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# **QX200 Droplet Reader and QX Manager Software Regulatory Edition**

**User Guide**

**Version 1.2**



**BIO-RAD**



**QX200 Droplet Reader  
and  
QX Manager Software  
Regulatory Edition**

**User Guide**

**Version 1.2**



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# Chapter 1 Safety and Regulatory Compliance

This section cites regulatory requirements for laboratory and electrical equipment, as well as requirements for working with chemicals and hazardous substances, and also explains safety precautions and recommendations.

## Regulatory Compliance

The QX200 Droplet Reader has been tested and found to be in compliance with all applicable requirements of the following safety and electromagnetic standards:

- IEC 61010-1:2010 (3rd ed.), EN61010-1:2010 (3rd ed.). Electrical equipment for measurement, control, and laboratory use - Part 1: General requirements
- IEC 61010-2-081:2015, EN 61010-2-081:2015. 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)
- IEC 61010-2-101:2015 (2nd ed.). Safety requirements for electrical equipment for measurement, control, and laboratory use. Particular requirements for in vitro diagnostic (IVD) medical equipment
- IEC 61326-1:2012 (Class A), EN 61326-1:2013 (Class A). Electrical equipment for measurement, control, and laboratory use. EMC requirements, Part 1: General requirements
- CAN/CSA 22.2 No 61010-1-04, Safety requirements for electrical equipment for measurement, control, and laboratory use, Part 1: General requirements
- Restriction of hazardous substances (ROHS) directive (European Union)
- Registration, evaluation, authorization and restriction of chemicals (REACH). European Chemicals Agency (ECHA) June 1, 2007
- Waste electrical and electronic equipment (WEEE) directive

This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of FCC rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment.



The CE symbol indicates that the manufacturer ensures the product conforms with the essential requirements of the applicable EN directives.



The CSA symbol indicates that a project has been tested to Canadian and U.S. standards, and it meets the requirements of those applicable standards.



The Waste Electrical and Electronic Equipment (WEEE) Directive symbol indicates that when the end-user wishes to discard this product, it must be sent to separate collection facilities for recovery and recycling.

## Safety Warning Labels

Warning labels posted on the instrument and in this manual warn you about sources of injury or harm. [Table 1](#) defines each safety warning label.

**Table 1. Meaning of safety warning labels**

Icon	Meaning
	<b>Warning about risk of harm to body or equipment</b> Operating the QX200 Droplet Reader 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.
	<b>Warning about handling biohazardous materials</b> When handling biohazardous samples, adhere to the recommended precautions and guidelines and comply with any local guidelines specific to your laboratory and location.

## Safe Use Specifications

For safe operation of the QX200 Droplet Reader, Bio-Rad Laboratories, Inc. strongly recommends that you comply with instructions listed in this section and in [Instrument Maintenance on page 185](#).

This instrument is intended for laboratory use only. Bio-Rad is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended, or by modifications to the instrument not performed by Bio-Rad or an authorized agent.

- This instrument is for use only by trained personnel.
- Use only the power cord supplied with the instrument, and the plug adapter corresponding to the electrical outlets in your region.
- Position the instrument on a solid, stable surface, with adequate room at the back and on each side so that users can easily reach the power cord and USB port.
- This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the provided instructional documentation, may cause harmful interference to radio communications. Operation of the systems in a residential area is likely to cause harmful interference, in which case users will be required to correct the interference at their own expense.

**Note:** Bio-Rad recommends maintaining a backup power source in case of power outages. A universal power supply (UPS) can protect from brown outs and power surges, while a regular backup generator does not.

[Table 2](#) lists the safe use specifications for Bio-Rad's QX200 Droplet Reader. The supplied shielded cables must be used with these instruments to ensure compliance with the Class A FCC limits.

**Table 2. Conditions for safe use**

Usage aspect	Conditions for safe use
Rated input power	<p>Input: 100–240 V, 50–60 Hz, 1A</p> <p>Voltage fluctuations shall not exceed +10% of ratings for the included external power supply.</p> <p>Use only the power cord supplied with the equipment.</p> <p>Fuse: 10A 250V SLOW BLOW</p>
Pollution degree/environment	2 (indoor use only)
Usage temperature	18–30°C

Usage aspect	Conditions for safe use
Relative humidity	85% maximum (non-condensing)
Altitude	0 to 6,560 ft (0 to 2,000 meters) above sea level
Installation category	II (external power supply plugs into a standard AC receptacle)
Ventilation requirements	<p>The following distances should be unobstructed for proper ventilation:</p> <ul style="list-style-type: none"><li>■ 5 in (12 cm) on the left and right sides of the instrument</li><li>■ 10 in (25 cm) behind the machine</li></ul>
Benchtop requirements	<p>The following requirements apply to the benchtop or table on which the instrument will be installed.</p> <p>The benchtop must be 76" W x 30" D (193cm x 76cm) single benchtop rated for more than 250 lbs (114 kg) load</p> <p><b>Important:</b> Do not place instrument over multiple benchtop surfaces.</p> <p>34" – 40" (86cm – 102cm); actual height is at the discretion of user for an ergonomic operation of the equipment</p>

## Hazards

The QX200 Droplet Reader is designed to operate safely when used in the manner prescribed by the manufacturer. If the instrument or any of its associated components is used in a manner not specified by the manufacturer, the inherent protection provided by the instrument may be impaired.

Bio-Rad Laboratories, Inc. is not liable for any injury or damage caused by the use of this equipment in any unspecified manner, or by modifications to the instrument not performed by Bio-Rad or an authorized agent. Only trained Bio-Rad personnel should perform service on the QX200 Droplet Reader.

## Biohazards

The QX200 Droplet Reader is a laboratory product. However, if biohazardous samples are present, adhere to the following guidelines and comply with any local guidelines specific to your laboratory and location.

**Note:** No biohazardous substances are expended during normal operations of this instrument.

## General Precautions

- Always wear laboratory coat, laboratory gloves, and safety glasses with side shields or goggles.
- Keep your hands away from your mouth, nose, and eyes.
- Completely protect any cut or abrasion before working with potentially infectious materials.
- Wash your hands thoroughly with soap and water after working with any potentially infectious material before leaving the laboratory.
- Store all infectious or potentially infectious material in unbreakable leak-proof containers.
- Before leaving the laboratory, remove protective clothing.
- Do not use a gloved hand to write, answer the telephone, turn on a light switch, or touch anything that other people may touch without gloves.
- Change gloves frequently. Remove gloves immediately when they are visibly contaminated.
- Do not expose materials that cannot be properly decontaminated to potentially infectious material.
- Upon completion of an operation involving biohazardous material, decontaminate the work area with an appropriate disinfectant (for example, a 1:10 dilution of household bleach).

## Surface Decontamination



**WARNING!** To prevent electrical shock, always turn off and unplug the instrument prior to performing decontamination procedures.

**Important:** Do not use abrasive or corrosive detergents or strong alkaline solutions. These agents can scratch surfaces and damage the system.

The following areas can be cleaned with 10% bleach solution:

- Outer area and chassis
- Inner plate holders
- Droplet reading surfaces

To prepare and apply the disinfectant, refer to the instructions provided by the product manufacturer. For more information on surface cleaning, see [Appendix C, Instrument Maintenance](#). For questions regarding the use of other cleaning agents, contact Bio-Rad Technical Support.

**Important:** Do not clean the handler Y-axis rail when the front door is open. This is a lubricated surface, and failures will occur if the lubrication is removed.

## Disposal of Biohazardous Material

Dispose of the following potentially contaminated materials in accordance with laboratory local, regional, and national regulations:

- Clinical samples
- Reagents
- Used reaction vessels or other consumables that may be contaminated

## Chemical Hazards

The QX200 Droplet Reader contains no potentially hazardous chemical materials.

## Explosive or Flammability Hazards

The QX200 Droplet Reader poses no uncommon hazard related to flammability or explosion when used in a proper manner as specified by Bio-Rad.

## Electrical Hazards

The QX200 Droplet Reader poses no uncommon electrical hazard to operators if installed and operated properly without physical modification and connected to a power source of proper specification.

## Decommissioning and Disposal

The QX200 Droplet Reader contains electrical materials that should be disposed of as unsorted waste and must be collected separately, according to European Union Directive 2012/19/EU on waste electrical and electronic equipment — WEEE Directive. The purpose of decommissioning is to make sure that the equipment is electrically and environmentally safe for disposal. Before disposal, contact your local Bio-Rad representative for country-specific instructions.

## Transport

Before moving or shipping the QX200 Droplet Reader, decontamination procedures must be performed. Always move or ship the instrument with the supplied packaging materials, which will protect the instrument from damage. If appropriate containers cannot be found, contact your local Bio-Rad office.

## Warranty

The QX200 Droplet Reader and its associated accessories are covered by a standard Bio-Rad warranty. Contact your local Bio-Rad office for the details of the warranty.

Use of unapproved supermixes or additives may harm the instrument, and voids the warranty.

Alteration of this instrument voids the warranty and safety certification, as it creates a potential safety hazard.

This instrument is intended for laboratory use only. Bio-Rad Laboratories is not responsible for any injury or damage caused by use of this instrument for purposes other than those for which it is intended, or by modifications of the instrument not performed by Bio-Rad Laboratories or an authorized agent. Follow the safety specifications listed here and throughout this manual. Use only the power cord supplied with the instrument, using only the plug adaptor that corresponds to the electrical outlets in your region.



## Chapter 2 Introduction to Droplet Digital PCR

Droplet digital polymerase chain reaction (ddPCR) is a digital PCR method based on water-oil emulsion droplet technology. ddPCR uses a combination of microfluidics and proprietary surfactant chemistries to divide each sample into water-in-oil droplets. The technology uses reagents and workflows similar to those used for most standard TaqMan probe-based assays, and provides absolute quantification of nucleic acid target sequences by counting nucleic acid molecules encapsulated in discrete, volumetrically defined water-in-oil droplet partitions.

ddPCR is highly effective in the following areas:

- **Absolute quantification** — ddPCR provides a concentration of target DNA copies per input sample without the need for running standard curves, making this technique ideal for target DNA measurements, viral load analysis, and microbial quantification.
- **Genomic alterations such as gene copy number variation (CNV)** — CNVs result in too few or too many dosage-sensitive genes responsible for phenotypic variability, complex behavioral traits, and disease. ddPCR enables measurement of 1.2x differences in gene copy number.
- **Detection of rare sequences** — researchers must amplify single genes in a complex sample, such as a few tumor cells in a wild-type background. ddPCR is sensitive enough to detect rare mutations or sequences.
- **Gene expression and microRNA analysis** — ddPCR provides stand-alone absolute quantification of expression levels, especially low-abundance microRNAs, with sensitivity and precision.
- **Next-generation sequencing (NGS)** — ddPCR quantifies NGS sample library preparations to increase sequencing accuracy and reduce run repeats. Validate sequencing results such as single nucleotide polymorphisms or copy number variations with absolute quantification.
- **Single cell analysis** — the high degree (10-fold to 100-fold) of cell-cell variation in gene expression and genomic content among homogeneous post-mitotic, progenitor, and stem cell populations drives a need for analysis from single cells. ddPCR enables low copy number quantification and gene expression of individual cells.
- **Genome edit detection** — ddPCR enables fast, precise, and cost-effective assessment of HDR (Homology directed repair) and NHEJ (non-homologous end joining) generated by CRISPR-Cas9 or other genome editing tools.

ddPCR has the following benefits for nucleic acid quantification:

- **Unparalleled precision** — The massive sample partitioning afforded by ddPCR enables small fold differences in target DNA sequence between samples to be reliably measured.
- **Increased signal-to-noise ratio** — High-copy templates and background are diluted, effectively enriching template concentration in target-positive partitions. This allows for the sensitive detection of rare targets and enables a  $\pm 10\%$  precision in quantification.
- **Removal of PCR efficiency bias** — Error rates are reduced by removing the amplification efficiency reliance of qPCR, enabling accurate quantification of targets to near zero.
- **Simplified quantification** — There is no requirement for a standard curve for absolute quantification.

## ddPCR Workflow

The ddPCR process adheres to the following workflow:

- You prepare your samples for PCR by combining DNA or RNA with primers, probes dye, and Bio-Rad ddPCR supermix.
- A droplet generator fractionates a sample into approximately 20,000 uniform nanoliter-sized droplets, with target and background DNA distributed randomly into the droplets during the partitioning process.
- Following droplet generation, the droplets are transferred to a standard 96-well PCR plate and amplified through a thermal cycler, which performs PCR amplification of the nucleic acid target in each individual droplet.
- A droplet reader reads each droplet to determine the fraction of positive droplets in the original sample. Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets.

# Chapter 2 QX200 Droplet Digital PCR System

## QX200 Droplet Digital PCR System

As part of its QX200 Droplet Digital PCR System, Bio-Rad provides the following instruments:

- QX200 Droplet Generator or the Automated Droplet Generator to generate the droplets from your samples
- QX200 Droplet Reader to read the generated and thermal-cycled droplets

**QX200 Droplet Generator**



**Automated Droplet Generator**



**QX200 Droplet Reader**



**Important:** Your QX200 Droplet Reader performs the post-PCR droplet reading function only. A droplet generator and a thermal cycler are required to perform a full ddPCR cycle. You can purchase additional instruments, including a thermal cycler, as well as associated accessories and consumables, from Bio-Rad. For information, see [Appendix G, Ordering Information](#).

**Important:** Your QX Droplet Generator performs the initial droplet generation function only. A thermal cycler and a droplet reader are required to perform a full ddPCR cycle. You can purchase additional instruments, as well as associated accessories and consumables, from Bio-Rad. For information, see [Appendix G, Ordering Information](#).

Using the ddPCR system, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include:

■ Copy number variation	■ Absolute quantification
■ Rare sequence detection	■ Rare mutant detection
■ Gene expression analysis	■ mRNA analysis
■ Next generation sequencing (NGS) library quantification	■ NGS sample preparation
■ Viral load determination	■ GMO detection
■ Single cell gene expression analysis	

This instrument guide describes the use of the QX200 Droplet Generator and preparation for PCR. For information on the QX200 Droplet Reader, see the QX200 Droplet Reader Instruction Manual (Item #10031906).

## Finding Out More

Click the Help tab (  ) on the left, and then click the Bio-Rad Website link to access links to technical notes, manuals, videos, product information, and technical support. The website also provides many technical resources on a wide variety of methods and applications related to PCR, droplet digital PCR, and gene expression.

## Chapter 3 About the QX200 Droplet Reader

Following the droplet generation and thermal cycling processes, the QX200 Droplet Reader collects binary data for analysis as it

- Singulates the droplets in each sample
- Streams the droplets in single-file past a two-color detector to determine which droplets contain one or more target molecules (positive reading) and which do not (negative reading)

Use the information in this chapter to correctly assemble your plate, connect the instrument, start up the instrument, and insert the plate for processing. Information on components and specifications is also included.



### LEGEND

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- 1 Button to open and close lid
- 2 Status indicator lights (for information, see [Status Indicator Lights on page 25](#))
- 3 On the back of the instrument, power and USB outlets
- 4 Oil and waste compartment (for information, see [Fluid Maintenance on page 185](#))

## Included Items and Additional Components

This section identifies the functional items included with your droplet reader purchase, as well as additional components and consumables. For information on items you may need for a full ddPCR cycle, see [Appendix G, Ordering Information](#).

[Table 3](#) explains the components that ship with the QX200 Droplet Reader.

**Table 3. QX200 Droplet Readeror Included Components**

Component	Description	Catalog Number
QX200 Droplet Reader	Droplet reading instrument, part of QX200 ddPCR system	1864003
USB cable and power cord	USB cable that connects the computer to the instrument and power cord that connects the instrument to the power source  <b>Note:</b> To obtain cables or power cords, contact Bio-Rad technical support.	Included
Droplet reader plate holders (2)	Positions and secures the 96-well plate in the droplet reader plate compartment	12006834

To ensure you have everything you need to complete runs on the QX200 Droplet Reader and analyze your results, [Table 4](#) identifies additional components and accessories that you can purchase separately.

**Table 4. Additional Accessories and Consumables**

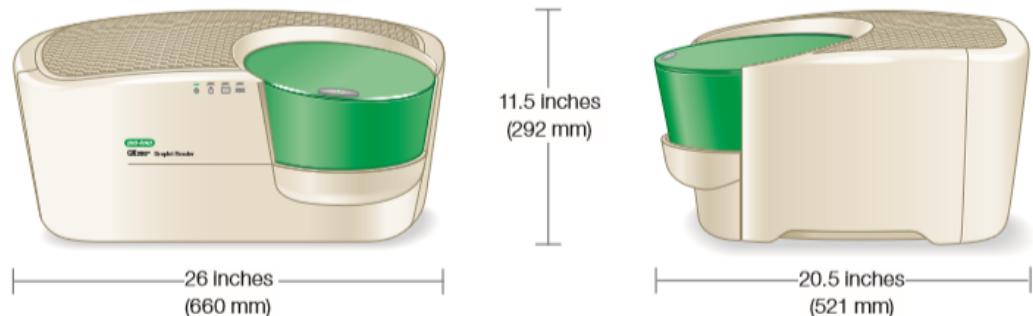
Item	Description	Catalog Number
Computer	Computer that connects to the QX200 Droplet Reader for data collection and analysis	17006483
Software purchase	Bio-Rad QX Manager Software software Regulatory Edition	12012172
96-well PCR plates	ddPCR 96-well plates	12001925
Rainin pipets	20 $\mu$ l for sample loading 50 $\mu$ l for droplet transfer 8-channel, 200 $\mu$ l for oil	L-20, L8-20 L-50, L8-50 L8-200

**Table 4. Additional Accessories and Consumables, continued**

Item	Description	Catalog Number
Rainin pipet tips	Filtered	GP-L10F GP-L200F
Foil plate seals	Pierceable foil plate seals	1814040
Plate sealer	PX1 PCR plate sealer	1814000
Droplet reader oil	ddPCR droplet reader oil	1863004
	<b>Note:</b> Use the empty oil bottle as your waste container bottle.	

## Instrument Specifications

This section contains information on the instrument size and weight of the instrument. Environmental and safe use information is explained in [Safe Use Specifications on page 11](#).

**Table 5. Instrument specifications**

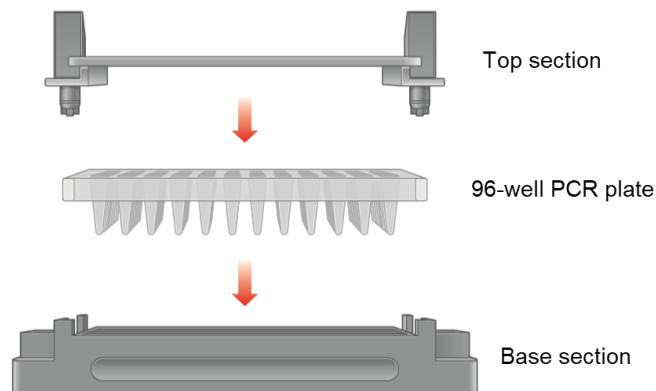
Element	Specifications
Weight	56.6 lbs (26 kg)
Size (W x H x D)	26 x 20.5 x 11.5" (660 x 521 x 292 cm)

## Assembling a Plate for the QX200 Droplet Reader

The QX200 Droplet Reader accepts a solid 96-well plate that fits into a secure holder. Three separate components comprise the plate:

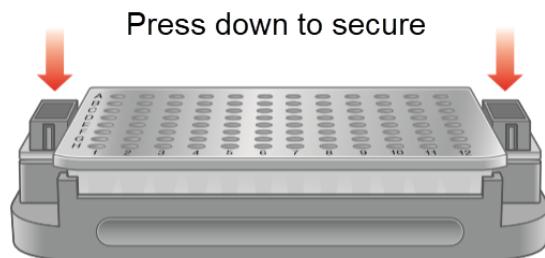
- A base section to hold the plate
- The plate itself
- A top section to secure the plate

You must assemble the plate components before you can insert the plate into the QX200 Droplet Reader.



### To assemble the plate

1. Place the plate into the base section.
2. Raise the release tabs on the top section.
3. Place the top section over the plate, and then lower the release tabs to secure the plate in the holder.



## Connecting and Starting the Instrument

To execute runs on the QX200 Droplet Reader, you must connect the instrument to a computer on which the QX Manager Software software is installed and configured.

**Tip:** Bio-Rad recommends purchasing at least one configured computer that can remain connected to the QX200 Droplet Reader and be used for adding and running plates in QX Manager Software. For information on additional items you can purchase for your instrument, see [Included Items and Additional Components on page 22](#).

### To connect the computer to the instrument

1. Connect the USB cable provided by Bio-Rad to the USB port on the back of the QX200 Droplet Reader, and then to the computer.
2. Press the Power button on the instrument..

The Power indicator light turns solid green to indicate that power is on.

3. Power up and log into the connected computer, and then open QX Manager Software and sign in.

For information, see [Signing Into the Software on page 31](#).

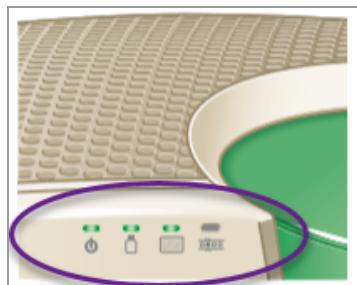
**Note:** Users must sign into the computer and software with the same secure Windows account.

After recognizing the connected instrument, QX Manager Software displays the Add Plate window.

**Note:** If the software does not recognize a connection to the instrument, it displays the Data Analysis window.

## Status Indicator Lights

This section describes the indicator lights on the front of the QX200 Droplet Reader.



Before you start a run, check the indicator lights to ensure the instrument is ready for droplet reading runs. For information, see [Table 6](#).

**Table 6. Droplet Reader Status Indicator Lights**

Indicator	 (Power)	 (Fluids)	 (Plate)	 (Run Status)
Solid Green		Power is on Fluid levels OK See Note (1)	Plate loaded properly	Run complete
Flashing Green		N/A <ul style="list-style-type: none"><li>■ Less than 30% oil is available</li><li>■ More than 70% waste in container</li></ul> See Note (2)	N/A	Run in progress
Flashing Amber		N/A <ul style="list-style-type: none"><li>■ Less than 10% oil available</li><li>■ More than 90% (700ml) waste in container</li></ul> See Note (3)	N/A	Error during run
No color		Power is off	N/A	No plate loaded Instrument is idle

**Notes:**

- (1) If the bottle indicator light is solid green, there is enough oil in the bottle to complete a run on 96 wells. You can configure the full plate in QX Manager Software for your run.
- (2) If the bottle indicator light is flashing green, there is enough oil in the bottle to start a run on a small number of wells. For example, you can run 24 wells with the oil bottle at a 19% level. If you configure your plate for more wells than the oil level allows, or if you fail to exclude the proper number of wells, the software does not enable the Start Run button.
- (3) You must add oil and remove waste before the instrument can start the run.

## Inserting an Assembled Plate into the Instrument

### To insert a plate into the QX200 Droplet Reader

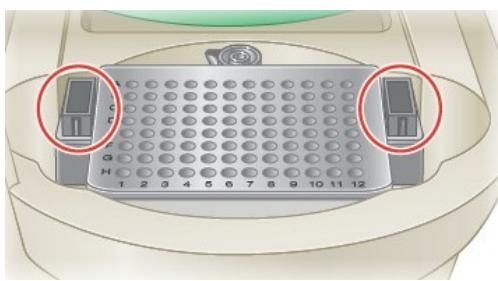
1. Press the button to open the compartment.



2. Fit the assembled plate into the compartment. Well A1 must be in the top-left position



**Important:** Make sure the tabs on the plate holder are securely clamped and the plate lays flat. Otherwise, the sampling needle can strike the lid and damage the instrument.



3. Press the button again to close the lid.

For information on adding your plate and starting runs in QX Manager Software, see [Running Experiments on page 41](#).



# Chapter 4 About QX Manager Software

## Regulatory Edition

QX Manager Software, when connected to your QX200 Droplet Reader provides all necessary functionality to create, run, and analyze experiments on your samples.

QX Manager Software Regulatory Edition provides features to permit operation in compliance with Title 21 of the U.S. Code of Federal Regulations Part 11 (21 CFR Part 11) within a closed system. A closed system is defined as “an environment in which system access is controlled by the persons who are responsible for the content of electronic records that are on the system” (Section 11.3(b) (4)).

### Important:

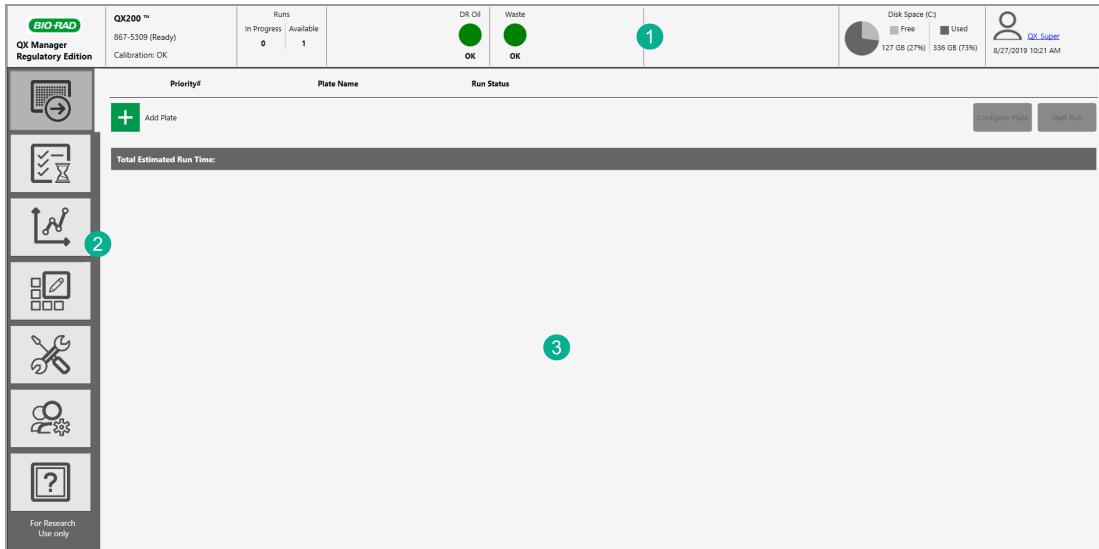
- The security controls built into QX Manager Software Regulatory Edition must be properly configured and administered by the software administrators in your organization in order to be secure and in compliance with 21 CFR Part 11.
- Bio-Rad makes no claim that QX Manager Software Regulatory Edition is CFR-compliant in and of itself, nor does the company guarantee compliance for the user. Your organization must establish policies and standard operating procedures that work in conjunction with the tools provided by Bio-Rad to ensure compliance with 21 CFR Part 11.

Using the instrument and software, you can

- Set up customized, real-time ddPCR experiments for plate runs
- Use the live analysis function during the droplet reading process
- Analyze your data files in a variety of charts and tables in the Analysis module
- Reprocess data with updated color calibration values
- Create and store plate and report templates, and produce reports on your data
- View system and experiment audit logs
- Produce an audit log report on old and new data values after reprocessing a file

**Tip:** If QX Manager Software goes into standby mode after being idle, click in the lockout screen to log into the software again and redisplay the last active window.

The following image highlights functional areas.



LEGEND

- 1 The status bar displays information about the instrument and user. For information on specific functionality, see [Instrument Status Bar on page 37](#).
- 2 Tabs provide access to the main functional windows.
- 3 The main pane displays the details of the selected tab.

# Chapter 5 Getting Started in QX Manager Software

Use the information in this chapter to

- Sign into the software or change users
- Understand your assigned user privileges and modify your personal preferences
- Become familiar with the functional windows
- Understand the compatible file types for your software edition

## Signing Into the Software

Your system administrator sets up users and privileges for the software, and communicates the information to you.

**Important:** In the Regulatory Edition, you are prompted to sign in again before the software can execute an auditable action.

### To sign in

1. To open the software, right-click the software icon on the Desktop and select Open.

The Sign in dialog box opens.



2. Enter your user name.
3. If the Sign in to: label shows the correct domain name, enter only the user name. If the label is blank or shows a different domain then enter it as **domain name\user name**

**Note:** The domain and computer names shown in the graphics are examples only. If you are unsure of the domain name, contact your system administrator.

4. Enter the password, and then click Sign in.

On first use, you must agree to the end user license agreement.



5. Clear the Show EULA on sign in checkbox, and then click I Agree.

**Note:** If you click I Disagree, the application closes immediately. If you leave the checkbox selected, you must agree to the EULA each time you log into the application.

When you agree to the EULA, the dialog box closes and the application opens. Your user name appears in the Instrument Status bar.

If you are connected to the QX200 Droplet Reader, the Add Plate window opens.

If you are opening the software on a standalone computer, the Data Analysis window opens.

## Viewing Your User Privileges

In the User Setup and Preferences window, your privileges are identified by a green check mark in the corresponding checkbox.

- Select the User Setup and Preferences tab.



You can view your assigned privileges at any time, but the check boxes in the display are not enabled unless you are assigned the Add/Manage Users privilege.

User Management

User Name: USPLECOTKYF2\QX\_User1

Full Name: QX\_User1 (USPLECOTKYF2)

Check Name

Privileges

- Add/Manage Users
- Create New Templates
- View Datafiles (created by other users)
- Overwrite Existing Datafile name
- System Settings
- Maintenance
- Data Archive

**Note:** Users who are assigned the Add/Manage Users privilege can also create users and update privileges and preferences for any user. Only the superuser can remove the Add/Manage user privilege from a user account.

User Management

User Name: USPLECOTKYF2\Mary\_Jones

Full Name: Mary Jones

Check Name

Privileges

- Add/Manage Users
- Create New Templates
- View Datafiles (created by other users)
- Overwrite Existing Datafile name
- System Settings
- Maintenance
- Data Archive

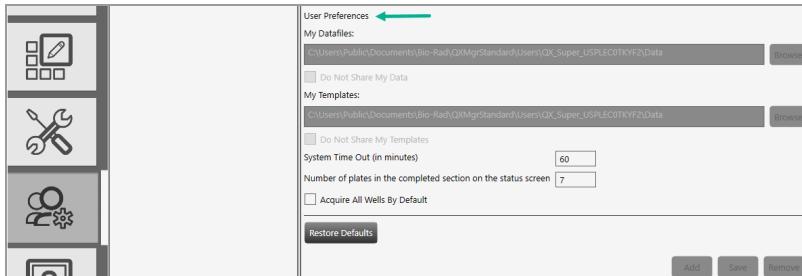
**Table 7** lists the available privileges, which can be assigned in any combination.

**Table 7. User privileges**

Privilege	Purpose
Add and manage users	<p>Add or remove users, set privileges, and change preferences.</p> <p><b>Note:</b> Only the superuser can remove this privilege from other users.</p>
Create new templates	Save a plate or analysis report design as a template.
View data files (created by other users)	View files created by other users.
Overwrite existing data file name	<p>Use Save or Save As capability.</p> <ul style="list-style-type: none"> <li>■ Selecting Save replaces the original file content with any changes made by the user, without changing the file name.</li> <li>■ Selecting Save As allows the user to save either existing or new content with a new file name.</li> </ul> <p>Whether or not changes are made, .qlps files are automatically saved as .ddpcrs files.</p> <p><b>Note:</b> Users without this permission can open files and perform analysis but cannot save their changes.</p>
System settings	<p>View logs, and view and modify the preferred or shared data file and template locations.</p> <p><b>Note:</b> All users can view the file locations.</p> <p><b>Important:</b> Your system administrator can set preferred locations in System Settings, which override individual user location preferences.</p>
Maintenance	<p>Perform software updates.</p> <p>View the event log.</p> <p><b>Note:</b> All users can view the maintenance log and maintenance reports.</p>
Data archive	Move raw data from the computer to free up disk space needed for runs on the instrument.

## Managing Your Preferences

Use the User Setup and Preferences window to modify your personal user preferences.



Default folder locations appear in your preferences, but you can change the storage locations for your templates and data files. You can also choose to keep all your data files and templates private. The software prompts you to choose either your personal folder or the shared folder each time you save a file or template.

**Important:** If your system administrator enables the Preferred Locations in System Settings, data file folder locations in User Preferences are overridden, and all data files are saved to the preferred file paths. Users can still choose where to store templates.

### To modify your preferences

1. Click the User Setup and Preferences tab, and then change any of the following preferences:
  - Enter a different file path for your data files and templates.
  - Select or clear the check boxes to change your data file and template privacy settings.

The paths and checkboxes are disabled if your administrator has set preferred locations in System Settings.

  - Enter a different system timeout period.
  - Enter a different total of completed plates to show in the Run Status window, up to a maximum of 100.
  - Select the Acquire all Wells By Default checkbox.

**Note:** If you do not select the checkbox, the default Plate Editor display is all wells excluded from droplet reading and you must manually include the wells for each droplet reading run.
2. Click Save.
3. When the confirmation message appears, click Yes to save the changes, and then click OK.

## Signing Out or Changing Users

While the software is running, one user can sign out and a different user can sign in.

**Note:** Where the screen is locked and the initial user left unsaved changes, an advisory prompt appears. The subsequent user has the option to save or discard the changes before completing the sign-out process.

### To sign out

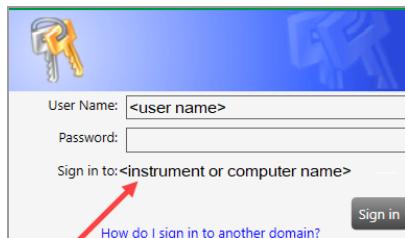
1. Click the user name link in the upper-right corner and select Sign Out.
2. Click Yes to confirm.

If there are unsaved changes, QX Manager Software displays a prompt.

- To discard the changes and proceed, click Yes.
- To cancel the sign out, click No. Save the changes, and then repeat Steps 1 and 2.

### To change users

1. Click anywhere in the lockout screen to display the Sign in window.
2. Enter a user name.
  - The domain name appears below the user login fields. If you are on the same domain as the previous user, you can enter your user name only.



- If the user is on a different domain, enter the domain name followed by a backslash and the user name.  
**<domain name>\<user name>** \*(for example, global\john\_smith)

3. Enter the password and click Sign in.

## Instrument Status Bar

An instrument status bar appears above all windows except the analysis displays, which open in a separate module.

When a computer is connected to the QX200 Droplet Reader, the status bar displays the following information:



### LEGEND

- 1 Software name

---

- 2 Instrument name and status information

---

- 3 Number of runs in progress and runs available

---

- 4 Oil and waste levels
 

For information on oil and waste level maintenance, see [Fluid Maintenance on page 185](#).

---

- 5 Used and free disk space in gigabytes (GB)
 

**Note:** When QX Manager Software detects low disk space, the software displays an advisory prompt to archive data. For information, see [Archiving Data on page 198](#).

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- 6 Name of the current user, along with the current date and time.

If a computer is not connected to the QX200 Droplet Reader, the status bar shows only the software, disk space, and user information.

## Functional Windows

This section briefly describes the functional areas in QX Manager Software. [Table 8](#) explains the primary window accessed from each tab, and includes references to specific chapters or sections in this document where you can find additional information on the functionality.

**Table 8. Window Tabs**

Tab	Name	Purpose
	Add Plate	<ul style="list-style-type: none"> <li>■ Add a plate.</li> <li>■ Access the Plate Configuration window, where you can set up your plate for the run.</li> </ul> <p>For information on how the functionality is used in this and subsequent windows, see <a href="#">Adding the Plate on page 41</a>.</p>
	Run Status	<ul style="list-style-type: none"> <li>■ In the top pane, view the run in progress.</li> <li>■ In the bottom pane, view up to 100 completed runs (successful, failed).</li> </ul> <p>For information on run status, see <a href="#">Running the Experiment on page 54</a> and <a href="#">Tracking the Run Status on page 55</a>.</p>
	Data Analysis	<p>Access the Data Analysis and Gene Study modules.</p> <p>For information on file analysis, see <a href="#">Data Analysis Module Overview on page 77</a> and <a href="#">Data Analysis Methodology on page 115</a>.</p>
	Template Setup	<ul style="list-style-type: none"> <li>■ Access windows where you can set up plate and report templates.</li> <li>■ Search for existing template files.</li> <li>■ Perform lot management for consumables and reagents.</li> </ul> <p>For information on creating or editing templates, see <a href="#">Creating or Editing Plate Templates on page 63</a> and <a href="#">Creating Report Templates on page 150</a>.</p> <p>For information on lot management, see <a href="#">Lot Management for Consumables and Reagents on page 59</a>.</p>

**Table 8. Window Tabs, continued**

Tab	Name	Purpose
	System Utilities	<ul style="list-style-type: none"> <li>■ Access file storage information, event and maintenance logs, maintenance reports, and tools.</li> <li>■ Archive and reprocess data.</li> </ul> <p>For information, see <a href="#">System Utilities on page 191</a>.</p> <p><b>Note:</b> Functionality available to you depends on your assigned user privileges.</p> <p><b>Important:</b> Instrument calibration functionality is available only to the Bio-Rad service engineer.</p>
	Users and Preferences	<ul style="list-style-type: none"> <li>■ View the user privileges you have been assigned, and view and modify your personal preferences.</li> <li>■ Create, edit, or remove QX Manager Software users (if you are assigned the Add/Manage Users privilege).</li> </ul> <p>For information on your user privileges and preferences, see <a href="#">Getting Started in QX Manager Software on page 31</a>. For information on setting up users in QX Manager Software, see <a href="#">Managing Users on page 179</a>.</p>
	Help	Access software version information, the End User License Agreement, the Bio-Rad website, open source software license information, and this document in PDF format.

## Compatible File Types

This section describes the file types you can open in QX Manager Software.

The following applies if your organization is using more than one edition of the software:

- Analysis files created in QX Manager Software Regulatory Edition open only in Regulatory Edition.
- Analysis files created in QX Manager Software Standard Edition open only in Standard Edition.

**Table 9. Compatible software file types**

File Type	Extension	Details
Plate	.ddplt	Plate template file containing setup details to perform experiments; this file type opens in the Plate Editor.
Data	.ddpcrs	Contains the results of an experiment run and data analysis performed in QX Manager Software Regulatory Edition; this file type opens in the Analysis module.
Data	.qlps	File type from older QuantaSoft regulatory versions, for two-channel experiment runs and data analysis; this file type opens in the Analysis module. <b>Note:</b> You can open .qlps files in QX Manager Software Regulatory Edition, but if you make any changes, the software prompts you to save the file as a .ddpcrs file.

# Chapter 6 Running Experiments

After the QX Manager Software recognizes the plate holder inserted in the QX200 Droplet Reader, you can open the Plate Configuration window where you can identify your plate, configure your plate layout, and start the run. The software enables each button sequentially (Add Plate, Configure Plate, Start Run) when you complete the prerequisite tasks.

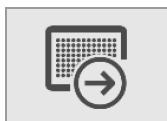


## Adding the Plate

This section explains how to add the plate in QX Manager Software. You can insert a plate into the instrument before or after you add the plate in the software. For information on how to properly insert a plate into the QX200 Droplet Reader, see [Inserting an Assembled Plate into the Instrument on page 27](#).

### To add a plate in QX Manager Software

1. Click the Add Plate tab.



If the Runs Available number in the Instrument Status Bar is 1, the Add Plate button is enabled.

**Note:** You cannot add a plate in the software if the QX200 Droplet Reader is currently processing a run.

2. Click  next to Add Plate.

When QX Manager Software recognizes the plate holder in the compartment, the software enables the Configure Plate button.

**Note:** If the software does not recognize the plate holder, the software displays an advisory prompt.

3. Click Configure Plate. The Plate Configuration window opens.

## Plate Configuration Window

After you click Configure Plate, the Plate Configuration window opens to the Plate Information display.

All users can start runs on selected plates using the initial configuration, an altered configuration, or just the plate and data file names and the supermix.

When configuring plates

- Users without the Create New Template user privilege can customize a plate design before starting a run but cannot save the file.
- Users with the Create New Template user privilege can save a new or modified plate design as a template before starting the run.

### Plate Information Tab

The Plate Information tab is selected by default when the Plate Configuration window opens, and provides functionality allowing you to quickly set up a plate for a run.

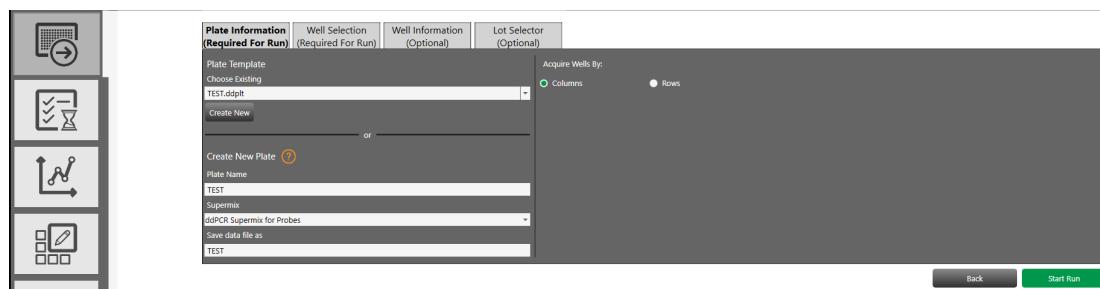
You can

- Select, modify (optional), and run an existing template
- Create and run a new plate design
- With the applicable user privilege, save existing or new plate designs as templates
- Identify a plate name, a supermix, and a data file name (optional) for a run

**Note:** At a minimum, you must enter a plate name and select a supermix to enable the Start Run button. The data file name field is automatically populated with the plate name, but you can change it. You can identify the remainder of your experiment parameters either in the Plate Editor before you start the run, or in the plate editor layout in the Analysis module after the run has concluded.

- Select the well acquisition method the instrument should use

### Identifying Plate Information



### To select an existing plate template

1. Under Plate Template, click the dropdown arrow under Choose Existing and select a template file. The software automatically populates the fields under Create New Plate.
2. (Optional) Under Create New Plate, change the plate or data file name.
3. (Optional) Under Acquire Wells By, select the alternative well acquisition method. The default acquisition is by column.
4. Click the Well Selection tab and include all sample wells for droplet reading. For information, see [Including or Excluding Wells in the Plate Layout on page 45](#).
5. Select the Well Information tab, and then select the wells to be processed in the run.
6. (Optional) Change the configuration for one or more wells. For information, see [Defining or Editing Well Information on page 47](#).
7. Click Apply.

The Start Run button is enabled.

### To create a new template

1. Under Plate Template, click Create New to open the corresponding editor window in template Edit mode.

**Important:** You must be assigned the Create New Template user privilege, which allows you to save the template. Otherwise, you must use an existing template or use the Create New Plate option and configure your plate from the Well Selection and Well Information tabs.

The Plate Editor opens in Edit mode.

2. Select the wells to be processed and click Include selected wells. For information, see [Including or Excluding Wells in the Plate Layout on page 45](#).

By default, all wells in the Plate Editor are automatically excluded, and you need to enable the wells to be run. If you typically run full plates, you can change the setting to Acquire all wells by default in your user preferences. For information, see [Managing Your Preferences on page 35](#).

**Important:** All empty wells must be excluded.

3. Configure experiment parameters for each well to be processed. For information see [Creating or Editing Plate Templates on page 63](#).
4. (Optional) Under Acquire Wells By, select the alternative well acquisition method. The default acquisition is by column, but you can direct the instrument to acquire wells by row.
5. Click Apply.
6. Click Save and select one of the following:
  - Shared Templates. The template is saved in the folder specified in the System Settings.
  - My Templates (saves the file to the path in your user preferences) or System Templates (saves the file to the preferred location specified by your system administrator).
  - If your system administrator has specified preferred locations for all users, your template must be saved to that location.

The Start Run button is enabled.

### To create a plate without parameters

1. Enter a plate name under Create New Plate.
2. Select a supermix.
3. Enter a file name for the data file that QX Manager Software generates at the end of the run.

The Start Run button is enabled.

## Well Selection Tab

When you select the Well Selection tab, a blank Plate Editor grid appears.

The QX200 Droplet Reader requires empty wells to be excluded from droplet reading; therefore, the QX Manager Software Plate Editor layout shows all wells as excluded by default, and you must select and manually include the wells to be run.

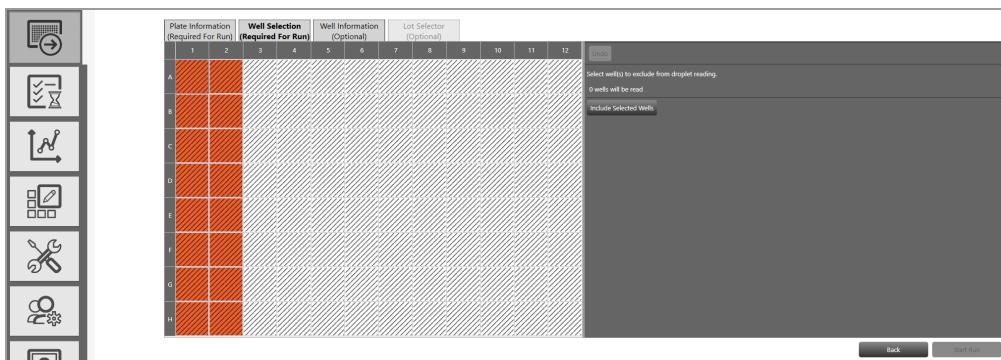
**Tip:** If you typically run full plates, you can change the setting in User Preferences to acquire all wells by default. For information, see [Managing Your Preferences on page 35](#).

### Including or Excluding Wells in the Plate Layout

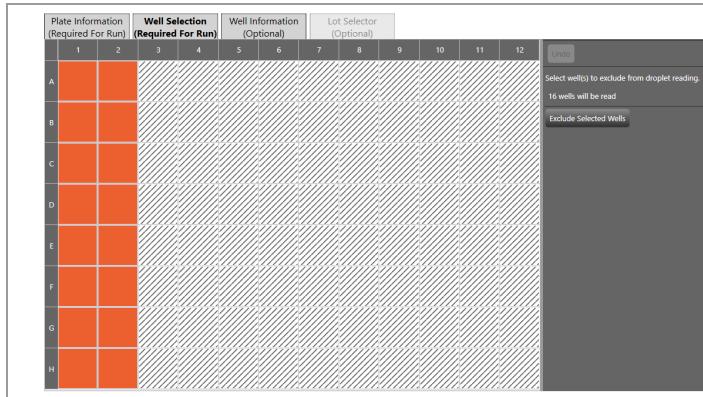
All wells are initially excluded by default.

#### To include wells in the experiment run

1. Select the Well Selection tab.
2. In the Plate Editor layout, select the wells containing sample.



3. Click Include Selected Wells.



On the right, QX Manager Software specifies the number of wells that will be included in the droplet reading run.

### To exclude wells

1. Select one or more included wells.
2. Click Exclude Selected Wells.

On the right, QX Manager Software specifies the number of wells that will be included in the droplet reading run.

## Well Information Tab

When you select the Well Information tab, the Plate Editor opens. The window is identical to the Edit window in Template Setup.

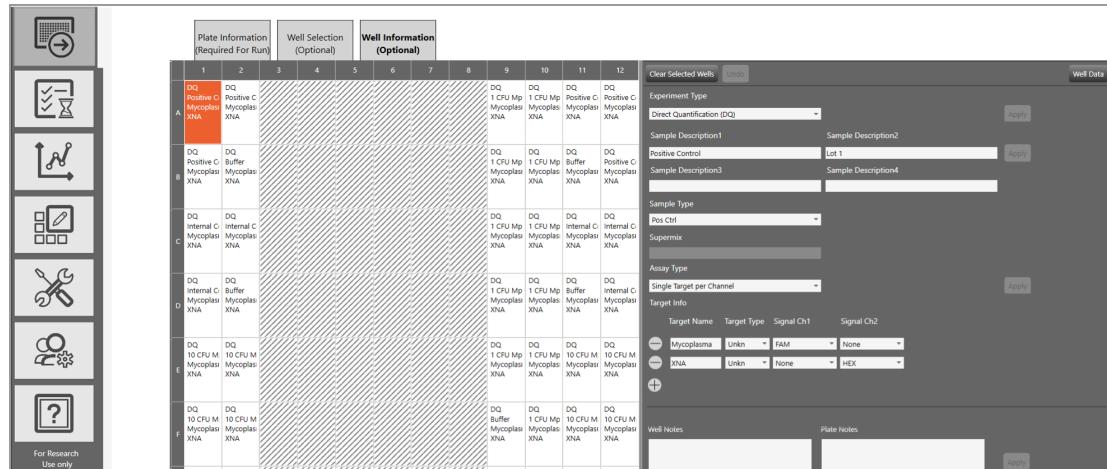
You can configure your experiment parameters for selected wells before or after the run. However, you must select a supermix, and identify the plate and file name before the Start Run button is enabled.

**Note:** Beginning in version 1.2, QX Manager Software can calculate automatic thresholds for positive control wells, but you must select Pos Ctrl from the Sample Type drop-down list. You can make the selection before or after the run. For information, see [Sample Types on page 73](#).

### Defining or Editing Well Information

You can assign different experiment parameters to each well or groups of wells.

**Note:** You must click Apply for the software to recognize your new or edited information.



You can apply your entries at any time.

**Note:** If you click the Save button before the plate layout is complete, the file closes and you must reopen it from the saved template location.

### To set up your wells in the plate

1. If you are accessing the Plate Editor from
  - the Add Plate tab, click Configure Plate and select the Well Information tab to display the Plate Editor.
  - the Template Setup tab, the Plate Editor opens with the Edit button selected by default.

If you are creating a new template, the grid is blank. If you are editing an existing template, information appears for the configured wells.

2. Select a well or group of wells.
3. Select an experiment type. For more information, see [Experiment Types on page 71](#).
4. In the Sample Description fields, enter up to four words or phrases that describe the sample. For more information, see [Sample Descriptions on page 72](#).
5. Select a sample type. For more information, see [Sample Types on page 73](#).

**Note:** For automatic thresholding of positive control wells, you must select Pos Ctrl.

6. Select a supermix. For more information, see [Supermixes on page 73](#).
7. Select an Assay Type. Available assay types vary, depending on the selected Experiment Type. For information, see the fluorophore options in [Assay Types and Fluorophores on page 74](#).
8. (Optional) Under Target Info, change the fluorophore assigned to a channel.

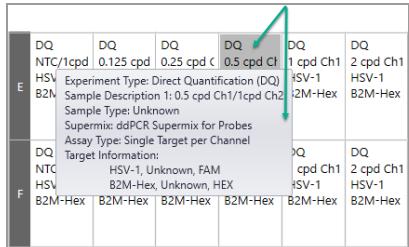
The fluorophore information populates automatically for each target, with the maximum number of rows allowed for the assay method, but you can modify the default fluorophore assignments, if applicable.

- a. Click the field dropdown arrow and select an alternative.
- b. To delete a row, click the minus (-) icon next to the target.
- c. To re-add a row, click the plus (+) icon.

9. (Optional) To clear one or more wells at any time, select the wells and click Clear Selected Wells.

## 10. Click Apply.

**Tip:** After you have applied your entries and selections, you can pause the cursor on a well to see the well information.



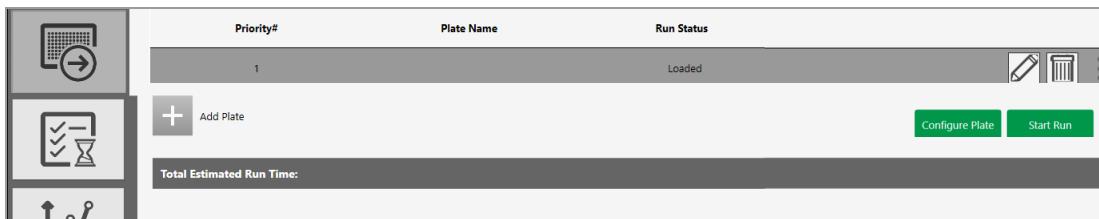
DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ
NTC/1cpd	0.125 cpd	0.25 cpd	0.5 cpd	Ch1	1 cpd Ch1	2 cpd Ch1	
HSV	Experiment Type: Direct Quantification (DQ)				HSV-1	HSV-1	
B2M	Sample Description 1: 0.5 cpd Ch1/1cpd Ch2				B2M-Hex	B2M-Hex	
	Sample Type: Unknown						
	Supermix: ddPCR Supermix for Probes						
	Assay Type: Single Target per Channel						
DQ	Target Information:						
NTC	HSV-1, Unknown, FAM						
HSV	B2M-Hex, Unknown, HEX						
B2M-Hex	B2M-Hex	B2M-Hex	B2M-Hex	B2M-Hex	B2M-Hex	B2M-Hex	B2M-Hex

## 11. If you are finished with your plate configuration, click Save.

## Starting the Run

When all required elements to set up a plate for a run in are satisfied, the QX Manager Software enables the Start Run button.

**Important:** Ensure there is sufficient QX200 Droplet Reader Oil available, and the waste bottle is less than 70% full.



- Tap Start Run.

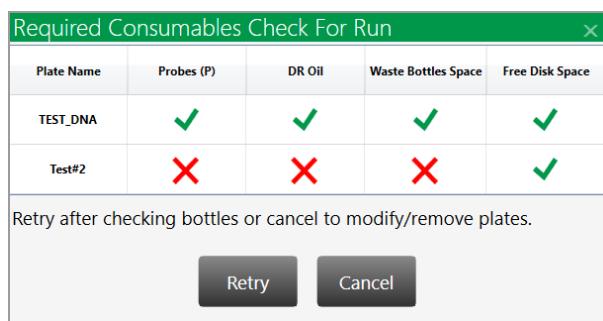
To reauthenticate your user credentials, you are prompted to sign in again.

Enter your user name and password, and then click Sign in.

The software performs a mandatory prerequisites check to ensure that the instrument is ready.

One of the following occurs:

- If the instrument passes the check, the run starts normally. Continue to [Running the Experiment on page 54](#).
- If the check finds anything amiss on the instrument, the software displays a dialog box in which the issues are identified. You must resolve each issue before continuing. For information, see [Run Prerequisites Checklist](#).

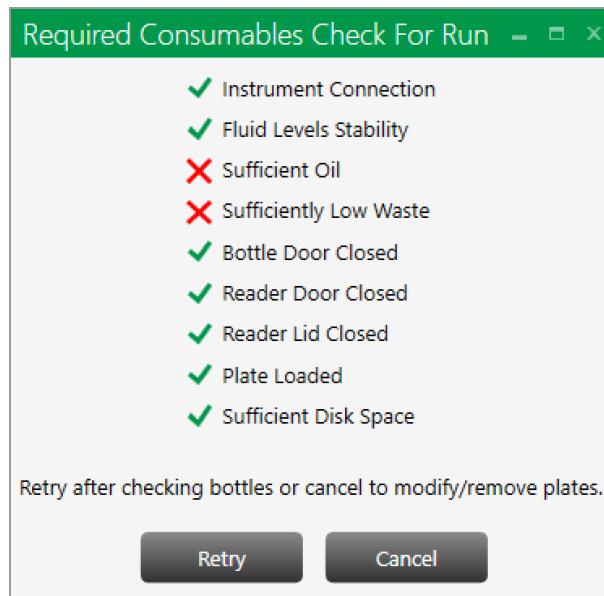


The instrument cannot start the run until all specified issues are resolved.

## Run Prerequisites Checklist

If the prerequisites check on the instrument status finds one or more problems with instrument readiness, QX Manager Software identifies them with an  in the Check for Run dialog box. You must resolve all issues before the instrument can start the run.

**Note:** If you encounter a problem with the instrument or software that is not described here, see [Troubleshooting on page 203](#).



For information on each issue that can prevent the instrument from starting the run, see [Table 10](#).

**Table 10. Pre-Run Error Indicators**

<b>Status Check Item</b>	<b>Issue</b>	<b>Suggested Resolution</b>
Instrument Connection	USB cable connection not recognized	Disconnect, and then reconnect, the USB cable between the instrument and the computer.
	Power connection not recognized	Ensure the power outlet is working. Disengage the power cord from the outlet, and then reinsert it into the outlet.
Fluid Levels Stability	The oil or waste is moving within the bottle so the level cannot be accurately determined.	Wait until the liquid is settled, then try again. Ensure the instrument rests on a solid, stable surface.
Sufficient Oil	Oil level is below the acceptable level for a run.	Remove the oil bottle and replace it with a full one.
Sufficiently Low Waste	Waste level is above the acceptable level for a run.	<ol style="list-style-type: none"> <li>1. Empty any remaining oil from the bottle and then place a Waste label (provided by Bio-Rad) over the Oil label.</li> <li>2. Insert the bottle into the Waste slot.</li> </ol> <p>For information on changing containers, see <a href="#">Instrument Maintenance on page 185</a>.</p>
Bottle Door Closed	Bottle door is not closed properly.	Open the bottle door and ensure there are no blockages, and then close the door again.
Reader Door Closed	Reader door is not closed properly.	Open the reader door and ensure there are no blockages, and then close the door again.
Reader Lid Closed	Reader lid is not closed properly.	Open the reader lid and ensure there are no blockages, and then close the lid again.

**Table 10. Pre-Run Error Indicators, continued**

Status Check Item	Issue	Suggested Resolution
Plate Loaded	Plate is not loaded properly.	<ol style="list-style-type: none"><li>1. Open the reader door and remove the plate.</li><li>2. Ensure the tabs of the plate cover are securely clamped.</li><li>3. Insert the plate again and ensure it lays flat.</li></ol> <p>For information on loading plates in the QX200 Droplet Reader, see <a href="#">Inserting an Assembled Plate into the Instrument on page 27</a></p>
Sufficient Disk Space	There is not enough storage space available for the run data.	<p>Archive older data files to clear space.</p> <p>For information on archiving data files, see <a href="#">Archiving Data on page 198</a>.</p>

After you resolve the issues, continue to [Running the Experiment](#).

## Running the Experiment

During and after the run, QX Manager Software displays run information in the Run Status window. For information, see [Tracking the Run Status](#).

- During the run, you can perform real-time analysis on the droplets in each well after the well has been read. For information, see [Using Live Analysis on page 57](#).
- You can cancel the run at any time. For information, see [Canceling a Run on page 58](#).
- When the run concludes and the plate has been removed from the outbox, QX Manager Software moves the run to the Completed list in the Run Status window, and saves the analysis data file.

Data files are always saved to one of the following locations:

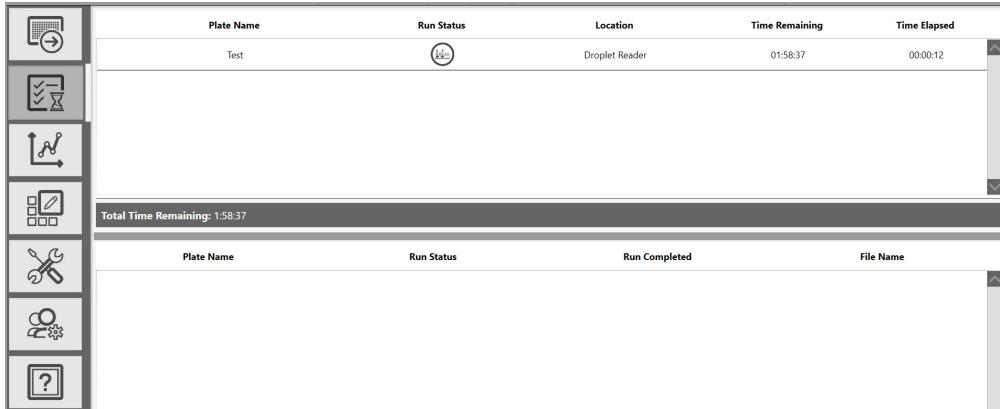
- Your personal file location specified in your user preferences
- The preferred location in System Settings

**Important:** if your system administrator has designated preferred locations for all users, these file paths override the file paths indicated in your preferences.

For information on file storage locations, see [System Utilities on page 191](#). For information on opening and analyzing your data file, see [Data Analysis Module Overview on page 77](#).

## Tracking the Run Status

You can track each run from the Run Status window.



The top pane shows the run in progress. The run remains in the top pane for the duration of the processing.

Icons displayed in the Run Status column indicate each process point, as shown in [Table 11](#).

**Table 11. Run status indicators**

Status Icon	Description
	Ready to begin droplet reading
	Droplet reading
	Run completed, plate in Outbox
	Run completed, plate removed from Outbox
	Run stopped or canceled by the system

When the run is finished, the software moves the run to the bottom pane, where up to 100 completed runs are visible.

**Tip:** You set the number of completed runs to display in your user preferences. See [Managing Your Preferences on page 35](#).

The content of each column is explained in [Table 12](#).

**Table 12. Run Information Columns**

Column Name	Location	Description
Plate name	Top and bottom panes	Name entered under Create New Plate <b>Note:</b> If you selected an existing template, the template name appears.
Run status	Top and bottom panes	Icons for each process step, as explained in <a href="#">Table 11 on page 55</a>
Time remaining	Top pane only	Hours and minutes until the run will be finished
Time elapsed	Top pane only	Time, in hours and minutes the run has been in progress
Total time remaining	Below the top pane	Total hours and minutes remaining for all in-progress runs
Run completed	Bottom pane only	Date and time stamp showing when the instrument completed the run
File name	Bottom pane only	Plate name and system-generated identifier

After run completion, the software saves the analysis data file in either your personal file location or to a preferred location specified by your system administrator.

**Important:** If your system administrator has specified preferred locations for all users in System Settings, files are saved there, rather than to the path specified in user preferences. For information, see [Preferred Location on page 192](#).

If your system is connected to a network, and shared folders are identified in the global preferences, the data file is also copied to a shared folder.

## Using Live Analysis

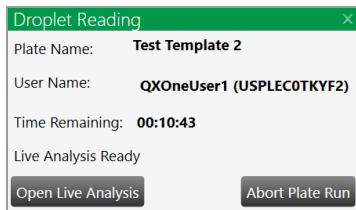
When the QX200 Droplet Reader initiates droplet reading, QX Manager Software provides an option for real-time analysis of the wells that have been read, while the process as a whole is still active.

QX Manager Software initially shows each well in the Plate Editor as disabled. As the software confirms that the instrument has acquired a well and read the droplets, the software enables the well for live analysis in read-only mode. The process continues until all wells are enabled and the process is finished.

### To use live analysis

1. Tap the droplet reading icon (⌚) for the run in progress.

The Droplet Reading dialog box opens.



2. Tap Open Live Analysis.
3. Select an analysis window.
4. In the well selector, select one or more wells that are enabled.

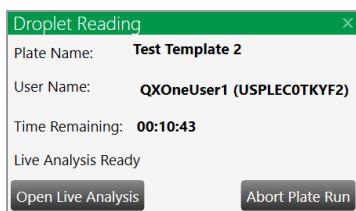
**Important:** QX Manager Software displays read-only data in the selected analysis window. You can view the data but to make changes, you must wait for the run to complete, and then view the data in the Analysis module.

## Canceling a Run

You can cancel a run at any time during the droplet reading process.

1. In the Run Status window, click the droplet reading icon (⌚) for the run to be canceled.

The Droplet Reading dialog box opens.



2. Click Abort Plate Run.
3. When the confirmation message appears, click Yes.

The instrument finishes reading the current well, stops the process, and reverts to its pre-run state.

The  icon appears in the Run Status column.

QX Manager Software then creates the data file for the wells that were processed, and the run end status in the Event Log is specified as “run aborted by user.”

**Note:** If you click No, or if you do not respond to the confirmation message within 30 sec, the process continues without interruption.

# Chapter 7 Lot Management for Consumables and Reagents

You can maintain an inventory and track the use of your consumables and reagents in the QX Manager Software.

For consumables, you can manage lots of the following items:

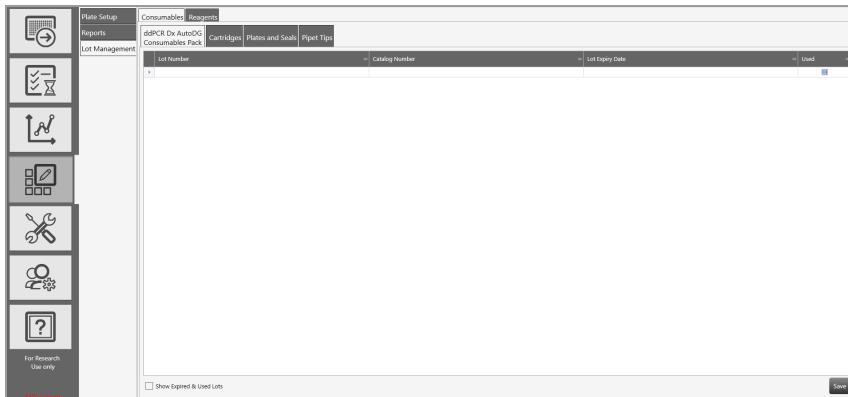
- ddPCR Dx AutoDG consumables packs
- Cartridges
- Plates and seals
- Pipet tips

For reagents, you can manage lots of the following items:

- Supermix
- Droplet generator oil
- Droplet reader oil
- Buffer controls
- Assays

Lots that have been fully used are not displayed.

## Managing Consumable Lots



### To enter a consumables lot

1. Select the Template Setup icon.



2. Select Lot Management, and then select the Consumables tab.

From the Consumables window, the following tabs appear above the lot information grid:

- ddPCR Dx AutoDG Consumables Pack (appears by default)
- Cartridges
- Plates and Seals
- Pipet Tips

3. Select a tab.

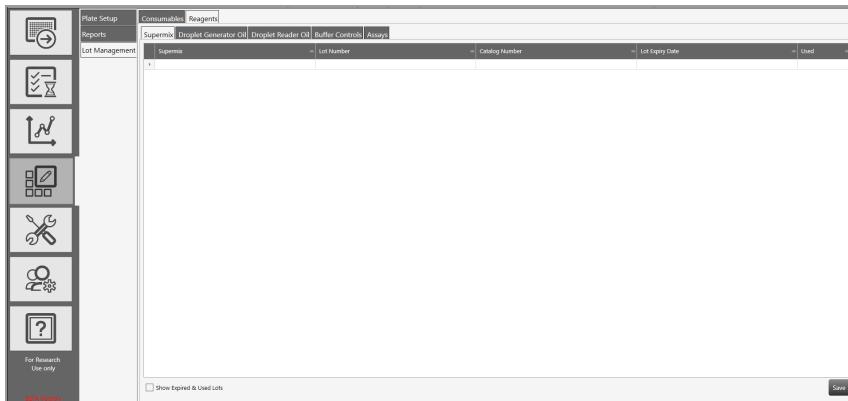
4. (Optional) To display expired or used lots, select the checkbox at the bottom-left side of the window.

5. Click in each field and enter the corresponding information as follows:

- Lot Number
- Catalog Number
- Lot Expiry Date

6. If the consumable was used in a run, select the Used checkbox.
7. Repeat until all lots are added.
8. Click Save.

## Managing Reagent Lots



### To enter a reagent lot

1. Select the Template Setup icon.



2. Select Lot Management, and then select the Reagents tab.

From the Reagents window, the following tabs appear above the lot information grid:

- Supermix (appears by default)
- Droplet Generator Oil
- Droplet Reader Oil
- Buffer Controls
- Assays

3. Select a tab.

4. (Optional) To display expired or used lots, select the checkbox at the bottom-left side of the window.
5. Click in each field and enter the corresponding information as follows:
  - Supermix (in Supermix grid only)
  - Lot Number
  - Catalog Number
  - Lot Expiry Date
6. If the consumable was used in a run, select the Used checkbox.
7. Repeat until all lots are added.
8. Click Save.

## Showing Lots in Run Information Output and Reports

After you have set up your lots, you can ensure that lots appear in the Run Information screen of your analysis files.

### To show the lots in Run Information

1. Click Add Plate, then click Configure Plate.
2. Select the Lot Selector tab.
3. For consumables and reagents
  - a. Select each applicable lot for the run
  - b. After each selection, click Select Lots.
4. Continue setting up the plate until the Start Run button is enabled.
5. Click Start Run.
6. When the run is completed, open the analysis file.
7. Click the Run Information tab to see the used lots.

### To show the lots in a report

► Ensure the Lot Information check box is selected in the report elements before running the report.

# Chapter 8 Creating or Editing Plate Templates

If you are assigned the Create New Templates user privilege, you can save a new or reconfigured plate as a template for reuse from the Add Plate and Template Setup windows.



Templates that are available to you appear in the default view of the Template Setup tab, as follows:

- Templates you created and saved, which are saved to one of the following paths:
  - The path specified in your user preferences; these appear under My Templates
  - The path specified under Preferred Locations in System Settings, if your system administrator has specified the path for all users, these appear under System Templates

**Important:** If your administrator has specified the Preferred Location for all users, the path overrides the path specified in user preferences. Preferred locations can be enabled only by users with the System Settings user privilege.

- Templates created and saved by any user and designated as shared; these appear under Shared Templates.

**Note:** From the Template Setup or Add Plate window, you can create or modify plate templates. From the Template Setup window, you can also modify reports.

## Plate Editor Window

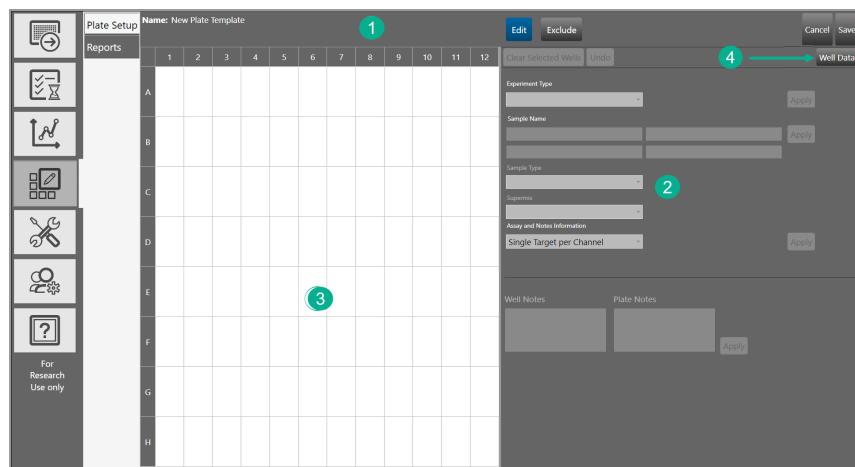
Use the Template Setup Plate Editor to

- Create new or edit existing plate template files
- Save new or modified templates for reuse (with Create New Templates permission only)
- Define or change experiment type, sample type, supermix, and assay type for each well

**Tip:** Except for the supermix, which you must select pre-run, you can configure the plate experiment parameters before or after a run.

- Set reference targets and control samples
- Exclude wells from droplet reading
- Add applicable plate or well notes

The Plate Layout view is the default template view.

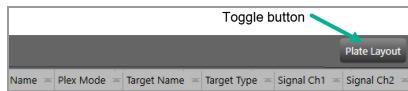


### LEGEND

- 1 You can access the Edit and Exclude buttons from the top of the screen.
- 2 The right pane contains the interface for defining your experiment parameters.
- 3 The left pane displays the plate grid and configuration information in each well.

## LEGEND , CONTINUED

4 The Well Data button toggles to the Well Data table, where you can see your plate setup in a tabular format, and then back to the Plate Editor. When the Well Data table is displayed, the button name changes to Plate Layout.



## Plate Editor Tools

Use the buttons described in [Table 13](#) to define your experiment in the Plate Template Editor.

**Table 13. Plate Editor Buttons**

Button	Description
Edit	<p>Allows you to</p> <ul style="list-style-type: none"> <li>■ Select wells or clear well selections</li> <li>■ Set the experiment type, sample name and type, supermix, and assay type</li> <li>■ Set the target name, type, and fluorophore for each channel</li> <li>■ Add well or plate notes</li> </ul> <p><b>Note:</b> This button is enabled by default when you open the window.</p>
Exclude	Allows you to exclude wells from droplet reading
Cancel	Allows you to cancel and return to the Template Setup window
Save	Opens a dialog box where you can enter a file name and specify a storage location
Clear Selected Wells	Allows you to delete information from selected wells
Undo	Allows you to reverse your last action
	<b>Note:</b> This button is enabled only after you have applied at least one experiment parameter (for example, the selection for Experiment Type).
Well Data or Plate Layout	Toggle button to access the Well Data table and return to Plate Layout view
Apply	Applies your entries and choices for selected wells

## Opening a New Plate Template

You can open a blank plate template from either the Template Setup window or the Add Plate window.

- Use Template Setup if you are creating one or more templates to store for future use.
- Use Add Plate if you are creating a template to correspond to a plate you are about to run.

### To open a new plate template from the Template Setup window

1. Tap the Template Setup tab.



2. Click Plate Setup.
3. Click Add New Plate.
4. Click Edit Plate Layout.

The Plate Editor opens, displaying a blank plate template.

5. To set up your plate, see [Template Setup — Setting Up Your Wells on page 68](#).

### To open a new plate template from the Add Plate window

1. Click the Add Plate tab.



2. Click the + icon next to Add Plate.
3. When the software enables the Configure Plate button, click Configure Plate.

The Plate Configuration window opens to the Plate Information tab by default.

4. Under Plate Template, click Create New.

The template Plate Editor opens, displaying a blank plate layout.

5. To set up your plate, continue to [Template Setup — Setting Up Your Wells on page 68](#).

## Opening an Existing Plate Template

You can open an existing plate template from either the Template Setup window or the Add Plate window.

- Use Template Setup if you are editing one or more templates to store for future use.
- Use Add Plate if you are editing a template to correspond to a plate you are about to run.

### To open an existing plate file from the Template Setup window

1. Click the Template Setup tab.



The Plate Setup window appears by default and a list of available plate templates appears.

2. Select a template and click Edit Plate Layout.

**Tip:** You can also double-click the template file to open it.

The Plate Editor opens, displaying the plate template as it is currently configured.

To make changes to the plate, continue to [Template Setup — Setting Up Your Wells on page 68](#).

### To open an existing plate template from the Add Plate window

1. Click the Add Plate tab.



2. Click the + icon next to Add Plate.

3. When the software enables the Configure Plate button, click Configure Plate.

The Plate Configuration window opens to the Plate Information tab by default.

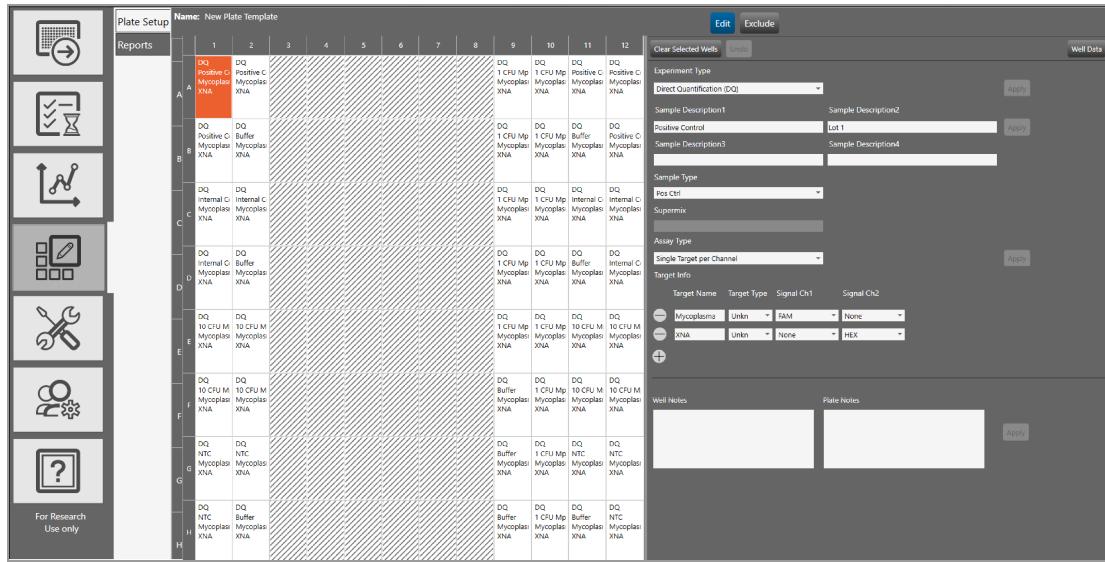
4. Under Plate Template, click the dropdown arrow and select a template from the list.

The template Plate Editor opens, displaying the configured layout.

You can run the plate without changing the template, or you can modify the configuration. To make changes to the plate, continue to [Template Setup — Setting Up Your Wells on page 68](#).

## Template Setup — Setting Up Your Wells

When you open the Plate Editor from the Template Setup tab, the Edit button is selected by default. You can assign different experiment parameters to each well or groups of wells.



For each well, or group of wells, you must

- Choose an experiment type
- Select a sample type

**Note:** For automatic thresholding of positive control wells, you must select Pos Ctrl.

- Select a supermix
- Select an assay method

Optionally, you can

- Enter up to four sample descriptors for each well
- Modify fluorophore assignments
- Add notes for each well
- Add notes for the plate

If you are creating a new template, the grid is blank. If you are editing an existing template, previously saved information appears when you select one or more configured wells.

## To define well parameters

1. Select a well or group of wells.
2. From the Experiment Type dropdown list, select a new or different experiment type. For more information, see [Experiment Types on page 71](#).
3. In the Sample Description fields, enter or change up to four words or phrases that describe the sample. For more information, see [Sample Descriptions on page 72](#).
4. From the Sample Type dropdown list, select a new or different sample type. For more information, see [Sample Types on page 73](#).
5. From the Supermix dropdown list, select or change the supermix. For more information, see [Supermixes on page 73](#).
6. The Assay Type field populates automatically based on your experiment selection. To change it, select a different assay type from the dropdown list. For information, see the fluorophore options in [Assay Types and Fluorophores on page 74](#).

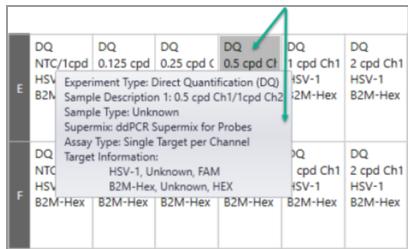
**Note:** Available assay types vary, depending on the selected experiment type.

7. (Optional) Under Target Info, change the fluorophore assigned to a channel.

The fluorophore information populates automatically for each target, with the maximum number of rows allowed for the assay method, but you can modify the default fluorophore assignments, if applicable.

- a. Click the field dropdown arrow and select an alternative.
- b. To delete a row, click the minus (-) icon next to the target.
- c. To re-add a row, click the plus (+) icon.
8. (Optional) To clear one or more wells at any time, select the wells and click Clear Selected Wells.
9. Click Apply.

**Note:** You must click Apply for the software to recognize your new or edited information, and before you can save the template. Once applied, you can pause on a well to see the corresponding information in a tool tip.



	DQ	DQ	DQ	DQ	DQ	DQ
	NTC/1cpd	0.125 cpd	0.25 cpd	0.5 cpd Ch1	1 cpd Ch1	2 cpd Ch1
E	HSV	Experiment Type: Direct Quantification (DQ)	B2N	Sample Description 1: 0.5 cpd Ch1/1cpd Ch2	HSV-1	HSV-1
	B2N	Sample Type: Unknown		Supermix: ddPCR Supermix for Probes	B2M-Hex	B2M-Hex
F	DQ	Target Information:	NTC	HSV-1, Unknown, FAM	DQ	DQ
	HSV		B2M-Hex	B2M-Hex, Unknown, HEX	1 cpd Ch1	2 cpd Ch1
	B2M-Hex	B2M-Hex	B2M-Hex	B2M-Hex	HSV-1	HSV-1
				B2M-Hex	B2M-Hex	B2M-Hex

10. Ensure sample wells are included for droplet reading. For information, see [Template Setup – Including or Excluding Wells](#).
11. If you are finished with your plate configuration, click Save in the upper-right corner and then in the Save dialog box, enter a new name for the template and click Save again.

**Note:** You must save the template with a new name. You cannot overwrite existing templates. If you click the Save button before the plate layout is complete, the file closes and you must reopen it from the saved template location.

## Experiment Types

QX Manager Software offers seven experiment types from which you can choose, each enabling different assay options and analysis tools. [Table 14](#) briefly explains each experiment type.

**Tip:** You can assign multiple experiment types within the plate, but you can assign only one experiment type to each well.

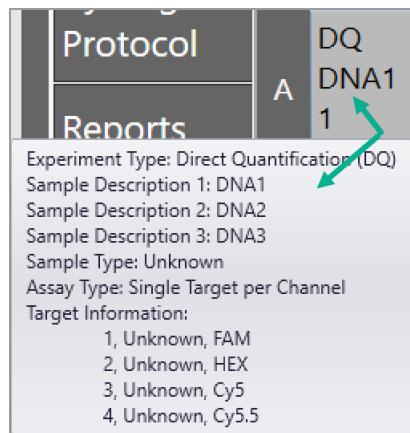
**Table 14. Experiment types**

Type	Description
Direct quantification (DQ)	DQ uses absolute quantification to determine the concentration (copies/μl) of target DNA copies in a sample. DQ measures the number of positive and negative droplets for each target in a well based on their fluorescence amplitude, and uses a Poisson algorithm to calculate the starting concentration of each target DNA molecule.  <b>Note:</b> The ABS experiment type created in QuantaSoft Software versions 1.4 through 1.7 is automatically mapped into the software as a DQ experiment type.
Copy number variation (CNV)	CNV determines concentration and calculates the copy number of an unknown (CNV) target relative to a known reference or references within the same well.
Mutation detection (MUT)	MUT determines concentration and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background.
Rare event detection (RED)	RED determines the concentration of a known mutant or a rare target species relative to a given reference species within a large pool of background DNA.
Drop-off (DOF)	DOF determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits; DOF is designed to support an assay strategy where one probe counts all alleles and one “drop-off” probe sits on top of a predicted cut site or mutation site.
Gene expression (GEX)	GEX determines concentration (as with DQ) and calculates relative expression levels of an unknown target relative to a known reference or references within the same well.
Residual DNA quantification (RDQ)	RDQ provides a precise method for residual host cell DNA quantification.  <b>Note:</b> Users can enter a conversion factor (1/C-value) for a given genome (human, mouse, etc.) to calculate the mass for a well based on the number of copies measured, up to three decimals. The software displays the result in pg/μl of the ddPCR well in the Molecular Weight column of the Data Table. Starting sample concentration can then be back-calculated based on dilution factor.

## Sample Descriptions

QX Manager Software allows you to enter descriptive words or phrases for your sample in up to four fields per well. Descriptors can include information such as research type, dilution factors, and so forth.

**Tip:** Only the information from the first field appears in the Plate Editor layout. However, you can see entries in the second, third, and fourth fields in the Well Data table, or you can pause on a well to display a tool tip, as shown in the following graphic.



## Sample Types

You can assign multiple sample types within the plate, but you can assign only one sample type to each well.

QX Manager Software provides four sample types for selection:

- Unknown
- NTC (no template control)
- Pos Ctrl (positive control) where one or more targets are expected
- Neg Ctrl (negative control), where no response is expected

**Important:** Beginning in v1.2, QX Manager Software can automatically calculate thresholds for positive control wells, but you must select Pos Ctrl from the Sample Type dropdown list. You can select Pos Ctrl during plate setup or in the Analysis module after the run is finished.

## Supermixes

**Important:** Use of unapproved supermixes can harm the instrument and void the warranty.

QX Manager Software offers five PCR supermixes. Each supermix is optimized to deliver maximum PCR efficiency and sensitivity for the amplification and detection of DNA and RNA targets.

- **ddPCR Multiplex Supermix** — For use in nucleic acid sample preparations to amplify and detect DNA targets in probe-based experiments involving multiple targets
- **ddPCR Supermix for Probes (No dUTP)** — For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probe-based assays
- **ddPCR Supermix for Probes** — For use in sample preparation with uracil N-glucosylase (UNG) decontamination protocols to prevent the reamplification of carryover PCR products between experiments
- **ddPCR Supermix for Residual DNA Quantification** — For use in residual DNA detection
- **One-Step RT ddPCR Advanced Kit for Probes** — For use in absolute quantification of target RNA molecules

## Assay Types and Fluorophores

QX Manager Software offers a total of five assay methods, which are explained in [Table 15](#).

**Table 15. Assay types**

Assay type	Description
Single target per channel	Method assuming one or two probe colors (FAM/EvaGreen® and HEX/VIC) and one or two targets per well, with a single target detected per channel. This is the default assay type, with the maximum four rows displayed. You can delete up to three rows.  This assay type is available for the DQ, CNV, MUT, and GEX experiment types.
Amplitude multiplex	Method to increase multiplexing up to four targets per well, with one or two targets detected per channel. The maximum of eight rows are displayed. You can delete up to seven rows.
Probe mix triplex	Triplex mode that allows exactly three targets per well, with one target detected in each of two channels and a third target detected across both channels.
Advanced classification	Method to increase multiplexing rate up to 20 targets per well in any combination of method targets detected per channel. This method requires manual classification of droplets.  This assay type is available only for the DQ experiment type.
Basic drop-off	This mode allows two groups of two targets each. Group 1 is shown by default, with one target in FAM <u>and</u> HEX/VIC and one target in FAM <u>or</u> HEX/VIC.  This assay type is available only for the DOF experiment type.

The experiment type you select determines the available assay methods. [Table 16](#) displays the sample type associated with each assay method.

**Table 16. Assay types by experiment type**

Assay type	Experiment type						
	DQ	CNV	MUT	DOF	GEX	RDQ	RED
Single target per channel	✓	✓	✓		✓	✓	✓
Amplitude multiplex	✓	✓	✓		✓	✓	✓
Probe mix triplex	✓	✓	✓		✓	✓	✓
Advanced classification	✓						✓
Basic drop-off					✓		

QX Manager Software automatically populates the fluorophore in each channel for each target but you can change the default selections

Use [Table 17](#) to select from the fluorophore options for each assay method listed.

**Table 17. Fluorophore options**

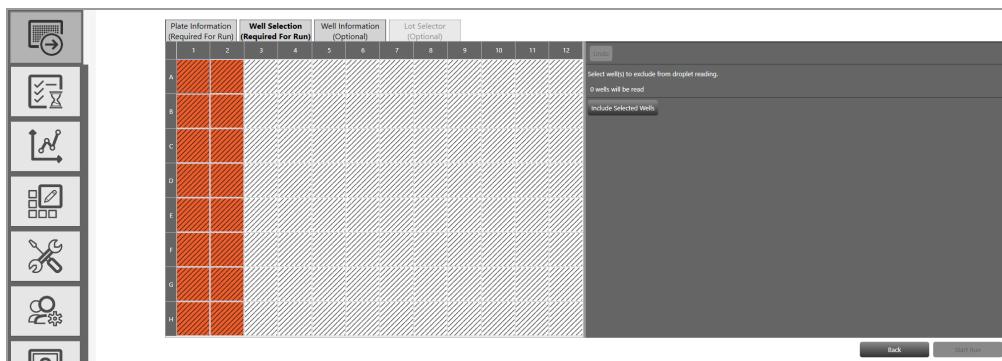
Assay method	Signal Ch1	Signal Ch2
Single target per channel, for 1 to 2 targets	FAM	HEX
	EvaGreen®	VIC
	None	None
Amplitude multiplex, for 1 to 4 targets	FAM Hi	HEX Hi
	FAM Lo	HEX Lo
	EvaGreen® Hi	VIC Hi
	EvaGreen® Lo	VIC Lo
	None	None
Probe mix triplex, for 3 targets	FAM	HEX
	EvaGreen®	VIC
	None	None
Advanced classification	FAM 1-10	HEX 1-10 VIC 1-10

## Template Setup – Including or Excluding Wells

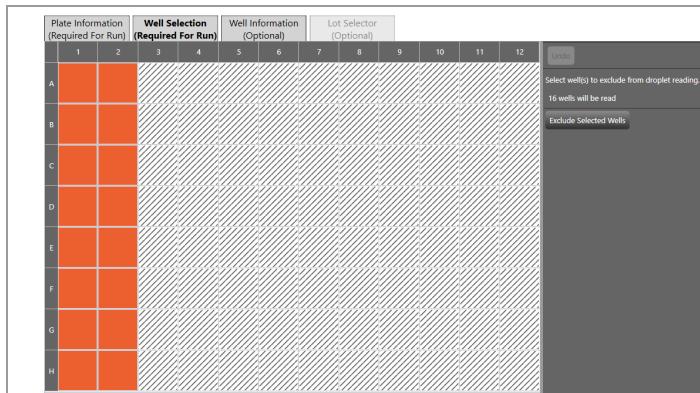
Optionally, you can include or exclude wells from droplet reading.

### To identify wells to include in the experiment run

1. Select the Well Selection tab.
2. In the Plate Editor layout, select the wells containing sample.



3. Click Include Selected Wells.



On the right, QX Manager Software specifies the number of wells that will be included in the droplet reading run.

# Chapter 9 Data Analysis Module Overview

As the instrument reads droplets in each well, the QX200 Droplet Reader collects raw data. At the end of each droplet reading run, QX Manager Software uses the raw data to produce a data file, which contains analysis information based on the number of positive and negative droplets identified for each target in each sample. The fraction of positive droplets is fit to a Poisson algorithm to determine the starting concentration of the target DNA molecule in units of copies/ $\mu$ l input.

When you open the data file in the Analysis module, you can view the data with the automatic analysis thresholds and calculations, or you can choose different options to recalculate, replot, or recolor the results.

When you select the Data Analysis tab, you can access the standard analysis and gene study modules.

- ddPCR Data Analysis
- Gene Study Analysis



The Data Analysis module functionality allows you to

- View your droplet data in 1D and 2D plots
- Analyze multiple targets within a well using amplitude multiplexing and probe mixing strategies
- Detect genomic editing events and non-wild type events with a “drop-off assay” analysis option
- Visualize and export data with improved flexibility
- Generate reports for different analysis types

The Gene Study module allows you to

- Collect data by adding one or more data files
- View gene expression calculations across multiple experiments

**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.

This section summarizes the analysis functionality, and also describes the general options governing your chart displays. More detailed information, including specific information and uses for each analysis window, as well as information on data calculations, is contained in [Chapter 10, Data Analysis Methodology](#).

For information on analysis reporting, see [Report Elements on page 145](#). For information on gene studies, see [Gene Study Module on page 153](#).

## Opening a Data File for Analysis

You can open the following file types in the Data Analysis module:

- .qlps

If you open this file type, the Plate Editor appears by default. When you save this file type in QX Manager Software, the software automatically saves the file as a .ddpcrs file. This creates a new file and does not overwrite the original.

- .ddpcrs

Analysis data files are typically stored in one of the following defined storage locations:

- Personal folder for the individual user on the connected computer or the system folder specified for Preferred locations in the System Settings window

**Note:** Your personal folder location is displayed in your user preferences. You can open the System Settings window to see the preferred or shared storage locations.

**Important:** If your system administrator has specified preferred location file paths for all users, the file paths specified in user preferences are overridden.

- Shared folder in a different location, such as a network path

**Note:** The software also saves data files to a shared folder if it is accessible.

To open data files, you must be on a computer on which QX Manager Software is installed and have access to the storage locations.

## To open a data file for analysis

1. Open QX Manager Software and log into the application.
2. Select the Data Analysis tab.



Available data files are displayed under the following headings.

- **Recent Datafiles**

Displays a list of files you have recently opened.

- **System Datafiles or My Datafiles**

Only one of the following locations is available:

- If your system administrator has enabled Preferred Locations for all users, all data files are stored in that folder, and files appear under System Datafiles.
- If preferred locations are not enabled for all users, your data files are saved to your personal folder and appear under My Datafiles.

- **Shared Datafiles**

Displays a list of data files saved to the shared location, if one has been specified in the System Settings window.

**Important:** Data files are always saved to either My Datafiles or System Datafiles, data files are also saved to the Shared location (such as a network directory) if it is accessible when the software saves the data file.

3. Click a file to open it in the Analysis module.

Tabs for each analysis and data window appear on the left of the Analysis window.

**Tip:** From the computer attached to the QX200 Droplet Reader, you can also open the data file from the Run Status window. In the completed files section, click the completed run icon (✓) for the file, and then click Launch Analysis.

## Saving a Data File

You can save data files with or without modifications, with the same or different file names, and in the same or different locations.

**Note:** To save a file with the same file name (Save button), you must be assigned the Overwrite Existing Datafile name user privilege.

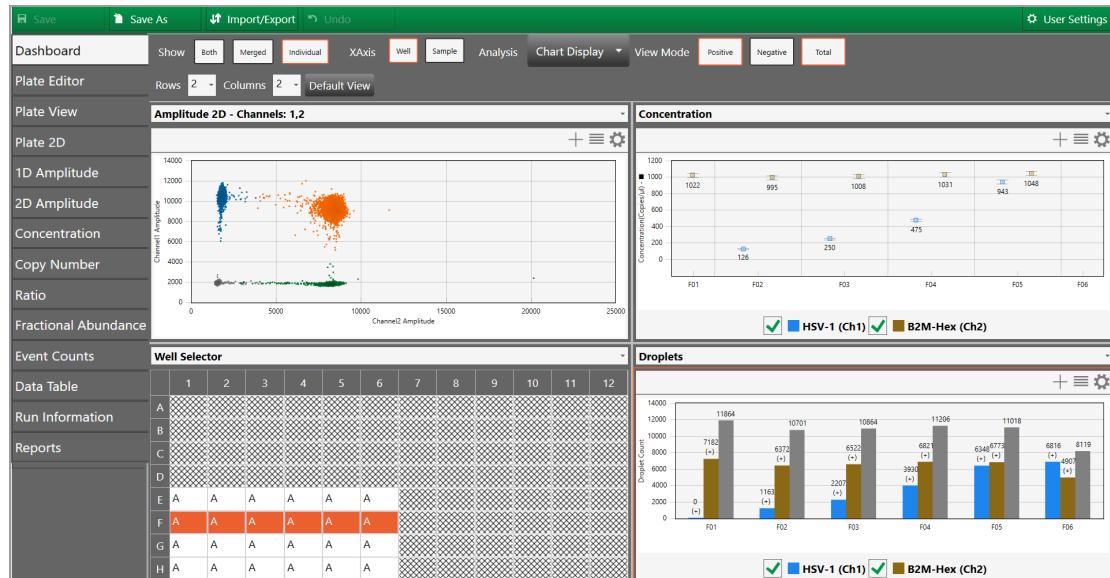
### To save a data file

1. Do one of the following:
  - Click Save to save the file under the same file name and in the same location.
  - Click Save as to save the file under a different file name or in a different location.
    - If you are saving the file under the same name and in the same location, QX Manager Software saves the file immediately.
    - If you are saving the file under a different file name, or in a different location, continue to Step 3.
2. Enter the Save Reason, and then click Save As.
3. Enter your user name and password, and then click Sign in.
  - If you are saving the file under the same name and in the same location, QX Manager Software saves the file immediately.
  - If you are saving the file under a different file name, or in a different location, continue to Step 3.
4. Navigate to the new location and/ or enter a new file name.
5. Click Save.

## Analysis Dashboard

When you open a data file, the Analysis module opens to a Dashboard window containing default analysis and data panes in a summary view. You can customize the view to increase or decrease the number of panes that appear in the Dashboard, and you can also change the selection of panes that appear.

For information on the available options in the Dashboard view, see [Dashboard Options on page 83](#).



**Note:** If you open a .qlps file, the Plate Editor opens as the default view and you must select the Dashboard tab to open it.

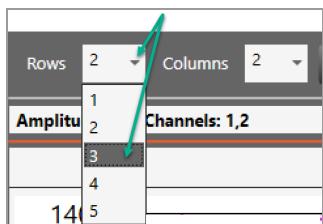
From the tabs on the left, you can access plate views, amplitude charts, Data Visualization distribution charts, droplet counts, and a complete data table. You can also view data specific to the run in the Run Information window, and run reports on any of the analysis charts from the Reports window.

## Modifying the Dashboard Display

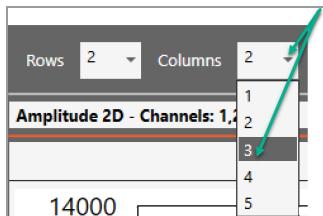
You can modify the column number and row number to display any or all of the analysis windows simultaneously.

### To change the number of windows in the display

1. Tap the dropdown arrow for Rows and then select a different number.



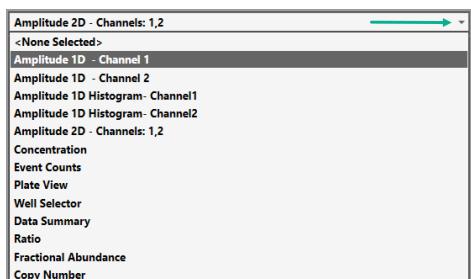
2. Tap the dropdown arrow for Columns, and then select a different number.



You can also use the dropdown menus to change the windows that appear in the Dashboard.

### To display a different window in a particular Dashboard slot

1. Tap the dropdown arrow in the upper-right corner to display the list of available windows, and then select a window.

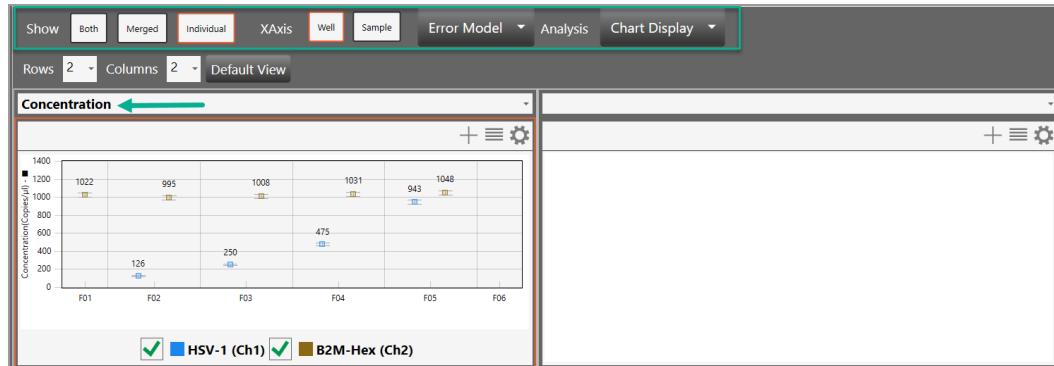


The dropdown list closes and the window changes immediately.

## Dashboard Options

From the Dashboard window, depending on the individual dashboard window you have selected, you can use a range of options as described below:

- When you select a window, the options that appear in the menu bar correspond to the options you see if you open the same window from the side menu. For example, if you select the Dashboard Concentration chart, the Concentration menu options appear.



- You can reset the entire dashboard display by adding or removing rows or columns to display more or fewer individual windows.



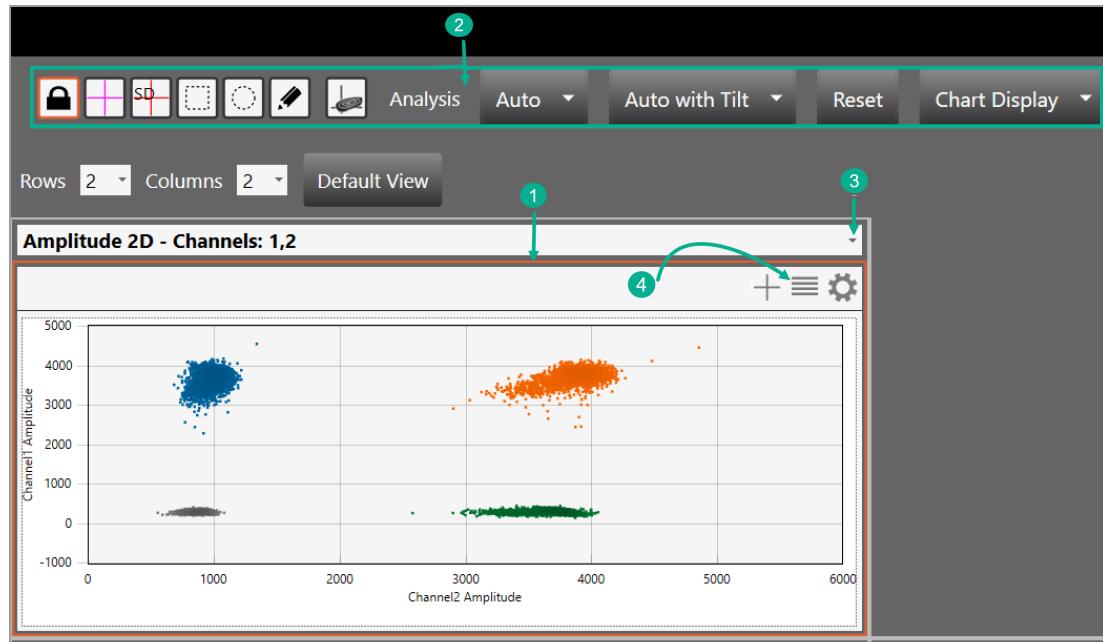
Click Default View to return to the default display of two rows and two columns.

- From any window you can
  - Expand or collapse the window
  - Copy, save, or print the image
  - Select display, scale, axis, and logarithmic options
  - Use the dropdown list to replace the current display with a different display
  - Use the menu bar options for the particular chart type

When you add the Droplets chart to the Dashboard (highlighted below). QX Manager Software adds the View Mode options.

**Note:** The Droplets window is identical to the Event Counts window.

Table 18 on page 84 describes the option sets identified in the following graphic.



**Table 18. Dashboard options**

Item	Description
1	Border to identify selected window
2	Toolbar corresponding to the selected window type in the Dashboard
3	Dropdown arrow to replace the current display with a new display
4	Chart options

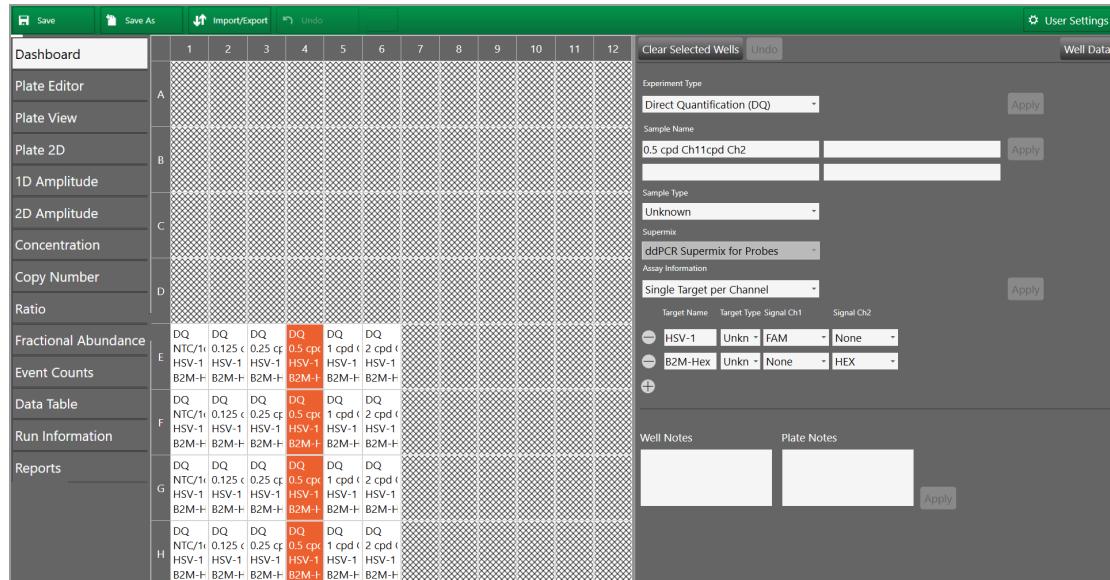
# Plate View Windows

In the QX Manager Software Analysis module you can open the following windows that contain data on all wells in the run:

- **Plate Editor (analysis view)** — Displays the experiment type, sample names, sample types, supermixes, and assay type for each well in the run (similar to the Template Setup Plate Editor)
- **Plate View** — Displays concentration results in a text format for each processed well
- **2D Plate View** — Displays a two-dimensional amplitude chart for each processed well

## Plate Editor — Analysis View

You can use the Plate Editor tab in the Analysis module to select wells, and then view or change the experiment parameters. Similar to other Plate Editor displays, the Analysis Plate Editor displays the experiment information for each well in the run.



**Important:** You can change all fields except the supermix.

Wells you select in the Analysis Plate Editor determine the data initially displayed in other analysis windows.

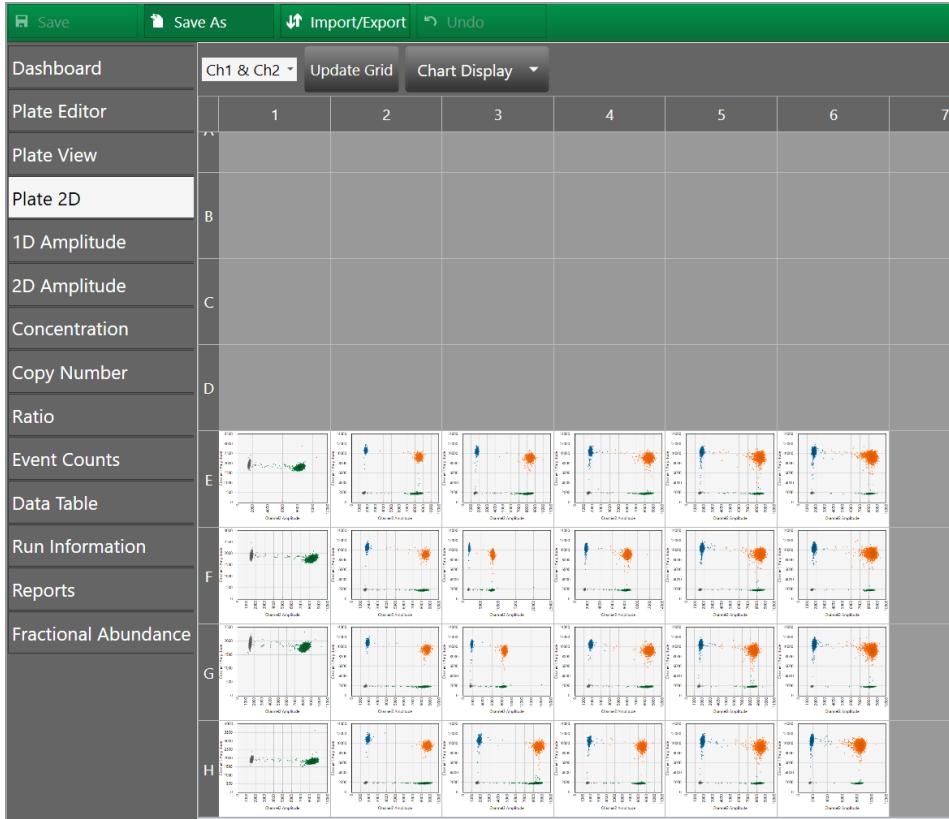
## Plate View

The Plate View tab provides, within the plate layout grid, a text view of the calculated concentration for each target in each well processed in the run. For information on the concentration results in chart format, see [Concentration on page 131](#).

	1	2	3	4	5	6	7	8	9	10	11	12
Dashboard												
Plate Editor	A											
Plate View												
Plate 2D	B											
1D Amplitude												
2D Amplitude												
Concentration												
Copy Number												
Ratio												
Event Counts												
Data Table	E	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ
	NTC/1cpd Ch2	0.125 cpd Ch1/1cpd Ch2	0.25 cpd Ch1/1cpd Ch2	0.5 cpd Ch1/1cpd Ch2	1 cpd Ch1/1cpd Ch2	2 cpd Ch1/1cpd Ch2						
	U HSV-1	No Call	U HSV-1	121.01	U HSV-1	238.41	U HSV-1	481.78	U HSV-1	956.36	U HSV-1	1939.3
	U B2M-Hex	1050.3	U B2M-Hex	1007.9	U B2M-Hex	1046	U B2M-Hex	1017.8	U B2M-Hex	1020	U B2M-Hex	996.9
Run Information	F	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ
	NTC/1cpd Ch2	0.125 cpd Ch1/1cpd Ch2	0.25 cpd Ch1/1cpd Ch2	0.5 cpd Ch1/1cpd Ch2	1 cpd Ch1/1cpd Ch2	2 cpd Ch1/1cpd Ch2						
	U HSV-1	No Call	U HSV-1	126.43	U HSV-1	249.55	U HSV-1	474.58	U HSV-1	943.27	U HSV-1	No Call
	U B2M-Hex	1021.7	U B2M-Hex	994.51	U B2M-Hex	1007.8	U B2M-Hex	1031.1	U B2M-Hex	1048.1	U B2M-Hex	No Call
Reports	G	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ
	NTC/1cpd Ch2	0.125 cpd Ch1/1cpd Ch2	0.25 cpd Ch1/1cpd Ch2	0.5 cpd Ch1/1cpd Ch2	1 cpd Ch1/1cpd Ch2	2 cpd Ch1/1cpd Ch2						
	U HSV-1	No Call	U HSV-1	124.21	U HSV-1	238.75	U HSV-1	497.89	U HSV-1	996.06	U HSV-1	1916.6
	U B2M-Hex	1012.2	U B2M-Hex	1042.8	U B2M-Hex	1026.1	U B2M-Hex	1040.4	U B2M-Hex	1053.3	U B2M-Hex	1039.7
Fractional Abundance	H	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ	DQ
	NTC/1cpd Ch2	0.125 cpd Ch1/1cpd Ch2	0.25 cpd Ch1/1cpd Ch2	0.5 cpd Ch1/1cpd Ch2	1 cpd Ch1/1cpd Ch2	2 cpd Ch1/1cpd Ch2						
	U HSV-1	No Call	U HSV-1	128.28	U HSV-1	243.35	U HSV-1	474.69	U HSV-1	974.42	U HSV-1	2026.4
	U B2M-Hex	1028.6	U B2M-Hex	1040.4	U B2M-Hex	1042.2	U B2M-Hex	1025.9	U B2M-Hex	1021.6	U B2M-Hex	1047.1

## 2D Plate View

To facilitate comparisons of amplitude in the processed wells, the Plate 2D tab provides a two-dimensional amplitude view in a separate chart for each well. To see a larger representation of a well, select the well in the 2D Amplitude view.



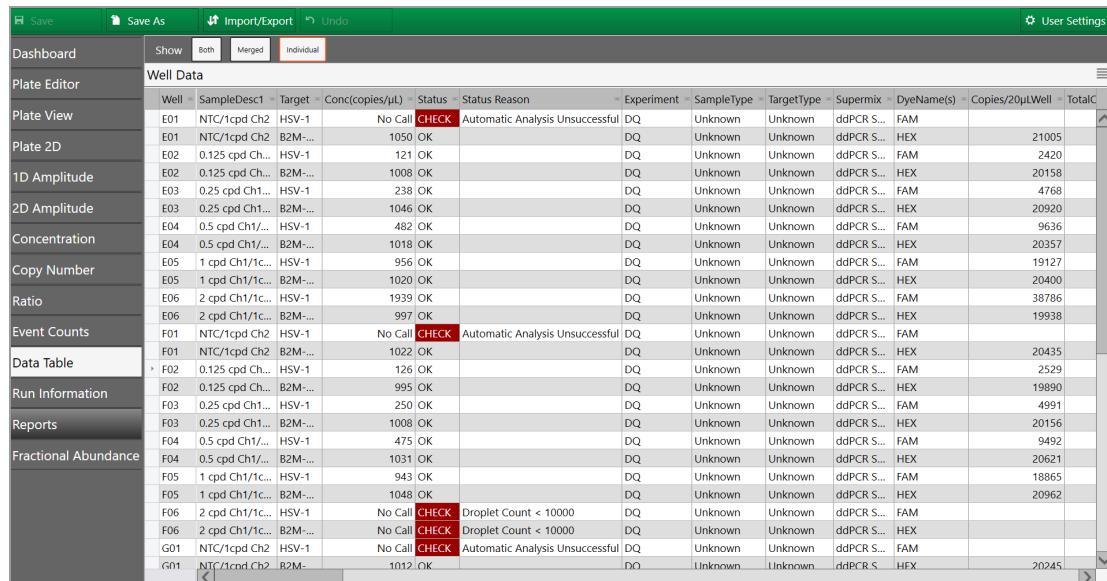
## Data Windows

QX Manager Software provides the following table views for your well and run data:

- **Data Table** — available from the main tabs on the left, shows your configured experiment parameters, plus all calculations from the run
- **Note:** You can display the data in individual well format, merged format, or both.
- **Plate Well Data Table** — available in plate setup windows (including the Analysis module Plate Editor), shows only your configuration information for each individual well
- **Analysis Well Data Table** — available in the Analysis module, shows a subset of configuration and run data for the wells selected in the Well Selector

### Data Table Tab

The Data Table window contains columns of data generated for each target in each well, as droplet clusters are identified through thresholding or manual clustering tools.



The screenshot shows the Data Table tab in the QX Manager Software. The interface includes a top menu with Save, Save As, Import/Export, Undo, and User Settings. Below the menu is a toolbar with buttons for Show (Both, Merged, Individual), a search bar, and a refresh icon. The main area is a data grid with the following columns:

Well	SampleDesc1	Target	Conc(copies/μL)	Status	Status Reason	Experiment	SampleType	TargetType	Supermix	DyeName(s)	Copies/20μLWell	TotalC
E01	NTC/1cpd Ch2	HSV-1		No Call	<span style="background-color: red;">CHECK</span>	Automatic Analysis Unsuccessful	DQ	Unknown	Unknown	ddPCR S...	FAM	
E01	NTC/1cpd Ch2	B2M...	1050	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	21005
E02	0.125 cpd Ch...	HSV-1	121	OK			DQ	Unknown	Unknown	ddPCR S...	FAM	2420
E02	0.125 cpd Ch...	B2M...	1008	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	20158
E03	0.25 cpd Ch1...	HSV-1	238	OK			DQ	Unknown	Unknown	ddPCR S...	FAM	4768
E03	0.25 cpd Ch1...	B2M...	1046	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	20920
E04	0.5 cpd Ch1/...	HSV-1	482	OK			DQ	Unknown	Unknown	ddPCR S...	FAM	9636
E04	0.5 cpd Ch1/...	B2M...	1018	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	20357
E05	1 cpd Ch1/1c...	HSV-1	956	OK			DQ	Unknown	Unknown	ddPCR S...	FAM	19127
E05	1 cpd Ch1/1c...	B2M...	1020	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	20400
E06	2 cpd Ch1/1c...	HSV-1	1939	OK			DQ	Unknown	Unknown	ddPCR S...	FAM	38786
E06	2 cpd Ch1/1c...	B2M...	997	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	19938
F01	NTC/1cpd Ch...	HSV-1		No Call	<span style="background-color: red;">CHECK</span>	Automatic Analysis Unsuccessful	DQ	Unknown	Unknown	ddPCR S...	FAM	
F01	NTC/1cpd Ch2	B2M...	1022	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	20435
F02	0.125 cpd Ch...	HSV-1	126	OK			DQ	Unknown	Unknown	ddPCR S...	FAM	2529
F02	0.125 cpd Ch...	B2M...	995	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	19890
F03	0.25 cpd Ch1...	HSV-1	250	OK			DQ	Unknown	Unknown	ddPCR S...	FAM	4991
F03	0.25 cpd Ch1...	B2M...	1008	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	20156
F04	0.5 cpd Ch1/...	HSV-1	475	OK			DQ	Unknown	Unknown	ddPCR S...	FAM	9492
F04	0.5 cpd Ch1/...	B2M...	1031	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	20621
F05	1 cpd Ch1/1c...	HSV-1	943	OK			DQ	Unknown	Unknown	ddPCR S...	FAM	18865
F05	1 cpd Ch1/1c...	B2M...	1048	OK			DQ	Unknown	Unknown	ddPCR S...	HEX	20962
F06	2 cpd Ch1/1c...	HSV-1		No Call	<span style="background-color: red;">CHECK</span>	Droplet Count < 10000	DQ	Unknown	Unknown	ddPCR S...	FAM	
F06	2 cpd Ch1/1c...	B2M...		No Call	<span style="background-color: red;">CHECK</span>	Droplet Count < 10000	DQ	Unknown	Unknown	ddPCR S...	HEX	
G01	NTC/1cpd Ch2	HSV-1		No Call	<span style="background-color: red;">CHECK</span>	Automatic Analysis Unsuccessful	DQ	Unknown	Unknown	ddPCR S...	FAM	
G01	NTC/1cpd Ch2	R2M-	1012	OK			DQ	Unknown	Unknown	ddPCR S...	HFX	20245

## Data Table Column Definitions

Table 19 defines each field in the Data Table.

**Table 19. Data Table columns**

Column name	Content description
Well	The well location in the plate <b>Note:</b> The instrument accepts 96-well plates only.
Sample description 1	Words or phrases to describe the sample in each well
Sample description 2	<b>Note:</b> Only the sample description 1 data appears in plate layout views but you can pause on a well in the Plate Editor to display a tool tip containing the entries in all completed Sample description fields.
Sample description 3	
Sample description 4	
Target	Target name from Plate Editor tab
Conc(copies/μl)	Concentration of the target molecules recorded as copies per microliter
Molecular Weight (pg/μl)	Mass concentration of DNA in the well in pg/μl
Status	Contains either Check, OK, Multi, or Manual <ul style="list-style-type: none"> <li>■ If Check, either the automatic analysis for the well failed or fewer than 10,000 droplets were counted in the well</li> <li>■ If OK, enough droplets were counted to trigger automatic analysis</li> <li>■ If Multi, the data was automatically analyzed as part of a multi-well selection</li> <li>■ If Manual, the droplets were analyzed manually</li> </ul>
Status Reason	Identifies the reason for the status (unsuccessful automatic analysis, not enough droplets counted, or droplets analyzed manually)
Experiment	Experiment type selected for the well in the Plate Editor
SampleType	Sample type selected for the well in the Plate Editor
TargetType	Target type selected for each dye (FAM or EvaGreen® and HEX or VIC) in the Plate Editor

**Table 19. Data Table columns, continued**

Column name	Content description
Supermix	Supermix selected for the well in Plate Editor
DyeName(s)	Dye assigned to channels 1 and 2, respectively, for the well in the Plate Editor
Copies/20 $\mu$ lWell	Concentration of the target normalized to a volume of 20 $\mu$ l
TotalConfMax	For merged wells the high error bar for the target concentration of the combined wells at a 95% confidence interval
TotalConfMin	For merged wells the low error bar for the target concentration of the combined wells at a 95% confidence interval
PoissonConfMax	Maximum target concentration normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval
PoissonConfMin	Minimum target concentration normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
Accepted Droplets	Total number of droplets accepted by quality algorithm
Positives	Number of droplets that contain the target
Negatives	Number of droplets that do not contain the target
Ch1+Ch2+	Number of droplets that contain both channel 1 and channel 2 targets
Ch1+Ch2-	Number of droplets that contain just the channel 1 target
Ch1-Ch2+	Number of droplets that contain just the channel 2 target
Ch1-Ch2-	Number of droplets that contain neither channel 1 nor channel 2 targets
Linkage	Shows the concentration of copies of two targets that are predicted to be targeting a linked DNA molecule, calculated by an overabundance of copies in the double-positive cluster versus the expected value in copies per $\mu$ l
CNV	Copy number calculated for the target relative to the reference
TotalCNVMax	For merged wells, the high error bar for the copy number of the combined wells at a 95% confidence interval

**Table 19. Data Table columns, continued**

Column name	Content description
TotalCNVMin	For merged wells, the low error bar for the copy number of the combined wells at a 95% confidence interval
PoissonCNVMax	Maximum copy number normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval
PoissonCNVMin	Minimum copy number normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
ReferenceCopies	Copy number identified for the reference target in the Plate Editor Default is 2, indicating 2 copies per diploid genome.
UnknownCopies	This field is currently unused in QX Manager Software
Threshold1	Threshold value of first threshold line from left to right and bottom to top
Threshold2	Threshold value of second threshold line from left to right and bottom to top
Threshold3	Threshold value of third threshold line from left to right and bottom to top
ThresholdSigmaAbove	Distance of the threshold from the mean of the negative cluster, as a multiple of the negative cluster standard deviation
ThresholdSigmaBelow	Distance of the threshold from the mean of the positive cluster, as a multiple of the positive cluster standard deviation
ReferenceUsed	Identifies which target was used as a reference
Ratio	The ratio of the target against the identified reference
TotalRatioMax	For merged wells the high error bar for the ratio of the unknown against the reference of the combined wells at a 95% confidence interval
TotalRatioMin	For merged wells the low error bar for the ratio of the unknown against the reference of the combined wells at a 95% confidence interval

**Table 19. Data Table columns, continued**

Column name	Content description
PoissonRatioMax	Maximum ratio of the unknown against the reference normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval
PoissonRatioMin	Minimum ratio of the unknown against the reference normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
FractionalAbundance	Calculation of fractional abundance of this unknown target vs. the reference target
TotalFractionalAbundanceMax	For merged wells the high error bar for the fractional abundance of the combined wells at a 95% confidence interval
TotalFractionalAbundanceMin	For merged wells the low error bar for the fractional abundance of the combined wells at a 95% confidence interval
PoissonFractionalAbundanceMax	Maximum fractional abundance normalized for the high error bar of the droplet Poisson distribution for the 95% confidence interval
PoissonFractionalAbundanceMin	Minimum fractional abundance normalized for the low error bar of the droplet Poisson distribution for the 95% confidence interval
MeanAmplitudeOfPositives	Mean amplitude value of all droplets that contain the target
MeanAmplitudeOfNegatives	Mean amplitude value of all droplets that contain no target
MeanAmplitudeTotal	Mean amplitude value of all droplets
ExperimentComments	Not used in QX Manager Software. Data populates this field only from files created in QuantaSoft versions 1.4 – 1.7, if comments were added.
MergedWells	Identifies which wells were merged together
TotalConfidenceMax68	For merged wells the high error bar for the target concentration of the combined wells at a 68% confidence interval
TotalConfidenceMin68	For merged wells the low error bar for the target concentration of the combined wells at a 68% confidence interval
PoissonConfidenceMax68	Maximum target concentration normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval

**Table 19. Data Table columns, continued**

Column name	Content description
PoissonConfidenceMin68	Minimum target concentration normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval
TotalCNVMax68	For merged wells the high error bar for the copy number of the combined wells at a 68% confidence interval
TotalCNVMin68	For merged wells the low error bar for the copy number of the combined wells at a 68% confidence interval
PoissonCNVMax68	Maximum target concentration normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval
PoissonCNVMin68	Minimum target concentration normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval
TotalRatioMax68	For merged wells the high error bar for the ratio of the unknown against the reference of the combined wells at a 68% confidence interval
TotalRatioMin68	For merged wells the low error bar for the ratio of the unknown against the reference of the combined wells at a 68% confidence interval
PoissonRatioMax68	Maximum ratio of the unknown against the reference normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval
PoissonRatioMin68	Minimum ratio of the unknown against the reference normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval
TotalFractionalAbundanceMax68	For merged wells the high error bar for the fractional abundance of the combined wells at a 68% confidence interval
TotalFractionalAbundanceMin68	For merged wells the low error bar for the fractional abundance of the combined wells at a 68% confidence interval
PoissonFractionalAbundanceMax68	Maximum fractional abundance normalized for the high error bar of the droplet Poisson distribution for the 68% confidence interval

**Table 19. Data Table columns, continued**

Column name	Content description
PoissonFractionalAbundanceMin68	Minimum fractional abundance normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval plate list after correcting the issue
TiltCorrected	Indicates if tilt correction was applied to the well <b>Note:</b> Tilt correction rotates the angles from the double-negative cluster centroid to each droplet, such that the two single positive cluster centroids are orthogonal with the double-negative cluster centroid.

## Plate Well Data Table

From the Plate Editor you can access a window containing the Well Data table, which displays configuration and run information on all wells containing sample.

**Note:** You cannot edit this table.

**Table 20. Plate Well Data Table columns**

Column name	Content description
Well	Well number (A01 through H12)
Perform Droplet Reading	Yes, if wells are read No, if they are not
Experiment Type	Experiment Type selected in the Plate Editor: <ul style="list-style-type: none"> <li>■ Direct Quantification (DQ)</li> <li>■ Copy Number Variation (CNV)</li> <li>■ Mutation Detection (MUT)</li> <li>■ Rare Event Detection (RED)</li> <li>■ Drop-off (DOF)</li> <li>■ Gene Expression (GEX)</li> <li>■ Residual DNA Quantification (RDQ)</li> </ul>
Sample description 1	Words or phrases to identify each sample, as entered in the Plate Editor
Sample description 2	<b>Note:</b> You can enter up to four sample descriptions for each well.
Sample description 3	
Sample description 4	
Sample Type	Sample type selected in the Plate Editor: <ul style="list-style-type: none"> <li>■ Unknown</li> <li>■ No template control (NTC)</li> <li>■ Positive control (Pos-Ctrl)</li> <li>■ Negative control (Neg-Ctrl)</li> </ul>

**Table 20. Plate Well Data Table columns, continued**

Column name	Content description
Supermix Name	Supermix selected in the Plate Editor: <ul style="list-style-type: none"> <li>■ ddPCR Multiplex Supermix</li> <li>■ ddPCR Supermix for Probes (no dUTP)</li> <li>■ ddPCR Supermix for Probes</li> <li>■ ddPCR Supermix for Residual DNA Quantification</li> <li>■ One-Step RT-ddPCR Advanced Kit for Probes</li> <li>■ ddPCR EvaGreen® Supermix</li> </ul>
Plex Mode	Assay method selected in the Plate Editor: <ul style="list-style-type: none"> <li>■ Single Target per Channel</li> <li>■ Amplitude Multiplex</li> <li>■ Probe Mix Triplex</li> <li>■ Advanced Classification Method</li> </ul>
Target Name	Target name entered in the Plate Editor tab
Target Type	Target type selected in the Plate Editor tab
Signal Channel 1	FAM, EvaGreen®, or None
Signal Channel 2	HEX, VIC, or None
Reference Copies	Copy number identified for the reference target in the Plate Editor tab
Well Notes	Entries by the user
Plot?	If selected, target will be used to calculate the copy number for the well
RDQ Conversion Factor	The Residual DNA Quantification conversion factor for the well  <b>Note:</b> RDQ is only available in Residual DNA Quantification experiments.

## Analysis Well Data Table

A well data table containing both configuration and run data appears in the analysis windows, next to the Well Selector. This table displays a subset of data columns for only the wells selected in the Plate Editor or Well Selector.

Well Data							
Well	SampleDesc1	Target	Conc(copies/µL)	Status	Experiment	SampleType	+
F01	NTC/1cpd Ch2	HSV-1		No Call	CHECK	DQ	Unknc
F01	NTC/1cpd Ch2	B2M-...		1022	OK	DQ	Unknc
F02	0.125 cpd Ch...	HSV-1		126	OK	DQ	Unknc
F02	0.125 cpd Ch...	B2M-...		995	OK	DQ	Unknc
F03	0.25 cpd Ch1...	HSV-1		250	OK	DQ	Unknc
F03	0.25 cpd Ch1...	B2M-...		1008	OK	DQ	Unknc
F04	0.5 cpd Ch1/...	HSV-1		475	OK	DQ	Unknc

Table 21 defines each column in the consolidated table.

Table 21. Fields in the Analysis Well Data table

Column name	Content description
Well	The well location in the plate the sample is from
Sample description 1	The sample name used to identify the well in the Plate Editor tab
Sample description 2	
Sample description 3	
Sample description 4	
Target	Target Name from Plate Editor tab
Conc (copies/µL)	Concentration identified using the Poisson calculation
Status	Contains either Check, OK, Multi, or Manual <ul style="list-style-type: none"> <li>■ If Check, either the automatic analysis for the well failed or fewer than 10,000 droplets were counted in the well</li> <li>■ If OK, enough droplets were counted to trigger automatic analysis</li> <li>■ If Multi, the data was automatically analyzed as part of a multi-well selection</li> <li>■ If Manual, the droplets were analyzed manually</li> </ul>
Experiment	Experiment type from the Plate Editor
SampleType	Sample type from the Plate Editor

**Table 21. Fields in the Analysis Well Data table, continued**

<b>Column name</b>	<b>Content description</b>
TargetType	Target type assigned to the target in the Plate Editor
Supermix	Supermix from the Plate Editor
DyeName(s)	Fluorophore assigned to Ch1 and Ch2 to a target in the Plate Editor
Accepted Droplets	Total number of droplets accepted by quality algorithms
Positives	Number of droplets that contain the target
Negatives	Number of droplets that do not contain the target

## Analysis Windows

In its Analysis module, QX Manager Software provides amplitude displays and Data Visualization charts in the Analysis module.

### Amplitude Charts

Amplitude charts illustrate the droplets by degree of dye fluorescence. QX Manager Software displays fluorescence for positive and negative targets identified with FAM/EvaGreen® and HEX/VIC fluorophores in the following analysis windows:

- 1D Amplitude
- 2D Amplitude

### Data Visualization Charts

Data visualization charts show calculations based on positive droplet concentrations. QX Manager Software counts the number of positive and negative droplets for each fluorophore in each sample, and then corrects the concentration based on a Poisson distribution to determine the concentration of the target DNA molecules in units of copies/ $\mu$ l in the following analysis windows:

- Concentration
- Copy Number
- Ratio
- Fractional Abundance

When you open the analysis data file in QX Manager Software and select one or more wells in the grid, the software plots each calculated Poisson distribution average as a data point in the grid. You can pause on the data point to see the high, median, and low numbers.

**Note:** You can display the data using a confidence level of either 68% or 95% (default setting).

### Event Counts

Event counts record the number of droplets in which the target was found (positive) and was not found (negative). The Event Counts tab shows a bar chart view of the number of positive, negative, and total droplets for each of the targets in each of the selected wells.

**Note:** In the Dashboard display, event counts are shown in the Droplets window.

For more detailed information, see [Chapter 10, Data Analysis Methodology](#).

## Analysis Display and Output Options

QX Manager Software provides the following miscellaneous option types in the Analysis module:

- **Universal Options** — available from the primary toolbar to save, import, or export data files
- **Chart Display Preferences** — to change how your chart appears in the analysis window
- **Chart Scale Options** — to change the scale represented on an axis
- **Chart Menu Options** — available in chart views (from the  icon) to copy, save, and print chart images. In Amplitude charts showing data points, you can show or hide
- **Table Menu Options** — available in table views (from the  icon) to copy data, export data to Excel or CSV files, and show or hide columns

You can also expand the chart display to full-screen size and then return to the original display.

### Universal Options



Universal options are explained in [Table 22](#), and are available in all Analysis module windows.

**Table 22. Universal options**

Option	Description
Save	Saves changes to the analysis file under its current file name or file extension
Save as	Saves the file as a different file extension (for example, .qlps to ddpcrs to .ddpcrs, or saves changes to the file under a different file name)
Import/export	Imports or exports plate setup data
Undo	Enabled in the Plate Editor only
User Settings	Allows you to restore your user preferences to the default settings and to show or hide color matrix change messages

### To save the data file

1. Click Save or Save As.
  - If you click Save, the software immediately saves any changes you made under the original file name.
  - If you click Save As, continue to Step 2.
2. In the Save As dialog box, enter a new file name.
3. (Optional) enter a file location.
4. Click Save.

The file is saved with the new file name.

### To import or export setup or analysis data

1. Click Import/Export.

[Table 23](#) describes the available import/export options.

**Note:** If there is unsaved data, the software prompts you to save the file before its contents can be imported or exported.

**Table 23. Import/export options**

Import/export option	Description
Import plate setup CSV	Import plate layout data from a CSV file to the Plate Editor  <b>Note:</b> This option is only available in the Plate Editor. The new plate layout overwrites the existing layout.
Export plate setup CSV	Export a configured plate layout to a CSV file  <b>Note:</b> This option is only available in the Plate Editor.
Import plate setup	Import a plate layout from a plate template (ddplt) file  <b>Note:</b> This option is only available in the Plate Editor.
Export plate setup	Export the plate layout to a plate template file.  <b>Note:</b> This option is only available in the Plate Editor.
Export visible data to CSV	Export only the data from the wells you selected in the Well Selector to a CSV file

**Table 23. Import/export options, continued**

Import/export option	Description
Export amplitude data	Exports the data to an Excel spreadsheet, and indicates whether or not the target was found (1 = yes, 0 = no)
Export cluster data	Exports the data to an Excel spreadsheet <b>Note:</b> Includes (for each well) whether or not the target was found (1 = yes, 0 = no), the droplet count, and the mean and standard deviation for each dye, and the cluster ID
Export linkage data	Exports to a CSV file the number of copies of a target that are present in overabundance, versus. the expected value in copies per $\mu$ l
Export visible charts	Exports all charts that are displayed
Print visible charts	Prints all charts that are displayed

[Table 24](#) shows where the options are available.

**Table 24. Option availability**

Import/export option	Plate editor	Plate view	Plate 2D	Amplitude plots	Data Visualization charts	Event counts	Data table
Import plate setup from CSV	✓						
Export plate setup to CSV	✓						
Import plate setup	✓						
Export plate setup	✓						
Export visible data to CSV				✓	✓	✓	✓
Export amplitude data	✓	✓	✓	✓	✓	✓	✓
Export cluster data	✓	✓	✓	✓	✓	✓	✓

**Table 24. Option availability, continued**

Import/export option	Plate editor	Plate view	Plate 2D	Amplitude plots	Data Visualization charts	Event counts	Data table
Export linkage data	✓	✓	✓	✓	✓	✓	✓
Export visible charts			✓	✓	✓	✓	
Print visible charts			✓	✓	✓	✓	

**Note:** Amplitude plots are available in 1D and 2D, and Data Visualization charts are Concentration, Copy Number, Ratio, and Fractional Abundance. In the Dashboard view corresponding options are available for each plot or chart displayed.

## Expanding or Reducing the Chart

You can expand or reduce the chart size in the following windows:

- Dashboard
- 1D Amplitude
- 2D Amplitude
- Concentration
- Copy Number
- Ratio
- Fractional Abundance
- Event Counts

If you expand an amplitude or Data Visualization chart to full size, the Well Selector and the Well Data table are hidden from view until you collapse the chart again.

### To expand or reduce the chart size

1. To increase the chart size to the full window, click the  icon in the upper-right corner of the chart.
2. To reduce the window to its original size, click the  icon.

## Selecting Chart Display Preferences

The Chart Display preferences primarily control font sizes and positioning. [Table 25](#) explains each preference, as well as the windows in which it is available.

**Table 25. Chart display preferences**

Preference	Window	Description
Channel or Dye	1D Amplitude	Select Channel or Dye to identify your chart accordingly.
	2D Amplitude	If you select Channel, the channel number appears as the chart title. If you select Dye, FAM or HEX appears as the chart title.
Axis Data Label Size	1D Amplitude	Select a different font size.
	2D Amplitude	
	Concentration	
	Copy Number	
	Ratio	
	Fractional Abundance	
	Event Counts	
Chart Data Label Size	Concentration	Select a different font size.
	Copy Number	
	Ratio	
	Fractional Abundance	
	Event Counts	
Chart Marker Size	Concentration	Select a different marker size.
	Copy Number	
	Ratio	
	Fractional Abundance	
Chart Marker Label Position	Concentration	Reposition the marker label.
	Copy Number	
	Ratio	
	Fractional Abundance	

### To select chart display preferences

1. Click the Chart Display button.

2. Select your options.

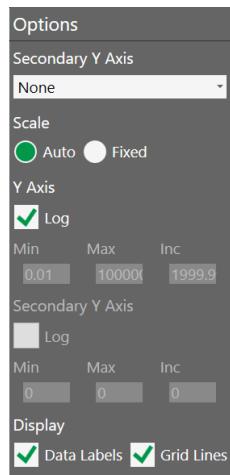
The display changes immediately to reflect your choices.

3. Do one of the following:

- To save your choices as your default settings, click Save as my default. When the success message appears, click OK to close the dialog.
- To close the menu without saving the settings, click anywhere in the chart display.

## Selecting Chart Scale Options

Chart scale options primarily control chart scale displays.



Each preference, as well as the windows in which it is available, is explained in [Table 26](#).

**Table 26. Chart scale options**

Option	Description
Scale	<p>Appears in options for all charts</p> <p>The Scale option determines the numerical range for your chart data:</p> <p>Auto — Selected by default; QX Manager Software automatically displays a scale to match the data distribution.</p> <p>Fixed — Selecting Fixed enables the Axis fields, and allows you to manually adjust the range and increment.</p>
Axis	<p>Appears in options for all charts</p> <p>When the Axis fields are enabled, you can manually adjust the minimum (Min) and maximum (Max) points for the data display on each axis, as well as the increments (Incr).</p> <p>X — Horizontal axis Y — Vertical axis</p> <p><b>Note:</b> You can modify the x axis scale in the 2D Amplitude chart only.</p>
Secondary Y Axis	<p>Appears in options for Concentration only</p> <p>From the dropdown list, you can select from Channel 2 concentration, ratio, copy number, or fractional abundance to display the corresponding data in a secondary row on the y axis.</p>
Log	<p>Appears in options for all Data Visualization charts (Concentration, Copy Number, Ratio, and Fractional Abundance)</p> <p>You can select the Log checkbox to display the y axis in base 10 logarithmic format.</p> <p><b>Note:</b> In the Concentration chart, you can also select Log for the secondary y axis.</p>
Inverse	<p>Appears in options for Ratio and Fractional Abundance only</p> <p>The Inverse option equals the opposing number of the Ratio data point to a total of 2, and the opposing number of the Fractional Abundance data point to a total of 100.</p>
Data labels	<p>Appears in options for all Data Visualization charts</p> <p>A data label appears by default for each point in the chart, but you can clear the checkbox to remove the labels.</p>
Grid Lines	<p>Appears in options for all charts</p> <p>Gridlines appear by default. You can clear the check box to remove them.</p>

### To select chart scale options

1. Click the  icon in the upper-left corner of the chart to display the corresponding chart options.
2. To manually define the chart scale, select Fixed.

**Note:** Auto is the default setting.

3. In the Min, Max, and Inc fields, enter your lower and upper limits, and the increment, for the y axis. For 2D Amplitude, you can also change the scale on the x axis.
4. For Concentration, Copy Number, Ratio, Fractional Abundance, and Event Counts, select an option for a secondary y axis display. To change to a base 10 logarithmic scale on the y axis, select the Log checkbox.
5. For Ratio and Fractional Abundance, to view an inverse calculation, select the Inverse checkbox.
6. To remove the data labels or grid lines from the display, clear the Data Labels or Grid Lines checkbox.

## Using Chart Menu Options

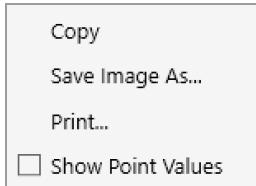
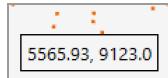


Chart menu options are explained in [Table 27](#).

**Table 27. Chart menu options**

Option	Description
Copy	Copies the chart to the clipboard, which you can paste into a separate document, such as an MS Word file
Save Image as	Saves the chart as an image file
Print	Prints the image
Show Point Values	Shows corresponding point values when you pause on a data point



**Tip:** Select the Show Point Values checkbox to see the data.

Chart menu options are available in the following windows:

- 1D Amplitude
- 2D Amplitude
- Concentration
- Copy Number
- Ratio
- Fractional Abundance
- Event Counts

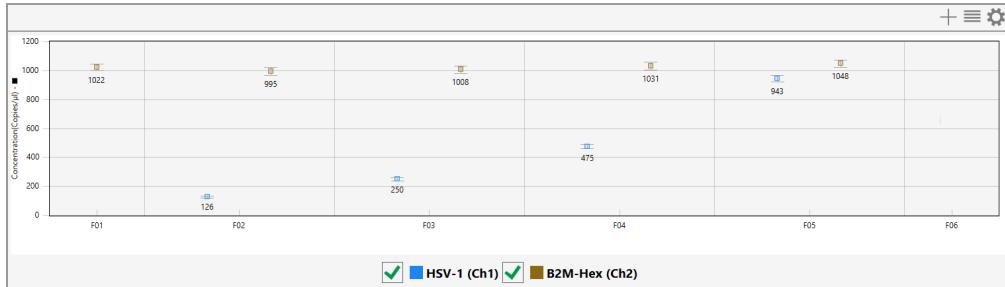
#### To use the chart menu options

1. Select wells in the Well Selector.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E	A	A	A	A	A	A						
F	A	A	A	A	A	A						
G	A	A	A	A	A	A						
H	A	A	A	A	A	A						

Corresponding data appears in the Well Data table and the associated chart.

Well Data						
Well	SampleDesc1	Target	Conc(copies/µL)	Status	Experiment	
F01	NTC/1cpd Ch2	HSV-1	No Call	CHECK	DQ	
F01	NTC/1cpd Ch2	B2M-...	1022	OK	DQ	
F02	0.125 cpd Ch...	HSV-1	126	OK	DQ	
F02	0.125 cpd Ch...	B2M-...	995	OK	DQ	
F03	0.25 cpd Ch1...	HSV-1	250	OK	DQ	
F03	0.25 cpd Ch1...	B2M-...	1008	OK	DQ	
F04	0.5 cpd Ch1/...	HSV-1	475	OK	DQ	



2. In the upper-right corner of the chart, click the menu icon (≡) and select from the dropdown list.
  - Select Copy to copy the chart to the Clipboard.  
**Note:** To paste the data, navigate to the location (for example, a Word file), then right-click and select Paste.
  - Select Save Image As and follow the prompts to save the file.
  - Select Print and follow the prompts to print the image.
3. Click anywhere in the display to close the dialog box.

## Using Table Menu Options

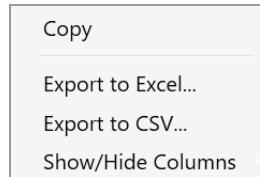


Table menu options are explained in [Table 28](#).

**Table 28. Table options**

Option	Description
Copy	Copies the information from the selected wells to the clipboard
Export to Excel	Exports the data to an .xlsx file
Export to CSV	Exports the data to a .csv file

**Table 28. Table options, continued**

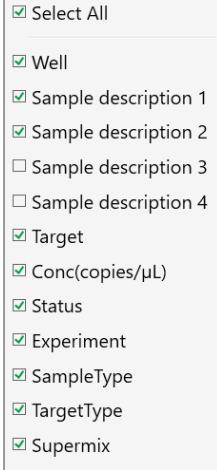
Option	Description
Show/Hide Columns	<p>Add or remove columns from a table display</p> <p>When you select Show/Hide Columns, the following dialog box opens. Select or clear check boxes to show or hide columns in the data table.</p> 

Table menu options are available in the following windows:

- 1D Amplitude
- 2D Amplitude
- Concentration
- Copy Number
- Ratio
- Fractional Abundance
- Event Counts

**Tip:** You can access the menu icon from the individual windows, or from the windows in the Dashboard display.

### To use the table menu options

If you are using these options in the Data Table, skip to Step 3.

1. Select wells in the Well Selector.

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E	A	A	A	A	A	A						
F	A	A	A	A	A	A						
G	A	A	A	A	A	A						
H	A	A	A	A	A	A						

Corresponding data appears in the Well Data table.

Well Data						
Well	SampleDesc1	Target	Conc(copies/µL)	Status	Experiment	
F01	NTC/1cpd Ch2	HSV-1	No Call	CHECK	DQ	
F01	NTC/1cpd Ch2	B2M-...	1022	OK	DQ	
F02	0.125 cpd Ch...	HSV-1	126	OK	DQ	
F02	0.125 cpd Ch...	B2M-...	995	OK	DQ	
F03	0.25 cpd Ch1...	HSV-1	250	OK	DQ	
F03	0.25 cpd Ch1...	B2M-...	1008	OK	DQ	
F04	0.5 cpd Ch1/...	HSV-1	475	OK	DQ	

2. In the upper-right corner of the table, click the menu icon (≡) and select from the dropdown list.

- To copy the data from the selected wells to the Clipboard, select Copy.

**Note:** To paste the data, navigate to the location (for example, an Excel file) and then right-click and select Paste.

- To export your data, select Export to Excel or Export to CSV, and then follow the prompts to save the file.

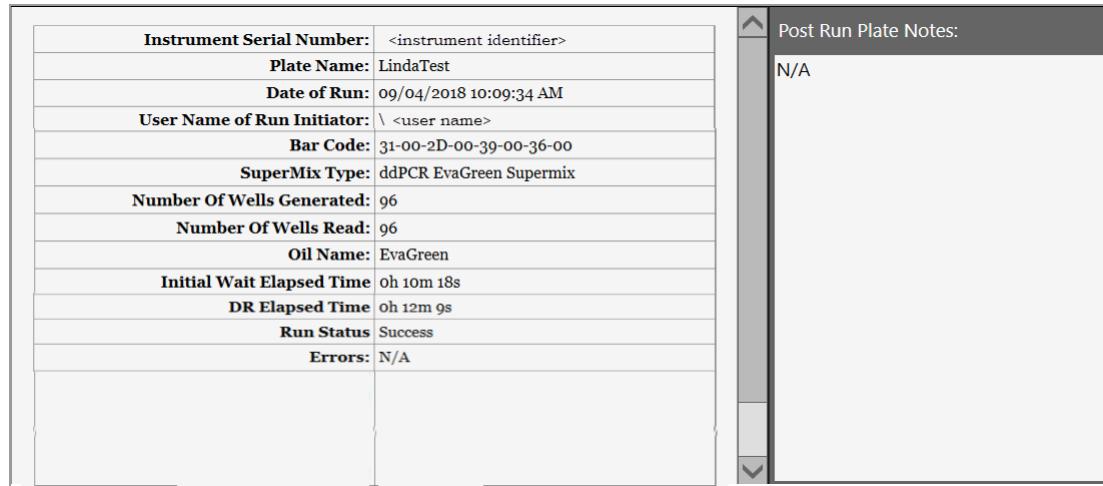
**Note:** Exporting to Excel exports the data in spreadsheet format, while Exporting to CSV exports the data in comma-delimited format.

- To show or hide the corresponding column in the table, select Show/Hide Columns, and then select or clear checkboxes.

3. Click anywhere in the display to close the dialog box.

## Viewing Run and Lot Information

Use the Run Information window to view information on the run and view or add post-run notes regarding the plate.



The screenshot shows a software interface for viewing run information. On the left is a table with various parameters and their values. On the right is a panel for post-run plate notes.

<b>Instrument Serial Number:</b>	<instrument identifier>
<b>Plate Name:</b>	LindaTest
<b>Date of Run:</b>	09/04/2018 10:09:34 AM
<b>User Name of Run Initiator:</b>	\ <user name>
<b>Bar Code:</b>	31-00-2D-00-39-00-36-00
<b>SuperMix Type:</b>	ddPCR EvaGreen Supermix
<b>Number Of Wells Generated:</b>	96
<b>Number Of Wells Read:</b>	96
<b>Oil Name:</b>	EvaGreen
<b>Initial Wait Elapsed Time</b>	0h 10m 18s
<b>DR Elapsed Time</b>	0h 12m 9s
<b>Run Status</b>	Success
<b>Errors:</b>	N/A

**Post Run Plate Notes:**

N/A

For information on displaying your used lots in the Run Information window, see [Showing Lots in Run Information Output and Reports on page 62](#).

# Chapter 10 Data Analysis Methodology

As summarized in [Chapter 9, Data Analysis Module Overview](#), the software presents analysis data in several different formats. When you open the data file in the Analysis module, you can view the data with the automatic analysis thresholds and calculations or you can choose different options to recalculate, replot, or recolor the results.

Data is shown in the following formats:

- Amplitude, which shows each droplet in each well in a scatter plot, in one-dimensional or two-dimensional views
- Data visualizations, which show estimated target molecule concentration, ratio, and fractional abundance of one target against another target based on selected error model and confidence interval

**Note:** Copy number variation is also calculated if the user ran a CNV experiment.

- Event counts, which are precise counts of positive and negative droplets for each target in each well

## Amplitude Plot Analysis Options

QX Manager Software provides two amplitude plot displays in which you can view the data with automatic thresholding where applicable, or choose different options to replot or recolor the results.

In the 1D amplitude and 2D amplitude plots, you can

- Show well calculations

**Note:** The 1D amplitude plot shows the data for each selected well in its own section, while the 2D amplitude plot shows all selected wells together.

- Modify automatic thresholds calculated by QX Manager Software
- View data in a heat map
- View histograms (1D amplitude tab only)
- Re-apply automatic analysis
- Apply tilt correction to automatic analysis

**Table 29** explains the analysis options that are available in both 1D amplitude and 2D amplitude plots.

**Table 29. 1D and 2D amplitude tool options**

Icon	Mode	Description
	View mode	<p>Data appears with the thresholds or clusters assigned by QX Manager Software.</p> <p>When this icon is selected, the view is locked and you cannot modify the thresholds.</p>
	Heat map	Shows the distribution of droplets, with higher density of droplets shown as hot.
	Automatic analysis	Disables tilt correction and automatically reanalyzes the data.
		Enables tilt correction and automatically reanalyzes the data.
	Automatic analysis with tilt correction	<p><b>Note:</b> Tilt correction rotates the angles from the double-negative cluster centroid to each droplet, such that the two single positive cluster centroids are orthogonal with the double-negative cluster centroid.</p>
	Reset	Resets the data to the original automatically analyzed data.
	Chart display options	Choose whether to display axis with channel number or fluorophore, and change the size of axis markers, data labels, and axis labels.

Table 30 explains the analysis options available only for 1D amplitude plots.

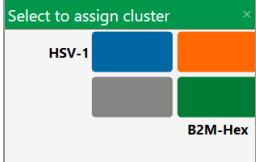
**Table 30. 1D amplitude tool options**

Icon	Mode	Description
	Automatic thresholding	<p>Individual Wells: Clusters are generated for each selected well separately.</p> <p>Combined Wells: Droplets from all selected wells are combined, and clusters are generated on that combined set.</p> <p>Positive Control Wells: Thresholds are generated for each channel based on any positive controls currently selected, and applies those thresholds to all currently selected wells.</p> <p><b>Note:</b> If you change the thresholds manually, you can click Reset to return to the automatic thresholds.</p>
 	<p>Threshold, single well</p> <p>Threshold, multiple wells</p>	<p>For a single well or multiple wells, the user can manually input thresholding values in the 1D amplitude plot.</p> <p>You can click anywhere in the plot to apply threshold lines to the data.</p> <p><b>To adjust the threshold values</b></p> <ul style="list-style-type: none"> <li>■ Drag the lines horizontally in the plot area</li> <li>■ Enter a new threshold value in the boxes at the end of each threshold line</li> <li>■ Click the up and down arrows to incrementally adjust the numbers</li> </ul>
 	<p>Threshold, single well SD</p> <p>Threshold, multiple wells SD</p>	<p>For a single well or multiple wells, QX Manager Software automatically calculates and populates thresholding values with standard deviations (SD).</p> <p><b>Important:</b> SD thresholds are not recommended for RMD, since these clusters are not typically located at right angles to each other.</p> <p><b>Note:</b> The SD thresholds are set at a limit that is less than the mean number of positive targets, and more than the mean number of negative targets. Starting from where the user clicks in the plot, SD locates the threshold between the positive and negative clusters such that the classification error is minimized.</p>
	Show histogram	Hides or displays histogram plots for 1D amplitude

**Table 31** explains the analysis options available only for 2D amplitude plots.

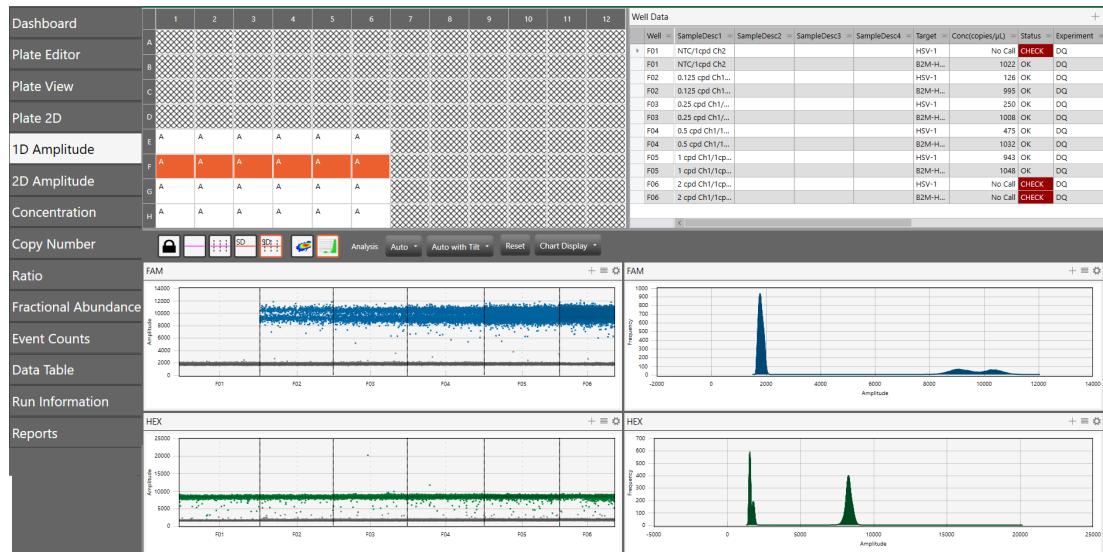
**Table 31. 2D amplitude tool options**

Icon	Mode	Description
Automatic thresholding	<p>Individual Wells: Clusters are generated for each selected well separately.</p> <p>Combined Wells: Droplets from all selected wells are combined, and clusters are generated on that combined set.</p> <p>Positive Control Wells: Thresholds are generated for each channel based on any positive controls currently selected, and applies those thresholds to all currently selected wells.</p> <p><b>Note:</b> If you change the thresholds manually, you can click Reset to return to the automatic thresholds.</p>	
	Threshold line	<p>Use the Threshold line icons to establish thresholds across a pair of channels, dividing droplets into distinct clusters separated by different colors.</p> <p>You can click anywhere in the plot to apply threshold lines to the data.</p> <p><b>To adjust the threshold values, you can</b></p> <ul style="list-style-type: none"> <li>■ Drag the lines horizontally or vertically in the plot area</li> <li>■ Enter a new threshold value in the boxes at the end of each threshold line</li> <li>■ Click the up and down arrows to incrementally adjust the numbers</li> </ul>
	Threshold line SD	<p>QX Manager Software automatically calculates and populates threshold line values with standard deviations (SD).</p> <p><b>Important:</b> SD thresholds are not recommended for RMD, since these clusters are not typically located at right angles to each other.</p> <p><b>Note:</b> The SD thresholds are set at a limit that is less than the mean number of positive targets, and more than the mean number of negative targets. Starting from where the user clicks in the plot, SD locates the threshold between the positive and negative clusters such that the classification error is minimized.</p>

Icon	Mode	Description
	Threshold cluster square	Use the Threshold Cluster icons to establish thresholds across a pair of channels, dividing droplets into distinct clusters separated by different colors.
	Threshold cluster circle	To classify your droplets, you can
	Threshold cluster freeform	<ul style="list-style-type: none"> <li>■ Click any of the three Threshold Cluster Mode buttons (square, circle, or free form)</li> <li>■ Draw the chosen shape around the cluster of droplets</li> </ul> <p><b>Note:</b> When using the cluster mode tools to draw a shape around a cluster of droplets, a dialog box with color selections by target appears in the window to help you select the correct target combination.</p>  <p>Target names are shown along the two axes based on the signal values assigned during plate setup. Click the colored box that best represents the location of the cluster of droplets selected to automatically classify the droplets as positive or negative for each given target.</p> <p><b>Note:</b> The Cluster dialog box display varies according to the plex mode.</p>

## 1D Amplitude

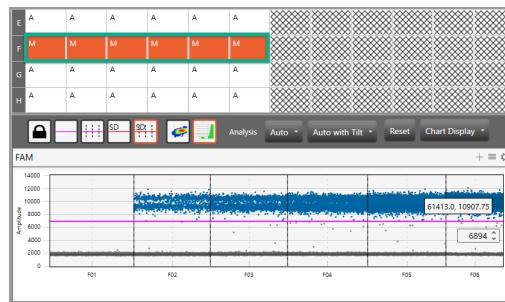
Use the 1D Amplitude analysis window to view droplet amplitudes for each channel, in selection order, for each well selected in the Well Selector.



The 1D Amplitude plots illustrate

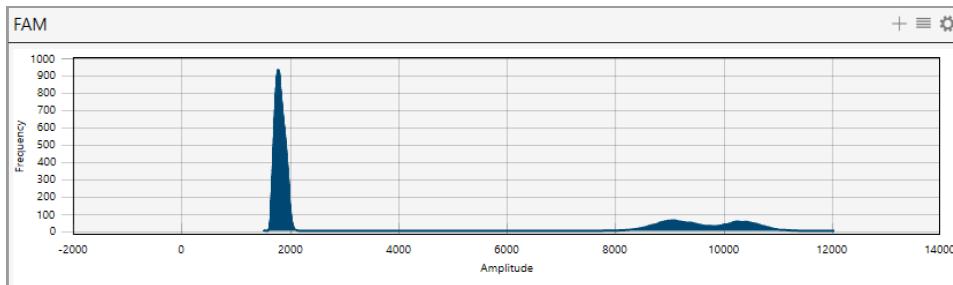
- All accepted droplets, with a visual representation of the positive and negative droplets in each well

Positive droplets appear in color, while negative droplets appear in gray. The Well Selector displays an A in each well where QX Manager Software automatically thresholded the droplet data. If you create a manual threshold, the Well Selector displays an M in the wells where the threshold changed.



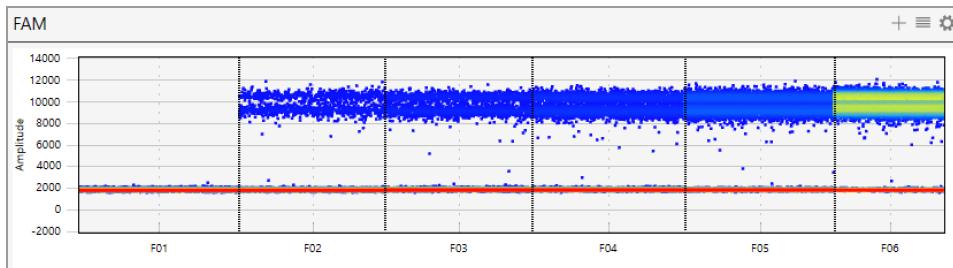
- Amplitude in a histogram plot

The histogram displays frequency of droplets at specific fluorescence amplitudes for each well or group of wells selected. The Channel 1 (FAM/EvaGreen®) histogram appears in blue and Channel 2 (HEX/VIC) histogram in green.



**Note:** The Histogram icon is enabled by default so a Histogram plot appears automatically for each fluorophore (channel).

- Amplitude in a Heat Map view



When you select the 1D Amplitude tab, the toolbar options that allow you to change thresholds and show or hide the heat map and histogram appear above the plots.



**Note:** For detailed information on each toolbar option, see [Amplitude Plot Analysis Options on page 115](#).

Default color assignments for 1D amplitude plots are

Positive FAM = Blue

Positive HEX = Green

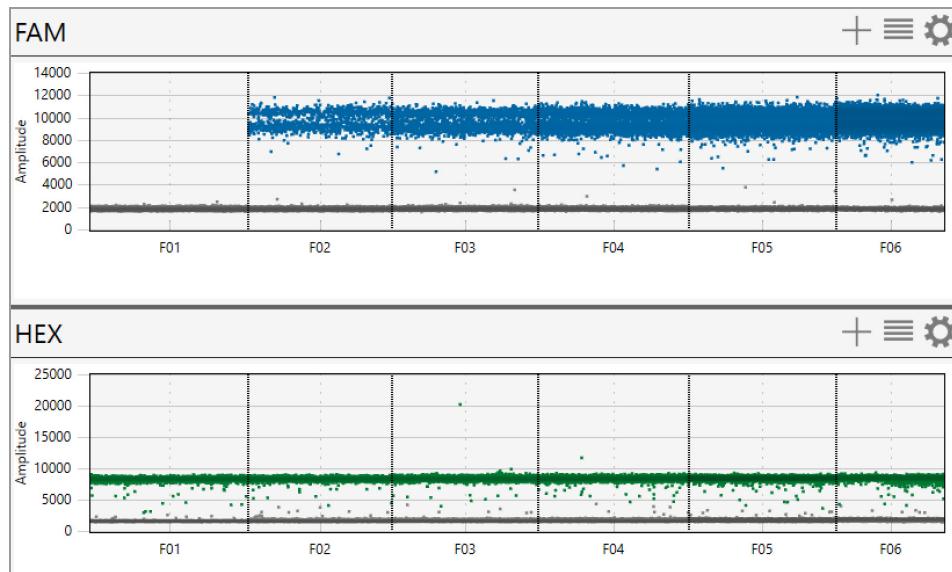
Negative = Gray

The images in this section display data from a direct quantification experiment involving two targets:

- The FAM plot (FAM dye in channel 1) shows the positive droplets for the first target in blue.

- The HEX plot (HEX dye in channel 2) shows the positive droplets for the second target in green.

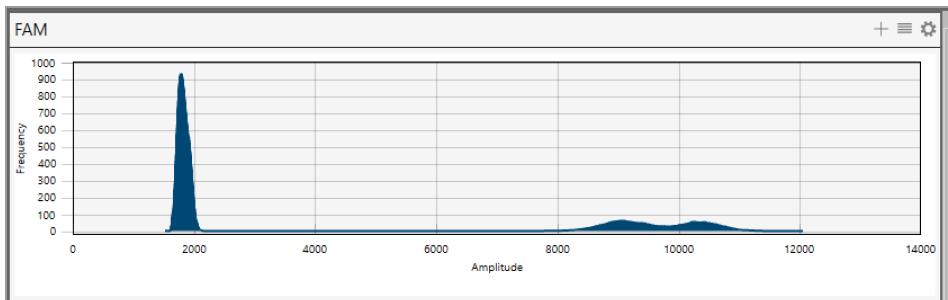
Negative droplets appear in grayscale in each plot.



For experiments where enough droplets are recognized, QX Manager Software automatically configures an amplitude threshold, which distinguishes between positive and negative droplets.

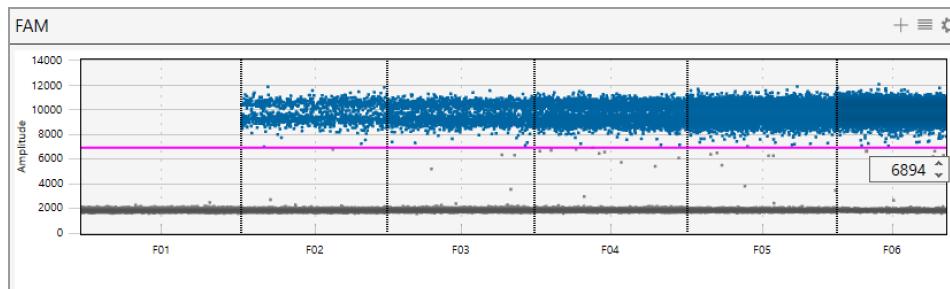
**Note:** Well data must meet certain quality metrics (minimum of 10,000 droplets) before the software will automatically calculate a threshold above which droplets are considered positive. You can use the manual thresholding tools to ensure correct quantification of positive and negative droplets.

By default, the Histogram icon (█) is enabled so the Histogram plot automatically appears with the amplitude plot.



### To manually adjust the thresholds

1. Select one or more wells.
2. Click any of the threshold icons (█, █, SD, SD) to set manual thresholds:
  - █ Regular threshold for a single well; when multiple wells are selected, you can set a different threshold for each well
  - █ Regular threshold for multiple wells, where you can set one threshold for all selected wells
  - █ Standard deviation threshold for a single well; when multiple wells are selected, you can set a different threshold for each well
  - █ Standard deviation for multiple wells, where you can set one threshold for all selected wells
3. Click on the location in the plot where you want to draw the new threshold.
4. When the line appears, use the up or down arrows, or click and drag the threshold, to adjust the location.



QX Manager Software displays the manual threshold and changes the color of the droplets that are now above or below the threshold.

**Note:** You can also manually enter a threshold number.

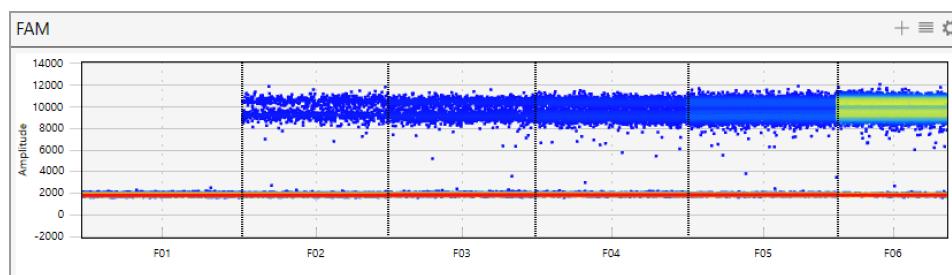
When you manually modify a threshold, the well selector displays an M for manual instead of the A for Automatic.

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	A	A	A	A	A	A	A	A	A	A
B	M	M	M	M	M	M	M	M	M	M	M	M
C	A	A	A	A	A	A	A	A	A	A	A	A
D	A	A	A	A	A	A	A	A	A	A	A	A
E												
F												
G												
H												

5. (Optional) To reset to the automatic threshold, click Auto.
6. (Optional) To adjust the automatic threshold to consider tilt correction, click Auto with Tilt.
7. (Optional) To reset everything to the original display, click Reset.

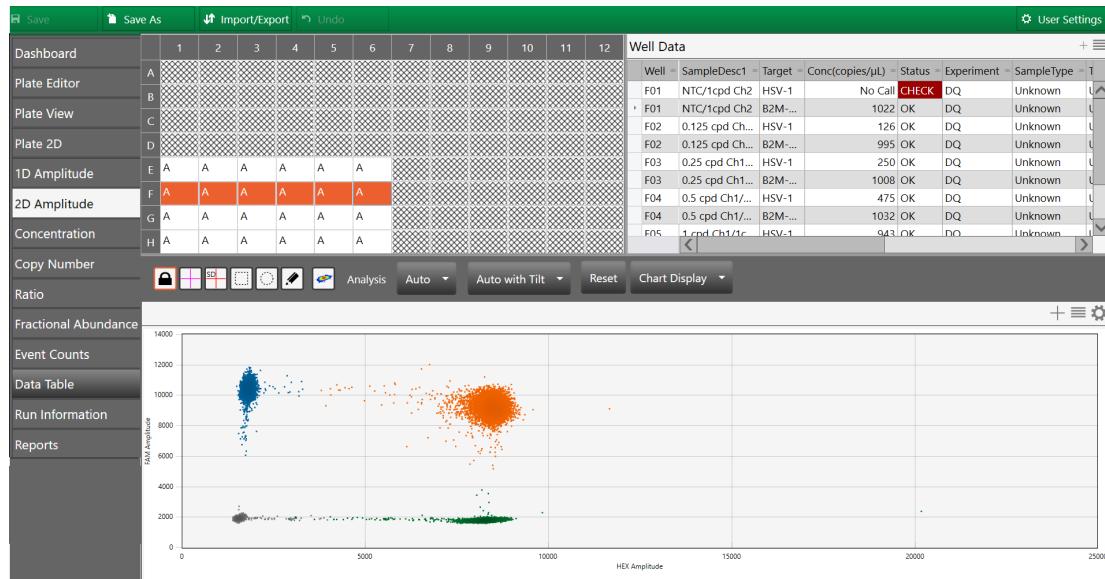
#### To view a heat map representation of the plot data

► Click the  icon.



## 2D Amplitude

For the wells you select in the analysis view of the Plate Editor, the 2D Amplitude window illustrates fluorescence for two targets.

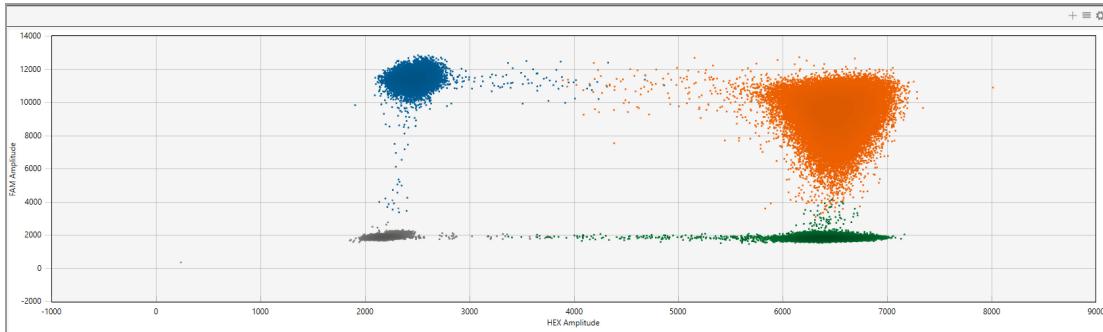


When you select the 2D Amplitude tab, the toolbar options allow you to change thresholds, manually cluster wells, and show or hide the heat map above the plots. For detailed information on each toolbar option, see [Amplitude Plot Analysis Options on page 115](#).



The images in this section display data from a direct quantification experiment involving two targets.

The plot shows individual cluster representations for first and second target positive droplets, droplets containing both targets, and droplets containing neither target.



Default color assignments for 2D amplitude plots are

Positive FAM	=	Blue
Positive HEX/VIC	=	Green
Positive FAM and HEX/VIC	=	Orange (may appear brown on some monitors)
Double negative	=	Gray

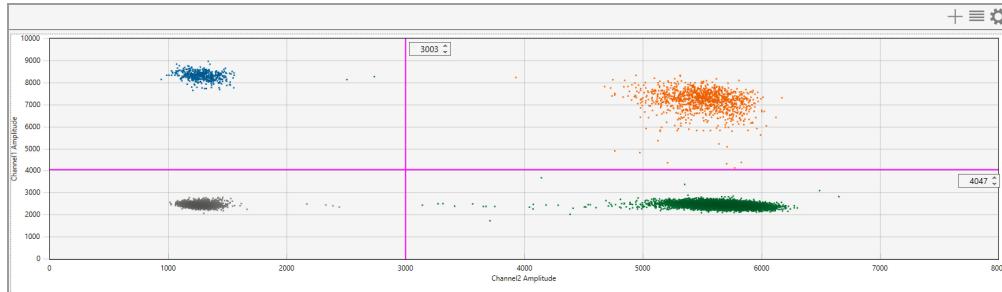
**Note:** Well data must meet certain quality metrics (minimum of 10,000 droplets) before QX Manager Software will automatically calculate a threshold above which droplets are considered positive. You can use the manual thresholding tools to ensure correct quantification of positive and negative droplets.

### To use the 2D amplitude tools

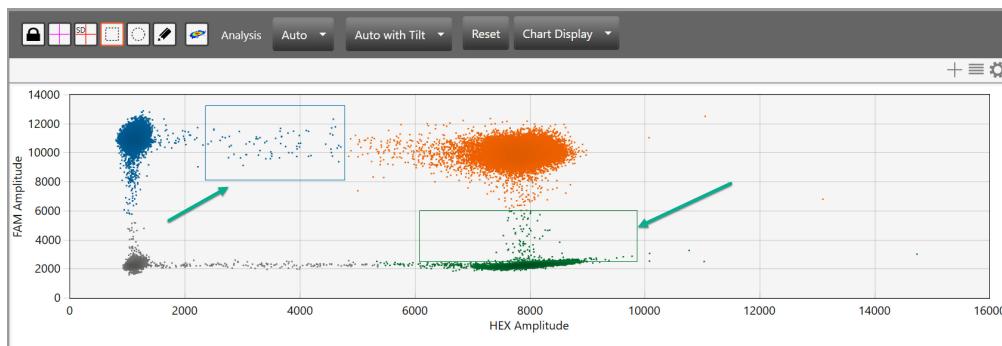
1. Open your data file in the Analysis module and select the 2D Amplitude tab.
2. Choose a threshold line or cluster option.

**Note:** Use the cluster option when the data is not orthogonal and the threshold lines do not adequately divide the populations of droplets.

- To adjust threshold lines, select a threshold line type (  ) and then click in the plot to apply crosshair lines. Use any of the following methods:
  - Drag the lines horizontally or vertically in the plot area.
  - Enter a new threshold value in the boxes at the end of each threshold line.
  - Tap the up and down arrows to incrementally adjust the numbers.



- To adjust thresholds using the cluster option, select a cluster type (□ ○ ↗) and draw the chosen shape around the cluster of droplets to classify.



- (Optional) To reset to the automatic threshold, click Auto.
- (Optional) To adjust the automatic threshold to consider tilt correction, click Auto with Tilt.
- (Optional) To reset everything to the original display, click Reset.

## Data Visualization Chart Analysis Options

QX Manager Software also provides chart displays where you can view the data from the original calculations, or choose different options to recalculate the results.

In charts reflecting Data Visualization distributions, you can

- Show individual or merged well data.
- Change the error model and confidence level.

**Note:** For event counts, you can also alter the view mode to see positive, negative, or all droplet counts.

Table 32 explains the available options.

**Table 32. Chart tool options for Data Visualization distribution charts**

Icon	Mode	Description
	Show both	Shows the calculated total or percentage as a merged calculation for all selected wells and as an individual calculation for each selected well
	Show merged	Shows the calculated total or percentage as a combined number for all selected wells with the same parameters <b>Note:</b> Well setup must match for each well before the software can merge them.
	Show individual	Shows the calculated total or percentage as an individual calculation for each selected well
	X-axis well	Shows the well number on the x axis
	X-axis sample	Shows a partial sample name on the x axis <b>Note:</b> You can select Well and Sample simultaneously.
	Error model and confidence interval	Select an error model and confidence level. The default choices are Poisson and 95%, to yield the highest confidence in the results.  
<p><b>Important:</b> The software uses the following multipliers in its calculations:</p> <ul style="list-style-type: none"> <li>■ For 95% confidence interval, the sigma multiplier is 1.96.</li> <li>■ For 68% confidence interval, the sigma multiplier is 1.0.</li> </ul>		

**Table 33** identifies the additional options available in the Event Count window. These options are available for event counts only.

**Table 33. Additional options for event counts**

Icon	Mode	Description
	View mode positive	Displays in a bar chart the number of positive droplets for each target in each well
	View mode negative	Displays in a bar chart the number of negative droplets for each target in each well
	View mode total	Displays in a bar chart the number of total droplets in each well

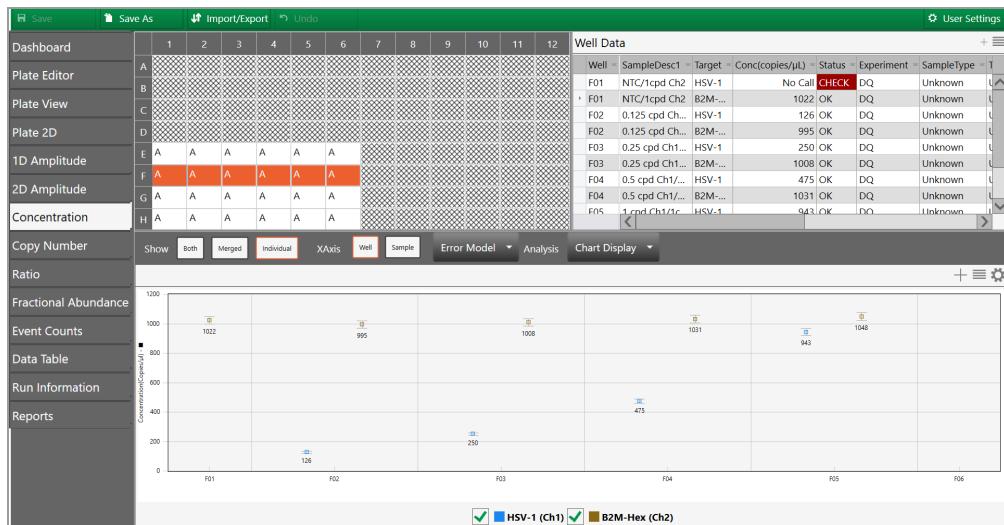
## Concentration

Concentration measures the number of copies of the target molecules in each  $\mu\text{l}$  of sample based on the default settings. Use the toolbar to merge or separate the calculated results for each well, and to recalculate your results based on a different error model and confidence interval.

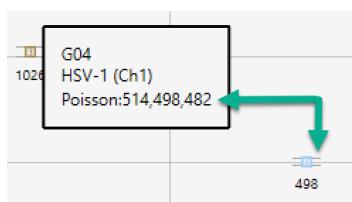
For information on toolbar options, see [Data Visualization Chart Analysis Options on page 128](#).



The following image displays concentration data, across a sequence of wells.



QX Manager Software plots the averaged concentration for each target as a data point. Pause on a data point to see the high, median, and low numbers.



The software uses the formula and variables shown below.

### Concentration Calculation

$$c = -\ln\left(\frac{N_{\text{neg}}}{N}\right) / V_{\text{droplet}}$$

**Where:**

<b>-ln</b>	Negative natural logarithm
<b>V<sub>droplet</sub></b>	Volume of droplet
<b>N<sub>neg</sub></b>	Number of negative droplets
<b>N</b>	Total number of droplets

### Confidence Interval Calculation

**Note:** If you select a 68% confidence interval, the multiplier is changed from 1.96 to 1.00.

$$p = \frac{N_{\text{neg}}}{N}$$

$$\partial_{N_{\text{neg}}} = \sqrt{Np(1-p)}$$

$$N_{\text{neg},\text{min}} = N_{\text{neg}} - 1.96 * \partial_{N_{\text{neg}}}$$

$$N_{\text{neg},\text{max}} = N_{\text{neg}} + 1.96 * \partial_{N_{\text{neg}}}$$

**Where:**

<b>V<sub>droplet</sub></b>	Volume of droplet
<b>N<sub>neg</sub></b>	Number of negative droplets
<b>N</b>	Total number of droplets
<b>p</b>	p-value (probability of finding the target in the sample)
<b>δ</b>	Delta

## Bayesian Calculation

**Note:** If you specify the lesser confidence interval when you define your error model, the software uses 68% in its calculations instead of 95%.

$$b1 = -\ln\left(\frac{N_{neg,min}}{N}\right) / V_{droplet}$$

$$b2 = -\ln\left(\frac{N_{neg,max}}{N}\right) / V_{droplet}$$

$$(c_{low\_95\%}, c_{high\_95\%}) = (\min(b1, b2), \max(b1, b2))$$

count	low	high
0	0	2.996
1	0.042	4.776
2	0.303	6.406
3	0.712	7.951
4	1.207	9.431
5	1.758	10.865
6	2.35	12.264
7	2.974	13.632
8	3.622	14.979
9	4.292	16.304
10	4.979	17.614
11	5.68	18.911
12	6.395	20.193
13	7.121	21.465
14	7.858	22.726
15	8.603	23.98
16	9.356	25.226
17	10.117	26.464
18	10.885	27.694
19	11.659	28.919
20	12.439	30.139
21	13.224	31.353
22	14.015	32.561
23	14.809	33.767
24	15.608	34.967
25	16.412	36.162
26	17.219	37.355
27	18.03	38.543
28	18.844	39.729
29	19.661	40.912
30	20.482	42.09
31	21.306	43.266
32	22.132	44.439

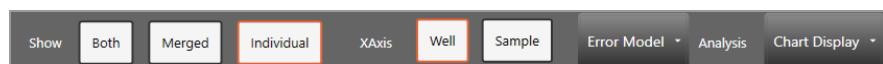
33	22.961	45.61
34	23.792	46.779
35	24.626	47.945
36	25.462	49.109
37	26.301	50.269
38	27.142	51.428
39	27.984	52.586
40	28.829	53.741
41	29.676	54.893
42	30.524	56.045
43	31.375	57.194
44	32.227	58.342
45	33.08	59.489
46	33.935	60.634
47	34.792	61.776
48	35.651	62.917
49	36.51	64.058
50	37.372	65.196
51	38.234	66.334
52	39.098	67.47
53	39.963	68.605
54	40.829	69.739
55	41.697	70.87
56	42.566	72.001
57	43.436	73.131
58	44.307	74.26
59	45.18	75.387
60	46.053	76.514
61	46.927	77.64
62	47.803	78.764
63	48.679	79.888
64	49.556	81.011
65	50.434	82.133
66	51.314	83.252

67	52.194	84.372
68	53.075	85.491
69	53.957	86.609
70	54.84	87.726
71	55.723	88.843
72	56.608	89.958
73	57.493	91.073
74	58.379	92.187
75	59.266	93.3
76	60.153	94.413
77	61.041	95.525
78	61.93	96.636
79	62.819	97.747
80	63.71	98.856
81	64.601	99.964
82	65.493	101.072
83	66.385	102.18
84	67.278	103.287
85	68.172	104.393
86	69.066	105.499
87	69.961	106.604
88	70.856	107.709
89	71.752	108.813
90	72.649	109.916
91	73.546	111.019
92	74.444	112.121
93	75.342	113.223
94	76.241	114.324
95	77.14	115.425
96	78.04	116.525
97	78.941	117.624
98	79.842	118.723
99	80.743	119.822

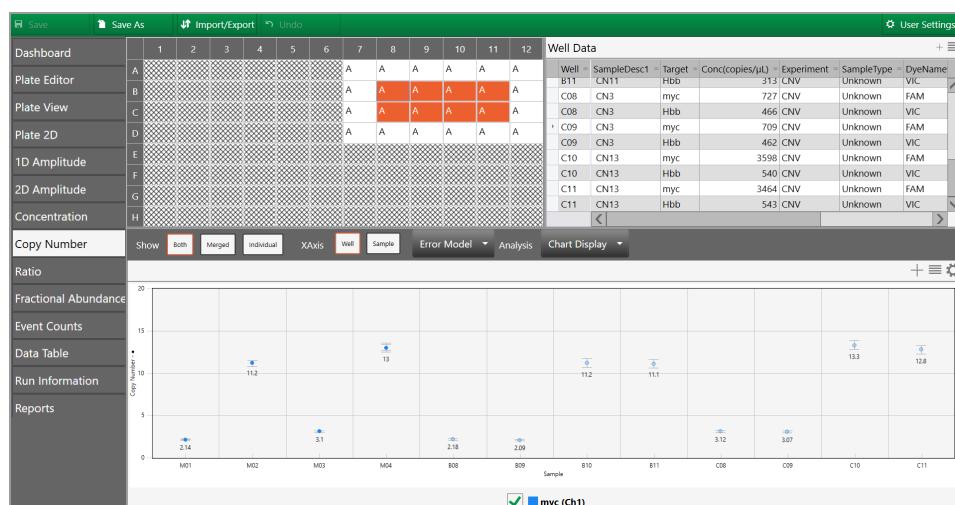
## Copy Number

**Important:** To see copy number variation data, you must run a copy number variation (CNV) ddPCR experiment.

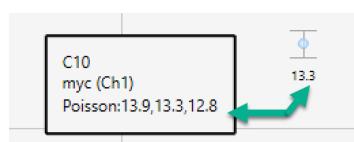
Copy number measures the count of the target gene, relative to the reference gene, to calculate the number gene targets per genome. Use the toolbar to merge or separate the calculated results for each well, and to recalculate your results based on a different error model and confidence interval. For information on toolbar options, see [Data Visualization Chart Analysis Options on page 128](#).



The following image displays copy number data, across a sequence of wells.



QX Manager Software plots each copy number variation as a data point. Pause on a data point to see the high, median, and low numbers.



The software uses the formula and variables shown below.

$$CNV = \frac{A}{B} N_B$$

**Where:**

**A** Target concentration

**B** Reference concentration

**N<sub>B</sub>** Number of reference copies in genome (usually 2)

### Confidence Interval Calculations

**Note:** If you specify the lesser confidence interval when you define your error model, the software uses 68% in its calculations instead of 95%.

$$CI_{CNV} = N_B \cdot \frac{A}{B} \sqrt{\frac{CI_A^2}{A^2} + \frac{CI_B^2}{B^2}}$$

$$(CNV_{low\_95\%}, CNV_{hi\_95\%}) = (CNV - (1/2)CI_{CNV}, CNV + (1/2)CI_{CNV})$$

**Where:**

**A** Target concentration

**B** Reference concentration

**N<sub>B</sub>** Number of reference copies in genome (usually 2)

**CI<sub>A</sub>** Poisson confidence interval of target (95% max - 95% min)

**CI<sub>B</sub>** Poisson confidence interval of reference (95% max - 95% min)

### Notes:

Copy Number analysis involves determining the number of copies of a given target DNA sequence as compared to an invariant reference DNA sequence. Use the CNV analysis to determine structural variations, such as ploidy changes (addition or deletion of chromosomes), deletions (missing partial chromosomes) or duplications (repeated target sequences) in the genome.

The ability to discriminate between consecutive CN states has been a technical challenge in copy number assessment. Fundamentally, as CN state increases, the percentage difference in target genomic material between states decreases. For example, for a given target locus, a CN of 3 is 50% more abundant in concentration per genome than a CN of 2, while a CN of 5 is only 25% more abundant per genome than a CN of 4, and so on.

Due to the massive partitioning of the reaction across up to 20,000 droplets per well, and the absolute nature and precision of the ddPCR concentration measurement, ddPCR CNV analysis enables the quantitative discrimination required to resolve consecutive copy number states beyond CN 3. For example, a 20% difference in target concentration between a CN 5 versus CN 6 sample can be reproducibly resolved using ddPCR. This discrimination is both a function of concentration differences between consecutive CN states, as well as the amount of DNA assayed per well.

## Ratio

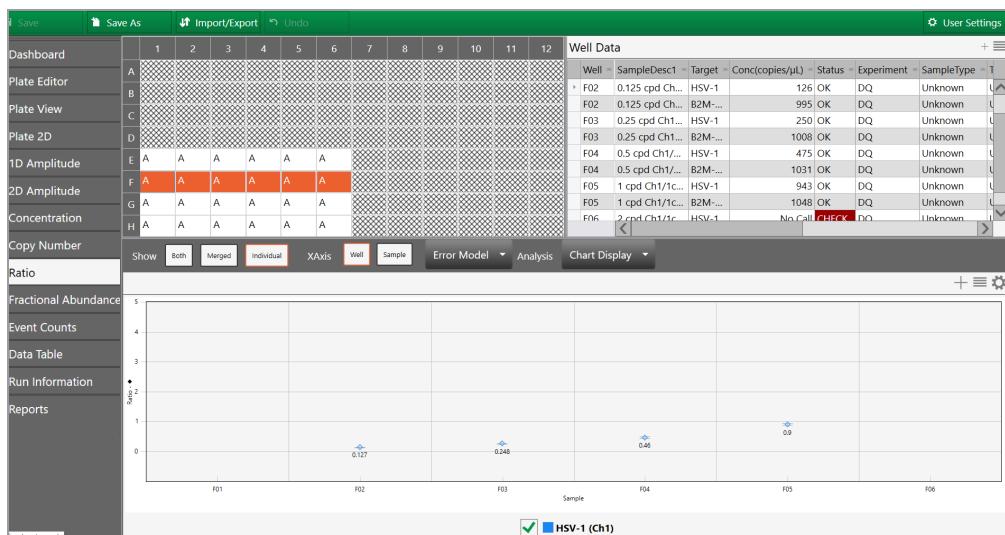
Ratio determines the quotient of two target molecules in each  $\mu$ l of sample based on the default settings.

### T1:T2

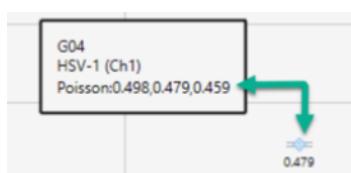
Use the toolbar to merge or separate the calculated results for each well, and to recalculate your results based on a different error model and confidence interval. For information on toolbar options, see [Data Visualization Chart Analysis Options on page 128](#).



The following image displays ratio data, across a sequence of wells.



QX Manager Software plots each ratio as a data point. To see the confidence level numbers, pause on the data point.



The software uses the formula and variables shown below.

$$r = \frac{A}{B}$$

**Where:**

<b>A</b>	Target concentration
<b>B</b>	Reference concentration

**Note:** If no reference is selected, the target with the highest dye concentration in the highest channel will automatically be considered as the reference. If multiple references are selected, the geometric mean is used.

### Confidence Interval Calculations

**Note:** If you specify the lesser confidence interval when you define your error model, the software uses 68% in its calculations instead of 95%.

$$CI_r = \frac{A}{B} \sqrt{\frac{CI_A^2}{A^2} + \frac{CI_B^2}{B^2}}$$

$$(r_{low\_95\%}, r_{hi\_95\%}) = (r - (1/2)CI_r, r + (1/2)CI_r)$$

**Where:**

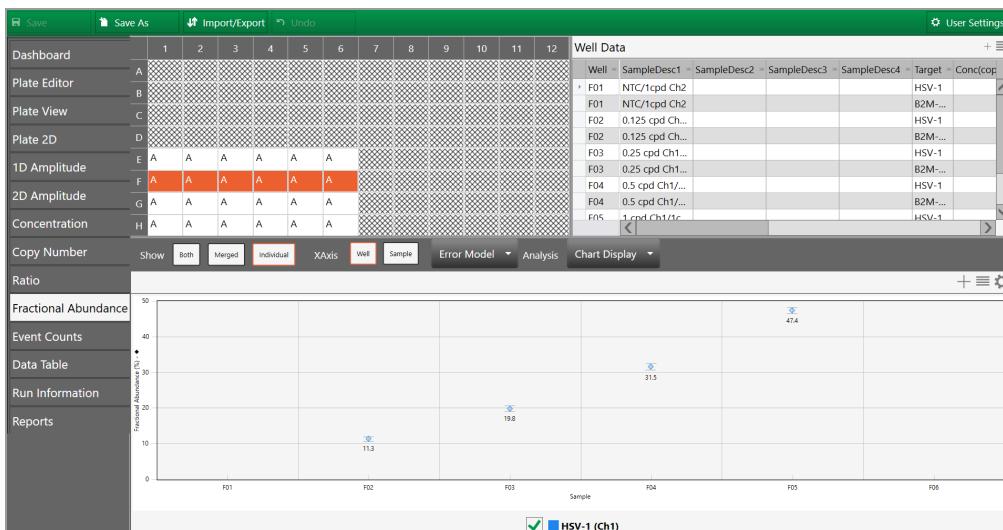
<b>A</b>	Channel 1 target concentration
<b>B</b>	Channel 2 target concentration
<b>CI<sub>A</sub></b>	Confidence interval of Channel 1 target (95% max - 95% min)
<b>CI<sub>B</sub></b>	Confidence interval of Channel 2 target (95% max - 95% min)

## Fractional Abundance

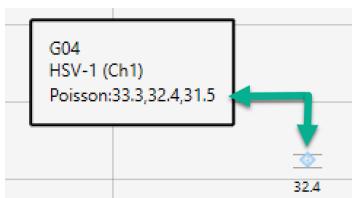
Fractional Abundance determines the relative abundance of your targets in each  $\mu$ l of sample based on the default settings. Use the toolbar to merge or separate the calculated results for each well, and to recalculate your results based on a different error model and confidence interval. For information on toolbar options, see [Data Visualization Chart Analysis Options on page 128](#).



The following image displays fractional abundance data across a sequence of wells.



QX Manager Software plots the fractional abundance as a data point. To see the high and low confidence interval numbers, pause on the data point.



The software uses the formula and variables shown below.

$$f = \frac{A}{A + B}$$

**Where:**

**A** Target concentration

**B** Reference concentration

**Note:** If no reference is selected, the target with the highest dye concentration in the highest channel will automatically be considered as the reference. If multiple references are selected, the geometric mean is used.

**Confidence Interval Calculations**

**Note:** If you specify the lesser confidence interval when you define your error model, the software uses 68% in its calculations instead of 95%.

$$CI_f = \frac{1}{(A+B)^2} \sqrt{B^2 CI_A^2 + A^2 CI_B^2}$$

$$(f_{low\_95\%}, f_{hi\_95\%}) = (f - (1/2)CI_f, f + (1/2)CI_f)$$

**Where:**

**A** Channel 1 target concentration

**B** Channel 2 target concentration

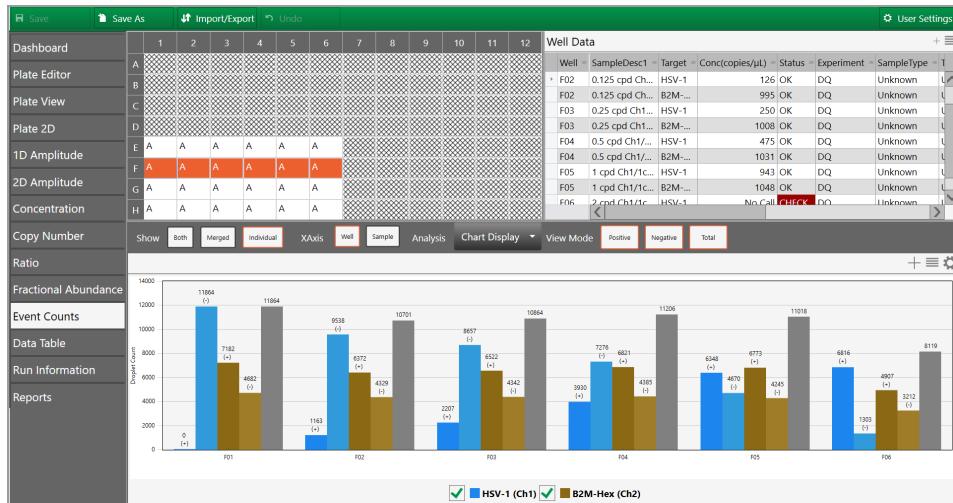
**CI<sub>A</sub>** Confidence interval of Channel 1 target (95% max - 95% min)

**CI<sub>B</sub>** Confidence interval of Channel 2 target (95% max - 95% min)

## Event Counts

The Event Counts tab shows a bar chart view of the number of positive and negative droplets for each of the targets in each of the selected wells.

**Note:** In the Dashboard display, event counts appear in the Droplets window.



Use the toolbar to change the display by combining or separating data for each well. You can also show data by well name or by sample name, and you can show any combination of positive, negative, or combined droplet counts.



## Linkage Analysis

In ddPCR, physical linkage of two targets can occur in one of the following patterns:

- Tandem repeats if the same target sequence
- Proximity of two target sequences physically linked on the same piece of DNA

QX Manager Software uses the formula and variables shown below.

$$\text{linkage} = \ln(N) - \ln(n_{00} + n_{01} + n_{10} + \frac{n_{01}n_{10}}{n_{00}})$$

**Where:**

<b>ln</b>	Natural logarithm
<b>-ln</b>	Negative natural logarithm
<b>N</b>	Total number of droplets
<b>n<sub>00</sub></b>	Number of negative droplets for both channels
<b>n<sub>01</sub></b>	Number of negative droplets in channel 1 and positive droplets in channel 2
<b>n<sub>10</sub></b>	number of positive droplets in channel 1 and negative droplets in channel 2

$$\sigma_{\text{linkage}} = \sqrt{\frac{(n_{00} + n_{01})^2 \sigma_{10}^2 + (n_{00} + n_{10})^2 \sigma_{01}^2 + (n_{00} - \frac{n_{10}n_{01}}{n_{00}})^2 \sigma_{00}^2}{(n_{00}^2 + n_{00}n_{10} + n_{00}n_{01} + n_{01}n_{10})^2}}$$

**Where:**

<b>N</b>	Total number of droplets
<b>n<sub>00</sub></b>	Number of negative droplets for both channels
<b>n<sub>01</sub></b>	Number of negative droplets in channel 1 and positive droplets in channel 2
<b>n<sub>10</sub></b>	number of positive droplets in channel 1 and negative droplets in channel 2

<b>p</b>	p-value (calculated probability)
$\delta$	Delta

### Confidence Interval Calculation

$$CI_{linkage} = (linkage - (1.96)\sigma_{linkage}, linkage + (1.96)\sigma_{linkage})$$

Where:

$\sigma$  Standard deviation



# Chapter 11 Analysis Module Reporting

Use the Reporting function to create a variety of reports in the QX Manager Software Analysis module.

You can create reports on the fly from any analysis file or, if you need to generate specific reports on a regular basis, you can set up report templates for reuse.

This section contains information on available report elements, how to create analysis reports, and how to create report templates.

## Report Elements

QX Manager Software allows you to generate reports for any or all of the following elements of the run and data.

### Run Setup

- Plate Setup
  - Well
  - Perform droplet reading
  - Experiment type
  - Sample description 1, 2, 3, and 4
  - Sample type
  - Supermix name
  - Plex mode
  - Target name
  - Target type
  - Signal channel 1 and 2
  - Reference copies
  - Well notes
  - Plot

- RDQ conversion factor
- Plate name
- Supermix information
- Number of wells read
- Selected wells for report
- Username of the person who did the run
- Run start time
- Run complete time
- Initial wait elapsed time
- DR elapsed time
- Errors
- Instrument serial number
- Firmware version
- Software version
- Software
- Run notes
- Lot information

## 1D Amplitude

- Threshold value
- Tilt correction
- Amplitude 1D chart
- Amplitude 1D chart for each well
- Amplitude 1D histogram chart
- Amplitude 1D histogram chart for each well

## 2D Amplitude

- Threshold value
- Tilt correction

- Amplitude 2D chart
- Amplitude 2D chart for each well

## Concentration

- Error model
- Confidence model
- Chart

## Copy Number

- Error model
- Confidence model
- Chart

## Ratio

- Error model
- Confidence model
- Inverse
- Chart

## Fractional Abundance

- Error model
- Confidence model
- Inverse
- Chart

## Event Counts

## Analysis Results

## Audit Logs

The audit log contains all of the original data from the plate run, and also records any changes that were made to the data file. The log records the change number, date, username for the user who made the change, the software version, full user name, computer name, and the reason for the change. Also recorded in a tabular format are the change type and description, as well as old and new values for all well settings. The description column includes the well number of any affected well, and may also include other descriptive information.

## Creating Analysis Reports

You can create a report using a template or you can create a custom report on the fly.

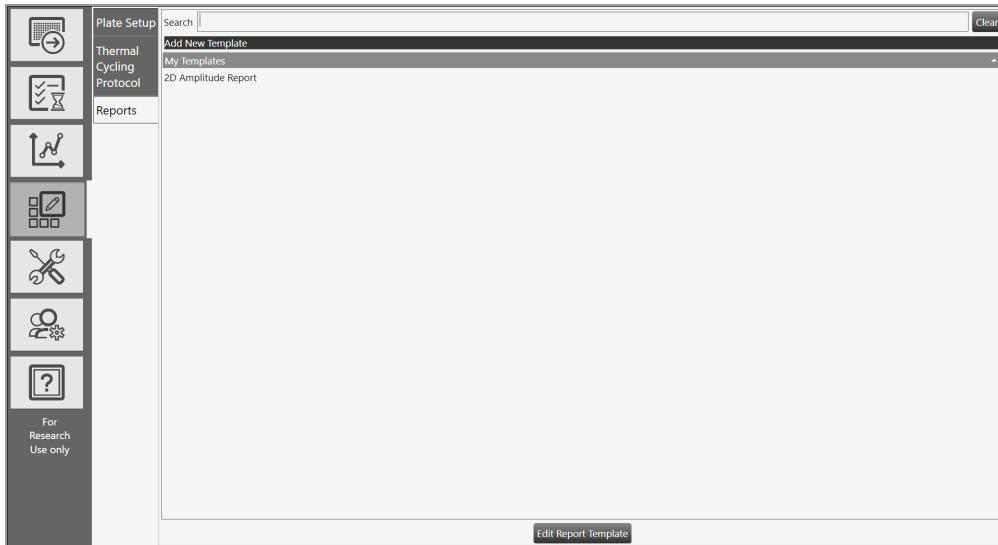
1. Select the Reports tab.
2. Do one of the following:
  - To create a report from a template, click the Template dropdown arrow and select a template from the list.
    - To change reporting elements in the template, go to Step 3.
    - To run the report without changing the template, go to Step 5.
  - To create a custom report, go to Step 3.
3. Click the arrow on the left to see expanded lists.
4. Select checkboxes for report elements to include.
5. Enter a report title.
6. (Optional) Select the Password Encrypt Report checkbox and then enter a password.
7. Click Generate Report.

The software creates a PDF report.

8. To save the report, click Save.

## Creating Report Templates

If you are assigned the Create New Templates user privilege, you can create and save a new report template by editing the default report structure or by opening and editing an existing report, and then saving the modified structure as a new template.



**Note:** For information on creating reports, see [Report Elements on page 145](#).

### To create a report template

1. Select the Template Setup tab and click Reports.



2. Do one of the following:

- To create a new template, click Add New Template.
- To open an existing template, select the template and then click Edit Report Template.

The default or selected layout appears.

3. Click the arrow to the left of each item to expand the list of reportable items.
4. Select and clear checkboxes to create the report structure.

**Tip:** Use the buttons at the bottom of the screen to expand or collapse all categories at once, or to select or clear all checkboxes at once. You can also drag and drop the section to rearrange the order of appearance in the report, and drag and drop items in the section to reorder them.



5. In the Report Title field, enter a name for your report template.
6. Click Save.
7. In the Save dialog box, enter a name for your template file.
8. Select a storage location.

Choose from the following:

- System Templates or My Templates

**Important:** Only one of these locations will be valid. If your administrator has specified preferred Locations in System Settings, you can save your templates to the System Templates folder or the Shared folder. If preferred locations are not specified, you can save them to the My Templates folder or the Shared Templates folder.

- Shared Templates

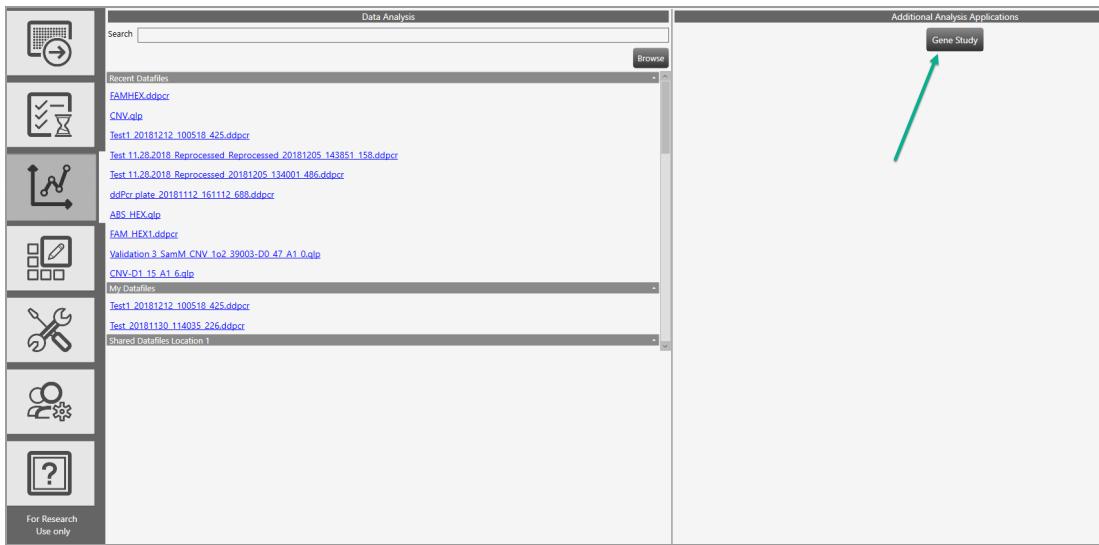
You have the option to save your templates to this folder to make them available to all users.

9. Click Save again to save the report format as a template.



# Chapter 12 Gene Study Module

You access the Gene Study module from the Data Analysis window.

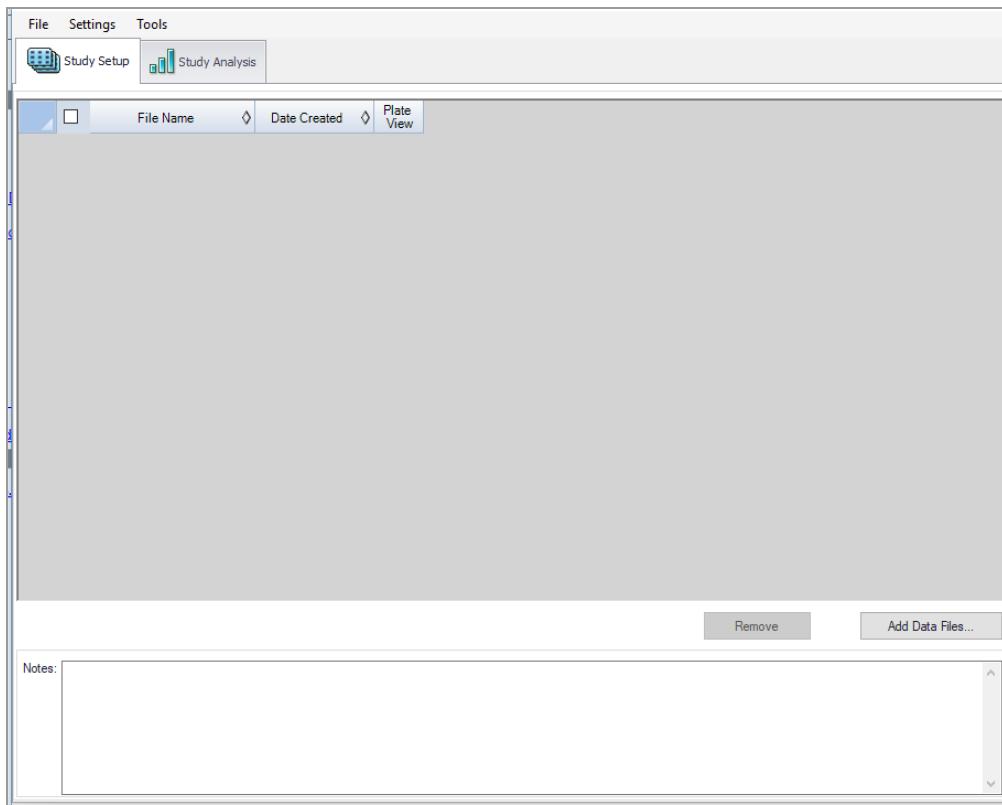


To create a gene study, you add one or more data files and then compare gene expression data from one or more ddPCR experiments.

**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.

## To open the Gene Study module

- Click Gene Study.



The Gene Study dialog box includes two tabs:

- **Study Setup** — manages the run files in the gene study

**Important:** Adding or removing data files in a gene study does not change the data in the original file.

- **Study Analysis** — displays the gene expression data for the combined runs.

From these tabs you can add files to set up a gene study, analyze your data in charts and plots, and run reports on the gene study data.

## Gene Study Options

The Gene Study module provides the menus and corresponding options described below.

- **File** — Open, save, and close the gene study, and add files
- **Settings** — Restore the default layout
- **Tools** — Create a report

### To open a gene study file

1. Click File → Open.
2. In the Open dialog box, navigate to and select the data file, and then click Open.

The file opens in the Gene Study module.

### To save a gene study file

1. Click File and select Save or Save As.
2. If prompted, enter a file name and click Save.
3. If prompted to overwrite an existing file, click Yes or No.

### To close a gene study file

► Click File → Close.

**Note:** If you have not saved the file, an advisory prompt appears.

### To add data files

1. Click File → Add Data Files.
2. In the Open dialog box, navigate to the data files to be added to the study.
3. Select one or more files and then click Open.

The files are added to the Study Setup window.

### To restore default settings

► Click Settings → Restore default settings.

**Note:** At least one modified data file must be open.

### To generate a report

► See [Gene Study Reports on page 163](#).

## Setting up the Gene Study

Use the Study Setup window to

- Add data files to include in the study
- Remove data files from the study
- Select data files for viewing and analysis
- Show the plate view for a data file

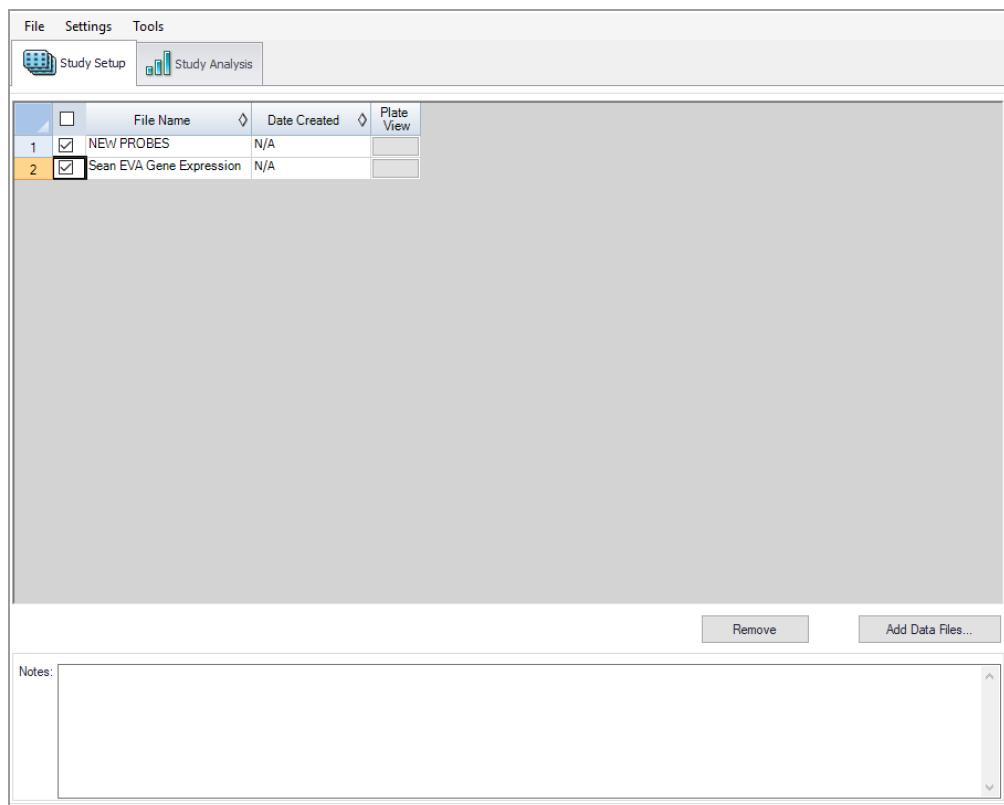


Table 34 defines each column in Study Setup.

**Table 34. Gene Study Setup Fields**

Column Title	Description
File Name	Name of the data file
Date Created	Date the file was imported into the Gene Study module
Plate View	Opens a map of the plate with the data for each dye in each well included in the Gene Study

Gene Study Plate View



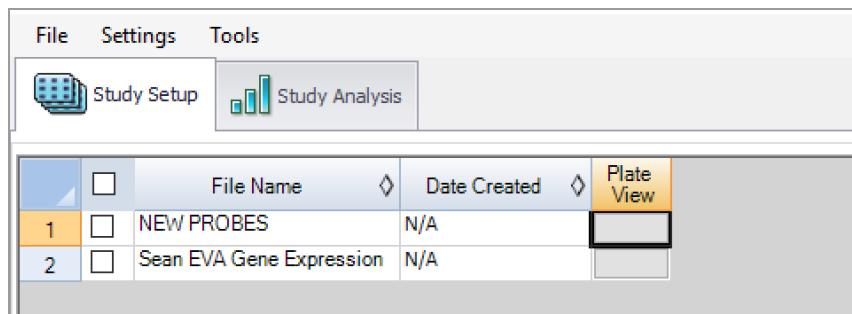
Close

## Adding Data Files

### To add data files

1. On the Study Setup tab, click Add Data Files.
2. In the Open dialog box, navigate to the data files to be added to the study.
3. Select one or more files and then click Open.

The files are added to the Study Setup window.



The screenshot shows the 'Study Setup' tab selected in the top navigation bar. Below the tabs, a table lists two data files: 'NEW PROBES' and 'Sean EVA Gene Expression'. The 'Plate View' column for 'NEW PROBES' is highlighted with an orange border.

	<input type="checkbox"/>	File Name	Date Created	Plate View
1	<input type="checkbox"/>	NEW PROBES	N/A	
2	<input type="checkbox"/>	Sean EVA Gene Expression	N/A	

## Removing Data Files

### To remove data files

- Select the checkbox next to the file and then click Remove.

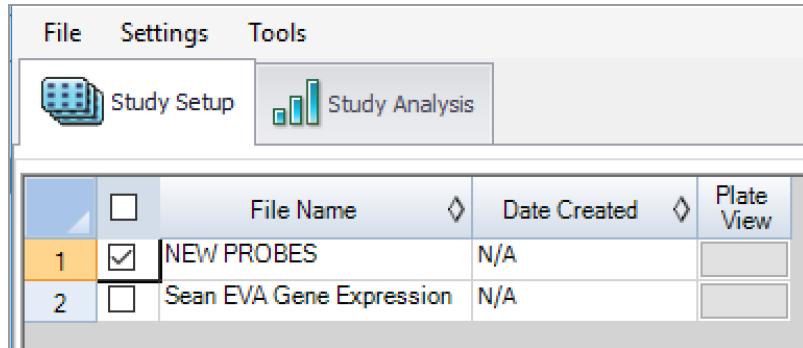
The file is removed from the Study Setup window.

**Note:** The Remove button is disabled until you select a file.

## Selecting Data Files for Analysis

### To select data files for analysis

1. Select the checkboxes next to the files to be included in the analysis.



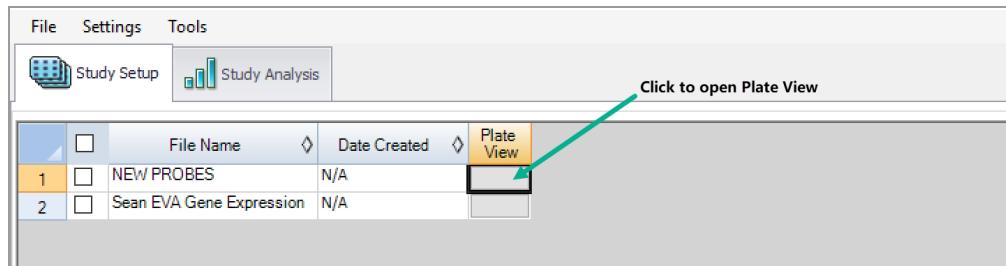
	<input type="checkbox"/>	File Name	Date Created	Plate View
1	<input checked="" type="checkbox"/>	NEW PROBES	N/A	[button]
2	<input type="checkbox"/>	Sean EVA Gene Expression	N/A	[button]

2. Select the Study Analysis tab to see the analysis results. For information, see [Using the Study Analysis Features on page 161](#).

## Showing the Plate View

### To show the plate view

1. In the Plate View column, click the button for the plate view to be displayed.



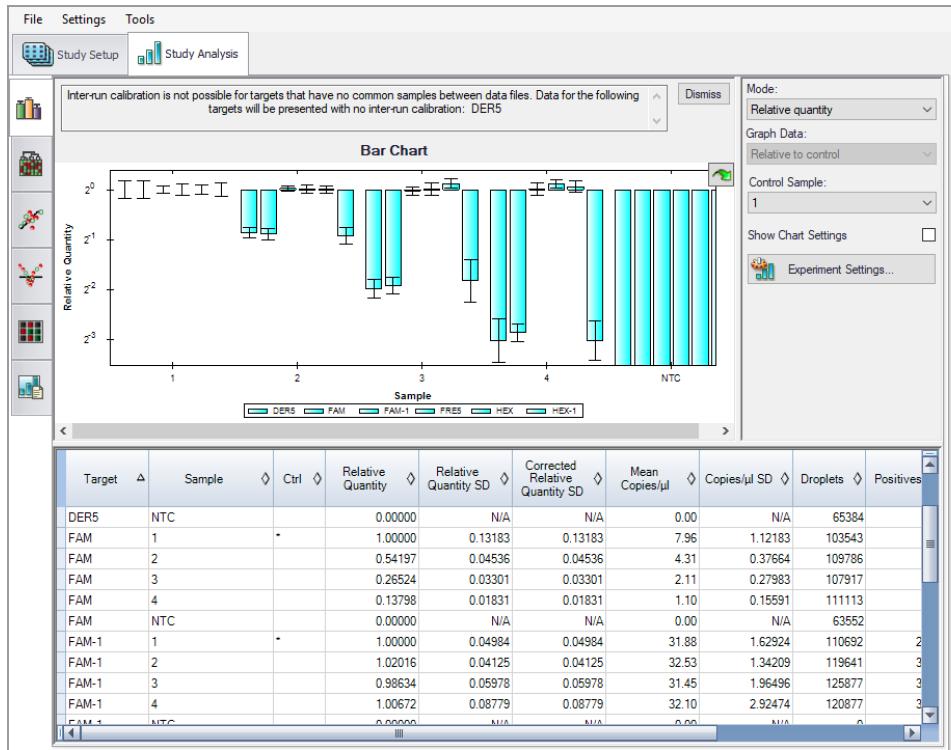
	<input type="checkbox"/>	File Name	Date Created	Plate View
1	<input type="checkbox"/>	NEW PROBES	N/A	[button]
2	<input type="checkbox"/>	Sean EVA Gene Expression	N/A	[button]

The Plate view appears.



- Click Close to close the view.

## Using the Study Analysis Features



The Study Analysis tab displays the data from all runs in the gene study in the views described in [Table 35](#).

**Table 35. Analysis Toolbar**

Button	Description
Bar Chart	Size of bars correspond to normalized expression or relative quantity
Clustergram	Data is arranged in a hierarchy based on the degree of similarity of expression for different targets and samples

**Table 35. Analysis Toolbar, continued**

Button	Description
 Scatter Plot	Dot plot of normalized expression of targets for a control versus an experimental sample
 Volcano Plot	Dot plot of change in expression (regulation) of a target for an experimental sample compared to a control
 Heat Map	Plate layout grid showing target regulation for an experimental compared to a control sample based on relative normalized expression <b>Note:</b> If the same targets are in the same location on multiple plates but with different samples, use the dropdown menu to select a particular plate for analysis.
 Results Table	Provides results in a tabular format

## Gene Study Reports

Use the Gene Study Report dialog box to arrange the gene study data into a report. Table 34 lists all the options available for a gene study report.

**Table 36. Gene Study Report Categories**

Category	Option	Description
<b>Header</b>		
	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
<b>Study Analysis Bar Chart</b>		
	Analysis Settings	List of the selected parameters
	Chart	Gene expression bar chart showing the data
	Target Names	List of targets in the gene study
	Sample Names	List of samples in the gene study
	Data	Spreadsheet that shows the data
	Target Stability	Target stability data
	Inter-run Calibration	Inter-run calibration data
<b>Study Analysis Clustergram, Scatter Plot, Volcano Plot, and Heat Map</b>		
	Analysis Settings	Settings for each chart type
	Chart	Gene expression chart showing the data
	Data	Spreadsheet listing the data in each target

## Creating a Gene Study Report

Before creating a report, make any needed adjustments to the gene study data and charts.

### To create a gene study report

1. Select Tools → Reports in the Gene Study menu to open the Report dialog box.
2. Select the options you want to include in the report.

The report opens with default options selected. Select or clear the checkboxes to change whole categories or individual options within a category.

For information on the available options to display, see [Gene Study Reports on page 163](#).

3. Change the order of categories and items in a report.

Drag the options to the required position. Note that items can be reordered only within the categories to which they belong.

4. Click Update Report to update the Report Preview window with any changes.
5. Print or save the report:
  - a. Click the Print Report button on the toolbar to print the current report.
  - b. Select File → Save to save the report in PDF, MHT or MHTML file format and select a location to save the file.

**Note:** MHT and MHTML are Microsoft formats.

- c. Select File → Save As to save the report with a new name or in a new location.
6. (Optional) To save the current report settings as a template, select Template → Save or Save As.

## Appendix A Experiment Examples

This section contains setup and analysis information for the following sample experiments:

- Direct Quantification, using the Amplitude Multiplex assay type
- Copy Number Variation, using the Probe Mix Triplex assay type
- Drop-Off, using the Basic Drop-off Assay type for genome edit detection
- Direct Quantification, using the Advanced Classification Method assay type

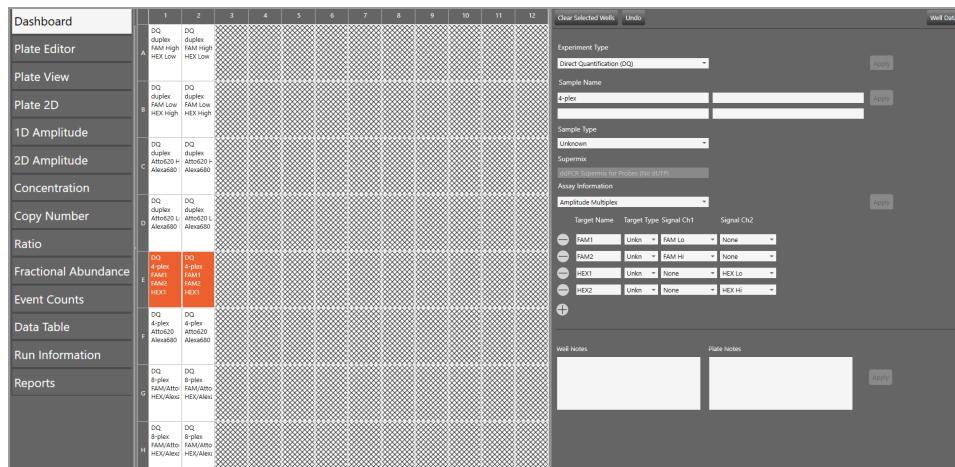
## Direct Quantification Multiplexed

You can increase the number of targets to be evaluated by using the Amplitude Multiplex option.

### Setting Up Experiment Parameters

#### To set experiment parameters

1. Open the analysis data file and select the Plate Editor tab.



2. Select one or more wells to be assigned well analysis attributes.
3. Change the experiment type to Direct Quantification (DQ), and then click Apply.
4. (Optional) Change the sample description identifiers.
5. (Optional) Change the sample type. The default type is Unknown.

**Tip:** For automatic thresholding of positive control wells, you must select Pos Ctrl.

**Note:** You cannot change the supermix that was used during data collection.

6. Under Assay Type, select Amplitude Multiplex and click Apply.

Four target rows appear.

7. Change the target name and type (unknown or reference) for each target in the well. The software defaults to FAM Lo, FAM Hi, HEX Lo, and HEX Hi, respectively, for each target.
8. If you select Reference, you can change the number of reference copies. The default is 2.

If no reference is selected, the target with the highest dye concentration in the highest channel is used as the reference.

If more than one reference is selected in the well, checkboxes appear to the right for using the reference in the plot.

**Note:** Each Reference target enables ratio calculations in the results.

9. (Optional) Change the high and low target identifiers.

Amplitude Multiplex allows for up to two assays in each channel, indicated by Lo and Hi labels. In this multiplex mode, each target may have a signal for only one channel. You can

- Select a different dye, if the channel supports two dyes (for example change HEX to VIC or VIC to HEX)
- Select a different channel for the target (for example, change FAM to HEX)
- Change Hi to Lo or Lo to Hi

10. Click Apply.

**Tip:** Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can click Undo to undo an applied well setting.

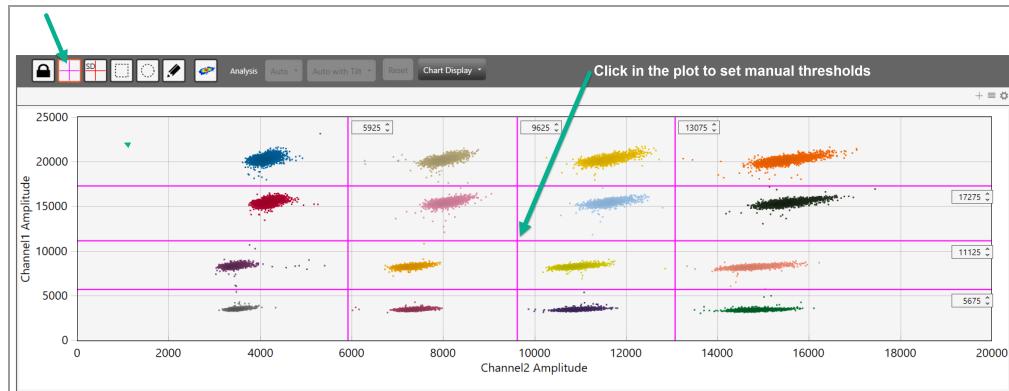
## Viewing and Adjusting Results

Automatic thresholding is not available for Amplitude Multiplex experiments.

For clusters that are easily identifiable across the chart, use Threshold Lines mode to set manual thresholds. If the data is not orthogonal and the threshold lines do not adequately divide the populations of droplets, use Threshold Clusters mode to set manual thresholds.

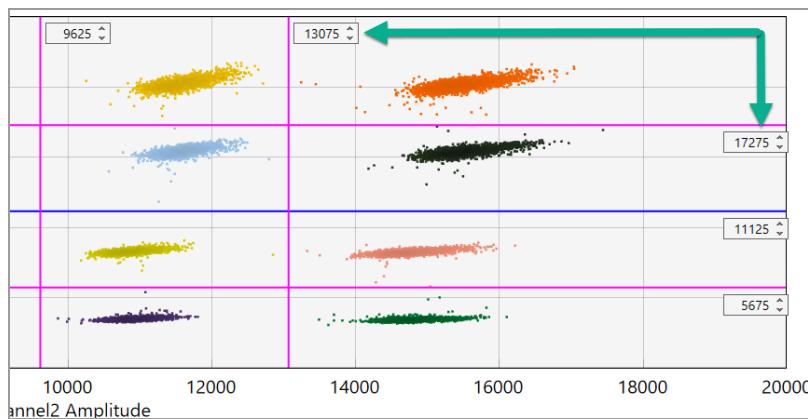
### To set thresholds in the 2D Amplitude window

1. Select the 2D Amplitude tab.



2. On the toolbar, click the Threshold Line Mode button.
3. Click anywhere on the plot to apply “best fit” threshold lines to the data.
4. To adjust the thresholds, drag the lines horizontally or vertically in the plot area.

**Tip:** You can also enter a specific threshold value in the boxes at the end of each threshold line.



### To apply and adjust manual clusters

1. Click any of the three cluster mode buttons (square, circle, or freehand).
2. Press the left mouse button and draw the chosen shape around the cluster of droplets to be classified first.

**Tip:** When you release the mouse button, a pop-up tool appears to help you select the correct target combination. Target names are shown along the two axis based on the signal values assigned during plate setup.



3. To classify droplets as positive or negative for each given target, select the colored box that best represents the location of the cluster of droplets.

## Copy Number Variation Triplexed

A CNV experiment with probe mix triplexing uses two targets and a reference to produce analysis data.

### Setting Up Experiment Parameters

#### To set experiment parameters

1. Open the analysis data file and select the Plate Editor tab.
2. Select one or more wells to be assigned well analysis attributes.
3. Set the experiment type to Copy Number Variation (CNV), and click Apply.
4. (Optional) Change the sample description identifiers.
5. (Optional) Change the sample type. The default type is Unknown.

**Tip:** For automatic thresholding of positive control wells, you must select Pos Ctrl.

**Note:** You cannot change the supermix that was used during data collection.

6. Click Apply.
7. From the Assay Type dropdown list, select Probe Mix Triplex and click Apply.

QX Manager Software displays three targets.

**Important:** You cannot add or delete triplex targets.

8. Enter target names and select the target type (unknown or reference) for each target in the well.
9. Assign the targets as follows:

Target	Target Type	Signal Ch1	Signal Ch2	Ref Copies	Plot?
Target 1	Unknown	FAM	None	N/A	N/A
Target 2	Unknown	None	HEX/VIC	N/A	N/A
Target 3	Reference	FAM	HEX/VIC	2	Yes

10. Click Apply.

**Tip:** Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can click Undo to undo an applied well setting.

## Viewing and Adjusting Results

**Note:** Linear thresholding is not available for Probe Mix Triplex experiments.

### To see the results

1. After all wells are configured, select the 2D Amplitude tab to evaluate the data and manually set clusters.
2. Click any of the three cluster mode buttons (square, circle, or freehand).
3. Press the left mouse button and draw the chosen shape around the cluster of droplets to be classified first.

**Tip:** When you release the mouse button, a pop-up tool appears to help you select the correct target combination. Target names are shown along the two axis based on the signal values assigned during plate setup.



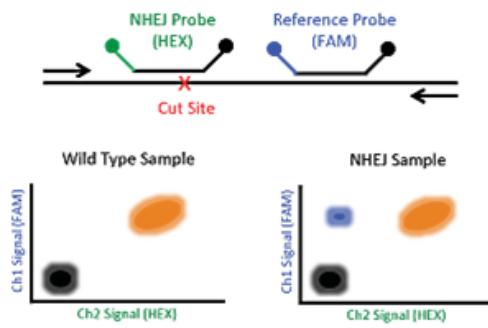
4. To classify droplets as positive or negative for each given target, select the colored box that best represents the location of the cluster of droplets.

## Drop-Off Assays for Genome Edit Detection

Two configurations of drop-off assays are possible and are shown below. Assign a signal (expected cluster position) to wild-type and NHEJ indel alleles. The signal produced by a wild-type allele will always be positive for both FAM and HEX, while the signal produced by an NHEJ indel allele depends on the assay configuration used (as shown below left and right).

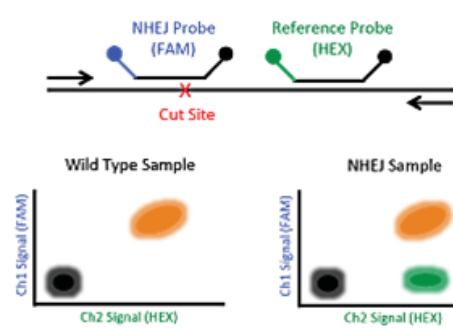
### Drop-Off Probe in HEX

*NHEJ Probe = HEX and Reference Probe = FAM*



### Drop-Off Probe in FAM

*NHEJ Probe = FAM and Reference Probe = HEX*



## Setting Up Experiment Parameters

### To set experiment parameters

1. Open the analysis data file and select the Plate Editor tab.
2. Select one or more wells to be assigned well analysis attributes.
3. Set the experiment type to Drop-Off (DOF) and click Apply.

Experiment Type  
Drop Off (DOF)

Sample Name  
Test

Sample Type  
Unknown

Supermix  
ddPCR Supermix for Probes (No dUTP)

Assay Information  
Basic Drop-Off

Target Name	Target Type	Signal Ch1	Signal Ch2	Ref Copies
WildType	Ref	FAM	HEX	2
NHEJEvent	Unkn	FAM	None	

4. (Optional) Change the sample description identifiers.
5. (Optional) Change the sample type. The default type is Unknown.

**Tip:** For automatic thresholding of positive control wells, you must select Pos Ctrl.

**Note:** You cannot change the supermix that was used during data collection.

6. Click Apply.

QX Manager Software automatically assigns the assay method as Basic Drop-Off.

7. Enter the target names, and then designate one target as Reference and the other as Unknown.

The Reference target enables ratio calculation of results.

8. Click Apply.

**Tip:** Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can click Undo to undo an applied well setting.

## Viewing and Adjusting Results

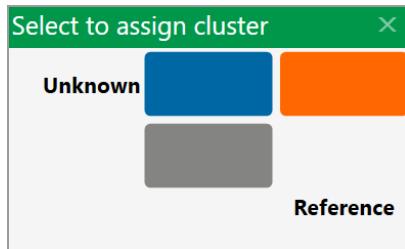
Thresholding is not available for Drop Off experiments.

### To apply and adjust clusters

1. After all wells are configured, select the 2D Amplitude tab to evaluate the data and manually set clusters.
2. Click any of the three cluster mode buttons (square, circle, or freehand).

3. Press the left mouse button and draw the chosen shape around the cluster of droplets to be classified first.

**Tip:** When you release the mouse button, a pop-up tool appears to help you select the available cluster assignments. Target names are shown along the two axis based on the signal values assigned during plate setup.



4. To classify droplets as positive or negative for each given target, select the colored box that best represents the location of the cluster of droplets.

# Direct Quantification with the Advanced Classification Method

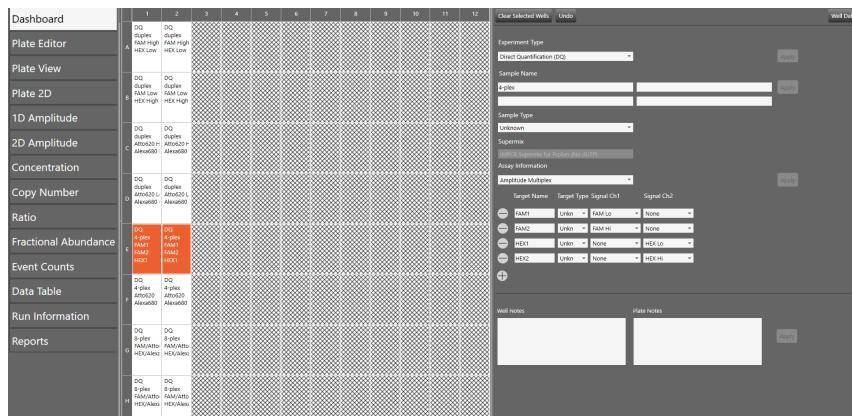
The Advanced Classification Method analysis mode allows the user complete control of droplet identification, and does not force or preassign any clusters. This allows a higher degree of multiplexing through radial dye mixing.

**Important:** This mode is intended for experienced users of droplet digital PCR.

## Setting Up Experiment Parameters

### To use the advanced classification method

1. Open the analysis file and select the Plate Editor tab.



2. Select one or more wells to be assigned well analysis attributes.
3. Set the experiment type to Direct Quantification (DQ) or RED and click Apply.
4. (Optional) Change the sample description identifiers.
5. (Optional) Change the sample type. The default type is Unknown.

**Tip:** For automatic thresholding of positive control wells, you must select Pos Ctrl.

**Note:** You cannot change the supermix that was used during data collection.

6. Click Apply.
7. Under Assay Type, select Advanced Classification Method and click Apply.
8. Use the + icon to add up to ten targets.

9. Enter the target name and type (unknown or reference) for each target in the well.
10. (Optional) If you select Reference, you can change the number of reference copies. The default is 2.
  - If no reference is selected, the target with the highest dye concentration in the highest channel is used as the reference.
  - If more than one reference is selected in the well, checkboxes appear to the right for using the reference in the plot.

**Note:** Each Reference target enables ratio calculations in the results.

11. Assign the first target an expected signal in a channel.

12. Assign your last target a signal in a channel that was not selected for your first target.

Each target in between will be assigned a signal depending on mixture ratio of the target.

13. Click Apply to confirm all well settings.

**Tip:** Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can click Undo to undo an applied well setting.

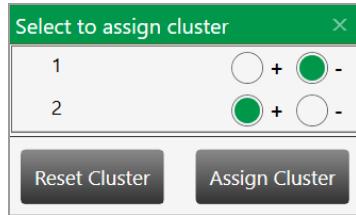
## Viewing and Adjusting Results

1. Click the 2D Amplitude tab.

All droplets are unclassified by default and are colored red. Only the Threshold Cluster mode is available for setting thresholds in Advanced Classification experiments in the 2D plot.

2. Use one of the Threshold Cluster mode tools (box, circle or freehand) to assign droplet clusters to targets.

When you select a droplet cluster, a cluster assignment box appears.



3. Select a radio button to identify the composition of the cluster.

For example, if the cluster is composed of positive droplets for Target-2, select the positive radio button for Target-2 and the negative radio buttons for the remainder.

**Important:** For calculations to occur, you must have at least one positive cluster for each target, as well as the assigned negative cluster.

**Note:** The software sets aside unassigned for calculation purposes. If you are unsure of droplet/cluster composition, leave it unassigned (red).

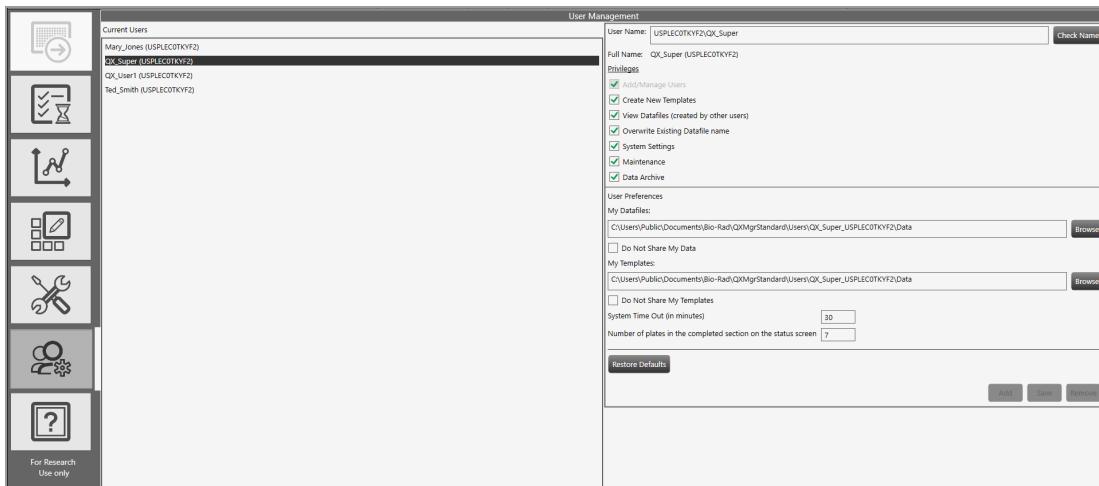
You can view the concentration results in the data table and in the Concentration tab.

If one of your samples was identified as a Reference under AssayType, you can view a ratio of concentration for unknown versus reference in the Ratio tab.

## Appendix A Experiment Examples

## Appendix B Managing Users

Use the User Setup and Preferences window to add, change, or remove QX Manager Software users, and set or change user privileges and preferences.



A superuser account, with all permissions, should be set up first. You will use the superuser account to create additional users and assign user privileges. If you need assistance, contact Bio-Rad Technical Support.

**Note:** Users who are not assigned any of the specified privileges can still execute runs and use the Analysis module.

You can set up local or domain accounts. If the computer connected to the QX200 Droplet Reader.

- Can access your company's network, QX Manager Software can recognize the Active Directory user names.
- Cannot access your company's network, your IT department can set up user names as local Windows accounts.

You can create user accounts with any user name convention and password character structure, as long as it complies with your organizational requirements. The software is designed to validate each user against the name it finds in either the Active Directory or local domain, so users must log in with the proper domain and user name.

However, QX Manager Software must be able to validate the user in either the local or domain user group before you can add the user in the software. Note the following:

- For local IDs, you must set up each user as a local Windows user account on each computer where the software is installed.
- For Active Directory IDs, your IT department must connect the computer connected to the QX200 Droplet Reader, as well as any separate computers running the software, to your network.

## Adding Users

Only a user who is assigned the Add/Manage Users privilege can add QX Manager Software users.

Before you begin, ensure that all users have IDs set up in Active Directory or as local accounts.

### To add a user

1. In the User Name field, enter the user name and then click Check Name.

One of the following occurs:

- If the software recognizes the user name, the software displays a validation message. Continue to Step 2.
- If the user is not recognized, an error message appears. Do one of the following:
  - For network users, ensure there is a working connection to the network and then verify your entry is accurate.
  - For local users, verify the entry is accurate. If the user is not set up locally or on the network, contact your system administrator for assistance.

2. When the successful validation message appears, click OK, and then click Add.

The software displays a confirmation message.

3. Click Yes to add the user, and then click OK to close the dialog box.

## Adding or Removing User Privileges

Only a user assigned the Add/Manage users privilege can add or remove user privileges.

By default, individual users without the privileges described in [Table 37](#) can set up and execute a run, open files, view and analyze results.

**Table 37. User Privileges**

Privilege	Description
Add and manage users	Add or remove users, set privileges, and change preferences. <b>Note:</b> Only the superuser can remove this privilege from other users.
Create new templates	Save a plate, thermal cycling protocol, or analysis report design as a template.
View data files created by other users	View files created by other users in the storage folders.
Overwrite existing data file name	Use Save or Save As capability. <ul style="list-style-type: none"> <li>■ Selecting Save replaces the original file content with any changes made by the user, without changing the file name.</li> <li>■ Selecting Save As saves the file with a new file name or location.</li> </ul> <b>Note:</b> Users without this permission can open files and perform analysis but cannot save their changes.
System settings	View event logs, and view and modify the shared data file and template locations. Two locations can be assigned for data files and one for templates. <b>Note:</b> You can also set preferred locations, which override all paths in individual preferences.
Maintenance	Perform software updates. View the event log. <b>Note:</b> All users can view the maintenance log and maintenance reports.
Data archive	Move raw data from the computer connected to the QX200 Droplet Reader to free up disk space needed for runs on the instrument.

**Table 38** contains a sample set of privileges assigned by user roles common to a laboratory. User roles can be defined and used in any scenario, but may be required in regulatory environments.

**Table 38. Example User Roles**

Privilege	Superuser	Lab Manager	Group Lead	Technician/ Student Intern
Add/manage users	✓	✓		
Create new templates*	✓	✓	✓	✓*
View data files created by other users	✓	✓	✓	✓
Override existing ddPCR file name	✓	✓	✓	
System settings	✓			
Maintenance	✓			
Archive data files	✓	✓	✓	✓

\*You can assign this privilege to non-administrative roles if anyone can create and save a template. If template formats are restricted, then you should assign this permission to administrative roles only.

**Note:** Bio-Rad recommends defining at least one additional superuser as a backup, as well as other administrative and standard roles based on your needs. Guest users can open shared templates, execute runs, and perform analysis but are not typically assigned other privileges.

### To add or modify privileges

1. Click the Add/Manage Users tab to open the User Management window.
2. Select a user from the Current Users list. The name appears in the User Name field.
3. Select or clear the checkbox for each privilege in accordance with the user's role in using the software.

You can assign user privileges in any combination.

4. When the confirmation message appears, click Yes to save the changes, and then click OK to close the pop-up.

## Modifying User Preferences

Users assigned the Add/Manage Users privilege can change the preferences for other users. Individual users can also change their own preferences.

### To modify a user's preferences

1. Click the Add/Manage Users tab, and then enter the user name.
2. Change any of the following preferences for the user:
  - Enter a different file path for user data files and templates.  
**Important:** You can also specify Preferred Locations in System Settings, which override all file paths specified in individual preferences. For information, see [System Settings Tab on page 192](#).
  - Select or clear the checkbox to change user data file and template privacy settings.
  - Enter a different default system timeout period.
  - Enter a different number of completed plates to show in the Status section, up to a maximum of 100.
3. Click Save.  
QX Manager Software displays a confirmation message.
4. Click Yes to save the changes, and then click OK to close the dialog box.

## Removing Users

Only a user assigned the Add/Manage Users privilege can remove users. Removing a user from the software does not remove the user from other systems or databases. The user still exists on your network, or in the Windows local user list.

### To remove a user

1. Click the Add/Manage Users tab to open the User Management window.
2. In the Current Users pane, select the user and click Remove.  
QX Manager Software displays a confirmation message.
3. Click Yes to remove the user, and then click OK to close the dialog box.



## Appendix C Instrument Maintenance

Bio-Rad recommends regular maintenance of your equipment, which includes

- Keeping the surface areas clean
- Regularly checking the fluid levels
- Inspecting the equipment for damaged parts or wiring

Bio-Rad also recommends semi-annual preventive maintenance on your instrument by a Bio-Rad service engineer. Contact Bio-Rad if your instrument requires service or recalibration.

## Fluid Maintenance

You must ensure there is enough oil for at least one full run, and you must empty the waste container when it approaches a full state.

You can check your fluid levels using any of the following methods:

- Check the QX Manager Software status bar, which shows oil as a percentage remaining and waste as percentage full
- Check the Indicator lights on the QX200 Droplet Reader, which flash amber when oil is low and waste is high
- Check the levels in the containers themselves

[Table 39](#) explains fluid level maintenance for your instrument.

**Table 39. Fluid Levels**

<b>QX200 Indicator Light Color</b>	<b>Fluid Level</b>	<b>Status</b>
Solid green	Oil greater than 30% or waste less than 70%	Bottle levels OK. You can start your run.
Flashing green	Oil less than 30% or waste greater than 70%	You may be able to start a run on fewer than 96 wells.  If there is not enough oil in the dispensers to perform a run on the number of plates you set up in the Plate Editor, the software prevents the run until you fill the oil dispensers and empty the waste container.
Flashing amber	Oil less than 10% or waste greater than 90%	The software prevents the run until you fill the oil dispenser.

## Replacing Droplet Oil

### To replace the oil bottle

1. Slide the container carriage out using the handle on the side of the instrument.
2. Remove the oil bottle and set it aside, to use when changing the waste bottle.
3. Insert a new bottle of oil, and tighten the cap into place.

## Managing Waste

You must apply standard Material Safety Data Sheet (MSDS) and OSHA practices when handling and disposing of generated waste. A typical waste profile should contain the following:

Fluorinated oils	95%
Water	5%
Bleach	Less than 0.5%
Proteins, nucleic acids, fluorescent dye	Less than 0.1%

Before disposal, Bio-Rad recommends collecting the waste bottles in a polyethylene container and discarding the container within one month of the oldest waste collected.

While Bio-Rad droplet reader fluids are nonflammable and inert, with low toxicity and environmental impact, they are based on fluorinated hydrocarbon chemistry and disposal should comply with institutional, state, and local regulations. Contact your local environmental health and safety specialists for guidance on proper disposal, including locations in your area.

Use the last empty oil bottle as the new waste bottle. Bio-Rad provides a new Waste label to cover the Oil label.

### To replace the waste bottle

1. Slide the container carriage out using the handle on the side of the instrument.
2. Remove the waste bottle.
3. Place the new Waste label over the Oil label on the oil bottle.
4. Insert the last empty oil bottle into the waste container slot.

**Important:** Use each empty oil bottle as a new waste bottle only once and then discard it. Before discarding, add 50ml of 10% bleach solution to the bottle to prevent microbial growth, and then label the bottle as waste.

## Flushing or Priming the Instrument

You should flush the instrument after you replace the waste bottle.

### To flush the instrument

1. Select the System Utilities tab.
2. Tap Flush.

A message appears, asking if you want to flush the instrument.

3. Tap Yes to begin.
4. A “Performing Flush” message appears.

When the flushing process is finished, an operation complete message appears.

5. Tap OK.

### To prime the instrument

You should prime the instrument after you replace the oil bottle.

1. Select the System Utilities tab.
2. Tap Prime.

A message appears, asking if you want to prime the instrument.

3. Tap Yes to begin.
4. A “Performing Prime” message appears.

When the priming process is finished, an operation complete message appears.

5. Tap OK.

## Instrument Maintenance Log

QX200 Droplet Reader System Maintenance Log		Month	Year																													
Droplet Reader Serial Number	Last Annual Maintenance Date																															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
<b>As necessary</b>																																
Replace droplet reader oil																																
Wipe droplet reader plate holder(s) (if wet)																																
Discard waste bottle after one-time use																																
Use empty oil bottle as new waste bottle																																
Add 50mL of 10% bleach to new waste bottle																																
Signature																																
<b>Daily</b>																																
Clean work area (before and after use)																																
Discard used consumables																																
<b>Weekly (at start of week)</b>																																
Check droplet reader oil expiration date																																
Restart the droplet reader																																
Restart the connected computer and software																																
<b>Monthly</b>																																
Clean droplet reader plate holder metal surface in the instrument with 80% ethanol or isopropanol																																
<b>Quarterly</b>																																
Prime instrument																																
Purge droplet reader oil																																
Backup the database																																
<b>Annual Maintenance</b>																																
Call Bio-Rad																																



## Appendix D System Utilities

From the System Utilities tab, you can access the following tabs for managing tasks and data related to the instrument, software, data, and storage:

- **System Settings** — View shared template and data file locations
- **Event Log** — View logged data about all software activities
- **Maintenance Log** — Record maintenance activities
- **Maintenance Reports** — PDF maintenance records from Bio-Rad maintenance
- **Archive Data** — Clear up space in your primary storage areas
- **Reprocess Data** — Reprocess a .data file using new calibration values
- **Tools** — Flush or prime the instrument

### Notes:

- The Instrument Calibration tab is enabled only for the Bio-Rad service engineer user account.
- If you are not connected to the QX200 Droplet Reader, only the System Settings and Event Log tabs are available.

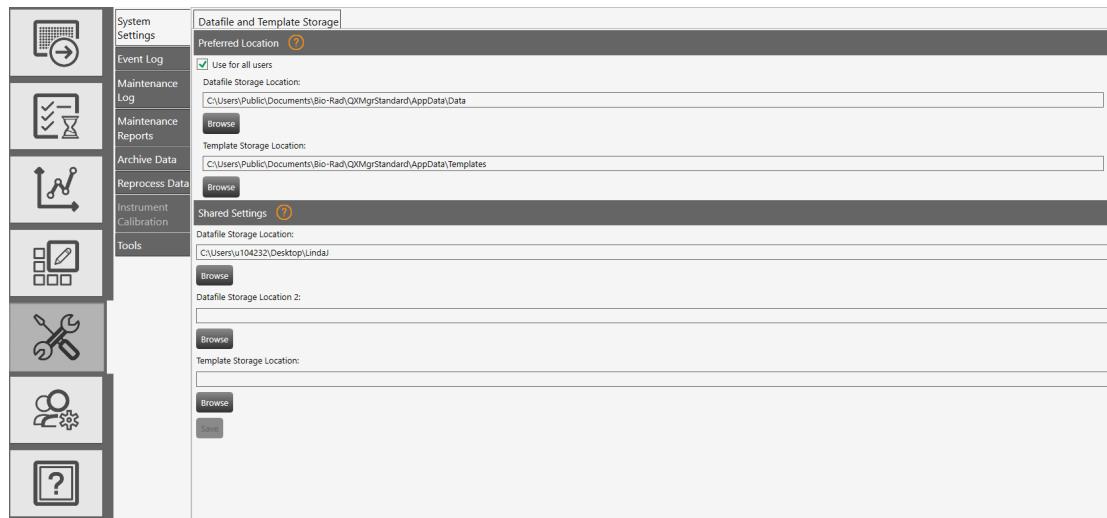
## System Settings Tab

The System Settings window opens by default when you select the System Utilities tab, and contains fields where you can enable and enter storage file paths.

In System Settings, you can set up the following storage areas:

- Preferred Location
- Shared Settings

**Important:** Primary storage locations are either the file paths specified in the user's individual preferences, or the file paths specified under Preferred Locations. If a system administrator enables the Preferred Location settings for all users, files paths in individual user preferences are overridden.



### Preferred Location

If Preferred Location file paths are used, then data files for all users are automatically saved to the specified folders on the instrument.

**Important:** Only users assigned the System Settings user privilege can set preferred locations. This setting overrides individual user storage settings.

**Note:** Preferred location restrictions apply to data files only; users are able to choose a location for template storage.

### To specify preferred locations

1. Select the Use for all users checkbox and click Save.

The file paths are identified in the Datafile Storage Location and Template Storage Location fields.

2. (Optional) Modify the file paths, and then click Save.

## Shared Settings

Under Shared Settings, you can specify two shared data file storage folders and one shared template storage folder. Because data files are often very large, the software allows you to set a primary storage folder and a backup storage folder. Bio-Rad recommends routinely checking available storage space and if necessary, using the Archive function.

**Important:** Only users assigned the System Settings user privilege can change storage locations in the System Settings window.

### To specify shared folders

1. In the Datafile Storage Location field, enter a file path or click Browse to search for the folder.
2. In the Datafile Storage Location 2 folder, enter a file path or click Browse to search for the folder.

**Note:** These paths point to secondary storage locations. Data files are always saved initially to the preferred location (if specified by your administrator), or to the path specified in the user's preferences.

3. In the Template Storage Location field, enter a file path or click Browse to search for the folder.
4. Click Save.

## System Log Files

From the System Utilities tab, you can access log files for system and software events (event log), and maintenance activities (maintenance log).

### Event Log

Users who are assigned the Maintenance user privilege can view the system event log, which contains timestamped information on all software activities.

Log File: QXManagerRegulatoryHistory.log					
		Search	Previous	Next	
		Date/Time	=	Message	=
System Settings		12/10/2018 10:31:44 AM		User Logged in. User Name: BRR&D. User Full Name: Bio-Rad Research and Development	Info
Maintenance Log		12/10/2018 10:31:22 AM		User Logged in. User Name: BRR&D. User Full Name: Bio-Rad Research and Development	Info
Archive Data		12/10/2018 10:31:21 AM		Application Startup. Application Version: 1.0.66. OS Version: Microsoft Windows 10 Enterprise : 10.0.17134. Locale: English (United States)	Info
Reprocess Data		12/7/2018 2:51:30 PM		Application Closed	Info
Instrument Calibration		12/7/2018 2:47:07 PM		Failed To Open Analysis File: Y:\QSDRegulatoryFiles\adRenamed\extension\ColorCalibration\Rate_20180815_202254_669_Repro.qbs	Warning
Tools		12/7/2018 2:44:47 PM		Analysis File Opened. Path: Y:\DemoData\QLB\Combined EvaGreen Experiments\Combined EvaGreen Experiments.qbs	Info
		12/7/2018 2:44:24 PM		User Logged in. User Name: BRR&D. User Full Name: Bio-Rad Research and Development	Info
		12/7/2018 2:44:17 PM		User Logged in. User Name: BRR&D. User Full Name: Bio-Rad Research and Development	Info
		12/7/2018 2:44:16 PM		Application Startup. Application Version: 1.0.62. OS Version: Microsoft Windows 10 Enterprise : 10.0.17134. Locale: English (United States)	Info
		12/6/2018 4:01:23 PM		Application Closed	Info
		12/6/2018 4:01:19 PM		Changes discarded for Datafile - Y:\DemoData\QLB\Combined EvaGreen Experiments\Combined EvaGreen Experiments.qbs	Info
		12/6/2018 4:01:19 PM		Bio-Rad Research and Development Shutdown the application and discarded 0 loaded plates and changes in Datafiles.	Info
		12/6/2018 1:26:30 PM		Analysis File Opened. Path: Y:\DemoData\QLB\Combined EvaGreen Experiments\Combined EvaGreen Experiments.qbs	Info
		12/6/2018 1:18:00 PM		User Logged in. User Name: BRR&D. User Full Name: Bio-Rad Research and Development	Info
		12/6/2018 1:17:34 PM		User Logged in. User Name: BRR&D. User Full Name: Bio-Rad Research and Development	Info
		12/6/2018 1:17:33 PM		Application Startup. Application Version: 1.0.62. OS Version: Microsoft Windows 10 Enterprise : 10.0.17134. Locale: English (United States)	Info
		12/5/2018 3:13:01 PM		Application Closed	Info
		12/5/2018 3:11:49 PM		User Logged in. User Name: BRR&D. User Full Name: Bio-Rad Research and Development	Info
		12/5/2018 3:11:40 PM		User Logged in. User Name: BRR&D. User Full Name: Bio-Rad Research and Development	Info
		12/5/2018 3:11:39 PM		Y:\QSDRegulatoryFiles\adRenamed\extension\ColorCalibration\Rate_20180815_202254_669_Repro.qbs	Info

### To view the Event Log

1. Select the System Settings tab.



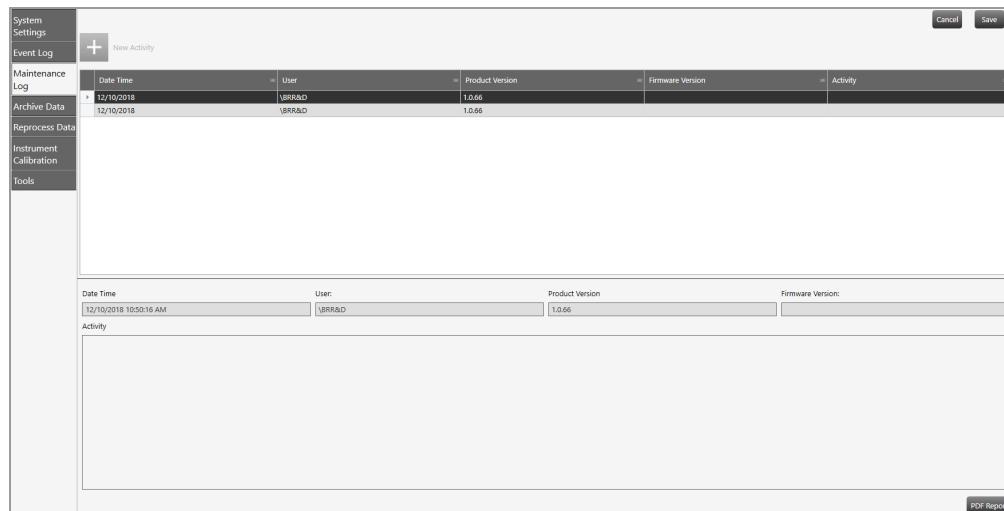
2. Select Event Log and do one of the following:
  - Scroll through the list of events displayed in the Event Log window.
  - Click Open Log and open the text file in Notepad.
3. (Optional) To automatically generate and display a PDF document, click PDF Report.

**Tip:** To restrict access to the file, you can assign a password. Select the PDF Password check box and then enter a password in the field. The file is saved in the Log Reports folder and can be opened only by entering the password.

## Maintenance Log

All users can view the maintenance log, which contains a list of maintenance records.

Users who are assigned the Maintenance user privilege can set up maintenance records, specify associated activities, and generate PDF reports.



The screenshot shows the Maintenance Log interface. On the left is a vertical menu with options: System Settings, Event Log (selected), Maintenance Log, Archive Data, Reprocess Data, Instrument Calibration, and Tools. The main area has a header 'New Activity' with 'Cancel' and 'Save' buttons. Below is a table with columns: Date Time, User, Product Version, Firmware Version, and Activity. The table shows two rows: one for 12/10/2018 with User \BR&D and Product Version 1.0.66; and another for 12/10/2018 with User \BR&D and Product Version 1.0.66. At the bottom, there is a detailed view of a single activity row with fields for Date Time (12/10/2018 10:50:16 AM), User (\BR&D), Product Version (1.0.66), Firmware Version, and Activity. A 'PDF Report' button is at the bottom right.

Date Time	User	Product Version	Firmware Version	Activity
12/10/2018	\BR&D	1.0.66		
12/10/2018	\BR&D	1.0.66		

### To view the Maintenance Log

1. Select the System Settings tab.



2. Select Maintenance Log.
3. To view the log entries, scroll through the list in the grid.

### To add a new activity

1. Click the plus sign (+) next to New Activity.

A line item appears in the grid and the Date Time, User, Product Version, and Firmware Version fields are automatically completed.

2. In the Activity field, enter a description of the planned activity.
3. Click Save.
4. Repeat to add another activity.

After saving, you can create a PDF report of the added activities.

5. (Optional) Click PDF Report.

## Maintenance Reports

Reports generated from the Bio-Rad service engineer when performing maintenance on your instrument are stored on the Maintenance Reports tab.

- To open a report, click the list item.

Following is an example of a Color Calibration report:

**BIO-RAD**

Color Calibration and Carryover Report

Username of the Person who did the Run:CHARLIE-1

Report Generation Date and Time:7/02/2019 09:03:29 PM

Instrument Serial Number:CHARLIE-1

Firmware Version:1.2700

Software Version:1.0.774.0628

SoftwareQX ONE Software Standard Edition

Lot Information

GCRC Cartridge:1821G

Droplet Generation Oil:HKDR101,JKDR101

Droplet Reader Oil:HKDR101,JKDR101

Calibration Effective Date:6/27/2019 01:20:11 PM

Color Calibration Result	Carryover Test Result	# of carryover events
Channel 1 (FAM) Pass	Fail	0
Channel 2 (HEX/VIC) Pass		
Channel 3 (CY5) Pass		
Channel 4 (CY5.5) Pass		
Low Hex	Fail	

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## Archiving Data

When the storage space on the computer connected to the QX200 Droplet Reader approaches a predefined limit, the software displays an advisory message at set intervals. Users can free up space by moving raw data to an archive storage folder in a different location.

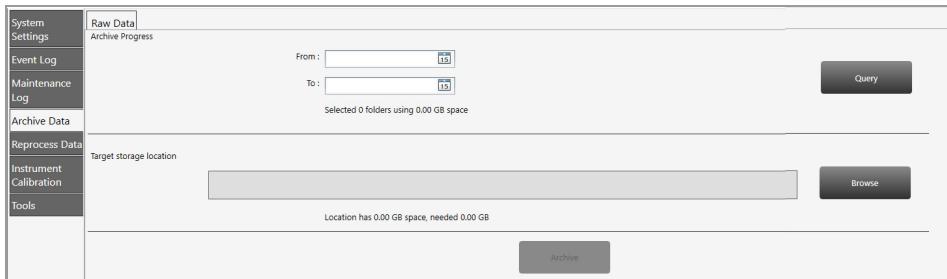
**Important:** You must be assigned the Data Archive user privilege to archive data.

### To archive files

1. Select the System Settings tab.



2. Select Archive Data.
3. Select a tab to archive a file type. For example, select Raw Data.



4. Under Raw Data Archive Progress, click the calendar icons and select a From date and a To date to define a date span.
5. Click Query to search for files dated within the date span.
6. Click Browse to search for a select the target storage location.
7. Click Archive.

## Reprocessing Data

After your instrument has been recalibrated, you can reprocess a data file from a run using different color calibration values.

**Note:** The instrument must be in an idle state before you can reprocess the data inqX Manager Software. If the file was created in an older software version, the software cannot reprocess the file and displays an advisory message.

### To reprocess a data file

1. Select the System Utilities tab.



2. Click Reprocess Data.
3. Click Browse and navigate to the analysis data file, and then in the Select Datafile dialog box, select the file and click Open.

The calibration values appear in the first Color Calibration table.

Channel	= FAM	= HEX	= VIC	= EvaGreen
1	262.302578125	7375.31396484375	0	13888.0962390625
2	5980.73046875	8777.4287109375	10000	3590.365234375

Channel	= FAM	= HEX	= VIC	= EvaGreen
1				
2				

4. Under the New calibration values heading, enter new values for the FAM, HEX, VIC, and EvaGreen® fluorophores.
5. Click Reprocess Data.

**Note:** When you reprocess a data file, the software creates an audit record containing the old and new calibration values.

## System Calibration and Tools

You cannot execute runs on the QX200 Droplet Reader unless the instrument is properly calibrated.

Only a Bio-Rad service engineer can calibrate your instrument and use the Instrument Calibration options in QX Manager Software.

If your instrument requires service, contact Bio-Rad Technical Support.

The Tools functions are currently applicable only to instrument maintenance. For information, see [Flushing or Priming the Instrument on page 188](#).

## Appendix E Additional Computers

For analysis purposes only, you can download purchased software from the Bio-Rad website and install it on computers provided by your company. This section explains the recommended system requirements for QX Manager Software, and provides instructions for downloading and installing or updating the software.

## Appendix E Additional Computers

# Appendix F Troubleshooting

This section contains information on troubleshooting and correcting minor issues you may experience with your instrument or software.

## Event Log

The Event Log contains records for all system and user activities, including system errors. If you do not understand, or cannot find an immediate remedy to, the error event, contact Bio-Rad Technical Support.

## Instrument Errors

If an error occurs during a run, the software cancels the run and displays an X in the Status column, along with a Run Aborted by System message as the Run End status. [Appendix F](#) contains error grouped by type.

1. Tap the X to display the corresponding error code and description.
2. Perform the steps in the solution column before starting a new run.
3. If the suggested solution does not resolve the problem, contact Bio-Rad Technical Support immediately.

**Note:** The error code and description are also displayed in the Run Information window .

Communication errors can be caused by instrument, connectivity, or power failures.

**Table 40. Communication errors**

Error Code	Description	Solution
102, 103, 104*, 110*, 111	Communication loss between the instrument and software, detector board error, loss during a run, or unable to reestablish a connection  <b>Note:</b> For codes marked with an asterisk (*), some data may be usable.	Disconnect and reconnect the USB cable, and then power cycle the instrument.
302, 308	Communication error involving pump  <b>Note:</b> Some data might be usable.	Power cycle the instrument.
502	Invalid checksum value	
900	Invalid command	

An electrical error is typically triggered by an invalid setting.

**Table 41. Electrical error codes**

Error Code	Description	Solution
406	Electrical Error; invalid power value setting.	Power cycle the instrument.
	<b>Note:</b> Some data might be usable.	

Mechanical errors can be caused by fluid issues (low oil, high waste), instrument operation errors, or binary data collection errors.

**Table 42. Mechanical error codes**

Error Code	Description	Solution
105, 106	Fluidics or motion response was invalid.  <b>Note:</b> Some data may be usable.	Power cycle the instrument.
201	Motors disabled  <b>Note:</b> Some data may be usable.	<ol style="list-style-type: none"> <li>1. Remove and reinsert the plate and plate lid.</li> <li>2. Check for incorrect or multiple heat seal foil layers.</li> <li>3. Check for strain on all motor axes.</li> <li>4. Power cycle the instrument.</li> </ol>

**Table 42. Mechanical error codes, continued**

Error Code	Description	Solution
205, 207, 210, 211, 212, 218	X, Y, or Z-axis, unable to reach target location or home in allotted time.  <b>Note:</b> Some data might be usable.	Check for motor obstructions, and then power cycle the instrument.
301, 303, 304, 305, 307, 312, 316, 317	Pump errors  <b>Note:</b> Some data might be usable.	Power cycle the instrument.

## Appendix F Troubleshooting

## Appendix G Ordering Information

This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad's ddPCR products, including your QX200 Droplet Reader.

### ddPCR System and Instrument Packages

[Table 43](#) contains information on the full QX200 Droplet Digital PCR system, as well as individual ddPCR instruments and included accessories.

**Table 43. ddPCR System and Instrument Packages**

Product	Description	Catalog Number
QX200 Droplet Digital PCR System	Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables	1864001
QX200 Droplet Generator	Includes the droplet generator (1), cartridges and gaskets (24 each), cartridge holders (2), and power cord (1)	1864002
QX200 Droplet Reader	Includes the droplet reader (1), plate holders (2), USB cable (1), and power cord (1)	1864003
Automated Droplet Generator	Includes the automated droplet generator (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1)	18641001
C1000 Thermal Cycler	Touch thermal cycler with 96-deep well reaction module	1851197

## QX200 Droplet Reader Accessories

Table 44 contains information on accessories for the QX200 Droplet Reader.

**Table 44. QX200 Droplet Reader Accessories**

Product	Description	Catalog Number
Computer	Computer that connects to the QX200 Droplet Reader for data collection and analysis	17006483
USB cable and power cord	Cable connecting the computer to the instrument and power cord connecting the instrument to the power source  <b>Note:</b> Contact Bio-Rad Technical Support for replacement information.	Included
Droplet reader plate holders (2)	Used to position the 96-well plate in the droplet reader plate compartment	12006834

**Note:** Reagents include the PCR supermix (for probes or EvaGreen®), droplet generation oil, and buffer control kit (for probes or EvaGreen®).

## QX200 Droplet Generator Accessories

Table 45 contains information on the accessories for the QX200 Droplet Generator.

**Table 45. QX200 Droplet Generator Accessories**

Product	Description	Catalog Number
DG8 droplet generator cartridges and gaskets	Microfluidic cartridges used to mix sample and oil for droplet generation  Gaskets to seal the cartridges  <b>Note:</b> You must seal the cartridge to prevent evaporation and to apply required pressure for droplet generation.	1864007
Droplet generator cartridge holder	Positions and holds the droplet generator cartridge in the instrument compartment	1863051
Power cord	Connects the QX200 Droplet Generator to the power source  <b>Note:</b> Contact Bio-Rad Technical Support for replacement information.	N/A

## Automated Droplet Generator Accessories

Table 46 contains information on accessories for the Automatic Droplet Generator.

**Table 46. Automated Droplet Generator Accessories**

Product	Description	Catalog Number
Cooling block accessory	Prevents evaporation during droplet generation	12002819
DG32 droplet generator cartridges	Microfluidic cartridge with gasket for mixing sample and droplet generation oil	1864108 1864109
Oil purge reservoir	Collects oil waste from priming and flushing	
Power cord	Connects the Automated Droplet Generator to the power source <b>Note:</b> Contact Bio-Rad Technical Support for replacement information.	N/A

## ddPCR Materials and Consumables

Table 47 contains information on accessories and consumables for Bio-Rad instruments.

**Table 47. Consumables and Other Materials**

Product	Description	Catalog Number
96-well PCR plates	ddPCR 96-well plates	12001925
DG32 droplet generator cartridges (AUTODG)	Microfluidic cartridge with gasket for mixing sample and droplet generation oil in the Automated Droplet Generator	1864108 1864109
Rainin Pipets	20 $\mu$ l for sample loading 50 $\mu$ l for droplet transfer 8-channel, 200 $\mu$ l for oil	L-20, L8-20 L-50, L8-50 L8-200
Rainin Pipet tips	Filtered	GP-L10F GP-L200F
Pipet tips (AUTODG)	Tips for Automated Droplet Generator	1864120 1864121

**Table 47. Consumables and Other Materials, continued**

Product	Description	Catalog Number	
Pipet tip bin (AUTODG)	Tip waste bins for Automated Droplet Generator	1864125	
Foil plate seals	Pierceable foil plate seals	1814040	
Plate sealer	PX1 PCR plate sealer	1814000	
Droplet generation oils	Droplet generation oil for probes	1863005	
	Automated droplet generator oil for EvaGreen®	1864112	
	Automated droplet generator oil for probes	1864110	
Buffer controls	QX200 buffer control kit for EvaGreen®	1864052	
	QX200 buffer control kit for probes	1863052	
Droplet reader oil	ddPCR droplet reader oil	1863004	
Droplet reader waste bottle	You can use an empty droplet reader oil bottle to collect the waste from droplet reading.	N/A	
PCR Supermixes			
<b>Note:</b> You can use these supermixes in both the QX200 Droplet Generator and the Automatic Droplet Generator.			
QX200 ddPCR EvaGreen® supermix <i>For use in nucleic acid sample preparation with the QX200 droplet generator</i>		1864033 1864034 1864035 1864036	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1ml) 50ml (50 x 1ml)
ddPCR Supermix for Probes (no dUTP) <i>2x supermix, for use in nucleic acid sample preparation with the QX100 or QX200 droplet generators</i>		1863023 1863024 1863025	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1)
ddPCR Supermix for Probes <b>Note:</b> <i>2x supermix, for use in sample preparation with the QX100 or QX200 droplet generators</i>		1863026 1863010 1863027 1863028	2ml (2 x 1ml) 5ml (5 x 1ml) 25ml (25 x 1ml) 50ml (50 x 1ml)

**Table 47. Consumables and Other Materials, continued**

Product	Description	Catalog Number
ddPCR Supermix for Residual DNA Quantification	1864037	2ml (2 x 1ml)
<b>Note:</b> 2x supermix, for use in residual DNA detection with the QX100 or QX200 droplet generators	1864038	5ml (5 x 1ml)
	1864039	25ml (25 x 1ml)
	1864040	1ml)
		50ml (50 x 1ml)
One-step RT-ddPCR Advanced Kit for Probes	1864033	2ml (2 x 1ml)
<b>Note:</b> 200 or 500 x 20 $\mu$ l reactions, 2x RT0-ddPCR mix, includes 1 or 2 manganese acetate tubes	1864034	5ml (5 x 1ml)
ddPCR Mutation Screening Kits		
ddPCR KRAS G12/G13 Screening Kit	1863506	
ddPCR KRAS Q61 Screening Kit	12001626	
ddPCR BRAF V600 Screening Kit	12001037	
ddPCR NRAS Q61 Screening Kit	12001006	
ddPCR NRAS G12 Screening Kit	12001094	
ddPCR NRAS G12/G13 Screening Kit	12001627	
ddPCR EGFR Exon 19 Deletions Screening Kit	12002392	
<b>Note:</b> All kits include 20x multiplex assay and 2x ddPCR supermix for probes (no DUTP)		
ddPCR Residual DNA Quantification Kits		
ddPCR CHO Residual DNA Quantification Kit	17000031	
ddPCR E. coli Residual DNA Quantification Kit	17000032	
<b>Note:</b> 200 x 20 $\mu$ l reactions, includes 20x CHO or E.coli/RDQ assay and 2X ddPCXR Supermix for Residual DNA Quantification		
ddPCR Copy Number Determination Kits		

**Table 47. Consumables and Other Materials, continued**

Product	Description	Catalog Number	
	ddPCR SMN1 Copy Number Determination Kit	1863500	
	ddPCR SMN2 Copy Number Determination Kit	1863503	
	<b>Note:</b> 200 x 20 $\mu$ l reactions, includes assay at 20x concentration, 2x ddPCR Supermix for Probes (no dUTP) and positive controls		
<hr/>			
ddPCR Library Quantification Kits			
	ddPCR Library Quantification Kit for Illumina TruSeq	1863040	
	<b>Note:</b> 200 x 20 $\mu$ l reactions, includes 1 vial of primers and probes at 20x concentration, 2x ddPCR Supermix for Probes (no dUTP) and positive controls		
<hr/>			
ddPCR Genome Edit Detection Assays			
	ddPCR HDR Gene Edit Assay	12002312	100 rxns
	ddPCR HDR Gene Edit Assay	12002313	500 rxns
	ddPCR HDR Gene Edit Package	12003796	1,000 rxns
	ddPCR HDR Ref Assay, Predesigned	12003805	100 rxns
	ddPCR HDR Ref Assay, Predesigned	12003806	500 rxns
	ddPCR HDR Ref Package, Predesigned	12003793	1,000 rxns
	ddPCR NHEJ Gene Edit Assay	12002314	100 rxns
	ddPCR NHEJ Gene Edit Assay	12002315	500 rxns
	ddPCR NHEJ Gene Edit Package	12003794	1,000 rxns





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