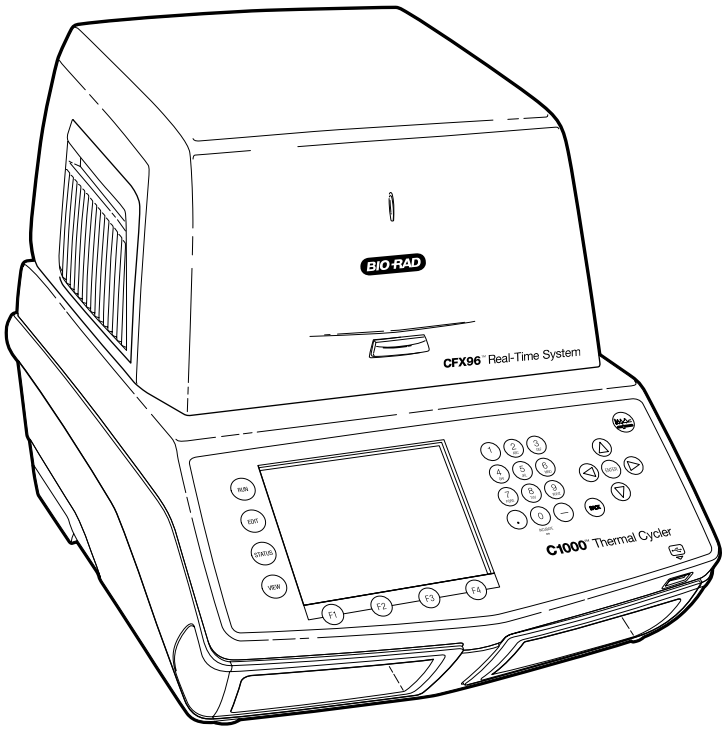
CFX96™ Real-Time PCR Detection System

Instruction Manual

Catalog # 184-5096
185-5096



BIO-RAD

CFX96™ Real-Time PCR Detection System

Instruction Manual

Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547 · 800-424-6723

10010000

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The CFX96 optical reaction module is intended for laboratory research applications only.

This CFX™ detection module, when combined with a C1000™ thermal cycler for which the applicable real-time thermal cycler royalty fee has been paid, constitutes a real time thermal cycler licensed under U.S. Patent No. 6,814,934 and corresponding claims in any Canadian counterpart patent thereof owned by Applera Corporation, for use solely in research and all applied fields except human and veterinary in vitro diagnostics. These license rights are effective only if this detection module is combined with a Bio-Rad thermal cycler for which the applicable real-time thermal cycler royalty fee has been paid and not with any other thermal cycler. No rights are conveyed expressly, by implication or estoppel to any patents on real time methods, including but not limited to 5' nuclease assays, or to any patent claiming a reagent or kit. For further information on purchasing license rights, contact the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California, 94404, USA."

Safety and Regulatory Compliance

The CFX96™ real-time PCR detection system heats, cools, and collects data very fast during operation. For safe operation, we strongly recommend that you follow the safety specifications listed in this section and throughout this manual.

NOTE: The CFX96 optical reaction module is used exclusively with the C1000™ thermal cycler, and the combined instrument is called the CFX96 system.

Safety Warning Labels

Warning labels posted on the instrument and in this manual warn you about sources of injury or harm. Refer to Table 1 to review the meaning of each safety warning label.

Table 1. 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 warnings labels shown in Table 2 also display on the instrument, and refer directly to the safe use of this CFX96 optical reaction module.

Table 2. Instrument Safety Warning Labels

Icon	Meaning
	**Warning about risk of harm to body or equipment**. Operating the CFX96 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

NOTE: For information about the C1000 thermal cycler, refer to the C1000™ Thermal Cycler instruction manual.

Safe Use Specifications and Compliance

Table 3 lists the safe use specifications for the CFX96 System. Shielded cables (supplied) must be used with this unit to ensure compliance with the Class A FCC limits.

Table 3. Safe Use Specifications

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

Regulatory Compliance

This instrument has been tested, and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- IEC 61010-1:2001 (2nd Ed.), EN61010-1:2001 (2nd Ed). Electrical Equipment For Measurement, Control, and Laboratory Use - Part 1: General Requirements
- IEC 61010-2-010:2005, EN61010-2-010:2003. Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-010: Particular requirements for laboratory equipment for the heating of materials
- IEC 61010-2-081:2001+A1, EN61010-2-081:2002+A1. Safety requirements for electrical equipment for measurement, control and laboratory use. Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes (includes Amendment 1)
- EN 61326-1:2006 (Class A) Electrical equipment for measurement, control and laboratory use. EMC requirements, Part 1: General requirements

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.

Table of Contents

1 Introduction

Congratulations on the purchase of a CFX96™ optical reaction module! This instrument is the newest addition to Bio-Rad Laboratories' real-time PCR systems.

- **Expand the C1000™ thermal cycler.** Run PCR and real-time PCR on the same instrument
- **Expand your throughput.** Run up to four instruments with one computer
- **True five-target multiplexing.** Discriminate five targets in each reaction well
- **Fast scan option.** Complete SYBR I and single-color FAM protocols faster
- **FRET channel.** Expand your experiment options using Fluorescence resonance energy transfer
- **Quick installation and intuitive software.** Factory calibrated optics and easy software interface let you quickly set up your system

The CFX96 real-time PCR optical reaction module installs in the C1000™ thermal cycler (Figure 1) to create a system for running real-time PCR experiments. The CFX96 system runs under the control of CFX™ Manager software, which collects and analyzes real-time PCR data.

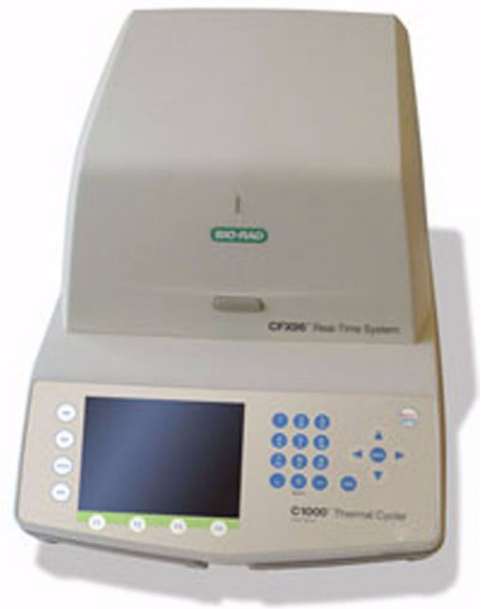


Figure 1. CFX96 system, with optical module mounted thermal cycler base.

Bio-Rad Resources

Bio-Rad Laboratories provides many resources for scientists. The following web sites contain useful information about running PCR and real-time PCR experiments:

- **Gene Expression Gateway (www.bio-rad.com/genomics/)**

  This site provides rich technical resources on a wide variety of methods and applications related to PCR, real-time PCR, and gene expression.

- **Life Science Research web site (discover.bio-rad.com)**

  This site includes links to technical notes, manuals, product information, and technical support.

Table 4 lists Bio-Rad resources and how to locate what you need:

Table 4. Bio-Rad resources

Resource	How to contact
Local Bio-Rad Laboratories representatives	Find local information and contacts on the Bio-Rad Laboratories web site 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 Laboratories web site (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 Laboratories provides quality technical support. We staff our Technical Support department with experienced scientists to provide our customers with practical and expert solutions. To find technical support on the web, go to the Bio-Rad Laboratories web site (www.bio-rad.com) or Gene Expression Gateway (www.bio-rad.com/genomics). To find local technical support, contact your nearest Bio-Rad Laboratories 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 is for scientists and technicians who run the CFX96 system and accessories. It explains how to safely set up and operate this system, and uses the writing conventions listed in Table 5, to quickly provide relevant information.

Table 5. 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 CFX96 system, see, “Safety and Regulatory Compliance” on page iii.

2 Get Started

The CFX96 optical reaction module ships as a complete unit that is ready to setup and start. Load the CFX96 module on the C1000 thermal cycler chassis to set up the CFX96 system. This chapter provides information about getting started:

- Unpacking the CFX96 optical reaction module (see next section on page 5)
- System requirements (page 5)
- Overview of the CFX96 module and the CFX96 system (page 7)
- Setting up the CFX96 system (page 10)
- Loading the block (page 15)

Unpacking the CFX96 Optical Reaction Module

Your CFX96 optical reaction module shipment includes these components:

- CFX96 optical reaction module (see Figure 2 on page 7)
- USB cable
- CFX Manager software installation CD
- CFX96 Real-Time PCR Detector instruction manual
- CFX Manager Software Installation, Protocol, Plate, Data Analysis, and Gene Expression Analysis quick guides

  NOTE: To run real-time PCR experiments, the CFX96 optical reaction module requires a C1000 base (Figure 7 on page 10) and CFX Manager software.

Remove all packing materials and store them for future use when moving the instrument. Place the CFX96 system on a flat, dry surface near a C1000 thermal cycler.

NOTE: Please check that all items were shipped. If any items are missing or damaged, contact your local Bio-Rad Laboratories office. For information about contacting Bio-Rad Laboratories, see “Bio-Rad Resources” on page 2, and the location of Bio-Rad offices listed on the back of this manual.

System Requirements

To install and run the CFX96 system, refer to the minimal power and computer specifications listed in these sections:

- Instrument requirements (page 6)
- Software requirements (page 6)

Instrument Requirements

To operate the CFX96 real-time PCR detection system, use the following power sources and cables:

- **Input power.** 100—240 VAC, 50—60 Hz
- **Indoor use.** Ambient temperature of 15°C—31°C. Relative humidity maximum of 80% (non-condensing)
- **USB cable.** The provided cable is sufficiently shielded. Bio-Rad Laboratories provides a well-shielded USB cable for use with this system

  NOTE: For a full list of the safety and compliance requirements for this instrument, see “Safety and Regulatory Compliance” on page iii.

Software Requirements

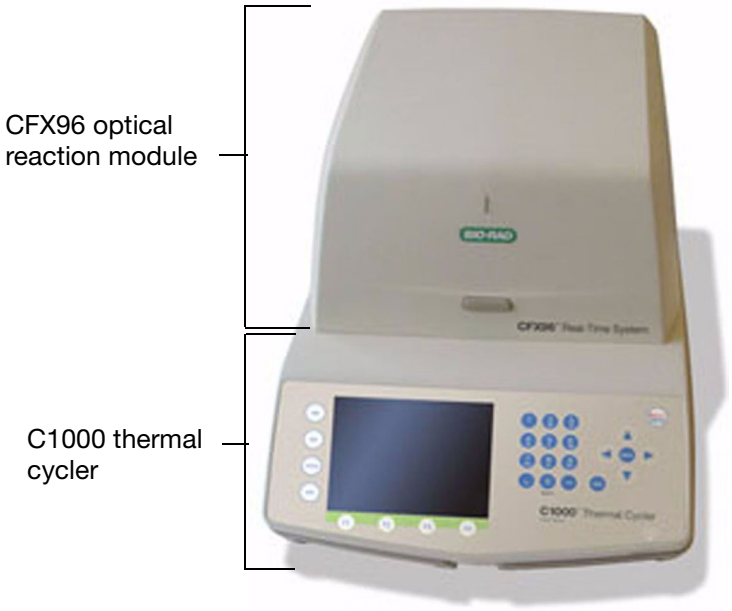
The CFX96 real-time PCR detection system requires CFX Manager software for instrument control and data collection. Table 6 lists the computer system requirements for running the software on Windows XP and Windows Vista.

Table 6. Software requirements for CFX Manager software

System	Minimum	Recommended
Operating system	Windows XP Professional SP2 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	1 GHz	2.0 GHz
RAM	1 GB RAM	2 GB
Screen resolution	1024 x 768 with true-color mode	1280 x 1024 with true-color mode
USB	USB 2.0, Hi-Speed port	USB 2.0 Hi-Speed port
Operating system	Windows XP, with Service Pack 2	Windows XP, with Service Pack 2
Internet browser	Internet Explorer	Internet Explorer
Software		Microsoft Office Suite

Overview of the CFX96 system

The CFX96 system includes two components to run real-time PCR experiments:



- **CFX96 optical reaction module.** This module includes the optical system to collect fluorescent data and a block (inside) that heats and cools the PCR reactions quickly. Each optical reaction module inserts into the thermal cycler base. For more information about setting up the system, see “Setting Up the CFX96 system” on page 10.
- **C1000 thermal cycler base.** The C1000 base includes the power button and ports (both on back panel) to connect to a computer.

  NOTE: For detailed information about the C1000 thermal cycler, refer to the instruction manuals for those instruments. Instruction manuals for all instruments, software, and reagents are available on the Bio-Rad Laboratories web site. For information about finding these documents, see “Bio-Rad Resources” on page 2.

The CFX96 optical reaction module (Figure 2) includes hand holds on both sides for lifting the module in and out of the C1000 thermal cycler base.

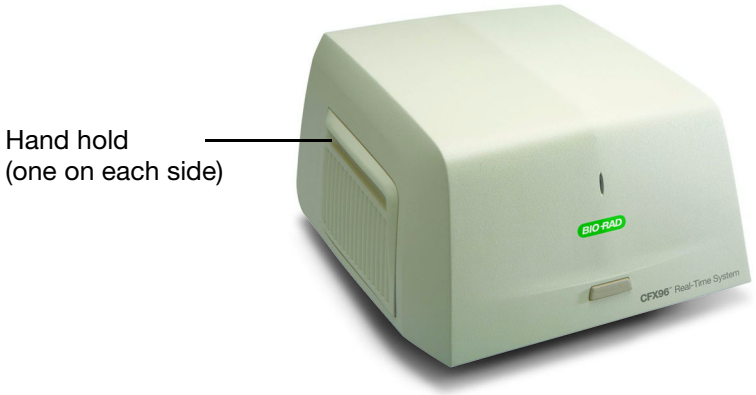


Figure 2. Front view of CFX96 optical reaction module.

The back of the CFX96 optical reaction module (Figure 3) includes these features:

- **Fins.** Keep the fins clean, and protect them from damage to insure fast and accurate performance

- **Serial number.** Note the serial number, which is also displayed in the instrument properties window (see “Main Software Window” (page 22).

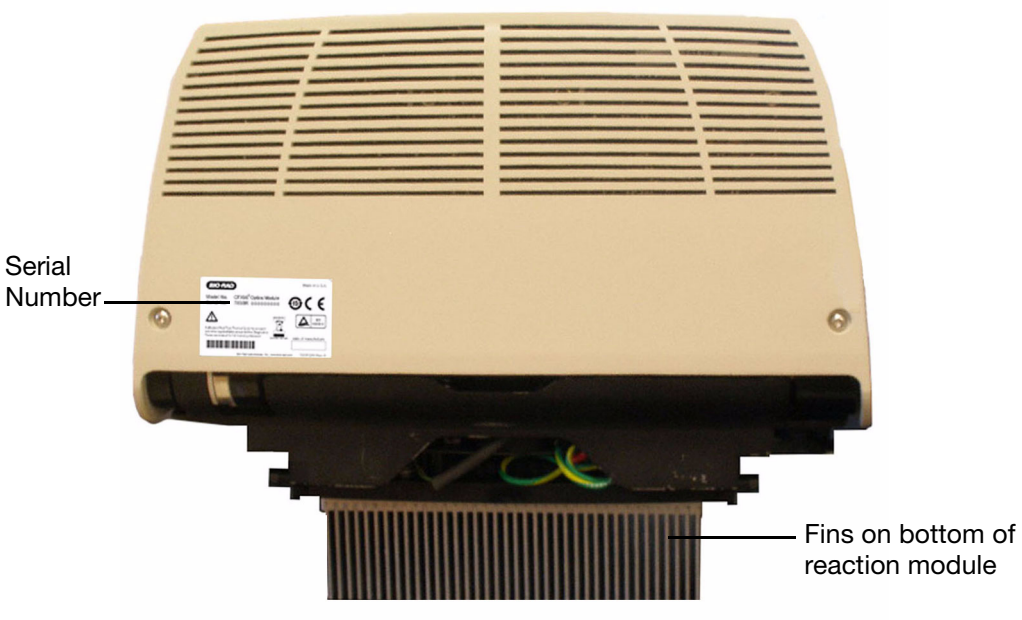


Figure 3. Back view of CFX96 optical reaction module.

Once the CFX96 optical reaction module is loaded into the C1000 thermal cycler base, the CFX96 system includes the features shown in Figure 4:

- **LED.** View the green light, which indicates that the CFX96 system is running an experiment
- **Open button.** Press the button on the outside of the lid to open the motorized lid
- **C1000 thermal cycler control panel.** When controlled by CFX Manager software, the screen displays the message “Under remote control” and most control panel keys are inactivated.

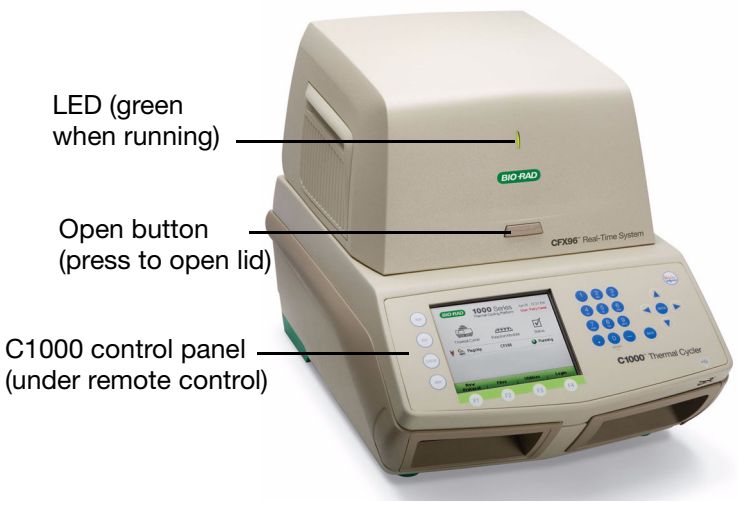


Figure 4. Front view of the CFX96 system with LED, lid button, and control panel.

The open lid in the CFX96 system includes the features shown in Figure 5:

- **Inner lid.** Heated inner lid to maintain temperature in the samples

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

- **Lid heated plate with holes.** Avoid touching or otherwise contaminating the heater plate. Never poke anything through the holes. These holes allow the shuttle system to collect fluorescent data. Any obstruction can damage the shuttle or interfere with data collection
- **Block.** Load samples in this block before the run
- **Close button.** Press this button on the inside the lid to close the motorized lid

Figure 5. Inside view of the CFX96 system.

WARNING! Never put anything through the heater plate holes in the inner lid. The shuttle system, located above this plate, could be damaged. When cleaning, use a damp, cloth and remove residual lint. Never use a wet sponge on the inner lid.

WARNING! Prevent contamination of the instrument by spills, and never run a reaction with an open or leaking sample lid. Both of these actions can result in contamination of the block, inner lid, and optical head in the shuttle system. Such contamination can dim the collected signal. Also, fluorecent contamination can create excessive background in the signal. The shuttle system must be cleaned by trained Bio-Rad service engineers. For more information about general cleaning and maintenance of the instrument, see “Cleaning the CFX96 Optical Reaction Module” (page 18).

The back panel of the CFX96 system includes these features (shown in Figure 6 on page 10):

- **Power switch.** Press the power switch to turn on the power to the system
- **Power input.** Plug in the power cord here
- **USB connections.** Use these ports to connect the CFX96 system to a computer. The system also connects to an S1000 thermal cycler through a USB connection. For

instructions about connecting the system to a computer see the C1000™ Thermal Cycler instruction manual

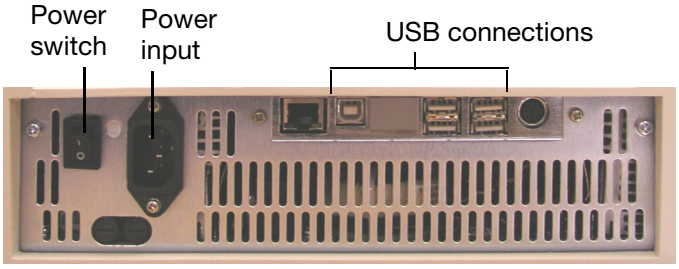


Figure 6. Back panel of CFX96 system.

WARNING! Avoid contact with the back panel of the CFX96 system during operation.

Setting Up the CFX96 system

To set up the CFX96 system, follow these instructions:

1. **Remove the reaction module from the C1000 thermal cycler, if it has one.**

   In order to install the CFX96 module, the reaction module bay in the C1000 base must be empty and the locking bar lowered (Figure 7).

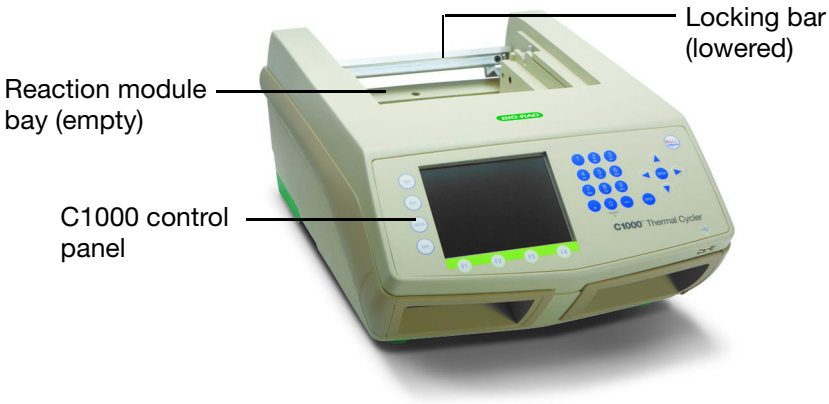


Figure 7. C1000 thermal cycler with bar and bay ready to load the CFX96 module.

2. **Carefully lift the CFX96 module, and place it in the C1000 bay so that it mostly covers the Bio-Rad label.**

   Note the location of the Bio-Rad logo in front of the bay (Figure 8). Then, using the hand holds on both sides (see Figure 2 on page 7**)**, lift the CFX96 module over the C1000

reaction module bay, and lower it into the bay (Figure 8) so that the Bio-Rad logo is mostly covered by the front of the module.

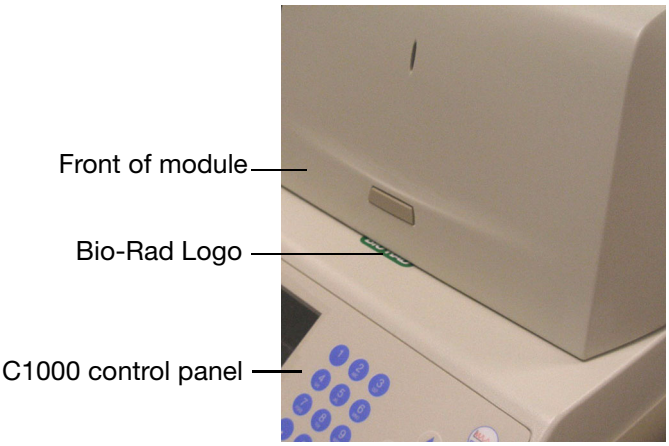


Figure 8. Place the module so that it mostly covers the Bio-Rad logo

WARNING! If you place the module over the Bio-Rad logo, the module will not install correctly, will not function, and will sit unevenly in the base.

3. **Push the module forward until it is difficult to push further.**

   Push gently on the back of the module to move it forward until there is resistance (Figure 9).

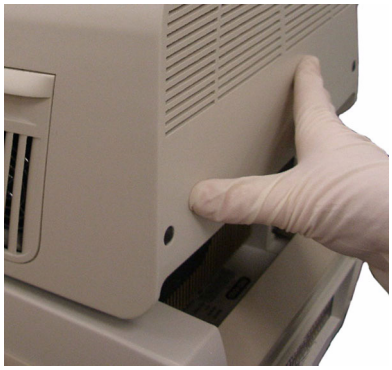


Figure 9. Push module forward until it stops moving or locks in place.

4. **Pull the locking bar all the way up to lock the module into the base, if it is not already locked.**

   NOTE: For full function, the locking bar must be flush with the housing of the C1000 base. When running the instrument, the locking bar should never be left below the housing.

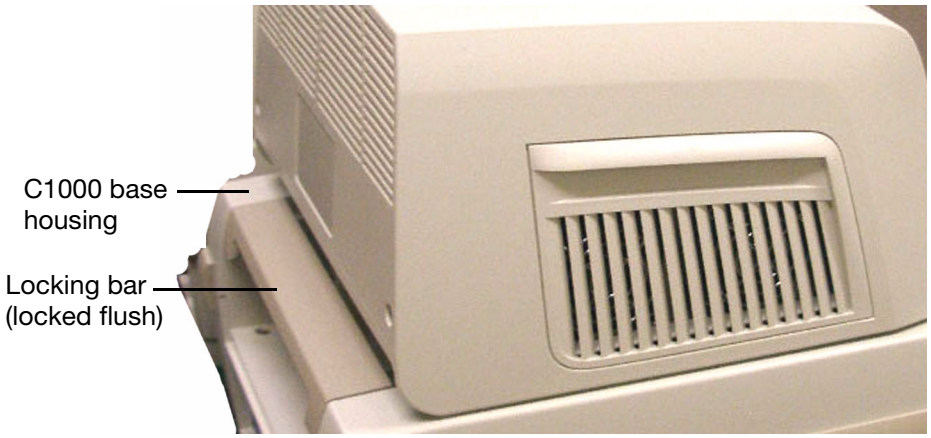


Figure 10. Locking bar in locked position and flush with housing.

5. **Check that the module is completely and evenly seated in the C1000 base.**

   As shown in Figure 11, check the space around the bottom of the module. There should be no extra space between the module and the base and the space should be even.

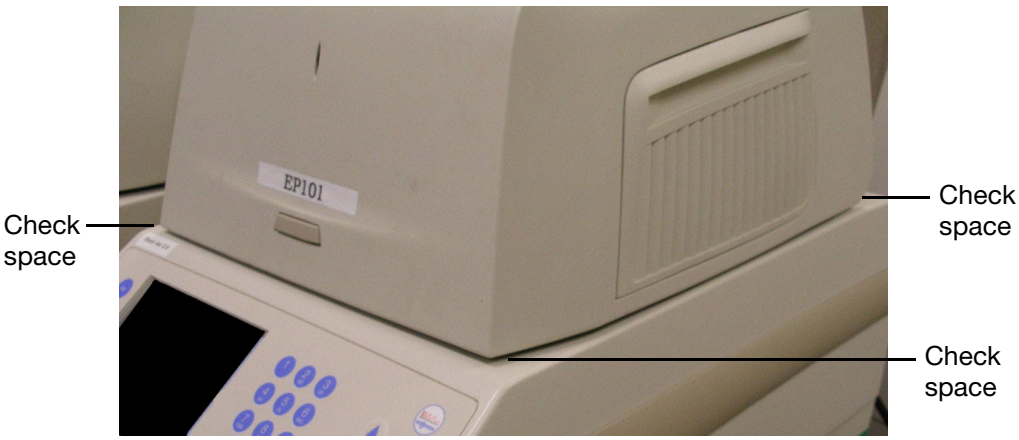


Figure 11. Check for even space between module and base, as shown here.

   NOTE: If the space between the module and the C1000 base is uneven, reinstall the module starting from Step 2 (page 10).

6. **Plug in the supplied power cord and USB cable.**

   Plug the power cord into the back of the C1000 base (Figure 6 on page 10), and into an appropriate three-pronged electrical outlet. Plug the USB 2.0 cable into the back of the instrument, and an appropriate computer port.

   NOTE: Use only the supplied power cord and USB cable. The power cord is three-pronged. The USB cable is sufficiently shielded to prevent data loss.

7. **Install the CFX Manager software.**

   Insert the software installation CD into a CD drive, then click **Install Software** to install the software.

   NOTE: If the software installation screen does not appear, see “Installing the Software Manually” on page 181.

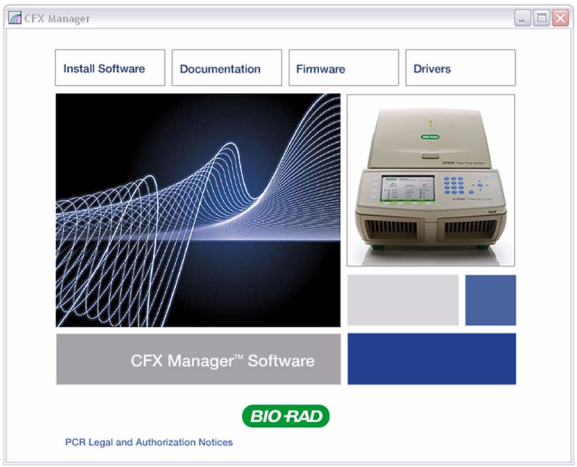


Figure 12. Software installation screen.

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

   NOTE: If the software drivers do not install automatically, insert the CD and click the **Drivers** button. See “Installing the Drivers Manually” on page 182 for full instructions.

8. **Open the software.**

   Place the CFX Manager software installation CD into a CD drive, and following the installation instructions shown on the screen. Start the software by selecting **Start > Bio-Rad > Bio-Rad CFX**.

9. **Turn on the CFX96 system.**

   Press the power switch on the back panel of the CFX 96 system (Figure 6 on page 10) to start it.

10. **Open the optical reaction module lid and remove the shipping screw.**

    Follow the instructions that appear in the software window (Figure 13) to remove the shipping screw. Press the button on the front of the lid (Figure 4 on page 8) to open the

optical reaction module lid and remove the shipping screw from the heater plate in the inner lid. Turn the screw counter-clockwise one quarter turn, and lift it out of the hole.

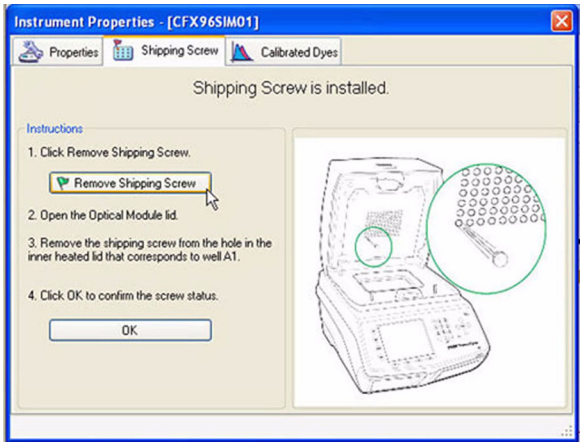


Figure 13. Software instructions for removing shipping screw.

TIP: The shipping screw must be in place when the module is shipped (Figure 14). Save this screw in a safe place for future shipping. For example, tape it to the back of the CFX96 module next to the serial number.

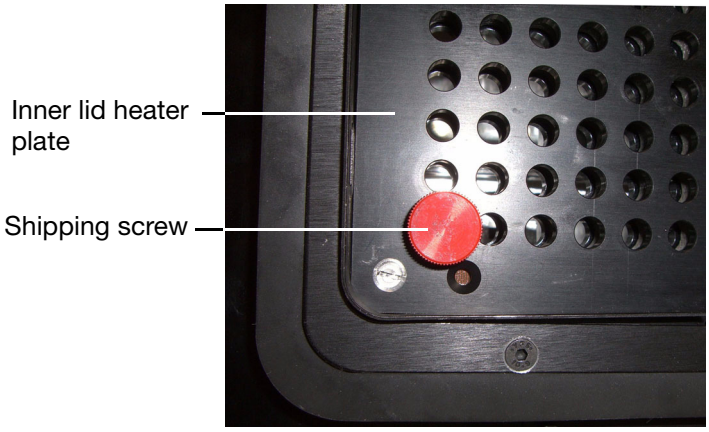


Figure 14. Remove shipping screw from the inner lid.

11. Begin running a real-time PCR experiment with the CFX96 system.

For quick guides about running experiments with CFX Manager software, refer to these CFX Manager Software quick guides:

- Installation Quick Guide
- Protocol Quick Guide
- Plate Quick Guide
- Data Analysis Quick Guide
- Gene Expression Analysis Quick Guide

Loading the Block

To load your reactions in the block, follow these suggestions:

- **Open the lid to expose the thermal block.** Click the **Open Lid** button located on software's Start Run tab (see "Start Run Tab" on page 34), or press the lid button on the front of the system(Figure 4) to start opening the motorized lid.

  WARNING! The lid moves slowly at first, and then increases speed when it opens or closes.

- **Place your samples in the block.** Place the 0.2 ml microplate or strip tubes with sealed lids in the block. Check that the tubes are completely sealed to prevent leakage.

  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 ("Plate Tab" on page 33). If needed, edit the well contents before, during or after the run. The fluorescence data is stored whether or not the well contents are complete. As shown in Figure 15, rotating the microplate by 180° causes the software to expect the reading for Std-1 sample to appear in well A10, rather than in well H3.

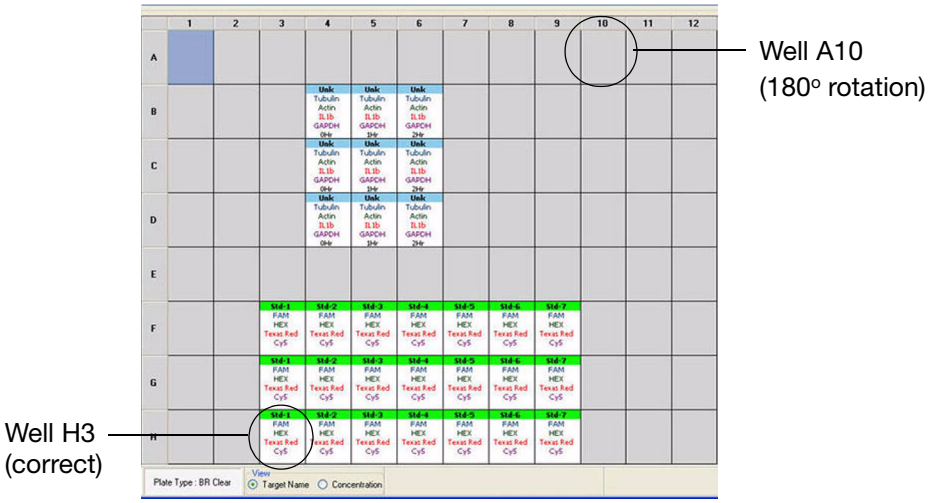


Figure 15. Plate Editor window showing plate orientation.

WARNING! Always balance the strip tubes or cut microplates in the wells (Figure 16). For example, if you run one strip tubes on the left side of the block,

then run an empty tube strip on the right side of the block to balance the pressure applied by the heated lid.

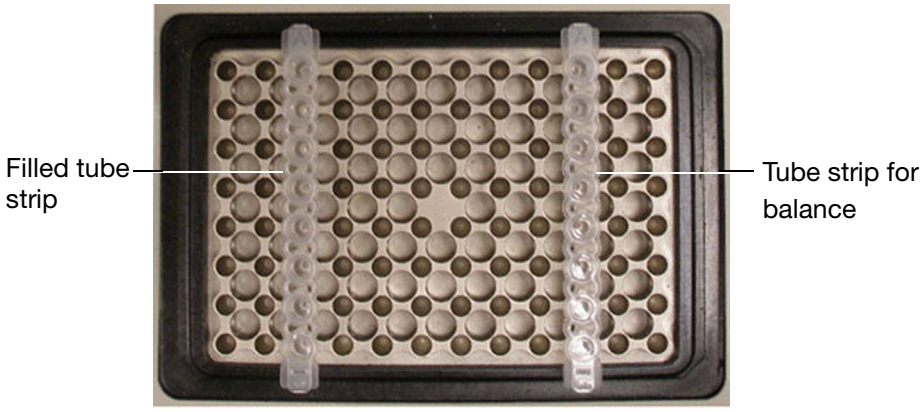


Figure 16. Balance the tube strips or cut microplates in the block.

- **Close the motorized lid after checking that nothing is blocking the lid.** Click the **Close Lid** button on the Start Run tab of the software window (see "Start Run Tab" on page 34), or push the lid button on the inside module (Figure 5 on page 9).

  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.

12. Start running a real-time PCR experiment in the Experiment Setup window.

Start the run by clicking the **Start Run** button in the CFX Manager software. Figure 17 shows the location of the Start Run button in the Experiment Setup window (see "Experiment Setup Window" on page 29).

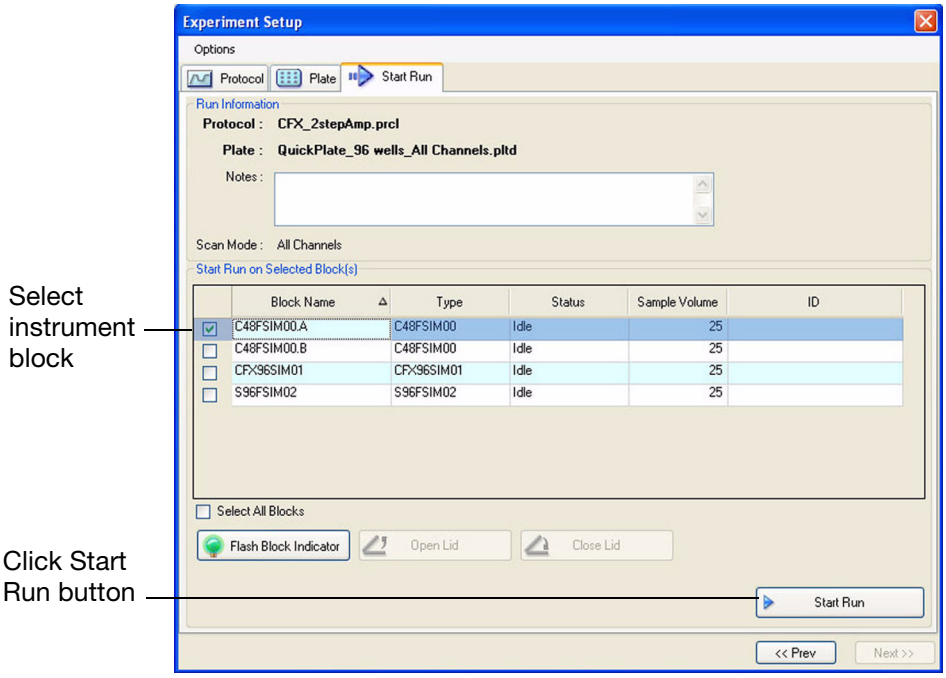


Figure 17. Start Run tab showing Start Run button.

3 Maintain the CFX96

To maintain the CFX96 optical reaction module follow these instructions:

- Avoiding contamination (see next section on page 17)
- Cleaning the CFX96 system (page 18)

For more information about running the CFX96 system, see the CFX Manager Software Protocol, Plate, Data Analysis, and Gene Expression Analysis quick guides.

Avoiding Contamination

Your CFX96 system includes a sensitive optical shuttle system that moves quickly during data collection, 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, and optical head in the shuttle system. Excessive dirt can dim the signal, and fluorecent contamination can create excessive background signal. The shuttle system cannot be cleaned, except by trained Bio-Rad service engineers.

Avoid contaminating the CFX96 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
- Clean the block and inner lid periodically to prevent the build up of dirt, biohazardous material, or fluorescent solutions (page 18)
- Never clean or otherwise touch the optical system behind the heater plate holes, that are in the inner lid (Figure 18 on page 18)
- Clean the outer lid and C1000 base on a regular schedule (for details see C1000™ Thermal Cycler instruction manual)

Cleaning the CFX96 Optical Reaction Module

The CFX96 optical reaction module should be cleaned, along with the C1000 thermal cycler base, 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. For more detailed information about cleaning the C1000 base, see the C1000™ Thermal Cycler instruction manual.

WARNING! Never use cleaning solutions that are corrosive to aluminium. Avoid scratching the surface of the C1000 reaction module bay. Scratches and damage to this surface interfere with precise thermal control.

WARNING! Never pour water or other solutions in the C1000 reaction module bay. Wet components can cause electrical shock when the thermal cycler is plugged in.

Clean the CFX96 optical reaction module as soon as you discover depris, 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 remove the reaction module from the thermal cycler base, or 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 (Figure 18).

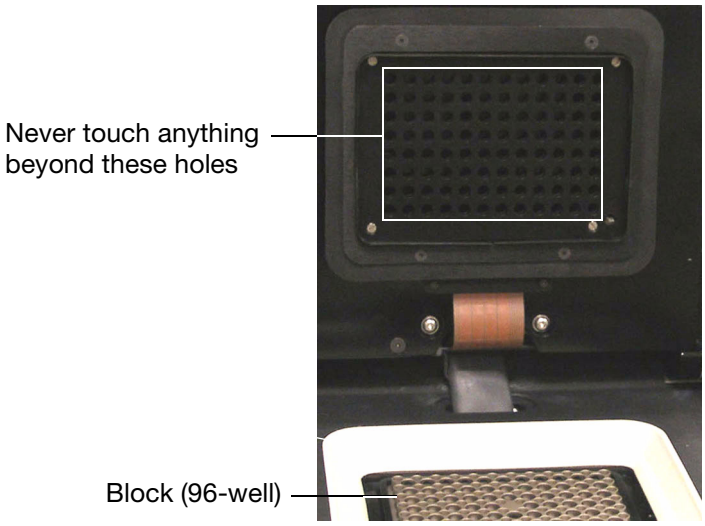


Figure 18. Heating plate holes in the inner lid.

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.
- **Clean the cooling fins.** Remove dust with a soft brush or damp cloth. Remove any heavy dust that is deep in the vents with a vacuum cleaner. Use water and a soft, lint-free cloth to remove debris that is stuck to the fins. Avoid scratching the surface. If needed,

use a mild soap solution and rinse well to remove residue completely. Cleaning the fins improves precise sample heating and cooling.

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 pipettes 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.

  WARNING! Never allow anything in the holes of the inner lid (Figure 18 on page 18). Sticking any physical object or liquid solution in the holes can damage the optical system.

4 Introduction to CFX Manager Software

The CFX Manager software creates and runs PCR and real-time PCR experiments with the CFX96 optical reaction module. Read this chapter for the following information about getting started:

With CFX Manager software, you can easily start running PCR and real-time PCR:

- **Start experiments quickly.** Open the Experiment Setup window to "Express Load" protocol and plate files, then start the run
- **Receive an email notification.** When the experiment finishes, you can receive an email notice with the attached data file
- **Customize data analysis.** Customize data analysis views and settings
- **Export charts and spreadsheets.** Export the data in charts and spreadsheets from right-click menu options
- **Group wells for analysis.** Analyze multiple experiments in a single plate with well groups
- **Run up to four instruments.** Manage real-time PCR systems, or a mix of real-time PCR systems and thermal cyclers

Getting Started

Get started running experiments with CFX Manager software by selecting one of these software windows:

- **Startup Wizard window (page 27).** Provides quick access to common software features, including creating a new experiment, opening a data file, or changing user preferences.
- **Experiment Setup window (page 29).** Provides easy access to the Protocol, Plate, and Start Run tabs to create an experiment, and edit or run an existing experiment.

To assist you in running successful experiments, Bio-Rad Laboratories provides resources with more information about PCR and real-time PCR. Also, the Resources chapter (page 177) provides information about contacting Bio-Rad Laboratories, FAQs, tips and tricks, references, and troubleshooting options.

Quick Guides

For instructions about running the system, refer to one of the CFX Manager Software quick guides that shipped with the system (see “Unpacking the CFX96 Optical Reaction Module” on page 5):

- Installation Quick Guide
- Protocol Quick Guide
- Plate Quick Guide
- Data Analysis Quick Guide
- Gene Expression Analysis Quick Guide

  TIP: See the software Help for more guides to get you started running experiments.

Main Software Window

Get started in the main software window by using these features:

- **Menu bar.** Select software commands (page 24), such as creating or opening files
- **Toolbar buttons.** Click these buttons (page 26) to open software files, the Startup Wizard (page 27), the Experiment Setup pane (page 29), and User Preferences window (page 157)
- **Startup Wizard window.** Access common software commands (page 27)
- **Detected Instruments pane.** View a list of attached instruments (page 145)
- **Status bar.** View the current software and instrument status (page 27)

Figure 19 shows the main software window and features:

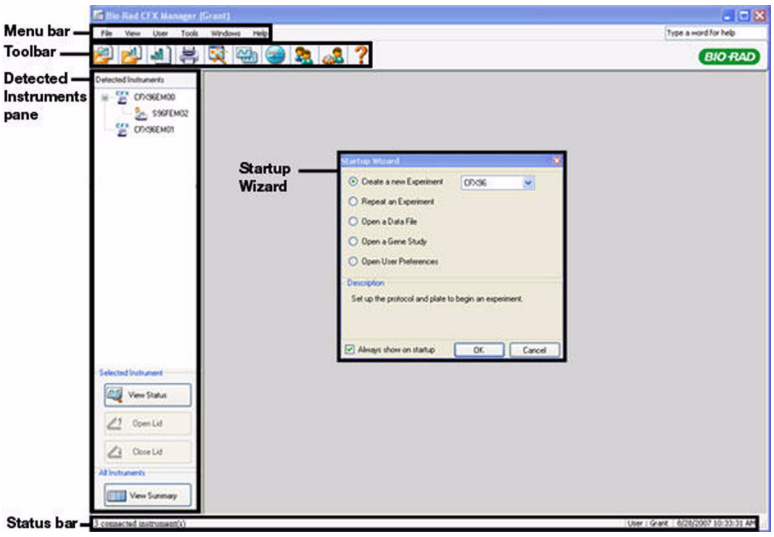


Figure 19. The main software window.

Menu Bar Items

The menu bar of the main software window provides the items listed in Figure 20:

Figure 20. 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
	Recent Data Files	View a list of the ten most recently viewed data files, and select a file to open in the Data Analysis window
	Repeat an Experiment	Open the protocol and plate from a completed run in the Experiment Setup window
	Exit	Exit the software program
View	Application Log	Display the application log for the software
	Run Reports	Display a list of run reports and select a report to review
	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 toolbar at the top of the main window
	Status Bar	Show or hide the status bar at the bottom of the window
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

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

Menu Item	Command	Function
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
	Zip Data and Log Files	Choose and condense selected files in a zipped file for storage or to email
	Application Data Folder	Open the Application Data folder to view software files
	User Data Folder	Open the Data folder to view data files and sample files
Windows	Cascade	Arrange the software windows on top of each other
	Tile Vertical	Arrange the software windows from top to bottom
	Tile Horizontal	Arrange the software windows from right to left
	Close All	Close all open 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 Web site	View a web site that lists Bio-Rad consumables for PCR and real-time PCR reagents
	PCR Plastic Consumables Web site	View a web site that lists Bio-Rad consumables for PCR and real-time PCR experiments
	Software Updates	Check for software updates from Bio-Rad Laboratories
	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 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	Action
	Open a Data File	Open a browser window to locate a data file (*.pcrd extension) and open it in the Data Analysis window (page 77)
	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 135)
	Create a New Gene Study	Open a empty Gene Study window (page 135) 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 27)
	Experiment Setup	Open the Experiment Setup window to run an experiment (page 29)
	Protocol AutoWriter	Open the Protocol AutoWriter window to create a new protocol (page 49)
	Select User	Open the Select User window to change software users (see “Log On and Change Password” on page 173)
	User Preferences	Open the User Preferences window (page 157)
	Help	Open the software Help window for more information about running PCR and real-time PCR

Status Bar

The status bar is at the bottom of the main software window. This bar shows the current status of the software.

NOTE: To show or hide the status bar, select **View > Status Bar** in the menu bar.

View the left side of the menu bar (Figure 21) to see the current status of instruments.

Figure 21. Left side of status bar in main software window.

View the right side of the menu bar (Figure 22) to see the current the user name, date, and time.

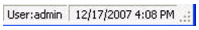


Figure 22. Right side of status bar in the main software window.

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

Startup Wizard

Click the Startup Wizard button in the toolbar of the main software window to open the Startup Wizard window. Then, select an option from the list of common software commands (Figure 23).

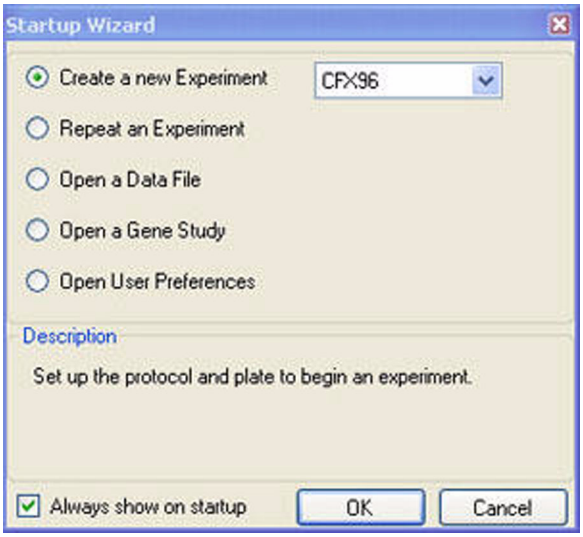


Figure 23. Startup Wizard window.

To begin using the software, choose one of the options in the Startup Wizard:

- **Create a new Experiment (page 36).** Set up a new experiment in the Experiment Setup window and select an instrument (listed in the pull-down menu)
- **Repeat an Experiment (page 41).** Open the protocol and plate from a completed run in the Experiment Setup window (page 29)
- **Open a Data file (page 77).** Open a data file to analyze results in the Data Analysis window
- **Open a Gene Study (page 135).** Open a Gene Study file to analyze results from multiple gene expression experiments in the Gene Study window analyze results from multiple gene expression experiments
- **Open User Preferences (page 157).** Open the User Preferences window to customize software settings

5 Run Experiments

To run an experiment in CFX Manager software, open the Experiment Setup window for quick access to all the files and settings needed to set up and run an experiment. The Experiment Setup window provides tabs for quick access to the experiment files and the run settings:

- **Protocol tab.** Select, edit or create a protocol (page 30)
- **Plate tab.** Select, edit or create a plate (page 33)
- **Start Run tab.** Select one or more instruments for the run (page 34)

  TIP: Each real-time PCR experiment requires a protocol file that includes a plate read, a plate file, and run settings. Each PCR experiment requires the protocol file and run settings.

For more information about experiments, see these sections:

- Experiment Setup window (next section on page 29)
- Protocol tab (page 30)
- Plate tab (page 33)
- Start Run tab (page 34)
- Experiment files (page 36)
- Create a new experiment (page 36)

Experiment Setup Window

The Experiment Setup window provides quick access to all 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 27)
- Click the **Experiment Setup** button in the toolbar (page 26)
- Select **File > New > Experiment** in the menu bar (page 24)

The Experiment Setup window includes these three tabs for setting up and starting an experiment:

- **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 40)
- **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 (see page 55; for real-time PCR experiments only)

- **Start run.** Click the Start Run tab (page 34) to check the run settings, select one or more instrument blocks, and begin the run

  NOTE: If the protocol 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 44) in at least one step in the protocol.

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

  TIP: To create an end point protocol (page 32), select **Options > End Point Only Run** in the Experiment Setup menu bar.

The Experiment Setup window opens with the Protocol tab in the front. To open another tab, click that tab. Alternatively, click **Prev** and **Next** buttons at the bottom of the window (Figure 24) to open the next or previous tab.

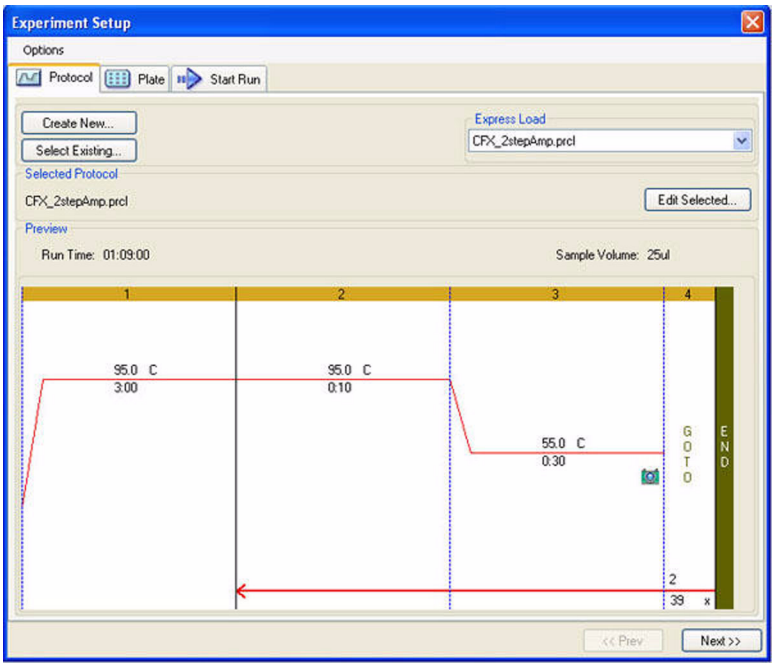


Figure 24. Experiment Setup window, including the Protocol, Plate, and Start Run tabs.

Protocol Tab

The Protocol tab shows a preview of the selected protocol file. Select a protocol from the **Express Load** menu. Alternatively, click one of the buttons on the Protocol tab (Figure 25 on page 31) to create a protocol, select an existing protocol, or edit the selected protocol. To preview the currently selected protocol, click the Protocol tab in the Experiment Setup window (page 29).

TIP: You can change the sample volume just prior to starting a run by typing a new sample volume in the block list on the Start Run tab.

A protocol file contains the instructions for the instrument temperature steps, as well as instrument options that control the lid temperature and ramp rate.

Select one of the following options to create and edit protocol files in the Protocol tab:

- **Create New button.** Open the Protocol Editor with a default protocol to create a new protocol

- **Select Existing button.** Open a browser window to select and load an existing protocol file (.prcl extension) in the Protocol tab
- **Express Load pull-down menu.** Select a protocol in this list to load it into the Protocol tab

  TIP: To add or delete the protocols listed in the **Express Load** menu, add or delete plate 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 button.** Open the currently selected protocol in the Protocol Editor
- **Options menu.** Select **Options > End Point Only Run** in the Experiment Setup menu bar to load and run an End Point Only protocol. Selecting this option a second time removes end point protocol from the Protocol tab

  TIP: To change the default parameters in a new plate, edit them in the User Preferences Plate tab (page 170).

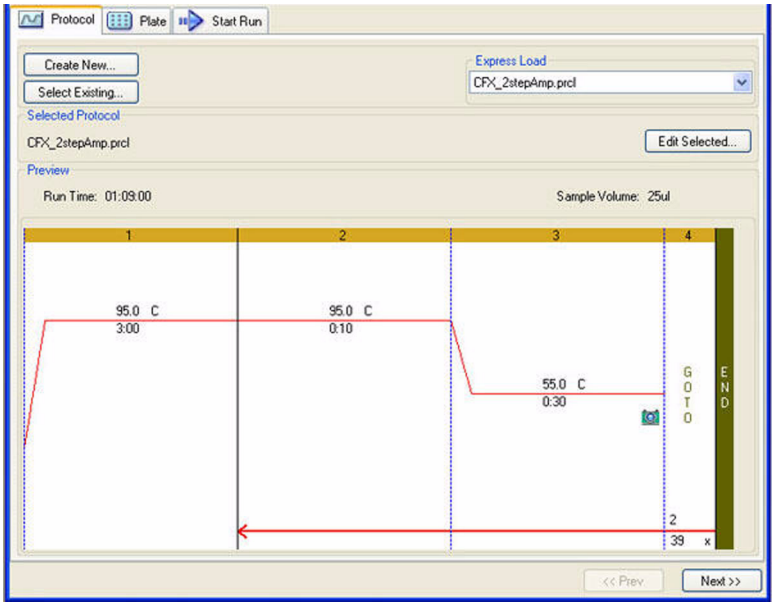


Figure 25. Protocol tab window.

TIP: To add or delete the protocols listed in the **Express Load** menu, add or delete plate 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.

End Point Only Protocol

To run a protocol that contains only an end point step, select **Options > End Point Only Run**. The Protocol tab opens the default end point protocol, which includes two cycles of 60.0°C for 30 seconds.

TIP: Change the end point parameters in the Selected Block(s) list on the Start Run tab (page 34).

Figure 26 shows the default end point protocol:

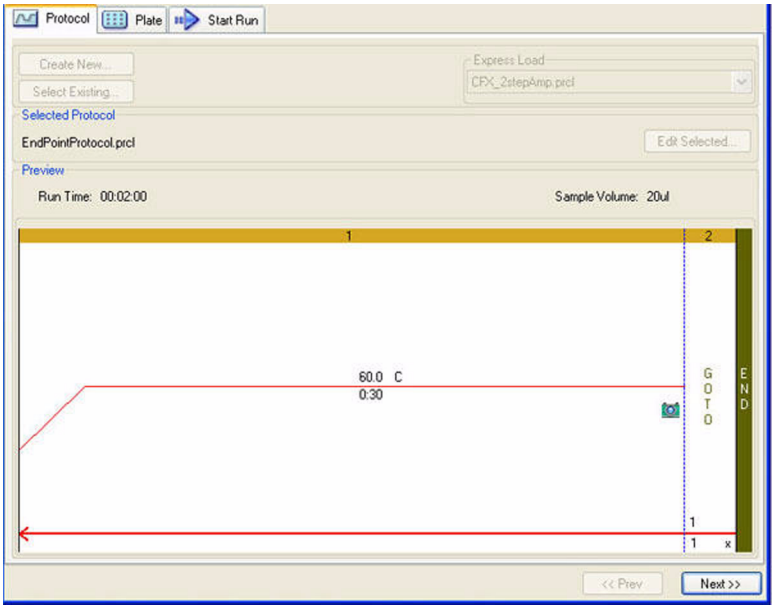


Figure 26. End Point Only protocol.

To change the temperature or sample volume, click the Start Run tab (page 71) and edit the **Sample Temperature** or **Sample Volume**. Figure 27 shows the sample temperature is changed to **55.0**.

Start Run on Selected Block(s)

	Block Name	Type	Status	Step Temperature	Sample Volume	ID
☐	CFX384SIM00	CFX384SIM00	Idle	55.0	25	
☐	CFX96SIM01	CFX96SIM01	Idle	60.0	25	

Figure 27. Change the sample volume on the Start Run tab.

TIP: View the data from an End Point Only run in the End Point tab (page 100) of the Data Analysis window.

Plate Tab

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

Click the Plate tab (Figure 28 on page 33) in the Experiment Setup window (real-time PCR experiments only), and select one of the following options to create or edit plate files:

- **Create New.** Open the Plate Editor with a new plate to create a new plate file.
- **Select Existing.** Open a browser window to select and load an existing plate file (.pltd extension) and load it into the Experiment Setup window
- **Express Load.** Select a plate from this list of existing plates. To get started quickly, select the QuickPlate file from the Express Load list, then load the well content during or after the run. This plate instructs the instrument to collect data from all channels in the Scan Mode

  TIP: To add or delete plate files from the Express Load menu, add or delete plate 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.** Open the selected plate in the Plate Editor

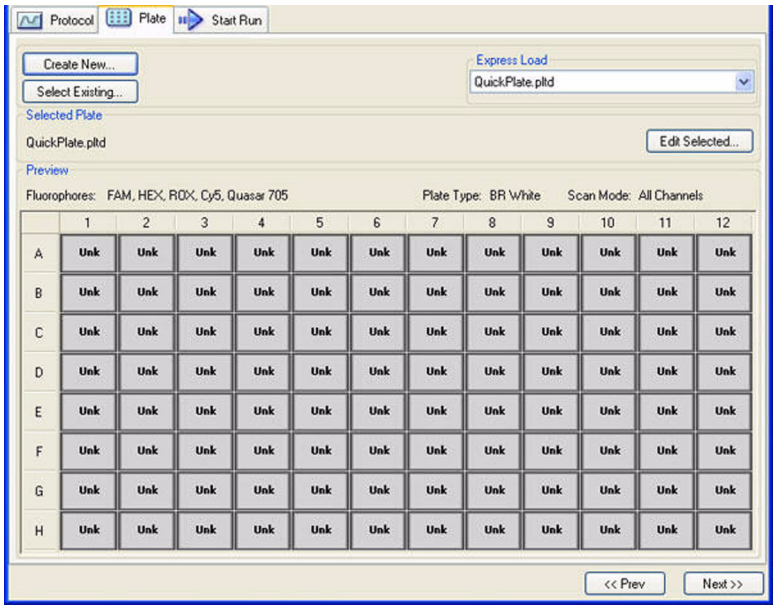


Figure 28. Plate tab window.

TIP: To change the default parameters in a new plate, edit them in the Plate tab in the User Preferences window.

Start Run Tab

The Start Run tab (Figure 29 on page 34) in the Experiment Setup window includes sections for checking information about the run and for selecting the instrument block:

- **Run Information pane.** View the selected protocol file, plate file, and scan mode. Enter optional notes about the experiment in the **Notes** box
- **Start Run on Selected Block(s) pane.** Select one or more blocks and load samples into those blocks, and then click the **Start Run** button to start the run from the software.

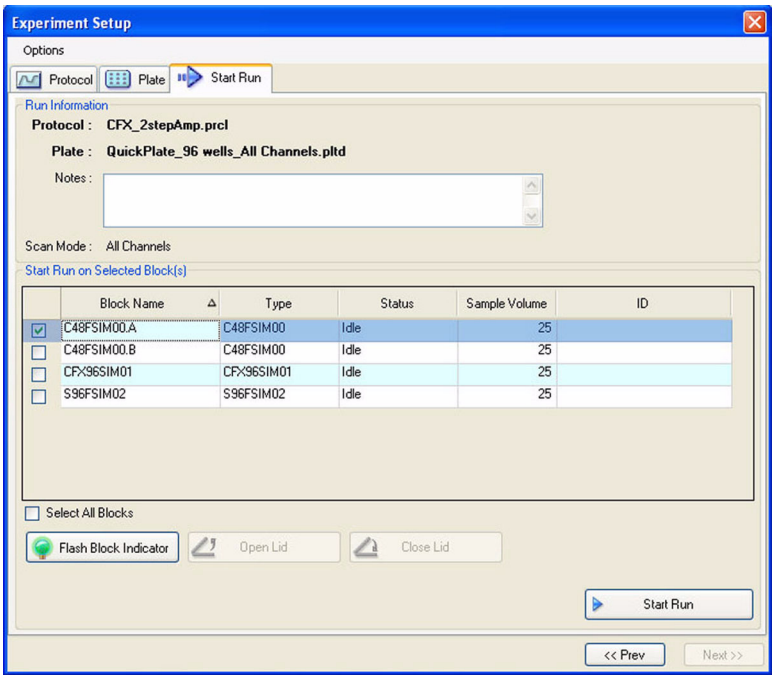


Figure 29. The Start Run tab.

TIP: To change parameters in the **Start Run on Selected Block(s)** list, click the text in a cell and edit the parameter. For example, to change the sample volume in one block, click the text in the Sample Volume column and type a new volume.

Selected Blocks List

The **Start Run on Selected Block(s)** pane shows the status of each instrument block detected by the software, and information about the selected protocol:

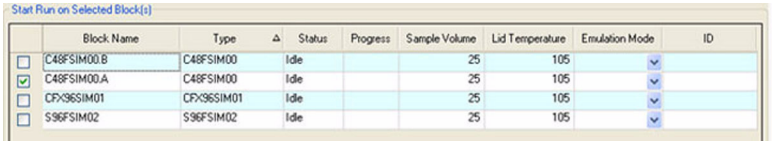


Figure 30. Start Run on Selected Block(s) in the Start Run tab.

Edit some of these parameters by clicking the text to select it. Then, type in the cell, or select a new parameter from the pull-down menu. To add or remove options, right-click on the list and select the option in the right-click menu.

Right-click Options in Selected Block(s) List

Table 9 shows information about each option in the **Start Run on Selected Block(s)** pane:

Table 9. Start Run on Selected Block(s) right-click menu options

Right-click Option	Function
Block Name (default)	Name of the instrument block. The default instrument block name is the serial number
Type (default)	Display type of instrument block
Status (default)	Current status of the block. Describes whether the block is running or idle
Sample Volume (default)	View or override the sample volume in the selected protocol. Override the sample volume for a run by selecting the text and typing a new volume
Lid Temperature	View the current temperature of the lid. Override that lid temperature by selecting the text and typing a new temperature
Emulation Mode	Select iCycler or DNA Engine. These selections instruct the instrument to emulate the ramp speed of these instruments
ID (default)	Enter an ID by hand, or by scanning it with a bar code reader.
Copy	Copy selected text
Copy as Image	Copy an image of the Start Run on Selected Block 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

Buttons for Controlling the Instrument

Click the following buttons in the Start Run tab to remotely operate the selected instruments:

- **Start Run.** Start the experiment on the selected instrument blocks
- **Flash Block Indicator.** Flash the LED on the selected instrument blocks to verify which instruments are currently selected
- **Open Lid.** Open motorized lid on selected instrument blocks
- **Close Lid.** Close motorized lid on selected instrument blocks

  NOTE: **Open Lid** and **Close Lid** buttons are only enabled when the selected blocks include a motorized lid.

  WARNING! Opening the lid during a run will pause the protocol and might alter the results of the experiment.

Software Files

CFX Manager software stores information about experiments in these files (Table 10):

Table 10. Open these file types with CFX Manager software

File type	Extension	How to view and edit file
Protocol	.prcl	Preview in Experiment Setup window and edit in Protocol Editor
Plate	.pltd	Preview in Experiment Setup window and edit in Plate Editor
Data	.pcrd	View and analyze in Data Analysis window
Gene Study	.mgxd	View and analyze in Gene Study window

When you create a new experiment in the software, you enter the protocol, plate, and run settings. After you run an experiment, all the information about that run is stored in the data file (.pcrd).

Create and Run an Experiment

To run an experiment, follow these instructions for an overview:

1. **Open the Experiment Setup window (page 29).**

   Open this window by clicking the **Experiment Setup** button on the toolbar in the main software window.

2. **Click the Protocol tab (page 30).**

   Open this tab to select an existing protocol to run or edit a protocol, or to create a new protocol in the Protocol Editor window (page 40).

3. **Click the Plate tab (**page 55**).**

   Open this tab to select an existing plate to run or edit, or you can create a new plate in the Plate Editor window (page 55; for real-time PCR experiments only).

   TIP: To get started quickly, select the QuickPlate file from the **Express Load** list, then load the well content during or after the run. This plate instructs the instrument to collect data from all channels in the Scan Mode (page 66).

4. **Click the Start Run tab (page 71).**

   Open this tab to enter notes (optional), check the run settings, select one or more instrument blocks, and begin the run.

5. **Load PCR reactions in the block (page 15).**

   Place the PCR reactions in 0.2 ml microplates or tube strips for your experiment into the selected instrument blocks.

   NOTE: When you load tube strips into the block, be sure to balance the load across the block (Figure 16 on page 16) to assure that the pressure on the lids is even. For example, if you load one tube strip on the left side of the block, be sure to load another tube strip (empty or full) on the right side of the block.

6. **Click the Start Run button (page 34).**

   Begin running the protocol on the selected instruments by clicking this button. When the run starts, the software opens the Run Details window (next).

7. **View the Run Details window (page 72).**

   Review the progress of the run and view the accumulating data in this window. When the run finishes, the software automatically opens the Data Analysis window (page 78).

8. **Analyze the data in the Data Analysis window (page 78).**

   Review the data in the Data Analysis window. Make adjustments to the well contents in the Plate Editor (page 55), then make adjustments to the data appearance in the Data Analysis window (page 78). Export and print the results.

6 Protocols

CFX Manager software uses the information from a selected protocol to run PCR. A software protocol instructs the selected instruments to control the temperature steps, lid temperature, and other instrument options. Protocols created in the Protocol Editor are available to load into the Protocol tab in the Experiment Setup window.

TIP: Refer to the CFX Manager Software Protocol Quick Guide to set up a protocol file.

For information about running protocols, see the following sections in this chapter:

- Protocol Editor window (page 40)
- Protocol Editor buttons (page 42)
- Protocol AutoWriter (page 49)
- Temperature control mode (page 51)

A software protocol contains the following parameters that instruct the instrument during a run:

- **Protocol steps.** Include temperature steps that instruct the thermal cycler to heat and cool for a specific amount of time
- **Lid temperature.** Instructs the instrument to maintain the specified lid temperature to prevent condensation
- **Sample volume.** Sets the temperature control mode according to the sample volume
- **Ramp rate.** Determines the heating and cooling rate of the block. The default ramp rate is the maximum rate of each block.
- **Plate Read.** Instructs the instrument to collect real-time PCR data by measuring fluorescence in the samples.

  WARNING! To collect real-time PCR data during a run, be sure to insert a Plate Read into at least one protocol step. If the protocol that is loaded in the Experiment Setup window does not include a plate read, then the Plate tab is hidden.

Protocol Editor Window

Open the Protocol Editor window to create a new protocol or to edit the selected protocol. Once a Protocol is created or edited in the Protocol Editor, click **OK** to load the protocol file in 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**
- To open or edit an existing protocol, select **File > Open > Protocol**
- In the Experiment Setup window, click the **Create New** button, or open an existing protocol by clicking the **Select Existing** or **Edit Selected** buttons

  TIP: To change the default settings in the Protocol Editor window, enter the changes in the User Preferences Protocol tab (page 169).

Protocol Editor Window

The Protocol Editor window (Figure 31) includes the following features:

- **Menu bar.** Select settings for the protocol
- **Toolbar.** Click buttons or select options for this protocol
- **Protocol.** View the selected protocol in a graphic (top) and text (bottom) view. Each view describes the protocol steps and includes different features
- **Protocol Editor buttons.** Create and edit a protocol by selecting a step, and then clicking one of the buttons to the left of the text view

  NOTE: To create an End Point Only protocol, select Options > End Point Only Run in the menu bar in the Experiment Setup window.

  TIP: Entering a Sample Volume determines the Temperature Control mode for each protocol.

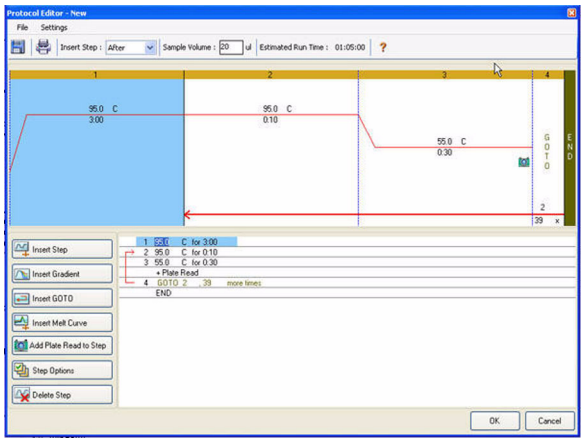


Figure 31. Protocol Editor window with buttons for creating and editing protocols.

Protocol Editor Menu Bar

The menu bar (Figure 32) in the Protocol Editor window provides these menu items:

Figure 32. Protocol Editor menu bar.

Table 11 lists the items in the menu bar:

Table 11. 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 the lid settings. Set the Lid Temperature and the Default Lid Temperatures
	Gradient Type	Select the block type for a gradient step. Choose from 96 Wells or 384 Wells

Protocol Editor Toolbar

The toolbar (Figure 33) in the Protocol Editor window provide quick access for important functions:

Figure 33. Protocol Editor toolbar.

TIP: To change the default sample volume and lid shut off temperature, enter the defaults in the Protocol tab of the User Preferences window.

Table 12 lists the function of the Protocol Editor toolbar buttons:

Table 12. Plate Editor toolbar buttons

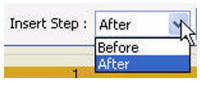
Toolbar Button and Menus	Name	Function
	Save	Save the current protocol file
	Print	Print the selected window
	Insert Step	Select **After** or **Before** to insert steps after or before the selected (highlighted) step
	Sample Volume	Enter a sample volume (in ul) between 0 and 50 (96 wells) or 0 and 30 (384 wells). The sample volume you enter determines the Temperature Control mode (page 51). Enter zero (0) to select Block mode

Table 12. Plate Editor toolbar buttons (continued)

Toolbar Button and Menus	Name	Function
	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 Buttons

The Protocol Editor window includes buttons for editing the protocol. First, select a step in the protocol by highlighting it. Then click one of the Protocol Editor buttons at the bottom left side of the Protocol editor window to change the protocol. Specify the location for inserting a new step, select Before or After in the Insert Step box at the top of the tab.

Table 13 describes the functions of each of the Protocol Editor buttons:

Table 13. Protocol Editor buttons and their functions

Button	Function
	Insert a temperature step before or after the selected step (page 43)
	Insert a gradient step before or after the selected step (page 45)
	Insert a GOTO step before or after the selected step (page 46)
	Insert a melt curve before or after the selected step. The default hold time is 5 seconds (page 47) NOTE: The Melt Curve step includes an extra 30 second hold.
	Add or remove a plate read in the selected step (page 44) NOTE: The button name changes, depending on the function that is available for the selected step
	Open the Step Options window to add or delete options in the selected step (page 48). Step Options include plate read, temperature, gradient range, increment, ramp rate, time, extend, and beep. TIP: To hold a step forever (an infinite hold), enter zero (0.00) for the time
	Delete the selected step (page 49)

Insert Step Button

To insert a temperature step before or after the selected step in the Protocol Editor window, follow these instructions:

To add and edit a temperature step Protocol Editor window:

1. Select a step next to the step you want to add by clicking on the step in the graphic or text view.
2. In the **Insert Step** option in the toolbar, select **Before** or **After** to insert the step before or after the selected step.
3. Insert a temperature step by clicking the **Insert Step** button.
4. (Optional) Edit the temperature or hold time by clicking the default temperature in the graphic or text view, and entering a new temperature.
5. (Optional) To insert a plate read in the step, click the **Add Plate Read to Step** button (page 44).
6. (Optional) Click the **Step Options** button to enter an increment or extend option in the Step Options window (page 48)

Figure 34 shows a new step was inserted after step 2.

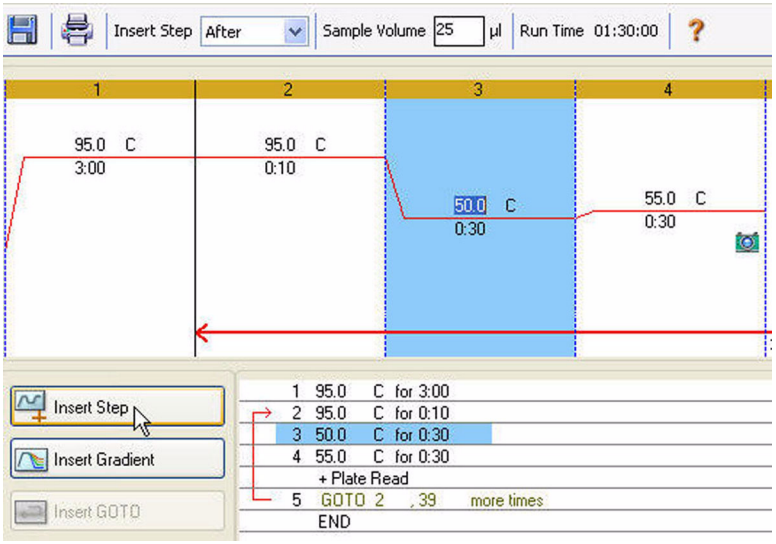


Figure 34. Protocol with inserted step.

Add or Remove a Plate Read

To add or remove a plate read, click the button which changes between these two names:

- **Add Plate Read to Step.** If the selected step does not include plate read, then you can add it
- **Remove Plate Read.** If the selected step already includes a plate read, then you can remove it

To add a plate read to a step or remove a plate read in the Protocol Editor window, follow these instructions:

1. Select the step where you want to add or remove the plate read.
2. Click the **Add Plate Read to Step** button to add a plate read to the selected step, or click the **Remove Plate Read** button to remove a plate read from the selected step.

   NOTE: To run a real-time PCR experiment, the protocol must contain one or more plate reads.

In Figure 35, step 4 includes a plate read. Notice that the camera icon in the graphic view (top) shows that step 4 includes a plate read.

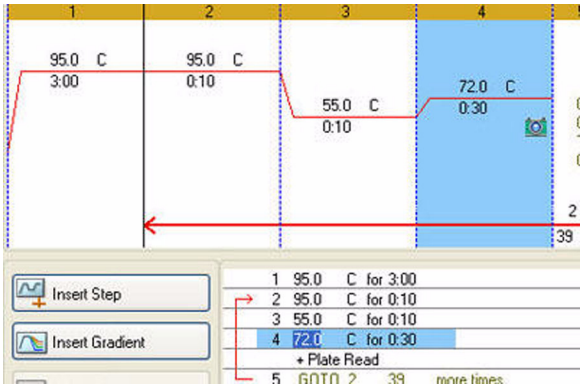


Figure 35. Protocol step with an added plate read.

Insert Gradient Button

To insert a gradient step before or after the selected step in the Protocol Editor window, follow these instructions:

1. Select a step next to the step you want to add by clicking on the step in the graphic or text view.
2. Select the plate size for the gradient by selecting **Options > Gradient Type** in the Protocol Editor menu bar.
3. In the **Insert Step** menu in the Protocol Editor toolbar, select **Before** or **After** to insert the step before or after the selected step.
4. Insert a temperature step by clicking the **Insert Gradient** button.
5. 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 48)
6. Edit the hold time by clicking the default time in the graphic or text view, and entering a new time.

Figure 36 shows the inserted gradient step. Notice the temperatures of each row in the gradient charted on the right side of the window.

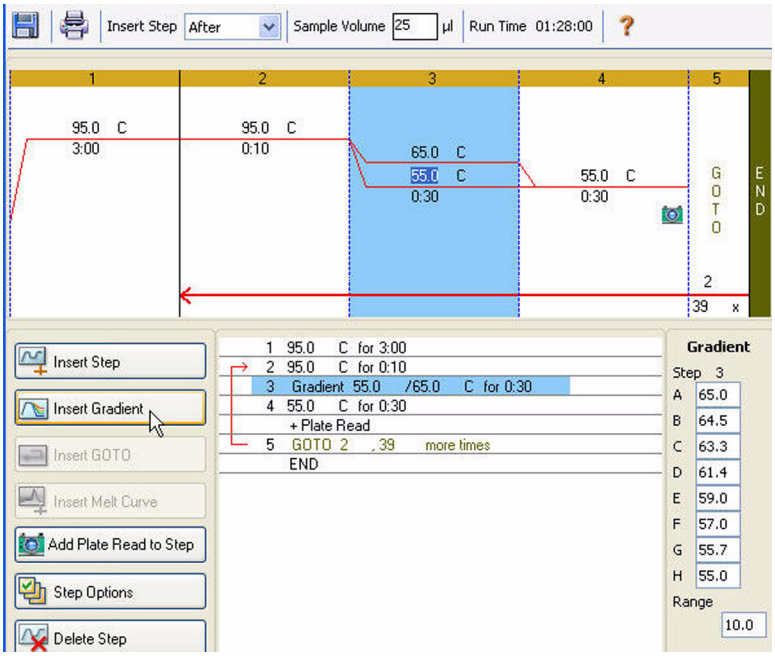


Figure 36. Protocol with inserted gradient step.

Insert GOTO Button

To insert a GOTO step before or after the selected step in the Protocol Editor window, follow these instructions:

1. Select a step next to the step you want to add by clicking on the step in the graphic or text view.
2. In the **Insert Step** option in the toolbar, select **Before** or **After** to insert the step before or after the selected step.
3. Insert a temperature step by clicking the **Insert GOTO** button.
4. (Optional) Edit the GOTO step or number of GOTO repeats by clicking the default number in the graphic or text view, and entering a new number.

Figure 37 shows an inserted GOTO step at the end of the protocol. Notice that the GOTO loop includes step 3 and 4

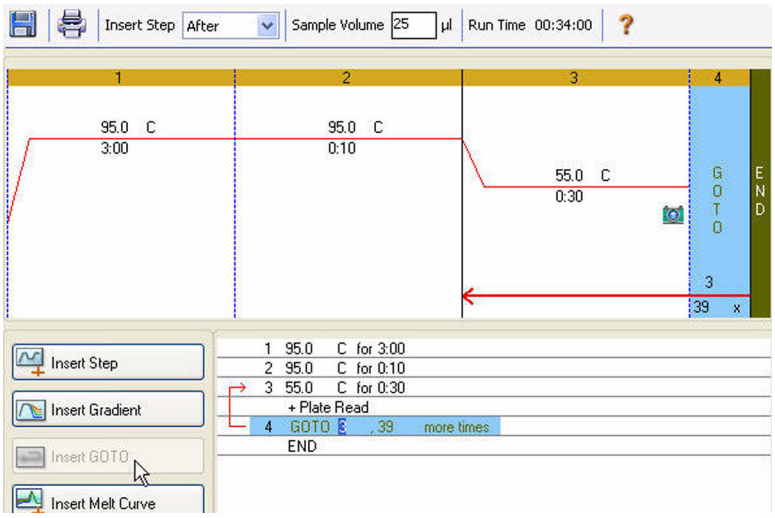


Figure 37. Protocol with inserted GOTO step.

Insert Melt Curve Button

To insert a melt curve step in the Protocol Editor window, follow these instructions:

1. Select a step next to the step you want to add by clicking on the step in the graphic or text view.
2. In the **Insert Step** option in the toolbar, select **Before** or **After** to insert the step before or after the selected step.
3. Insert a temperature step by clicking the **Insert Melt Curve** button.
4. Edit the melt temperature range or increment time by clicking the default number in the graphic or text view, and entering a new number. Alternatively, click the **Step Options** button to enter the gradient range in the Step Options window (page 48)

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

Figure 38 shows a melt curve step added after step 6:

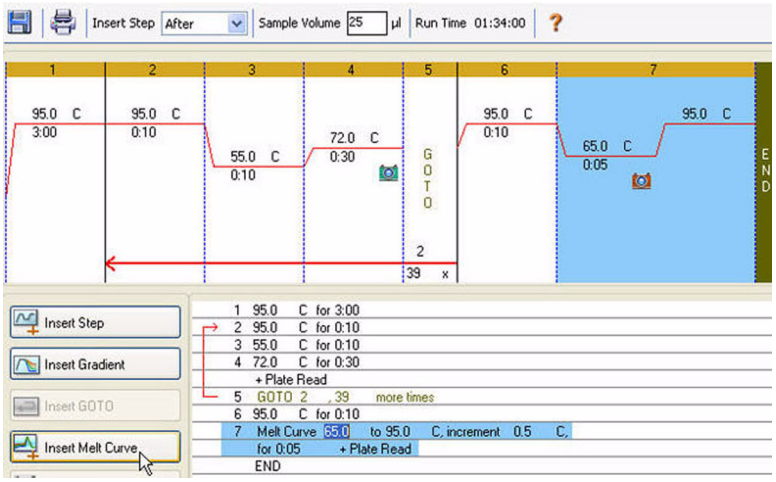


Figure 38. Protocol with inserted melt curve step.

Change Step Options

To change a step option for the selected step in the Protocol Editor window, follow these instructions:

1. Select a step by clicking on the step in the graphic or text view.
2. Click the **Step Options** button to open the Step Options window.
3. Add or remove options by entering a number, editing a number, or clicking a check box.

Figure 39 shows step 3 (selected) with an extend time of "**1**" sec/cycle.

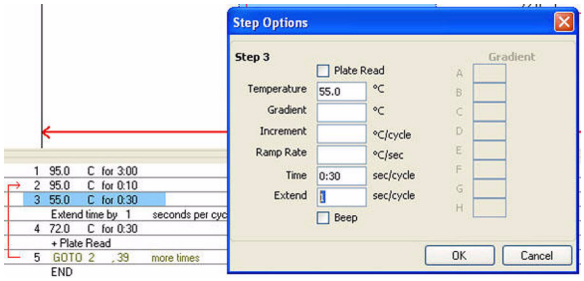


Figure 39. Protocol step 3 includes an extend time of 1 second/cycle.

Figure 40 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.

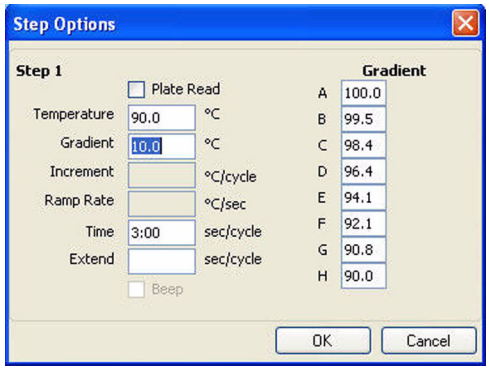


Figure 40. Step option for a gradient.

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 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. The range is added to the target temperature to create a thermal gradient across the block
- **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 to decrease the rate that the thermal cycler heats and cools. The range of possible ramp rates 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. To find the highest or lowest end of the range, enter any number outside the range for the Step Option to have the software automatically fill in the nearest number within the range.

Delete Step Button

To delete a step in the Protocol Editor window, follow these instructions:

1. Select (highlight) a protocol step in the graphic or text view.
2. Click the **Delete Step** button to delete the selected step.

   WARNING! You cannot undo this function.

Protocol AutoWriter

Open the Protocol AutoWriter to quickly write protocols for PCR and real-time PCR experiments:

- Protocol AutoWriter overview
- Create a protocol with the Protocol AutoWriter

To open the Protocol AutoWriter, select on 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 41 shows a protocol (bottom of window) written by the Protocol AutoWriter.

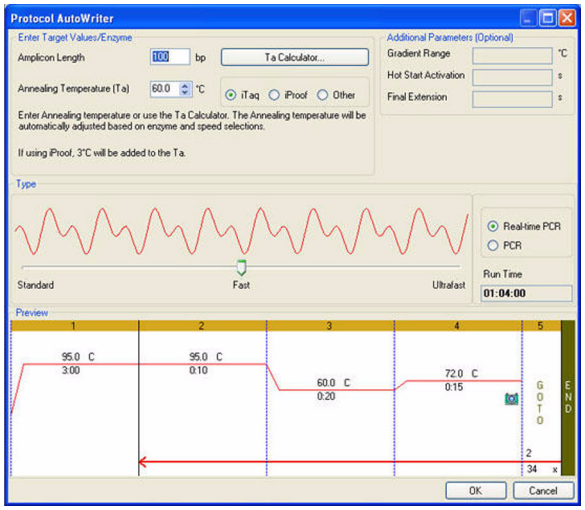


Figure 41. Protocol AutoWriter window with a new protocol.

Protocol AutoWriter Overview

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 melting temperature is unknown, click the **Ta Calculator** button to

enter the primer sequence to calculate the melting temperature in the Ta Calculator window

- **Amplicon length (bp).** Enter the expected length of the PCR product
- **Enzyme type.** Select the DNA polymerase enzyme (iTaq, iProof, or Other), enter additional information including the hot start activation time, and enter the final extension time
- **Run time (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 actual 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 sec.); 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 2-3 basepairs (bp), or redesign them to adjust the melting temperature.

Create a Protocol With the Protocol AutoWriter

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

1. Click the **Protocol AutoWriter** button 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 the **Ta Calculator** button to enter the primer sequences and calculate the annealing temperature. For information about the calculations used in the Ta Calculator see the Reference by 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 in the **Preview** pane and total run time. Make changes as needed.
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 40).

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

Temperature Control Mode

The instrument uses one of two temperature control modes to determine when the sample reaches the target temperature in a protocol. 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 (96-well block), or between 1 and 30 µl (384-well block) 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.

  TIP: For an End Point Only Run protocols, edit the sample volume in the Selected Block(s) list on the Start Run tab.

7 Plates

CFX Manager software uses the information you enter in the Plate tab of the Experiment Setup window to collect and analyze data (only real-time PCR experiments). Enter well contents in the Plate Editor before or after the run. Once a plate is set up and saved in the Plate Editor, it is available to load into the Plate tab in the Experiment Setup window (Figure 24 on page 30).

NOTE: When you load strip tubes into the block, be sure to balance the load to assure that the pressure on the lids is even. For example, if you load one strip of tubes on the left side of the block, be sure to load another strip (empty or full) on the right side of the block (Figure 16 on page 16).

TIP: Refer to the CFX Manager Software Plate Quick Guide to set up a plate file.

Read the sections in this chapter for more information about creating and editing plates files:

- Plates Contents, including the plate-wide parameters and well contents (next section)
- Plate Editor window (page 55)
- Experiment Settings window (page 63)
- Select Fluorophores window (page 65)
- Scan mode (page 66)
- Well Group Manager window (page 68)

Plate Contents

To create a plate, open the Plate Editor window and enter information about the samples in each well of a plate file for one experiment. In the Plate Editor you can create a new plate or edit an existing plate. Once a plate is created, load that plate file into the Plate tab in the Experiment Setup by clicking OK at the bottom of the Plate Editor.

Enter the parameters into the plate file in the Plate Editor window. Load the following types of information in the plate file:

- Plate-wide parameters (page 54)
- Contents of wells (page 54)

Plate-Wide Parameters

Open the Plate Editor to enter plate-wide parameters. The software applies these parameters to all the wells in a plate:

- **Plate Size.** Select a plate size that represents the size of the reaction module block (see “Plate Editor Menu Bar” on page 61)
- **Plate Type.** Select clear or white wells (see “Plate Editor Menu Bar” on page 61)
- **Scan Mode.** Select the channels you want to scan (see “Plate Editor Toolbar” on page 62)
- **Number Convention.** Select scientific notation (see “Plate Editor Menu Bar” on page 61)
- **Units.** Select the units of measure to use when describing the concentration of the well contents (see “Plate Editor Menu Bar” on page 61)
- **Well Groups.** Create and select well groups (see “Create Well Groups” on page 68)

Contents of Wells

A CFX Manager software plate can contain the following information about the contents of each well. Enter and edit the contents of wells before, during, or after you run the experiment. After the run, the software links the well contents to the fluorescence data collected in that well. Each well of the plate can contain the following information:

TIP: Enter and edit the contents of wells before, during, or after you run the experiment. After the run, the software links the well contents to the fluorescence data collected in that well.

- **Sample Type.** Type of sample, including unknown, standard, NTC (no template control), positive control, negative control, and NRT (no reverse transcriptase)
- **Fluorophores.** One or more calibrated fluorophores. Each fluorophore corresponds to one or more targets
- **Target Name.** One or more targets of interest (genes or sequences) in each loaded well. Each target is assigned to one fluorophore. To create a consistent and permanent list, enter target names in the Target Names library in the User Preferences Plate tab (page 170)
- **Sample Name.** One sample (identifier or condition), such as "0 hr", "1 hr", or "2 hr". To create a consistent and permanent list, enter sample names in the Sample Names library in the User Preferences Plate tab (page 170)
- **Replicate #.** Samples that contain identical well contents. Select the wells and enter a Replicate Series to quickly add a pattern of replicates in a set of wells
- **Concentration.** Standard sample types require a concentration value, or a Dilution Series value. Enter the numbering convention and the units for the concentration by selecting **Settings > Number Convention** and **Settings > Units** in the Plate Editor menu bar (page 61)
- **Well Notes.** Optional notes. Use well notes to enter sample identifiers or other useful information for data analysis

  TIP: Target names and sample names must match within and between plates to compare data in the Gene Expression tab (in Data Analysis window) or in a Gene Study. Each name must contain the same punctuation and spacing. For example, the target name "Actin" is not the same as "actin", and the sample name "2hr" is not the same as "2 hr". To facilitate consistency in the names, enter all the names in the Target and Sample Names Libraries within the User Preferences Plate tab (page 170).

Plate Editor Window

Open the Plate Editor window to enter information about the samples in each well of a plate for one experiment. Open the Plate Editor window to create a new plate or to edit an existing plate. Once a Plate is created, load it into the Experiment Setup in the Plate tab.

Open the Plate Editor

To open the Plate Editor window (Figure 42), 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 33)
- To open an existing plate, select **File > Open> Plate**, or click the **Open Existing** button in the Plate tab (page 33)
- To edit the current plate in the Plate tab, click the **Edit Selected** button (page 33)
- To open the plate associated with that data file, in the Data Analysis window (page 78), click **View/Edit Plate** to open the plate associated with that data file

  TIP: Plate settings can be changed before and after the experiment is run, except for the scan mode and plate size. To change the default plate settings, enter the changes in the User Preferences Plate tab (page 170).

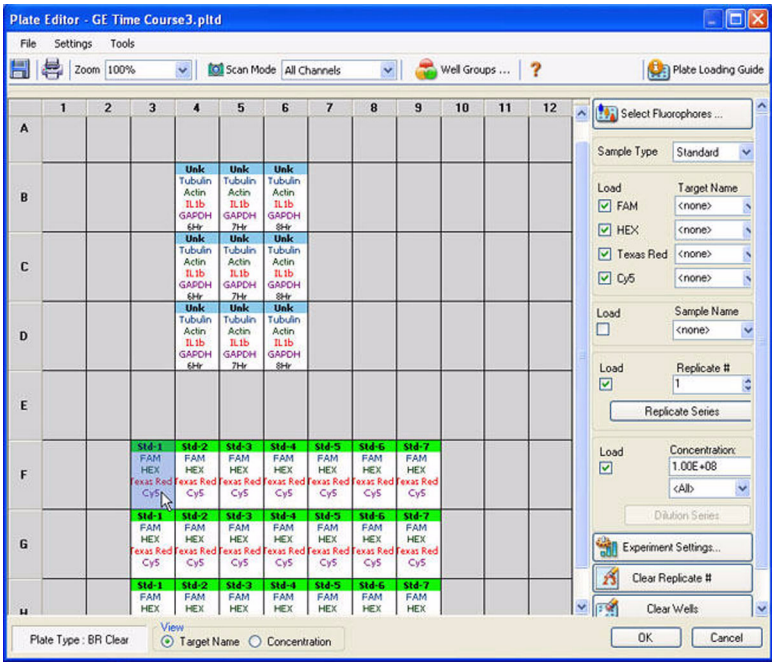


Figure 42. Plate Editor window.

Enter the plate parameters and the contents of the wells in the Plate Editor window, and instruct the instrument what type of data to collect. The Plate Editor displays this plate view of the contents in each well.

TIP: To run a real-time PCR experiment, you must load the minimal required information in the Plate Editor (page 55).

TIP: Change the plate settings before, during, and after running the experiment. However, the scan mode and plate size cannot be changed during or after the run. To change the default settings for the plate, enter the changes in the User Preferences Plate tab.

Plate Editor Features

The Plate Editor window includes the following features:

- **Menu bar.** Select settings for the plate size, plate type, number conventions, and units (page 61)

  NOTE: When you change the Plate Size, the associated Analysis mode (channels) and numbers of wells change. A 96-well plate collects data for up to 5 channels (All Channels option). A 384-well plate collects data for up to four channels (All Channels option). Refer to the Calibration Wizard for more details about the channels and fluorophores you can use with each instrument.
- **Toolbar.** Click buttons and select settings for the Scan Mode (page 62)
- **Plate view.** View the current well contents. Load wells by using the plate loading options on the upper right corner of the plate view (Figure 43 on page 57)
- **Plate Spreadsheet.** View a spreadsheet layout of the plate by selecting Tools > Show Spread Sheet View to open the Plate Spreadsheet window (page 67)

  NOTE: Select the units for the standard samples (**Std**) by selecting **Settings > Units**. These units must match the actual unit of measure for known standards in your wells. 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.

The Plate Editor shows this plate view of the contents in each well. This example shows some loaded wells with unknowns (**Unk**) and standards (**Std**) contents.

TIP: For a description of how to set up a plate, refer to the CFX Manager Software Plate Quick Guide. To change the default settings in the Protocol Editor window, enter the changes in the Protocol tab of the User Preferences window.

NOTE: CFX96 and CFX384 instruments are factory-calibrated for many fluorescent dye and plate combinations. 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.

Figure 43 shows the Plate Editor window. The bottom of the plate lists the plate type (**BR Clear**) and a selector for the **View** (Target Name or Concentration of the standard sample types).

Figure 43. Plate Editor plate view with Target Name selected.

TIP: Change the colors of each fluorophore name by clicking the Select Fluorophores button to open the Select Fluorophores window, and clicking the Color box.

To view the concentration of wells that contain standard samples, click **Concentration** at the bottom of the Plate Editor window (Figure 44)

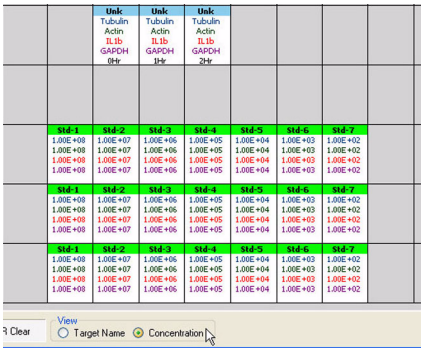


Figure 44. Plate Editor plate view with Concentration selected.

Select a well to load the contents in the plate. Each well you load corresponds to a PCR reaction in your experiment. The buttons and lists on the right side of the wells include all the options needed to load the wells.

TIP: Adjust the Zoom setting in the toolbar to increase or decrease the magnification of the plate view.

Well Loading Options

Table 14 lists the selections for loading the wells in the Plate Editor. Select an option to create or edit the contents of the wells.

Table 14. Options for loading the plate and wells in the Plate Editor

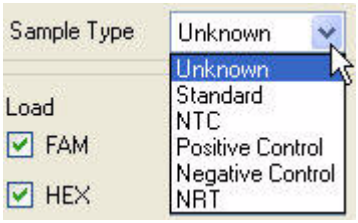
Option	Function
	Click the **Select Fluorophores** button to open the Select Fluorophores windows (page 65). TIP: Click a **Color** box in the Select Fluorophores window to change the color of the fluorophore in the plate. NOTE: To calibrate a new combination of fluorescent dye and plate type on an instrument, select Tools > Calibration Wizard (page 151)
	Select a **Sample Type** from the pull-down menu to load it in the selected wells. The sample type is used during data analysis in the Data Analysis window. Sample types include Unknown, Standard, NTC (no template control), Positive Control, Negative Control, and NRT (no reverse transcriptase)
	Click the **Load** box to add a fluorophore to the selected wells. Select a name in the **Target Name** menu (optional) and press the **Enter** key on your keyboard to load the target name in the well. To add fluorophores to the Load list, select them by clicking the **Select Fluorophores** button. The number of fluorophores available also depends on the selected Scan Mode. Select a **Target Name** (optional) to assist in data analysis. To add the target name to the selected wells, select a name in the pull-down list. To delete a target name, select it, press the **Delete** key and press **Enter** on your keyboard. TIP: To add a new target name to the pull-down menu in the current plate only, type a new name in the pull-down menu and press the **Enter** key. To permanently add target names to all plates, add them to the Library in the User Preferences Plate tab, or enter them in the Experiment Settings window (page 63).

Table 14. Options for loading the plate and wells in the Plate Editor (continued)

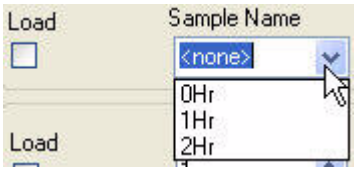
Option	Function
	Select a **Sample Name** from the pull-down menu to load that sample name in the selected wells. To delete a sample name, select it in the menu, press the **Delete** key on your keyboard, and then press **Enter**. TIP: To add a new sample name to the pull-down menu in the current plate, type a new name in the pull-down menu and press the **Enter** key on your keyboard. and will not appear in another plate. To delete a sample name, select it, press **Back Space** and then **Enter** on your keyboard. Tip: To permanently add sample names to all plates, add them to the Libraries in the User Preferences Plate tab (page 170), or enter them in the Experiment Settings window (page 63).
	Click the **Load** box to add a Replicate # to the selected wells. Click the Clear Replicate # to clear the replicate from selected cells. TIP: Click the **Replicate Series** button to open the Replicate Series pane
	Click the **Replicate Series** button to load a replicate series in a set of selected wells. Enter the **Replicate Group** size by selecting a number that represents the number of samples (wells) in each group of replicates. For each replicate group you load, select a **Starting Replicate #** to add replicate numbers. 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 typing a number in the **Concentration** box. To apply the concentration to one fluorophore in the well, select a single fluorophore from the drop-down menu (**<All>**) under the Concentration box. Select multiple wells with a Standard sample type to activate the Dilution Series button. Click the **Dilution Series** button to open the **Dilution Series** pane (next cell in table). To add the concentration to the selected wells, edit or type the concentration in the box. To delete a concentration, select it, press the **Back Space** key on your keyboard and then press **Enter**. TIP: To view the concentration listed in each well, select **Concentration** instead of Target Name (below the wells).

Table 14. Options for loading the plate and wells in the Plate Editor (continued)

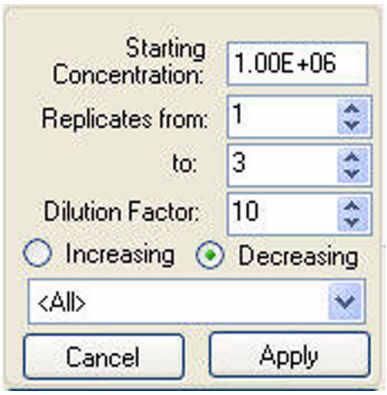
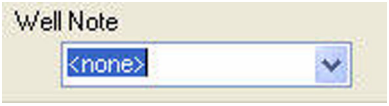
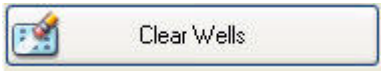
Option	Function
	Click the **Dilution Series** button to enter a dilution series for the concentration of Standard samples, and load a standard curve that appears in the Quantitation tab of the Data Analysis window (page 78) Enter the **Starting Concentration** for the dilution series, the **Replicates** from (starting replicate number) and to (ending replicate number), 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**. TIP: To view the concentration listed in each well, select **Concentration** instead of Target Name (below the wells)
	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 appear in the spreadsheet on the Quantitation Data tab (page 82)
	Click the **Experiment Settings** button to open the Experiment Settings window to manage the lists of names for Targets and Samples, and to set up a gene expression experiment. TIP: To add multiple target and sample names, add them to the Libraries in the User Preferences Plate tab (page 170)
	Click the **Clear Replicate #** button to clear the replicates #s in the selected wells
	Click the **Clear Wells** button to clear all content in the selected wells NOTE: Clicking the Clear Wells button permanently removes the content of the wells, but does not remove the fluorescence data collected during the plate read

Plate Editor Menu Bar

The menu bar in the Plate Editor window provides these menus:

Table 15 shows the functions of the items in the menu bar.

Table 15. Menu bar items in the Plate Editor

Menu Item	Command	Function
File	Save	Save the plate files
	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 384-well for the CFX384, 96-well for the CFX96 NOTE: The Plate Size must be the same as the block size in the instrument
	Plate Type	Choose the type of wells in the plate that holds your samples, including BR White and BR Clear. NOTE: If you use a new plate type, it must be calibrated (page 152). NOTE: For accurate data analysis, the Plate Type must be the same as the plate used to hold the samples in the experiment
	Number Convention	Select or cancel the selection for Scientific Notation
	Units	Select the units to show in the spreadsheets. 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 load the wells in a plate
Help	Help Contents	Open the software Help for more information about plates

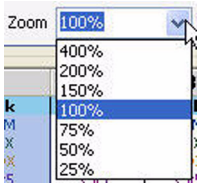
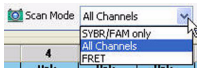
Plate Editor Toolbar

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

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
	Scan Mode	Select a scan mode to instruct the instrument what channels to collect fluorescence data from during a run. Select All Channels (default), SYBR/FAM only, or FRET (channel 6 only)
	Well Groups	Open the Well Group Manager window and set up well groups for this plate
	Help	Open the software Help for information about plates

Experiment Settings Window

Open the Experiment Settings window to change the list of Targets and Samples listed in the Plate Editor:

- **Targets.** A list of target names for each PCR reaction, such as a genes or sequences of interest. Click the Reference column for all the targets that serve as 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 for all the samples that contain controls for an experiment

  TIP: It is critical to write target and sample names consistently. These names appear in the Gene Expression tab of the Data Analysis window, and they also influence how the data are analyzed. To add a consistent list of names, type them in the Library box within the User Preferences Plate tab.

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

- While setting up an experiment, click the **Experiment Settings** button in the Plate Editor
- While analyzing data in the Data Analysis window, click the Edit/View Plate button in the tool bar to open the Plate Editor, or click the **Experiment Settings** button in the Gene Expression tab

Change the Experiment Settings to add information for data analysis. For example, select reference gene names, control samples, and efficiencies. Also, if researchers commonly uses specific targets as reference genes and specific samples as controls, then add those targets and samples in the Experiment Settings window.

Click the **Targets** tab (Figure 45) to view the list of targets and select one or more targets as a **Reference** (Figure 46 on page 64). Click the **Samples** tab to view the list of sample names and select one or more samples as a **Control** (Figure 47 on page 65).

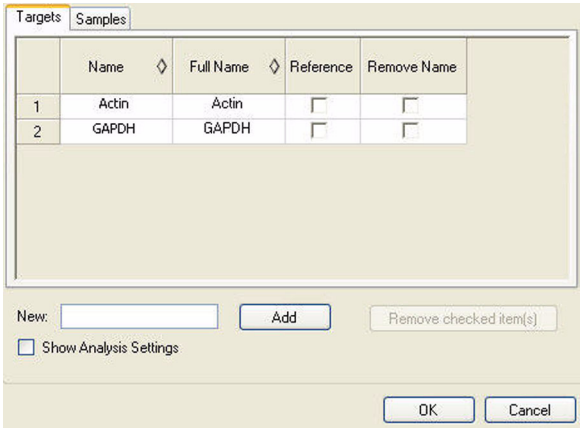


Figure 45. Experiment Settings window with Targets tab selected.

TIP: Click **Show Analysis Settings** (page 64) to select how the software shows information about the targets and samples in the Data Analysis window.

To adjust the settings in this window, use the following functions:

- Add a new target or sample name by typing the name in the **New** box, and clicking **Add** to add the target name to the list
- Remove a target or sample name from the list by clicking in the **Remove Name** box for that row, and then click the **Remove checked items(s)** button

- Select the target as a reference sample, click the box in the **Reference** column next to the Name for that target. Select reference targets when you want to use these targets as references in gene expression data analysis
- Select the sample as a control sample, click the box in the **Control** column next to the name for that sample. Select control samples when you want to use these samples as controls in gene expression data analysis
- Click the **Show Analysis Settings** box to show selections used in the Gene Expression analysis (next section)

Show Analysis Settings in Experiment Settings

Click the **Show Analysis Settings** box in the Experiment Settings window to view more parameters for each target or sample name. Adjust the analysis settings parameters to change information in the Data Analysis window.

To adjust the target parameters, do one of the following actions:

- Click a color in the **Color** column to change the color of the targets graphed in the Gene Expression chart
- Enter an number for the efficiency of each target. Calculate the relative efficiency for each target using one of the following two methods. Select **Auto Efficiency** if the data include a standard curve, and the software calculates the efficiency. Alternatively, type an value in the chart when you calculate the efficiency outside of the software

Figure 46 shows the efficiency of all the targets, which appear if **Auto Efficiency** is selected:

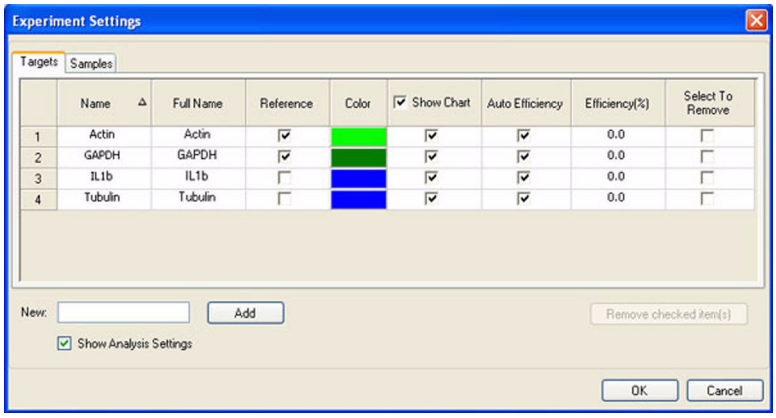


Figure 46. Targets tab in Experiment Settings window with Analysis Settings selected.

To adjust the settings for a sample in the Samples tab, do one of these actions:

- 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 Graph** column to show the sample in the Gene Expression chart using a color that is selected in the Color column

Figure 47 shows all the samples with the **Show Graph** option selected so that they appear in the Gene Expression tab.

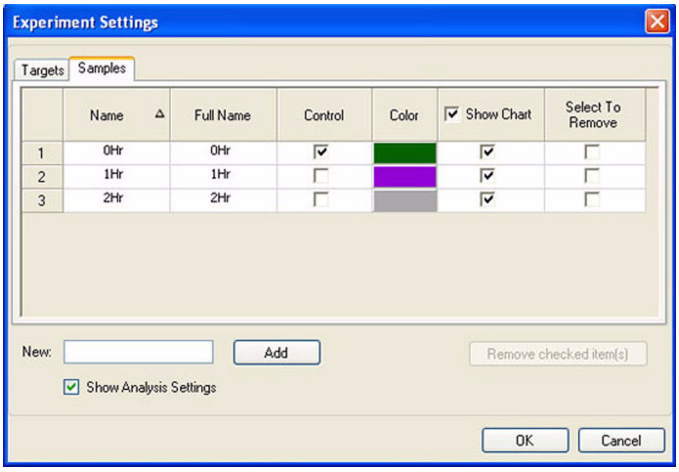


Figure 47. Samples tab in Experiment Settings window with Analysis Settings selected.

Select Fluorophores Window

The Select Fluorophores window lists the current and calibrated fluorophores in the Plate Editor window by channel. To open the Select Fluorophores window, click the Select Fluorophores button on the right side of the Plate Editor. The number of fluorophores listed in the Select Fluorophores window depends on the Scan Mode, which is selected in the Plate Editor. You cannot change the scan mode once the experiment is run.

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

Open the Select Fluorophores window to make these changes in the list of fluorophores:

- Click the **Selected** check box next to the fluorophore name to add or remove the fluorophores to the plate. Adding the fluorophore adds the name to the list on the right side of the Plate Editor 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 Standard Curve chart (Quantitation window)

  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.

The Select Fluorphores window (Figure 48) lists the available and selected fluorophores. In this example, SYBR is selected.

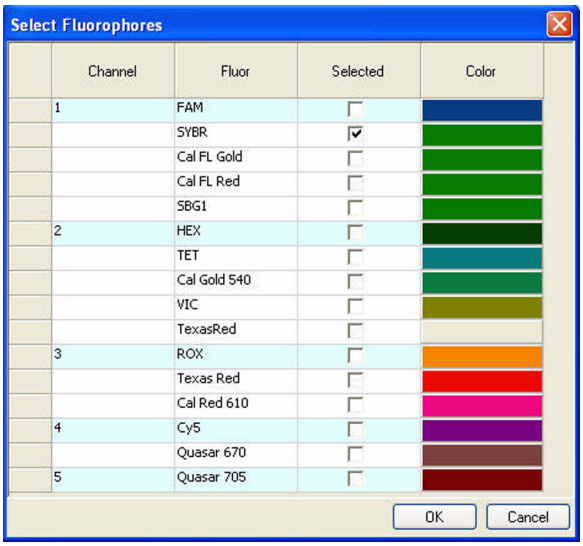


Figure 48. Select Fluorophores window.

TIP: To change the color of traces in the Amplification, Melt Curve, and Melt Peak charts, open the Trace Styles window in the Data Analysis window.

The list of fluorophores in the Select Fluorophores window includes fluorophores from one of these sources:

- **Factory calibrated fluorophores.** The default list of fluorophores in CFX Manager software includes all the factory calibrated fluorophores for the CFX96 and CFX394 reaction modules (page 153)
- **Additional calibrated fluorophores.** The software adds fluorophore names to the default list when you use the Calibration Wizard to calibrate a new fluorophore on an instrument

Scan Mode

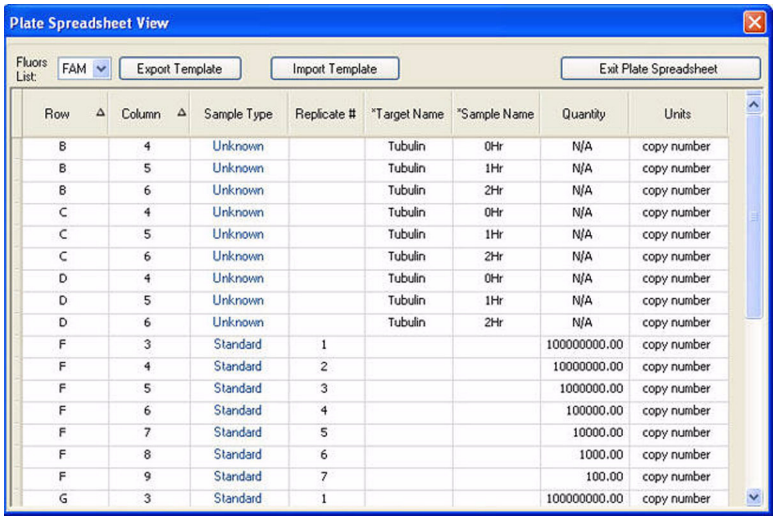
The CFX96 and CFX384 optical reaction modules excite and detect fluorophores in five (CFX384) or six (CFX96) channels. Select the Scan Mode in the Plate Editor choose the channels the instrument will scan during a run.

Select one of these scan modes to detect the calibrated fluorophores in the following channels:

- **All Channels.** Includes channels 1-5 on the CFX96, and channels 1-4 on the CFX384, but does not include channel 6 (FRET)
- **SYBR/FAM only.** Includes channels 1, and provides a fast scan
- **FRET.** Includes channel 6 only, and provides a fast scan

Plate Spreadsheet Window

The Plate Spreadsheet window shows the contents of a plate in the Plate Editor. Open the Plate Spreadsheet window (Figure 49) by selecting **Tools > Show Spreadsheet View** in the Plate Editor menu bar.



Row	Column	Sample Type	Replicate #	*Target Name	*Sample Name	Quantity	Units
B	4	Unknown		Tubulin	0Hr	N/A	copy number
B	5	Unknown		Tubulin	1Hr	N/A	copy number
B	6	Unknown		Tubulin	2Hr	N/A	copy number
C	4	Unknown		Tubulin	0Hr	N/A	copy number
C	5	Unknown		Tubulin	1Hr	N/A	copy number
C	6	Unknown		Tubulin	2Hr	N/A	copy number
D	4	Unknown		Tubulin	0Hr	N/A	copy number
D	5	Unknown		Tubulin	1Hr	N/A	copy number
D	6	Unknown		Tubulin	2Hr	N/A	copy number
F	3	Standard	1			100000000.00	copy number
F	4	Standard	2			10000000.00	copy number
F	5	Standard	3			1000000.00	copy number
F	6	Standard	4			100000.00	copy number
F	7	Standard	5			10000.00	copy number
F	8	Standard	6			1000.00	copy number
F	9	Standard	7			100.00	copy number
G	3	Standard	1			100000000.00	copy number

Figure 49. 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)

Sort or edit a column by selecting it and using 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 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 appears 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

Well Group Manager Window

The Well Groups Manager window shows the well groups for one plate. Set up well groups when you want to analyze sets of wells within the same plate in the Data Analysis window. For example, create well groups when you want to find the best primer set by comparing the data in different standard curves.

NOTE: The default well group is All Wells.

Open Well Groups Manager Window

To open the Well Groups Manager window, open the Plate Editor and click the **Well Groups** button in the toolbar. Use well groups to do the following functions:

- Create well groups in the Plate Editor by opening the Well Groups Manager window
- Analyze well groups separately in the Data Analysis window

Figure 50 on page 68 shows the two colors of wells in the Well Group Manage window:

- Wells in the selected group are blue (Group 2)
- Unselected wells are light gray, whether or not they contain well contents. These wells are not included in the Group 2

Figure 50 shows Group 2 selected. Notice that the wells in Group 2 are blue.

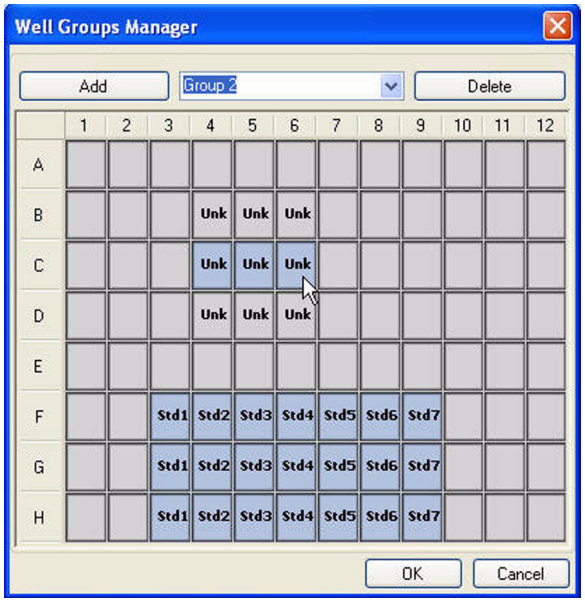


Figure 50. Color of wells in the Well Group Manager window.

Create Well Groups

To create well groups in the Well Group Manager, follow these instructions:

1. Click **Add** to create a new group. The drop-down menu shows the group name as **Group 1** for the first group.

2. Select the wells that will compose the well group in the plate view. Selected wells change color (turn blue). To add more wells, click the wells you want to add. To remove wells, click the well. To add or remove a group of wells, click and drag across the group of wells.

3. (Optional) Change the name of the group by selecting the group name in the pull-down menu and typing a new name.
4. (Optional) Create more well groups by repeating steps 1 and 2.
5. (Optional) Review the groups by selecting the group name in the drop-down list.
6. Optional) Delete well groups by selecting the group name in the drop-down list, and clicking the **Delete** button.
7. Click **OK** to finish and close the window, or click **Cancel** to close the window without making changes.

   NOTE: Once you create well groups, the Data Analysis window lists those groups in the **Well Group** pull-down menu.

Using Well Groups in Data Analysis

The well groups divide a single plate into subsets of wells. Once well groups get set up, select one of the 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 that were run in one plate.

Create well groups in your plate to use them for independent data analysis:

- Analyze each well group with a different baseline threshold method
- Analyze each well group with a different standard curve

8 Start and Monitor the Run

To start the run click the Start Run tab (next section), select an instrument, and click the **Start Run** button. When the run starts, the Run Details window (page 72) opens to show the progress of the run.

Starting a Run in the Start Run Tab

Start a run in the Start Run tab (page 34). This tab contains the final steps you need to start running PCR or real-time PCR experiments. Click the **Start Run** tab (Figure 51) in the Experiment Setup window to check the run information, select instrument blocks, and begin running an experiment.

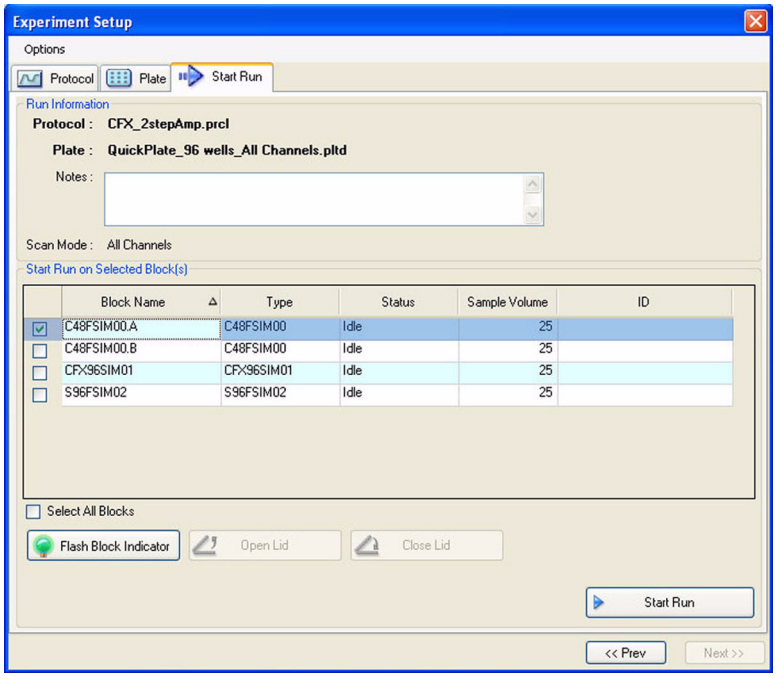


Figure 51. Start Run tab.

TIP: To add or remove columns in the Start Run on Selected Block(s) list, right-click on the table and select the options (page 35) from the menu.

Follow these instructions to start running an experiment once you have loaded a protocol and plate:

1. Check the information in **Run Information** pane. This information includes the protocol name, the plate name, and optional added notes. Return to the Protocol tab to change the protocol. Return to the Plate tab to change the Plate. Type optional notes about the experiment into the Notes box.
2. Select one or more blocks in the **Start Run on Selected Block(s)** list by clicking the check box to the right of the instrument block name. Click **Select All** to select all the blocks and run them at the same time.
3. (Optional) Change the parameters for the selected blocks, including the sample volume, lid settings, and emulation mode. To change a parameter, click the text and enter a new parameter or select an option in the menu. Right-click on the block list to select the parameters that show in this list.
4. (Optional) Click **Flash Block Indicator** to flash the LED on the lid of the selected block, and confirm the identity of all selected blocks. Click **Open Lid** or **Close Lid** to open and close the lid on the selected blocks.
5. Click the **Start Run** button to begin running the experiment on the selected blocks.

The Run Details window (next) appears as soon as the instrument starts running an experiment. View this window to monitor the run.

Run Details Window

When you click the Start Run button to begin running an experiment, CFX Manager software opens the Run Details window. Review the information in this window during a run to monitor the run or change the protocol during 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 count-down timer for the protocol

Figure 52 shows the features of the Run Details window:

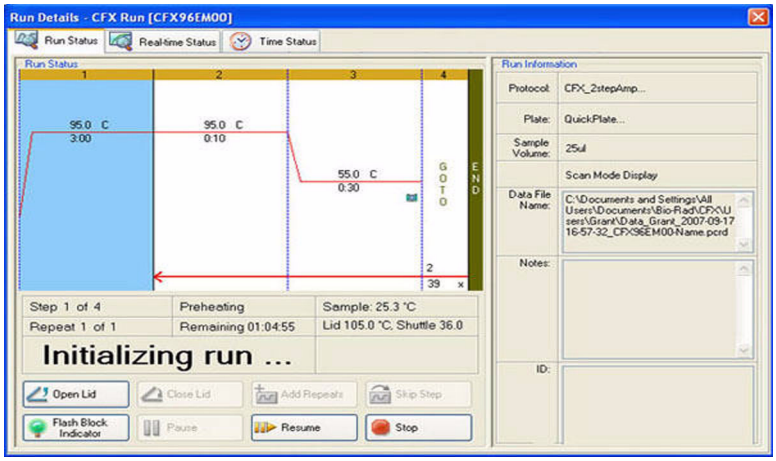


Figure 52. Run Details window.

Run Status Tab

The Run Status tab (Figure 53) shows the current status of a run in progress in the Run Details window and provides buttons (page 74) for controlling the lid and changing the run that is in progress. View information about the current run in these panes:

- **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 the details about the Experiment

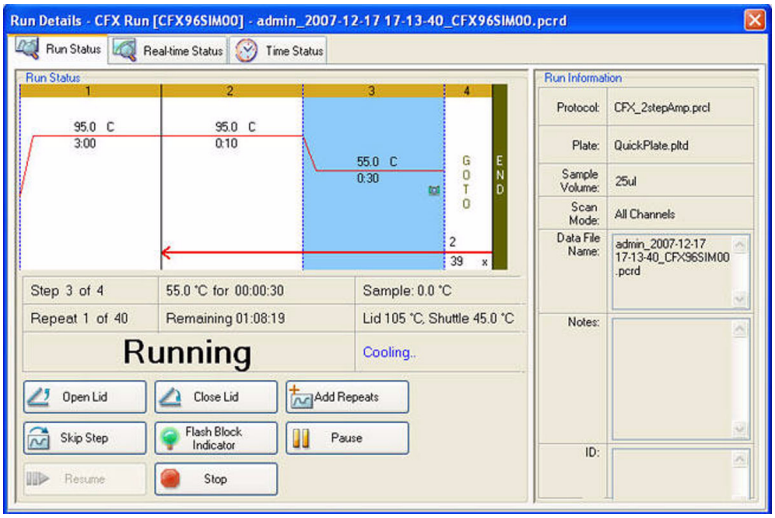
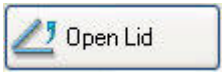
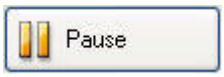
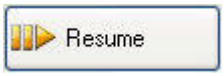
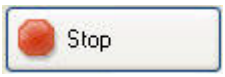


Figure 53. Run Status tab in the Run Details window.

Run Status Tab Buttons

Click one of the buttons listed in Table 17 to operate each instrument block from the software, or change the run that is in progress. These buttons appear in the Run Status tab (page 73).

Table 17. Run Status buttons and their functions

Button	Function
	Open the motorized lid on selected instruments **WARNING!** Opening the lid during a run will pause the run during the current step and might alter the data.
	Close the motorized lid on selected instruments
	Add more repeats to the current GOTO step (cycle) in the protocol. This button is only available when a GOTO step is running. NOTE: The added repeats change the run, but does not change the protocol file associated with the run. This action is recorded in the Run Log
	Skip the current step in the protocol. If you skip a GOTO step, the software verifies that you want to skip the entire GOTO step and proceed to the next step in the protocol. NOTE: Skipping the current step changes the run, but does not change the protocol file associated with the run. This action is recorded in the Run Log
	Flash the LED on the selected instrument to identify the selected blocks
	Pause the protocol. NOTE: Pausing the current step changes the run by lengthening the hold time, but does not change the step in the protocol file associated with the run. This action is recorded in the Run Log
	Resume a protocol that was paused
	Stop the run, which can change your data. NOTE: Stopping the protocol changes the run, but does not change the protocol file associated with the run. This action is recorded in the Run Log

Real-time Status Tab

The Real-Time Status tab shows the real-time PCR data as the software collects them in the amplification graph in the Run Details window. This tab also shows the well selector and text describing the protocol status at the bottom of the window.

NOTE: Real time data does not begin to appear until after the first two plate reads in the protocol.

TIP: Click the View/Edit Plate button to open the Plate Editor window. During the run you can enter more information about the contents of each well in the plate. Information about the contents of each well is critical to data analysis.

During the run, the Real-Time Status tab (Figure 54) shows the fluorescence data as they are collected with each cycle.

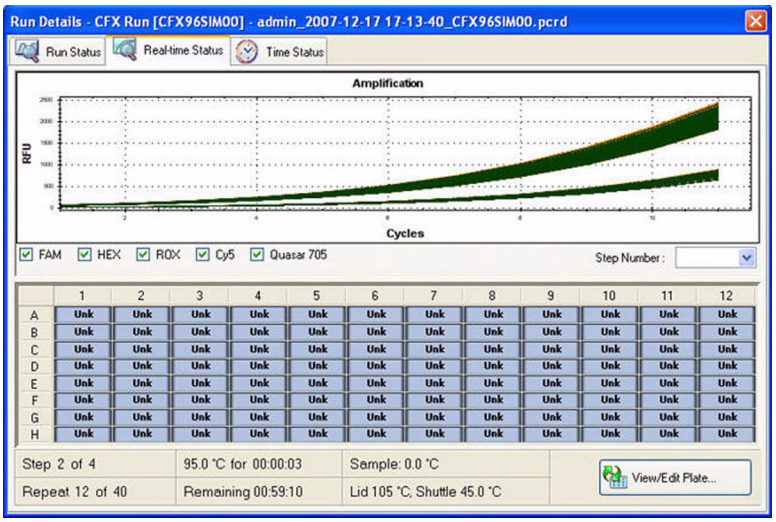


Figure 54. The Real-time Status tab displays the data as it accumulates during a run.

TIP: Click the **View/Edit Plate** button to open the Plate Editor (page 55), and finish adding well contents in the plate (page 31).

Time Status Tab

The Time Status tab in the Run Details window shows a count-down timer for the current run in a full-screen view. Click this tab to view the time remaining in the run.

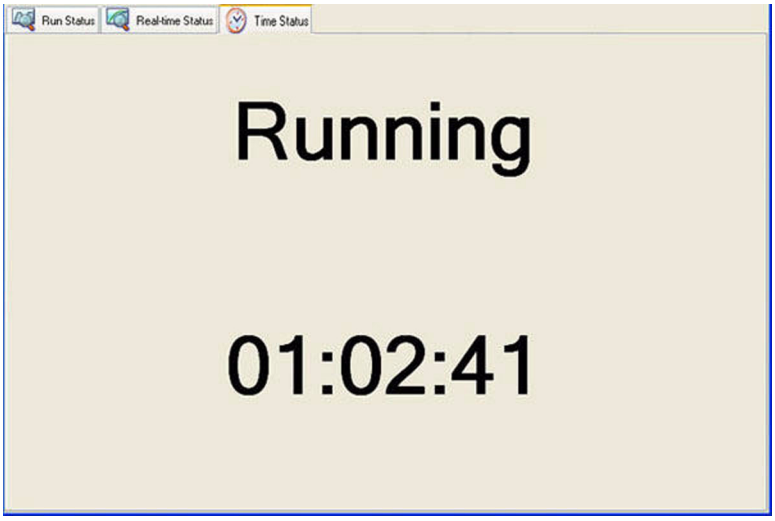


Figure 55. The Clock tab displays a count-down timer for the current run.

9 Data Analysis Window

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. This window shows a series of tabs that include charts, plates, and spreadsheet views of these data and the well contents:

- Open the Data Analysis window (next section on page 77)
- About Data Analysis (page 78)
- The Data Analysis window and tabs (page 78)
- Reports for data files (page 106)

Opening the Data Analysis Window

The Data Analysis window opens automatically at the end of a run to show the data from that run. 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 menu bar and open a data file
- Click the **Data Analysis** button in the main software window toolbar and open a data file
- 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 eight tabs (Figure 56). Each tab shows the analyzed data for a specific analysis method:

Figure 56. All the tabs that can display in the Data Analysis window.

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

About Data Analysis

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

To change the content of wells after a run, click the **Edit/View Plate** button at the top of the Data Analysis window and open the Plate Editor. Enter the contents of each well in the Plate Editor 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 change after the run.

TIP: During data analysis, select those data and well contents that you want to view (see “Selecting Wells in Data Analysis Window” on page 115).

Data Analysis Window

The Data Analysis window includes a menu bar, toolbar, and up to eight tabs. These tabs shows analyzed real-time PCR data from one experiment (a protocol and plate file run on one instrument). The software only displays a tab if the data are available for that type of analysis:

- **Quantitation tab (page 79).** Shows the amplification chart, standard curve chart (if data are available), well selector, and spreadsheet with the amplification data for each loaded well in the plate. Use the data in this tab to set the analysis conditions, including the baseline settings for individual wells and the threshold settings for the plate. For experiments with standards of known quantities, the Quantification tab shows the calculated reaction efficiency for the standard curve
- **Quantitation Data tab (page 82).** Shows a spreadsheet or plate map of the Quantitation data, including the selections for Results, Plate, and Amplification Data (RFU) spreadsheets that show the quantitation data in different formats
- **Melt Curve tab (page 85).** Shows a melt curve chart, melt peak chart, well selector, and spreadsheet view of the melt curve data for each well. Use the data shown in this tab to measure the melting temperature (Tm) of PCR products
- **Melt Curve Data tab (page 87).** Shows a spreadsheet view of the data, including the selections for Melt Peaks, Plate, Amplification, RFU, and -d(RFU)/dT (melt peak data) spreadsheets that show the melt curve data in different formats
- **Gene Expression tab (page 90).** Shows the data in a gene expression chart that graphs relative or normalized C(t), and a spreadsheet view of the gene expression data. This analysis requires expression data from at least two targets (genes)
- **End Point tab (page 100).** Shows spreadsheet view of the end point data in each well. Adjust the settings to change the positive and negative calls
- **Allelic Discrimination tab (page 102).** Shows a chart that graphs data from known and unknown samples to identify the genotypes in the unknown samples. The tab shows a well selector and spreadsheet view of these data. Adjust the settings to assign genotypes to unknown samples by comparisons with the genotype of known samples

- **Run Information tab (page 105).** Shows information about the experiment, including the protocol, optional notes, optional ID, and run log

  TIP: Change data analysis options, data viewing, or export data by selecting options in the Data Analysis window menu bar. Right-click any chart, spreadsheet, or well selector for more options. Click the **View/Edit Plate** button to open the Plate Editor and change the contents of the wells.

Step Number Selector

The step number selector is located below the Standard Curve chart on the Quantitation tab. The software displays this selector 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. Figure 57 shows the selected data collection step is **3** for all the data in the Quantitation and Melt Curve tabs:

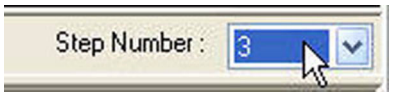


Figure 57. Step Number selection in the Data Analysis window.

Quantitation Tab

The Quantitation tab shows the amplification chart, which shows the relative fluorescence for each well at every cycle. This tab shows the amplification data in these four views:

- **Amplification Chart.** Shows the relative fluorescence (RFU) for each well at every cycle. Each trace in the chart represents data from a single fluorophore in one well. To add or remove fluorophores from view, click the fluorophore boxes below the chart
- **Standard Curve.** Shows a standard curve 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.** Select the wells with the fluorescence data you want to show
- **Well spreadsheet.** Shows a spreadsheet of the quantitation data collected in the selected wells

  TIP: Right-click any chart, spreadsheet, or well selector for more options.

  TIP: Select the wells that you want to see and to analyze in the Plate Editor and the Data Analysis window. For more information see “Selecting Wells in Data Analysis Window” on page 115.

Tab Layout for Quantitation

The software links the data in the four panes of the Quantitation tab. For example, highlighting a well in the well selector view highlights the data in all the other panes.

The Quantitation tab includes the Amplification and Standard Curve charts (Figure 58).

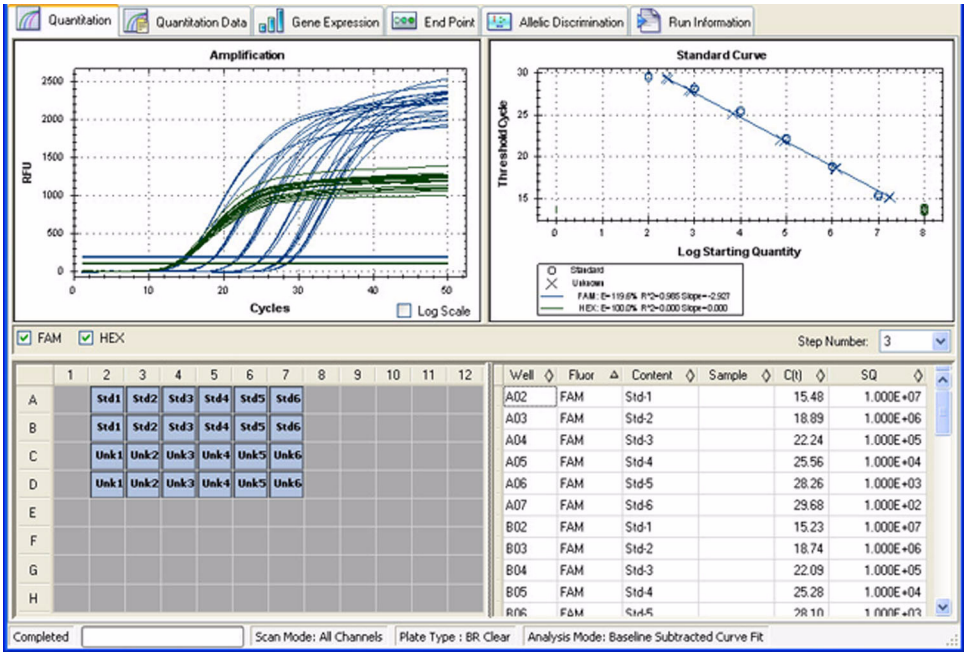


Figure 58. Layout for the Quantitation tab in Data Analysis window.

TIP: To change the colors of the traces in the Amplification chart, open the Trace Styles window (page 122) by clicking the **Trace Styles** button in the toolbar, or right-clicking the chart and selecting from that menu. To change the color of the lines in the Standard Curve chart, open the Plate Editor and click the **Select Fluorophores** button to open the Select Fluorophores window.

NOTE: If the protocol included more than one data collection step (camera icon), select the step with the data you want to view in the Step Number option below the Standard Curve chart (page 79).

NOTE: Changing the selected wells in the well selector, or clicking the fluorophore selector does not change the calculations for data analysis, but does change the well content you see in the spreadsheet and charts. To change calculations for data analysis, exclude wells from data analysis in the Plate Editor. Click the **View/Edit Plate** button to open the Plate Editor.

Data Analysis for Quantitation

The software automatically sets the baseline individually for each well. The software also calculates a threshold value for each fluorophore, and shows that threshold in the Amplification chart. Once you select the wells for analysis, check the baseline and threshold settings in these wells. If needed, make adjustments in the baselines and thresholds in the Quantitation tab (page 79), or in the Baseline Threshold window (page 120).

Quantitation data analysis does not require a standard curve. To create a standard curve, the data must contain at least one standard with a known concentration that is greater than zero.

Select 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:

- **Not Baseline Subtracted.** 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

  TIP: To manually set the baseline or the threshold for the selected fluorophore in one well, open the Baseline Thresholds window.

Adjust Data Display for Quantitation Tab

To adjust the data you see in the Quantitation tab, follow these options:

- If the protocol collected data in more than one step, select a number in the step in the Step Number selector (page 79) to view the data for that protocol step
- Select the fluorophore traces you want to view by clicking the fluorophore selection box below the amplification chart. Figure 59 shows **FAM** selected, so only FAM traces will show in the quantitation and melt curve tabs

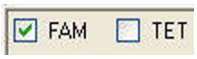


Figure 59. Selecting fluorophores in the Data Analysis window.

- Open the Trace Styles window to change the color of the traces in Amplification chart. To open this window, click the Trace Styles button in the toolbar
- Open the Select Fluorophores window to change the color of the lines in the Standard Curve chart. To open this window, click the **View/Edit Plate** button in the Data Analysis window, and then click the **Select Fluorophores** button in the Plate Editor
- Click **Log Scale** to change the amplification chart between linear (default) and semi-logarithmic scales
- Click and drag the threshold line on the Amplification chart to change the default setting. A good threshold is often half-way between the noise and the fluorescence plateau
- Set the baseline by changing the Analysis Mode (page 81) if needed
- Check that the standard curve efficiency has a sufficiently high R^2 value (as close to 0.99 as possible)
- Select wells (page 115) in the well selector to focus on subsets of the data
- Select a well group (page 116) to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group drop-down menu in the toolbar

- Exclude wells (page 117) from analysis temporarily in the Plate Editor window. For example, exclude wells that contain data that might skew the analysis, and view the changes immediately
- Clear the wells to delete the contents of the wells in the Plate Editor window (page 119). For example, clear the wells that contain contaminated or inaccurate well contents. Deleting the content does not remove the fluorescence data that were collected during a run

Data Description for Quantitation

Table 18 shows the types of data shown in the spreadsheet at the bottom right side of the Quantitation tab:

Table 18. Description of the types of data in the spreadsheet

Data Type	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	A combination of the Sample Type (required) and Replicate Number (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 the **View/Edit Plate** button.

Quantitation Data Tab

The Quantitation Data tab shows a spreadsheet that describes the quantitation data collected in each well for each cycle. Select one of the three options to show the data in different formats:

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

  TIP: Right-click any spreadsheet for options. Sort the data in any spreadsheet by right-clicking and choosing the Sort option. Click the **View/Edit Plate** button to open the Plate Editor, and change the contents of any wells in the plate.

  TIP: Select the wells that you want to see and to analyze in the Plate Editor and the Data Analysis window. For more information see “Selecting Wells in Data Analysis Window” on page 115.

Results Spreadsheet

Select the **Results** spreadsheet (Figure 60) to see the columns of data shown in Figure 60 for each well in the plate.

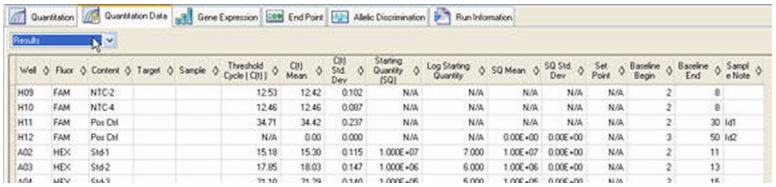


Figure 60. Quantitation Data tab with Results spreadsheet selected.

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.

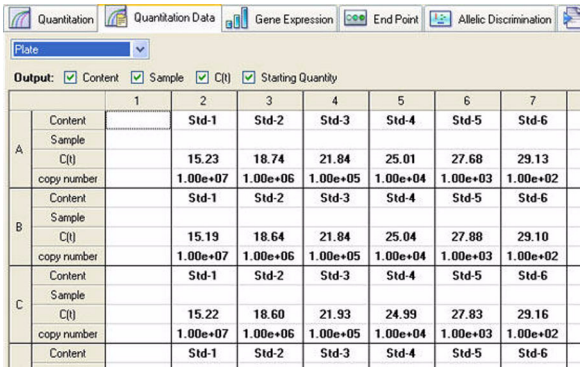
The Results spreadsheet includes the experiment results listed in Table 19.

Table 19. Data shown in 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
Set Point	Temperature of sample in the well for a gradient step
Sample Note	One round of denaturation, annealing, and extension, or one round of annealing and extension steps in a protocol

Plate Spreadsheet

Select the **Plate** spreadsheet o 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 61 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
	Sample							
	C(t)		15.23	18.74	21.84	25.01	27.68	29.13
	copy number		1.00e+07	1.00e+06	1.00e+05	1.00e+04	1.00e+03	1.00e+02
B	Content		Std-1	Std-2	Std-3	Std-4	Std-5	Std-6
	Sample							
	C(t)		15.19	18.64	21.84	25.04	27.88	29.10
	copy number		1.00e+07	1.00e+06	1.00e+05	1.00e+04	1.00e+03	1.00e+02
C	Content		Std-1	Std-2	Std-3	Std-4	Std-5	Std-6
	Sample							
	C(t)		15.22	18.60	21.93	24.99	27.83	29.16
	copy number		1.00e+07	1.00e+06	1.00e+05	1.00e+04	1.00e+03	1.00e+02
	Content		Std-1	Std-2	Std-3	Std-4	Std-5	Std-6

Figure 61. Plate spreadsheet in Quantitation Data tab shows a plate map.

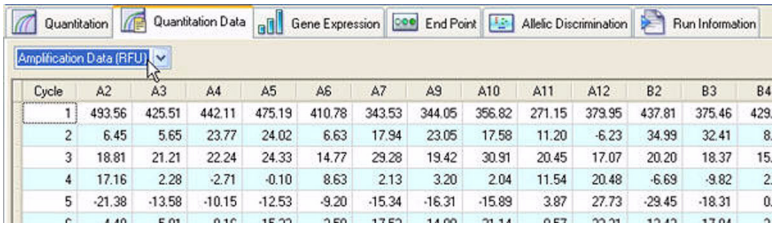
The Plate spreadsheet includes the information shown in Table 20, including the selected fluorophore in the plate map.

Table 20. Plate spreadsheet contents

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

Amplification Data (RFU) Spreadsheet

Select the **Amplification Data (RFU)** spreadsheet to see the individual RFU readings for each well and each cycle. Select individual fluorophores by clicking a tab at the bottom of the spreadsheet. The well number appears at the top of each column (Figure 62), and the cycle number appears down the rows.



Cycle	A2	A3	A4	A5	A6	A7	A9	A10	A11	A12	B2	B3	B4
1	493.56	425.51	442.11	475.19	410.78	343.53	344.05	356.82	271.15	379.95	437.81	375.46	429.
2	6.45	5.65	23.77	24.02	6.63	17.94	23.05	17.58	11.20	-6.23	34.99	32.41	8.
3	18.81	21.21	22.24	24.33	14.77	29.28	19.42	30.91	20.45	17.07	20.20	18.37	15.
4	17.16	2.28	-2.71	-0.10	8.63	2.13	3.20	2.04	11.54	20.48	-6.69	-9.82	2.
5	-21.38	-13.58	-10.15	-12.53	-9.20	-15.34	-16.31	-15.89	3.87	27.73	-29.45	-18.31	0.

Figure 62. Amplification Data (RFU) spreadsheet in the Quantitation tab.

The Amplification Data (RFU) spreadsheet includes the data shown in Table 21.

Table 21. Amplification Data (RFU) spreadsheet contents

Data Source	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

Open the Melt Curve tab to determine the melting temperature (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 relative fluorescence units (RFU) per temperature for each well
- **Melt Peak.** View the negative derivative of the RFU data per temperature for each sample in a chart that shows the peaks. Adjust the melt threshold line by dragging the horizontal bar up and down
- **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 87)

  TIP: Right-click any chart, spreadsheet, or well selector for more options

  TIP: Select the wells that you want to see and to analyze in the Plate Editor and the Data Analysis window. For more information see “Selecting Wells in Data Analysis Window” on page 115.

Tab Layout for Melt Curve

The software links the data in the four panes of the Melt Curve tab. Highlighting a peak in a chart highlights the associated well. As shown in Figure 63, highlighting a trace in the first peak of the Melt Peak chart simultaneously highlights the controls (NTC1), showing that the traces in the first peak belong to the NTC samples:

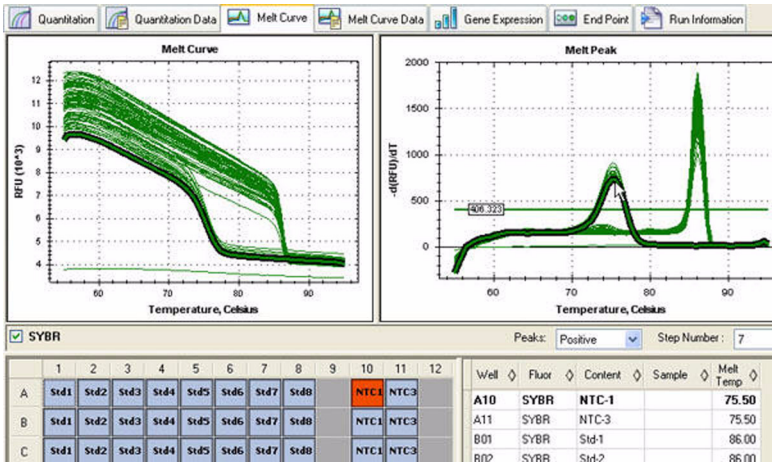


Figure 63. Layout of the Melt Curve tab in the Data Analysis window.

NOTE: Changing the selected wells in the well selector does not change the data analysis calculations, but changes the data you see in the spreadsheet and charts. To change calculations, exclude wells from data analysis in the Plate Editor. Click the **View/Edit Plate** button to open the Plate Editor.

Data Analysis for Melt Curve

For DNA-binding dyes and non-cleavable hybridization probes, their 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 derivative of fluorescence versus temperature (-dRFU/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. For example, previously unidentified peaks can list in the spreadsheet by dragging the threshold bar downward, which increases the distance between the threshold bar and the highest peak. Similarly, dragging the threshold bar up can cause previously identified minor peaks to drop off the spreadsheet.

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 (page 79) 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 (page 115) in the well selector to focus on subsets of the data
- Select a well group (page 116) to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group drop-down menu in the toolbar
- Exclude wells (page 117) from analysis temporarily in the Plate Editor window. For example, exclude wells that contain data that might skew the analysis, and view the changes immediately
- Clear the wells to delete the contents of the wells in the Plate Editor window (page 119). For example, clear the wells that contain contaminated or inaccurate well contents. Deleting the content does not remove the fluorescence data that were collected during a run

Data Spreadsheet for Melt Curve

Table 22 shows the types of information shown in the spreadsheet at the bottom right side of the Melt Curve tab.

Table 22. Melt Curve tab spreadsheet contents

Information	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	A combination of Sample Type (required) and Replicate Number (optional) loaded in the Plate Editor
Sample	Sample Name loaded in the Plate Editor
Melt Temp	The temperature of the two highest melt peaks. To see all melt peaks, click the Melt Curve Data tab and select the Melt Peaks spreadsheet
Peak Height	The highest point of the melt peak (-d(RFU)/dT)

Melt Curve Data Tab

The Melt Curve Data tab shows the data from the Melt Curve tab in 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 formats:

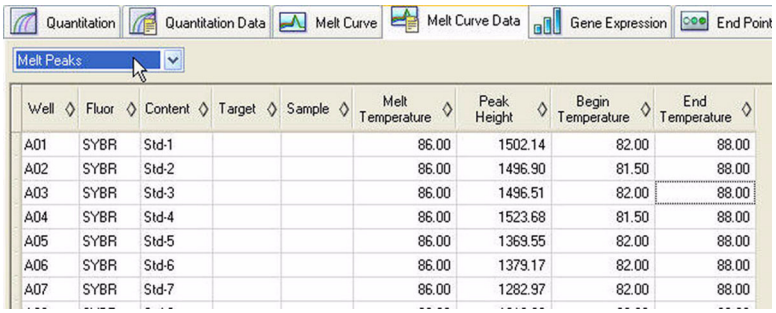
- **Melt Peaks spreadsheet.** List all the data, including all the melt peaks, for each trace
- **Plate spreadsheet.** List a view of the data and contents of each well in the plate
- **Amplification Data (RFU) spreadsheet.** List the RFU (relative fluorescence units) quantities at each temperature for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

  TIP: Right-click any spreadsheet for options. Sort the data in any spreadsheet by right-clicking and choosing the Sort option. Click the **View/Edit Plate** button to open the Plate Editor, and change the contents of any wells in the plate.

  TIP: Select the wells that you want to see and to analyze in the Plate Editor and the Data Analysis window. For more information see “Selecting Wells in Data Analysis Window” on page 115.

Melt Peaks Spreadsheet

Select the **Melt Peaks** spreadsheet (Figure 64) to view this information about these 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 64. Melt Peaks spreadsheet in Melt Curve Data tab.

The Melt Peaks spreadsheet includes the information shown in Table 23.

Table 23. Information in the Melt Peaks spreadsheet

Information	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	Sample Type
Target	Amplification target (gene)
Sample	Sample Name
Melt Temperature	The melting temperature 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 the **Plate** spreadsheet (Figure 65) to view these data from each well in the plate:

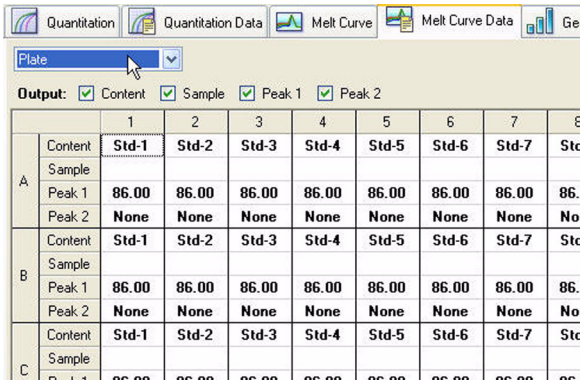


Figure 65. Plate spreadsheet in Melt Curve Data tab.

NOTE: To adjust the peak that the software calls, adjust the threshold line in the Melt Peak chart on the Melt Curve tab. The software calls up to two peaks for each trace in the well.

The Plate spreadsheet includes the information shown in Table 24 for the selected fluorophore in the plate layout.

Table 24. Plate spreadsheet in Melt Curve Data tab

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

Amplification Data (RFU) Spreadsheet

Select the **Amplification Data (RFU)** spreadsheet (Figure 66)to view these data:

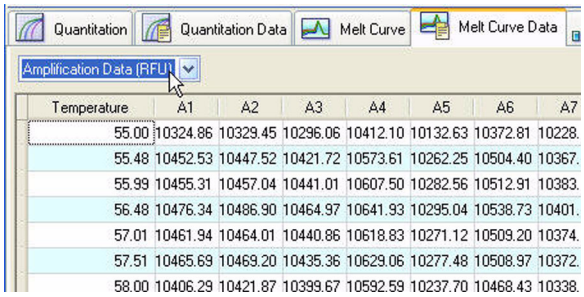


Figure 66. Amplification Data (RFU) spreadsheet in Melt Curve Data tab.

Table 25 lists the data shown in the Application Data (RFU) spreadsheet:

Table 25. Amplification Data (RFU) in the Melt Curve Data tab

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 spreadsheet (Figure 67) in the Melt Curve Data tab to view these data:

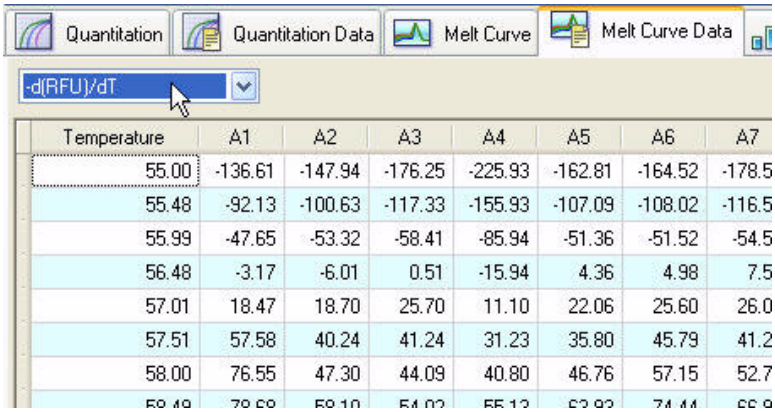


Figure 67. -d(RFU)/dT spreadsheet in the Melt Curve Data tab.

The -d(RFU)/dT spreadsheet includes the information listed in Table 26.

Table 26. -d(RFU)/dT spreadsheet in Melt Curve Data 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

Adjusting Melt Curve Data

Adjust the data shown in the Melt Curve Data tab by any of these methods:

- Select wells (page 115) in the well selector to focus on subsets of the data
- Select a well group (page 116) to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group drop-down menu in the toolbar
- Exclude wells (page 117) from analysis temporarily in the Plate Editor window. For example, exclude wells that contain data that might skew the analysis, and view the changes immediately
- Clear the wells to delete the contents of the wells in the Plate Editor window (page 119). For example, clear the wells that contain contaminated or inaccurate well contents. Deleting the content does not remove the fluorescence data that were collected during a run

Gene Expression Tab

The Gene Expression tab layout shows the relative expression of targets in these two views:

- **Gene Expression chart.** Shows the real-time PCR data as normalized expression ($\Delta\Delta C(t)$) or relative quantity ($\Delta C(t)$)
- **Target spreadsheet.** Shows a spreadsheet listing of the gene expression data for each target

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

  TIP: Combine and analyze multiple Gene Expression experiments that contain common targets in a Gene Study (page 135)

  TIP: Select the wells that you want to see and to analyze in the Plate Editor and the Data Analysis window. For more information see “Selecting Wells in Data Analysis Window” on page 115.

To open the Gene Expression tab, open a data file that includes a Gene Expression experiment in the Data Analysis window. For information about data analysis, see these sections:

- Gene expression tab layout (page 91)
- Gene expression data analysis (page 91)
- Adjusting gene expression data (page 92)
- Gene expression data description (page 93)
- Show details option (page 93)

For more information about running and analyzing data in a gene expression experiment, see the following sections:

- Creating a gene expression experiment (page 94)
- Requirements for gene expression analysis (page 94)
- Gene Expression tab options (page 96)

Gene Expression Tab Layout

The software links the data in the panes of the Gene Expression tab (Figure 68). For example, when you highlight a bar in the graph, the software highlights the corresponding data in the spreadsheet.

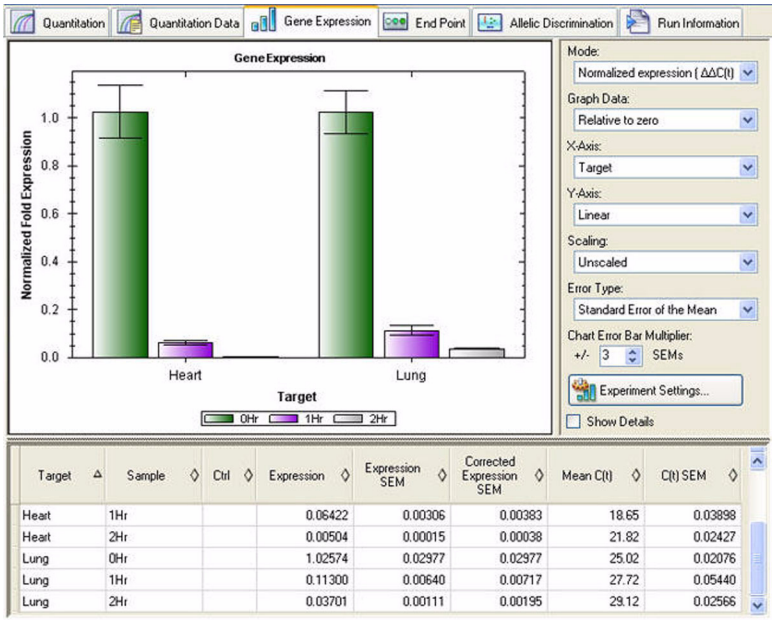


Figure 68. Layout of the Gene Expression tab in the Data Analysis window.

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.

Gene Expression Data Analysis

Open the Gene Expression tab to evaluate relative differences between PCR reactions in two or more wells of a plate. 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.

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. The most common use of this application is evaluating target concentration in cDNA samples to infer steady state mRNA levels. Typically, one or more reference gene's message levels 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.

In order to run gene expression analysis, the contents the wells of the plate can include the following information:

- **Two or more targets (required).** The contents of the wells must include at least two targets in two wells (multiplex experiment), or at least two wells with one target in each (singleplex experiments). These targets represent different amplified genes or sequences in your samples. For more details, see the instructions in Requirements for a Gene Expression Run about setting up a singleplex or multiplex experiment

- **One or more reference targets.** At least one target must be a reference target for normalized expression
- **Common samples (required).** To view your data plotted in the Gene Expression tab, your reactions must include common samples for all targets in the reaction. These samples represent different treatments or conditions for each of your target sequences
- **One or more control samples.** Quantify the expression levels by running control samples of known quantity

  TIP: For more information about data analysis in gene expression experiments, see Requirements for a Gene Expression Run (page 94).

The software calculates the relative expression level of a target with one of these scenarios when you select Relative quantity (ΔC(t)):

- 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

  NOTE: Without stringently quantified controls in every experiment, this software cannot correctly evaluate the relative expression of two samples.

Adjusting Gene Expression Data

To adjust the data you view in the Gene Expression tab, follow these methods:

- Select the Analysis Mode in the menu bar by selecting **Settings > Analysis Mode**
- Change the order of the bars in the chart by right-clicking and selecting the **Sort** option
- Select a Mode to analyze the data using Normalized expression (ΔΔC(t)) or Relative quantity (ΔC(t))
- Select a Graph Data option to graph the data Relative to control, or Relative to zero. When you select a control Sample in the Experiment Setup window, the software automatically defaults to calculate the data relative to that control
- Select an X-Axis option to show data from the Target or the Sample on that axis
- Select a Y-Axis option to graph the data as Linear, Log 2, or Log 10 scale
- Select a Scaling option to choose unscaled and leave the data unscaled. Select **Highest** to scale the expression data to the highest value or select **Lowest** to scale the expression data to the lowest value
- Select an Error Type to choose Standard Error of the Mean (default, SEMs), or Standard Deviation (Std Devs)
- Select a Chart Error Bar Multiplier to adjust the error bars for SEMs or Std Devs
- Click the **Experiment Settings** button to open the Experiment Settings window and change information about the targets or samples listed in this experiment
- Click **Show Details** to show more columns of data in the spreadsheet
- Select a well group (page 116) to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group drop-down menu in the toolbar
- Exclude wells (page 117) from analysis temporarily in the Plate Editor window. For example, exclude wells that contain data that might skew the analysis, and view the changes immediately

- Clear the wells to delete the contents of the wells in the Plate Editor window (page 119). For example, clear the wells that contain contaminated or inaccurate well contents. Deleting the content does not remove the fluorescence data that were collected during a run

Gene Expression Data Description

Table 27 describes the information shown in the spreadsheet at the bottom of the Gene Expression tab:

Table 27. 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 28 also shows this information:

Table 28. 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

Creating a Gene Expression Experiment

Create and run a Gene Expression experiment by following these steps:

1. **Check the requirements for a gene expression experiment.**

   Review the requirements for a gene expression experiment (page 94), including how to run singleplex or multiplex, and when to choose normalized or relative expression.

2. **Create a plate in the Plate Editor.**

   Before running an experiment, load the wells in the plate with at least the minimum required information in the Plate Editor (page 55). Then load the remaining contents of each well before, during, or after the run.

   NOTE: Be sure to select the correct scan mode in the toolbar (page 112) for the fluorophores in your experiment, otherwise the instrument might not collect accurate fluorescence data.

3. **Assign any reference targets and a control sample in the Plate Editor (optional).**

   Assign all reference targets (required) and a control sample (optional) in the Experiment Settings window (page 63).

4. **Load your reactions in the block and start the run.**

   Load the microplates or tubes that contain your reactions into the block. Then, select the block and click the Start Run button on the Start Run tab (page 71).

5. **Monitor the run (optional).**

   Once the run starts, the Run Details window opens to show the progress of the run. When the run finishes, the Data Analysis window opens (page 78).

6. **Select a gene expression mode (optional).**

   In the Data Analysis window, select the Analysis Mode in the menu bar. (page 113)

7. **Select the graphing options (optional).**

   Adjust the graph by selecting options in the Gene Expression tab (page 90).

8. **Adjust the spreadsheet (optional).**

   Organize or export the spreadsheet of data in the Data Analysis window (page 126).

Requirements for Gene Expression Analysis

The requirements for Gene Expression depend on the contents of your reactions:

- **Singleplex PCR.** Run a singleplex experiment with one fluorophore in the reactions
- **Multiplex PCR.** Run a multiplex experiment with more than one fluorophore in the reactions

To run a singleplex gene expression experiment, load the wells with the following required and optional contents:

- **Two or more targets.** Two targets are required (Figure 69 on page 95)
- **One or more reference targets (optional).** Select reference targets in the Experiment Settings window to analyze the data in Normalized Expression mode ($\Delta\Delta C(t)$), which normalizes expression data to the references. Experiments that do not contain a reference must be analyzed using Relative Expression mode ($\Delta C(t)$), which calculates the relative quantity
- **Two samples.** Two samples are required (Figure 69 on page 95)

- **One control sample (optional).** Quantify the expression levels by running a control sample of known quantity. Select a control sample in the Experiment Settings window

  TIP: Select reference targets and control samples in the Experiment Settings window. Click the **Experiment Settings** button to open this window (page 63).

For example, Figure 69 shows the minimum contents of the wells (and your PCR reactions) for a singleplex gene expression experiment:

Figure 69. Example of well contents in a singleplex gene expression experiment.

To run a multiplex gene expression experiment, load the wells with the following required and optional contents:

- **Two or more targets in each well.** Two targets are required (Figure 70 on page 95)
- **Select one or more reference targets (optional).** Select reference targets in the Experiment Settings window to analyze the data in Normalized Expression mode ($\Delta\Delta$C(t)). Experiments that do not contain a reference must be analyzed using Relative Expression mode (ΔC(t))
- **Two samples.** Two samples are required (Figure 70 on page 95)
- **Select one control (optional).** Quantify the expression levels by running a control sample of known quantity. Select a control sample in the Experiment Settings window

  TIP: Select reference targets and control samples in the Experiment Settings window. Click the **Experiment Settings** button to open this window (page 63).

For example, Figure 70 shows the minimum contents of the wells (and your PCR reactions) for a multiplex gene expression experiment:

Figure 70. Example of well contents in a multiplex gene expression experiment.

Gene Expression Tab Options

The Gene Expression tab evaluates the relative differences between any target concentrations. The software calculates the following quantified values using standard formulas (see “Data Analysis Formulas” on page 128).

Generally, it is recommended to adjust the data in the Quantitation tab before viewing the Gene Expression tab. To set up Gene Expression analysis, choose from the following options:

- **Gene Expression Mode options.** Select Normalized Gene Expression (ΔΔC(t)) (default), or Relative Quantity (ΔC(t))
- **Graph Data.** Select Relative to Control or Relative to Zero
- **X-Axis.** Select Target or Sample
- **Y-Axis.** Select Linear, Log 2, or Log 10
- **Scaling.** Select Unscaled, Highest, or Lowest
- **Error Type.** Select Standard Error of the Mean (SEM), or Standard Deviation (Std Devs)
- **Chart Error Bar Multiplier.** Select ± 1, 2, or 3 (for SEMs or Std Devs)
- **Right-click menus.** Select an option in the chart right-click menu or the spreadsheet right-click menu

Normalized Gene Expression

To normalize data (ΔΔC(t)), 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.

The formulas for calculating normalized gene expression include:

- Normalized expression (page 131)
- Normalized expression with a control sample selected (page 131)
- Normalization factor (page 130)
- Standard deviation for the normalized expression (page 131)
- Standard deviation of the scaled normalized expression (page 132)

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). The relative quantity is the concentration of one target relative to other target. Typically researchers who do not use reference genes are confident in one of the following considerations when they set up their experiment:

- Each sample represents the same amount of template in each of your biological samples. Typically researchers choose to load the same mass of RNA or cDNA in each well, because the mass of nucleic acid is thought to be an effective way of normalizing the resulting data. Therefore, no modification of relative quantity data are needed to obtain normalized data, because the data are normalized by the experimental design
- Any variance in the amount of biological sample loaded will be 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. Options include, but are not limited to the mass of nucleic acid loaded for each sample (relative quantity/quantity of RNA represented in each sample), number of cells from which the nucleic acid was isolated (relative quantity/number of cells represented in each sample), and mass of tissue from which nucleic acid was isolated (relative quantity/mass of tissue represented in each sample)

Normalize the loading by dividing the relative quantity value by a normalizing factor in this list of examples:

- Mass of nucleic acid loaded for each sample. Use relative quantity/quantity (ng) of RNA represented in each sample as a normalization factor
- Number of cells from which nucleic acid was isolated. Use relative quantity/number of cells represented in each sample as a normalization factor
- Mass of tissue from which nucleic acid was isolated. Use relative quantity/mass of tissue represented in each sample as a normalization factor

  NOTE: This list does not include all possible normalization factors.

The calculation for relative quantity uses these formulas:

- Relative quantity (page 129)
- Relative quantity with a control sample (page 130)
- Standard deviation of relative quantity (page 130)

To run a Relative Quantity (ΔC(t)) analysis, follow these steps:

1. Open a data file (.pcrd extension).
2. Assess the threshold and baseline information in the data, and make changes if necessary.
3. Click the **Gene Expression** tab.
4. Change the selected Targets and Samples in the Experiment Settings window.
5. Select **Relative Quantity** (ΔC(t)) from the pull-down menu.

   TIP: To compare results to data from other gene expression experiments, open a new Gene Study (page 135), or add a data file to an existing Gene Study.

Graph Data

The graph data options allow you to present the data in a Gene Expression 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 up-regulation and down-regulation of the target.
- **Relative to zero.** Graph the data with the origin at zero.

  TIP: Edit the target and sample names in the Experiment Settings window (page 63).

X-Axis Options

The x-axis option allows you to select the name that appears on the x-axis 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

  TIP: Change the target and sample selections in the Experiment Settings window (page 63).

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** ($\Delta\Delta C(t)$) 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 options 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

Right-click Menu Items for Gene Expression Graph

Right-click on the chart view of the Gene Expression graph to select the items shown in Table 29.

Table 29. 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 Gene Expression Chart Options window to adjust the scale on the graph
Sort	Sort the order that samples or targets appear on the chart axes
User Corrected Std Devs	Calculate the error bars using the corrected standard deviation formula

TIP: You can also right-click on the spreadsheet to select options.

End Point Tab

The End Point tab shows the average RFU (relative fluorescence unit) values to determine whether or not the target amplified by the last (end) cycle. Use these data to determine if a specific target sequence is present (positive) in a sample. Positive targets have higher RFU values than the cutoff 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 a tab layout that includes:

- **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

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

  TIP: Select the wells that you want to see and to analyze in the Plate Editor and the Data Analysis window. For more information see “Selecting Wells in Data Analysis Window” on page 115.

To analyze the end point data, the plate must contain negative controls, or the software cannot make the call. Then 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. To view end point data in a quantitation experiment, the data in the Quantification tab can be analyzed in either the Base Line Subtracted or the Base Line Subtracted Curve Fit analysis mode
- **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

Tab Layout for End Point Analysis

The software links the data shown in the End Point tab (Figure 71). For example, highlighting a well in the well selector (bottom left side of tab) highlights these data in the spreadsheet on the right side of the tab:

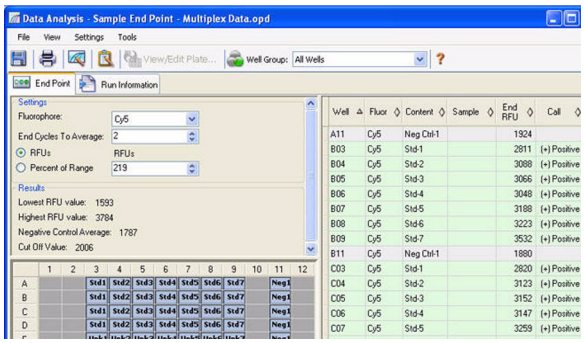


Figure 71. Layout of the End Point Analysis tab in the Data Analysis window.

NOTE: Changing the selected wells in the well selector (page 114) does not change the data analysis calculations, but does change the well content you see in the spreadsheet. To change calculations for Lowest RFU, Highest RFU, Negative

Control Average, and Cut Off Value in the Results pane, exclude wells from data analysis in the Plate Editor. Click the **View/Edit Plate** button to open the Plate Editor.

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

Data Analysis for End Point Analysis

Open the End Point tab to analyze final relative fluorescence units (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.

NOTE: End Point analysis requires at least one negative control well.

The Cut Off Value defines the tolerance for determining if an unknown sample is positive. 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).** t 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 (page 115) in the well selector to focus on subsets of the data
- Select a well group (page 116) to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group drop-down menu in the toolbar
- Exclude wells (page 117) from analysis temporarily in the Plate Editor window. For example, exclude wells that contain data that might skew the analysis, and view the changes immediately

- Clear the wells to delete the contents of the wells in the Plate Editor window (page 119). For example, clear the wells that contain contaminated or inaccurate well contents. Deleting the content does not remove the fluorescence data that were collected during a run

Data Description for End Point Analysis

Table 30 describes the information shown in the spreadsheet at the right side of the End Point tab.

Table 30. End Point data in spreadsheet

Information	Description
Well	Well position in the plate
Fluor	Fluorophore detected
Content	A combination of the Sample type and Replicate Number
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

Open the Allelic Discrimination tab to assign genotypes to unknown samples. The software compares samples with known genotypes to samples with unknown genotypes to make a call (assign a genotype to unknown samples). The software displays these allelic discrimination data in these layouts:

- **RFU or C(t) chart.** View the real-time PCR data in a graph of relative fluorescence units (RFU) or threshold cycle (C(t)) for Allele1/Allele2. 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

  TIP: Select options in menu bar and toolbar of the Data Analysis window to select data analysis options. Also, right-click any chart, spreadsheet, or well selector for options. Click the **View/Edit Plate** button to open the Plate Editor, and change the contents of any wells in the plate.

  TIP: Select the wells that you want to see and to analyze in the Plate Editor and the Data Analysis window. For more information see “Selecting Wells in Data Analysis Window” on page 115.

Tab Layout for Allelic Discrimination

The software links the information in the chart, spreadsheet, and well selector views in the Allelic Discrimination tab (Figure 72). The software takes an average C(t) or RFU for the positive controls to automatically set the threshold lines for discriminating the alleles. Adjust these thresholds, by clicking and dragging the threshold lines:

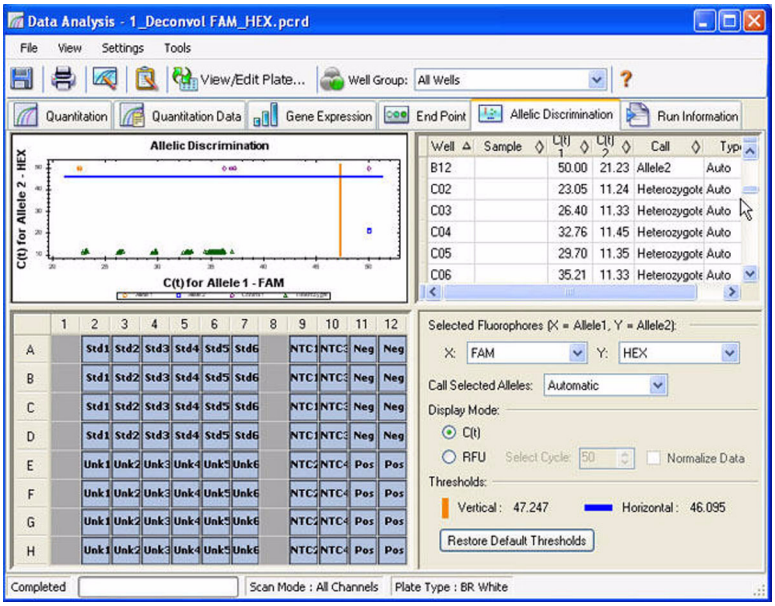


Figure 72. Layout of the Allelic Discrimination tab in the Data Analysis window.

NOTE: Changing the selected wells in the well selector does not change the data analysis calculations, but changes the well content you see in the spreadsheet and chart. To change the data used for data analysis, exclude wells from data analysis in the Plate Editor. Click the View/Edit Plate button to open the Plate Editor

Data Analysis for Allelic Discrimination

The Allelic Discrimination tab assigns the genotypes to wells with unknown samples using the RFU or threshold cycle (C(t)) of positive control samples. 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 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.

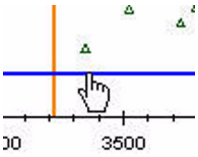
Adjust the position of the threshold bars by clicking and dragging them, and the software automatically adjusts the calculations to make new genotype assignments:

- 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 threshold cycle values 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.

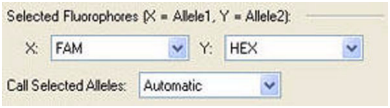
Adjusting Data for Allelic Discrimination

Adjust Allelic Discrimination data by following any of these methods. Refer to Figure 72 on page 103 for an image of the Allelic Discrimination tab.

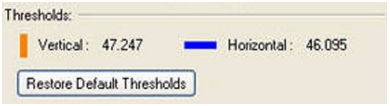
- Click and drag the threshold bars in the Allelic Discrimination chart to adjust the calls in the spreadsheet



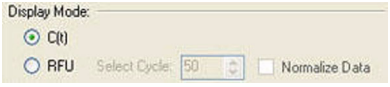
- Select a fluorophore for each axis in the chart (X-Axis and Y-Axis)



- 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



- Select wells in the well selector (page 115) in the Quantitation tab to focus on subsets of the data
- Select a well group (page 116) to view and analyze a subset of the wells in the plate. Select each well group by name in the Well Group drop-down menu in the toolbar
- Exclude wells (page 117) from analysis temporarily in the Plate Editor window. For example, exclude wells that contain data that might skew the analysis, and view the changes immediately
- Clear the wells to delete the contents of the wells in the Plate Editor window (page 119). For example, clear the wells that contain contaminated or inaccurate well contents. Deleting the content does not remove the fluorescence data that were collected during a run

Data Description for Allelic Discrimination

The Allelic Discrimination spreadsheet at the top right side of the Allelic Discrimination tab shows the information shown in Table 31.

Table 31. Information in 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 Allele1, Allele2, Heterozygote, None, Unknown, Control1, Control2
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.

Run Information Tab

The Run Information tab (Figure 73) 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. Enter or edit the ID for the run by typing in the ID box, or enter the ID automatically at the beginning of a run by using a bar code scanner.
- View the Other sections to see events, such as error messages, that might have occurred during the run. View these messages to help troubleshoot a run.

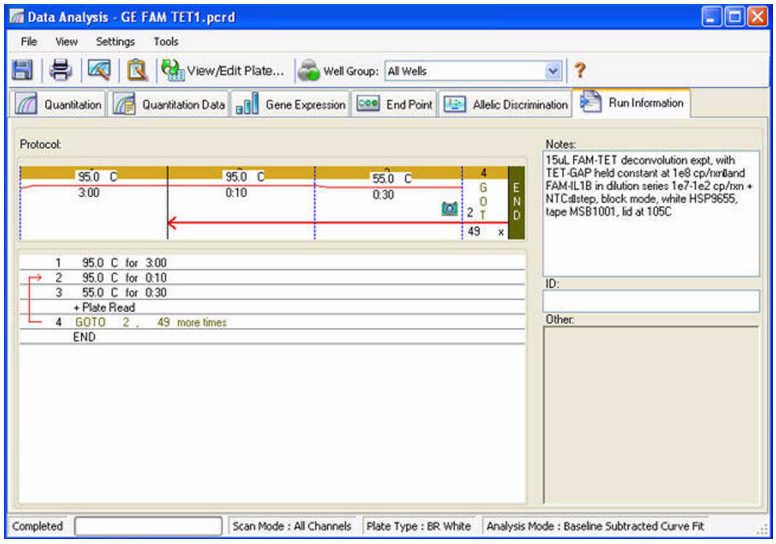


Figure 73. Layout of the Run Information tab in the Data Analysis window.

TIP: Right-click the Protocol to copy, export or print it. Right-click the Notes, ID, or Other panes to undo, cut, copy, paste, delete, or select the text.

NOTE: The Melt Curve step includes an extra 30 second hold.

Reports for Data Files

The Report window (Figure 74) shows information about the current data file in the Data Analysis window. The report links the data, protocol, plate, and other information about the run. 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:

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

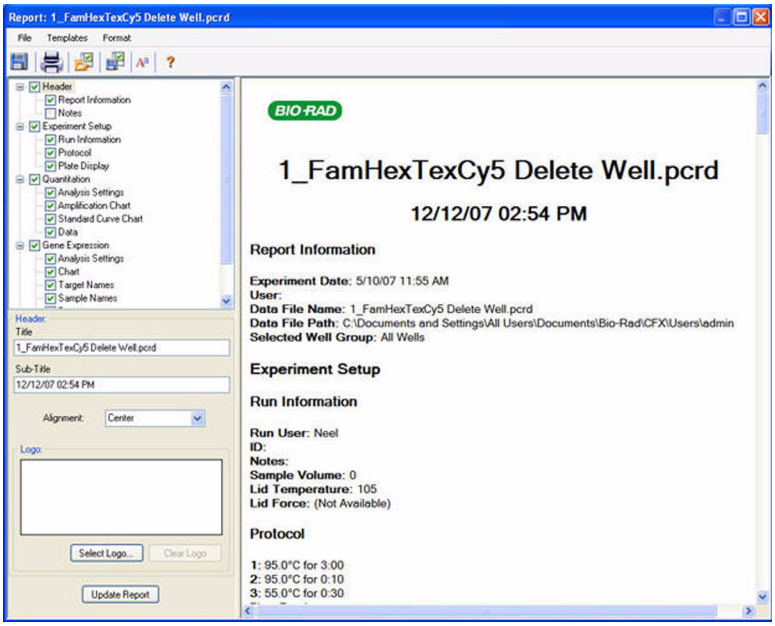


Figure 74. Example of a Report window for a data file.

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.

Create 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 the **Report** button in the Data Analysis toolbar to open the Report window.
3. (Optional) 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.
4. (Optional) Click the **Update Report** button to update the Report Preview with any changes to the report.

5. (Optional) Repeat steps 1 to 5 to further adjust the report.
6. Print or save the report. Click the **Print** button in the toolbar to print the current report. Select **File > Save** to save the report as a PDF (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. (Optional) Create a report template with the information you want. To save the current report settings in a template, select **Template > Save As**. Then load the report template the next time you want to make a new report.

Data Analysis Report Options List

A data report includes options that appear in a list, with check boxes to select or remove each option. The options that appear in this list depend on the type of data that appear in the Data Analysis window. For example, in Figure 75 the options do not include melt curve data because the data do not include melt curve data.

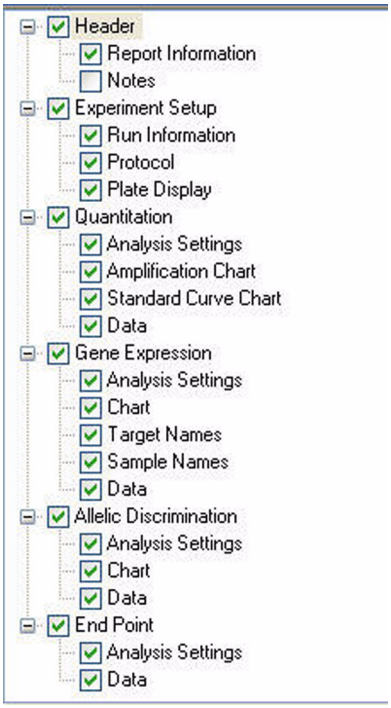


Figure 75. Example of the options list in the data Report window for Data Analysis.

The options that appear in this list depend on the data available in the experiment. For example, an **End Point Only Run** only contains end point data, so the quantitation tab does not appear in the Data Analysis window, and the option to show the quantitation data are not included in this list.

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 report.

Data Analysis Report Categories

A report can include any of the options in each category described in Table 32. The categories and options in the list depend on the type of data in Data Analysis window:

Table 32. 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
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

Table 32. Data analysis report categories in the options list (continued)

Category	Option	Description
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

Data Analysis Report Pane

The name of this pane changes depending on the option you highlighted in the report options list. Make selections and enter information in this pane. Then, click the **Update Report** button to update the report in the report preview pane. When the Standard Curve Chart selected in the report options list, the Standard Curve Chart information (Figure 76) appears in the report pane.

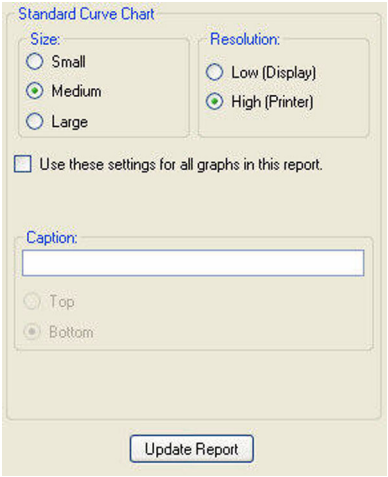


Figure 76. Standard Curve pane in the data Report window.

Report Preview

The Report preview pane (Figure 77) shows the current view of the Report, after you click the Update Report button. To make changes to the report, select report options in the list and enter information in the report options pane:

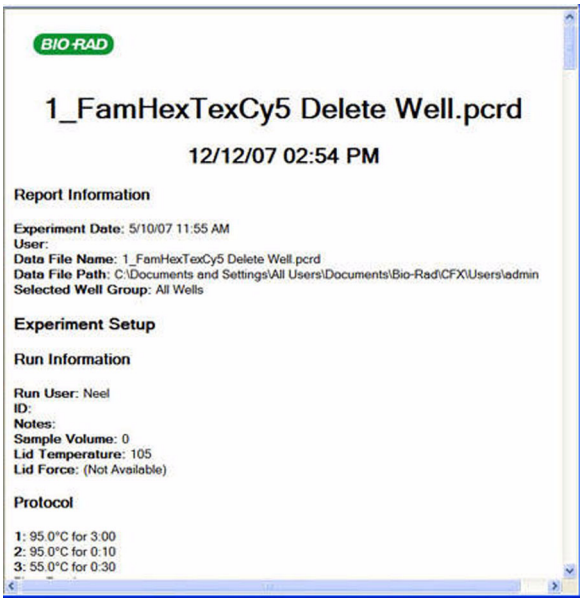


Figure 77. Preview panes in the data Report window.

10 Data Analysis Details

This chapter describes detailed information about the Data Analysis window, including these topics:)

Data Analysis Toolbar

The toolbar in the Data Analysis window (Figure 78) provide a quick access to important data analysis functions.

Figure 78. Toolbar in the Data Analysis window.

Table 33 lists the functions of buttons in the toolbar.

Table 33. Toolbar in the Data Analysis window

Toolbar button	Name	Function
	Save	Save the current data file
	Print	Print the selected window
	Trace Style	Open 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 drop-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 79) provides these menu items:

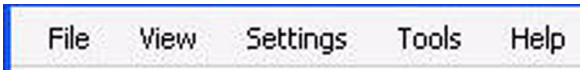


Figure 79. Menu bar in the Data Analysis window.

Table 34 lists the functions of items in the menu bar.

Table 34. 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
	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 the data with the mouse cursor. TIP: If the 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
	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

Well Selectors

The well selector allows you to select the data that appear in each Data Analysis tab. The selectors show all the wells in the plate, including the wells with content (entered in the Plate Editor) and empty wells. The content and selection of wells changes, depending on the following rules:

- **Loaded wells.** The wells loaded with content in the Plate Editor appear blue for selected wells, or light gray for unselected wells. The content is indicated by text that lists the sample type you entered
- **Empty wells.** The wells that do not contain text, indicated by a dark gray color.

  NOTE: Wells that do not contain text might contain fluorescence data collected by the instrument during the run, but the software will not display those data until you load content into that well in the Plate Editor

In Figure 80, 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 selected 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
- **Unselected unloaded wells (dark gray).** These wells do not contain content that was loaded in the Plate Editor window

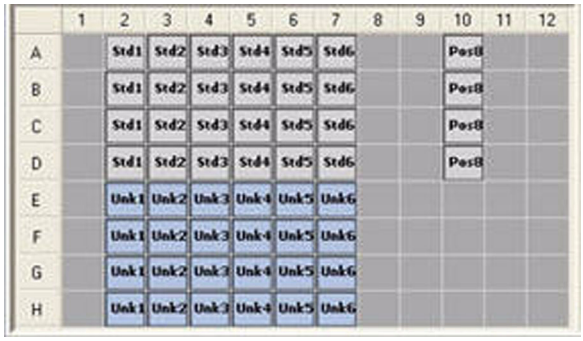


Figure 80. Three types of wells in the well selector.

Right-Click Menu Items

Right-click any well selector view to select the items listed in Table 35.

Table 35. Right-click menu items in the well selectors

Item	Function
Copy	Copy the content of the well to a clipboard, including the 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

Selecting Wells in Data Analysis Window

The well selector in the Data Analysis window shows all the wells in the plate. The selector includes both empty wells and loaded wells. Loaded wells contain content entered in the Plate Editor.

To change the data that appear in the Data Analysis window, change the selected wells by one of these methods:

- Select wells (page 115) in the well selector in the Data Analysis window to focus on subsets of the data (run results). The selected wells are blue and unselected wells are gray
- Select a well group (page 116) in the Data Analysis window to view and analyze a subset of the wells in the plate. Select each Well Group by name in the Select Well Groups drop-down menu in the toolbar. Only wells in the well group appear in the well selector
- Exclude wells (page 117) from analysis temporarily in the Plate Editor window. For example, exclude wells that contain data that might skew the analysis, and view the changes immediately. Excluded wells do not appear in the well selector
- Clear the wells (page 119) to delete the contents of the wells in the Plate Editor window. For example, clear the wells that contain contaminated or inaccurate well contents. Deleting the content does not remove the fluorescence data that were collected during a run. Cleared wells do not appear in the well selector

Select Wells in the Data Analysis Window

Click the wells in the well selectors of the Data Analysis window. Select one or more wells to show or to hide the data. Hidden wells turn a lighter color, and the software does not display these data in the charts or spreadsheets throughout the Data Analysis window. However, these hidden data are included in any data analysis calculations.

> TIP: To remove data from data analysis calculations, exclude wells from data analysis, select Well Groups, or clear the wells.

To hide or show the data associated with one or more well, follow these options:

- 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

In Figure 81 the data from the wells in rows E through H (darker color) shows in charts and spreadsheets, and the data from wells in rows A through D (light color) are hidden:

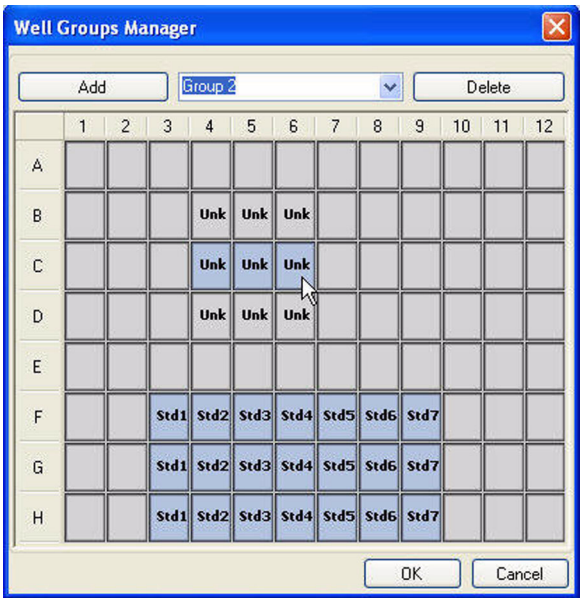


Figure 81. Well Groups Manager window showing Group 2.

Create Well Groups in the Plate Editor

Group any wells in one plate into subsets for independent data analysis. When you create Well Groups in the Well Group Manager window, the group names appear in the Data Analysis window within the Select Well Groups list.

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

For example, in Figure 82 All Wells is selected in the Well Groups menu, and well 7H is highlighted. All the data in all the wells with content are shown in the charts and spreadsheet:

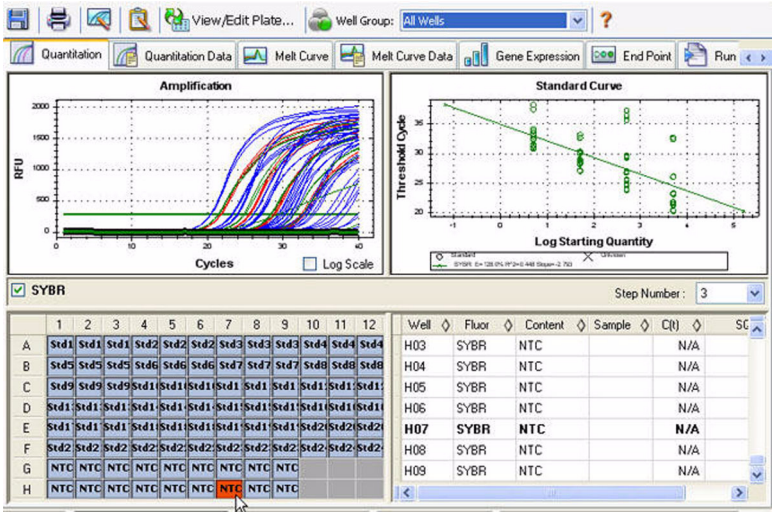


Figure 82. Data Analysis window with the All Wells selected.

Figure 83 shows Group 2 selected in the Well Groups menu. Notice that when Group 2 is selected, only the wells in that well group appear loaded with content in the well selector. The data from these wells are shown in the charts and spreadsheet with well 1B highlighted:

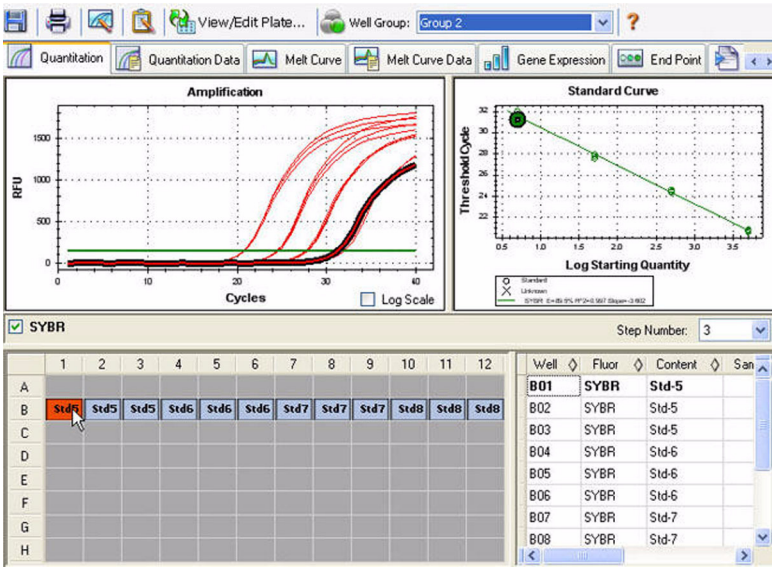


Figure 83. Data Analysis window with Group 2 selected.

NOTE: The wells that are not within the well group called Group 2 do not appear in the plate, and data for these wells are not included in the calculations for data analysis.

Exclude Wells Temporarily in the Plate Editor

Exclude wells from data analysis temporarily in the Plate Editor.

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

To exclude wells, follow these instructions:

1. Select one or more wells in the well selector view.

2. Click **Exclude Wells in Analysis** (Figure 84) to exclude the selected wells. This checkbox is at the bottom of the Plate Editor controls.

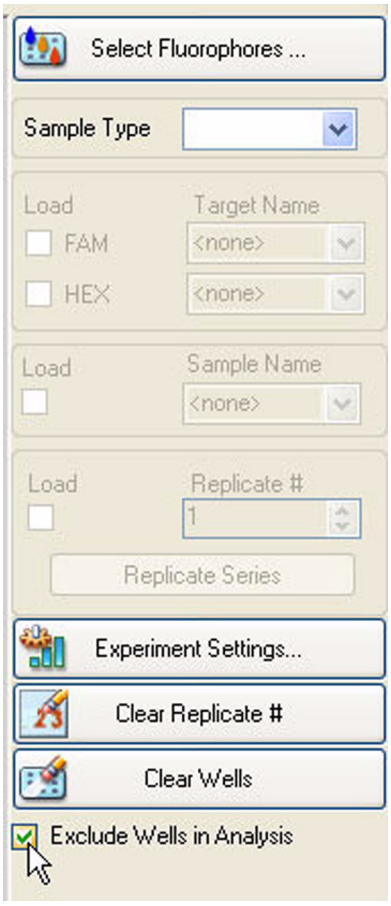


Figure 84. Exclude Wells in Analysis checkbox at bottom of the pane.

To select one or more wells, use these methods:

- Click an individual well to select that well
- Click the top left corner of the plate to select all the wells in the well selector view
- Click a column or row to select those wells

In Figure 85, one well (under the pointer) was excluded from data analysis in the Plate Editor. Notice that the excluded well is marked with an asterisk (*).

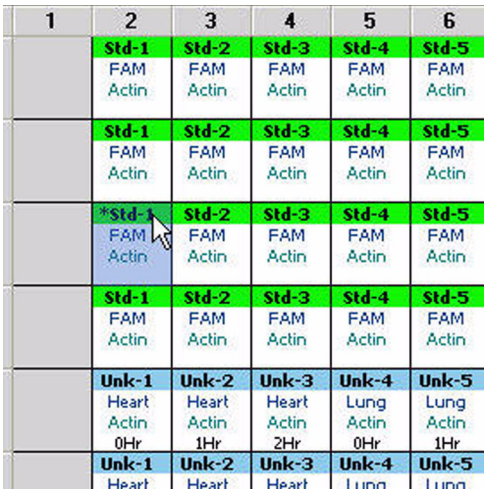


Figure 85. Excluded well (*) after clicking Exclude Wells in Analysis checkbox in Plate Editor.

The well that is excluded does not appear in the well selector (Data Analysis window shown in Figure 86) after it is excluded in the Plate Editor window, and data from this well are not included in data analysis calculations.

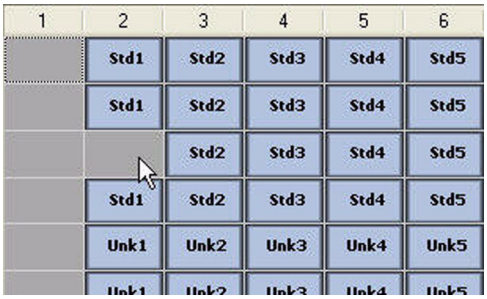


Figure 86. Excluded well (under pointer) in Data Analysis window.

Clear the Wells in the Plate Editor

Clear the contents from wells from in the Plate Editor.

> **WARNING!** Clearing wells in the Plate Editor deletes all records of the content of the well. You cannot retrieve deleted content. However, deleting the content does not remove the fluorescence data that were collected during a run.

To clear the contents of a well, follow these instructions:

1. Select one or more wells in the well selector view of the Plate Editor.
2. Click the **Clear Wells** button. Notice that cleared wells appear empty.

   NOTE: Clearing wells does not remove the fluorescence data that the instrument collected during the run.

To clear one or more wells, select the wells with one of these methods:

- Click an individual well to select that well
- Click the top left corner of the plate to select all the wells in the well selector view

- Click a column or row to select those wells

Figure 87 shows that the cleared well (under the pointer) is empty in the Plate Editor window.

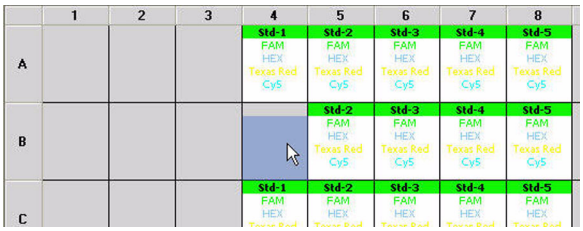


Figure 87. Cleared well in the Plate Editor window after clicking the Clear Wells button.

Baseline Thresholds Window

Open the Baseline Thresholds window (Figure 88) to change the default baseline for selected wells, or to change the threshold for each fluorescence curve in the amplification chart. To open this window, follow these steps:

1. In the Quantitation tab, select the fluorophore in the fluorophore selector (Figure 92 on page 123) below the Amplification chart.
2. Select **Settings > Baseline Thresholds** to open the window.

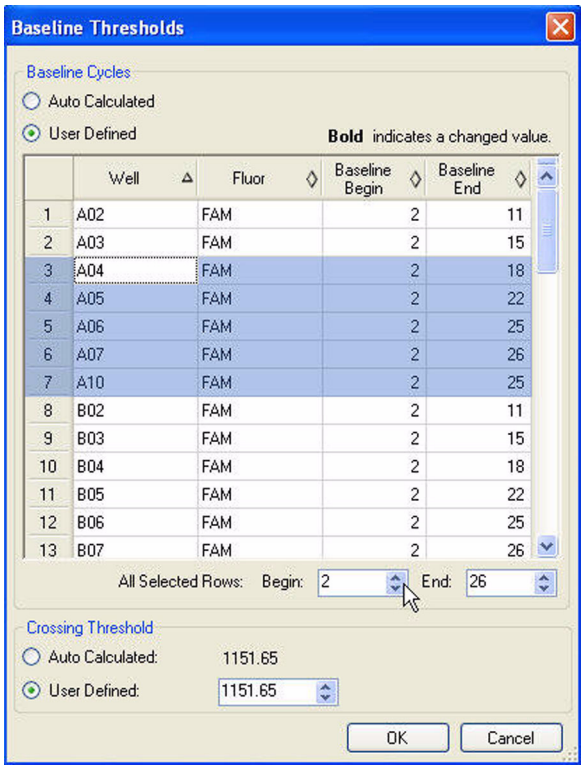


Figure 88. Baseline Thresholds window for adjusting the baselines and thresholds of one fluorophore.

This window includes two panes which provide the following functions for the selected fluorophore:

- **Baseline Cycles.** Adjust the begin and end baseline cycle for each well

- **Crossing Threshold.** Adjust the threshold manually by typing in a value, or by clicking **User Defined** and selecting a threshold level from the list

Adjusting the Baseline

To adjust baseline, follow these instructions:

1. Select one fluorophore in the fluorophore selector in the Quantitation tab (Figure 92 on page 123) by clicking the boxes next to the fluorophore name located under the Amplification chart.
2. Select **Settings > Baseline Threshold** in the menu bar to open the Baseline Threshold window.
3. Select one or more wells, and adjust the beginning and ending baseline for each selected well by changing the **Begin** and **End** cycle number (Figure 89).

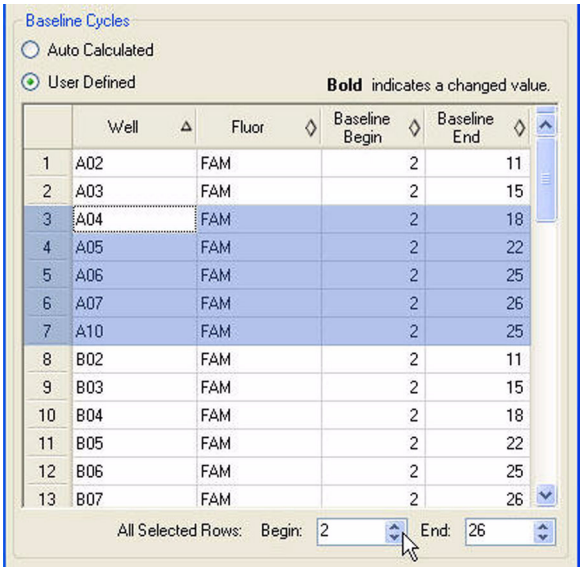


Figure 89. Adjust the Begin and End cycle number.

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

To select one or more rows in the Baseline Thresholds window, choose from the following methods:

- Select one row by clicking the row number
- Select all rows by clicking the top left corner of the list
- Select multiple individual wells by holding down the control key
- Select multiple wells in a row by holding down the shift key

Adjusting the Threshold

Adjust the threshold for a fluorophore by specifying an exact crossing threshold for the selected fluorophore. To adjust baseline, follow these instructions:

1. Select one fluorophore in the fluorophore selector in the Quantitation tab (Figure 92 on page 123) by clicking the boxes next to the fluorophore name located under the Amplification chart.

2. Select **Settings > Baseline Threshold** in the menu bar to open the Baseline Threshold window.
3. Adjust the crossing threshold (Figure 90) for the fluorophore by clicking **User Defined** and entering a threshold number.

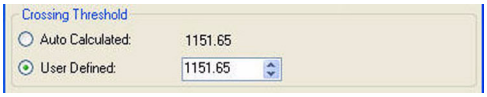


Figure 90. Adjust the crossing threshold.

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

Trace Styles Window

Open the Trace Style window (Figure 91) to adjust the appearance of traces in the amplification and melt curve charts on the Quantitation and Melt Curve tabs.

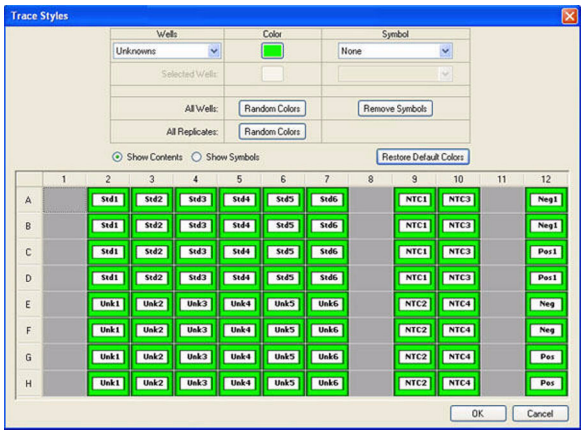


Figure 91. Trace Styles window.

To open this window, follow these two steps:

1. Select one fluorophore from the fluorophore selection boxes (Figure 92 on page 123) under the Amplification chart.

   NOTE: The Trace Styles window displays the trace for one fluorophore at a time.

2. Click the **Trace Styles** button in the Data Analysis toolbar, or select **Settings > Trace Styles** in the Data Analysis menu bar.

Define Trace Styles

Use the tools in the Trace Styles window to adjust appearance of traces in an amplification chart, 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 trace and show that color in the well selector
- 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

Charts

Each chart in the Data Analysis window displays the data in a different graph. The charts also include some selectors and options for adjusting how you view the data:

- Fluorophore selector for selecting the fluorophores that appear in the chart (Figure 92 on page 123)
- Log Scale option to change the scale of the Y-axis
- Right-click menu items in all charts, including changing the scale and the order of the displayed data
- Magnifying function to magnify an area of the chart and view the details
- Standard Curve chart to view the standards

  TIP: To select the data that appear in a chart, follow the methods described in “Selecting Wells in Data Analysis Window” on page 115.

Fluorophore Selector

To select one or more fluorophores, click the fluorophore selector (Figure 92) below Amplification chart on the Quantitation tab. Click the box next to the fluorophore name to include or exclude that fluorophore in the quantitation and melt curve tabs.

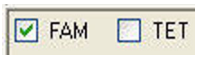


Figure 92. Fluorophore selector with FAM selected.

For example, when only FAM is selected in the selector below the chart (Figure 93 on page 124), then FAM is the only fluorophore that appears in the Quantitation (page 79) and Melt Curve (page 85) tabs.

TIP: To adjust the baseline or the threshold for a fluorophore, select that fluorophore in the fluorophore selector, then select **Settings > Baseline Threshold** to open the Baseline Thresholds window.

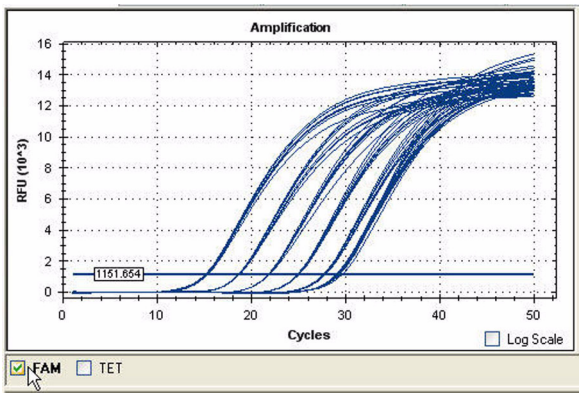


Figure 93. FAM selected in Amplification chart in the Quantitation tab.

Log Scale Option

The software displays a log scale option on the Amplification chart in the Quantitation tab. Click the **Log Scale** box at the bottom of the chart (Figure 94) to view a semi-log scale, as shown in this image:

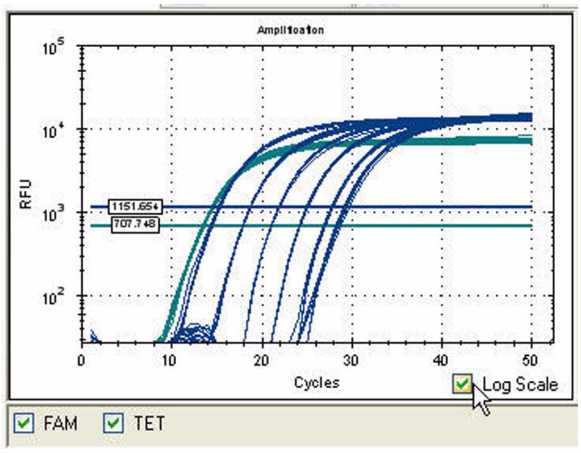


Figure 94. Log Scale option selected in Amplification chart.

TIP: To magnify any area of the Standard Curve chart, click and drag the mouse across an area of the chart. To return the chart to a full view, right-click and select **Set Scale to Default** from the menu.

Magnify an Area of the Chart

Magnify any chart in the Data Analysis window to view details in one area, or to change the shown scale. To magnify an area of the chart, select an area by clicking and dragging the mouse across that area. 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.

Standard Curve Chart

The software creates Standard Curve chart (Figure 95) in the Quantitation tab (page 79) if the data include sample types defined as standard (Std) for one fluorophore in the experiment.

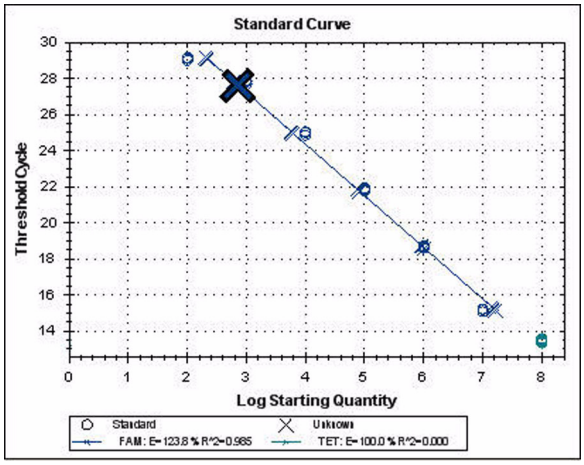


Figure 95. Standard Curve chart.

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
- Coefficient of determination (R^2). Use this statistic to determine how correctly the line describes the data (goodness of fit)

Right-Click Menu Items

Right-click the Amplification, Standard Curve, Melt Curve, or Melt Peak chart to select the items shown in Table 36. Some of these items apply to all charts, and others apply only to a specific chart.

Table 36. Right-click menu options 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. Selecting this option shows C(t) values in the Amplification chart, and shows relative quantity in the Gene Expression 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, including changing the title of the chart, selecting limits for the X and Y axes, showing grid lines, and showing minor ticks in the axes
Amplification chart only	
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)
Gene Expression chart only	
Sort...	Open the Gene Expression Chart Sorting window to change the order of Target and Sample names in the chart (only appears in Gene Expression tab)
Use Corrected Std Devs	Change the chart to show corrected standard deviation or standard deviation (only appears in Gene Expression tab)

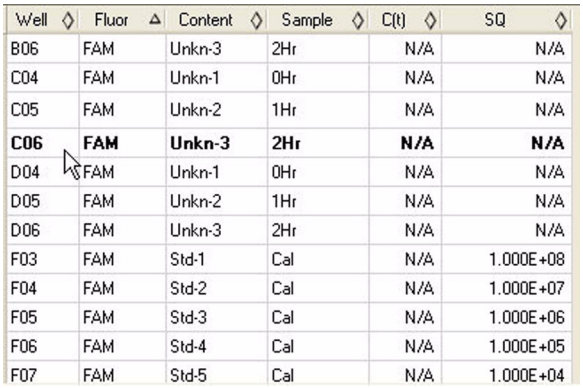
Spreadsheets

The spreadsheets shown in the CFX Manager software include the following options for sorting and transferring the data:

- Highlight the data (next section) on the associated charts and well selector by holding the mouse pointer over a cell
- Click a right-click menu item (page 127), including copying, printing, exporting, and sorting the data
- Sort up to three columns (page 127) in the spreadsheet to rearrange the data for viewing

Highlight the Data

The Quantitation tab shows data in a spreadsheet (Figure 96).



Well	Fluor	Content	Sample	C(t)	SQ
B06	FAM	Unkn-3	2Hr	N/A	N/A
C04	FAM	Unkn-1	0Hr	N/A	N/A
C05	FAM	Unkn-2	1Hr	N/A	N/A
C06	**FAM**	**Unkn-3**	**2Hr**	**N/A**	**N/A**
D04	FAM	Unkn-1	0Hr	N/A	N/A
D05	FAM	Unkn-2	1Hr	N/A	N/A
D06	FAM	Unkn-3	2Hr	N/A	N/A
F03	FAM	Std-1	Cal	N/A	1.000E+08
F04	FAM	Std-2	Cal	N/A	1.000E+07
F05	FAM	Std-3	Cal	N/A	1.000E+06
F06	FAM	Std-4	Cal	N/A	1.000E+05
F07	FAM	Std-5	Cal	N/A	1.000E+04

Figure 96. Spreadsheet in Quantitation tab.

Right-click Menu Items

Right-click any spreadsheet view to select the items shown in Table 37.

Table 37. Right-click menu items for spreadsheets

Item	Function
Copy	Copy the contents of the selected wells to a clipboard. TIP: Paste the well 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
Find...	Search for text
Sort...	Sort the data in up to three columns TIP: To change the order of the columns, click the top of the column and drag it to a new location

Sort Columns

Sort the columns by one of these methods:

- Click and drag a column to a new location in the selected table
- Right-click and select **Sort** to open the Sort window

  TIP: To sort one column, click and drag it to a new location.

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.

2. Select **Sort** from the right-click menu to open the Sort window.
3. In the Sort window, select the first column title to sort. Sort the data in Ascending or Descending order.
4. (Optional) 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.
5. Click **OK** to sort the data, or click **Cancel** to stop sorting.

Data Analysis Formulas

CFX Manager software calculates formulas automatically and displays the resulting information in the Data Analysis tabs. The software uses these formulas and definitions for quantitation, gene expression, and allelic discrimination data analysis:

- Efficiency of the reaction for the Quantitation tab and Gene Expression tab (page 128)
- Relative quantity ($\Delta C(t)$) for the Gene Expression tab (page 129)
- Relative quantity with a control sample selected for the Gene Expression tab (page 130)
- Standard deviation of relative quantity for the Gene Expression tab (page 130)
- Normalization factor for the Gene Expression tab (page 130)
- Normalized expression ($\Delta\Delta C(t)$) for the Gene Expression tab (page 131)
- Normalized expression with a control sample is selected for the Gene Expression tab (page 131)
- Standard deviation for the normalized expression for the Gene Expression tab (page 131)
- Normalized expression scaled to highest sample for the Gene Expression tab (page 132)
- Normalized expression scaled to lowest sample for the Gene Expression tab (page 132)
- Standard deviation of scaled normalized expression for the Gene Expression tab (page 132)
- Corrected values formulas for the Gene Expression tab (page 133)
- Cut off formula for the End Point tab (page 133)
- Normalized RFU for the Allelic Discrimination tab (page 134)

Efficiency of the Reaction for Quantitation and Gene Expression

The reaction efficiency (E) 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. Evidence suggests that using accurate measure of efficiencies for each primer and probe sets will give you more accurate results when analyzing data with the mathematical formulas used in the Gene Expression tab.

The default value of efficiency used in the gene expression calculations is 100%. During assay development, the efficiency for each primer and probe set is typically evaluated and recorded. 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, then the software automatically calculates the efficiency and displays it under the Standard Curve on the Quantitation tab (Data Analysis window).

The efficiency (E) in the efficiency formulas refers 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 this 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

If you do not want the software to calculate the efficiency, or you do not include a standard curve, then enter the recorded efficiency for your experiment in the Targets tab on the Experiment Settings window:

- **Auto Efficiency.** For this setting, software uses efficiency values calculated from the standard curve in your experiment so that you do not have to type them in yourself. To use Auto Efficiency, your experiment must have standards that result in valid standard curves in the Quantitation tab
- **Auto Efficiency Standards.** For this efficiency setting the software requires a minimum of two standards at different concentrations. Generally, the recommendation is to use standards that include at least four samples in triplicate across a relevant dynamic range

Relative Quantity

The relative quantity ($\Delta C(t)$) for any sample (GOI) is calculated with this formula:

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

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 (MIN) = Average C(t) for the Sample with the lowest average C(t) for GOI
- C_T (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 (GOI) with a gene of interest 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 (control) = Average C(t) for the control sample
- C_T (sample) = Average C(t) for any samples with a GOI
- GOI = Gene of interest (one target)

Standard Deviation of 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) sample = Standard deviation of the C(t) for the sample (GOI)
- Relative Quantity = 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 (ΔΔC(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 (1). This normalized expression is equivalent to the unscaled normalized expression analysis when a control is chosen.

Standard Deviation for the Normalized Expression

Re-scaling 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 levels, depending on the Scaling Option you choose. The standard deviation (SD) of the normalization factor is calculated with this formula:

$$\text{SD NF}_n = \text{NF}_n \times \sqrt{\left(\frac{\text{SD RQ}_{\text{sample (Ref 1)}}}{n \times \text{RQ}_{\text{sample (Ref 1)}}}\right)^2 + \left(\frac{\text{SD RQ}_{\text{sample (Ref 2)}}}{n \times \text{RQ}_{\text{sample (Ref 2)}}}\right)^2 + \ldots + \left(\frac{\text{SD RQ}_{\text{sample (Ref n)}}}{n \times \text{RQ}_{\text{sample (Ref n)}}}\right)^2}$$

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 re-scaling function on the standard deviation, as shown here:

Where:

- NE = Normalized Expression

$$\text{SD NE}_{\text{sample (GOI)}} = \text{NE}_{\text{sample (GOI)}} \times \sqrt{\left(\frac{\text{SD NF}_{\text{sample}}}{\text{NF}_{\text{sample}}}\right)^2 + \left(\frac{\text{SD RQ}_{\text{sample (GOI)}}}{\text{RQ}_{\text{sample (GOI)}}}\right)^2}$$

- RQ = Relative quantity of a sample
- SD = standard deviation
- GOI = Gene of interest (one target)

Normalized Expression Scaled to Highest

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:

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

Where GOI = Gene of interest (target).

Normalized Expression Scaled to Lowest

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

Re-scaling 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 which scaling option you choose.

> NOTE: When a control sample is assigned, you do not need to perform this re-scaling 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 non-corrected values will only be seen if a standard curve was 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:

$$\text{SE NF}_\text{n} = \text{NF}_\text{n} \times \sqrt{\left(\frac{\text{SE RQ}_{\text{sample (Ref 1)}}}{\text{n} \times \text{SE RQ}_{\text{sample (Ref 1)}}}\right)^2 + \left(\frac{\text{SE RQ}_{\text{sample (Ref 2)}}}{\text{n} \times \text{SE RQ}_{\text{sample (Ref 2)}}}\right)^2 + \ldots + \left(\frac{\text{SE RQ}_{\text{sample (Ref n)}}}{\text{n} \times \text{SE RQ}_{\text{sample (Ref n)}}}\right)^2}$$

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:

$$\text{SE GOI}_\text{n} = \text{GOI}_\text{n} \times \sqrt{\left(\frac{\text{SE NF}_\text{n}}{\text{NF}_\text{n}}\right)^2 + \left(\frac{\text{SE GOI}}{\text{GOI}}\right)^2}$$

Where:

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

Cut Off Formula for End Point Analysis

The Cut Off Value formula that appears in the End Point tab is calculated using the average of the negative control data in the following formula:

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

Where the tolerance is calculated by one of these methods:

- **RFUs.** 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

Normalized RFU for Allelic Discrimination

Select the Normalize check box to normalize the RFU in the Allelic Discrimination chart. 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

11 Gene Study Window

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.

> 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 63).

Create a Gene Study by adding data from one or more data files (.pcrd extension) to the Gene Study. Once the data files are included in a Gene Study, the software groups them into a single file (.mgxd extension). To distribute a Gene Study file to a colleague, simply email the file. To store a Gene Study for security, save the file on a back-up drive.

> 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.

Before importing data into a Gene Study, you must 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 samples
- 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 Gene Study window includes two tabs:

- **Study Setup tab.** Click this tab to manage the Experiments in the Gene Study file. 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 97 shows the Gene Study window, including the Study Setup and Study Analysis tabs.

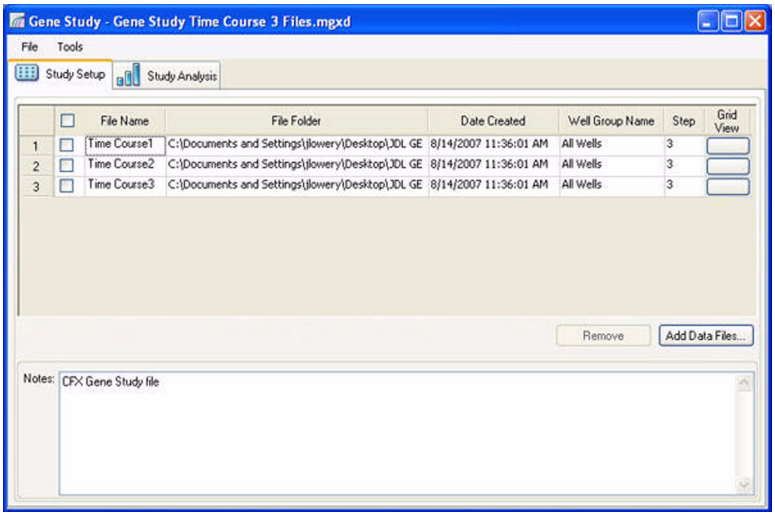


Figure 97. Gene Study window.

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. This dominant calibrator is then used to adjust all C(t) values within 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 multi-tiered 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 targets have 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 targets have an identical range as the ΔC(t) values, then 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 targets have identical Average ΔC(t) absolute values, then set the dominant calibrator to the replicate group with the smallest ΔC(t).

   NOTE: The first data file imported into the Gene Study will always serve as the "hub" file for pair-wise data comparison during Inter-run Calibration.

Study Setup Tab

The Study Setup tab (Figure 98) shows a list of all the Experiments in the Gene Study.

- **Add Experiments.** Click the **Add Data Files** button 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**. Removing files from a Gene Study does not change the data in the file
- **Add Notes about the Gene Study.** Type in the Notes box to add comments about the files and analysis in this Gene Study

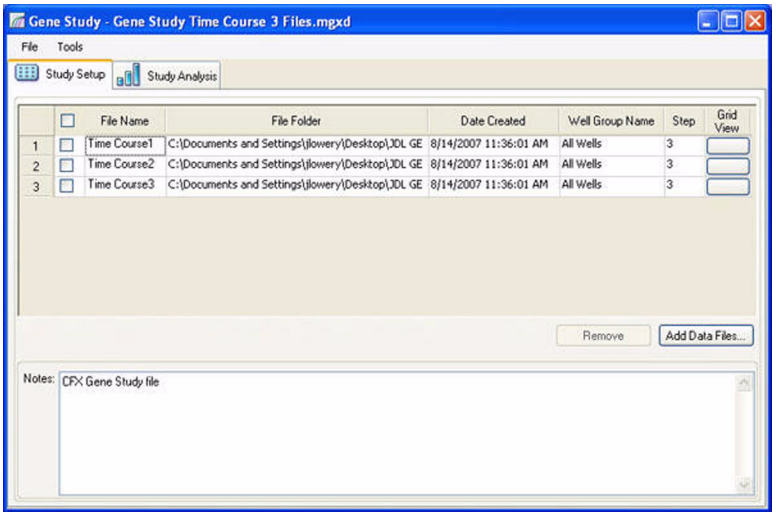


Figure 98. Study Setup tab in Gene Study window.

The Study Setup tab lists the data files in the Gene Study, as described in Table 38.

Table 38. 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.** Display Normalized Expression (ΔΔC(t)) or Relative Quantity (ΔC(t))
- **Graph Data.** Display Relative to normal or Relative to control in the graph
- **X-Axis Options.** Change 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 value, 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 99).

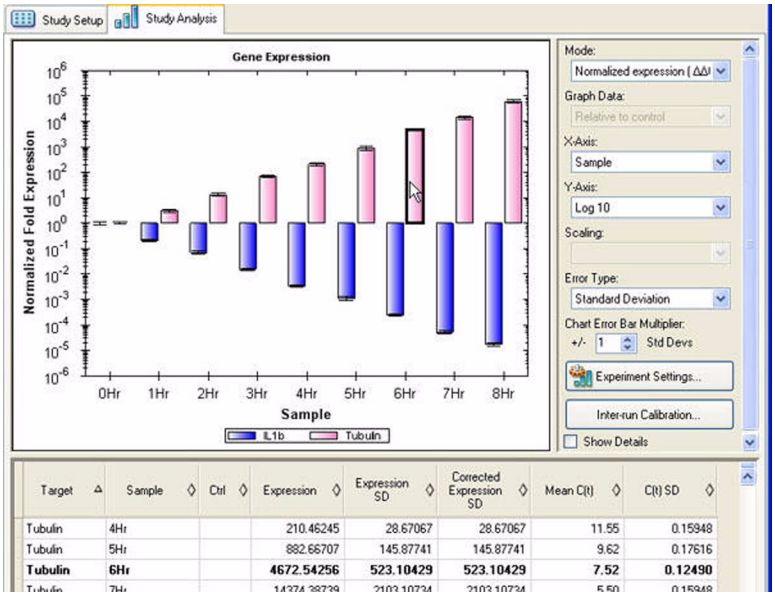
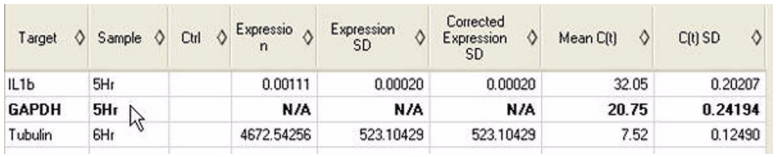


Figure 99. Study Analysis tab in Gene Study window.

Gene Study Data Spreadsheet

The data spreadsheet in the Gene Study window lists information about each target and sample in the Gene Study (Figure 100).



Target	Sample	Ctrl	Expression	Expression SD	Corrected Expression SD	Mean C(t)	C(t) SD
IL1b	5Hr		0.00111	0.00020	0.00020	32.05	0.20207
GAPDH	**5Hr**		**N/A**	**N/A**	**N/A**	**20.75**	**0.24194**
Tubulin	6Hr		4672.54256	523.10429	523.10429	7.52	0.12490

Figure 100. Data spreadsheet in the Gene Study window.

TIP: Right-click on the spreadsheet to select from a menu of options.

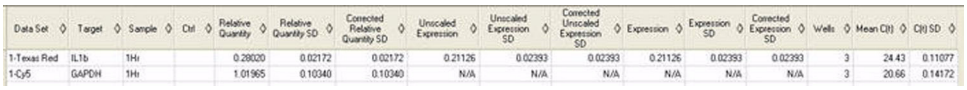
Table 39 describes the information shown in the Gene Study spreadsheet.

Table 39. 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 ($\Delta\Delta$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

Gene Study Show Details Data

Click the Show Details check box to show additional columns of information in the Gene Study spreadsheet (Figure 101).



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 101. Show Details data in the Gene Study tab.

When you click the Show Details check box, the spreadsheet adds the information in the columns listed in Table 40.

Table 40. 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

Open the Gene Study Report window to arrange the Gene Study data into a report. To open a report from the Gene Study window, select **Tools > Reports**. The Gene Study Report window includes these three panes:

- Report options list pane (page 141)
- Report option pane (page 142)
- Report preview pane (page 142)

  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.

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. Select the content of the report by clicking the check boxes in the report options list to select and remove options.
4. (Optional) Edit each report option in the report options pane.
5. Click the **Update Report** button to update the report preview pane.
6. (Optional) Repeat steps 1 to 4 to adjust the report.

7. Print or save the report. Click the **Print** button in the toolbar to print the current report. Select **File > Save** to save the report as a PDF (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.
8. (Optional) 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 As** to save the current report as a template.

Gene Study Report Options List

The Gene Study report includes a list of options. The content of the list depend on the type of data that are available in the Gene Study. Change the content of the report by selecting and removing options in the list (Figure 102).

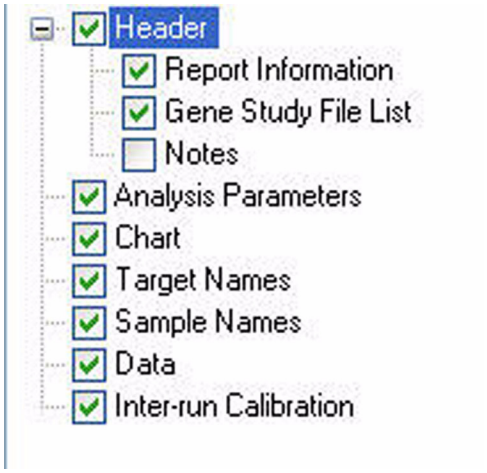


Figure 102. Example of options list for a Gene Study report.

The Gene Study report includes a list of options for creating a report. The options in the list depend on the type of data that are available in the Gene Study. Change the content of the report by selecting and removing options in the list shown in Table 41.

Table 41. 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 parameters selected for data analysis
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
Inter-run Calibration		Inter-run calibration data

Gene Study Report Option

Fill in the text for the report by entering text and images in option panes. In Figure 103, the pane shows the report Header and Logo.

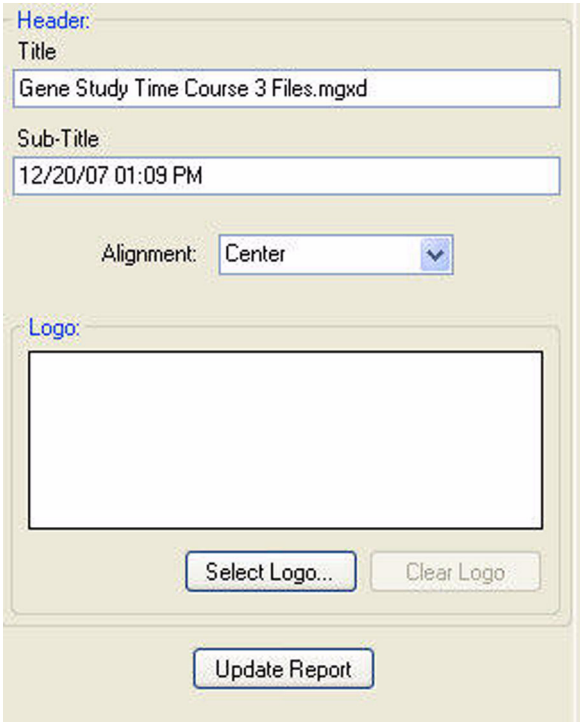


Figure 103. Example of Header and Logo options in a Gene Study report.

Gene Study Report Preview

The report preview pane shows a view of the Report (Figure 104). The report preview pane shows a view of the Report. This view changes as you select and edit the report options.

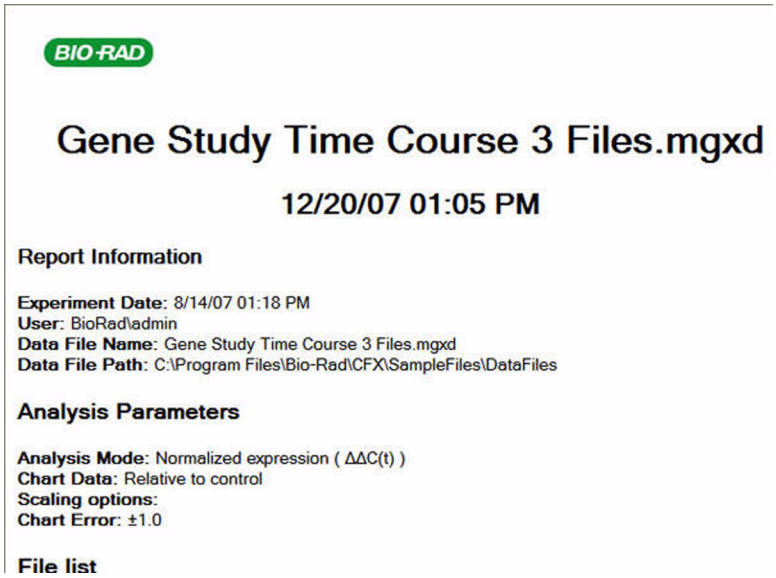


Figure 104. Example of preview pane in a Gene Study report.

12 Manage Instruments

CFX Manager software manages multiple instruments with combinations of reaction modules (page 144). Read these sections for information about managing one or more instruments with the software:

- Run multiple instruments and reaction modules (next section)
- Detected Instruments pane (page 145)
- Instrument Summary window (page 147)
- Instrument Properties window (page 149)
- Calibration Wizard (page 151)

  TIP: For easy identification, name the instruments (page 150). The default name for any instrument is the serial number.

Run Multiple Instruments

CFX Manager software runs these 1000-series instruments:

- **CFX96 and CFX384 real-time PCR systems.** These systems both include a C1000 thermal cycler and an optical reaction module.
- **C1000 thermal cycler.** The C1000 thermal cycler is the most versatile of the instruments run by CFX Manager software. For more information, see C1000 Thermal Cycler instruction manual
- **S1000 thermal cycler connected to a C1000 thermal cycler.** For more information, see S1000 and C1000 Thermal Cycler instruction manuals

  TIP: Locate PDF copies of the instruction manuals by opening the Documents folder on the installation CD, or searching the Literature section of the Bio-Rad Laboratories web site (www.bio-rad.com). See “Bio-Rad Resources” on page 2 for detailed information about finding instruction manuals on the web site or on the installation CD.

Reaction Modules

The 1000-series instruments run reaction modules with three block sizes for PCR and real-time PCR experiments. Add and remove these reaction modules in a few minutes (no tools required):

- 96-well fast reaction module, gradient enabled
- 384-well reaction module
- Dual 48/48 fast reaction module, gradient enabled
- CFX96 optical reaction module, gradient enabled (requires C1000 thermal cycler chassis). Use this module to collect fluoresence data from real-time PCR experiments
- CFX384 optical reaction module (requires C1000 thermal cycler chassis). Use this module to collect fluoresence data from real-time PCR experiments

Viewing Multiple Instruments

CFX Manager software easily controls multiple thermal cyclers and real-time PCR instruments through USB connections. Open these windows, panes and viewers to manage detected instruments:

- **Detected Instruments pane.** View this pane to see all the instruments that the software detects (page 145)
- **Instrument Summary window.** Open this window to see a list of the detected instruments with a summary of their status and buttons to control the run (page 147)
- **Instrument Properties window.** Open this window to remove the shipping screw, to rename instruments and modules, and to view calibration files (page 149)
- **Calibration Wizard.** Open this window to see the currently calibrated fluorophores in the Calibration Viewer (page 153), and to calibrate a new fluorophore and plate combination in the Calibration Wizard (page 151)

  TIP: For easy identification, rename the connected instrument (page 150). The default name for any instrument is the serial number.

  TIP: Locate PDF copies of the instruction manuals by opening the Documents folder on the installation CD, or searching the Literature section of the Bio-Rad Laboratories web site (www.bio-rad.com). See “Bio-Rad Resources” on page 2 for detailed information about finding instruction manuals on the web site or on the installation CD.

CFX Manager software controls 1000-series instruments through USB connections. For example, you can simultaneously run the following combinations of instruments:

- Connect up to four real-time PCR instruments. For example, run CFX96 and CFX384 systems at the same time
- Combine up to four instruments when running a mix of real-time PCR systems and thermal cyclers. For example, connect one CFX96 real-time PCR system, two C1000 thermal cyclers, and one S1000 thermal cycler at the same time

  NOTE: To prevent data loss when connecting an instrument to another instrument or to a computer, use a sufficiently shielded USB cable (catalog #184-8000).

  NOTE: Instruments that are not connected or not turned on will not appear in the lists of detected instruments. To connect an instrument, follow the instructions in these instruction manuals or in the software Help. See “Bio-Rad Resources” on page 2 for more information about finding copies of instruction manuals.

Detected Instruments Pane

The Detected Instruments pane provides a list of all detected instruments. This list shows each instrument as an icon named with the serial number (default).

TIP: Right-click any instruments in the Detected Instruments pane to open the Instrument Properties window and rename the instrument to replace the serial number.

The list of instruments also shows individual blocks (Block A and Block B) for each dual block reaction module.

NOTE: To show or hide the Detected Instruments pane, select **View > Detected Instruments** in the menu bar.

Figure 105 shows three detected instruments:

- One C1000 thermal cycler (C48FEM00) with a Dual 48 reaction module (shown as block A and block B)
- One S1000 thermal cycler (S96FEM01) with a 96-well block, which is connected to the C1000 thermal cycler called C48FEM00
- One CFX96 system (CFX96M02)

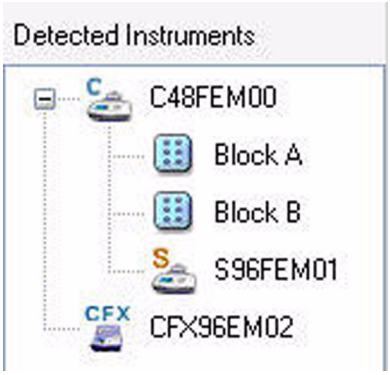


Figure 105. Instruments listed at the top of the Detected Instruments pane.

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

- **View Status.** Open the Run Details window
- **Flash Block Indicator.** Flash the block on the instrument
- **Open Lid.** Close a motorized lid on the selected instrument block
- **Close Lid.** Close a motorized lid on the selected instrument block
- **Rename.** Change the name of the instrument
- **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

To connect an instrument, follow these instructions:

1. Connect a C1000, CFX96 instrument, or CFX384 instrument to the computer with a sufficiently shielded USB cable (184-8000).
2. Connect an S1000 thermal cycler to a C1000, CFX96 instrument, or CFX384 instrument with a sufficiently shielded USB cable (184-8000).

3. Turn on the instruments. The icon for the connected instruments appears within seconds.

   NOTE: If the instrument does not appear in the Detected Instrument pane within a minute, see the troubleshooting options. To prevent data loss and connection problems, use a sufficiently shielded USB cable (catalog # 184-8000) when connecting instruments to the computer or to another instrument.

Select one or more blocks in the top of the pane by clicking the icon for the instrument block and then clicking one of buttons in Figure 106.

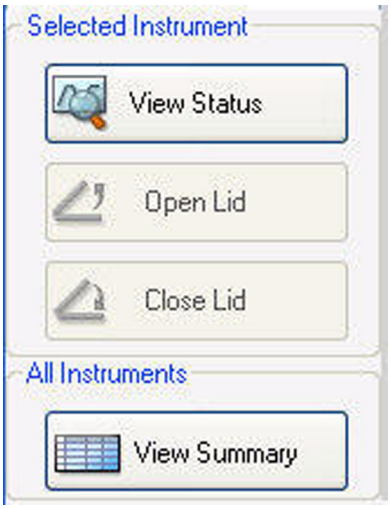


Figure 106. Buttons at the bottom of the Detected Instruments pane.

- Click **View Status** to view the current status of the selected instrument (block)
- Click **Open Lid** to open the motorized lids on the selected real-time PCR instrument
- Click **Close Lid** to close the motorized lids on the selected real-time PCR instrument
- Click **View Summary** to open the Instrument Summary window

  NOTE: If only one instrument is detected, then the View Summary button does not appear. To view the Instrument Summary window for a single instrument, select **View > Instrument Summary** or double-click the block icon.

Instrument Summary Window

The Instrument Summary window shows a list of the detected instruments and their status. Open the instrument summary by clicking the **View Summary** button (Figure 106) in the Detected Instrument pane.

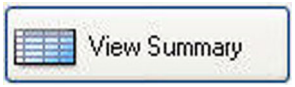


Figure 107 shows the Instrument Summary window, including the Block Name list which shows the current status of all detected instruments by Block Name. Select one or more blocks and click the buttons in the tool bar to change the status of each instrument. Right-click in the window to make changes in the Block Name list.

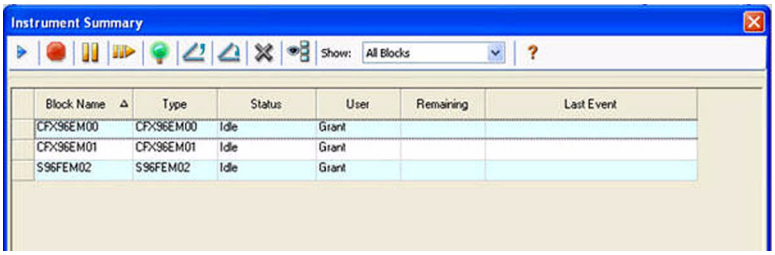


Figure 107. Instrument Summary window.

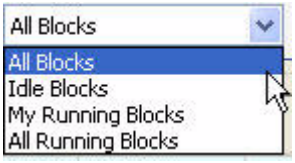
Instrument Summary Toolbar

The toolbar in the Instrument Summary window includes the buttons and functions listed in Table 42.

Table 42. Toolbar buttons in the Instrument Summary window

Button	Button Name	Function
	Set up Experiment	Set up an experiment with the selected block. Select one or more blocks, and click the **Experiment Setup** button to open the Experiment Setup window.
	Stop	Stop the current run on the selected blocks
	Pause	Pause the current run on the selected blocks
	Resume	Resume the run on the selected blocks
	Flash Block Indicator	Flash the LED on the lid of the selected blocks. Use this button to identify the selected blocks
	Open Lid	Open a motorized lid on the selected blocks

Table 42. Toolbar buttons in the Instrument Summary window (continued)

Button	Button Name	Function
	Close Lid	Close a motorized lid on the selected blocks
	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 in the Start Run on Selected Block(s) pane. Select one of these options in the right-click menu:

- **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

Instrument Properties Window

The Instrument Properties window provides an easy way to view information about one instrument. Open this window to view or change this information:

- Change the instrument name that shows in this window and in the Detected Instruments pane
- View the serial number for the instrument and reaction modules
- Install or remove the shipping screw when you need to transport the instrument
- View the list of calibrated fluorophores (dyes), and open the Fluorophore Calibration Viewer

To open the Instrument Properties window, right-click the instrument icon in the Detected Instruments pane (page 145). The window includes these three tabs as shown in Figure 108:

- **Properties (page 150).** View the serial numbers and names for the thermal cycler or reaction module
- **Shipping Screw (page 150).** Remove the shipping screw to run the instrument, or install the shipping screw when you want to transport the instrument
- **Calibrated Dyes (page 153).** Check the calibrated dyes for the instrument. To calibrate more fluorophores, open the Calibration Wizard

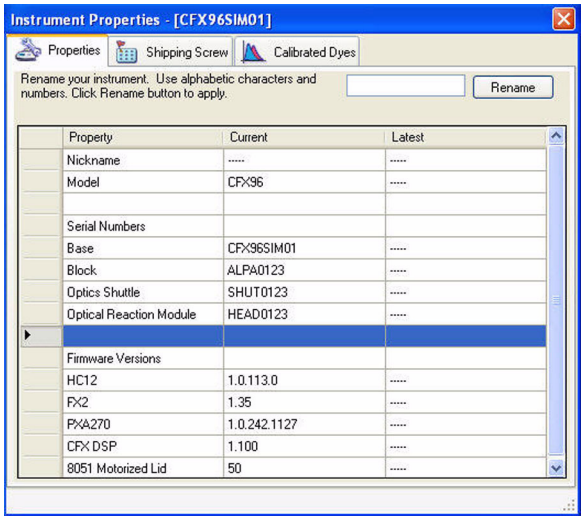


Figure 108. Instrument Properties window.

Naming an Instrument in the Properties Tab

The default name for an instrument and reaction module is the serial number. The name of the instrument appears in many locations, including the Detected Instruments pane (page 145), Instrument Summary window (page 147), and Instrument Properties (page 149) window. Figure 109 shows the software detects two CFX96 instruments by name:

Figure 109. Two CFX96 instruments listed in the Instrument Summary window.

To rename an instrument for ease of identification, follow these instructions:

1. In the Detected Instrument pane, right-click the instrument icon and select **Rename** or **Properties** from the menu to open the Instrument Properties window.
2. In the Instrument Properties window, type a name in the **Rename** box at the top of the Properties tab.

   NOTE: If the instrument cannot be renamed by the user who is logged in to the software, then the Rename button is not active.
3. Click **Rename** to save the new name.

Shipping Screw Tab

The Shipping Screw tab includes instructions for installing and removing the shipping screw. To prevent damage to the optical reaction modules, install a shipping screw any time you ship the CFX96 or CFX384 systems.

To install the shipping screw, click the Install Shipping Screw button and follow the instructions in the tab shown in Figure 110.

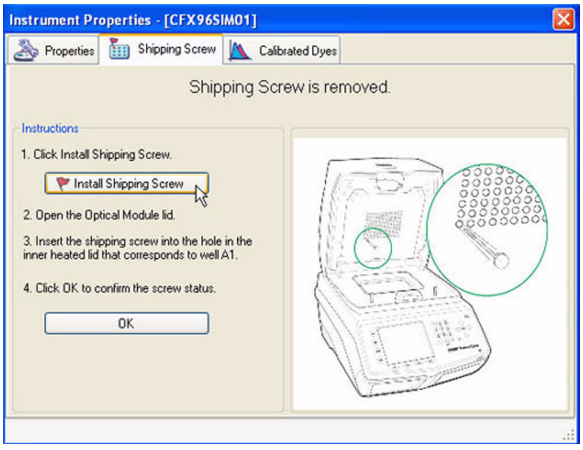


Figure 110. Instructions for installing the shipping screw in the Shipping Screw tab.

To remove the shipping screw, click the Remove Shipping Screw button and follow the instructions in the tab shown in Figure 111.

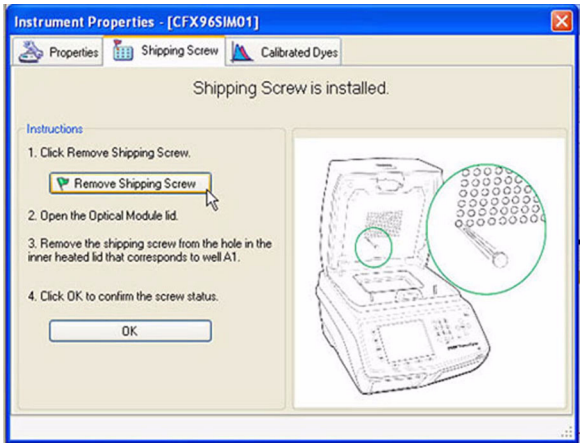


Figure 111. Instructions for removing the shipping screw in the Shipping Screw tab.

Calibration Wizard

The CFX96 and CFX384 real-time PCR systems are factory calibrated for commonly used fluorophores. To correctly read the fluorophore during a run, each instrument must be calibrated for that fluorophore in the same plate type that you use to run your experiments. Each instrument maintains a list of calibrated fluorophores and associated plate types, which the software opens in the Calibration Wizard.

Open the Calibration Wizard to calibrate the CFX96 and CFX384 real-time PCR systems (page 152).

To open the Calibration Wizard, follow these steps:

1. Select an instrument in the Detected Instruments pane.
2. Select **Tool > Calibration Wizard** to open the window and calibrate new dye and plate combinations (page 152).

Figure 112 shows an example of the Dye Calibration wizard window.

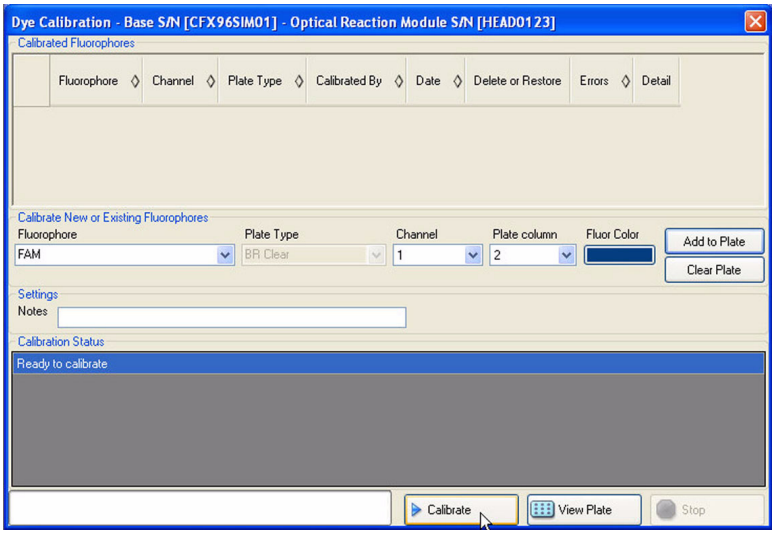


Figure 112. Calibration Wizard window.

Calibrating the CFX96 and CFX384 Systems

Each instrument maintains a list of the calibrated fluorophores and plates. Those calibrations are specific to each instrument, and each instrument maintains a list of calibrated fluorophores and associated plate types.

To calibrate the CFX96 and CFX384 real-time PCR systems, follow these steps:

1. In the Detected Instruments pane list, click the instrument that you want to calibrate to highlight it.
2. Select the **Tools > Calibration Wizard** to open the Calibration Wizard window and review the list of Calibrated Fluorophores.
3. In the Calibrate New or Existing Fluorophores pane, 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.
4. Select the Plate Type. If the plate type is not included in the list, type the name in the box to add it to the list.
5. Select a Channel for the fluorophore. For information about the excitation and detection range of each channel, see Table 43 on page 153.
6. Click the **Add to List** button to add the fluorophore. To clear the plate, click **Clear List** to remove all the fluorophores.
7. (Optional) Repeat steps 1-6 to add each fluorophore you plan to calibrate to the plate.
8. When you finish adding fluorophores, click **View Plate** to open the Dye Plate Display. Use this window as a guide for loading dyes into the plate.
9. Begin preparing a 96- or 384-well plate for dye calibration by pipetting dye solution into each well, following the pattern shown in the Pure Dye Plate Display. For each fluorophore, fill 4 wells with 50 µl (96-well plate) or 30 µl (384-well plate) of 300 nM dye solution. Notice that at least half the plate contains blank wells.
10. Seal the plate using the sealing method you will use in your experiment.
11. Place the calibration plate in the block and close the lid. Then click **Calibrate**, and click **OK** to confirm that the plate is in the block.
12. When the CFX Manager software completes the calibration run, a dialog box appears. Click **Yes** to finish calibration and open the Dye Calibration Viewer.

Factory Calibrated Fluorophores and Channels

All the instruments are factory calibrated in each channel for commonly used fluorophores. During a run, the instruments excite and detect the following factory calibrated fluorophores in white-welled and clear-welled plates on the instruments listed in Table 43:

Table 43. Factory calibrated fluorophores, channels, and instruments

Factory calibrated fluorophores (white-welled and clear-well plates only)	Channel	Instruments that collects data in the channel
FAM™, SYBR™ Green I	1	All instruments: CFX96, CFX384
VIC®, HEX™, TET™, Cal Gold 540™	2	All instruments: CFX96, CFX384
ROX™, TEXAS RED®, Cal Red 610™	3	CFX96 and CFX384
CY5, Quasar 670™	4	CFX96 and CFX384
Quasar 705™	5	CFX96 only
FRET	6	CFX96 and CFX384

Viewing the Calibrated Dyes

Open the Fluorophore Calibration Viewer window to view the list of calibrated fluorophores and plates for one instrument. To open this window, follow these instructions:

1. Right-click on one instrument icon in the Detected Instruments (page 145) pane.
1. Select **Properties** in the right-click menu to open the Instrument Properties window.
2. Click the **Calibrated Dyes** tab (Figure 113).
3. Click an **Info** button to see detailed information about a previous calibration.

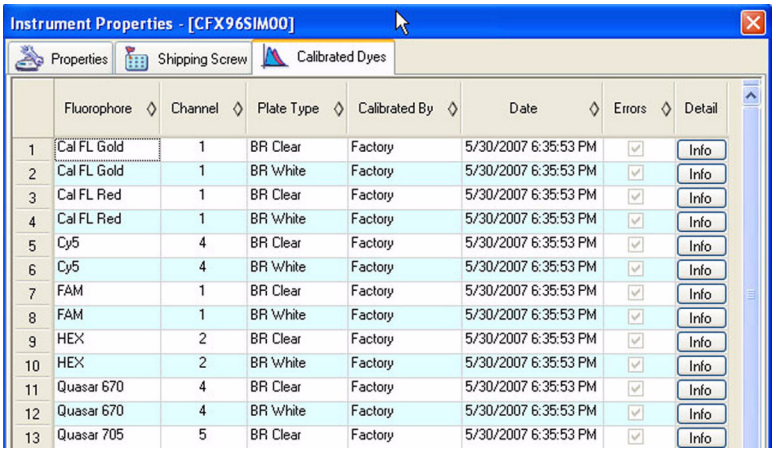


Figure 113. Calibrated Dyes tab in the Instrument Properties window.

13 Software Logs

The CFX Manager software tracks information about the state of an instrument during a run. Use these logs to track events that occur on instruments and in the software and for troubleshooting:

- **Application Logs.** A list of event logs that record the state of the software during all runs
- **Run Logs.** A list of logs that shows information about events that occurred on an instrument during each run

  TIP: The software creates customizable data Reports (page 106) and a Gene Study Reports (page 140) about the data in the Data Analysis and Gene Study windows.

Application Logs

CFX Manager software provides a log (Figure 114) of all the events that occurred on one instrument during all runs.

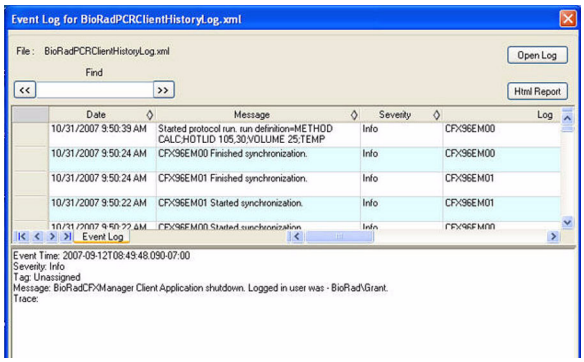


Figure 114. Example of an Event Log file.

Open this log to track information about the runs on one instrument, including the following columns of information about each event:

- **Date.** The date and time of the event
- **Message.** A description of the recorded event
- **Severity.** A description of the severity of the event. Most events contain only information (Info)
- **Log.** Instrument name, serial number, or software window that recorded the event

Run Log

The run log lists all the events that occurred on each instrument during one run. Open this log to track information about the events that occurred during one run.

To open a run log (Figure 115) in the Data Analysis window, select **View > Run Log**.

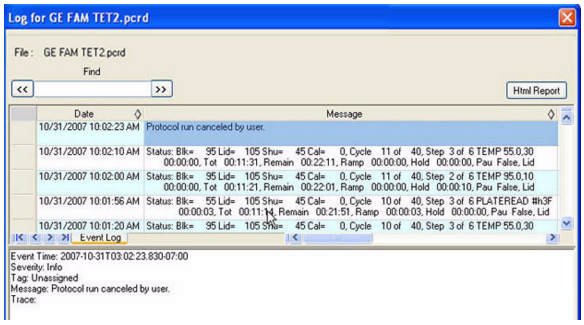


Figure 115. Example of a Run Log from a Data Analysis file.

14 Users and Preferences

CFX Manager software includes tools to manage software users and select your user preferences:

- **User Preferences (page 157).** Open this window to edit your user preferences, including setting up preferences for email (page 159), data analysis (page 171), and gene expression analysis (page 172)
- **Log on (page 173).** Open this window to log in to the software or change your password. This window opens automatically when you start the software.

  NOTE: The software does not open this window if there is only one user listed in the User Administration window
- **Change Password (page 174).** Open this window to change your password

  NOTE: A password is not required to run the software. If you need to log on to the software, contact your software administrator.
- **User Preferences (page 157).** Open this window to view and edit your user preferences
- **User Administration (page 174).** Open this window to manage users and user rights. The Administrator is the only software user who can open this window and manage user rights

User Preferences Window

CFX Manager software tracks the preferences of each User that logs onto the software in the Login window (Figure 116 on page 158).

To change User Preferences, open the User Preferences window using one of these methods:

- Click the **User Preferences** button in the main software window toolbar
- Select **User > User Preferences** in the main software window menu bar

The User Preferences window includes a series of tabs for editing preferences.

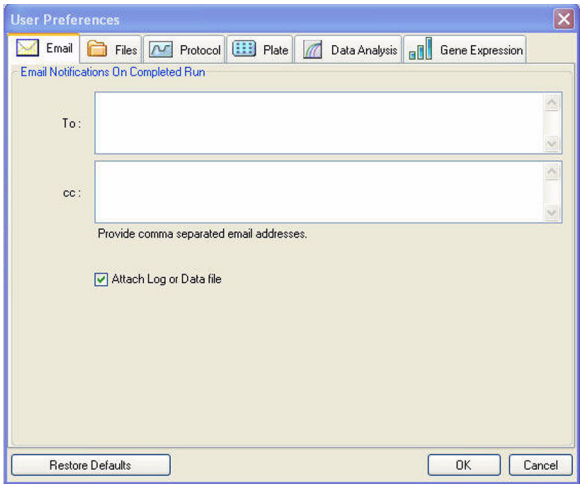


Figure 116. User Preferences with tabs.

Click one of the tabs in the User Preferences window (Figure 116) to view or change preferences:

- **Email (page 159).** Enter the email addresses where you want to receive confirmation of the completion of the run. The software can also send an attached data file with the email

  NOTE: To set up a Windows computer for email notification, see page 159.
- **Files (page 168).** Specify where default files are located and where files should be saved
- **Protocol (page 169).** Specify default settings for the Protocol Editor
- **Plate (page 170).** Specify default settings for the Plate Editor, including Target and Sample name libraries
- **Data Analysis (page 171).** Specify default settings for the Data Analysis window
- **Gene Expression (page 172).** Specify default settings for Gene Expression data

Email Preferences Tab

View the email preferences by opening the User Preferences window using one of these methods and clicking the **Email** tab:

- Click the **User Preferences** button in the main software window toolbar
- Select **User > User Preferences** in the main software window menu bar

  NOTE: To set up the computer so that it can send email to a server, follow the instructions in “Set Up Email Notification” on page 159.

Select the Email Tab in the User Preferences window (Figure 117) to specify the default settings for sending email notices when a run completes, and to choose to attach logs or data files to the email.

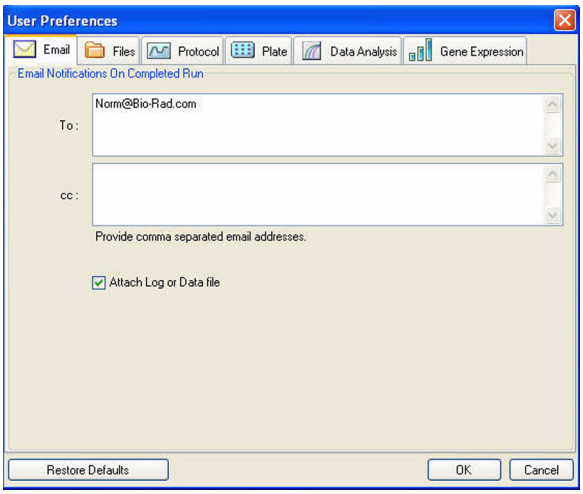


Figure 117. Email tab in User Preferences window.

Set Up Email Notification

To set up email notification from CFX Manager software, install and configure the SMTP server on the computer that is running the software:

- Setting up email notification on Windows XP computers (next section)
- Setting up email notification on Windows Vista computers (page 165)

  NOTE: Always check with the local IT staff who manage your computer, intranet, and internet access before making changes to your computer.

Setting Up Email Notification on Windows XP

To set up email notification, set up the SMTP server on the Windows XP computer running CFX Manager software. Always check with the local IT staff who manage your computer, intranet, and internet access before making changes to your computer.

NOTE: To install a new Windows component, you need the Windows XP installation CD or access to the I386 on the main drive before setting up the email notification from the computer running CFX Manager software.

To set up the computer for email notification on XP:

1. Install the SMTP server (page 160).
2. Configure the SMTP server (page 161).

3. (Optional) If needed, specify a Smart Host (page 163).

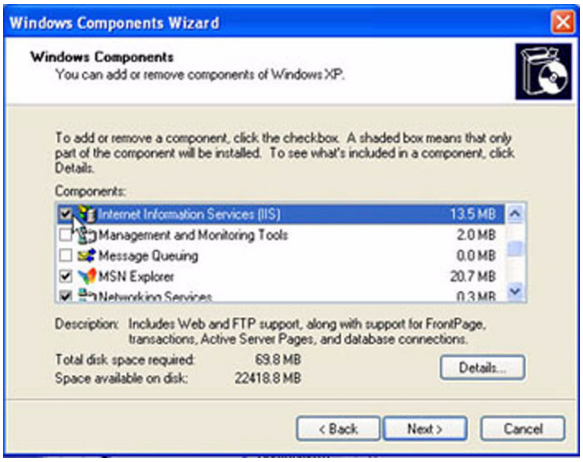
   NOTE: If the email is not working, check the list of troubleshooting options (page 165).

INSTALL THE SMTP SERVER FOR WINDOWS XP

Follow these steps to install the SMTP server on the computer that is running CFX Manager software:

1. Check with your local IT staff for special instructions about installing the SMTP server on your computer.
2. Select **Start > Control Panel > Add or Remove Programs** to open the Add or Remove Programs window.
3. Click the **Add/Remove Windows Components** button on the left side of the window to open the Windows Components wizard.

4. Click **Internet Information Services (IIS)** in the Windows Components Wizard, and click **Next** to install it.



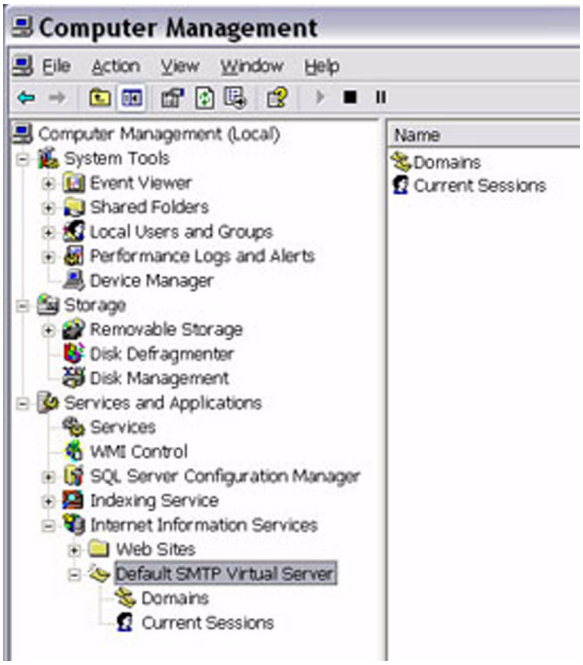
   NOTE: Insert the Windows XP installation CD if you do not have access to the I386 folder.

5. Check for updates from Microsoft after the installation is complete.

CONFIGURE THE SMTP SERVER FOR WINDOWS XP

Follow these steps to configure the SMTP server to receive email from the computer that is running CFX Manager software:

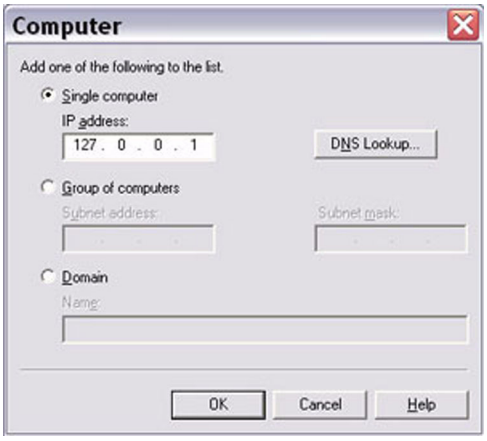
1. Select **Start > Control Panel > Administrative Tools > Internet Information Services** to open the Computer Management window.
2. Right-click the **Default SMTP Virtual Server** item in the list, and select **Properties**.



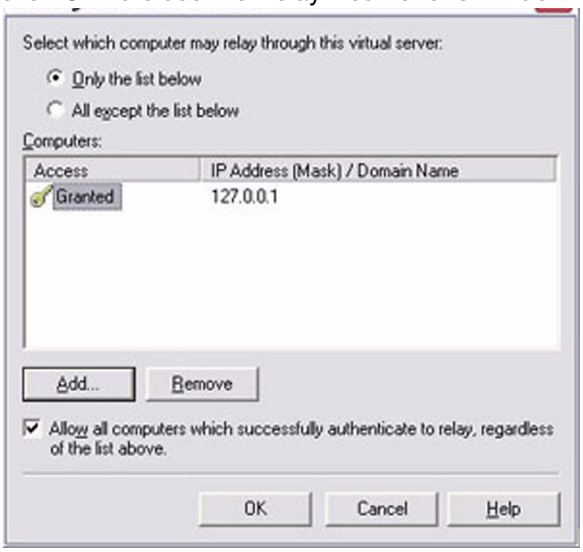
3. Click the **Access** tab in the Default SMTP Virtual Server Properties window.

4. Click the **Relay** button to open the Relay Restrictions window.

5. Click **Add** to add the local computer to open the Computer window.
6. Select Single computer and type **127.0.0.1** in the IP address box.



7. Click **OK** to accept the changes and close the Computer window. The Relay Restrictions window will display the IP address of the computer in the IP Address (Mask)/Domain Name list. Then, click **OK** to close the Relay Restrictions window.



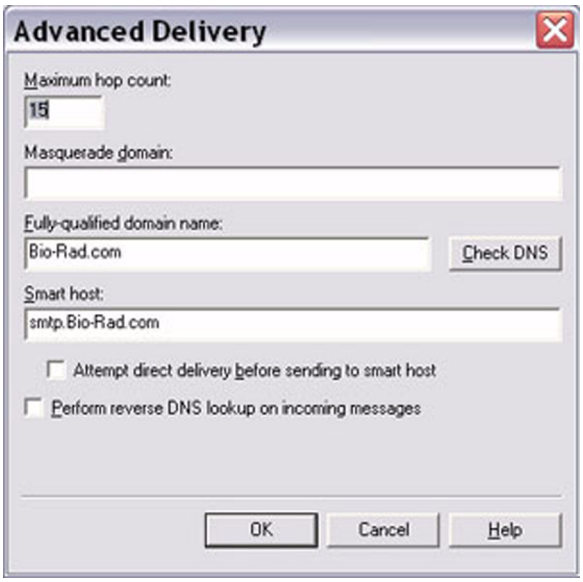
8. (Optional) Specify a smart host (next section), if needed for sending the email to the next SMTP server.
9. Click **OK** to close the window.

Specify a Smart Host for Windows XP

Follow these steps to specify a smart host for the SMTP server:

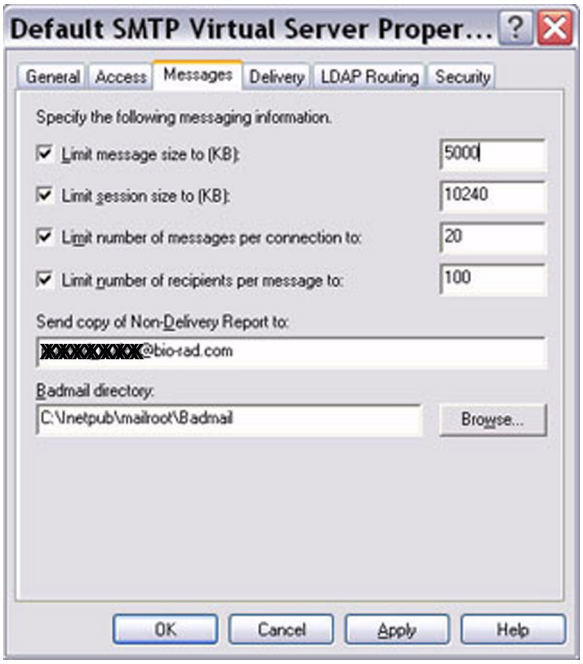
1. Open the Default SMTP Virtual Server Properties window if it is not already open. See instructions in steps 1-2 of the Configure the SMTP Server.
2. Click the **Delivery** tab in the Default SMTP Virtual Server Properties window.

3. Click the **Advanced** button to open the Advanced Delivery window.



4. Enter the server name in the Smart host box, and click **OK** to close the window. In the example shown in step 3, the domain name is smtp.Bio-Rad.com.

5. Click the **Message** tab to change the size limit for email messages. Type **5000** in the Limit message size to (KB) box. Enter an email address where you want to send notices about emails that were not delivered.



6. Click **OK** to close the window.

7. Verify that the SMTP server is installed. **Select Start > Control > Administrative > Services** to open the services. Then find the line that starts with Simple Mail Transfer and verify that the Startup Type is Automatic and that the Status is Started. Double-click the **Startup Type** to change (start or stop) the service.

Troubleshooting Email Notification for Windows XP

If you have any troubles with your email notification settings, examine these folders and logs for information about the source of the problem:

- Check with your IT department for any important information about changing the settings and sending emails from the computer running CFX Manager software
- Check the folder C:\Inetpub\mailroot to manage outgoing email
- Check the folder C:\WINDOWS\system32\Logfiles\SMTPSVC1 for SMTP server logs
- View the system event viewer. Open the event viewer by selecting **Start > Control Panel > Administrative Tools > Event Viewer**, then select **System** to see the log

Setting Up Email Notification on Windows Vista

To set up email notification, set up the SMTP server on the Windows Vista computer running CFX Manager software.

> NOTE: To install a new Windows component, you need the Windows XP installation CD or access to the I386 on the main drive before setting up the email notification from the computer running CFX Manager software.

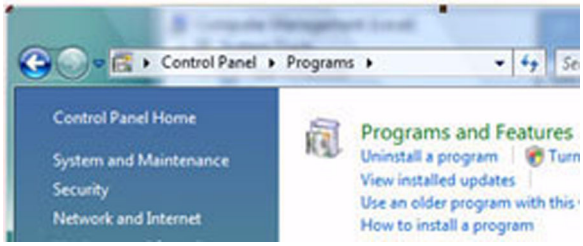
To set up the computer for email notification on Windows Vista:

1. Install the SMTP server (page 165).
2. Configure the SMTP server (page 166).
3. (Optional) If needed, edit the BioRadCFXManager.exe.config file (page 167).

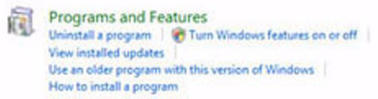
Install the SMTP Server for Windows Vista

Follow these steps to install the SMTP server on the computer that is running CFX Manager software:

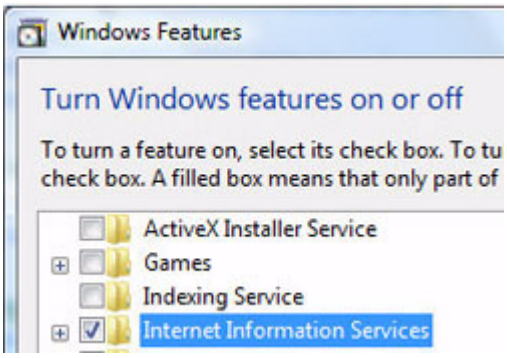
1. Check with your local IT staff for special instructions about installing the SMTP server on your computer
2. Select **Start > Control Panel** to open the Control Panel window, then click **Programs**.



3. Click **Turn Windows features on or off**.



4. Click **Internet Information Services** and then click **OK** to begin the installation.

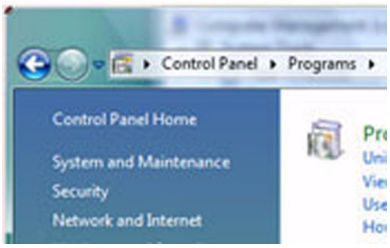


5. After the installation is complete, check for updates from Microsoft to assure proper function.
6. Configure the SMTP Server (next section).

Configure the SMTP Server for Windows Vista

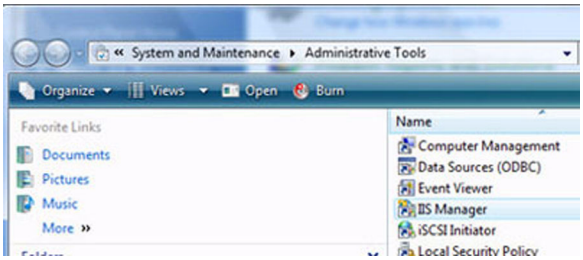
Follow these steps to configure the SMTP server:

1. Select **Start > Control Panel** to open the Control Panel window, then click **System and Maintenance**.

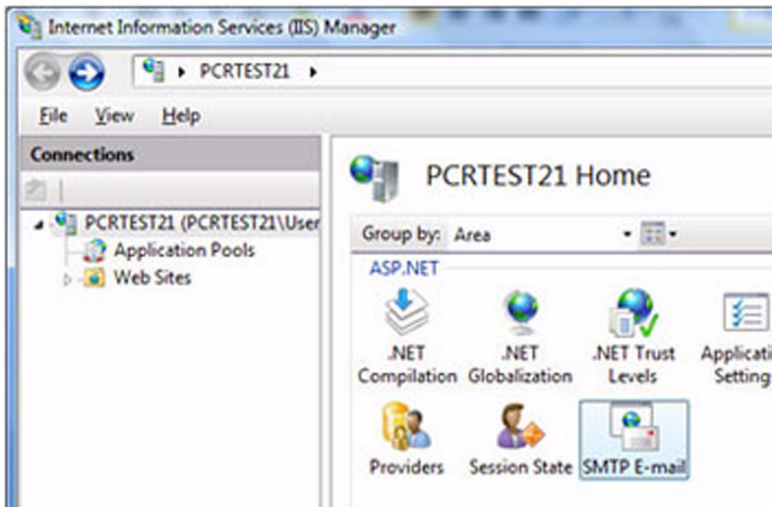


2. Scroll through the menu to find Administrative Tools and click on it.

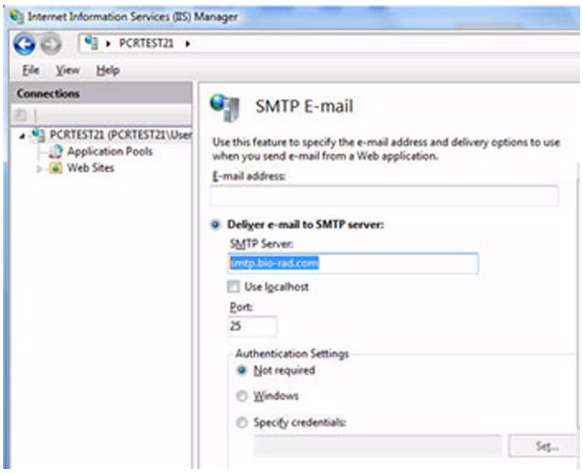
3. Double-click **IIS Manager** to open it.



4. Select **SMTP E-mail**.



5. Type the server name in the SMTP Server box, and click **Apply** to accept the change. In this example, the SMTP Server is smtp.Bio-Rad.com:



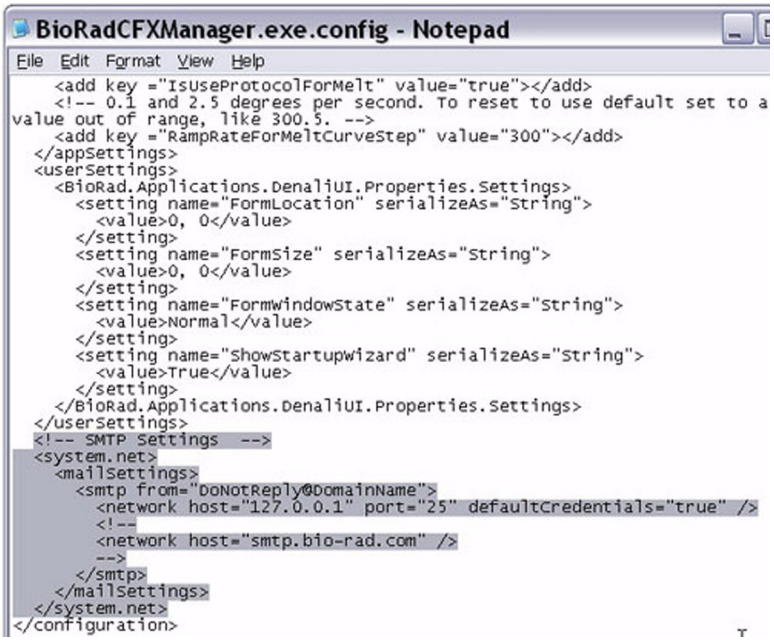
NOTE: Due to security settings, entering the server name might not be sufficient. If this happens, edit the BioRadCFXManager.exe.config file (next section).

EDIT THE BIORADCFXMANAGER.EXE.CONFIG FILE FOR VISTA

Follow these steps to edit the BioRadCFXManager.exe.config file:

1. Select the BioRadCFXManager.exe.config file in the file path: C:\Program Files\Bio-Rad\CFXSEC1000\.
2. Open the file in the Notepad program.

3. Locate the highlighted section (shown in image) and replace **127.0.0.1** with the actual name of your server. Keep the quotes around the server name.



4. Save the changes, and close the file.

Files Preferences Tab

View the file preferences by opening the User Preferences (Figure 118 on page 168) window using one of these methods and clicking the **Files** tab:

- Click the **User Preferences** button in the main software window toolbar
- Select **User > User Preferences** in the main software window menu bar

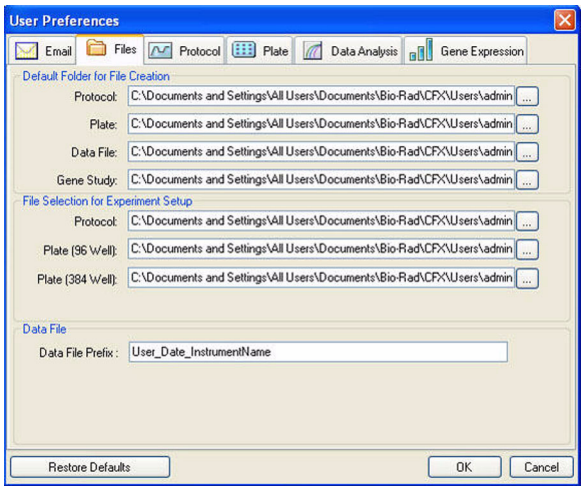


Figure 118. Files Preferences tab in the User Preferences window.

Select the Files tab in the User Preferences window to list the default location for opening a file and saving a file. Notice that you can list the location for each type of file:

- **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 creates a file name that starts with the User (user name of the user who is logged on to software), Date (file creation date), and Instrument Name (instrument serial number, or instrument name)

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

Protocol Preferences Tab

View the protocol preferences by opening the User Preferences window (Figure 119 on page 169) using one of these methods and clicking the **Protocol** tab:

- Click the **User Preferences** button in the main software window toolbar

  Select **User > User Preferences** in the main software window menu bar

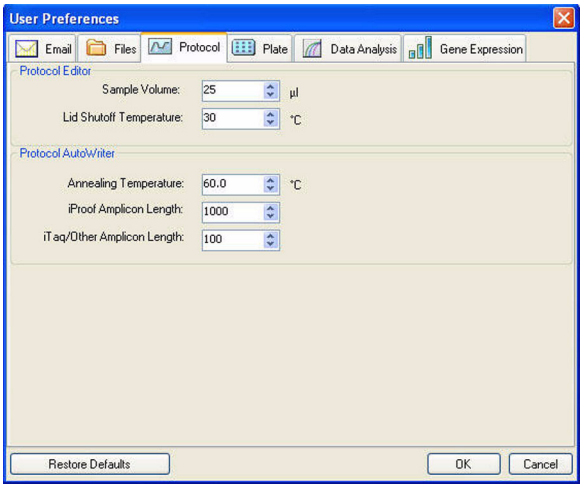


Figure 119. Protocol Preferences tab in the User Preferences window.

Select the **Protocol** tab in the User Preferences window 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 of the plate (in microliters), and select a Lid Shutoff Temperature to set the temperature at which the lid heater will turn off during a run
- **AutoWriter.** Selects default settings that appears in the Protocol AutoWriter. Enter the default Annealing Temperature. Enter a default amplicon length for experiments that use iProof polymerase, and experiments that use iTaq or Other polymerases

Plate Preferences Tab

View the plate preferences by opening the User Preferences window using one of these methods and clicking the **Plate** tab:

- Click the **User Preferences** button in the main software window toolbar
- Select **User > User Preferences** in the main software window menu bar

Select the **Plate** tab in the User Preferences window (Figure 120) to specify the following default settings for a new Plate file in the Plate Editor window:

- **Plate Type.** Select the default well type in a plate from the list
- **Plate Size.** Select the default plate size from the list
- **Units.** Select the units used to describe the concentration of the content of 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. Select All Channels to collect data from every channel
- **Fluorophores.** Click check boxes to select the default fluorophores for every well in a plate. Each plate must include at least one fluorophore. This list of fluorophores appears in the Select Fluorophores windows
- **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. This list of names appears in the list of Targets and Samples tabs in the Experiment Settings window

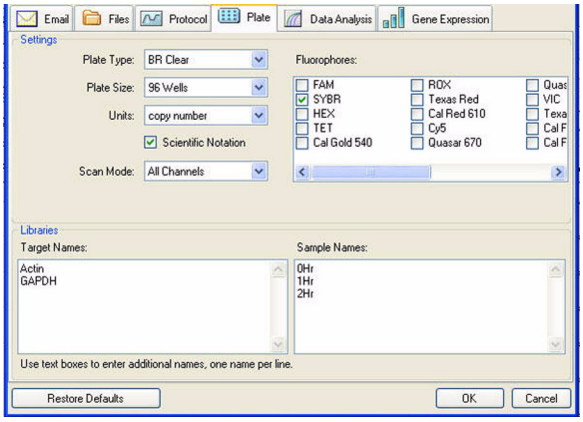


Figure 120. Plate tab in the User Preferences window.

Data Analysis Preferences Tab

View the data analysis preferences by opening the User Preferences (Figure 121 on page 171) window using one of these methods and clicking the **Data Analysis** tab:

- Click the **User Preferences** button in the main software window toolbar

  Select **User > User Preferences** in the main software window menu bar

  NOTE: The software applies the user preferences for data analysis at the end of the run.

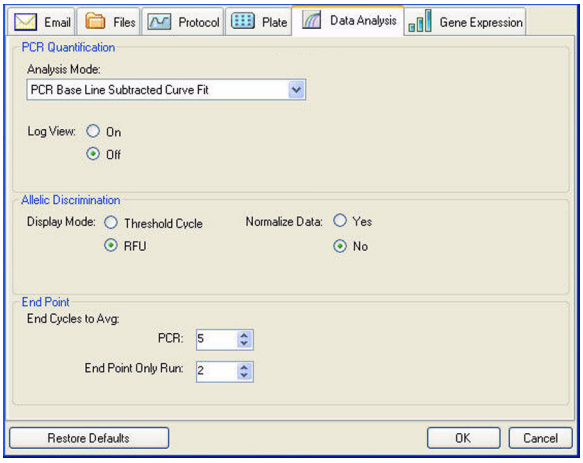


Figure 121. Data Analysis tab in the User Preferences window.

Select the **Data Analysis** Tab in the User Preferences window to change the default settings for new Quantitation, Allelic Discrimination, and End Point data that appear in the Data Analysis window.

For the quantification data, select the following settings:

- **Analysis Mode.** Select the default baselining method for the analysis mode. Choose Baseline Subtracted Curve Fit, Not Baseline Subtracted, or Baseline Subtracted
- **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 un-normalized 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 Preferences Tab

View the gene expression preferences by opening the User Preferences window (Figure 122 on page 172) using one of these methods and clicking the **Gene Expression** tab:

- Click the **User Preferences** button in the main software window toolbar
- Select **User > User Preferences** in the main software window menu bar

  NOTE: The software applies the user preferences for gene expression during data analysis, at the end of the run.

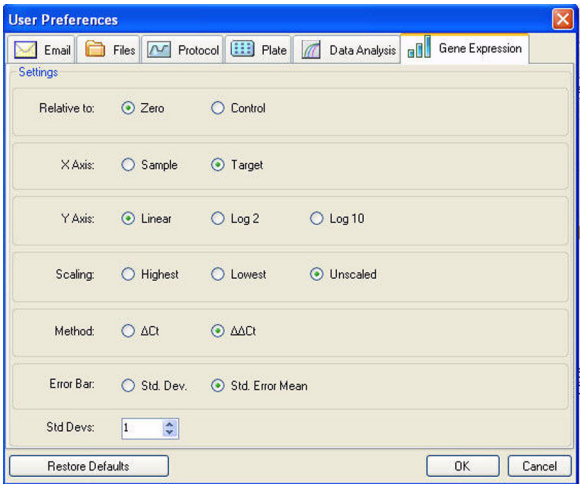


Figure 122. Gene Expression tab in the User Preferences window.

Select the Gene Expression Tab in the User Preferences window to specify the default settings for a new Gene Expression data file:

- **Relative to.** Select a control or zero. 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 Relative to zero to instruct the software to ignore the controls, which is the default selection when no control sample is assigned in the Experiment Settings window
- **X-Axis.** Graph the Target or the Sample 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 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

  TIP: Click the **Restore Default** button to restore all settings to the default settings shown in this image. Then click **OK** to save the settings, and close the window.

  NOTE: The software applies the user preferences for gene expression during data analysis, at the end of the run.

Log On and Change Password

CFX Manager software manages multiple users through two software dialog boxes:

- **Login dialog box.** Open this dialog box to log on to the software (Figure 124 on page 174)

  NOTE: When you start the software, it automatically opens the Login dialog box if there are two or more software users listed in the User Administration window. If there is only one user, then the Login dialog box does not open unless you select User > Select User.

- **Change Password dialog box.** Open this dialog box to change your password (Figure 125 on page 174)

  NOTE: Depending on how the software is set up, you might not need a password.

To view the current software user, look at the top of the main software window (Figure 123). When a user logs on to the software, the main software window lists the software name followed by the user name. In this example the user name is Grant:

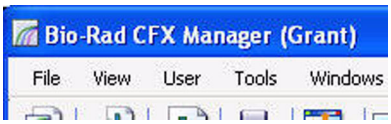


Figure 123. User name displays on the top of the main software window.

Log On to the Software or Switch Software User

To log on or to switch to another software user, open the Login dialog box (Figure 124 on page 174) by one of these methods:

- Click the Bio-Rad CFX Manager icon on the computer desktop.
- If the software is running, select **User > Select User**.

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

1. Open the Login dialog box, if it is not already open. This dialog box opens automatically when the software starts. If the software is already running, then open the dialog box 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 user name is "admin" (administrator).
3. Type a password in the **Password** box.

4. Click **OK** to open the software, and close the Login dialog box.

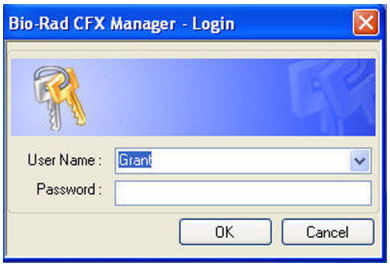


Figure 124. Login dialog box.

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

NOTE: When you switch software users by logging in as a different user, then the previous software user will be logged off the software.

Change a Password

To change a password, open the Change Password dialog box (Figure 125).

Figure 125. Change your password in the Change Password window.

Change a password by following these steps:

1. Select **User > Change Password** to open the Change Password dialog box.
2. Enter the old password in the Old Password box.
3. Enter the new password in the New Password box, and enter it again in the Confirm New Password box.
4. Click **OK** to confirm the change.

User Administration

Open the User Administration window in the main software window:

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

If you log on as an Administrator, open the User Administration window to manage users and manage 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 126). In this example, the Guest user is given the added right to save files.:

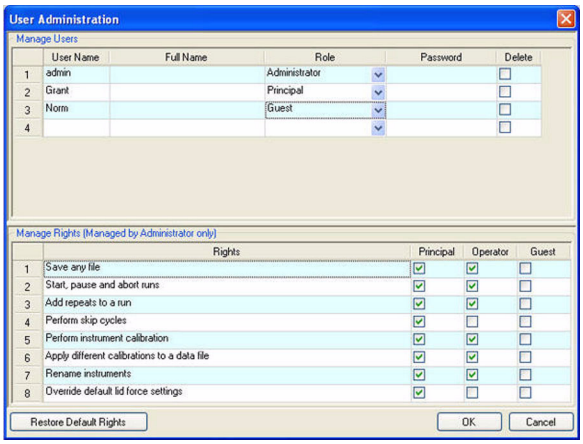


Figure 126. User Administration window with three users.

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 role 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.

Assign 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.

15 Resources

Bio-Rad Laboratories provides many resources for researchers. These resources contain useful information about running PCR or real-time PCR experiments using CFX Manager software and 1000-Series instruments:

- **Frequently asked questions (page 177).** Lists FAQs about data analysis
- **Tips and tricks (page 179).** Lists useful information about using the software
- **References (page 178).** Lists literature about data analysis
- **Glossary.** Lists terms specifically used in the software and on the instruments
- **Troubleshooting options (page 181).** Lists options for troubleshooting problems with the software and instruments
- **Software Help tools (page 184).** Lists resources available in the software to find information about running the software
- **Instruments, parts and accessories (page 184).** Lists catalog numbers and descriptions, including instruments, reaction modules, reagents and plastic consumables for running PCR and real-time PCR experiments.

Frequently Asked Questions

The following list is a series of questions (**Q**) and answers (**A**) about real-time PCR data 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 when a control is selected vary from that outlined in the geNorm web site?

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 for that example.

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 as 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 outline on the geNorm web site and will give 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 the **View/Edit Plate** button 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 (xef) to add names to the Targets or Samples tabs, where you can enter also enter the full names and remove names from the lists. Alternatively, add long lists of names to the Libraries for target and sample names in the User Preferences Plate tab (page 170). These names appear on the axis in various chart views, including gene expression.

Q: How do I determine 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.

References

The CFX96 System instruction manual refers to the references in this list:

Breslauer KJ, Frank R, Blöcker H, and Marky LA. Predicting DNA duplex stability from the base sequence, Proc. Nat. Acad. Sci., 83,3746-50 (1986)

Livak JL, Marmaro J and Todd JA. Towards fully automated genome-wide polymorphism screening, Nature Genetics, 9, 341-342 (1995)

Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Research, 29(9), 2002-2007 (2001)

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, and Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes, Genome Biology, 3(7), 1-12 (2002)

Tips and Tricks

CFX Manager Software functions the resources listed in these tips and tricks listed in page 179.

Table 44. Tips and tricks for using CFX Manager software

Software window	Tips and tricks
General	Open any protocol, plate, data, or Gene Study file by dragging it from a Windows folder to an open software window
	Print or export the information shown in many windows by right-clicking a chart, spreadsheet, or well selector
	Export data in multiple formats, including spreadsheets, images, and text
	Change the order of open windows in the main software window by selecting options from the Windows menu in the software menu bar
	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 on to the software
	Add data files to a Gene Study by dragging from a Windows folder to an open Gene Study window
	Open multiple Data Analysis and Gene Study files at the same time
	Click the **Settings** or **Tools** menus to find advanced functions in every software window
Instruments	Rename any instrument listed in the Detected Instrument pane by right-clicking on the instrument icon, selecting **Rename** in the right-click menu in the Instrument Properties window. Then enter a name in the Rename box and click the **Rename** button
Protocol Editor	To specify the location of the inserted step in the **Protocol Editor**, select **Before** or **After** in the Insert Step box at the top of the tab.
	To add or delete protocol files from the Express Load menu, add or delete the protocol 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.

Table 44. Tips and tricks for using CFX Manager software (continued)

Software window	Tips and tricks
Plate Editor	To view all the information loaded into one well in a plate, double-click the well to open the Well Info window
	To add or delete plates from the Express Load menu, add or delete plate 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.
	Enter the minimum required information in the plate before starting the run. Then enter the remaining contents of the wells during or after the run
	In order to compare data in the Data Analysis or Gene Study window, the target names and sample names must match within and between plates. Each name must contain the same punctuation and spacing
	To temporarily add a new sample name or target name to the pull-down menu in the current plate, type a new name in the pull-down menu and press the **Enter** key on your keyboard. To permanently add sample names to all plates, add them to the Library in the User Preferences Plate tab, or enter them in the Experiment Settings window
Data Analysis	Right-click any graph, chart, or view to change viewing and data analysis options
	Highlight all the data for one fluorophore in the Data Analysis window by holding the mouse over the fluorophore name
	Select wells in the well selector to focus on subsets of the data (results)
	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 drop-down menu in the toolbar
	Exclude wells from analysis temporarily in the Plate Editor window. For example, exclude wells that contain data that might skew the analysis, and view the changes immediately
	Clear the wells to delete the contents of the wells in the Plate Editor window. For example, clear the wells that contain contaminated or inaccurate well contents. Deleting the content does not remove the fluorescence data that were collected during a run

Troubleshooting Options

If you have a specific problem with the software or an instrument, refer to these sources:

- For hardware problems read the installation and troubleshooting sections in the instruction manual for that instrument. To get another copy of the manual, see “Bio-Rad Resources” on page 2.
- For software problems read further in this troubleshooting section

CFX Manager software includes the following sections for tips on troubleshooting options:

- Communication errors (next section, page 181)
- Installing driver manually (page 182)
- Review Run and Application logs (page 182)
- Options during and after a Power failure (page 182)

Communication Errors

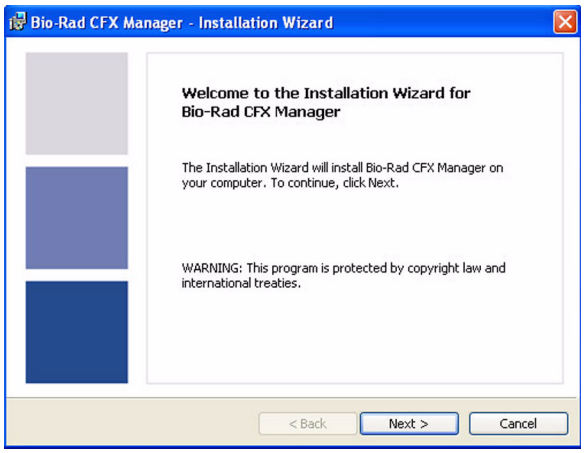
Typically, software and instrument communication problems can be resolved by using the following options:

- Check that your computer has sufficient RAM and free hard drive space. The minimum RAM is 1 GB, and the minimum hard drive space is 10 GB
- Restart your computer. Be sure to save any work in progress before restarting

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**.

Installing the Drivers Manually

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

1. Insert the software CD. If the CD is not available, then locate the drivers folder in the file path C:\Program Files\Bio-Rad\Drivers on your hard drive.
2. Click the **Drivers** button software installation screen (Figure 12).
3. Click the **BaseUnit** folder to open it.

Figure 127. BaseUnit folder.

4. For Windows XP computers, double-click **BioRadC1000DriverInstall.exe** to launch the installation window. For Windows Vista, right-click **BioRadC1000DriverInstall.exe** and select **Run as Admin** to launch the installation window.

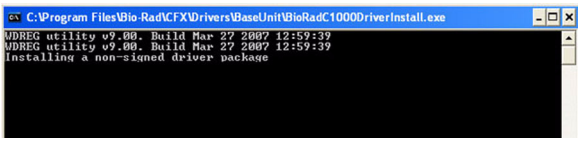


Figure 128. Driver installation window.

When installation is complete, the installation window closes.

NOTE: If the drivers do not install with manual installation, please contact the technical support team in your local Bio-Rad office (page 2).

Run and Application Logs

Before starting a new run, each 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.

Power Failure Options

In a power failure, the instrument and computer will shut down. If the power failure is short, then the instrument will resume running a protocol, but the Application log will note the power failure. Depending on the length of time that the power is off and the computer settings, the instrument and software attempt to continue running depending on the protocol step:

- If the protocol is in a step with no plate read, then the protocol continues running as soon as the instrument gets power again
- If the protocol is in a step with a plate read, then the instrument waits for the software to restart and resume communication to collect the data. In this situation, the protocol only continues if the software is not shut down by the computer. When the computer and software start up again, then the protocol continues

If you experience an extended power failure, you might want to open a motorized lid on a reaction module to remove your samples. Follow these steps to manually open the module by removing the locking plate:

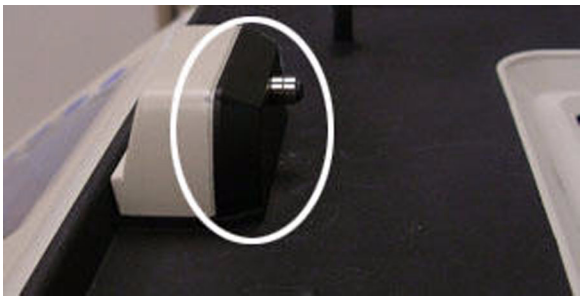
1. Remove the reaction module from the C1000 chassis. Refer to the instrument instruction manual for instructions about removing a module. For more information about finding the manual, see “Bio-Rad Resources” on page 2.

2. Position the module on the front of a desk, so that the front of the module extends 2 inches over the edge of the desk as shown in this image:

3. With a screw driver, remove the two large screws from the under the front edge of the reaction module (below the button for opening the lid). Do not remove the two small screws that are located at the front edge of the module. You should hear the locking latch release from inside the module. This image shows the two large screws:

4. Push the reaction module lid open. Notice that the latch (dark plastic) is no longer attached. Remove your samples from the block.

5. Reassemble the reaction module with the lid open by replacing the locking latch and securing it with the large screws. This image shows the locking latch in place:



Software Help Tools

This CFX Manager software provides Help with 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 the **F1** key 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**
- Click the www.bio-rad.com link at the bottom of each Help page to open the Bio-Rad Laboratories web site for links to information and resources

Open a PDF copy of instruction manuals on the software installation CD, or download a copy of the manual from the Bio-Rad Laboratories web site (see page 2)

Instruments, Parts and Accessories

Bio-Rad Laboratories offers the following instruments, accessories, and plastics. The 1000-series includes the instruments, software, and accessories listed in Table 45.

Table 45. 1000-series instruments, reaction modules, and accessories catalog numbers.

Catalog Number	Description
Instruments and Reaction Modules	
184-1000	C1000 Thermal Cycler Chassis
184-2000	S1000 Thermal Cycler Chassis
184-0048	Dual 48/48 Fast Reaction Module
184-0096	96-Well Fast Reaction Module
184-0384	384-Well Reaction Module
184-5096	CFX96 Optical Reaction Module
185-5096	CFX96 Real-Time PCR Detection System
185-1096	C1000 Thermal Cycler With 96-Well Fast Reaction Module
185-1048	C1000 Thermal Cycler With Dual 48/48 Fast Reaction Module
185-1384	C1000 Thermal Cycler With 384-Well Reaction Module
185-2096	S1000 Thermal Cycler With 96-Well Fast Reaction Module
185-2048	S1000 Thermal Cycler With Dual 48/48 Fast Reaction Module
185-2384	S1000 Thermal Cycler With 384-Well Reaction Module
Software and Accessories	
184-5000	CFX Manager Software
184-5001	CFX Manager Software, Security Edition, 1 user license
184-5005	CFX Manager Software, Security Edition, 5 user licenses
184-5010	CFX Manager Software, Security Edition, 10 user licenses
184-8000	USB Cable *

*To prevent data loss, use a sufficiently shielded USB cable (catalog # 184-8000) when connecting instruments to the computer or to another instrument.

Table 46 lists the recommended reagents and plastics for PCR and real-time PCR.

NOTE: If you do not completely fill the block with tubes or a full-sized microplate, always balance the block (page 15) with empty tube strips and cut microplates before starting the run.

Table 46. Recommended plastics for 1000-series instruments

Catalog Number	Description
Reagents for Reverse Transcription	
170-8890	iScript cDNA Synthesis Kit, 25 reactions
170-8896	iScript Select cDNA Synthesis Kit, 25 reactions
170-8892	iScript One-Step RT-PCR Kit with SYBR Green, 50 reactions
170-8894	iScript One-Step RT-PCR Kit for Probes, 50 reactions
Real-Time PCR Supermixes	
172-5848	iQ Multiplex Powermix, 50 reactions
170-8860	iQ Supermix, 100 reactions
170-8880	iQ SYBR Green Supermix, 100 reactions
Amplification Plastics	
HSP-9601	Hard-Shell® Thin-Wall 96-Well Skirted PCR Plates, clear wells, white shell, 50
HSP-9655	Hard-Shell Thin-Wall 96-Well Skirted PCR Plates, white wells, white shell, 50
HSP-3801	Hard-Shell Thin-Wall 384-Well Skirted PCR Plates, clear wells, clear shell, 50
HSP-3865	Hard-Shell Thin-Wall 384-Well Skirted PCR Plates, white wells, black shell, 50
HSP-3901	Hard-Shell Thin-Wall 384-Well Skirted PCR Plates, clear wells, clear shell, 50
TLS-0801	Low-Profile 0.2 ml 8-Tube Strips Without Caps, clear, 120
TLS-0851	Low-Profile 0.2 ml 8-Tube Strips Without Caps, white, 120
TCS-0803	Optical Flat 8-Cap Strips, for 0.2 ml tubes and plates, ultraclear, 120
MLL-9601	Multiplate™ Low-Profile 96-Well Unskirted PCR Plates, clear, 25
TLS-0801	Low-Profile 0.2 ml 8-Tube Strips Without Caps, clear, 120
TLS-0851	Low-Profile 0.2 ml 8-Tube Strips Without Caps, white, 120
TCS-0803	Optical Flat 8-Cap Strips, for 0.2 ml tubes and plates, ultraclear, 120
MLL-9651	Multiplate Low-Profile 96-Well Unskirted PCR Plates, white, 25
MSB-1001	Microseal® ‘B’ Adhesive Seals, optically clear, 100

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