NTC** (no template control), **Positive Control**, **Negative Control**, or **NRT** (no reverse transcriptase control) sample types
- Click the box in the Color column to select a color for the wells
- Select a symbol from the pull-down menu in the Symbol column
- Click **Show Contents** to show the sample types in each well, or click **Show Symbols** to show the selected Symbols in each well
- Click **Remove Symbols** to remove all the added symbols from all wells
- Click **Restore Default Colors** to return to the default trace colors

Standard Curve Chart

The software creates a Standard Curve chart (Figure 53) in the Quantitation tab if the data include sample types defined as standard (Std) for one fluorophore in the experiment.

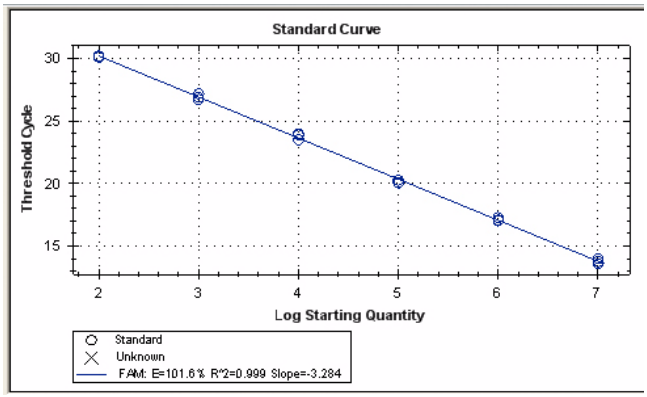


Figure 53. Standard Curve chart.

The Standard Curve chart displays the following information:

- Name for each curve (the fluorophore name)
- Color of each fluorophore
- Reaction efficiency (E). Use this statistic to optimize a multiplex reaction, and equalize the data for a standard curve

  NOTE: The reaction efficiency describes how much of your target is being produced with each cycle in the protocol. An efficiency of 100% means that you are doubling your target with each cycle.
- Coefficient of determination, R^2 (written as R^2). Use this statistic to determine how correctly the line describes the data (goodness of fit)

Chart Right-Click Menu Options

In addition to the common right-click menu options to copy, print and export charts, Table 23 lists the menu options available only on the Amplification chart.

Table 23. Amplification chart specific right-click menu options

Menu Option	Function
Show Threshold Values	Display the threshold value for each amplification curve on the chart

Trace Styles...	Open the Trace Styles window to change trace styles that appear on the Quantitation and Melt Curve tabs
Baseline Thresholds...	Open the Baseline Thresholds window to change baseline or thresholds of each fluorophore (changes appear in Amplification chart in Quantitation tab)

Quantitation Tab Spreadsheet

Table 24 shows the type of data shown in the spreadsheet at the bottom right side of the Quantitation tab:

Table 24. Contents of the quantitation tab

Information	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	A combination of the Sample Type (required) and Replicate # (optional) loaded in the Plate Editor
Sample	Sample Name loaded in the Plate Editor wells
C(t)	Threshold cycle for each trace

TIP: To make changes to the Content and Sample, open the Plate Editor by clicking **View/Edit Plate**.

Quantitation Data Tab

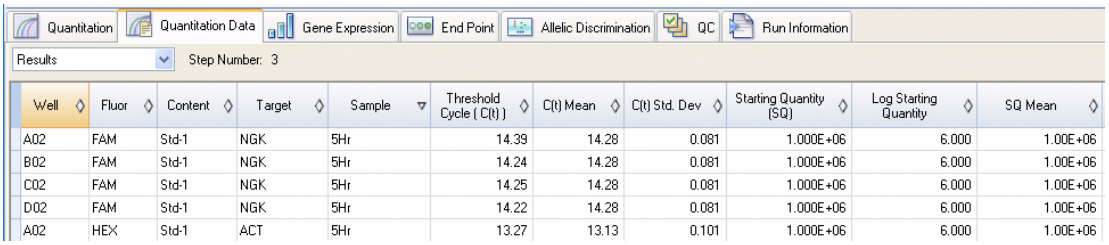
The Quantitation Data tab shows spreadsheets that describe the quantitation data collected in each well. Select one of the three options to show the data in different formats:

- **Results.** Displays a spreadsheet view of the data
- **Plate.** Displays a view of the data in each well as a plate map
- **RFU.** Choose this spreadsheet to show the RFU quantities in each well for each cycle

TIP: Right-click any spreadsheet for options, including the sort option.

Results Spreadsheet

Select a **Results** spreadsheet (Figure 54) to see data for each well in the plate.



Well	Fluor	Content	Target	Sample	Threshold Cycle (C(t))	C(t) Mean	C(t) Std. Dev	Starting Quantity (SQ)	Log Starting Quantity	SQ Mean
A02	FAM	Std-1	NGK	5Hr	14.39	14.28	0.081	1.000E+06	6.000	1.00E+06
B02	FAM	Std-1	NGK	5Hr	14.24	14.28	0.081	1.000E+06	6.000	1.00E+06
C02	FAM	Std-1	NGK	5Hr	14.25	14.28	0.081	1.000E+06	6.000	1.00E+06
D02	FAM	Std-1	NGK	5Hr	14.22	14.28	0.081	1.000E+06	6.000	1.00E+06
A02	HEX	Std-1	ACT	5Hr	13.27	13.13	0.101	1.000E+06	6.000	1.00E+06

Figure 54. Quantitation Data tab with Results spreadsheet selected.

NOTE: All Std. Dev (standard deviation) calculations apply to the replicate groups assigned in the wells in the Plate Editor window. The calculations average the C(t) value for each well in the replicate group.

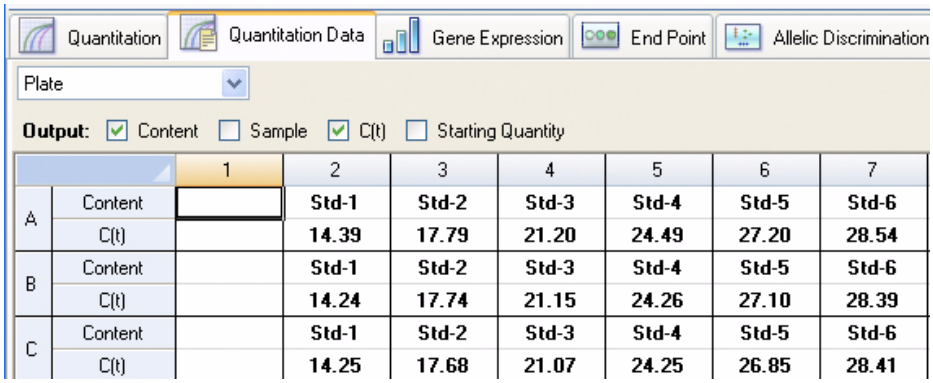
The Results spreadsheet includes the type of information listed in Table 25.

Table 25. Contents of the results spreadsheet

Information	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	Sample type and replicate number
Target	Amplification target name (gene)
Sample	Sample description
Threshold Cycle (C(t))	Threshold cycle
C(t) Mean	Mean of the threshold cycle for the replicate group
C(t) Std. Dev	Standard deviation of the threshold cycle for the replicate group
Starting Quantity (SQ)	Estimate of the starting quantity of the target
Log Starting Quantity	Log of the starting quantity
SQ Mean	Mean of the starting quantity
SQ Std. Dev	Standard deviation of the starting quantity

Plate Spreadsheet

Select the **Plate** spreadsheet to see a plate map of the data for one fluorophore at a time. Select each fluorophore by clicking a tab at the bottom of the spreadsheet. Figure 55 shows the Plate spreadsheet as plate map.



		1	2	3	4	5	6	7
A	Content		Std-1	Std-2	Std-3	Std-4	Std-5	Std-6
	C(t)		14.39	17.79	21.20	24.49	27.20	28.54
B	Content		Std-1	Std-2	Std-3	Std-4	Std-5	Std-6
	C(t)		14.24	17.74	21.15	24.26	27.10	28.39
C	Content		Std-1	Std-2	Std-3	Std-4	Std-5	Std-6
	C(t)		14.25	17.68	21.07	24.25	26.85	28.41

Figure 55. Plate spreadsheet in Quantitation Data tab.

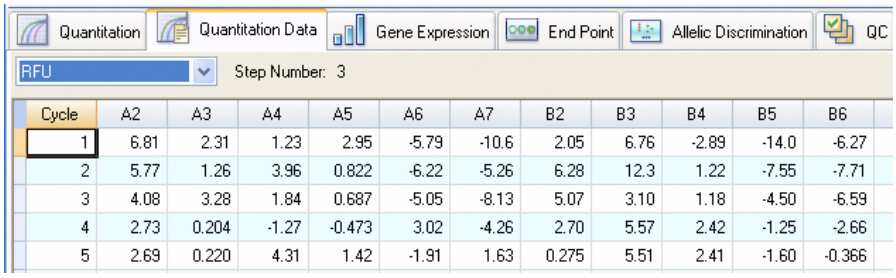
The Plate spreadsheet includes the type of information shown in Table 26, including the selected fluorophore in the plate map.

Table 26. Contents of the plate spreadsheet

Information	Description
Content	Sample type and replicate number
Sample	Sample description
Copy Number	Starting number of targets in the sample
RFU	Relative fluorescence units

RFU Spreadsheet

Select the **RFU** spreadsheet to see the RFU readings for each well acquired at each cycle of the experiment. Select individual fluorophores by clicking a tab at the bottom of the spreadsheet. The well number appears at the top of each column, and the cycle number appears to the left of each row (Figure 56).



Cycle	A2	A3	A4	A5	A6	A7	B2	B3	B4	B5	B6
1	6.81	2.31	1.23	2.95	-5.79	-10.6	2.05	6.76	-2.89	-14.0	-6.27
2	5.77	1.26	3.96	0.822	-6.22	-5.26	6.28	12.3	1.22	-7.55	-7.71
3	4.08	3.28	1.84	0.687	-5.05	-8.13	5.07	3.10	1.18	-4.50	-6.59
4	2.73	0.204	-1.27	-0.473	3.02	-4.26	2.70	5.57	2.42	-1.25	-2.66
5	2.69	0.220	4.31	1.42	-1.91	1.63	0.275	5.51	2.41	-1.60	-0.366

Figure 56. RFU spreadsheet in the Quantitation Data tab.

The RFU spreadsheet includes the type of information shown in Table 27.

Table 27. Contents of the RFU spreadsheet

Information	Description
Well number (A2, A3, A4, A5, A6...)	Well data, listed by position in the plate for all the loaded wells
Cycle	One round of denaturation, annealing, and extension, or one round of annealing and extension steps in a protocol

Melt Curve Tab

For DNA-binding dyes and noncleavable hybridization probes, the fluorescence is brightest when the two strands of DNA anneal. Therefore, as the temperature rises towards the melting temperature (Tm), fluorescence decreases at a constant rate (constant slope). At the Tm, there is a dramatic reduction in the fluorescence with a noticeable change in slope. The rate of this change is determined by plotting the negative first regression of fluorescence versus temperature (-d(RFU)/dT). The greatest rate of change in fluorescence results in visible peaks and represents the Tm of the double-stranded DNA complexes.

The software plots the RFU data collected during a melt curve as a function of temperature. To analyze melt peak data, the software assigns a beginning and ending temperature to each peak by moving the threshold bar. The floor of the peak area is specified by the position of the melt threshold bar. A valid peak must have a minimum height relative to the distance between the threshold bar and the height of the highest peak.

Open the Melt Curve tab (Figure 57) to determine the Tm of amplified PCR products. This tab shows the melt curve data in these four views:

- **Melt Curve.** View the real-time data for each fluorophore as RFUs per temperature for each well
- **Melt Peak.** View the negative regression of the RFU data per temperature for each well
- **Well Selector.** Select wells to show or hide the data
- **Peak spreadsheet.** View a spreadsheet of the data collected in the selected well

  NOTE: This spreadsheet only shows as many as two peaks for each trace. To see more peaks, click the **Melt Curve Data** tab (page 68).

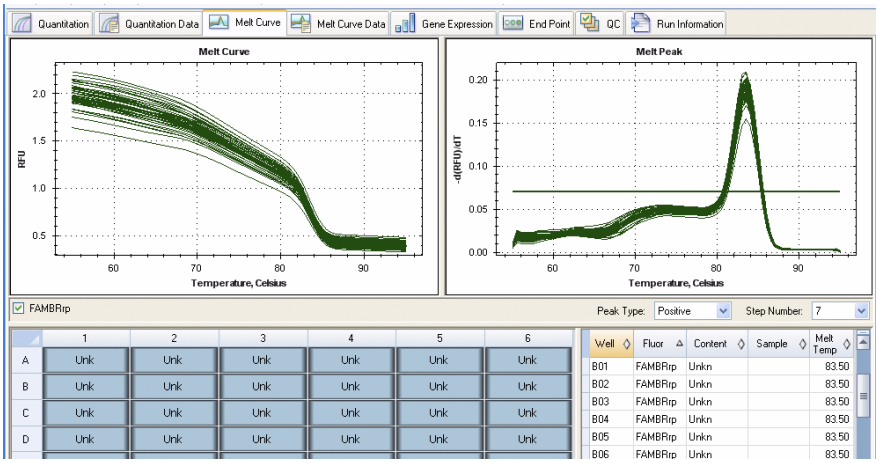


Figure 57. Layout of the Melt Curve tab in the Data Analysis window.

Adjusting Melt Curve Data

Adjust the melt curve data by any of these methods:

- Click and drag the threshold bars in the Melt Peak chart to include or exclude peaks in data analysis
- Select **Positive** in the Peaks pull-down menu to show the spreadsheet data for the peaks above the Melt Threshold line, or select **Negative** to view the spreadsheet data for the peaks below the Melt Threshold line
- Open the Trace Styles window to change the color of the traces in Melt Curve and Melt Peak charts
- Select a number in the Step Number selector to view the Melt Curve data at another step in the protocol. The list shows more than one step if the protocol includes plate read (camera icon) in two or more melt curve steps
- Select wells in the well selector to focus on subsets of the data
- Select a well group (page 53) to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group pull-down menu in the toolbar

Melt Curve Tab Spreadsheet

Table 28 shows the type of information in the spreadsheet at the bottom right side of the Melt Curve tab.

Table 28. Contents of the Melt Curve tab spreadsheet

Information	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	A combination of Sample Type and Replicate #
Sample	Sample Name loaded in the Plate Editor
Melt Temp	The temperature of the melt peak for each well. Only the two highest peaks are displayed in this spreadsheet.
Peak Height	The highest point of the melt peak (-d(RFU)/dT)

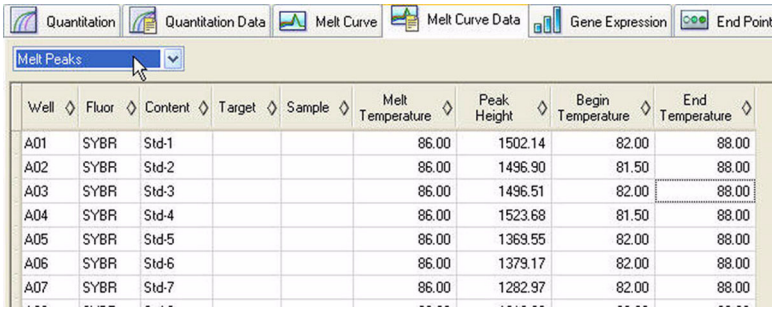
Melt Curve Data Tab

The Melt Curve Data tab shows multiple spreadsheets that include all the melt peaks for each trace. Select one of these four options to show the melt curve data in different spreadsheets:

- **Melt Peaks.** List all the data, including all the melt peaks, for each trace
- **Plate.** List a view of the data and contents of each well in the plate
- **RFU.** List the RFU quantities at each temperature for each well
- **-d(RFU)/dT.** List the negative rate of change in RFU as the temperature (T) changes. This is a first regression plot for each well in the plate

Melt Peaks Spreadsheet

Select **Melt Peaks** from the pull-down menu (Figure 58) to view melt curve data.



Well	Fluor	Content	Target	Sample	Melt Temperature	Peak Height	Begin Temperature	End Temperature
A01	SYBR	Std-1			86.00	1502.14	82.00	88.00
A02	SYBR	Std-2			86.00	1496.90	81.50	88.00
A03	SYBR	Std-3			86.00	1496.51	82.00	88.00
A04	SYBR	Std-4			86.00	1523.68	81.50	88.00
A05	SYBR	Std-5			86.00	1369.55	82.00	88.00
A06	SYBR	Std-6			86.00	1379.17	82.00	88.00
A07	SYBR	Std-7			86.00	1282.97	82.00	88.00

Figure 58. Melt Peaks spreadsheet in Melt Curve Data tab.

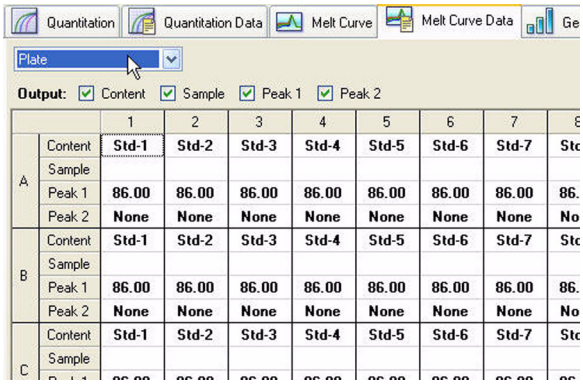
The Melt Peaks spreadsheet (Figure 58) includes the type of information shown in Table 29.

Table 29. Contents of the Melt Peaks spreadsheet

Information	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	Sample Type listed in the Plate Editor window
Target	Amplification target (gene)
Sample	Sample Name listed in the Plate Editor window
Melt Temperature	The melting temperature of each product, listed as one peak (highest) per row in the spreadsheet
Peak Height	Height of the peak
Begin Temperature	Temperature at the beginning of the peak
End Temperature	Temperature at the end of the peak

Plate Spreadsheet

Select **Plate** from the pull-down menu (Figure 59) to view melt curve data in a plate format.



		1	2	3	4	5	6	7	8
A	Content	Std-1	Std-2	Std-3	Std-4	Std-5	Std-6	Std-7	Std
	Sample								
	Peak 1	86.00	86.00	86.00	86.00	86.00	86.00	86.00	86.
	Peak 2	None	None	None	None	None	None	None	No
B	Content	Std-1	Std-2	Std-3	Std-4	Std-5	Std-6	Std-7	Std
	Sample								
	Peak 1	86.00	86.00	86.00	86.00	86.00	86.00	86.00	86.
	Peak 2	None	None	None	None	None	None	None	No
C	Content	Std-1	Std-2	Std-3	Std-4	Std-5	Std-6	Std-7	Std
	Sample								

Figure 59. Plate spreadsheet in Melt Curve Data tab.

NOTE: To adjust the peak that the software calls, adjust the threshold line in the Melt Peak chart on the Melt Curve tab.

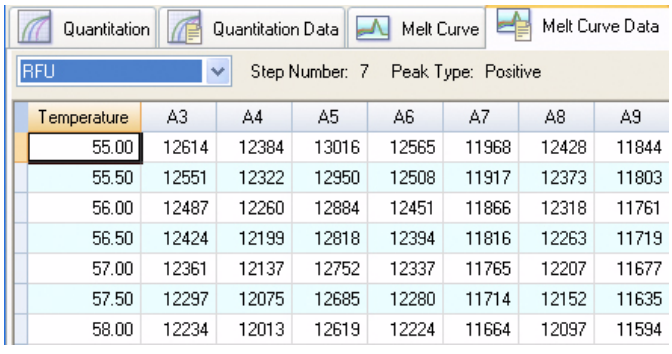
The Plate spreadsheet includes the information shown in Table 30.

Table 30. Contents of the Plate spreadsheet

Information	Description
Content	A combination of Sample Type (required) and Replicate # (optional)
Sample	Sample description
Peak 1	First melt peak (highest)
Peak 2	Second (lower) melt peak

RFU Spreadsheet

Select **RFU** from the pull-down menu to view the fluorescence for each well at each cycle acquired during the melt curve (Figure 60).



Temperature	A3	A4	A5	A6	A7	A8	A9
55.00	12614	12384	13016	12565	11968	12428	11844
55.50	12551	12322	12950	12508	11917	12373	11803
56.00	12487	12260	12884	12451	11866	12318	11761
56.50	12424	12199	12818	12394	11816	12263	11719
57.00	12361	12137	12752	12337	11765	12207	11677
57.50	12297	12075	12685	12280	11714	12152	11635
58.00	12234	12013	12619	12224	11664	12097	11594

Figure 60. RFU spreadsheet in Melt Curve Data tab.

Table 31 lists the type of information shown in the RFU spreadsheet.

Table 31. RFU spreadsheet content

Information	Description
Well number (A1, A2, A3, A4, A5...)	Well position in the plate for the loaded wells
Temperature	Melting temperature of the amplified target. Plotted as one well per row, and multiple wells for multiple products in the same well

-d(RFU)/dT Spreadsheet

Select **-d(RFU)/dT** from the pull down menu to view the type of data shown in Figure 61.

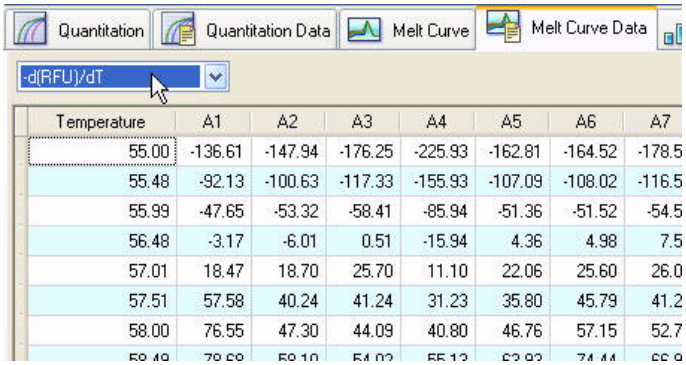


Figure 61. The -d(RFU)/dT spreadsheet in the Melt Curve Data tab.

Table 32 lists the type of information shown in the -d(RFU)/dT spreadsheet.

Table 32. Contents of the -d(RFU)/dT spreadsheet

Information	Description
Well number (A1, A2, A3, A4, A5...)	Well position in the plate for the loaded wells
-d(RFU)/dT	Negative rate of change in RFU as temperature (T) changes

End Point Tab

Open the End Point tab to analyze final RFUs for the sample wells. The software compares the RFU levels for wells with unknown samples to the RFU levels for wells with negative controls, and “calls” the unknown as a Positive or Negative. Positive samples have an RFU value that is greater than the average RFU value of the negative controls plus the Cut Off Value.

To analyze the end point data, the plate must contain negative controls, or the software cannot make the call. Run one of these two types of protocols:

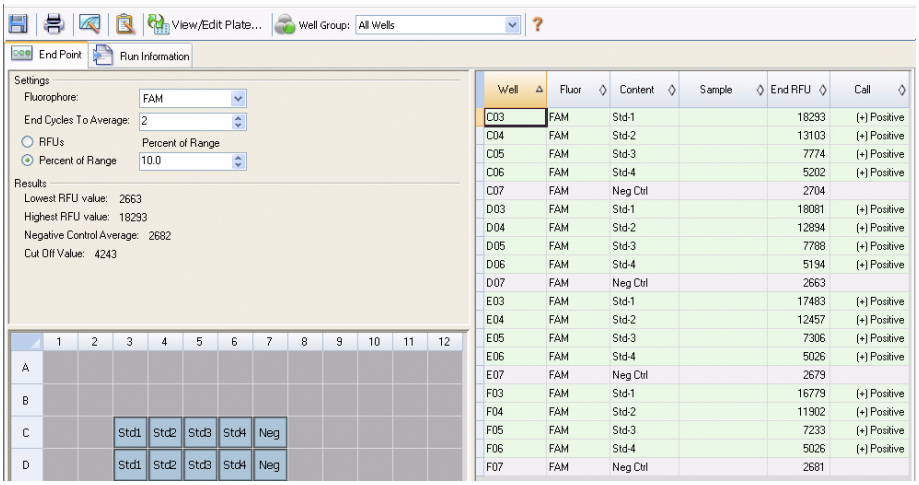
- **Run a Quantitation protocol.** Set up a standard protocol. After running the experiment, open the Data Analysis window, adjust the data analysis settings in the Quantitation tab, and then click the End Point tab to pick an end point cycle
- **Run an End Point Only protocol.** Load the End Point Only protocol in the Plate tab of the Experiment Setup window, select or create a plate, and run the experiment

The End Point tab shows the average RFU values to determine whether or not the target was amplified by the last (end) cycle (Figure 62). Use these data to determine if a specific target sequence is present (positive) in a sample. Positive targets have higher RFU values than the cut off level you define.

> TIP: To create an end point protocol, open the Protocol tab (Experiment Setup window) and select **Options > End Point Only Run**.

The software displays these data in the End Point tab:

- **Settings.** Adjust data analysis settings
- **Results.** Shows the results immediately after you adjust the settings
- **Well Selector.** Select the wells with the end point data you want to show
- **Well spreadsheet.** Shows a spreadsheet of the end RFU collected in the selected wells



Well	Fluor	Content	Sample	End RFU	Call
C03	FAM	Std-1		18293	(+) Positive
C04	FAM	Std-2		13103	(+) Positive
C05	FAM	Std-3		7774	(+) Positive
C06	FAM	Std-4		5202	(+) Positive
C07	FAM	Neg Ctrl		2704	
D03	FAM	Std-1		18081	(+) Positive
D04	FAM	Std-2		12894	(+) Positive
D05	FAM	Std-3		7788	(+) Positive
D06	FAM	Std-4		5194	(+) Positive
D07	FAM	Neg Ctrl		2663	
E03	FAM	Std-1		17483	(+) Positive
E04	FAM	Std-2		12457	(+) Positive
E05	FAM	Std-3		7306	(+) Positive
E06	FAM	Std-4		5026	(+) Positive
E07	FAM	Neg Ctrl		2679	
F03	FAM	Std-1		16779	(+) Positive
F04	FAM	Std-2		11902	(+) Positive
F05	FAM	Std-3		7233	(+) Positive
F06	FAM	Std-4		5026	(+) Positive
F07	FAM	Neg Ctrl		2681	

Figure 62. Layout of the End Point analysis tab.

The Results list includes this information:

- **Lowest RFU value.** Lowest RFU value in the data
- **Highest RFU value.** Highest RFU value in the data
- **Negative Control Average.** Average RFU for the wells that contain negative controls
- **Cut Off Value.** Calculated by adding the tolerance (RFU or Percentage of Range listed in the Settings) and the average of the negative controls. Samples with RFUs that are greater than the cut off value will be called “Positive”. To adjust the cut off value, change the RFU or Percentage of Range

The Cut Off Value is calculated using this formula:

$$\text{Cut Off Value} = \text{Negative Control Average} + \text{Tolerance}$$

Select a tolerance by one of these methods:

- **RFUs (default).** Select this method to use an absolute RFU value for the tolerance. The minimum RFU tolerance value is 2. The maximum is the absolute value of the highest RFU value minus the absolute value of the lowest RFU value. The default RFU tolerance value is 10% of the total RFU range
- **Percent of Range.** Select this method to use a percentage of the RFU range for the tolerance. The minimum percent of range is 1 percent. The maximum percent of range is 99 percent. The default percent of range is 10 percent

Adjusting the End Point Data Analysis

Adjust the information shown in the End Point tab by following these methods:

- Choose a **Fluorophore** from the pull-down list to view the data
- Choose an **End Cycle to Average** value to set the number of cycles that the software uses to calculate the average end point RFU
- Select **RFUs** to view the data in relative fluorescence units
- Select **Percentage of Range** to view the data as a percentage of the RFU range
- Select wells in the well selector to focus on subsets of the data
- Select a well group (page 53) to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group pull-down menu in the toolbar

Data Description for End Point Analysis

Table 33 list the type of information shown in the spreadsheet in the End Point tab.

Table 33. Contents of the End Point spreadsheet

Information	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	A combination of the Sample type and Replicate #
End RFU	RFU at the end point cycle
Call	Positive or Negative, where positive samples have an RFU value greater than the average RFU of the negative controls plus the Cut Off Value
Sample	Sample Name loaded in the Plate Editor

Allelic Discrimination Tab

The Allelic Discrimination tab assigns the genotypes to wells with unknown samples using the RFU or C(t) of positive control samples (Figure 63). Use this data to identify samples with different genotypes, including Allele 1, Allele 2, Heterozygote, Unknown, Control 1, or Control 2.

> NOTE: The data for allelic discrimination must come from multiplex experiments with at least two fluorophores. Each fluorophore identifies one allele in all samples.

Allelic discrimination analysis requires the following minimal well contents:

- Two fluorophores in each well, except the wells that contain positive controls can contain only one fluorophore
- One fluorophore that is common to all wells in the well group
- NTC (no template control) samples if you want to normalize the data

The software displays allelic discrimination data in these layouts:

- **RFU or C(t) chart.** View the data in a graph of RFU or C(t) for Allele 1/Allele 2. Each point in the graph represents data from a single fluorophore in one well
- **Well spreadsheet.** Shows a spreadsheet listing the allelic discrimination data collected in each well of the plate
- **Well selector.** Select the wells with the end point data you want to show

- **Well spreadsheet.** Shows a spreadsheet listing the allelic discrimination data collected in the selected wells

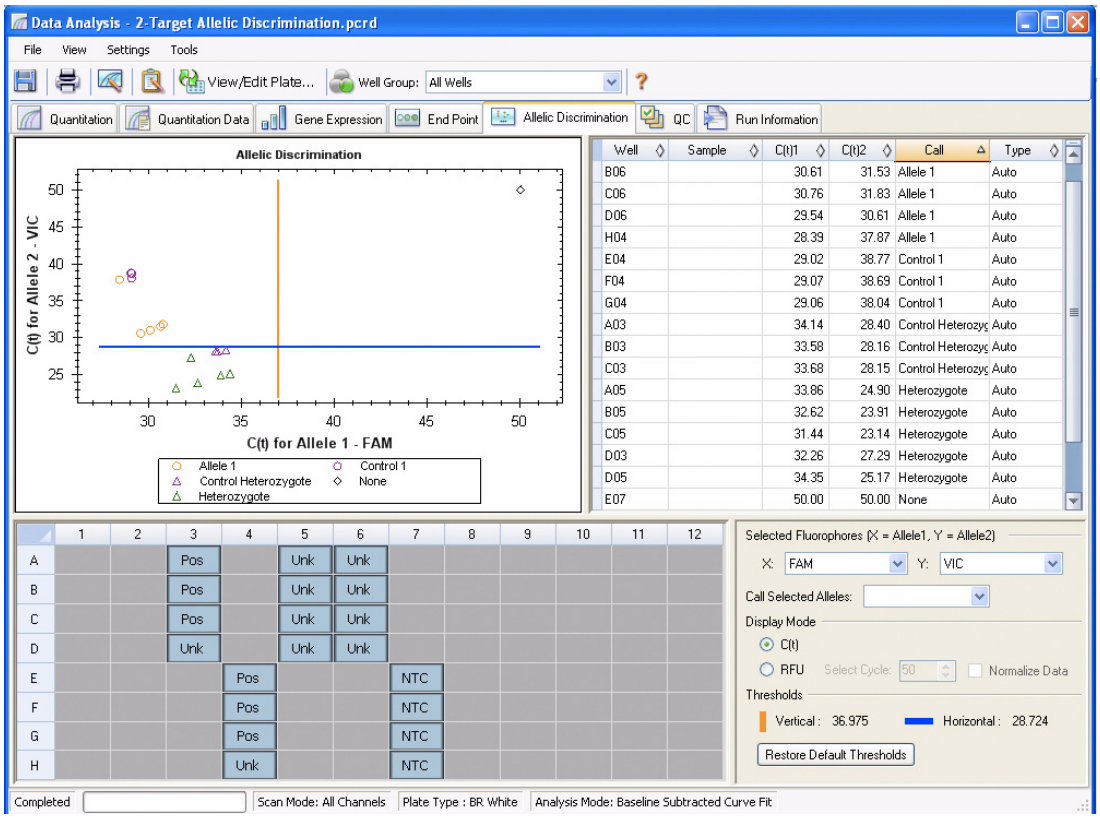


Figure 63. Layout of the Allelic Discrimination tab in the Data Analysis window.

Adjusting Data for Allelic Discrimination

The software automatically assigns a genotype to wells with unknown samples based on the positions of the vertical and horizontal threshold bars, and then lists genotype calls in the spreadsheet view. To automatically call genotypes, the software uses positive controls (when available), or estimates the thresholds. The software takes an average C(t) or RFU for the positive controls to automatically set the threshold lines for discriminating the alleles.

Adjust the position of the threshold bars by clicking and dragging them, and the software automatically adjusts the calculations to make new genotype assignments:

- If the experiment contains three controls in the plate, then the position of the threshold bars is based on the mean and standard deviation of the RFU or C(t) of the controls
- If the number of controls is less than three, then the position of the threshold bars is determined by the range of RFU or threshold cycle values in the selected fluorophore

Adjust allelic discrimination data by following any of these methods:

- Click and drag the threshold bars in the Allelic Discrimination chart to adjust the calls in the spreadsheet
- Select a fluorophore for each axis in the chart (**X:** and **Y:**) in the settings options on the bottom right of the window

- Change a call manually by highlighting a row in the spreadsheet, and then selecting an option in the Call Selected Alleles list (including Allele 1, Allele 2, Heterozygote, None, Unknown, Control 1, or Control 2)
- Click the **Restore Default Thresholds** button to restore the vertical and horizontal bars to their original position, which are indicated by the numbers next to the bars
- Select the **C(t) Display Mode** to view the data as threshold levels. Select **RFU Display Mode** to view the data in relative fluorescence units at the selected cycle
- Select **Normalize Data** to normalize the RFU data shown in the chart and spreadsheet

Normalization changes the data on the chart to a range from 0 to 1 on both axes. To normalize the data, the plate must contain wells with "no template control" (NTC) sample types for both Allele 1 and Allele 2. For this plot, the RFU data are normalized to the NTC values as a linear combination of Allele 1- and Allele 2-specific RFUs. This plot is an effective way to present RFU data.

The calculations for normalized RFU follows the formulas presented in Livak et al. (1995).

$$\text{Normalized } A_1 = \frac{A_1}{A_1 + A_2 + \bar{x}(NTC_{A1+A2})}$$

Where:

- A_1 represents RFU for Allele 1
- A_2 represents RFU for Allele 2
- $\bar{x}$ represents the mean RFU
- NTC_{A1+A2} represents the sum of RFUs for the NTC sample of Allele 1 and Allele 2

Allelic Discrimination tab Spreadsheet

The Allelic Discrimination spreadsheet at the top right side of the Allelic Discrimination tab shows the information shown in Table 34.

Table 34. Contents of the Allelic Discrimination spreadsheet

Information	Description
Well	Well position in the plate
RFU1 or C(t)1	RFU or C(t) for Allele1
RFU2 or C(t)2	RFU or C(t) for Allele2
Call	Identity of the allele, including automatic Allele 1, Allele 2, Heterozygote, None, Unknown, Control 1, Control 2
Type	Auto (Automatic) or Manual. Describes the way the call was made. Automatic means the software selected the call. Manual means the call was chosen by the user

QC Tab

Open the **QC** tab to quickly assess the quality of the experimental data based on the rules defined in the QC tab in the User Preferences window (see "QC Tab" on page 108).

The software displays QC information in these layouts (Figure 64).

- **Amplification chart.** Shows the RFU for each well at every cycle. Each trace in the chart represents data from a single fluorophore in one well
- **QC rules.** Shows the currently applied QC rules and the settings that define each rule

  NOTE: You can turn on or turn off rules by clicking the check box next to the rule in the Use Rule column.
- **Well selector.** Selects the wells with the fluorescence data you want to show
- **Rule Description.** Shows the selected QC rule and highlights wells that fail the rule

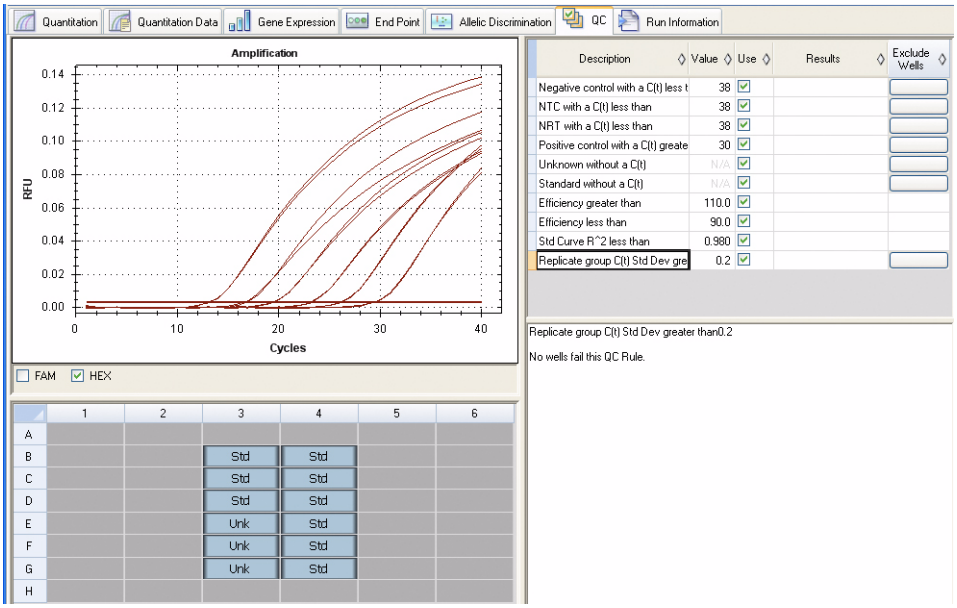


Figure 64. QC tab layout.

Run Information Tab

The Run Information tab (Figure 65) shows the protocol and other information about the run for each experiment. Open this tab for the following options:

- View the protocol
- Enter and edit the Notes. Enter or edit notes about the experiment and run by typing in the Notes box
- Enter and edit the data ID for the run by typing in the ID box

- View the **Other** section to see events, such as error messages, that might have occurred during the run. View these messages to help troubleshoot a run

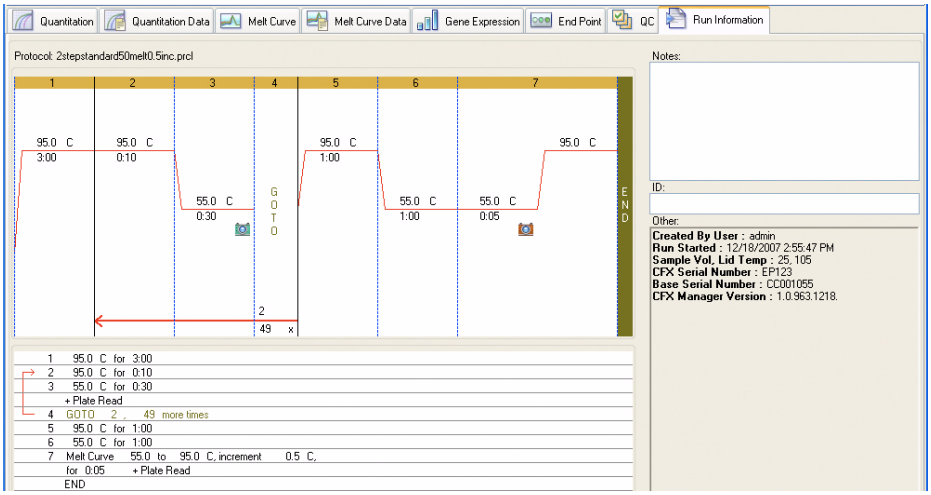


Figure 65. Layout of the Run Information tab in the Data Analysis window.

Reports for Data Files

The Report window (Figure 66) shows information about the current data file in the Data Analysis window. To open a report, select **Tools > Reports**, or click the **Reports** button on the toolbar in the Data Analysis window.

The Report window shows these three sections:

- **Menu and toolbar.** Select options to format, save and print the report or template
- **Options list (top, left side of window).** Select options to show in the report
- **Options pane (bottom, left side of window).** Enter information about a selected option
- **Preview pane (right side of window).** View the current report in a preview

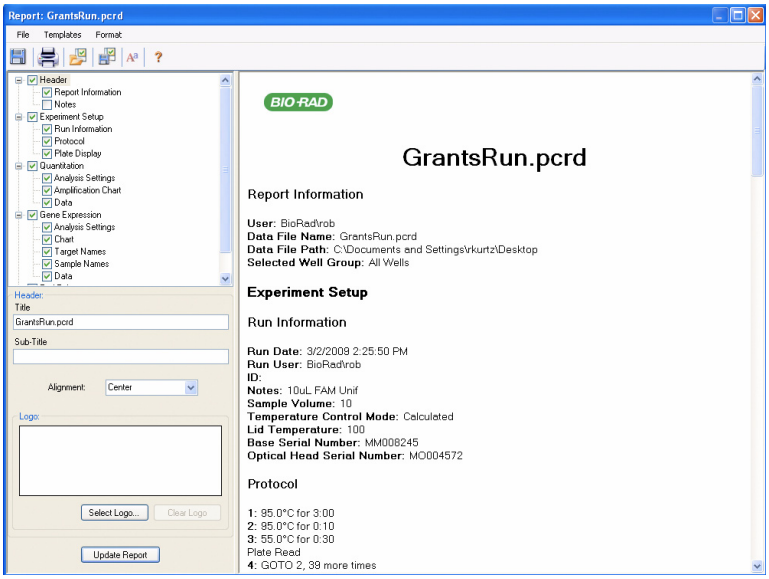


Figure 66. Example of a Report window for a data file.

TIP: The layout of the report can define the type of information that appears in any report if you save the report as a template. Select **Template > Save** or **Save As** to save the layout of the current report as a template.

Creating a Data Analysis Report

To create a report in the Data Analysis window, follow these steps:

1. Make final adjustments to the well contents, selected wells, charts, and spreadsheets in the Data Analysis window before creating the report.
2. Click **Report** in the Data Analysis toolbar to open the Report window.
3. Change the options you want to include in the report. The report opens with default options selected. Click the check boxes in the report options list to change whole categories or individual options within a category.

   NOTE: The data that appear in the report are dependent on the current selections within the tabs of the Data Analysis window. For example, a quantitation experiment might not contain a standard curve, and therefore those data do not appear in the Data Analysis window or in the data report.
4. Click **Update Report** to update the Report Preview with any changes.
5. Print or save the report. Click **Print** in the toolbar to print the current report. Select **File > Save** to save the report as a PDF (Adobe Acrobat Reader file), MHT (Microsoft document), or MHTML (Microsoft document) formatted file and select a location to store the file. Select **File > Save As** to save the report with a new name or in a new location.
6. (Optional) Create a report template with the information you want. To save the current report settings in a template, select **Template > Save** or **Save As**. Then load the report template the next time you want to make a new report.

Data Analysis Report Categories

A report can include any of the options in each category described in Table 35, depending on the type of data in Data Analysis window.

Table 35. Data analysis report categories in the options list

Category	Option	Description
Header		Title, subtitle and logo for the report
	Report Information	Experiment date, user name, data file name, data file path, and selected well group
	Notes	Notes about the data report
Experiment Setup		
	Run Information	Includes the experiment date, user, data file name, data file path, and the selected well group
	Protocol	Text view of the protocol steps and options
	Plate Display	Show a plate view of the information in each well of the plate

Table 35. Data analysis report categories in the options list (continued)

Category	Option	Description
Quantitation		
	Analysis Settings	Includes the step number when data were collected, the analysis mode, and the baseline subtraction method
	Amplification Chart	Copy of the amplification chart for experiments that include quantitation data
	Standard Curve Chart	Copy of the standard curve chart
	Data	Spreadsheet listing the data in each well
Gene Expression		
	Analysis Settings	Includes the analysis mode, chart data, scaling option, and chart error
	Chart	Copy of the gene expression chart
	Target Names	Chart of the names
	Sample Names	Chart of the names
	Data	Spreadsheet listing the data in each well
Melt Curve		
	Analysis Settings	Includes the melt step number and threshold bar setting
	Melt Curve Chart	Copy of the melt curve chart
	Melt Peak Chart	Copy of the melt peak chart
	Data	Spreadsheet listing the data in each well
Allelic Discrimination		
	Analysis Settings	Includes display mode, fluorophores, cycle, thresholds, and normalized data
	Chart	Copy of the allelic discrimination chart
	Data	Spreadsheet listing the data in each well
End Point		
	Analysis Settings	Includes fluorophore, end cycles to average, mode, lowest RFU value, highest RFU value, and cut off value
	Data	Spreadsheet listing the data in each well

8 Gene Expression Analysis

Read this chapter for information about performing Gene Expression Analysis:

Overview of Gene Expression Analysis

With the use of stringently qualified controls in your reactions, you can run a gene expression experiment to normalize the relative differences in a target concentration between samples. Typically, message levels for one or more reference genes are used to normalize the expression levels of a gene of interest. Reference genes take into account loading differences or other variations represented in each sample, and they should not be regulated in the biological system being studied.

Open the Gene Expression tab to evaluate relative differences between PCR reactions in two or more wells. For example, you can evaluate relative numbers of viral genomes, or relative number of transfected sequences in a PCR reaction. The most common application for gene expression study is the comparison of cDNA concentration in more than one reaction to estimate the levels of steady state messenger RNA.

The software calculates the relative expression level of a target with one of these scenarios:

- Relative expression level of a target sequence (Target 1) relative to another target (Target 2). For example, the amount of one gene relative to another gene under the same sample treatment
- Relative expression level of one target sequence in one sample compared to the same target under different sample treatments. For example, the relative amount of one gene relative to itself under different temporal, geographical, or developmental conditions

Plate Setup for Gene Expression Analysis

To perform gene expression analysis, the contents of the wells must include the following:

- **Two or more targets.** The two targets that represent different amplified genes or sequences in your samples
- **One or more reference targets.** At least one target must be a reference target for normalized expression. Assign all reference targets in the Experiment Settings window (page 44) to analyze the data in Normalized Expression mode (ΔΔC(t)). Experiments that do not contain a reference must be analyzed using Relative Expression mode (ΔC(t))
- **Common samples.** Your reactions must include common samples (minimum of two required) to view your data plotted in the Gene Expression tab. These samples represent different treatments or conditions for each of your target sequences. Assign a control sample (optional) in the Experiment Settings window (page 44)

The requirements for Gene Expression setup in the Plate Editor depend on whether reaction contents are **singleplex PCR** with one fluorophore in the reactions, or **multiplex PCR** with more than one fluorophore in the reactions.

Figure 67 shows an example of the minimum contents of the wells for a singleplex gene expression experiment.

Unk	Unk
Target1	Target1
Sample1	Sample2
Unk	**Unk**
Target2	Target2
Sample1	Sample2

Figure 67. Example of well contents in a singleplex gene expression experiment.

Figure 68 shows an example of the minimum contents of the wells for a multiplex gene expression experiment.

Unk	Unk
Target1	Target1
Target2	Target2
Sample1	Sample2

Figure 68. Example of well contents in a multiplex gene expression experiment.

Gene Expression Tab

The Gene Expression tab (Figure 69) in the Data Analysis window shows the relative expression of targets in these two views:

- **Gene Expression chart.** Shows the real-time PCR data as normalized expression (ΔΔC(t)) or relative quantity (ΔC(t))
- **Spreadsheet.** Shows a spreadsheet of the gene expression data

  TIP: Right-click any chart or spreadsheet for options. Click **View/Edit Plate** to open the Plate Editor, and change well contents in the plate.

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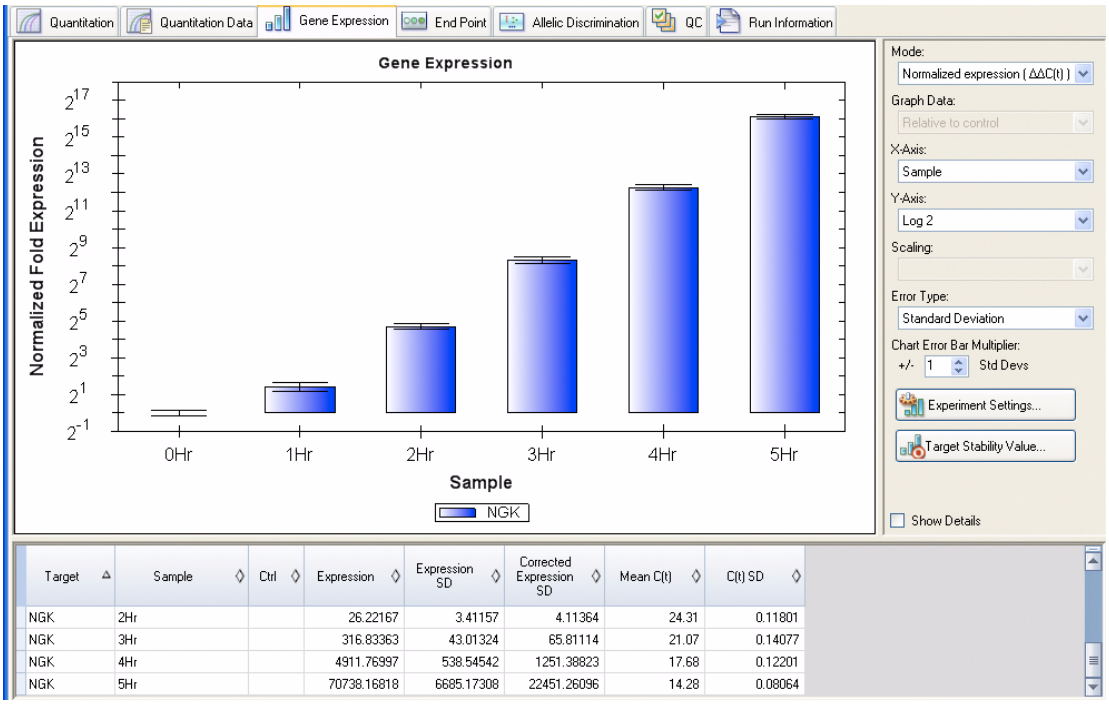


Figure 69. Layout of the Gene Expression tab in the Data Analysis window.

TIP: Right-click on the chart to select right-click menu options. Select **Sort** from this menu to rearrange the order of the Target and Sample names in the chart.

Normalized Gene Expression

To normalize data use the measured expression level of one or more reference genes (targets) as a normalization factor. Reference genes are targets that are not regulated in the biological system being studied, such as actin, GAPDH, or histone H3.

To set up normalized gene expression (ΔΔC(t)) analysis, follow these steps:

1. Open a data file (.pcrd extension).
2. Review the data in the Quantitation tab of the Data Analysis window. Make adjustments to the data, such as changing the threshold and the Analysis Mode.
3. Click the **Gene Expression** tab.
4. Choose a control in the **Samples** tab of the Experiment Settings window. If a control is assigned, the software normalizes the relative quantities for all genes to the control quantity, which is set to 1.
5. Select reference genes for this experiment in the Target tab of the Experiment Settings window. Gene expression analysis requires one reference among the targets in your samples.
6. Select **Normalized Expression (ΔΔC(t))** if it is not already selected, and then view the expression levels in the Gene Expression tab.

Relative Quantity

By definition, relative quantity (ΔC(t)) data are not normalized. This method is used to quantitate samples that do not include any reference genes (targets). Typically, researchers are confident in one of the following considerations when they set up their experiment:

- Each sample represents the same amount of template in each biological sample, possibly the same mass of RNA or cDNA in each well
- Any variance in the amount of biological sample loaded is normalized after the run by some method in the data analysis outside of the software. For example, a researcher might choose to simply divide the relative quantity value by the normalizing factor, possibly the mass of nucleic acid loaded for each sample, or the number of cells from which the nucleic acid was isolated

Select **Relative Quantity (ΔC(t))** from the pull-down menu in the chart controls of the Gene Expression tab to run a Relative Quantity (ΔC(t)) analysis.

> TIP: To compare results to data from other gene expression experiments, open a new Gene Study (page 90), or add a data file to an existing Gene Study.

Adjusting Gene Expression Data

After selecting your analysis method, adjust the data you view in the Gene Expression tab by changing the settings options to the right of the chart.

Graph Data

Graph data options allow you to present the data in the graph with one of these two options:

- **Relative to control.** Graph the data with the axis scaled from 0 to 1. If you assign a control in your experiment, select this option to quickly visualize upregulation and downregulation of the target
- **Relative to zero.** Graph the data with the origin at zero

X-Axis Options

The x-axis option allows you to select the x-axis data of the Gene Expression graph:

- **Target.** Select this option to graph the target names on the x-axis
- **Sample.** Select this option to graph the sample names on the x-axis

Y-Axis Options

The y-axis option allows you to show the Gene Expression graph in one of these three scales:

- **Linear.** Select this option to show a linear scale
- **Log 2.** Select this option to evaluate samples across a large dynamic range
- **Log 10.** Select this option to evaluate samples across a very large dynamic range

Scaling Options

Select **Normalized Gene Expression (ΔΔC(t))** to activate the scaling options in the Gene Expression graph. Select one of these scaling options to calculate and present your data in a manner that best suits your experimental design:

- **Unscaled expression.** This option presents the unscaled normalized gene expression

- **Highest expression.** Scale the normalized gene expression to the highest for each target by dividing the expression level of each sample by the highest level of expression in all the samples. This scaling option uses the scaled to highest formula
- **Lowest expression.** Recalculate the normalized gene expression for each target by dividing the expression level of each sample by the lowest level of expression in all the samples. This scaling option uses the scaled to lowest formula

Error Type

Select an option for the type of error calculations (error bars) in the Gene Expression graph:

- Standard Error of the Mean (default, SEMs)
- Standard Deviation (Std Devs)

Chart Error Bar Multiplier

Select a multiplier for the error bars in the Gene Expression graph. Select one of these integers: +/- 1 (default), 2, or 3. The type of multiplier changes when you select the Error Type:

- SEMs for Standard Error of the Mean
- Std Devs for Standard Deviations

Target Stability Value

Open this window whenever more than 1 reference gene is used. The software calculates two quality parameters for the reference genes:

- **Coefficient of Variation (CV)** of normalized reference gene relative quantities. Lower CV values denotes higher stability
- **M-value**. A measure of the reference gene expression stability

Right-Click Menu Options for Gene Expression Graph

Right-click on the Gene Expression graph to select the items shown in Table 36.

Table 36. Right-click menu items

Item	Function
Copy	Copy the chart to a clipboard
Save as Image	Save the graph in the chart view as an image file. The default image type is PNG. The other selections for image file types include GIF, JPG, TIF, and BMP
Page Setup...	Select a page setup for printing
Print...	Print the chart view
Show Point Values	Display the relative quantity of each point on the graph when you place the cursor over that point
Set Scale to Default	Set the chart view back to the default settings after magnifying it
Chart Options...	Open the Chart Options window to adjust the graph
Sort	Sort the order that samples or targets appear on the x-axis
User Corrected Std Devs	Calculate the error bars using the corrected standard deviation formula
Use Solid Bar Colors	Display solid bars in the graph
x-axis labels	Choose to display x-axis labels horizontal or angled

Gene Expression Spreadsheet

Table 37 describes the information shown in the Gene Expression spreadsheet.

Table 37. Description of information in the spreadsheet on the Gene Expression tab

Information	Description
Target	Target Name (amplified gene) selected in the Experiment Settings window
Sample	Sample Name selected in the Experiment Settings window
Ctrl	Control sample, when the Sample Name is selected as a control in the Experiment Settings window
Expression	Normalized Gene Expression ($\Delta\Delta$C(t)) or Relative quantity (ΔC(t)) depending on the selected mode
Expression SEM (or SD)	Standard Error of the Mean or Standard Deviation, depending on the selected option
Corrected Expression SEM (or SD)	Corrected value calculation for Standard Error of the Mean (SEM) or Standard Deviation (SD) of the relative expression, depending on the selected option
Mean (C(t))	Mean of the threshold cycle
C(t) SEM (or SD)	Standard Error of the Mean or Standard Deviation of the threshold cycle, depending on the selected option

Show Details Option

When you click the Show Details check box, Table 38 also shows this information.

Table 38. Information in Gene Expression spreadsheet with Show Details selected

Information	Description
Data Set	Fluorescence data from one fluorophore in the data file
Relative Quantity	Calculated relative quantity of samples
Relative Quantity SD	Standard deviation of the relative quantity calculation
Corrected Relative Quantity SD	Calculated standard deviation of the corrected relative quantity
Unscaled Expression	Calculated unscaled expression
Unscaled Expression SD	Calculated standard deviation unscaled expression
Corrected Unscaled Expression SD	Calculated standard deviation of the unscaled expression
Expression	Relative expression level
Wells	Well number in the plate

Experiment Settings Window

Open the Experiment Settings window by clicking **Experiment Settings** in the Gene Expression tab. In this window, view or change the list of Targets and Samples, select reference genes, select control samples, or set the Gene Expression Analysis sample group to be analyzed if Collection Names have been added to the wells (Figure 70).

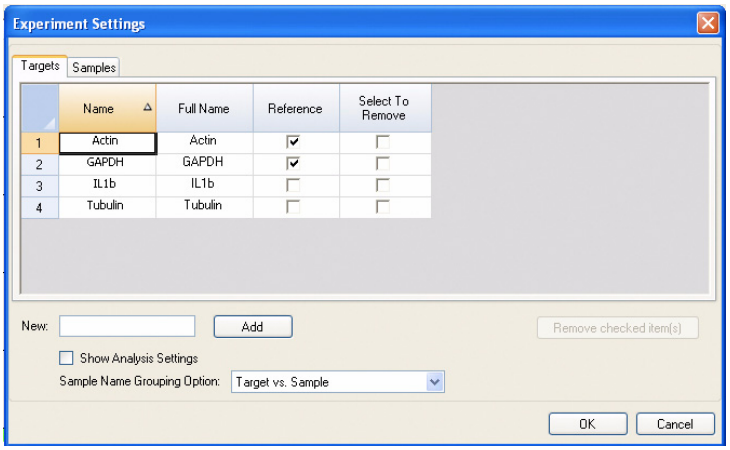


Figure 70. Experiment Settings window with Targets tab selected.

To adjust the lists in these tabs, use the following functions:

- Add a target or sample name by typing a name in the **New** box and clicking **Add**
- Remove a target or sample name from the list by clicking the **Remove Name** box for that row, and then clicking the **Remove checked item(s)** button
- Select the target as a reference for gene expression data analysis by clicking the box in the **Reference** column next to the Name for that target
- Select the sample as a control sample for gene expression data analysis by clicking the box in the **Control** column next to the name for that sample

Sample Name Grouping Option

Loading **Collection Names** in the wells enables samples to be analyzed in one of four configurations defined by the **Sample Name Grouping Option**, providing an option to analyze samples based on biological replicates. For example, a study may include a time course analysis (0 hr, 1 hr, 2 hr, etc) across biological samples (mouse 1, mouse 2, mouse 3, etc.). Researchers may want to analyze results based on the time course, based on the biological samples or by a combination of the two.

These options are available from the pull-down menu in the Experiment Settings tab.

- **Target vs. Sample.** Only the well sample name is used in the gene expression calculations. In the results shown in Figure 71, only the sample name is used in the analysis (0 hr, 1 hr, 2 hr, etc)

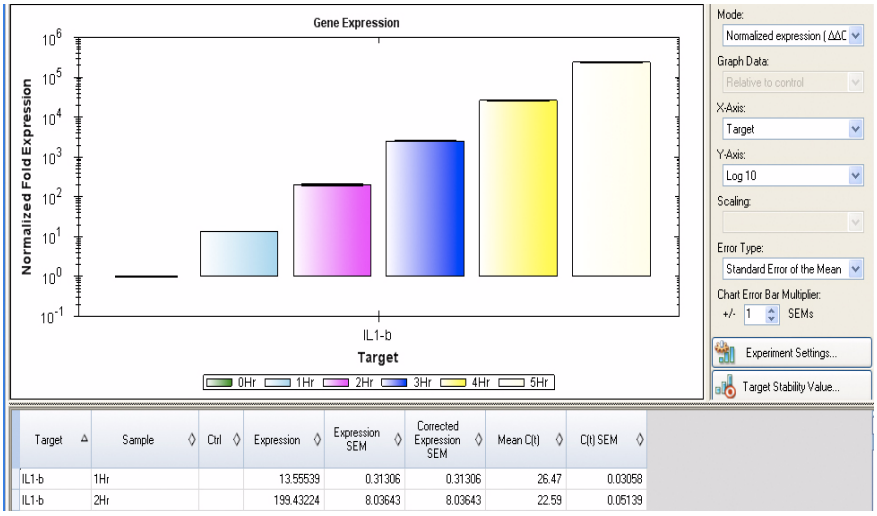


Figure 71. Target vs. Sample option results.

- **Target vs. Collection.** Only the well collection name is used in the calculations
- **Target vs. Sample_Collection.** The sample name and collection name are combined to make a single name that is used in the calculations. The end-result of this analysis is the separation of each sample name to include the collection name (

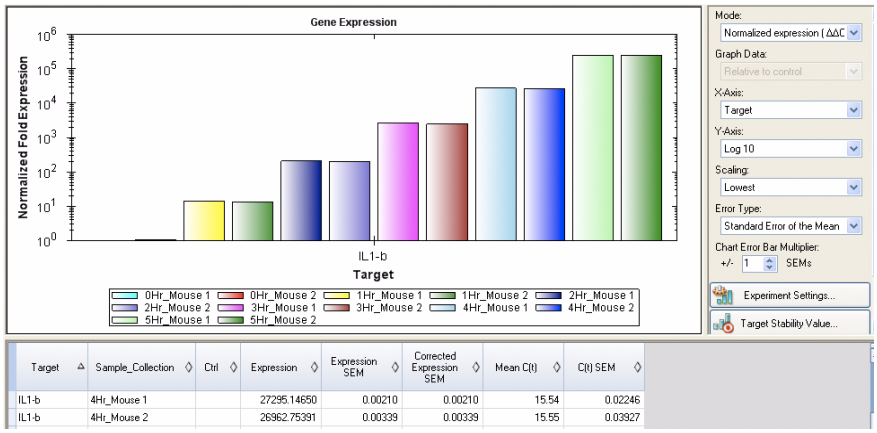


Figure 72. Target vs. Sample_Collection results.

- **Target vs. Collection_Sample.** The collection name and sample name are combined to make a single name that is used in the calculations

Show Analysis Settings in Experiment Settings

Click the **Show Analysis Settings** box in the Experiment Settings window to view or change analysis parameters applied in the Gene Expression tab:

- Click a cell in the **Color** column to change the color of the targets graphed in the Gene Expression chart
- Enter a number for the efficiency of a target. The software calculates relative efficiency for a target using **Auto Efficiency** if the data for a target includes a standard curve. Alternatively, type a previously determined efficiency

Figure 73 shows the efficiency of all the targets, which appear if **Auto Efficiency** is selected.

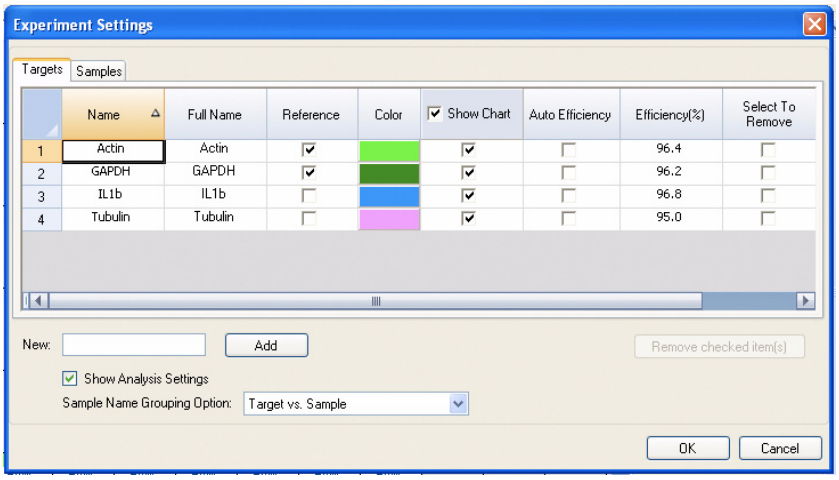


Figure 73. Targets tab in Experiment Settings with Show Analysis Settings selected.

To adjust the settings for a sample in the Samples tab:

- Click a color in the **Color** column to change the color of the samples graphed in the Gene Expression chart
- Click a box in the **Show Chart** column to show the sample in the Gene Expression chart using a color that is selected in the Color column

Figure 74 shows the samples with the **Show Chart** option selected.

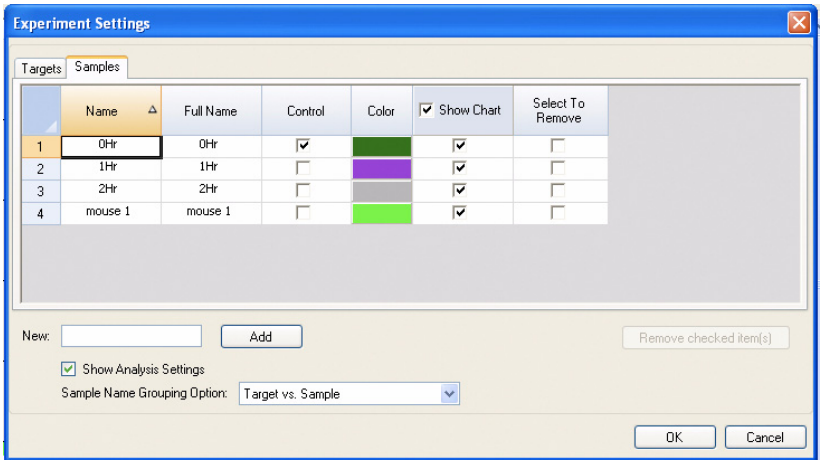


Figure 74. Samples tab in Experiment Settings with Show Analysis Settings selected.

Performing a Gene Study

Create a Gene Study to compare gene expression data from one or more real-time PCR experiments using an inter-run calibrator to normalize between the experiments. Create a Gene Study by adding data from one or more data files (.pcrd extension) to the Gene Study, the software groups them into a single file (.mgxd extension).

NOTE: The gene expression data must include a common sample in every data file to create a Gene Study. The software uses the common sample to normalize the

data between experiments. Select the sample names in the Experiment Settings window (page 44).

NOTE: The maximum number of samples you can analyze in a Gene Study is limited by the size of the computer's RAM and virtual memory.

Gene Study Inter-Run Calibration

All data within the Gene Study are normalized by inter-run calibrator to calculate the smallest average ΔC(t) value. When the data files within the Gene Study include more than one inter-run calibrator, then the calibrator with the smallest average ΔC(t) value becomes the dominant inter-run calibrator. The dominant calibrator is used to adjust all C(t) values in the Gene Study.

To find the dominant inter-run calibrator, the software calculates the average of the ΔC(t) values for all inter-run calibrators of a given target (gene), and then uses a multitiered algorithm to determine the dominant inter-run calibrator within all the data. The algorithm for finding the dominant inter-run calibrator includes the following hierarchy:

1. Set the dominant calibrator to the target with the highest number of common replicate groups in a given pair-wise comparison.
2. If any target has the same number of common replicate groups, then set the dominant calibrator to the target with the smallest range of ΔC(t) values in pair-wise comparisons. The range is examined by comparing the absolute value of the difference between the maximum and minimum ΔC(t) for the inter-run calibrators of a given target.
3. If any target has an identical range as the ΔC(t) values, set the dominant calibrator to the target with the smallest absolute value of average ΔC(t) for eligible inter-run calibrator samples.
4. If any target has identical average ΔC(t) absolute values, set the dominant calibrator to the replicate group with the smallest ΔC(t).

   NOTE: The first data file imported into the Gene Study always serves as the “hub” file for pairwise data comparison during inter-run calibration.

Gene Study Window

The Gene Study window includes two tabs:

- **Study Setup tab.** Click this tab to manage the experiments in the Gene Study. Adding or removing data files in a Gene Study does not change the original data in that file
- **Study Analysis tab.** Click this tab to view the gene expression data for the combined experiments

Figure 75 shows the Gene Study window, including the Study Setup and Study Analysis tabs.

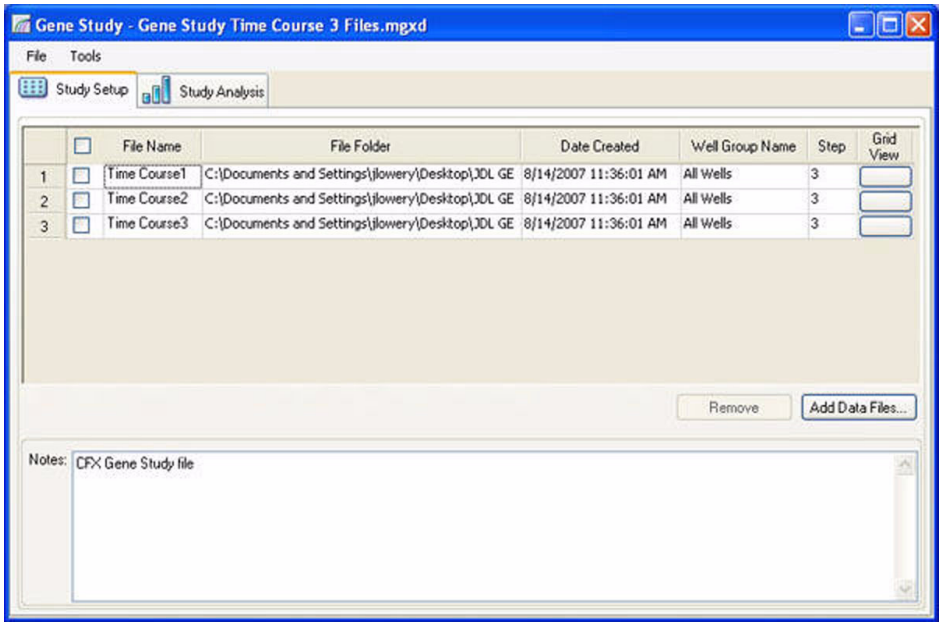


Figure 75. Gene Study window.

Study Setup Tab

Before importing data into a Gene Study, do the following in the Data Analysis window:

- Check that samples that contain the same content are named with the same name. In a Gene Study, the software assumes that wells with the same Target or Sample name contain the same sample
- Adjust the baseline and threshold (C(t)) in the Quantitation tab to optimize the data in each experiment before you add them to a Gene Study
- Select the well group you want to include in the Gene Study

The Study Setup tab (Figure 75) shows a list of all the experiments in the Gene Study.

- **Add experiments.** Click **Add Data Files** to select a file from a browser window. To quickly add experiments to a Gene Study, drag the data files (.pcrd extension) to the Gene Study window

  TIP: In order to show data from one well group in the Gene Study, that group must be selected before importing the Data file.
- **Remove experiments from this Gene Study.** Select one or more files in the list and click **Remove**
- **Add notes about the Gene Study.** Type in the Notes box to add comments about the files and analysis in this Gene Study

The Study Setup tab lists the data files in the Gene Study, as described in Table 39.

Table 39. Study Setup tab in Gene Study window

Column Title	Description
File Name	Name of the experiment data file (.pcrd extension)
File Folder	Directory that stores the data file for each experiment in the Gene Study
Date Created	Date the run data were collected
Well Group Name	Name of the well group that was selected when the file was added to the Gene Study TIP: In order to analyze one well group in the Gene Study, that well group must be selected in the Data Analysis window before importing the data file into the Gene Study
Step	Protocol step that included the plate read to collect real-time PCR data
Grid View	Open a plate map of the plate with the data in each of the experiments included in the Gene Study

Study Analysis Tab

The Study Analysis tab shows the data from all experiments that are added to the Gene Study. Open this tab to analyze the data, and select these options for the Gene Expression chart:

- **Mode.** Select **Normalized Expression (ΔΔC(t))** or **Relative Quantity (ΔC(t))**
- **Graph Data.** Select **Relative to normal** or **Relative to control** in the graph
- **X-axis options.** Select the labels on the x-axis of the graph, including Sample or Target
- **Y-axis options.** Change the labels on the y-axis of the graph, including Linear, Log 2, or Log 10
- **Scaling Options.** Choose **Highest** or **Lowest** value, or leave the data **Unscaled**. This option is only available when your samples do not contain controls
- **Graph Error.** Select the multiplier for standard deviation bars in the graph, including ±1, 2, or 3
- **Experiment Settings button.** Choose the show options for targets and samples in the Experiment Settings window
- **Show Details check box.** Click **Show Details** to add more columns of data to the chart

Highlighting a sample in the Gene Expression chart highlights the corresponding cell in the spreadsheet below the chart (Figure 76).

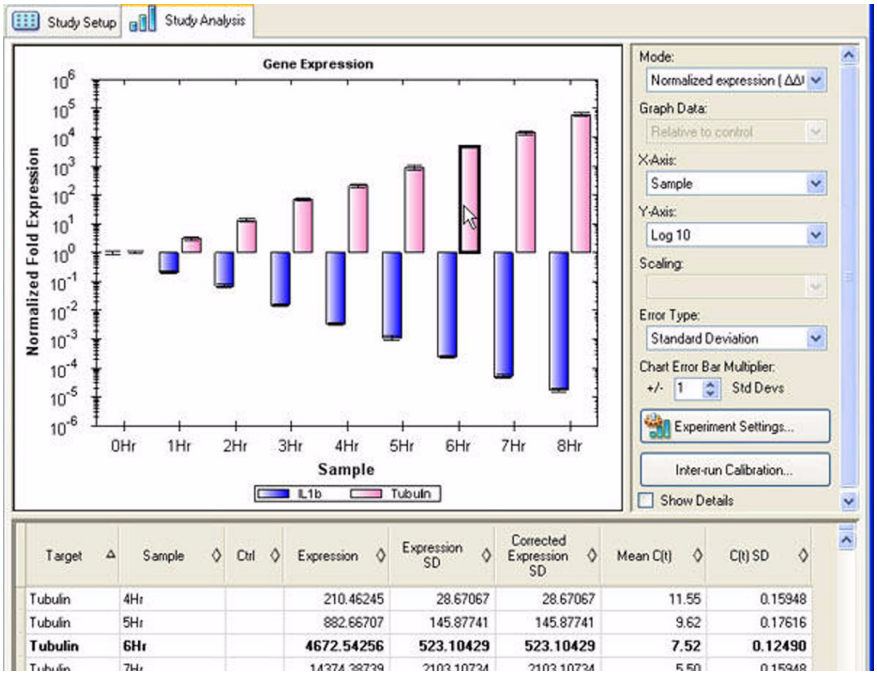


Figure 76. Study Analysis tab in Gene Study window.

Gene Study Data Spreadsheet

The data spreadsheet in the Gene Study window lists information about each target and sample in the Gene Study (Figure 76).

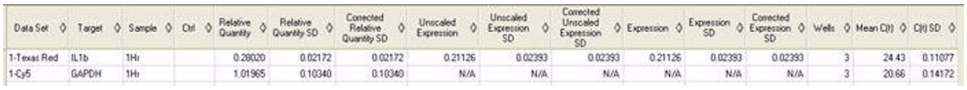
Table 40 describes the information shown in the Gene Study spreadsheet.

Table 40. Information in the spreadsheet on the Study Analysis tab

Information	Description
Target	Target Name (amplified gene) selected in the Experiment Settings window
Sample	Sample Name selected in the Experiment Settings window
Ctrl	Control sample, when the sample name is selected as a control in the Experiment Settings window
Expression	Normalized Gene Expression (ΔΔC(t)) or Relative Quantity (ΔC(t)) depending on the selected mode
Expression SEM (or SD)	Standard Error of the Mean or Standard Deviation, depending on the selected option
Corrected Expression SEM (or SD)	Corrected value calculation for Standard Error of the Mean (SEM) or Standard Deviation (SD) of the relative expression, depending on the selected option
Mean (C(t))	Mean of the threshold cycle
C(t) SEM (or SD)	Standard Error of the Mean or Standard Deviation of the threshold cycle, depending on the selected option

Show Details Data

Click the Show Details check box to show additional information (Figure 77).



Data Set	Target	Sample	Ctrl	Relative Quantity	Relative Quantity SD	Corrected Relative Quantity SD	Unscaled Expression	Unscaled Expression SD	Corrected Unscaled Expression SD	Expression	Expression SD	Corrected Expression SD	Wells	Mean Ct(t)	Ct(t) SD
1-Texas Red	IL1b	1Hr		0.28020	0.02172	0.02172	0.21126	0.02393	0.02393	0.21126	0.02393	0.02393	3	24.43	0.11077
1-Cy5	GAPDH	1Hr		1.01965	0.10340	0.10340	N/A	N/A	N/A	N/A	N/A	N/A	3	20.66	0.14172

Figure 77. Show Details data in the Gene Study tab.

The spreadsheet adds the information in the columns listed in Table 41.

Table 41. Information added to the spreadsheet when Show Details selected

Information	Description
Data Set	Fluorescence data from one fluorophore in one data file
Relative Quantity	Calculated relative quantity of samples
Relative Quantity SD	Standard deviation of the relative quantity calculation
Corrected Relative Quantity SD	Calculated standard deviation of the corrected relative quantity
Unscaled Expression	Calculated unscaled expression
Unscaled Expression SD	Calculated standard deviation unscaled expression
Corrected Unscaled Expression SD	Corrected standard deviation of the unscaled expression
Expression	Relative expression
Wells	Well number in the plate

Gene Study Report Window

Open the Gene Study Report window to arrange the Gene Study data into a report. To create a gene study report, follow these steps:

1. Adjust the Gene Study report data and charts, as needed, before creating a report.
2. Select **Tools > Reports** to open the Gene Study report window.
3. Click the check boxes in the report options list to select and remove options to choose the data to display. Select the options shown in Table 42.

Table 42. Categories for a Gene Study report

Category	Option	Description
Header		Title, subtitle, and logo for the report
	Report Information	Date, user name, data file name, data file path, and the selected well group
	Gene Study File List	List of all the data files in the Gene Study
	Notes	Notes about the data report
Analysis Parameters		A list of the selected analysis parameters
Chart		Gene Expression chart showing the data
Target Names		List of targets in the Gene Study
Sample Names		List of samples the Gene Study
Data		Spreadsheet that shows the data

Table 42. Categories for a Gene Study report (continued)

Category	Option	Description
Inter-Run Calibration		Inter-run calibration data

4. Fill in the text for the report by entering text and images in option panes (Figure 78).

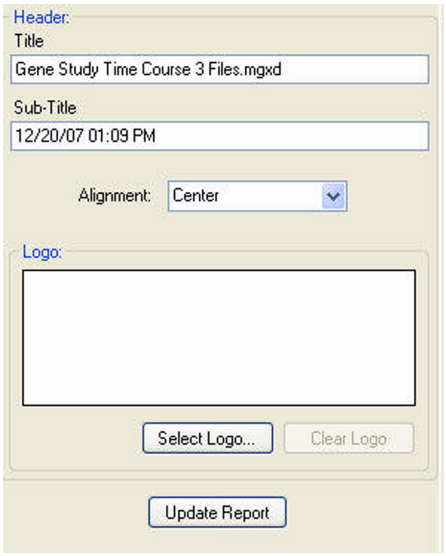


Figure 78. Example of Header and Logo options in a Gene Study report.

5. Click **Update Report** to update the report preview pane. The report preview pane shows a view of the Report.
6. Print or save the report. Click **Print** in the toolbar to print the current report. Select **File > Save** to save the report as a PDF (Adobe Acrobat Reader file), MHT (Microsoft document), or MHTML (Microsoft document) formatted file and select a location to store the file. Select **File > Save As** to save the report with a new name or in a new location.
7. Create a report template once you create a report with the content you want to include in all reports. To create a template, select **Template > Save** or **Save As** and save the current report as a template.

Frequently Asked Questions

The following list is a series of questions (**Q**) and answers (**A**) about gene expression analysis in CFX Manager software.

Q: Why should I normalize my data?

A: Relative quantity data that is not normalized by some means is difficult to interpret. Imagine the case where you load 1 µg of RNA in one well and 10 ng in the other well. If you perform a relative quantity analysis on the results from such an assay, then the fact that the 10 ng sample has a smaller relative quantity value is irrelevant. It is likely the result of using less RNA and not the result of some biological response.

Q: Why does the formula for relative quantity vary from that outlined in the geNorm web site when a control is selected?

A: This is where the CFX Manager software calculations differ from those outlined on the geNorm web site. In the example on that web site, the results are not scaled to the control until normalized expression is calculated. This is referred to as “re-scaled normalized expression” in the spreadsheet.

Q: How does normalized expression as calculated by CFX Manager software compare to the model introduced by M. Pfaffl (2001)?

A: If you only evaluate one reference gene and one gene of interest, you will get exactly the same results using the CFX Manager software as you would using the model introduced in M. Pfaffl (2001). However, standard deviations might be slightly different.

Q: How does normalized expression calculated by this software compare to the model outlined by Dr. Jo Vandesompele on the geNorm web site?

A: The CFX Manager software uses the models outlined on the geNorm web site and gives you the same results.

Q: Why would I have to assign Target Names (genes) in the Gene Expression tab?

A: If you have not assigned Target Names in your initial plate setup or if you are studying more than five genes, click **View/Edit Plate** to open the Plate Editor and assign target names to the wells in the plate.

Q: Can I customize my target (gene) and sample (condition) names?

A: Yes. Open the Experiment Settings window (page 44) to add names to the Targets or Samples tabs, where you can also enter or remove the full names from the lists. Alternatively, permanently add long lists of names to the Libraries for target and sample names in the Plate tab in the User Preferences window (page 105). These names appear on the axis in various chart views, including gene expression.

Q: How do I determine reaction efficiencies?

A: Typically the efficiency for each primer (or primer/probe) set is evaluated and recorded during assay development. Generate a standard curve using serial dilutions of a representative sample across a relevant dynamic range, and then record the efficiency for subsequent gene expression analysis.

Gene Expression Calculations

CFX Manager software calculates formulas automatically and displays the resulting information in the Data Analysis tabs.

Reaction Efficiency

Evidence suggests that using accurate measure of efficiencies for each primer and probe sets gives you more accurate results when analyzing gene expression data. The default value of efficiency used in the gene expression calculations is 100%. To evaluate the reaction efficiency, generate a standard curve using serial dilutions of a representative sample across a relevant dynamic range, and then record the efficiency for subsequent gene expression analysis. If your experiment includes a standard curve, the software automatically calculates the efficiency and displays it under the Standard Curve on the Quantitation tab when Auto Efficiency is checked in the Targets tab in the Experiment Settings window.

The efficiency formulas refer to the “efficiencies” as described by Pfaffl (2001) and Vandesompele et al. (2002). In these publications, an efficiency of 2 (perfect doubling with every cycle) is equivalent to 100% efficiency in CFX Manager software. You have the option to convert your efficiency calculations to those used in the software by using the following mathematical relationships:

- E = (% Efficiency * 0.01) + 1
- % Efficiency = (E - 1) * 100

Relative Quantity

The relative quantity (ΔC(t)) for any sample (GOI) is calculated with this formula:

$$\text{Relative Quantity}_{\text{sample (GOI)}} = E_{\text{GOI}}^{(C_{T\,(\text{MIN})} - C_{T\,(\text{sample})})}$$

Where:

- E = Efficiency of primer and probe set. This efficiency is calculated with the formula (% Efficiency * 0.01) + 1, where 100% efficiency = 2
- $C_{T\,(\text{MIN})}$ = Average C(t) for the Sample with the lowest average C(t) for GOI
- $C_{T\,(\text{sample})}$ = Average C(t) for the Sample
- GOI = Gene of interest (one target)

Relative Quantity When a Control Is Selected

When a control sample (control) is assigned, then the relative quantity (RQ) for any sample with a gene of interest (GOI) is calculated with this formula:

$$\text{Relative Quantity}_{\text{sample (GOI)}} = E_{\text{GOI}}^{(C_{T\,(\text{control})} - C_{T\,(\text{sample})})}$$

Where:

- E = Efficiency of primer and probe set. This efficiency is calculated with the formula (% Efficiency * 0.01) + 1, where 100% efficiency = 2
- $C_{T\,(\text{control})}$ = Average C(t) for the control sample
- $C_{T\,(\text{sample})}$ = Average C(t) for any samples with a GOI
- GOI = Gene of interest (one target)

Standard Deviation of the Relative Quantity

The standard deviation of the relative quantity is calculated with the following formula:

$$\text{SD Relative Quantity} = \text{SD C(t)}_{\text{GOI}} \times \text{Relative Quantity}_{\text{Sample X}} \times \text{Ln}\,(E_{\text{GOI}})$$

Where:

- SD Relative Quantity = standard deviation of the relative quantity
- SD C(t) GOI = Standard deviation of the C(t) for the sample (GOI)
- Relative Quantity Sample X = Relative quantity of the sample
- E = Efficiency of primer and probe set. This efficiency is calculated with the formula (% Efficiency * 0.01) + 1, where 100% efficiency = 2
- GOI = Gene of interest (one target)

Normalization Factor

The denominator of the normalized expression equation is referred to as the normalization factor. The normalization factor is the geometric mean of the relative quantities of all the reference targets (genes) for a given sample, as described in this formula:

$$\text{Normalization Factor}_{\text{sample (GOI)}} = (\text{RQ}_{\text{sample (Ref 1)}} \times \text{RQ}_{\text{sample (Ref 2)}} \times \ldots \times \text{RQ}_{\text{sample (Ref n)}})^{\frac{1}{n}}$$

Where:

- RQ = Relative quantity
- n = Number of reference targets
- GOI = Gene of interest (one target)

Normalized Expression

Normalized expression ($\Delta\Delta$C(t)) is the relative quantity of your target (gene) normalized to the quantities of the reference targets (genes or sequences) in your biological system. To select reference targets, open the Experiment Settings window and click the reference column for each target that serves as a reference gene.

The calculation for normalized expression is described in the following formula, which uses the calculated Relative Quantity (RQ) calculation:

$$\text{Normalized Expression}_{\text{sample (GOI)}} = \frac{\text{RQ}_{\text{sample (GOI)}}}{(\text{RQ}_{\text{sample (Ref 1)}} \times \text{RQ}_{\text{sample (Ref 2)}} \times \ldots \times \text{RQ}_{\text{sample (Ref n)}})^{\frac{1}{n}}}$$

Where:

- RQ = Relative quantity of a sample
- Ref = Reference target in an experiment that includes one or more reference targets in each sample
- GOI = Gene of interest (one target)

Provided that reference targets do not change their expression level in your biological system, the calculation of normalized expression will account for loading differences or variations in cell number that is represented in each of your samples.

Normalized Expression When a Control Is Selected

When you select a control sample in the Experiment Settings window, the software sets the expression level of the control sample to 1. In this situation, the software normalizes the relative quantities of all target (gene) expression to the control quantity (a value of 1). This normalized expression is equivalent to the unscaled normalized expression analysis when a control is chosen.

Standard Deviation for the Normalized Expression

Rescaling the normalized expression value is accomplished by dividing the standard deviation of the normalized expression by the normalized expression value for the highest or lowest individual expression level, depending on the scaling option you choose. The standard deviation (SD) of the normalization factor is calculated with this formula:

$$SD\ NF_n = NF_n \times \sqrt{\left(\frac{SD\ RQ_{sample\ (Ref\ 1)}}{n \times RQ_{sample\ (Ref\ 1)}}\right)^2 + \left(\frac{SD\ RQ_{sample\ (Ref\ 2)}}{n \times RQ_{sample\ (Ref\ 2)}}\right)^2 + \ldots + \left(\frac{SD\ RQ_{sample\ (Ref\ n)}}{n \times RQ_{sample\ (Ref\ n)}}\right)^2}$$

Where:

- RQ = Relative quantity of a sample
- SD = Standard deviation
- NF = Normalization factor
- Ref = Reference target
- n = Number of reference targets

When a control sample is assigned, you do not need to perform this rescaling function on the standard deviation, as shown in the following formula:

$$SD\ NE_{sample\ (GOI)} = NE_{sample\ (GOI)} \times \sqrt{\left(\frac{SD\ NF_{sample}}{NF_{sample}}\right)^2 + \left(\frac{SD\ RQ_{sample\ (GOI)}}{RQ_{sample\ (GOI)}}\right)^2}$$

Where:

- NE = Normalized Expression
- RQ = Relative quantity of a sample
- SD = Standard deviation
- GOI = Gene of interest (one target)

Normalized Expression Scaled to Highest Expression Level

When the experiment does not include controls, scale the normalized expression (NE) for each target (gene) by dividing the expression level of each sample by the highest level of expression in all the samples. The software sets the highest level of expression to a value of 1, and re-scales all the sample expression levels. The highest scaling is calculated by this formula:

$$\text{Scaled Normalized Expression}_{sample\ (GOI)} = \frac{\text{Normalized Expression}_{sample\ (GOI)}}{\text{Normalized Expression}_{Highest\ sample\ (GOI)}}$$

Where:

- GOI = Gene of interest (target)

Normalized Expression Scaled to Lowest Expression Level

When the experiment does not include controls, scale the normalized expression (NE) for each target (gene) by dividing the expression level of each sample by the lowest level of expression in all the samples. The software sets the lowest level of expression to a value of 1, and re-scales all the sample expression levels. The lowest scaling is calculated by this formula:

$$\text{Scaled Normalized Expression}_{\text{sample (GOI)}} = \frac{\text{Normalized Expression}_{\text{sample (GOI)}}}{\text{Normalized Expression}_{\text{Lowest sample (GOI)}}}$$

Where:

- GOI = Gene of interest (target)

Standard Deviation for the Scaled Normalized Expression

Rescaling the scaled normalized expression (NE) value is accomplished by dividing the standard deviation (SD) of the normalized expression by the normalized expression value for the highest (MAX) or lowest (MIN) expression level, depending on the scaling option.

> NOTE: When a control sample is assigned, you do not need to perform this rescaling function on the standard deviation.

The formula for this calculation is shown here:

$$\text{SD Scaled NE}_{\text{sample (GOI)}} = \frac{\text{SD NE}_{\text{sample (GOI)}}}{\text{NE}_{\text{MAX or MIN (GOI)}}}$$

Where:

- NE = Normalized expression
- SD = Standard deviation
- GOI = Gene of interest (target)
- MAX = Highest expression level
- MIN = Lowest expression level

Corrected Values Formulas

A difference between corrected values and noncorrected values is only seen if a standard curve is created as part of the real-time PCR experiment. The software uses three equations in determining the error propagation:

- Standard error
- Standard error for normalized expression
- Standard error for the normalized gene of interest (target)

The formula for standard error is shown here:

$$\text{Standard Error} = \frac{\text{SD}}{\sqrt{\text{n}}}$$

Where

- n = Number of reference targets (genes)
- SD = Standard deviation

The standard error for the normalization factor in the normalized expression formula is shown here:

$$SE\ NF_n = NF_n \times \sqrt{\left(\frac{SE\ RQ_{sample\ (Ref\ 1)}}{n \times SE\ RQ_{sample\ (Ref\ 1)}}\right)^2 + \left(\frac{SE\ RQ_{sample\ (Ref\ 2)}}{n \times SE\ RQ_{sample\ (Ref\ 2)}}\right)^2 + \ldots + \left(\frac{SE\ RQ_{sample\ (Ref\ n)}}{n \times SE\ RQ_{sample\ (Ref\ n)}}\right)^2}$$

Where:

- n = Number of reference targets
- SE = Standard error
- NF = Normalized expression
- RQ = Relative quantity

The standard error for normalized gene of interest (GOI) formula is shown here:

$$SE\ GOI_n = GOI_n \times \sqrt{\left(\frac{SE\ NF_n}{NF_n}\right)^2 + \left(\frac{SE\ GOI}{GOI}\right)^2}$$

Where:

- SE = Standard error
- GOI = Gene of interest (one target)
- NF = Normalization factor
- n = Number of reference targets

9 Users and Preferences

Read this chapter to learn more about managing software users and their preferences:

- Logging in and Selecting a User (below)
- User Preferences window (page 102)
- Configuring email notification (page 103)
- User Administration (page 109)

Logging in and Selecting a User

CFX Manager software manages multiple users and their preferences. The current, logged in software user is displayed at the top of the main software window (Figure 79).

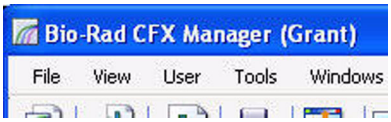


Figure 79. User name displayed.

CFX Manager software manages who logs in to the software through the Login dialog box (Figure 80). When you start the software, the Login dialog box opens automatically if there are two or more users listed in the User Administration window.

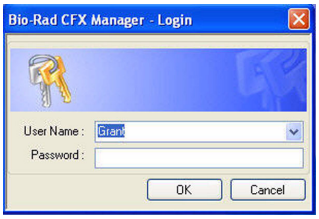


Figure 80. Login dialog box.

Log in to the software, or switch users by following these steps:

1. Open the Login dialog box, if it is not already open, by clicking the **Select User** button in the toolbar or selecting **User > Select User** in the menu bar.
2. Select a name from the **User Name** pull-down list. The default is “Admin” (administrator).
3. Type a password in the **Password** box.
4. Click **OK** to close the Login dialog box and open the software.

5. To add a new user name and password, contact your software administrator.

Changing a Password

Change a password by following these steps:

1. Select **User > Change Password** from the main software window menu to open the Change Password dialog box (Figure 81).
2. Enter the old password in the Old Password box.
3. Enter the new password in the New Password and the Confirm New Password boxes.
4. Click **OK** to confirm the change.

Figure 81. The Change Password window.

User Preferences Window

CFX Manager software tracks the preferences of each user that logs in to the software. To change user preferences, open the User Preferences window using one of these methods:

- Click **User Preferences** in the main software window toolbar
- Select **User > User Preferences** in the main software window menu bar
- Click one of the tabs (Figure 82) to view or change preferences

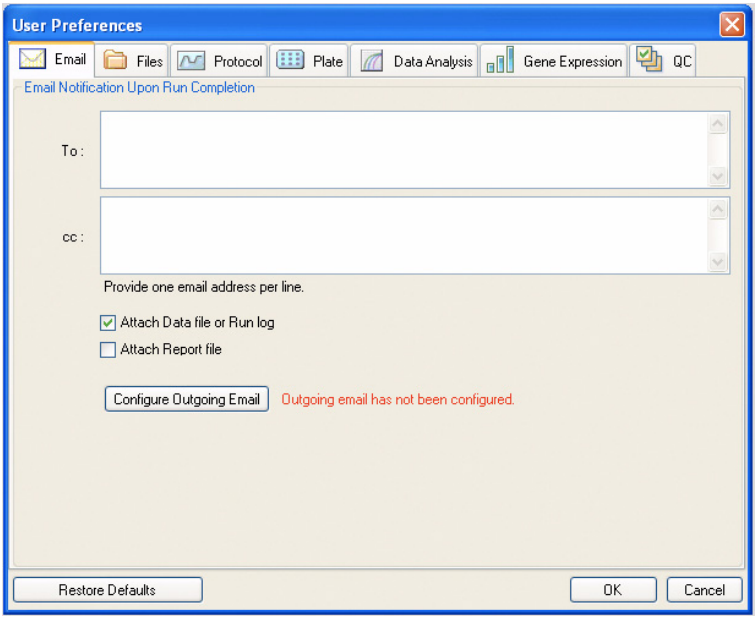


Figure 82. User Preferences window with tabs.

Email Tab

Select the **Email** tab (Figure 82) to enter the email addresses where you want to receive confirmation of the completion of the run. The software can send an attached data file or report file with the email when the check boxes next to these options are checked.

Configuring Email Notification

Click **Configure Outgoing Email** to open the Options window (Figure 83), configure the SMTP server, and send a test email from the computer. Input the following:

- **SMTP Server Name.** The name of the SMTP server as provided by your ISP
- **Port.** The port number of your SMTP server, as provided by your ISP; this is usually 25
- **Use SSL.** Whether to use Secure Sockets Layer. Some SMTP servers require this to be used, others require that it not be used
- **Use Default "From" Address.** This can usually be left in the default checked state. However, some SMTP servers require all sent email to have a "from" address that is from a certain domain, i.e.<name>@YourCompany.com. If that is the case, this checkbox must be unchecked, and a valid "from" email address must be supplied in the box labeled "From" Address:
- **Authentication Required.** Many SMTP servers require authentication. If so, this checkbox must be checked, and a User Name and Password must be supplied
- **Test email.** To test the email settings, enter one or more email addresses in **Test Email Address** text box. Multiple email addresses can be separated by a comma. Then click the **Test Email** button

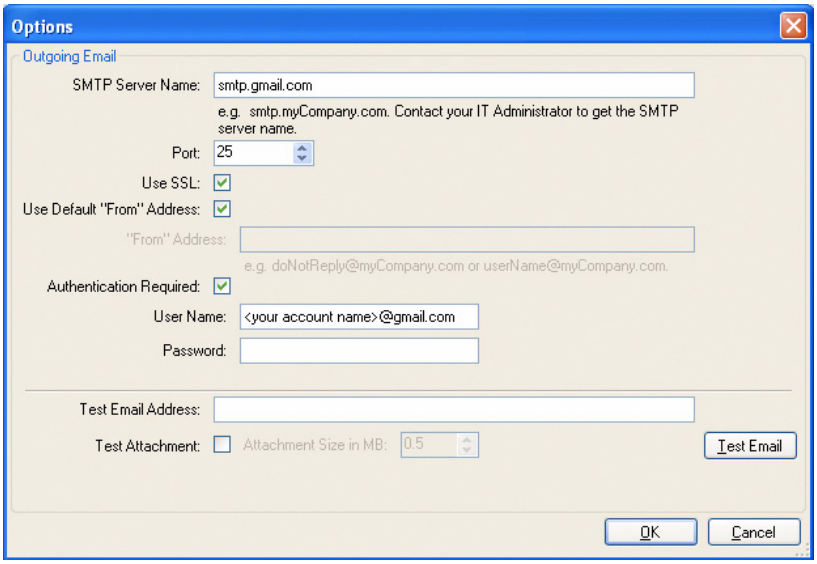


Figure 83. Options to configure email.

NOTE: Some SMTP servers do not allow attachments, and others allow attachments only up to certain sizes. If you are going to use CFX Manager software to email Data Files and/or Reports, you may want to test your server's ability to email attachments by checking the Test Attachment box, and setting the Attachment Size to 5 megabytes (MB) or more.

Files Tab

Select the **Files** tab to list the default locations for opening and saving files (Figure 84).

- **Default Folder for File Creation.** Select a default folder where you want to save new files. Select a location for each file type (Protocol, Plate, Data, or Gene Study file)
- **File Selection for Experiment Setup.** Select the default protocol and plate files that appear when you open the Experiment Setup window
- **Data File Prefix.** Define the beginning text of the file name for data files. The default setting instructs the software to create a file name that starts with the User (user name of the user who is currently logged on to the software), Date (file creation date), and Instrument Name (instrument serial number or name)

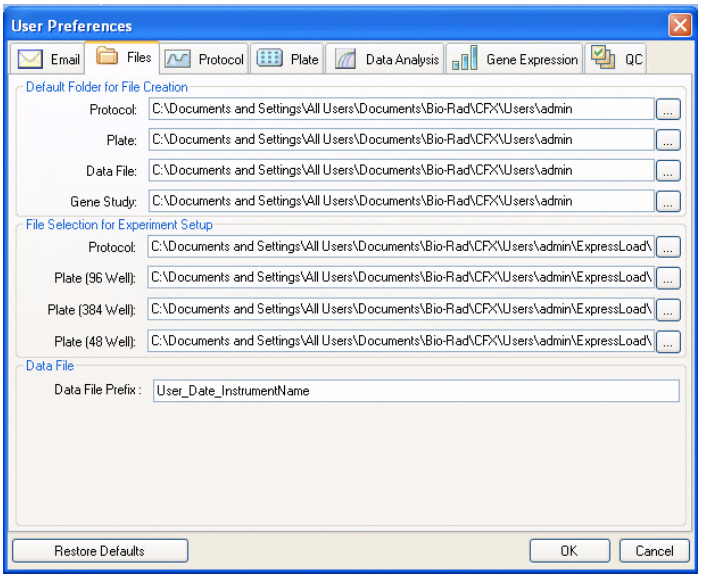


Figure 84. Files tab in the User Preferences window.

TIP: Click the "**...**" button to the right of each box to open a browser window and locate a folder.

Protocol Tab

Select the **Protocol** tab in the User Preferences window (Figure 85) to specify the default settings for a new protocol file in the Protocol Editor window:

- **Protocol Editor.** Set the default settings that appear in the Protocol Editor. Select a default Sample Volume to describe the volume of each sample in the wells (in µl), and select a Lid Shutoff Temperature at which the lid heater turns off during a run

- **Protocol AutoWriter.** Selects default settings that appear in the Protocol AutoWriter, including default annealing temperature for experiments that use iProof, iTaq, or Other polymerases and the default amplicon length

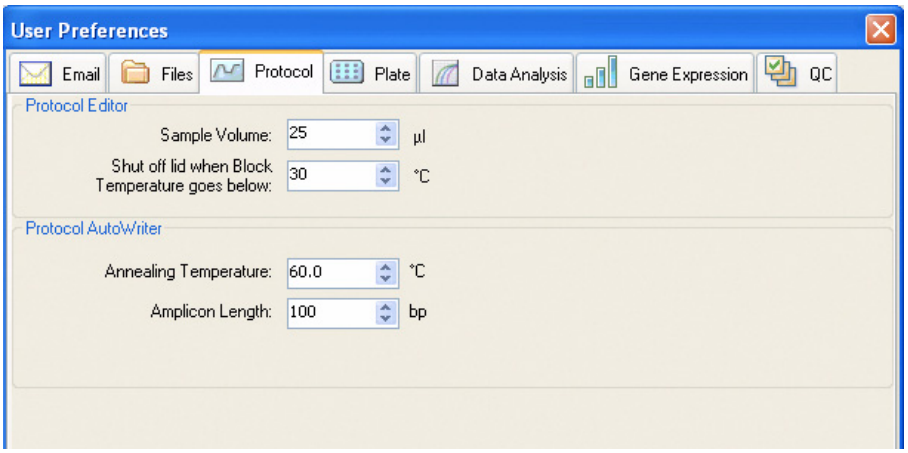


Figure 85. Protocol tab in the User Preferences window.

Plate Tab

Select the **Plate** tab in the User Preferences window (Figure 86) to specify the following default settings for a new Plate file in the Plate Editor window:

- **Plate Type.** Select the default plate well type from the list

  NOTE: The plate size default that is shown when the Plate tab is first opened is linked to the instrument pull down-menu in the Startup Wizard. Make sure you select MiniOpticon from the list.
- **Plate Size.** Select the default plate size from the list
- **Units.** Select the units used to describe the concentration of the starting template for wells that contain standards. The software uses these units to create a standard curve in the Data Analysis Quantitation tab
- **Scientific Notation.** Select scientific notation to view concentration units in that notation
- **Scan Mode.** Select a default scan mode to set the number of channels to scan during a run
- **Fluorophores.** Click check boxes to select the default fluorophores that appear in the Plate Editor well loading controls
- **Libraries.** Enter the target and sample names that you typically use in your experiments. Enter target names to list genes and sequences, and enter sample names to list conditions for experiment samples. These names appear in the lists of in the Targets tab and Samples tab in the Experiment Settings window

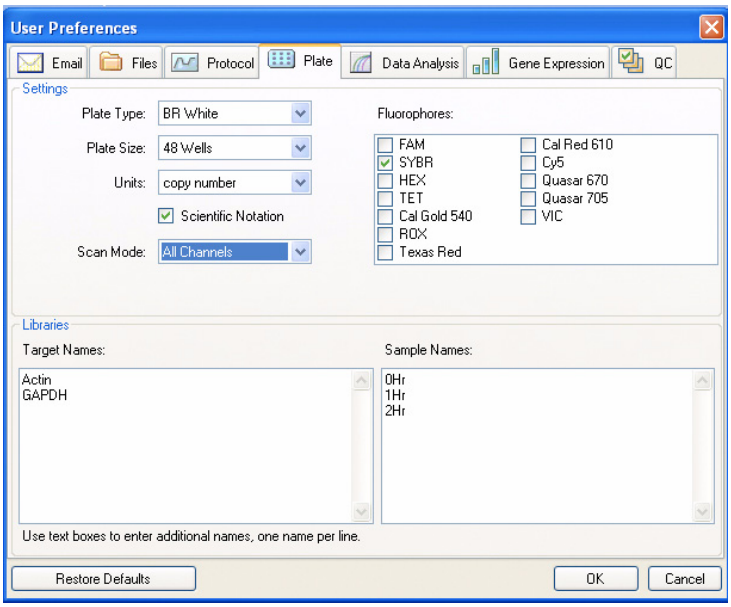


Figure 86. Plate tab in the User Preferences window.

Data Analysis Tab

Select **Data Analysis** in the User Preferences window (Figure 87) to change the default settings for data that are displayed in the Data Analysis window.

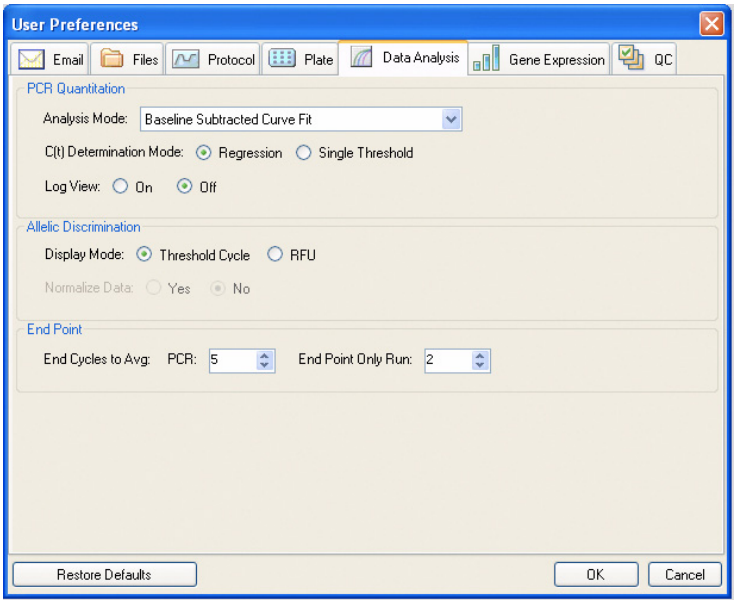


Figure 87. Data Analysis tab in the User Preferences window.

For the quantification data, select the following settings:

- **Analysis Mode.** Select the default base lining method for the analysis mode. Choose Baseline Subtracted Curve Fit, No Baseline Subtraction, or Baseline Subtracted

- **C(t) Determination Mode.** Select between **Regression** mode or **Single Threshold** mode to determine how C(t) values are calculated for each fluorescence trace
- **Log View.** Select **On** to show a semi-logarithmic graph of the amplification data. Select **Off** to show a linear graph

For the allelic discrimination data, select the following settings:

- **Display Mode.** Select **RFU** to show the data as a graph of the RFU, or select **Threshold Cycle** to show a graph of threshold cycles
- **Normalize Data.** This selection is only available when RFU is selected. Select **No** to show unnormalized data. Select **Yes** to normalize the data to the control sample

For the end point data, select the following settings. Select the number of end cycles to average when calculating the end point calculations:

- **PCR.** Enter a number of cycles for PCR to average the end cycles for quantitation data (default is 5)
- **End Point Only Run.** Enter a number of cycles for End Point Only Run to average the end cycles for end point data (default is 2)

Gene Expression Tab

Select **Gene Expression** in the User Preferences window (Figure 88) to specify the default settings for a new Gene Expression data file.

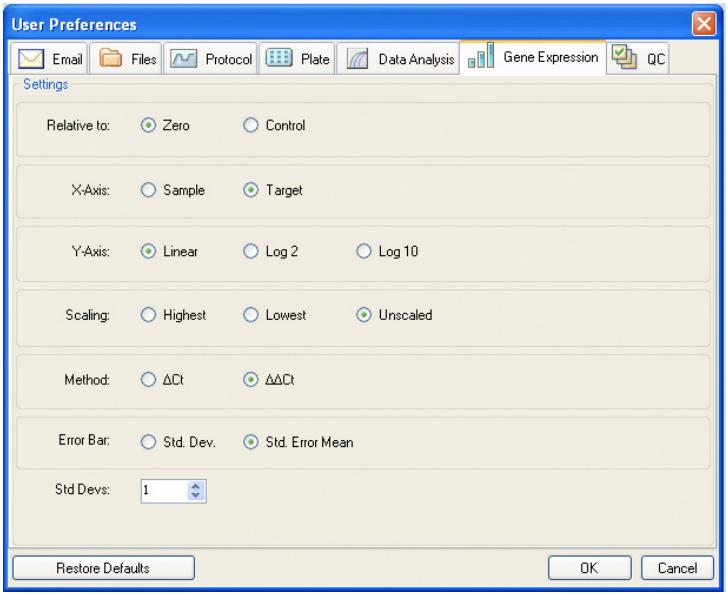


Figure 88. Gene Expression tab in the User Preferences window.

Specify the default settings for a new Gene Expression data file:

- **Relative to.** Select **Zero** or **Control**. To graph the gene expression data originating at 1 (relative to a control), select **Control**. When you assign a control sample in the Experiment Setup window, the software automatically defaults to calculate the data relative to that control. Select **Zero** to instruct the software to ignore the control, which is the default selection when no control sample is assigned in the Experiment Settings window
- **X-Axis.** Graph the **Sample** or **Target** on the x-axis

- **Y-Axis.** Graph **Linear**, **Log 2**, or **Log 10** scale on the y-axis
- **Scaling.** Select a scaling option for the graph. Leave the graph **Unscaled**. Alternatively, choose a scaling option to scale to the **Highest** value or to the **Lowest** value
- **Method.** Set the default analysis mode, including relative expression (ΔCt) or normalized expression (ΔΔCt) or relative expression (ΔCt)
- **Error Bar.** Select Std Dev. for standard deviation, or Std. Error Mean for the standard error of the mean
- **Std Devs.** Select the standard deviation multiplier to graph the error bars. The default is 1. Change the multiplier to either 2 or 3

QC Tab

Select the **QC** tab in the User Preferences window to specify QC rules to apply to data in Data Analysis Module. The software validates the data against the enabled tests and the assigned values (Figure 89).

NOTE: Wells that fail a QC parameter can easily be excluded from analysis in the QC module of the Data Analysis Window using the right-click menu option.

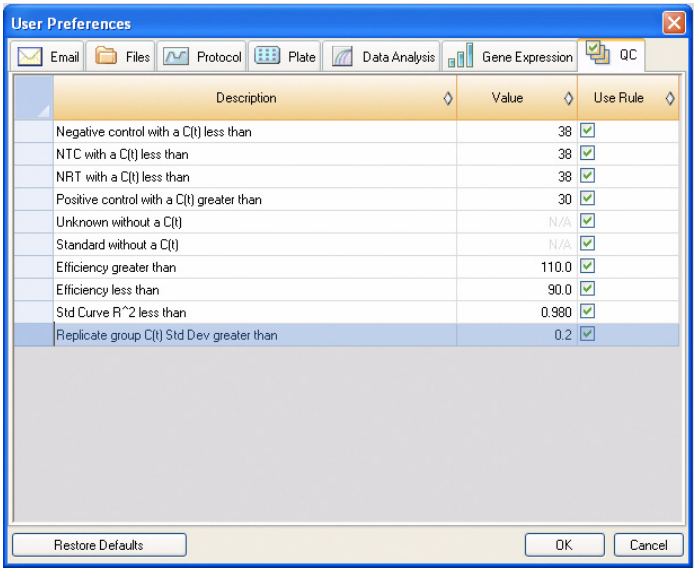


Figure 89. QC tab in User Preferences.

Specify to add cut off values and to enable the following QC rules:

- Negative control with a C(t) less than XX. Input a C(t) cut-off value
- NTC (no template control) with a C(t) less than XX. Input a C(t) cut-off value
- NRT (no reverse transcriptase control) with a C(t) less than XX. Input a C(t) cut-off value
- Positive control with a C(t) greater than XX. Input a C(t) cut-off value
- Unknown without a C(t)
- Standard without a C(t)
- Efficiency greater than XX. Input a reaction efficiency cut-off value that is calculated for the standard curve
- Efficiency less than XX. Input a reaction efficiency cut-off value that is calculated for the standard curve

- Std Curve R^2 less than XX. Input a cut-off R^2 value for the standard curve
- Replicate group C(t) Std Dev greater than XX. Input a cut-off standard deviation that is calculated for each replicate group

User Administration

Open the User Administration window in the main software window:

- Select **Users > User Administration**
- Click **User Administration** in the menu bar

If you log in as an Administrator, open the User Administration window to manage users and user rights:

- **Manage Users.** Add or remove users, and assign each user a role
- **Manage Rights.** Change rights for user roles (Principal, Operator, or Guest)

  NOTE: Only users who are Administrators can edit this window. Other users can only view it.

To assign a role to each user, select from the list of roles in the User Administration window (Figure 90). In this example, the Guest user is given the added right to save files.

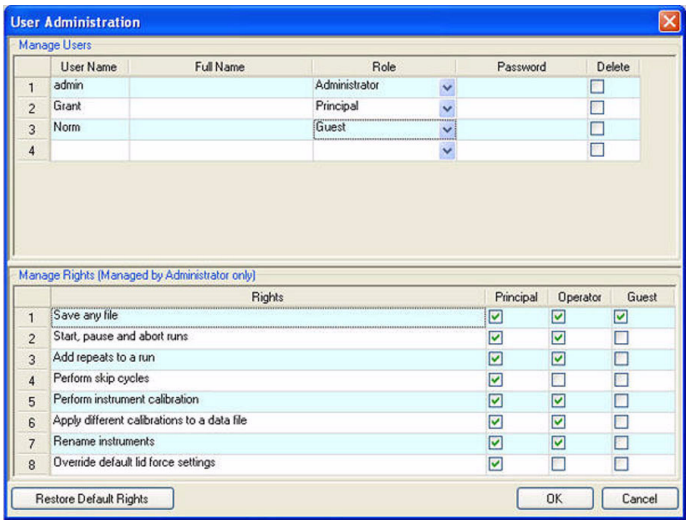


Figure 90. User Administration window with three users.

Adding and Removing Software Users

Only a software Administrator can add and remove users. To add software users in the Manage Users pane, follow these steps:

1. Enter a user name for the new software user.
2. Select a user role. These roles restrict the rights of each user. The default is Principal.
3. (Optional) Enter a full name and password for the new software user.
4. Click **OK** to open a dialog box and confirm that you want to close the window.
5. Click **Yes** to close the dialog box and window.

To remove a software user, follow these steps:

1. In the Manage Users pane, click the box in the Delete list for each software user you want to remove.
2. Click **OK** to open a dialog box and confirm that you want to close the window.
3. Click **Yes** to close the dialog box and window.

   NOTE: The list of software users must always include one Administrator.

Assigning Rights for User Roles

The User Administration window provides access to user roles and rights. The software includes these four roles:

- **Administrator (required).** Each Administrator has all rights, and you cannot change those rights. The Administrator can also add and remove software users, and change the rights for each role
- **Principal.** By default each Principal has all rights
- **Operator.** By default each Operator has all rights except skipping cycles and creating a Gene Study
- **Guest.** By default each Guest has no additional rights, and can only read files

To specify the rights for each role, follow these steps. Only a software Administrator can change the rights for any role:

1. In the Manage Rights pane, click a box under the name of the role to add or remove that right. Click one or more rights in the list. To change all the rights for all the roles to the default list, click **Restore Default Rights**.
2. Click **OK** to open a dialog box and confirm that you want to close the window.
3. Click **Yes** to close the dialog box and window.

To view your current user role and rights, select **User > User Administration**. Contact a software administrator to modify the user settings, rights, and roles listed in the User Administration window. A Principal, Operator, or Guest user can only view their user settings, rights, and roles.

10 Resources

Read this chapter to learn more about resources for the MiniOpticon system:

- Calibration Wizard (below)
- Instrument maintenance (page 112)
- Application Log (page 114)
- Troubleshooting (page 114)
- References (page 115)

Calibration Wizard

The MiniOpticon system is factory calibrated for commonly used fluorophores in Bio-Rad white-welled plates (Table 43).

Table 43. Factory calibrated fluorophores, channels, and instruments

Fluorophores	Channel
FAM, SYBR® Green I	1
HEX	2

To open the Calibration Wizard to calibrate the MiniOpticon system:

1. Select an instrument in the Detected Instruments pane.
2. Select **Tool > Calibration Wizard** to open the Dye Calibration window and calibrate new dye and plate combinations (Figure 91).

Figure 91 shows an example of the Dye Calibration window.

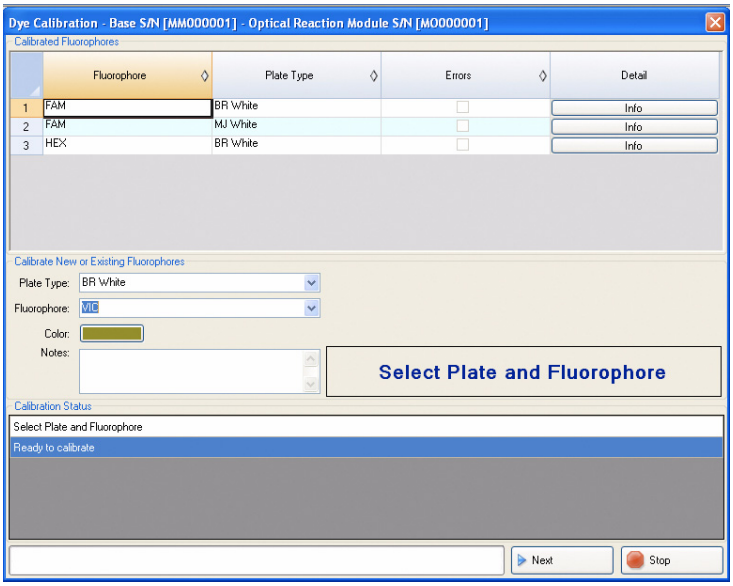


Figure 91. Dye Calibration window.

Calibrating the MiniOpticon System

To calibrate the MiniOpticon system in the Dye Calibration window:

1. Select the Plate Type in the Calibrate New or Existing Fluorophores pane. If the plate type is not included in the list, type the name in the box to add it to the list.
2. Select the fluorophore you want to calibrate from the pull-down list. If the fluorophore name is not included in the list, type the name in the box to add it to the list.
3. Begin preparing a 48-well plate for dye calibration by pipetting dye solution into each well. For each fluorophore, fill each well with 50 µl of 300 nM dye solution.
4. Seal the plate using the sealing method you will use in your experiment.
5. Click **Next** and follow the on-screen instructions. First, place an empty plate in the MiniOpticon system for the first procedure of the calibration. Next, place the calibration plate in the block and close the lid for the second procedure of the calibration. Click **OK** to confirm that the plate is in the block.
6. When the CFX Manager software completes the calibration run, a dialog box appears. Click **Yes** to finish calibration and open the Dye Calibration Viewer.
7. Click **OK** to close the window.

Instrument Maintenance

The MiniOpticon system includes a sensitive optical detector system and a sample block that must heat and cool very fast. Contamination of these components can interfere with thermal cycling and data collection.

WARNING! Never allow a reaction to run with an open or leaking sample lid. The reagents could escape and coat the block, inner lid, or optical detection system.

Excessive dirt can dim the signal, and fluorescence contamination can create excessive background signal.

Avoid contaminating the MiniOpticon system by following these suggestions:

- Always clean the outside of any containers before placing them in the block
- Never run a reaction with a seal that is open, loose, punctured, or otherwise damaged because you could contaminate the block, inner lid, and optical system
- Never run a PCR or real-time PCR reaction with volatile reagents that could explode and contaminate the block, inner lid, and optical system
- Never clean or otherwise touch the optical system behind the heater plate holes that are in the inner lid

Cleaning the Sample Block

The thermal block of the MiniOpticon system should be cleaned on a regular schedule to remove any debris or dirt that might interfere with proper function. Clean as soon as you discover debris and spilled liquids with a soft, lint-free cloth that is dampened with water. Cleaning the instrument allows precise instrument function.

WARNING! Never use cleaning solutions that are corrosive to aluminum. Avoid scratching the surface of the thermal block. Scratches and damage to this surface interfere with precise thermal control.

WARNING! Never pour water or other solutions in the reaction module bay. Wet components can cause electrical shock when the thermal cycler is plugged in.

Clean the MiniOpticon system as soon as you discover debris, dirt, or contamination in the block or on the inner lid. Any dirt can interfere with the ability of the block to change temperature quickly and collect accurate fluorescent data. To clean the reaction module, follow these guidelines. Follow these suggestions for cleaning:

WARNING! To prevent electrical shock, always unplug the base before cleaning the instrument.

WARNING! Never touch or allow solutions to touch the optical system that is located behind the heated plate holes in the inner lid.

TIP: For instructions on handling and cleaning radioactive or biohazardous materials, consult the guidelines for radiation safety and biosafety provided by your institution. These guidelines include cleaning, monitoring, and disposal methods for hazardous materials.

- **Clean the outer surface.** Use a damp cloth or tissue to clean spills off the outside case. If needed, use a mild soap solution, and then rinse the surface with a damp cloth. Cleaning the cover will prevent corrosion

  NOTE: Never use cleaning solutions that are corrosive to aluminum, such as bleach or abrasive cleansers.
- **Use of oil in the wells is not recommended.** If oil is used, the wells must be cleaned thoroughly and often. Remove the oil when it is discolored or contains dirt. Use a solution of 95% ethanol to clean oil. Do not allow oil to build up in the block
- **Clean the wells in the block.** Clean spills immediately to prevent them from drying. Use disposable plastic pipets with water (recommended), 95% ethanol, or a 1:100 dilution of bleach in water. Also use a soft, lint-free cloth or paper towel and water to clean the block. Always rinse the wells with water several times to remove all traces of cleaning reagents

  WARNING! Never clean the block with strong alkaline solutions (strong soap, ammonia, or high-concentration bleach). Never use corrosive or abrasive cleaning

solutions. These cleaning agents can damage the block and prevent precise thermal control.

WARNING! Bleach, ethanol, or soap that is left in the blocks could corrode the block and destroy plastics during a run. After cleaning, always rinse the wells thoroughly with water to remove all traces of cleaning reagents.

WARNING! Never heat the block after adding a cleaning solution. Heating the block with cleaning solution will damage the block, reaction module, and thermal cycler base.

- **Clean the inner lid.** Use a soft, lint-free cloth and water to remove debris and solutions from the inner lid surface. Never use abrasive detergents or rough material that will scratch the surface. Cleaning the inner lid improves precise sample heating and cooling

Application Log

Before starting a new run, the instrument initiates a self-diagnostic test to verify that it is running within specifications. The software records results of this test in the Run log and Application log file. If you notice a problem in one or more experiments, open the run and application logs to find out when the problem started happening.

CFX Manager software tracks information about the state of an instrument during a run in the **Application Log** (Figure 92). Use these logs to track events that occur on instruments and in the software and for troubleshooting.

To open the Application log in the main software window, select **View > Application Log**.

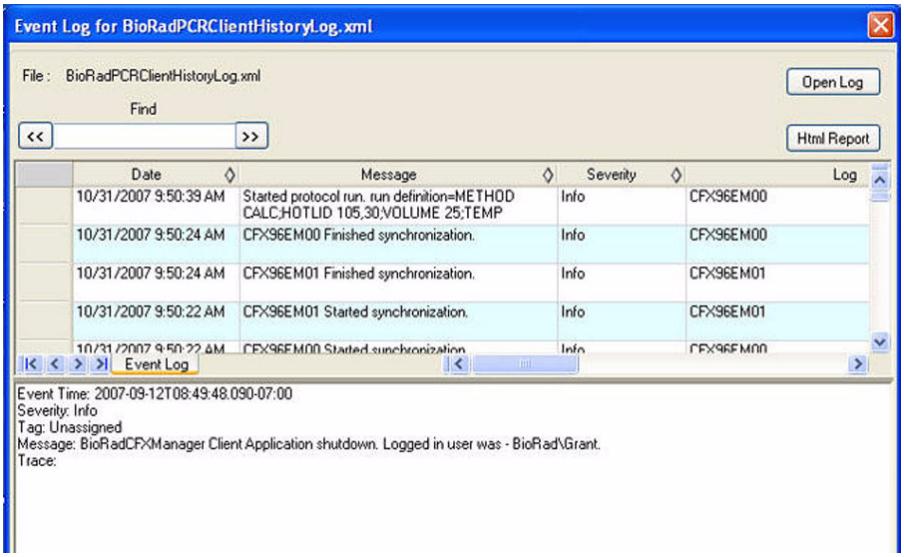


Figure 92. Example of an Event Log file.

Troubleshooting

Typically, software and instrument communication problems can be resolved by restarting your computer and the system. Be sure to save any work in progress before restarting.

NOTE: Check that your computer has sufficient RAM and free hard drive space. The minimum RAM is 2 GB, and the minimum hard drive space is 20 GB.

Installing the Software Manually

If needed, install the software manually by following these instructions:

1. Insert the software CD.
2. Right-click the software CD icon, and select **Explore** to open the CD window.
3. Double-click the **CFX_Manager** folder to open the folder, and then double-click **setup.exe** to start the software installation wizard.
4. Follow the instructions on the wizard to install the software, and then click **Finish**.

Power Failure Options

In a power failure, the instrument and computer will shut down. If the power failure is short, the instrument will resume running a protocol, but the Application log will note the power failure. Depending on the computer settings and the length of time that the power is off, the instrument and software attempt to continue running the experiment when they are restarted.

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