# QX200<sup>™</sup> Droplet Reader and QX Manager Software Standard Edition

**User Guide** 





## QX200 Droplet Reader and

## QX Manager Software Standard Edition

**User Guide** 

Version 2.1

For Research Use Only



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## **Revision History**

| Document   | Date              | Description of Change  |
|--|-------------------|--|
| QX200 Droplet Reader and QX Manager<br>Software Standard Edition | September<br>2023 | Add EcoTank functionality to<br>Instrument Maintenance section |
| Software version 2.1   |                   |  |
| DIR No. 10000107223 Ver G  |                   |  |
| QX200 Droplet Reader and QX Manager                              | July              | Release new SAP version to align                               |
| Software Standard Edition User Guide                             | 2023              | with general edits made to other                               |
| Software version 2.1   |                   | QX200 and QX600™ Droplet<br>Reader/QX Manager user guides      |
| DIR No. 10000107223 Ver F  |                   | after Ver E was released                                       |
| QX200 Droplet Reader and QX Manager                              | May               | Update the user guide with new and                             |
| Software Standard Edition User Guide                             | 2023              | improved features for Version 2.1;                             |
| Software version 2.1   |                   | add new legal statement and<br>Managing Waste section          |
| DIR No. 10000107223 Ver E  |                   |  |
| QX200 Droplet Reader and QX Manager                              | August 2022       | Update document with new                                       |
| Software Standard Edition User Guide                             |                   | features in QX Manager with the                                |
| Software version 2.0   |                   | QX200 instrument   |
| DIR No. 10000107223 Ver D  |                   |  |

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## 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.

**Important:** Only trained personnel should use this instrument.

## **Regulatory Compliance**

The instrument 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:2016, EN61010-1:2010 (3rd ed.). Electrical equipment for measurement, control, and laboratory use - Part 1: General requirements
- 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
- IEC 61010-2-081:2015, 3.0 edition, UL 61010-2-081:2015, CAN/CSA C22.2 No. 61010-2-081:19. 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)
- CAN/CSA 22.2 No 61010-1-04, Safety requirements for electrical, equipment for measurement, control, and laboratory use, Part 1: General requirements
- CAN/CSA C22.2 No. 61010-2-101:2019 IEC 61010-2-101: 2018 (3rd ed.)
- 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.

Table 1. Regulatory symbols

| Symbol                                      | Definition  |
|---|---|
| ( €   | The CE symbol indicates that the manufacturer ensures the product conforms with the essential requirements of the applicable EN directives.   |
| (F)   | 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.  |
| c • us                                      | This equipment has been tested and found to comply with the limits for a Class A digital device pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. |
| 2012/19/EU<br>2012/19/EU<br>www.bio-rad.com | 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 alert you about sources of injury or harm. Table 2 defines each safety warning label.

#### Table 2. Meaning of safety warning labels

#### Icon

#### Meaning



#### Warning about risk of harm to body or equipment

Operating the system before reading this manual can constitute a personal injury hazard. For safe use, do not operate this instrument in any manner unspecified in this manual. Only qualified laboratory personnel trained in the safe use of electrical equipment should operate this instrument. Always handle all components of the system with care and with clean, dry hands.



#### Warning about handling biohazardous materials

When handling biohazardous samples, adhere to the recommended precautions and guidelines and comply with any local guidelines specific to your laboratory and location.



#### Warning about risk of electric shock

In order to prevent electric shock, use caution when plugging and unplugging the instrument. Always turn off and unplug the instrument when performing maintenance procedures.

## Safe Use Specifications

For safe operation of the instrument, Bio-Rad™ strongly recommends that you comply with instructions listed in this section and in Appendix B, Instrument Maintenance.

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, power switch, and USB port 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.

For information on environmental requirements, see Environmental Requirements on page 26.

#### **Personal Protective Equipment**

Proper use of gloves is recommended with use of oils and sample plates. OSHA requirements for personal protective equipment (PPE) are set forth in the Code of Federal Regulations (CFR) at 29 CFR 1910.132 (General requirements); 29 CFR 1910.138 (Hand protection); 29 CFR 1926.95 (Criteria for standard personal protective equipment). Any gloves with impaired protective ability should be discarded and replaced. Consider the toxicity of the chemicals and factors such as duration of exposure, storage, and temperature when deciding to reuse chemically exposed gloves.

Listed below are features to aid glove selection for handling of machines, assays, oils, and cleaning solvents:

- Butyl gloves are made of a synthetic rubber and protect against peroxide, hydrofluoric acid, strong bases, alcohols, aldehydes, and ketones
- Natural (latex) rubber gloves are comfortable to wear and feature outstanding tensile strength, elasticity, and temperature resistance
- Neoprene gloves are made of synthetic rubber and offer good pliability, finger dexterity, high density, and tear resistance; they protect against alcohols, organic acids, and alkalis
- Nitrile gloves are made of copolymer and provide protection from chlorinated solvents such as trichloroethylene and tetrachloroethene; they offer protection when working with oils, greases, acids, and caustic substances

#### **Hazards**

The instrument 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 system.

#### **Biohazards**

The instrument 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.
- Change gloves frequently. 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. 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).

#### **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 instrument contains no potentially hazardous chemical materials.

#### **Explosive or Flammability Hazards**

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

#### **Electrical Hazards**

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

#### **Surface Decontamination**



WARNING! To prevent electrical shock, always turn off and unplug the instrument before 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 questions regarding the use of other cleaning agents, contact Bio-Rad Technical Support.

For more information on surface cleaning, see Appendix B, Instrument Maintenance.

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.

## **Decommissioning and Disposal**

The instrument 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**

You must perform the specified decontamination procedures before moving or shipping the system. 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**

Important: The instrument is for research use only, and not for use in diagnostic procedures.

The instrument and its associated accessories are covered by a standard Bio-Rad warranty. Contact your local Bio-Rad office for the details of the warranty. Follow the safety specifications listed in this chapter and throughout this guide.

The instrument is intended for laboratory use only. Bio-Rad Laboratories, Inc. 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. Alteration of this instrument voids the warranty and safety certification, as it creates a potential safety hazard.

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

Use only the power cord supplied with the instrument, using only the plug adaptor that corresponds to the electrical outlets in your region.

Safety and Regulatory Compliance

## Chapter 1 Introduction to Droplet Digital PCR

Droplet Digital™ PCR (ddPCR™) is a digital polymerase chain reaction 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 ±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 ~20,000 nanoliter-sized droplets with target and background DNA distributed randomly into the droplets during the partitioning process.
- Following droplet generation, the droplets are run 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 and uses Poisson statistical formulas to determine the absolute quantity.

Note: Positive droplets containing at least one copy of the target DNA molecule exhibit increased fluorescence compared to negative droplets.

### **Finding Out More**

Click the Help tab (2) 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 2 Using the QX200 Droplet Reader

Using the QX200<sup>™</sup> Droplet Reader, you can detect rare DNA target copies, determine copy number variation, and precisely measure gene expression levels. Applications and uses include:

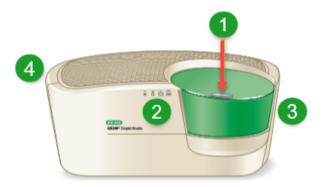
- Copy number variation
- Rare sequence detection
- Next generation sequencing (NGS) library quantification
- Viral load determination, including SARS-CoV-2 assays
- mRNA analysis
- Gene expression analysis and single cell gene expression analysis
- Absolute quantification
- Rare mutant detection
- NGS sample preparation
- GMO detection
- Contaminant testing

Following the droplet generation and thermal cycling processes, the QX200 Droplet Reader collects binary data for analysis as it singulates the ~20,000 nanoliter-sized droplets in each sample, and then streams them in single-file past a two-color detector to determine which droplets contain target molecules (positive reading) and which do not (negative reading).

The QX200 Droplet Reader detects targets in two selected fluorescent dyes, FAM or EvaGreen® and HEX or VIC.

**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 a droplet generator and thermal cycler, as well as associated accessories and consumables, from Bio-Rad. For information, see Appendix D, Ordering Information.

The following graphic and legend illustrate the functional areas on the instrument exterior:



#### LEGEND

- 1 Button to open and close lid
- 2 Status indicator lights (see Status Indicator Lights on page 32)
- On the back of the instrument, power and USB outlets
- Oil and waste compartment (see Instrument Maintenance on page 199)

## **Instrument Specifications**

This section contains information on the instrument size and weight of the instrument. Safety information is cited in Safe Use Specifications on page 14 and the proper environmental maintenance is cited in Environmental Requirements on page 26.



Table 3. Instrument specifications

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

## **Environmental Requirements**

Table 4 lists the environmental requirements for the QX200 Droplet Reader.

Important: You must use the supplied shielded cables with your instrument to ensure compliance with the Class A FCC limits.

Table 4. Conditions for safe use

| Usage aspect             | Requirements   |  |
|--------------------------|--|--|
| •                        | •  |  |
| Rated input power        | 100–240 V, 50–60 Hz, 1A 90W (plugs into standard AC receptacle)        |  |
| Fuses                    | 10A 250 V SLOW BLOW  |  |
| Voltage fluctuations     | ± 10% for the included external power supply                           |  |
|                          | <b>Note:</b> Use only the power cord supplied with the equipment.      |  |
| Pollution degree         | 2  |  |
| Usage temperature        | 18–30° C   |  |
| Relative humidity        | 85% max non-condensing   |  |
| Altitude                 | 0 to 6,560 ft (0 to 2,000 m) above sea level                           |  |
| Installation category    | II (external power supply plugs into a standard AC receptacle)         |  |
|                          | Indoor use only  |  |
| Ventilation requirements | The following distances should be unobstructed for proper ventilation: |  |
|                          | ■ 5 in (12 cm) on the left and right sides of the instrument           |  |
|                          | ■ 10 in (25 cm) behind the instrument                                  |  |

#### **Included Items**

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 D, Ordering Information.

Table 5 explains the components that ship with the QX200 Droplet Reader.

Table 5. QX200 Droplet Reader and Included Components

| Component                        | Description  | Catalog<br>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 | Included          |
|                                  | <b>Note:</b> To obtain cables or power cords, contact Bio-Rad technical support.                                       |                   |
| Droplet reader plate holders (2) | Positions and secures the 96-well plate in the droplet reader plate compartment  | 12006834          |

## **Additional Components**

The following tables identify additional components you can purchase for use with your system or instrument.

Table 6. Accessories and consumables

| Component               | Description  | Catalog Number                                     |
|-------------------------|--|--|
| Computer                | Computer that connects to the QX200 Droplet Reader for data collection and analysis  | 12017458   |
| Software purchase       | Bio-RadQX Manager Software, Premium Edition  Note: Standard Edition is included with your droplet reader purchase.                         | 12012172   |
| ddPCR 96-Well<br>Plates | Clear well/clear shell semi-skirted plates   | 12001925 (pkg of 25)                               |
| Droplet Reader<br>Oil   | ddPCR droplet reader oil for QX600 and QX200 instruments and systems   | 1863004  |
| Rainin Pipets           | <ul> <li>■ 20 µl pipet for sample loading</li> <li>■ 50 µl pipet for droplet transfer</li> <li>■ 8-channel 200 µl pipet for oil</li> </ul> | Rainin L-20<br>Rainin L-50, L8-50<br>Rainin L8-200 |
| Rainin Pipet Tips       | Filtered   | Rainin GP-L10F<br>Rainin GP-L200F                  |

Table 7. Thermal cyclers and plate sealer

| Product   | Description   | Catalog<br>Number |
|---|---|-------------------|
| C1000 Touch Thermal Cycler with 96-Deepwell Reaction Module | Includes the C1000 Touch Thermal Cycler chassis, 96-deepwell reaction module, USB flash drive   | 1851197           |
| PTC Tempo Deepwell Thermal Cycler                           | Includes the thermal cycler, power cord, USB cable, and Ethernet cable  | 12015392          |
| PX1 PCR Plate Sealer  | PCR plate sealer, includes heat sealing instrument, plate support block that holds 96-well and 384-well plates, sealing frame, power cord | 1814000           |
| Pierceable Foil Heat Seal                                   | Heat seal for the PX1 PCR Plate Sealer  | 1814040           |

Table 8. ddPCR supermixes

| Supermix                                  | Description  | Catalog number |                       |
|---|--|----------------|-----------------------|
| ddPCR<br>EvaGreen®                        | 2x supermix, for use in nucleic acid sample preparation with the QX600 and QX200 ddPCR systems   | 1864033        | 2ml (2 x 1ml)         |
|   |  | 1864034        | 5ml (5 x 1ml)         |
| Supermix                                  |  | 1864035        | 25ml (25 x 1ml)       |
|   |  | 1864036        | 50ml (50 x 1ml)       |
| ddPCR Supermix<br>for Probes (no<br>dUTP) | 2x supermix, for use in nucleic acid sample preparation with the QX600 and QX200 ddPCR systems   | 1863023        | 2ml (2 x 1ml)         |
|   |  | 1863024        | 5ml (5 x 1ml)         |
|   |  | 1863025        | 25ml (25 x 1)         |
| ddPCR Supermix<br>for Probes              | 2x supermix, for use in sample preparation with the QX600 and QX200 ddPCR systems  | 1863026        | 2ml (2 x 1ml)         |
|   |  | 1863010        | 5ml (5 x 1ml)         |
|   |  | 1863027        | 25ml (25 x 1ml)       |
|   |  | 1863028        | 50ml (50 x 1ml)       |
| ddPCR Multiplex<br>Supermix               | 4x supermix especially suited for probe-<br>based detection of multiple targets in DNA<br>samples using the QX600 and QX200<br>ddPCR systems | 12005909       | 1.2ml (2 x 0.6ml)     |
|   |  | 12005910       | 3 ml (5 x 0.6 ml)     |
|   |  | 12005911       | 12.5 ml (5 x 2.5 ml), |

Table 8. ddPCR supermixes, continued

| Supermix   | Description   | Catalog number                           |  |
|--|---|--|--|
| ddPCR Supermix<br>for Residual DNA<br>Quantification | 2x supermix, for use in residual DNA detection with the QX600 and QX200 ddPCR systems   | 1864037<br>1864038<br>1864039<br>1864040 | 2ml (2 x 1ml)<br>5ml (5 x 1ml)<br>25ml (25 x 1ml)<br>50ml (50 x 1ml) |
| One-step RT-<br>ddPCR Advanced<br>Kit for Probes     | 200 or 500x 20 µl reaction kit, for absolute quantification of target RNA in a one-step format with the QX600 and QX200 ddPCR systems | 1864021<br>1864022                       | 2ml (2 x 1ml)<br>5ml (5 x 1ml)                                       |

Table 9. Buffer controls

| Buffer                                 | Catalog<br>Number |
|--|-------------------|
| ddPCR Buffer Control Kit for Probes    | 1863052           |
| QX200 Buffer Control Kit for EvaGreen® | 1864052           |

## **Compatible ddPCR Process Instruments**

Along with the QX600 Droplet Reader, you can purchase the droplet generators shown below from Bio-Rad.

QX200 Droplet Generator



**Automated Droplet Generator** 



QX200 Droplet Reader



Important: A thermal cycler, which can be purchased separately, is required to perform a full ddPCR cycle. For information on additional instruments, accessories, and consumables, see Appendix D, Ordering Information.

## **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 is installed and configured. 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 Additional Components on page 28.

#### To connect and start the instrument

- 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. Turn on the connected computer and log in with a Windows account. For information, see your system administrator.
- 4. Open and log into QX Manager Software.

Important: The first time you log into the software, the Instrument window appears and you must select the instrument you are using. For information on selecting the correct instrument in QX Manager Software, see Selecting the Instrument and Droplet Volume on page 40.

## **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 information, see Table 10.

**Table 10. Droplet Reader Status Indicator Lights** 

| Indicator         | (Power)      | (Fluids)  | (Plate)               | (Run<br>Status)     |
|-------------------|--------------|---|-----------------------|---------------------|
| Solid Green       | Power is on  | Fluid levels OK   | Plate loaded properly | Complete            |
| Flashing Green    | N/A          | <ul><li>Less than 40% oil is available</li><li>More than 70% waste in container</li></ul> | N/A                   | In progress         |
| Flashing<br>Amber | N/A          | <ul><li>Less than 10% oil available</li><li>More than 90% waste in container</li></ul>    | N/A                   | Error during<br>run |
| No color          | Power is off | N/A   | No plate loaded       | Instrument is idle  |

If the bottle indicator light is solid green or flashing 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. Once the low level sensor is triggered, the Add Plate button is disabled and you cannot continue to run plates (less than 10%). You must replace the oil and waste bottles with new ones before the instrument can start the run.

## Chapter 3 About QX Manager Software

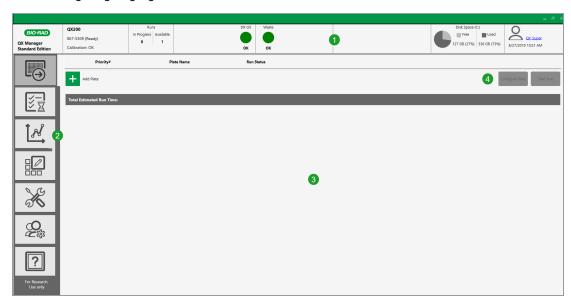
QX Manager Software, when connected to your QX200 Droplet Reader, provides the necessary functionality to create, run, and analyze droplet reading experiments on your samples.

During the installation of Standard Edition, you can enable the User Management module for all user management functions and preferences. If you do not enable User Management, you can use the software with a single generic user.

Using the instrument and software, you can

- Set up customized, real-time ddPCR experiments for plate runs
- Read standard droplets
- Choose between two droplet volumes
- See the available disk space and number of plates you can process
- 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

The following image highlights functional areas.



#### **LEGEND**

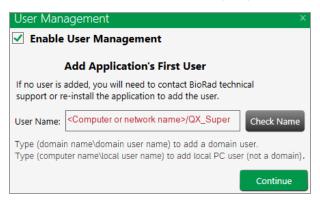
- 1 The status bar displays information about the instrument and user. For information on specific functionality, see Instrument Status Bar on page 46.
- 2 Tabs provide access to the main functional windows.
- 3 The main pane displays the details of the selected tab.
- 4 The buttons allow you to configure the plate and start the run.

# **Enabling User Management**

During the installation of QX Manager Software, Standard Edition, you can choose to enable or bypass the User Management module:

- If the Enable User Management checkbox is selected, all user functions are included in the installation.
- If the checkbox is not selected, User Management is not enabled and the corresponding tab is not included in the side pane. A single generic user, with a small set of user preferences, is always logged in. For information, see Partial Preferences on page 44.

Important: Before you can set up users in the software, all users must be set up on the computer as local Windows users or in Active Directory on your network.



#### To bypass the User Management module

Ensure the checkbox is cleared in the User Management dialog box, and then click Continue to complete the installation.

#### To enable the User Management module

- Ensure the Enable User Management checkbox is selected.
- Enter the user name and click Check Name to validate the user.
- Click Continue to add the user and complete the installation.

Chapter 3 About QX Manager Software

# **Chapter 4 Getting Started**

Use the information in this chapter to

- Sign into the software or change users
- Select the instrument
- Become familiar with the instrument status bar and main functional windows
- Understand the compatible file types for your software edition

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

# Signing In, Viewing Privileges, and Changing Preferences

Important: If User Management was not enabled during the software installation, QX Manager Software creates a single generic user, which is already logged in and is available to all users for setting up and running plates, and analyzing the results. The user can access a set of partial preferences using the generic user link, as shown in the following graphic. See Partial Preferences on page 44 for more information.

Typically, a system or software administrator sets up users and privileges for QX Manager Software, and communicates the information to you.

#### Note the following:

- When you sign in the first time after the software is installed, you must select the instrument to which the computer is connected. See Selecting the Instrument and Droplet Volume on page 40 for information.
- You cannot change your user privileges unless you are assigned the Add/Manage Users privilege. For information, see Viewing User Privileges on page 41.
- You can change your user preferences, which are set to defaults. For information, see Managing Your Preferences on page 43.



# Signing into the Software

Important: If User Management was not enabled during the installation, the generic user is automatically signed in when you open the software.

#### To sign in

- Right-click the software icon on the desktop and select Open to display the Sign in dialog box.
- Do one of the following to enter your user name:
  - If the Sign in to: label shows the correct domain name, enter only the user name and password.



If the Sign in to: label is blank or shows a different domain, then enter it as <computer or domain name\user name>, followed by the password.



For more information, click the How do I sign into another domain? link.



Note: If you are logging into a network domain, but don't know the name, contact your system administrator.

Ensure the Do not show EULA on sign in checkbox is selected and tap I Agree.



Each user must agree to the end user license agreement on initial login in order to user the software. If you clear the checkbox, the End User License Agreement appears the next time you log into the software, and you must agree again to the terms before the software opens. If you do not agree to the EULA, the application closes immediately.

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 computer not connected to an instrument, the Data Analysis window opens.

### Selecting the Instrument and Droplet Volume

When you open the software and sign in for the first time, the instrument connection window appears and you must select the connected instrument and a droplet volume.

Note: This procedure assumes the instrument is connected to the computer and QX Manager Software is installed. You can use QX Manager Software with the QX200 Droplet Reader and the QX600 Droplet Reader. If you are using both instruments, you can change instruments and reset the droplet volume at any time.

After QX Manager Version 2.0, the droplet volume size that QX Manager Software uses to calculate concentration by default was changed from 0.85 nL to 0.795 nL. The change resulted in a 7-8% higher concentration for experiments where all other variables were identical, thereby improving the accuracy on the QX200 Droplet Reader. System precision remains unchanged.

For QX Manager Version 2.1 and above, you can switch between the historical droplet size of 0.85 nL and the new droplet size of 0.795 nL. This is useful if you want to compare results across software versions and maintain consistent calculations.

#### To select the instrument and droplet volume

- Select QX200, and then click Save.
- Select a droplet volume and click Save.



## **Viewing User Privileges**

Important: If you did not enable the User Management module during the installation, the related functions are not available.

In the User Management window, your privileges are identified by the selected checkboxes.

### To view your assigned privileges

Select the User Management tab.



Unless you are assigned the Add/Manage Users privilege, checkboxes for your assigned privileges are selected but not enabled, as shown in the following graphic. To request changes to your privileges, contact your software administrator.



Table 11 lists the available privileges, which can be assigned in any combination.

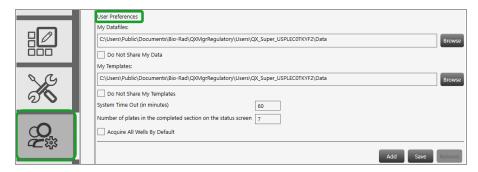
Table 11. User privileges

| Privilege                                      | Purpose   |  |
|--|---|--|
| Add and manage                                 | Add or remove users, set privileges, and change preferences.  |  |
| users  | <b>Important:</b> Users who are assigned the Add/Manage Users privilege can create users and update privileges and preferences for any user. Only the superuser can remove this privilege from user accounts. |  |
| Create new templates                           | Save a design for a plate or analysis report as a template.   |  |
| View data files<br>(created by other<br>users) |   |  |
| Overwrite existing data file                   | Save the file with a new file name. Files are always saved with the extension for the application you are using. For information, see Compatible File Types on page 48.                                       |  |
| name   | <b>Note:</b> Users without this permission can open files and perform analysis but cannot create a new file.  |  |
| System settings                                | View logs, reprocess data with new calibration values, and change file locations. All users can view the file locations.  |  |
|  | Important: If your administrator selected the Preferred Locations checkbox in   |  |
|  | System Settings, individual user location preferences are overridden.   |  |
| Maintenance                                    | View the event log and the system data settings.  |  |
|  | Note: All users can reprocess data, view system folder settings, as well as the   |  |
|  | maintenance log and maintenance reports.  |  |
| System Data                                    | Move raw data from the computer to free up disk space.  |  |
|  | Access troubleshooting data.  |  |
| Manage Plate                                   | Cancel a run while it is in process on the QX200 Droplet Reader.  |  |
| ·  |   |  |

### **Managing Your Preferences**

Important: If you did not enable User Management during the software installation, the User Management module is not included and a truncated set of preferences is available. See Partial Preferences on page 44 for information.

Use the User Management window to modify your personal user preferences. Default folder locations appear in your preferences, but you can change them. You can also choose to keep all 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.



#### To change your preferences

- 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.

Important: 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. If you do not select the checkbox, all wells are excluded by default and you must manually include the wells for each droplet reading run.
- Do one of the following:
  - If you are setting preferences for a new user, click Add.
  - If you are updating preferences, click Save.
- When the confirmation message appears, click Yes, and then click OK.

### **Partial Preferences**

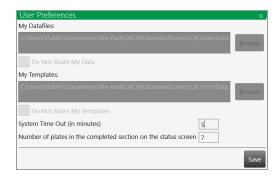
If User Management was not enabled during the software installation, you can still access partial functionality.

### To open the menu

1. Click the user name link in the upper-right corner to display the pop-up.

Important: The Service Sign in option is used by Bio-Rad service engineers only.

2. Click User Preferences to display the dialog box.



- 3. You can change the following:
  - System time out in minutes (default is 5 minutes)
  - Number of plates in the completed section of the status screen (default is 7)
- 4. After you have made your changes, click Save.

## **Signing Out or Changing Users**

While the instrument is idle, one user can log out and another user can log in.

Important: If User Management was not enabled during the software installation, the sign in, sign out, and change users functions are not available.

#### Note the following:

- Do not change users while a run is in progress or data might be affected.
- If the screen is locked and the initial user left unsaved changes, an advisory prompt appears. Click Save before proceeding or the previous user's work is lost.

#### To sign out

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

#### 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.

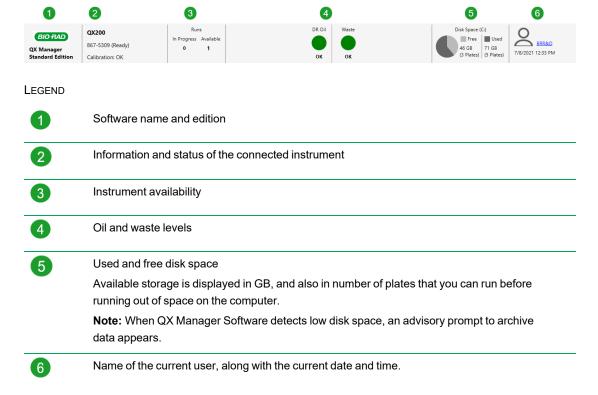
<domain name>\<user name> (for example, global\john\_smith)

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 instrument, the status bar appears as shown in the following graphics. If a computer is not connected to the instrument, the status bar shows only the software, disk space, and user information.



Important: If User Management was not enabled during the installation, the generic user name appears on the far right.

# **Tabs to Functional Windows**

Table 12 describes the functional areas and primary windows in QX Manager Software.

Table 12. Window Tabs

| Tab         | Name          | Purpose  |
|-------------|---------------|--|
|             | Add Plate     | Add and configure a plate for droplet reading  |
| <u>\$</u> _ | Run Status    | ■ In the top pane, view the run in progress  |
|             |               | ■ In the bottom pane, view up to 100 completed runs  |
| <u></u>     | Data Analysis | ■ Access the Data Analysis and Gene Study modules  |
|             | Templates     | <ul> <li>Set up templates for plates and reports, and search for existing<br/>template files</li> </ul>  |
| Q.G         | System        | ■ View storage locations, logs, and maintenance reports  |
| 20          |               | <ul> <li>Archive data, reprocess data with new calibration values, access<br/>troubleshooting information</li> </ul>   |
|             |               | Important: Available functionality depends on your assigned  |
|             |               | user privileges. Instrument calibration is available only to the Bio-Rad service engineer.   |
| <b>Q</b>    | Users         | View the user privileges you have been assigned, and view and<br>modify your personal preferences  |
|             |               | <ul> <li>Create, edit, or remove QX Manager Software users (if you are<br/>assigned the Add/Manage Users privilege)</li> </ul>   |
|             |               | Important: In Standard Edition, user functionality is available only if User Management was enabled during the installation.   |
| ?           | Help          | <ul> <li>Access software version information, current calibration values,</li> <li>End User License Agreement company website, and open source software license information</li> </ul> |
|             |               | <b>Tip:</b> You can also link the user and instrument guides to the labeled buttons.   |

# **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:

- You can open files created using Standard Edition only in Standard Edition.
- If you open a .qlp file in QX Manager, Standard Edition, the file opens and is saved as a .ddpcr file.

Table 13. Compatible file types

| File Type         | Extension | Details  |
|-------------------|-----------|--|
| Plate<br>template | .ddplt    | Plate template file containing setup details to perform experiments; this file type opens in the Plate Editor.                               |
| Plate<br>template | .csv      | Importable plate template file containing setup details in CSV format; this file type opens in the Plate Editor.                             |
| Data              | .ddpcr    | Contains the results of an experiment run performed on the droplet reading instrument and opened in QX Manager Software, Standard Edition.   |
| Data              | .qlp      | File type from earlier QuantaSoft™ versions, for two-channel experiment runs and data analysis; this file type opens in the Analysis module. |

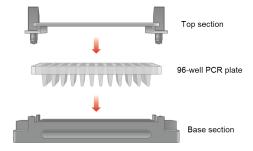
# **Chapter 5** Running Experiments

When the Start Run button is enabled, you can start the run. You can also cancel a run after it has started if you are assigned the Manage Plate user privilege. For information, see Canceling a Run on page 65.

# **Assembling a Plate**

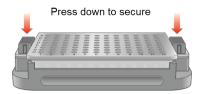
The QX200 Droplet Reader accepts a solid 96-well plate that fits into a secure holder. You must assemble the plate components before you can insert the plate into the QX200 Droplet Reader. Three separate components comprise the plate:

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



#### To assemble the plate

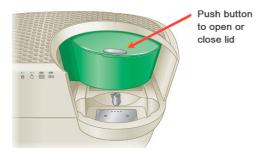
- Place the plate into the base section, and then raise the release tabs on the top section.
- Place the top section over the plate, and then lower the release tabs to secure the plate in the holder.



# **Inserting an Assembled Plate into the Instrument**

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

1. Press the button to open the compartment.



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.



Press the button again to close the lid.

# Adding the Plate in QX Manager Software

This section explains how to add the plate in QX Manager Software, Standard Edition when using the QX200 Droplet Reader for your droplet reading run. You can insert an assembled plate into the instrument before or after you add the plate in the software.

### To add a plate in QX Manager Software

Click the Add Plate tab.



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

2. Click 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. Remove and reinsert the plate. If necessary, contact Bio-Rad Technical Support.

Click Configure Plate. The Plate Configuration window opens.

# **Configuring the Plate**

After you click Configure Plate, the Plate Configuration window opens to the Plate Information display. The window features the following tabs:

- Plate Information
- Well Selection
- Well Information

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. Users with the Create New Template user privilege can save the configuration as a template before the droplet reading 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

Note: CSV template files appear in the dropdown list on the Plate Information tab, but not in the Templates window. For information, see Creating and Storing Plate Templates from CSV Files on page 79.

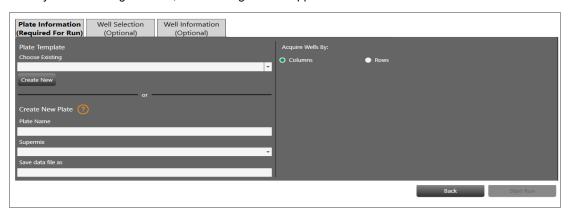
- Create and run a plate design using a manual configuration or a template
- With the Create New Templates user privilege, save plate designs as templates for reuse
- For an expedited run, identify a plate name and a supermix, and then start the run

Note: The data file name field is automatically filled with the plate name, but you can to 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 to acquire wells by columns or rows

### **Identifying Plate Information**

When you click Configure Plate, the following window appears.



### To identify the plate

- 1. Do one of the following:
  - Use an existing template click the dropdown arrow under Choose Existing and select a template file. The software automatically populates the fields under Create New Plate.
  - Create a new template click Create New, and see Creating or Editing Templates on page 67.
  - Create a new plate enter a plate name and select a supermix. When you enter the plate name, the Save data file as field is automatically populated, but you have the option to change it.
- (Optional) Under Acquire Wells By, select the alternative well acquisition method. The default is Column.

### **Well Selection Tab**

When you select the Well Selection tab, the Plate Editor grid appears. The wells in the plate layout are automatically excluded and you must manually include the wells to be processed. The QX200 Droplet Reader processes the entire 96-well plates by default. If you have added sample to fewer than 96 wells, you must exclude the remainder. For information, continue to Including or Excluding Wells in the Plate Layout.

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 43.

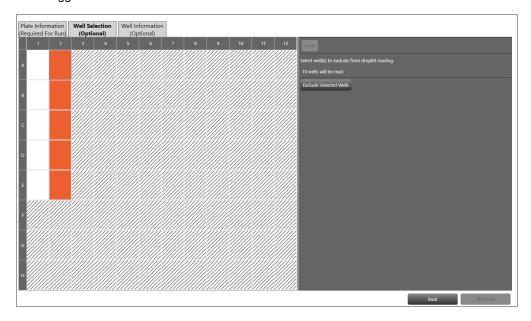
Note: The Well Selection window is identical to the Exclude window in Template Setup.

### **Including or Excluding Wells in the Plate Layout**

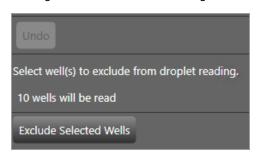
All wells are excluded by default when the plate editor appears.

#### To include wells in the run

- 1. Select the Well Selection tab.
- In the Plate Editor layout, select the wells containing sample and then click the Include Selected Wells toggle button.



3. On the right, QX Manager Software specifies the number of wells that will be included in the droplet reading run and the button text changes to Exclude Selected Wells.



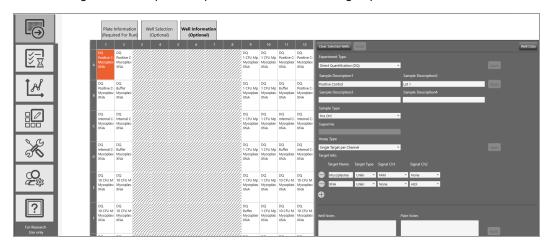
#### Well Information Tab

When you select the Well Information tab, the Plate Editor opens. You can configure your experiment parameters for selected wells before or after the run.

Note: 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 74.

### **Defining or Editing Well Information**

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



You can apply your entries at any time. You must click Apply for the software to recognize your new or edited information. If you enter information in a field and need to apply earlier changes first, QX Manager Software displays an advisory message.

### To define the configuration for the wells

- Select a well or group of wells. 1.
- 2. Select an experiment type; see Experiment Types on page 73.
- In the Sample Description fields, enter up to four words or phrases that describe the sample; see Sample Descriptions on page 74.
- Select a sample type; see Sample Types on page 74.

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

Select a supermix; see Supermixes on page 75.

- 6. Select an assay type.
- 7. Available assay types vary, depending on the selected experiment type; see the fluorophore options in Assay Types and Fluorophores on page 76.
- (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. However, you can modify the default fluorophore assignments, if applicable.
  - a. Click the dropdown arrow and select an alternative.
  - To delete a row, click the minus (-) icon next to the target.
  - c. To add a row, click the plus (+) icon.
- (Optional) To clear one or more wells at any time, select the wells and click Clear Selected Wells.
- 10. Click Apply. After you have applied your entries and selections, you can pause the cursor on a well to see the well information.



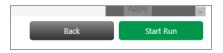
11. When you finish configuring the plate, click Save.

# Starting the Run

When all required elements to set up a plate for a run are satisfied, QX Manager Software enables the Start Run button. You can configure the elements of the plate before you start the run, or you can run the plate with just the plate name and supermix, and define the configuration from the Analysis module.

#### To start the droplet reading run

Tap Start Run.



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 61.
- If the check finds issues with the instrument, the software displays a dialog box in which the issues are identified. The instrument cannot start the run until all issues are resolved. For information, see Run Prerequisites Checklist on page 59.



# **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  $\times$  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 Appendix C, Troubleshooting.

For information on each issue that can prevent the instrument from starting the run, see Table 14 on page 60.

**Table 14. Pre-Run Error Indicators** 

| Status Check Item      | Issue  | Suggested Resolution  |
|------------------------|--|---|
| Instrument Connection  | Power connection not recognized.   | Ensure the power outlet is working. Disengage the power cord from the outlet, and then reinsert it into the outlet. |
|                        | USB cable connection not recognized.   | Disconnect, and then reconnect, the USB cable between the instrument and the computer.                              |
|                        | Instrument connection not recognized.  | Ensure the correct instrument is selected in the System Settings tab > Instrument tab.                              |
| 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.                                       | Empty any remaining oil from the bottle and then place a Waste label (provided by Bio-Rad) over the Oil label.      |
|                        |  | Insert the bottle into the Waste slot.  |
| 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 14. Pre-Run Error Indicators, continued

| Status Check Item     | Issue   | Suggested Resolution   |
|-----------------------|---|--|
| Plate Loaded          | Plate is not loaded properly.                                 | Open the reader door and remove the plate.                                     |
|                       |   | <ol><li>Ensure the tabs of the plate cover<br/>are securely clamped.</li></ol> |
|                       |   | <ol><li>Insert the plate again and ensure it lays flat.</li></ol>              |
| Sufficient Disk Space | There is not enough storage space available for the run data. | Archive older data files to clear space.                                       |

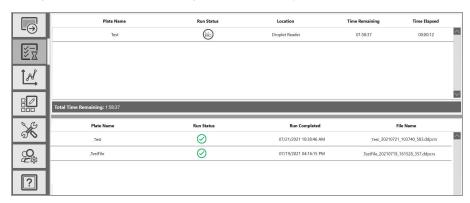
# **Running the Experiment**

The instrument allows you to do the following:

- During the run, perform real-time analysis on the droplets in each well after the well has been read
- Cancel a run (with the Manage Plate permission).
- When the run concludes, 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 your personal file location or a preferred location, and can also be saved to a shared location. For information, see Managing Your Preferences on page 43.

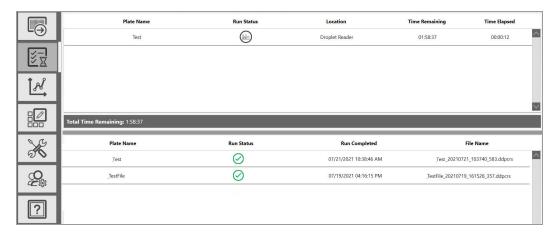
Important: if your system administrator has designated Preferred Locations in System Settings, they override any file paths indicated in your preferences.

During and after the run, QX Manager Software displays run information in the Run Status window.



# **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 15.

Table 15. Run status indicators

| Status Icon         | Description  |
|---------------------|--|
| $\overline{\Theta}$ | Ready to begin droplet reading   |
|                     | Droplet reading run  |
| $\odot$             | Run completed  |
| $\odot$             | Run completed, plate removed   |
| (!)                 | Run stopped or canceled by the system, or canceled by the user before it began |

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 43.

The content of each column is explained in Table 16.

**Table 16. Run Information Columns** 

| Column Name          | Location             | Description   |
|----------------------|----------------------|---|
| Plate name           | Top and bottom       | Name entered under Create New Plate   |
|                      | panes                | <b>Note:</b> If you selected an existing template, the template name appears. |
| Run status           | Top and bottom panes | Icons for each process step (see Table 15 on page 62)                         |
| 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 a location designated by you or a system administrator.

## **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

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

The Droplet Reading dialog box opens.



- 2. Click Open Live Analysis.
- 3. Select an analysis window.
- 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 change the data in the Analysis module.

# **Canceling a Run**

You can cancel a run if you are assigned the Manage Plate user privilege.

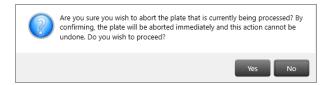
#### To cancel the run

In the Run Status window, click the X on the right to cancel the run.



You can also click the icon under Run Status before the run begins. The run cannot be canceled after droplet reading has commenced.

When the Are you sure message appears, click Yes.



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

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

The icon appears in the Run Status column.

QX Manager Software creates the data file for the wells that were processed on the canceled plate and the status in the Event Log is specified as "run aborted by user."

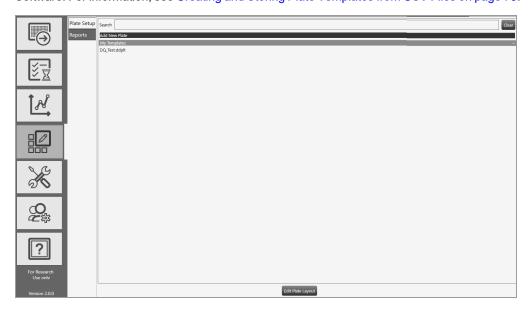
Chapter 5 Running Experiments

# **Chapter 6 Creating or Editing Templates**

If you have been assigned the Create New Templates user privilege, you can create, edit, and save plate and report templates for your common experiments. Templates that are available to you appear in the dropdown list in the Plate Information window, and also in the default view of the Template Setup tab. If the list is long, you can use the Search field to narrow the results.

For information on report templates, see Creating or Modifying Report Templates on page 81.

All users can configure a plate template in a CSV file, and then import the template into QX Manager Software. For information, see Creating and Storing Plate Templates from CSV Files on page 79.



Templates you created are saved to one of the following folders:

- The path specified in your user preferences; these appear under My Templates.
- The paths specified by your administrator in the shared or preferred location fields in System Settings.

**Important:** If a shared location is specified in System Settings, you can save your templates there or in your personal storage location. Preferred locations override personal folder settings so that templates from all users are saved there.

# **Creating or Modifying Plate Templates**

Important: You must be assigned the Create New Template user privilege to create or edit, and then save, a template.

#### To create or edit a template

- 1. Do one of the following:
  - Click Create New under Plate Template in the Plate information window, or you can select the Templates tab.
  - Click Edit Plate Layout in the main Templates window.
  - Search for and select a template under Plate Template in the Plate Information window.
  - Select the Templates tab, and then double-click an existing template under My Templates.

Note: Templates imported from a CSV file are available from the dropdown list only.

The Plate Editor opens in Edit mode. The Instrument Type field is grayed out.

2. Click the Exclude button, and select the wells to include in the plate layout, and then click Include selected wells.

By default, all wells in the Plate Editor are automatically excluded. You must select and include new wells in the template. 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 43.

- 3. Click the Edit button to display the layout for experiment and sample identification.
  - The fields are enabled when you include wells.
- 4. For one or more wells at a time, complete any or all of the following substeps to include the selections and entries that should be static. Leave blank any field that should be completed at the time of the experiment run.
  - Select an experiment and sample type, supermix, assay type, and enter sample descriptions.
    - Experiment Types on page 73
    - Sample Types on page 74
    - Sample Descriptions on page 74
    - Supermixes on page 75
    - Assay Types and Fluorophores on page 76

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

- b. If you selected Residual DNA Quantification as the experiment type, enter a conversion factor.
- Enter up to four sample descriptions, and any relevant well or plate notes.
- d. Edit the Target Name and Target Type fields.
- 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, templates are automatically saved to that location.

# **Template Plate Editor Window**

Use the Template Plate Editor to

- Create new or edit existing plate template files, and save new or modified templates for reuse
- Add up to four sample descriptions per well
- Exclude wells from droplet reading

Important: When you create an experiment from a template with wells excluded in the plate layout, the instrument pierces the foil for all wells in the physical plate, but reads droplets for active wells only.

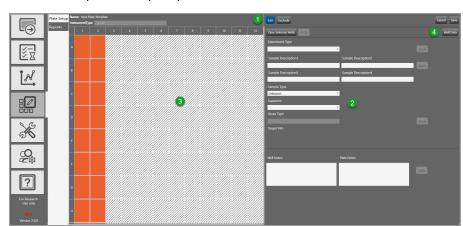
### To open the template plate editor

1. Click the Templates tab on the left.



In the Templates window, click Edit Plate Layout





You can also open the template plate editor from the Add Plate window.

#### LEGEND

- 1 The Edit and Exclude buttons are accessible 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.
- 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 appears, the button name changes to Plate Layout.



### **Plate Editor Tools**

Use the buttons described in Table 17 when you are creating a template or defining an experiment in the Plate Template Editor.

**Table 17. Plate Editor Buttons** 

| Button                    | Description   |
|---------------------------|---|
| Edit                      | Allows you to   |
|                           | Set the experiment type, sample name and type, supermix, and assay<br>type                  |
|                           | ■ Set the target name, type, and fluorophore for each channel                               |
|                           | Add well or plate notes   |
| Exclude                   | Toggle button that allows you to include or exclude wells from droplet reading              |
|                           | Note: Wells in the plate editor are initially excluded by default.                          |
| 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 storage location                     |
| Clear Selected Wells      | Allows you to delete information from selected wells  |
| Undo                      | Allows you to reverse your last action  |
|                           | Note: 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 that allows you to access the Well Data table and return to Plate Layout view |
| Apply                     | Applies your entries and choices for selected wells   |

### **Experiment Types**

QX Manager Software offers the experiment types described in Table 18, each enabling different assay options and analysis tools. You can assign multiple experiment types across the plate, but you can assign only one experiment type to each well.

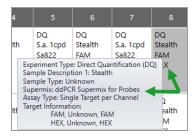
Table 18. Experiment types

| Туре                                    | Description  |
|---|--|
| Direct<br>quantification<br>(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.  Note: 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<br>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<br>(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<br>quantification<br>(RDQ) | RDQ provides a precise method for residual host cell DNA quantification.  Note: 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/ul 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

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**

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.

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

| ddPCR Supermix                           | Description   |
|--|---|
| Multiplex Supermix                       | For use in nucleic acid sample preparations to amplify and detect DNA targets in probe-based experiments involving multiple targets.                                  |
| Supermix for Probes (No dUTP)            | For use in nucleic acid sample preparations, with sensitivity for the amplification and detection of DNA targets using hydrolysis probebased assays.                  |
| 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. |
| Supermix for Residual DNA Quantification | For use in residual DNA detection.  |
| One-Step RT Advanced Kit for Probes      | For use in absolute quantification of target RNA molecules.   |
| EvaGreen® Supermix                       | For use in gene expression.   |

### **Assay Types and Fluorophores**

QX Manager Software offers the assay methods explained in Table 19.

Table 19. 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 two rows displayed. You can delete up to three rows.   |
| Amplitude<br>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.   |
|                           | Triplex mode that allows one groups of three targets each.  |
|                           | Group 1 has one target in FAM, one target in HEX/VIC, and one target in both channels. You can delete a group, but not the individual targets within the group.   |
|                           | <b>Note:</b> The channels within a group are not interchangeable; therefore FAM is always paired with HEX/VIC and so forth.   |
| Advanced classification   | Method to increase multiplexing rate in any combination of targets detected per channel. This method requires manual classification of droplets.  |
| Basic drop-off            | This mode allows two groups of two targets each. The first group appears by default, with one target in FAM and HEX/VIC and one target in FAM or HEX/VIC.   |
|                           | This assay type is available only for the DOF experiment type.  |
|                           | <b>Note:</b> The channels within a group are not interchangeable; therefore FAM is always paired with HEX/VIC and so forth.   |
| Double drop-off           | This assay type is available only for the DOF experiment type, and allows one groups of three targets.  |
|                           | The first group appears by default, and looks for one target in FAM <u>and</u> in HEX/VIC (target type reference) one target in FAM (target type unknown), and one target in HEX/VIC (target type unknown). |
|                           | By default, all reference copies are set to 2.  |
|                           | The channels are static. (Channel 1 FAM is always paired with Channel 2 HEX/VIC.  |
|                           |   |

The experiment type you select determines the available assay methods. Table 20 displays the sample type associated with each assay method.

Table 20. Assay types by experiment type

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

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

Use Table 21 to select from the fluorophore options for each assay method listed.

Table 21. Fluorophore options

| Assay method                                  | Signal<br>Ch1    | Signal<br>Ch2 |
|---|------------------|---------------|
| Single target per channel, for 1 to 2 targets | FAM<br>EvaGreen® | HEX<br>VIC    |
|   | None             | None          |
| Amplitude multiplex, for                      | FAM Hi           | HEX Hi        |
| 1 to 4 targets                                | FAM Lo           | HEX Lo        |
|   | EvaGreen® Hi     | VIC Hi        |
|   | EvaGreen® Lo     | VIC Lo        |
|   | None             | None          |

Table 21. Fluorophore options, continued

| Assay method                     | Signal<br>Ch1                                | Signal<br>Ch2                            |
|----------------------------------|--|--|
| Probe mix triplex, for 3 targets | FAM<br>None                                  | HEX<br>VIC<br>None                       |
| Advanced classification          | FAM<br>+1-10<br>EvaGreen®<br>1 to 10<br>None | HEX<br>1 to 10<br>VIC<br>1 to 10<br>None |

### **Creating and Storing Plate Templates from CSV Files**

You can create a plate template in a spreadsheet application, save it in CSV format, and then store it in a personal or shared folder for use in your droplet reading runs. Note the following:

- The headings for the columns containing the experiment information match the fields in the Plate Editor.
- Once you specify the information in row 1, the field entries must remain static until you install a new software version or edition.
- CSV template files appear in the Plate Setup dropdown list only.

#### To set up and save a CSV template

Open a spreadsheet program and enter the run settings into the template in the format shown below.

Fig. 1: Template CSV Layout Example, Columns A through H

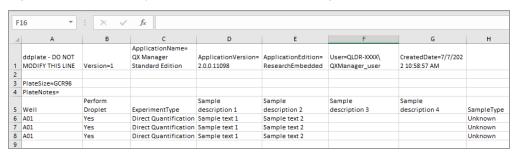
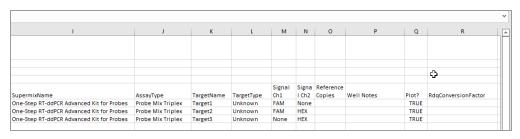


Fig. 2: Template CSV Layout Example, Columns I through R



- 2. When the configuration is finished, navigate to one of the following locations and save the .csv file to ensure it is available for your runs:
  - The Templates folder location specified in your user preferences. For information on your personal storage locations, see Managing Your Preferences on page 43.
  - The shared or preferred Templates location determined by your system administrator. For information on shared folders, including preferred locations, see Shared Settings on page 168.

Important: The .csv file must be in one of these locations to be available for selection from the dropdown list in the Plate Information window. CSV templates are not listed with standard templates in the Plate Templates window.

## **Creating or Modifying Report Templates**

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

### To create or edit a report template

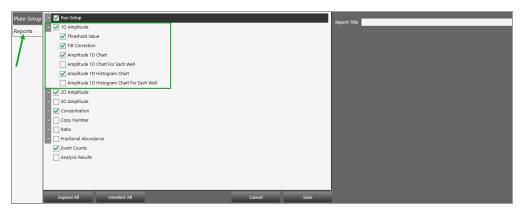
1. Select the Template Setup tab.



- Click Reports and do one of the following:
  - To create a new template, click Add New Template. and click Edit Report Template.
  - To open an existing template, select the template and click Edit Report Template.



3. Click the arrow to the left of each category to expand the list of reportable items.



Select and clear checkboxes to create the report structure.

Note: 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. Enter a name for your report in the Report Title field and click Save.



6. When the Save dialog box appears, enter a file name.



- 7. Select a storage location. Choose from the following:
  - System Templates (preferred location, if specified by the administrator)
  - My Templates (personal storage location specified in user preferences)
  - Shared Templates (files saved to this location are available to all users)
- Click Save again to save the report format as a template.

Chapter 6 Creating or Editing Templates

# Chapter 7 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/µl input.

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 8, Data Analysis Methodology.

When you select the Data Analysis tab, you can access the Analysis module and the Gene Study module.



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.

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

Important: The Gene Study module is accessible from a standalone computer only. The maximum number of samples you can analyze in a gene study is limited by the size of the RAM and virtual memory of the standalone computer.

### **Opening a Data File for Analysis**

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

- .qlp.
- .ddpcr

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. In QX Manager Software, 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

Files are always saved to one of the following locations:

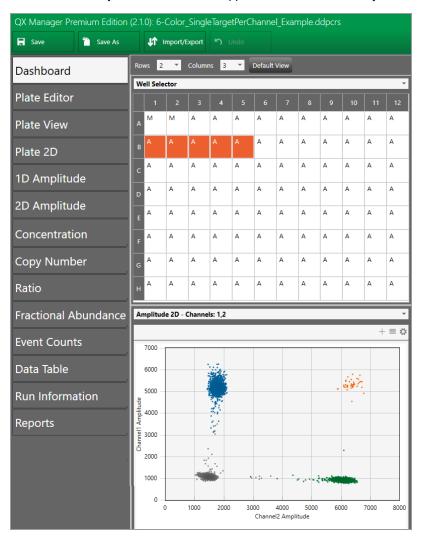
- ☐ If preferred locations are enabled, then files for all users are saved there and they appear under System Datafiles.
- ☐ If preferred locations are not enabled for all users, your files are saved to your personal folder and appear under My Datafiles.

### Shared Datafiles

You can also save your data files to a shared location; these appear under Shared Datafiles.

2. 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.

**Important:** When you save a .qlp.qlps file type in QX Manager Software, the software automatically saves the file as a .ddpcr file. This creates a new file and does not overwrite the original.

#### To save a data file

- 1. Do one of the following:
  - To save the file under the same file name and in the same location, click Save.
  - To save the file under a different file name or in a different location, click Save as, and then do one of the following:
    - ☐ 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, navigate to the new location (if applicable) and enter the new file name. You must be assigned the Overwrite existing file name user privilege to change the file name.
- 2. Click Save.

### **Analysis Display and Output Options**

QX Manager Software provides the following option that you can choose or set:

- 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 appear on the top menu bar, and are available from all windows, although functionality within the main option might differ according to the analysis window that appears.



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 with a different name  |
|               | If you open a file in the latest application that was created in an earlier application, the file assumes the file extension of the new application. See Saving a Data File on page 89 for information. |
| Import/export | ■ Imports or exports plate setup data to a CSV file   |
|               | Imports or exports plate setup data to a plate template file  |
|               | Exports visible data to a CSV file  |
|               | <ul><li>Exports amplitude, cluster, and linkage data</li></ul>  |
|               | Exports and prints the visible charts for the 2D plate layout   |
| Undo          | Enabled in the Plate Editor only, after you have made a change  |
| User Settings | Allows you to restore your user preferences to the default settings and to show or hide color matrix change messages  |

### To import or export setup or analysis data

► 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  |
| Import plate setup         | Import a plate layout from a plate template (.ddplt) file   |
|                            | Note: This option is only available in the Plate Editor.  |
| Export plate setup         | Export the plate layout to a plate template file  |
|                            | Note: 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   |
| Export amplitude data      | Export the data to an Excel spreadsheet, and indicates whether or not the target was found (1 = yes, 0 = no)  |
| Export cluster data        | Export 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 µL   |
| 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<br>editor | Plate<br>view | Plate<br>2D | Ampl<br>plots | Data<br>visual<br>charts | Event counts | Data<br>table |
|-----------------------------|-----------------|---------------|-------------|---------------|--------------------------|--------------|---------------|
| Import plate setup from CSV | ✓               |               |             |               |                          |              |               |
| Export plate setup to CSV   | <b>√</b>        |               |             |               |                          |              |               |
| Import plate setup          | ✓               |               |             |               |                          |              |               |
| Export plate setup          | ✓               |               |             |               |                          |              |               |
| Export visible data to CSV  |                 |               |             | ✓             | ✓                        | ✓            | <b>√</b>      |
| Export amplitude data       | <b>√</b>        | ✓             | <b>√</b>    | ✓             | <b>√</b>                 | <b>√</b>     | ✓             |
| Export cluster data         | <b>√</b>        | ✓             | <b>√</b>    | ✓             | <b>√</b>                 | <b>√</b>     | ✓             |
| Export linkage<br>data      | <b>√</b>        | ✓             | <b>√</b>    | ✓             | <b>√</b>                 | <b>√</b>     | ✓             |
| Export visible charts       |                 |               | <b>√</b>    | ✓             | ✓                        | ✓            |               |
| 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  $\pm$  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     | Plate 2D                     | Select Channel or Dye to identify your chart accordingly                |
|                    | 1D Amplitude<br>2D Amplitude | If you select Channel, the channel number appears as<br>the chart title |
|                    |                              | If you select Dye, FAM or HEX appears as the chart<br>title             |
| Axis Data Label    | 1D Amplitude                 | Select a different font size  |
| Size               | 2D Amplitude                 |   |
|                    | Concentration                |   |
|                    | Copy Number                  |   |
|                    | Ratio                        |   |
|                    | Fractional Abundance         |   |
|                    | Event Counts                 |   |
| Chart Data Label   | Concentration                | Select a different font size  |
| 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 | Concentration                | Reposition the marker label   |
| Position           | Copy Number                  |   |
|                    | Ratio                        |   |
|                    | Fractional Abundance         |   |

1. Click the Chart Display button.

#### 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 and Label Options**

Chart scale options primarily control chart scale and label displays.



Each preference, as well as the windows in which it is available, is explained in Table 26 on page 97.

Table 26. Chart scale options

| Option           | Description  |
|------------------|--|
| Scale            | Appears in options for all charts  |
|                  | The Scale option determines the numerical range for your chart data:   |
|                  | Auto — Selected by default; QX Manager Software automatically displays a scale to match the data distribution.   |
|                  | Fixed — Selecting Fixed enables the Axis fields, and allows you to manually adjust the range and increment.  |
| Axis             | Appears in options for all charts  |
|                  | 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).            |
|                  | X — Horizontal axis<br>Y — Vertical axis   |
|                  | Note: You can modify the x axis scale in the 2D Amplitude chart only.  |
| Secondary Y Axis | Appears in options for Concentration only  |
|                  | 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. |
| Log              | Appears in options for all Data Visualization charts (Concentration, Copy Number, Ratio, and Fractional Abundance)   |
|                  | You can select the Log checkbox to display the y axis in base 10 logarithmic format.   |
|                  | <b>Note:</b> In the Concentration chart, you can also select Log for the secondary y axis.   |
| Inverse          | Appears in options for Ratio and Fractional Abundance only   |
|                  | 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.             |
| Data labels      | Appears in options for all Data Visualization charts   |
|                  | A data label appears by default for each point in the chart, but you can clear the checkbox to remove the labels.  |
| Grid Lines       | Appears in options for all charts  |
|                  | Gridlines appear by default. You can clear the check box to remove them.   |
|                  |  |

### To select chart scale and label options

- Click the icon in the upper-left corner of the chart to display the corresponding chart options.
- 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**

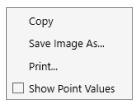


Chart menu options are explained in Table 27.

Table 27. Chart menu options

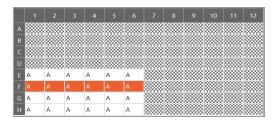
| Option            | Description   |
|-------------------|---|
| Сору              | Copies the chart to the clipboard, which you can paste into a separate document, such as an MS Word file                                      |
| Save Image as     | Saves the chart as an image file  |
| Print             | Prints the image  |
| Show Point Values | Shows corresponding point values when you pause on a data point  5565.93, 9123.0  Tip: Select the Show Point Values checkbox to see the data. |

Chart menu options are available in the following windows:

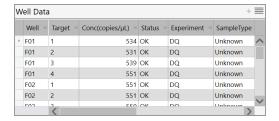
- 1D Amplitude
- 2D Amplitude
- Concentration
- Copy Number
- Ratio
- Fractional Abundance
- **Event Counts**

#### To use the chart menu options

Select one or more wells in the Well Selector.



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



- 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. To paste the data, navigate to the location (such as a Word file), 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.
- Click anywhere in the display to close the dialog box.

# **Using Table Menu Options**

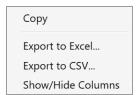


Table menu options are explained in Table 28.

Table 28. Table options

| Option               | Description   |  |
|----------------------|---|--|
| Сору                 | 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   |  |
| Show/Hide<br>Columns | Add or remove columns from a table display  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.     |  |
|                      | ✓ Select All  ✓ Well  ✓ Sample description 1  ✓ Sample description 2  □ Sample description 4  ✓ Target  ✓ Conc(copies/μL)  ✓ Status  ✓ Experiment  ✓ SampleType  ✓ TargetType  ✓ Supermix |  |

Table menu options are available in the following windows:

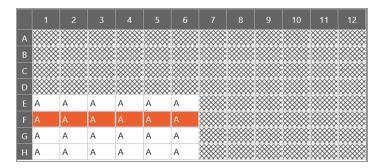
- 1D Amplitude
- 2D Amplitude
- Concentration
- Copy Number
- Ratio
- Fractional Abundance
- **Event Counts**

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

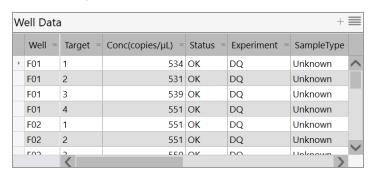
#### To use the table menu options

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

1. Select wells in the Well Selector.



Corresponding data appears in the Well Data table.



- 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.

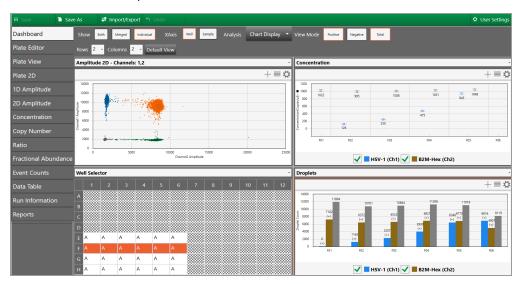
To export your data, select Export to Excel or Export to CSV, and then follow the prompts to save

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.

### **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 change panes, or increase or decrease the number of panes.



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

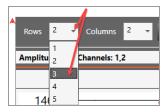
From the tabs on the left, you can access plate views, amplitude charts, data visualization distribution charts, droplet counts, and a complete data table. You can also view data specific to the run in the Run Information window, and run reports on any of the analysis charts from the Reports window.

### **Modifying the Dashboard Display**

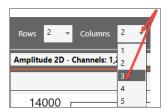
You can modify the column number and row number to display any or all of the analysis windows simultaneously.

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

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



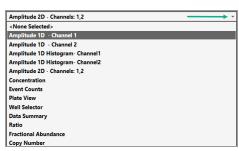
Click the dropdown arrow for Columns, and then select a different number.



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

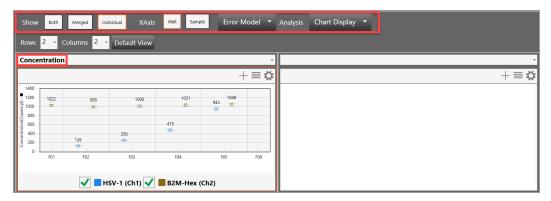
1. Click the dropdown arrow in the upper-right corner to display the list of available windows, and then select a window. The dropdown list closes and the window changes immediately.



### **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 Concentration chart in the Dashboard, 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. For information, see Modifying the Dashboard Display on page 104.



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

Chart Display ▼ Rows 2 T Columns 2 T Amplitude 2D - Channels: 1,2 5000 4000 2000 -1000 1000 3000 Channel2 Amplitude 5000 2000 4000

Table 29 describes the option sets identified in the following graphic.

Table 29. 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**

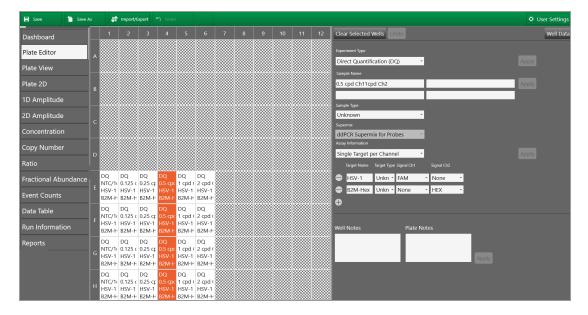
In the QX Manager Software Analysis module you can open the following windows that contain data on all wells in the run:

- 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.

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 138.

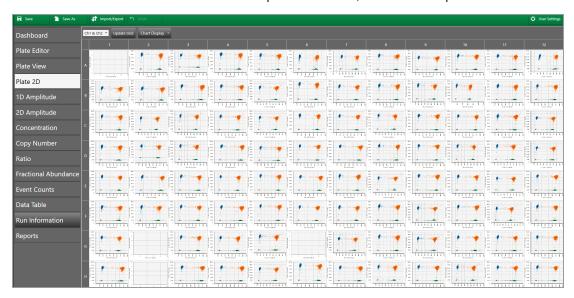


#### **2D Plate View**

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 in a single display. To see a larger representation of a well, select the well in the 2D Amplitude view.

#### To view different color arrays

Select the channel combination from the dropdown on the left, and then click Update Grid.



### **Data Windows**

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

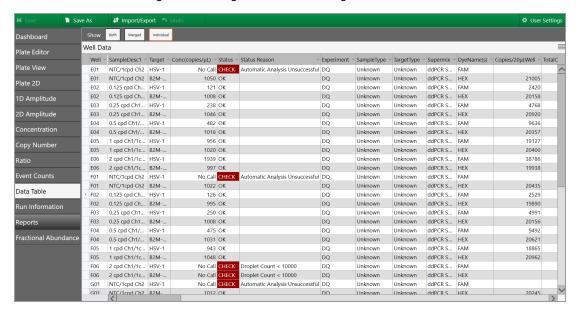
■ **Data Table** — available from the main tabs on the left, shows your configured experiment parameters, plus all calculations from the run

**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.



#### **Data Table Column Definitions**

Table 30 defines each field in the Data Table.

Table 30. 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 | Note: Only the sample description 1 data appears in plate                               |  |
| Sample description 3 | layout views but you can pause on a well in the Plate                                   |  |
| Sample description 4 | Editor to display a tool tip containing the entries in all                              |  |
|                      | completed Sample description fields.  |  |
| Target               | Target name from Plate Editor tab   |  |
| Conc(copies/µL)      | Concentration of the target molecules recorded as copies per                            |  |
|                      | microliter  |  |
| pg/µL                | Mass concentration of DNA in the well in pg/μL  |  |
| Status               | Contains either Check, OK, Multi, or Manual   |  |
|                      | If Check, either the automatic analysis for the well failed or                          |  |
|                      | fewer than 10,000 droplets were counted in the well                                     |  |
|                      | <ul><li>If OK, enough droplets were counted to trigger automatic<br/>analysis</li></ul> |  |
|                      | If Multi, the data was automatically analyzed as part of a<br>multi-well selection      |  |
|                      | If Manual, the droplets were analyzed manually  |  |
| 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 30. 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/20µLWell            | 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  |  |
| Copies/µl linked molecules | Shows number of copies of a target that are present in overabundance versus the expected value in copies per µL                    |  |
| 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                        |  |
| 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           |  |

Table 30. Data Table columns, continued

| Column name         | Content description  |  |
|---------------------|--|--|
| 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                 |  |
| 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  |  |

Table 30. Data Table columns, continued

| Column name                   | Content description  |  |
|-------------------------------|--|--|
| 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            | 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 |  |
| PoissonConfidenceMin68        | Minimum target concentration normalized for the low error bar of the droplet Poisson distribution for the 68% confidence interval  |  |

Table 30. Data Table columns, continued

| Column name                     | Content description   |
|---------------------------------|---|
| 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                                      |
| 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 |

Table 30. Data Table columns, continued

| Column name   | Content description  |  |
|---------------|--|--|
| TiltCorrected | Indicates if tilt correction was applied to the well   |  |
|               | <b>Note:</b> Tilt correction rotates the angles from the double-<br>negative cluster centroid to each droplet, such that the<br>two single positive cluster centroids are orthogonal with<br>the double-negative cluster centroid. |  |
| 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  |  |

#### **Plate Editor 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. Table 31 defines each column in the consolidated table.

Table 31. Fields in the Analysis Well Data table

| Column name              | Content description  |  |
|--------------------------|--|--|
| Well                     | The well location in the plate the sample is from                              |  |
| Perform Droplet Reading  | Yes or No  |  |
| Experiment Type          | Experiment type from the Plate Editor  |  |
| Sample description 1 – 4 | The sample name used to identify the well in the Plate Editor tab              |  |
| SampleType               | Sample type from the Plate Editor  |  |
| Supermix Name            | Supermix from the Plate Editor   |  |
| Assay type               | Assay type selected in the Plate Editor  |  |
| Target Name              | Target Name from Plate Editor tab  |  |
| TargetType               | Target type assigned to the target in the Plate Editor                         |  |
| Signal Ch1               | Selected fluorophore in Channel 1  |  |
| Signal Ch2               | Selected fluorophore in Channel 2  |  |
| 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 Windows**

In its Analysis module, QX Manager Software provides amplitude displays and data visualization charts in the Analysis module. For more information on each chart, see Chapter 8, Data Analysis Methodology.

#### **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 fits the fraction of positive droplets to a Poisson algorithm to determine the starting concentration of the target DNA molecule in units of copies/µl input 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.

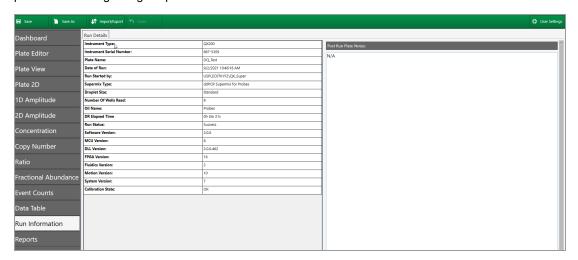
Note: You can display the data using a confidence level of either 68% or 95% (default setting).

#### **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.

# **Viewing Run Information**

Use the Run Information window to view the information about the plate and run. You can also view or add post-run notes regarding the plate. .



Chapter 7 Data Analysis Module Overview

# **Chapter 8 Data Analysis Methodology**

As summarized in Chapter 7, 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 twodimensional 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 32 explains the analysis options that are available in both 1D amplitude and 2D amplitude plots.

Table 32. 1D and 2D amplitude tool options

| Icon             | Mode                                    | Description   |
|------------------|---|---|
|                  | View mode                               | Data appears with the thresholds or clusters assigned by QX Manager Software.  When this icon is selected, the view is locked and you cannot modify the thresholds.   |
|                  | Heat map                                | Shows the distribution of droplets, with higher density of droplets shown as hot.   |
| Auto 🕶           | Automatic analysis                      | Disables tilt correction and automatically reanalyzes the data.   |
| Auto with Tilt ▼ | Automatic analysis with tilt correction | Enables tilt correction and automatically reanalyzes the data.  Note: 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. |
| Reset            | Reset                                   | Resets the data to the original automatically analyzed data.  |
| Chart Display ▼  | 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 33 explains the analysis options available only for 1D amplitude plots.

Table 33. 1D amplitude tool options

| lcon                   | Mode  | Description  |  |
|------------------------|---|--|--|
| Automatic thresholding |   | Individual Wells: Clusters are generated for each selected well separately.  |  |
|                        |   | Combined Wells: Droplets from all selected wells are combined, and clusters are generated on that combined set.  |  |
|                        |   | 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. |  |
|                        |   | <b>Note:</b> If you change the thresholds manually, you can click Reset to return to the automatic thresholds.   |  |
|                        | Threshold, single well                              | For a single well or multiple wells, the user can manually input thresholding values in the 1D amplitude plot.   |  |
|                        |   | You can click anywhere in the plot to apply threshold lines to the data  |  |
|                        | Threshold, multiple wells                           | To adjust the threshold values   |  |
|                        |   | Drag the lines horizontally in the plot area   |  |
|                        |   | Enter a new threshold value in the boxes at the end of each<br>threshold line  |  |
|                        |   | ■ Click the up and down arrows to incrementally adjust the numbers   |  |
| SD SD                  | Threshold, single<br>well SD<br>Threshold, multiple | For a single well or multiple wells, QX Manager Software automatically calculates and populates thresholding values with standard deviations (SD).                                 |  |
|                        | wells SD  | Important: SD thresholds are not recommended for RMD, since these clusters are not typically located at right angles to each other.  |  |
|                        |   | <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.   |  |
|                        | Show histogram                                      | Hides or displays histogram plots for 1D amplitude   |  |

Table 34 explains the analysis options available only for 2D amplitude plots.

Table 34. 2D amplitude tool options

| lcon                   | Mode              | Description  |  |  |
|------------------------|-------------------|--|--|--|
| Automatic thresholding |                   | Individual Wells: Clusters are generated for each selected well separately.  |  |  |
|                        |                   | Combined Wells: Droplets from all selected wells are combined, and clusters are generated on that combined set.  |  |  |
|                        |                   | Positive Control Wells: The software generates thresholds for each channel based on any positive controls currently selected, and applies those thresholds to all currently selected wells.  |  |  |
|                        |                   | <b>Note:</b> If you change the thresholds manually, you can click Reset to return to the automatic thresholds.   |  |  |
| Threshold              | Threshold line    | Use the Threshold line icons to establish thresholds across a pair of channels, dividing droplets into distinct clusters separated by different colors.  |  |  |
|                        |                   | You can click anywhere in the plot to apply threshold lines to the data.   |  |  |
|                        |                   | To adjust the threshold values, you can  |  |  |
|                        |                   | ■ Drag the lines horizontally or vertically in the plot area   |  |  |
|                        |                   | Enter a new threshold value in the boxes at the end of each<br>threshold line  |  |  |
|                        |                   | ■ Click the up and down arrows to incrementally adjust the numbers   |  |  |
| SD                     | Threshold line SD | QX Manager Software automatically calculates and populates threshold line values with standard deviations (SD).  |  |  |
|                        |                   | <b>Important</b> : SD thresholds are not recommended for RMD, since these clusters are not typically located at right angles to each other.  |  |  |
|                        |                   | <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 |  |  |

#### Description **Icon** Mode Use the Threshold Cluster icons to establish thresholds across a pair Threshold cluster of channels, dividing droplets into distinct clusters separated by square different colors. Threshold cluster circle

Threshold cluster freeform

To classify your droplets, you can

- Click any of the three Threshold Cluster Mode buttons (square, circle, or free form)
- Draw the chosen shape around the cluster of droplets

Note: 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.

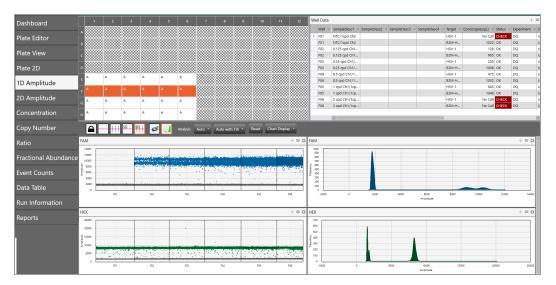


Target names are shown along the two axes based on the signal values assigned during plate setup. Click the colored box that best represents the location of the cluster of droplets selected to automatically classify the droplets as positive or negative for each given target.

Note: The Cluster dialog box display varies according to the plex mode.

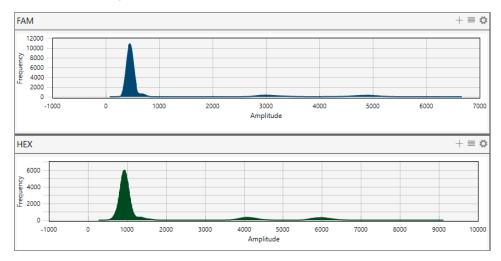
# **1D Amplitude**

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



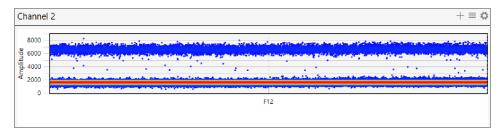
#### The 1D Amplitude plots illustrate

- 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. An M appears in any well in which you manually changed the threshold.
- Amplitude in a histogram plot



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.



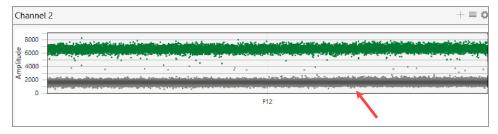
**Note:** For detailed information on each toolbar option, see Amplitude Plot Analysis Options on page 122.

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:

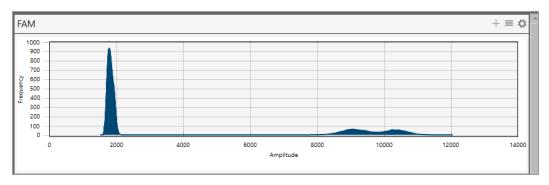
Negative droplets appear in grayscale in each plot.



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

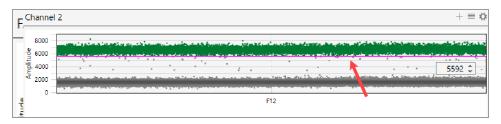
**Note:** Well data must meet certain quality metrics (minimum of 10,000 droplets) before the software will automatically calculate a threshold above which droplets are considered positive. You can use the manual thresholding tools to ensure correct quantification of positive and negative droplets.

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



#### To manually adjust the thresholds

- Select one or more wells.
- Click any of the threshold icons ( ) 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
- Click on the location in the plot where you want to draw the new threshold.
- 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.

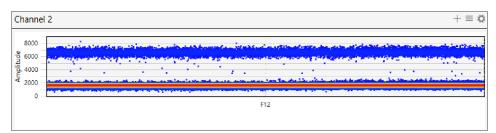
When you manually modify a threshold, the well selector displays an M for manual instead of the A for Automatic.



- 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.

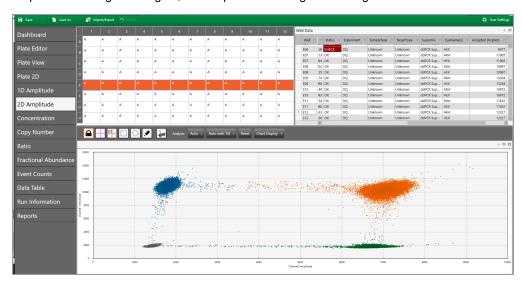
#### To view a heat map representation of the plot data

► Click the heat map ( ) 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. The images in this section display data from an 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.



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 122.

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

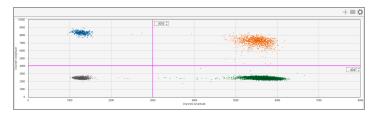
Note: Well data must meet certain quality metrics (minimum of 10,000 droplets) before QX Manager Software will automatically calculate a threshold above which droplets are considered positive. You can use the manual thresholding tools to ensure correct quantification of positive and negative droplets.

#### To use the 2D amplitude tools

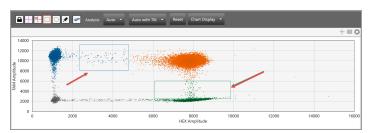
- Open your data file in the Analysis module and select the 2D Amplitude tab.
- Choose a threshold line or cluster option.

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.



- 3. (Optional) To reset to the automatic threshold, click Auto.
- 4. (Optional) To adjust the automatic threshold to consider tilt correction, click Auto with Tilt.
- 5. (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 35 explains the available options.

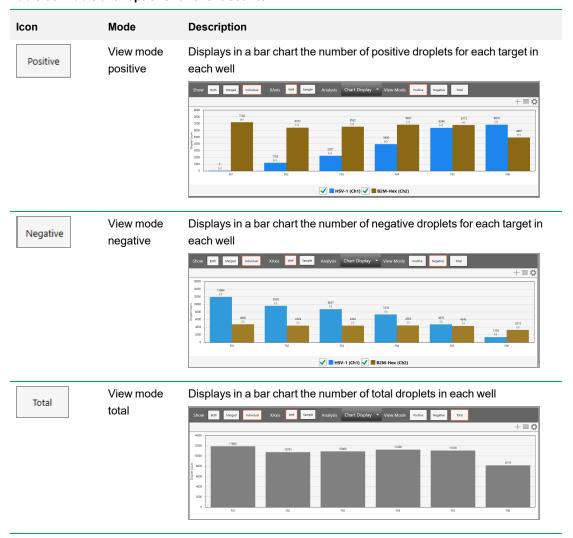
Table 35. Chart tool options for Data Visualization distribution charts

| Icon       | Mode            | Description   |
|------------|-----------------|---|
| Both       | 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 |
| Merged     | 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.  |
| Individual | Show individual | Shows the calculated total or percentage as an individual calculation for each selected well  |
| Well       | X-axis well     | Shows the well number on the x axis   |

| Icon          | Mode                                | Description   |
|---------------|-------------------------------------|---|
| Sample        | X-axis sample                       | Shows a partial sample name on the x axis                   |
|               |                                     | <b>Note:</b> You can select Well and Sample simultaneously. |
| Error Model 🔻 | Error model and confidence interval | Select an error model and confidence level                  |
|               |                                     | Note: The default choices are                               |
|               |                                     | Poisson and 95%, to yield the                               |
|               |                                     | highest confidence in the results.                          |
|               |                                     | Total Poisson  Confidence Interval  95% 68%                 |
|               |                                     | Important: The software uses the                            |
|               |                                     | following multipliers in its                                |
|               |                                     | calculations:   |
|               |                                     | ■ For 95% confidence interval, the sigma multiplier is 1.96 |
|               |                                     | ■ For 68% confidence interval, the sigma multiplier is 1.0  |

Table 36 identifies the additional options available in the Event Count window. These options are available for event counts only.

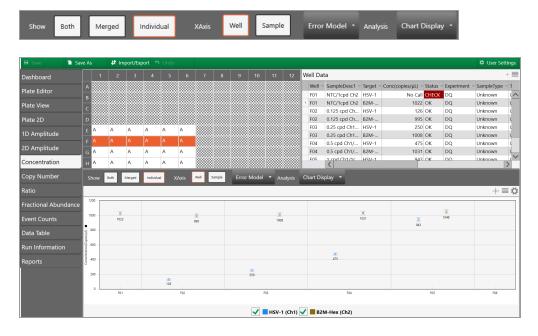
Table 36. Additional options for event counts



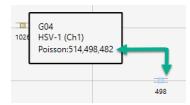
#### Concentration

Concentration measures the number of copies of the target molecules in each µl 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 135.



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.



#### **Concentration Calculation**

The software uses the formula and variables shown below.

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

#### Where:

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

#### Note the following:

- The droplet volume used by QX Manager Software, v1.2 and earlier, is defined as 0.85 nL.
- The droplet volume used by QX Manager Software, v2.0 is updated to 0.795 nL.
- Both volumes are available in QX Manager Software, v2.1.

This droplet volume change provides improved accuracy of measurements on the QX200 Droplet Reader, and can result in concentrations that are 7% to 8% higher in experiments where all other variables are identical. System precision is unchanged.

#### **Confidence Interval Calculation**

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

$$\begin{split} p &= \frac{N_{neg}}{N} \\ \partial_{N_{neg}} &= \sqrt{Np(1-p)} \\ N_{neg, \min} &= N_{neg} - 1.96 * \partial_{N_{neg}} \\ N_{neg, \max} &= N_{neg} + 1.96 * \partial_{N_{neg}} \end{split}$$

#### Where:

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

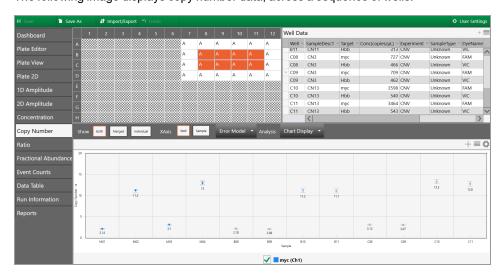
## **Copy Number**

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 135.



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.



#### **Copy Number Calculation**

The software uses the formula and variables shown below.

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

#### Where:

| Α              | Target concentration                             |
|----------------|--|
| В              | 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:

| Α              | Target concentration   |
|----------------|--|
| В              | Reference concentration                                      |
| N <sub>B</sub> | Number of reference copies in genome (usually 2)             |
| CIA            | Poisson confidence interval of target (95% max - 95% min)    |
| CIB            | 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**

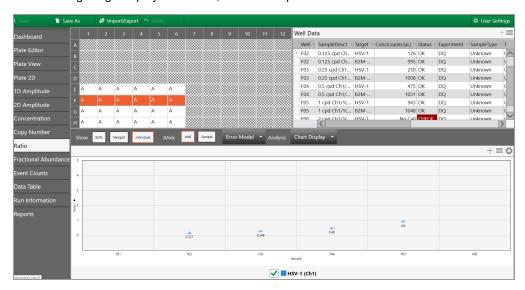
Ratio determines the quotient of two target molecules in each µl of sample based on the default settings.

#### 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 135.



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.



#### **Ratio Calculation**

The software uses the formula and variables shown below.

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

#### Where:

| Α | Target concentration    |
|---|-------------------------|
| В | 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: f 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:

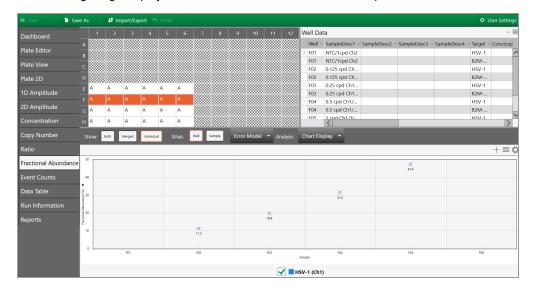
| A   | Channel 1 target concentration                              |
|-----|---|
| В   | Channel 2 target concentration                              |
| CIA | Confidence interval of Channel 1 target (95% max - 95% min) |
| CIR | Confidence interval of Channel 2 target (95% max - 95% min) |

#### **Fractional Abundance**

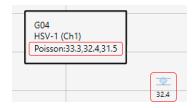
Fractional Abundance determines the relative abundance of your targets in each μl of sample based on the default settings. Use the toolbar to merge or separate the calculated results for each well, and to recalculate your results based on a different error model and confidence interval. For information on toolbar options, see Data Visualization Chart Analysis Options on page 135.



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.



#### **Fractional Abundance Calculation**

The software uses the formula and variables shown below.

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

#### Where:

| Α | Target concentration    |
|---|-------------------------|
| В | 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%.

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

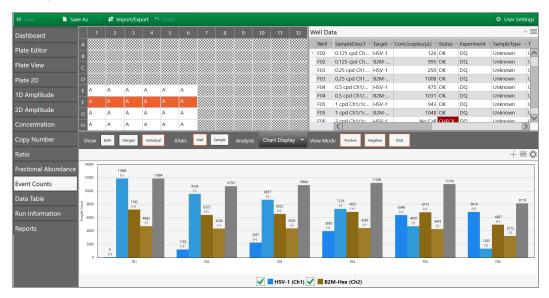
$$(f_{low_{95\%}}, f_{hi_{95\%}}) = (f - (1/2)CI_f, f + (1/2)CI_f)$$

#### Where:

| Α   | Channel 1 target concentration                              |
|-----|---|
| В   | Channel 2 target concentration                              |
| CIA | Confidence interval of Channel 1 target (95% max - 95% min) |
| CIB | 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.



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 formulas and variables shown below.

The following formula presents the linkage in copies per  $\mu L$ :

$$linkage = \ln(N) - \ln\left(n_{00} + n_{01} + n_{10} + \frac{n_{01}n_{10}}{n_{00}}\right) \left(\frac{1000}{droplet\ volume}\right)$$

The following formula presents the linkage in copies per droplet.

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

#### Where:

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

#### Where:

#### **Confidence Interval Calculation**

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

#### Where:

Standard deviation

Chapter 8 Data Analysis Methodology

# **Chapter 9 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.

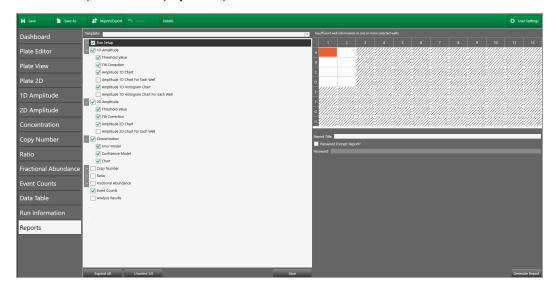
This section contains information on how to create analysis reports.

## **Creating Analysis Reports**

You can create reports with or without a template. For information on creating templates for reports you generate often, see Creating or Modifying Report Templates on page 81.

#### To create a report

Select the Reports tab to display the Reports window.



#### 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 create a custom report, click the arrows to expand reporting categories, and then select the checkbox for each report element to include, and clear checkboxes for elements to eliminate.

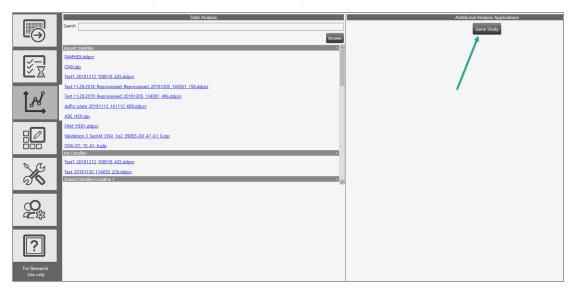
Note: Use the buttons at the bottom of the screen to expand or collapse all categories at once, or to select or clear all checkboxes at once. You can also drag and drop the section to rearrange the order of appearance in the report, and drag and drop items in the section to reorder them.



- 3. Enter a report title.
- 4. (Optional) To restrict viewing, select the Password Encrypt Report checkbox and then enter a password.
- 5. Click Generate Report to generate a PDF report.
- To save the report, click Save.

# Chapter 10 Gene Study Module

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

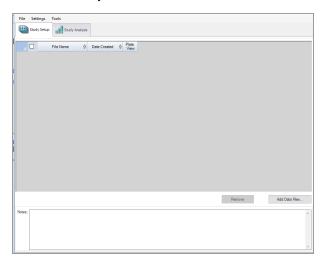


To create a gene study, you add one or more data files and then compare gene expression data from one or more ddPCR experiments.

**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.

#### 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 to add the files to the Gene Study 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 162.

## **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

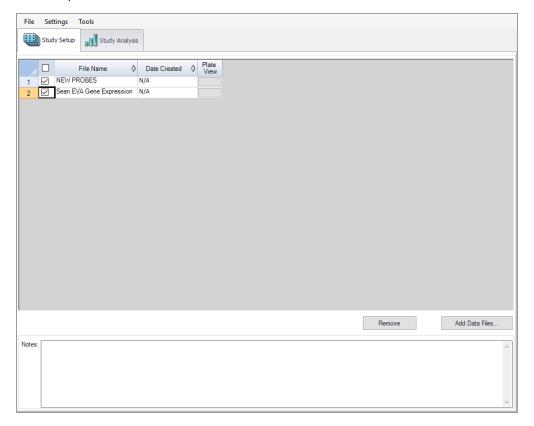


Table 37 defines each column in Study Setup.

Table 37. Gene Study Setup Fields

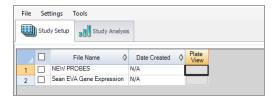
| umn Title | De  | script            | tion           |         |         |          |          |              |       |        |       |          |    |      |
|-----------|-----|-------------------|----------------|---------|---------|----------|----------|--------------|-------|--------|-------|----------|----|------|
| Name      | Na  | me of             | the da         | ta file |         |          |          |              |       |        |       |          |    |      |
| e Created | Dat | te the            | file wa        | ıs imp  | orted i | nto th   | e Gen    | e Stud       | dy mo | dule   |       |          |    |      |
| e View    | Ge  | ens a I<br>ne Stu | dy             | f the p | late w  | vith the | e data   | for ea       | ch dy | e in e | ach w | ell incl |    | in t |
|           |     | 1                 | 2              | 3       | 4       | 5        | 6        | 7            | 8     | 9      | 10    | 11       | 12 | H    |
|           |     | FAM               | FAM            | FAM     | FAM     | FAM      | FAM      | FAM          |       | 1      | 10    | T        | 12 |      |
|           |     | 8.014             |                |         | 9.344   | 7.082    | 9.086    | 0.000        |       |        |       |          |    | Ш    |
|           | A   | 1                 | 1              | 1       | 1       | 1        | 1        | NTC          |       |        |       |          |    | Ш    |
|           |     | Unk               | Unk            | Unk     | Unk     | Unk      | Unk      | Unk          |       |        |       |          |    |      |
|           |     | FAM               | FAM            | FAM     | FAM     | FAM      | FAM      | FAM          |       |        |       |          |    |      |
|           | В   | 4.705             | 3.816          | 3.995   | 4.353   | 4.447    | 4.647    | 0.000        |       |        |       |          |    | ш    |
|           | "   | 2                 | 2              | 2       | 2       | 2        | 2        | NTC          |       |        |       |          |    | ш    |
|           |     | Unk               | Unk            | Unk     | Unk     | Unk      | Unk      | Unk          |       |        |       |          |    |      |
|           |     | FAM               | FAM            | FAM     | FAM     | FAM      | FAM 1747 | FAM<br>0.000 |       |        |       |          |    | Ш    |
|           | l c | 2.369             | 2.169          | 2.223   | 1.885   | 2.357    | 1.747    |              |       | -      | -     |          | -  |      |
|           |     | Unk               | Unk            | Unk     | Unk     | Unk      | Unk      | NTC<br>Unk   |       |        |       |          |    | Ш    |
|           |     | FAM               | FAM            | FAM     | FAM     | FAM      | FAM      | FAM          |       |        |       |          |    |      |
|           |     | 1.000             |                |         | 1.298   |          | 0.929    |              |       |        |       |          |    |      |
|           | D   | 4                 | 4              | 4       | 4       | 4        | 4        | NTC          |       |        |       |          |    |      |
|           |     | Unk               | Unk            | Unk     | Unk     | Unk      | Unk      | Unk          |       |        |       |          |    |      |
|           |     | FAM-1             | FAM-1          | FAM-1   | FAM-1   | FAM-1    | FAM-1    | FAM-1        |       |        |       |          |    |      |
|           | _   | 32.904            | 31.401         | 29.440  | 32.486  | 31.268   | 33.993   | 0.000        |       |        |       |          |    |      |
|           | E   | 1                 | 1              | 1       | 1       | 1        | 1        | NTC          |       |        |       |          |    |      |
|           |     | Unk               | Unk            | Unk     | Unk     | Unk      | Unk      | Unk          |       |        |       |          |    |      |
|           |     | FAM-1             | FAM-1          | FAM-1   | FAM-1   | FAM-1    | FAM-1    | FAM-1        |       |        |       |          |    |      |
|           | F   | 33.789            | 33.305         | 33.518  | 32.269  | 30.299   | 32.104   |              |       |        |       |          |    |      |
|           |     | 2                 | 2              | 2       | 2       | 2        | 2        | NTC          |       |        |       |          |    |      |
|           |     | Unk               | Unk            | Unk     | Unk     | Unk      | Unk      | Unk          |       |        |       |          |    |      |
|           |     | FAM-1             | FAM-1<br>M HEX | FAM-1   | FAM-1   | FAM-1    | FAM-1    | FAM-1        |       |        |       |          |    |      |

## **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.
- Select one or more files and then click Open.

The files are added to the Study Setup window.



## **Removing Data Files**

#### To remove data files

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

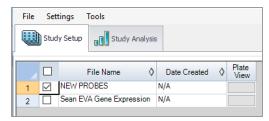
The file is removed from the Study Setup window.

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

## **Selecting Data Files for Analysis**

#### To select data files for analysis

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



Select the Study Analysis tab to see the analysis results.

## **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.



The Plate view appears.



2. 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 38.

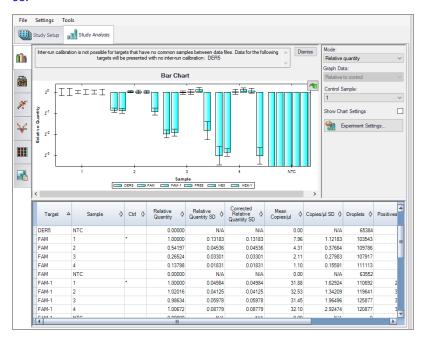


Table 38. Analysis Toolbar

| 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 |
| Scatter Plot | Dot plot of normalized expression of targets for a control versus an experimental sample                          |

Table 38. Analysis Toolbar, continued

| Button        | Description  |
|---------------|--|
| 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  Note: 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 39. Gene Study Report Categories

| Category     | Option                       | Description  |
|--------------|------------------------------|--|
| Header       | •                            | •  |
|              | 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  |
| Study Analy  | sis Bar Chart                |  |
|              | 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   |
| Study Analys | is Clustergram, Scatter Plot | , Volcano Plot, and Heat Map   |
|              | 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 162.

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.

Chapter 10 Gene Study Module

# **Chapter 11 System Utilities**

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

| Dat | afile and Template 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  |
|     | <b>System Data</b> — archive raw data to clear up space in primary storage areas, access troubleshooting data, and use the data recovery function |
|     | Reprocess Data — Reprocess a .data file using new calibration values  |
|     | Tools — Flush or prime the instrument   |
| Ins | trument   |
|     | Change the instrument   |
|     | Change the droplet volume   |

**Note:** The Instrument Calibration tab is enabled only for the Bio-Rad service engineer user account.

## **System Settings Tab**

When you select the System tab, the System Settings window opens by default.



The window contains the following data file and template storage options set by your software administrator:

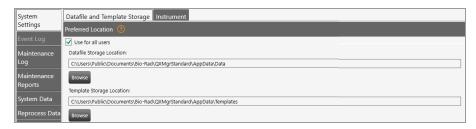
- Preferred Locations if the checkbox selected, storage for all users is limited to a single folder for data files and a single folder for templates.
- Shared Settings if the Preferred Locations checkbox remains cleared, then shared locations can be defined, but remain optional. Users can choose to save their files to their personal folders specified in User Preferences or to the shared location.

Note: The software administrator can also define a backup location for data file storage if the priority folder is full.

#### **Preferred Location**

If the administrator sets Preferred Location file paths, then data files and templates for all users are automatically saved to the specified folders. Preferred Locations override selections in individual user preferences.

Important: Only users assigned the System Settings user privilege can set preferred locations.



#### To specify preferred locations

- 1. Select the Use for all users checkbox.
- 2. (Optional) Keep the default paths or modify them to alternative secure storage locations.
- 3. Click Save.
- 4. When the confirmation message appears, click OK.

## **Shared Settings**

Under Shared Settings, you can choose to save your files to locations that are available to all users.



The software allows the software administrator 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 (typically your software administrator) can change storage locations in the System Settings window.

#### To specify shared folder locations

- 1. In each of the storage path fields, enter a file path or click Browse to search for the folder.
- 2. Click Save.
- 3. When the confirmation message appears, click OK.

## **Changing Instruments and Droplet Volume**

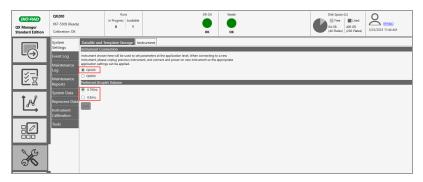
When you open QX Manager Software for the first time after installation, the Instrument connection window appears and you must select the instrument you will be using. If both the QX200 and QX600 droplet readers are used in your laboratory, you can select the alternate instrument on the Instrument tab in System Settings.

#### To change the instrument in the software

Select the System Settings tab.



In the System Settings window (default), select the Instrument tab.



Select the instrument and then click Save.

Features associated with the selected instrument become available when the instrument is connected.

From the Instrument tab, you can also select an alternate droplet volume. The 0.795 nL droplet volume is selected by default, but you can change it to 0.85 nL.

#### To change the droplet volume

Select the alternate button to change the volume.

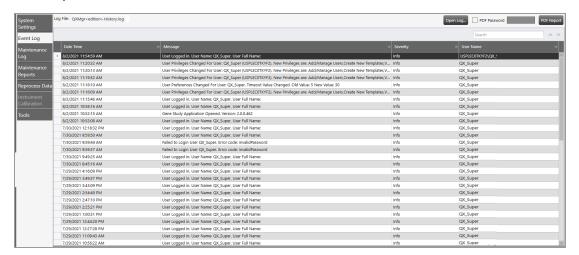
Your droplet volume selection persists until you change it again.

## **System Log Files**

From the System 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.



#### To view the Event Log

1. Select the System Utilities tab.



- 2. Select Event Log.
- 3. Click Open Log to choose either the debug log or the history log.
- 4. Review the log entries.
- 5. (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.

Tip: This guide also contains a form on which you can document completion of maintenance activities. For information, see Instrument Maintenance Log on page 212.



#### To add or view an activity

Select the System Utilities tab.



- 2. Select Maintenance Log. Note that activities that are already scheduled appear in the grid, sorted by newest activity first.
- Do one of the following:
  - Scroll to view a particular activity.

Important: You can view or add static activities; you cannot modify them.

Add an activity; click the + icon next to New Activity.

A line item appears in the bottom section, with all fields except Activity completed automatically.

- In the Activity field in the bottom section, type the activity into the Activity field, and click Save.
- Repeat to add another activity.
- (Optional) Click PDF Report to create a report of the added activities.

## **Maintenance Reports**

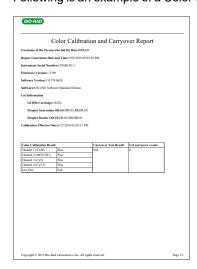
Reports generated from the Bio-Rad service engineer when performing maintenance on your instrument are stored on the Maintenance Reports tab.

#### To open a report

Click the list item.



Following is an example of a Color Calibration report:



## **System Data**

Select the System Data tab to archive raw data and access troubleshooting data.

## **Archiving Raw Data**

When the storage space on the computer connected to the QX200 Droplet Reader approaches the limit displayed under Disk Space, you can free up space by moving data to an archive storage folder in a different location.

Important: You must be assigned the System Data user privilege to archive data.

#### To archive raw data

Select the System Utilities tab.



2. Select System Data.

The Archive Raw Data tab is selected by default.



- 3. Under Archive Progress, click the calendar icons and select a From date and a To date to define a date span.
- Click Query to search for files dated within the date span.
- Click Browse to search for a select the target storage location.
- Click Archive.

## **Troubleshooting Data**

If issues occur with the instrument or software, you can access data to assist in troubleshooting the problem.

Important: You must be assigned the System Data user privilege to access troubleshooting data.

#### To collect data for troubleshooting

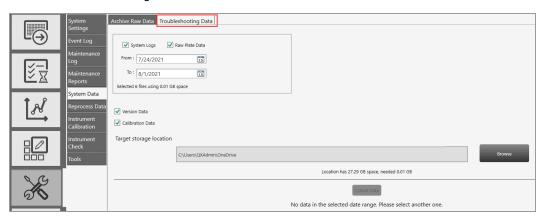
1. Select the System Settings tab.



Select System Data.

The window opens to the Archive Raw Data screen by default.

Select the Troubleshooting Tab.



Click the From and To calendar icons to select a date range.

- 5. Select any of the following checkboxes:
  - System Logs
  - Raw Plate Data
  - Version Data
  - Calibration Data
- 6. Click Browse to navigate to a folder where the data will be stored.

QX Manager Software advises how much space you need and how much is available, and also advises if there is no data from your specified date range.

7. Click Collect Data.

A progress bar appears.



QX Manager Software creates a subfolder containing the data you specified and places it in the target location.

## **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.

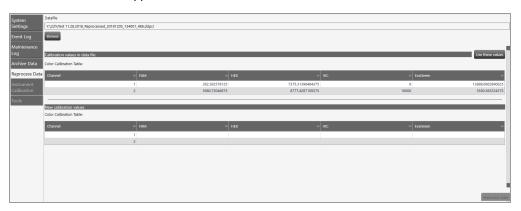
#### To reprocess a data file

1. Select the System Utilities tab.



- 2. Click Reprocess Data.
- 3. Under Datafile, click Browse and navigate to the analysis data file.
- 4. In the Select Datafile dialog box, select the file and click Open.

The calibration values appear in the first Color Calibration table.



- 5. Under the New calibration values heading, enternew values for the fluorophores.
- 6. Click Reprocess Data.

## **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.

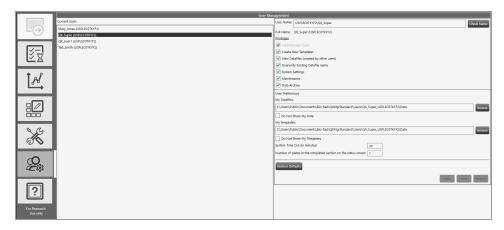
If your instrument requires service, contact Bio-Rad Technical Support.

The Tools functions are currently applicable only to instrument maintenance. For information, see Appendix B, Instrument Maintenance.

# **Chapter 12 Managing Users**

Important: This chapter applies only if you enabled the User Management module during the installation of QX Manager Software Standard Edition. If you did not enable the module, QX Manager Software creates a generic user with the ability to set up, run, and analyze experiments.

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



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

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

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.

# **Adding Users**

This section applies only if the User Management module was enabled during the installation of QX Manager Software Standard Edition.

Before you can add users to QX Manager Software, you must be logged in with the initial superuser account, or another account that is assigned the Add/Manage Users privilege.

Important: Before you begin, ensure that all users either have local Windows user accounts on the local computer or user accounts in Active Directory (network domain).

#### To add a user

- 1. Open QX Manager Software and sign into the application.
- Click the User Management tab on the left



- In the User Name field on the right, enter the user name as follows:
  - If the computer is connected to a network and you are using Active Directory accounts, enter <domain name>/<user name>. For example, global/johnsmith.

Important: Active Directory users must log in every time with the domain name preceding the user name.

If the computer is not connected to a network, enter <local computer name>/<user name>. For example, analysiscomputer/johnsmith.

Important: To locate the computer name, right-click on the yellow folder in the lower menu bar and select File Explorer. When the dialog box appears, the computer name is located at the top of the left panel. Local users are not required to reenter the computer name after the initial login.

#### 4. Click Check Name.

One of the following occurs:

- If the software recognizes the network domain or computer name and the user name, the software displays a validation message.
- 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.
- 5. When the This user is valid message appears, click OK, and then click Add at the bottom of the pane.

  An Are you sure you want to add this user? message appears.
- 6. Click Yes.

A confirmation message appears.

- 7. Click OK.
- 8. Continue until all users are added.

# **Adding or Removing User Privileges**

This section applies only if the User Management module was enabled during the installation of QX Manager Software Standard Edition.

Only a user assigned the Add/Manage users privilege can add or remove user privileges. By default, individual users without privileges can set up and execute a run, open files, view and analyze results.

For descriptions of all user privileges, see Viewing User Privileges on page 41.



Table 40 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 might be required in regulatory environments.

Table 40. Example User Roles

| Privilege                              | Superuser | Lab<br>Manager | Group<br>Lead | Technician/<br>Student Intern |
|--|-----------|----------------|---------------|-------------------------------|
| Add/manage users                       | ✓         | ✓              |               |                               |
| Create new templates                   | ✓         | ✓              | ✓             |                               |
| View data files created by other users | <b>√</b>  | ✓              | ✓             | ✓                             |
| Override existing ddPCR file name      | <b>√</b>  | ✓              | ✓             |                               |
| System settings                        | ✓         |                |               |                               |
| Maintenance                            | ✓         |                |               |                               |

Table 40. Example User Roles, continued

| Privilege                       | Superuser | Lab<br>Manager | Group<br>Lead | Technician/<br>Student Intern |
|---------------------------------|-----------|----------------|---------------|-------------------------------|
| Archive data files              | ✓         | ✓              | ✓             | ✓                             |
| Access the module recovery tool | ✓         | ✓              | ✓             | <b>√</b>                      |
| Manage Plates                   | ✓         | ✓              | ✓             |                               |

You can assign the Create new templates 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 or supervisory 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.
- Select a user from the Current Users list. The name appears in the User Name field. A network or computer name and backslash might appear before the user name.
- 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**

This section applies only if the User Management module was enabled during the installation of QX Manager Software Standard Edition. If User Management was not enabled, only the options for system timeout duration and the number of completed plates displayed in the Runs window are available. For information, see Partial Preferences on page 44.

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, and choose to keep them private.
    - **Important:** You can also specify Preferred Locations in System Settings, to override all file paths specified in individual preferences. For information, see System Settings Tab on page 166.
  - 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**

This section applies only if the User Management module was enabled during the installation of QX Manager Software Standard Edition.

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, such as Windows, or databases.

#### To remove a user

- 1. Click the Add/Manage Users tab to open the User Management window.
- 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 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. 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. You cannot change the supermix that was used during data collection.

### **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.

- Under Assay Type, select Amplitude Multiplex and click Apply. Four target rows appear.
- 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. (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
- 9. Click Apply.

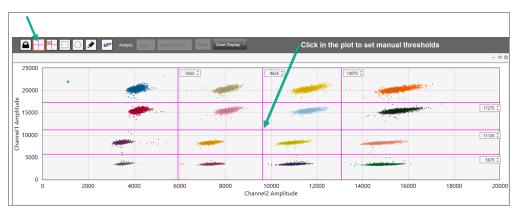
# **Viewing and Adjusting Results**

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.

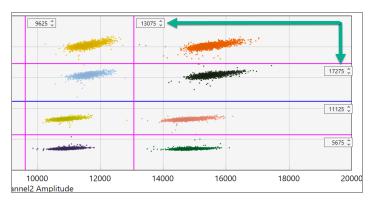
### To set thresholds in the 2D Amplitude window

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.

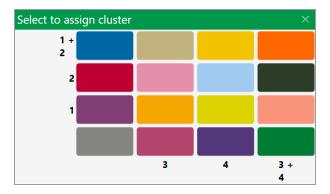
Tip: You can also enter a specific threshold value in the boxes at the end of each threshold line.



### 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.



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 in one groups of three to produce analysis data. 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.

## Setting Up Experiment Parameters

#### To set experiment parameters

- Open the analysis data file and select the Plate Editor tab.
- Select one or more wells to be assigned well analysis attributes. 2.
- 3. Set the experiment type to Copy Number Variation (CNV), and click Apply.
- (Optional) Change the sample description identifiers.
- (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.
- Enter target names and select the target type (unknown or reference) for each target in the well.

This creates one group of three targets.

### 9. Assign the targets as follows:

# Group 1

| Target   | Target Type | Signal<br>Ch1 | Signal Ch2 | Ref<br>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.

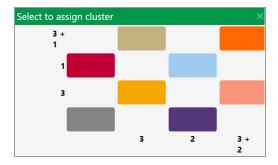
# **Viewing and Adjusting Results**

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).
- 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**

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.

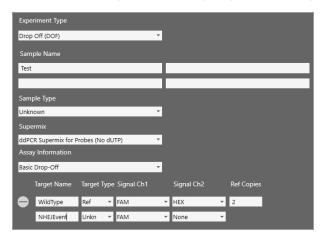
Two configurations of drop-off assays are possible and are shown below. Assign a signal (expected cluster position) to wild-type and NHEJ indel alleles. The signal produced by a wild-type allele will always be positive for both FAM and HEX, while the signal produced by an NHEJ indel allele depends on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

#### **Drop-Off Probe in HEX Drop-Off Probe in FAM** NHEJ Probe = HEX and Reference Probe =FAM NHEJ Probe = FAM and Reference Probe =HEX NHEJ Probe Reference Probe **NHEJ Probe** Reference Probe (FAM) (HEX) **Cut Site** Wild Type Sample NHEJ Sample Wild Type Sample **NHEJ Sample** Ch1 Signal (FAM) Ch2 Signal (HEX) Ch2 Signal (HEX) Ch2 Signal (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.



- 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.

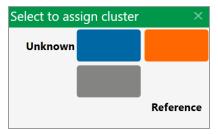
# **Viewing and Adjusting Results**

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.
- Click any of the three cluster mode buttons (square, circle, or freehand).
- 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.



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**

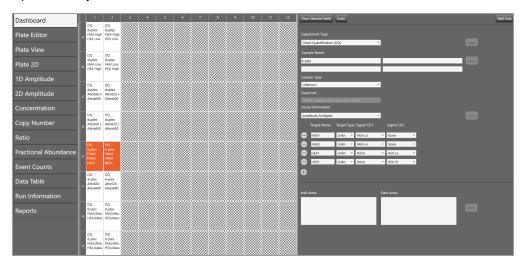
The Advanced Classification Method analysis mode allows the user complete control of droplet identification, and does not force or preassign any clusters. This allows a higher degree of multiplexing through radial dye mixing. Optionally, to expedite plate setup you can copy and paste data from one well to another, and you can click Undo to undo an applied well setting.

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.

- 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
  - 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.

## **Viewing and Adjusting Results**

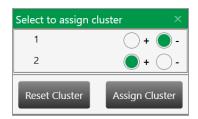
#### To view and adjust results

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 Instrument Maintenance**

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

Keeping the surface areas clean, post-run or as necessary

**Note:** Wipe down external panels and the plate holder with 70% ethanol. See Cleaning or Decontaminating the Instrument on page 211 for information.

- Regularly checking the fluid levels
- Inspecting the equipment for damaged parts or wiring

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

# **Fluid Maintenance and Waste Management**

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 verify your fluid levels by checking the following:

- Instrument status bar
- Instrument indicator lights
- The oil and waste bottles or EcoTank

Table 41 explains fluid level maintenance for your instrument.

Table 41. Fluid Levels

| Fluid Level                                 | Status  |
|---|---|
| Oil greater than 40% or waste less than 70% | Bottle levels OK. You can start your run.   |
| Oil less than 40% or waste                  | You may be able to start a run with fewer than 96 wells.  |
| greater than 70%                            | If there is not enough oil in the dispensers to perform a run on the  |
|   | number of wells you set up in the Plate Editor, the software prevents the run until you fill the oil dispenser and empty the waste container. |
|   | Pay attention to the fluid level by checking instrument status bar in the   |
|   | QX Manager Software or by checking the oil bottles.   |
| Oil less than 10% or waste                  | The software prevents the run until you fill the oil dispenser and empty  |
| greater than 90%                            | the waste container.  |

## **Changing the Droplet Reader Oil Bottle**

#### 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.

Once opened, a pop-up appears on your screen asking you to indicate which type of bottle you are mounting on the system.



**Note:** If using an EcoTank, see Using an EcoTank Oil and Waste Container on page 203 for information.

- Insert a new bottle of oil and tighten the cap into place.
- 4. Prime and flush the instrument at least once after the new oil bottle is installed. See Priming and Flushing the Instrument on page 209 for information.

If you are replacing reagents in the middle of a run, the run resumes when the bottle door is closed and you have clicked OK in the dialog box.

**Note:** If you are replacing the bottles mid-run, you can only replace with the same type of bottle. For example, if the run started with an EcoTank loaded, the software only allows you to replace that EcoTank with another EcoTank.

Once an EcoTank is loaded into the system, the software tracks the number of plates that have been run with the EcoTank. Once you have run 40 plates, the software begins to warn you to replace the EcoTank. After you have run 50 plates, you are required to replace the EcoTank before proceeding with a new run.

# **Emptying the Waste Container**

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

If you are using an EcoTank, see Using an EcoTank Oil and Waste Container on page 203 for information.

### To replace the waste bottle

- 1. Slide the container carriage out using the handle on the side of the instrument and remove the waste bottle.
- 2. Place the new Waste label over the Oil label on the oil bottle.
- Insert the last empty oil bottle into the waste container slot.
- 4. Flush the instrument after the new waste bottle is installed. See Priming and Flushing the Instrument on page 209 for information.

For information on disposing of waste, see Managing Waste on page 210.

# **Using an EcoTank Oil and Waste Container**

EcoTanks are high-capacity reservoirs that you can use instead of individual oil and waste bottles in your QX200 Droplet Reader. The ddPCR Droplet Reader Oil EcoTank for Probes distributes droplet reader oil and collects waste generated by the instrument into a single partitioned container.

The EcoTank can be used to read up to 50 plates. It ensures that floating waste droplets remain on the waste side of the bottle, while the droplet reader oil can recirculate through the instrument for another droplet reading cycle.

#### **Usage Advisories**

Note the following when using the Droplet Reader Oil EcoTank for Probes:

- Do not add bleach to the EcoTank before installation or while it is installed in the instrument. You can add bleach after you remove the EcoTank from the instrument and before storage or disposal.
- The unused EcoTank is stable for up to 12 months at room temperature (25°C).
- The EcoTank must be replaced after 6 months of use.
- The EcoTank for Probes is not compatible with QX200 ddPCR EvaGreen® Supermix (Catalog No. 1864033, 1864034, 1864035, 1864036).
- Do not reuse a spent EcoTank.

### Installing an EcoTank Bottle in the QX200 Droplet Reader

#### To install an EcoTank bottle

- 1. Open QX Manager Software.
- 2. Open the reagent door on the left side of the QX200 Droplet Digital PCR System and place an EcoTank in front of the currently installed oil bottles.

Note: The EcoTank is symmetrical and new bottles can be loaded in either orientation. Bio-Rad does not recommend changing the EcoTank orientation after it has been installed in the droplet reader.

- Remove the caps from the EcoTank.
- 4. Remove from the currently installed bottles the caps that are attached to the instrument, and attach them to the EcoTank.
- 5. Cap and remove the currently installed bottles.
- 6. Place the EcoTank in the bottle tray. Maintain the orientation to prevent twisting of the oil lines.

After the bottle door has opened, QX Manager Software prompts you to select the action taking place. Continue to the next section.

### Selecting the EcoTank in QX Manager Software

#### To specify an EcoTank is being used

- 1. Select New EcoTank is being loaded into instrument.
- 2. Enter the batch number, and click OK.



In the Instrument Status bar, QX Manager displays the EcoTank for the droplet reader and waste oils, as well as the number of plates remaining.



**Important:** If the instrument has been idle for more than a week, you must prime the instrument before running a plate. From the Setup menu, click Tools > Prime to fill the lines with oil before starting a run.

### **Reinstalling the Standard Bottles**

You can return the QX200 Droplet Reader to the initial two bottle configuration at any time, except when a run is in progress.

Complete the steps in the following sections to change the bottle configuration.

#### To exchange the EcoTank for the two-bottle configuration on the QX200 Droplet Reader

- 1. Open QX Manager Software.
- Complete the following substeps to prepare a new 1 L waste bottle for the QX200 Droplet Reader:
  - a. Add 50 ml 10% bleach to the Waste bottle to prevent microbial growth.
  - b. Place an identifying label on the Waste bottle.
- Open the reagent door on the left, and then place a droplet reader oil bottle and the new waste bottle into the space in front of the EcoTank bottle.
- 4. Remove the caps from the EcoTank and bottles.
- Replace the oil and waste caps on the used EcoTank bottle, and then use the handle on the EcoTank bottle to move it out of position and remove it from the instrument.
- 6. Place the oil and waste bottles into the corresponding slots and close the reagent door.

After the bottle door has opened, QX Manager Software prompts you to select the action taking place. Continue to the next section.

#### To specify that separate oil and waste bottles are being used in QX Manager Software

▶ Select New Oil and/or Waste bottle is being loaded into the instrument and click OK.



QX Manager displays the DR Oil and Waste indicators.



**Important:** If the instrument has been idle for more than a week, you must prime the instrument before running a plate. From the Setup menu, click Tools > Prime to fill the lines with oil before starting a run.

#### **Disposing of Waste**

Droplet generation and reader fluids from Bio-Rad are based on fluorinated hydrocarbon chemistry and should be disposed of in accordance with institutional, state, and local regulations. For information, see Managing Waste on page 210.

#### Disposing of the EcoTank and EcoTank Waste

When QX Manager indicates 50 plates have been run, you must replace the used EcoTank bottle. After removing the EcoTank from the instrument, you can install a new EcoTank or reinstall the standard twobottle configuration. If the EcoTank bottle is removed before 50 plates are run, discard the EcoTank bottle and oil in accordance with institutional, state, and local regulations.

#### To remove and dispose of the EcoTank and EcoTank Waste

- 1. Uncap one side of the EcoTank and take it to the liquid waste receptacle.
- 2. Hold the uncapped opening slightly lower than the capped opening and slowly pour out as much of the contents from the uncapped side as possible.
- 3. Return the bottle to its upright position to allow the fluid trapped in the closed cap side of the bottle to move to the open cap side, and repeat the process.
- 4. Pour entire contents of spent bottle into the waste receptacle.
- Place the cap on the empty EcoTank bottle and then discard per waste disposal regulations.

#### **Cleaning the EcoTank Waste Cap**

Repeat the following process once per month at a minimum.

#### To clean the waste cap

- Dampen a lint-free wipe with fresh 10% bleach solution.
- Remove the cap on the waste side of the EcoTank.
- Wipe down anywhere microbial growth might occur, including along the threading and inside of the cap.
- If the cleaning leaves excess liquid on the cap, dry the cap using a dry lint-free wipe.
- After the cleaning is done and the cap is dry, place and tighten the cap on the EcoTank.

# **Priming and Flushing the Instrument**

You should prime the instrument after you replace the oil bottle and you should prime and flush the instrument when you change the waste bottle.

**Important:** Before you initiate a prime or flush, *the lid must be closed*. Do NOT press the button to open the lid while a prime or flush is in progress.

#### To prime the instrument

- 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.

#### 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.

## **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:

**Table 42. Managing Waste** 

| Chemical name  | Weight % |
|--|----------|
| Fluorinated fluids   | >50%     |
| Water  | <50%     |
| Bleach   | <10%     |
| Other materials (proteins, nucleic acids, fluorescent dye) | <10%     |

Note: The chemical identities and exact percentages in the above table are withheld as trade secrets.

Bio-Rad droplet generation and reader fluids are based on fluorinated hydrocarbon chemistry, and should be disposed of in accordance with institutional, state and local regulations. Avoid release to the environment and prevent entry into sewer systems or bodies of water. As a disposal alternative, incinerate in a permitted high-temperature waste incineration facility.

These nonflammable fluids are inert and have low environmental impact and low toxicity.

#### **Toxicological Information**

Chemical name: (mixture) Oral LD50: >5000 mg/kg (Rat) Dermal LC50: >5000 mg/kg (Rabbit) Inhalation LD50: >8838 ppm (Rat/8-hr)

#### **Ecological Information**

Acute aquatic hazard: LC50 > 500 mg/l (96-hr./static; Pimephales promelas/Fathead minnow)

Chronic aquatic hazard: No information available (GHS Chronic 4)

# **Recovery From Idle Mode**

Complete the steps below if the instrument has been idle:

- 1. Check the expiration date on the oil bottle. If expired, replace the oil bottle.
- 2. Ensure the waste bottle is not full.
- 3. If needed, add 50 ml of 10% bleach to the waste bottle.
- 4. If you have changed the bottles, or if you have not used the system for a week or more, perform the prime and flush processes as specified in Priming and Flushing the Instrument on page 209.

# **Cleaning or Decontaminating the Instrument**

Use the protocols specified below for the following:

- Regular post-run instrument cleanup
- Instrument decontamination, as needed

If you have questions regarding the use of cleaning agents other than those specified, contact Bio-Rad Technical Support.

#### To clean the instrument

- 1. Remove the plate after a run is complete and ensure it is properly discarded.
- 2. Using 70% ethanol, wipe down the external panels, as well as the plate holder and its compartment.

#### To decontaminate the instrument

- 1. Remove the plate after a run is complete and ensure it is properly discarded.
- 2. Wipe down the plate holder and its compartment with 10% bleach.

Important: Do not use bleach inside the system.

- 3. Wipe down all external surfaces with 10% bleach.
- 4. Identify the equipment as Decontaminated.

# **Instrument Maintenance Log**

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

# **Appendix C 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. Table 43 contains error grouped by type.

- 1. Click the X to display the corresponding error code and description.
- Perform the steps in the solution column before starting a new run.
- 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 43. Communication errors** 

| Error Code                   | Description  | Solution   |
|------------------------------|--|--|
| 102, 103, 104*,<br>110*, 111 | Communication loss between the instrument and software, detector board error, loss during a run, or unable to reestablish a connection | Disconnect and reconnect the USB cable, and then power cycle the instrument. |
|                              | <b>Note:</b> For codes marked with an asterisk (*), some data may be usable.   |  |
| 302, 308                     | Communication error involving pump   | Power cycle the instrument.  |
|                              | Note: Some data might be usable.   |  |

Table 43. Communication errors, continued

| Error Code | Description                            | Solution |
|------------|--|----------|
| 502<br>900 | Invalid checksum value Invalid command | Retry.   |

An electrical error is typically triggered by an invalid setting.

Table 44. Electrical error codes

| Error Code | Description  | Solution                    |
|------------|--|-----------------------------|
| 406        | Electrical Error; invalid power value setting.  Note: Some data might be usable. | Power cycle the instrument. |

Mechanical errors can be caused by fluid issues (low oil, high waste), instrument operation errors, or binary data collection errors.

Table 45. Mechanical error codes

| Error Code     | Description                              | Solution   |
|----------------|--|--|
| 105, 106       | Fluidics or motion response was invalid. | Power cycle the instrument.  |
|                | Note: Some data may be usable.           |  |
| 201            | Motors disabled                          | 1. Remove and reinsert the plate and   |
|                | Note: Some data may be usable.           | plate lid.   |
|                |  | <ol><li>Check for incorrect or multiple heat<br/>seal foil layers.</li></ol> |
|                |  | 3. Check for strain on all motor axes.                                       |
|                |  | 4. Power cycle the instrument.   |
| 205, 207, 210, | X, Y, or Z-axis, unable to reach target  | Check for motor obstructions, and then                                       |
| 211, 212, 218  | location or home in allotted time.       | power cycle the instrument.  |
|                | Note: Some data might be usable.         |  |
| 301, 303, 304, | Pump errors                              | Power cycle the instrument.  |
| 305, 307, 312, | Note: Some data might be usable.         |  |
| 316, 317       |  |  |

# **Appendix D** Ordering Information

This appendix contains descriptions and catalog numbers for new or replacement instruments, accessories, and consumables for Bio-Rad ddPCR products, including your QX200 Droplet Reader.

### ddPCR System and Instrument Packages

Table 46. ddPCR system and instrument packages

| Product  | Description   | Catalog<br>Number |
|--|---|-------------------|
| QX200 Droplet Digital<br>PCR System            | Includes the QX200 Droplet Generator, QX200 Droplet Reader, computer, software, and consumables                 | 1864001           |
| QX600 Droplet Digital<br>PCR System            | Includes the QX200 Droplet Generator, QX600 Droplet Reader, computer, software, and consumables                 | 17007769          |
| QX200 AutoDG™<br>Droplet Digital PCR<br>System | Includes the AutoDG, QX200 Droplet Reader, computer, and QX Manager Software Standard Edition                   | 1864100           |
| QX600 AutoDG Droplet<br>Digital PCR System     | Includes the AutoDG, QX600 Droplet Reader, computer, QX<br>Manager Software Standard Edition, and consumables   | 17008371          |
| Automated Droplet<br>Generator                 | Includes the AutoDG (1), cooling block accessory (1), oil purge reservoir (1), and power cord (1)               | 1864101           |
| QX200 Droplet<br>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, two plate holders, USB cable, and power cord                                       | 1864003           |
| QX600 Droplet Reader                           | Includes the droplet reader, two plate holders, USB cable, and power cord                                       | 12013328          |

# **Thermal Cyclers and Plate Sealer**

Table 47. Thermal cyclers and plate sealer

| Product   | Description   | Catalog<br>Number |
|---|---|-------------------|
| C1000 Touch Thermal Cycler with 96-Deepwell Reaction Module | Includes the C1000 Touch Thermal Cycler chassis, 96-deepwell reaction module, USB flash drive   | 1851197           |
| PTC Tempo Deepwell Thermal Cycler                           | Includes the thermal cycler, power cord, USB cable, and Ethernet cable  | 12015392          |
| PX1 PCR Plate Sealer  | PCR plate sealer, includes heat sealing instrument, plate support block that holds 96-well and 384-well plates, sealing frame, power cord | 1814000           |
| Pierceable Foil Heat Seal                                   | Heat seal for the PX1 PCR Plate Sealer  | 1814040           |

### **AutoDG Accessories**

**Table 48. Automated Droplet Generator accessories** 

| Product                 | Description  | Catalog<br>Number |
|-------------------------|--|-------------------|
| Cooling block accessory | Prevents evaporation during droplet generation   | 12002819          |
| Oil purge reservoir     | Collects oil waste from priming and flushing  Note: Contact Bio-Rad Technical Support for replacement information. | N/A               |
| Power cord              | Connects the AutoDG to the power source  Note: Contact Bio-Rad Technical Support for replacement information.      | N/A               |

# **QX200 Droplet Generator Accessories**

| Product                                      | Description  | Catalog<br>Number |
|--|--|-------------------|
| DG8 droplet generator cartridges and gaskets | Microfluidic cartridges used to mix sample and oil for droplet generation, and gaskets to seal the cartridges  Note: 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  Note: Contact Bio-Rad Technical Support for replacement information.   | N/A               |

# **QX600 Droplet Reader Accessories**

Table 49. QX600 Droplet Reader accessories

| Product                          | Description  | Catalog<br>Number |
|----------------------------------|--|-------------------|
| Computer                         | Computer that connects to the QX600 Droplet Reader for data collection and analysis  | 12017458          |
| USB cable and power cord         | Cable connecting the computer to the instrument and power cord connecting the instrument to the power source  Note: 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          |

# **QX200 Droplet Reader Accessories**

Table 50. QX200 Droplet Reader accessories

| Product                          | Description  | Catalog<br>Number |
|----------------------------------|--|-------------------|
| Computer                         | Computer that connects to the QX200 Droplet Reader for data collection and analysis  | 12017458          |
| USB cable and power cord         | Cable connecting the computer to the instrument and power cord connecting the instrument to the power source  Note: 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          |

### ddPCR Consumables and Other Materials

Table 51. Consumables and other materials

| Description   | Catalog<br>Number   |
|---|---|
| Cear well/clear shell semi-skirted plates   | 12001925  |
|   | (pkg of 25)   |
| Microfluidic cartridge with gasket for the AutoDG   | 1864108   |
|   | (pkg of 30)   |
|   | 1864109   |
|   | (pkg of 60)   |
| Pipet tips for the AutoDG   | 1864120   |
|   | (pkg of 20)   |
|   | 1864121   |
|   | (pkg of 40)   |
| Pipet tip waste bins for the AutoDG   | 1864125   |
| Automated droplet generation oils for the AutoDG:   |   |
| Automated Droplet Generation Oil for Probes   | 1864110   |
| ■ Automated Droplet Generation Oil for EvaGreen®  | 1864112   |
| Droplet generation oil for the QX200 Droplet Generator:                                   |   |
| ■ Droplet Generation Oil for Probes, 10 x 7 ml  | 1863005   |
| ■ Droplet Generation Oil for EvaGreen®, 2 x 7 ml  | 1864005   |
| ■ Droplet Generation Oil for EvaGreen®, 10 x 7 ml   | 1864006   |
| ■ 20 µl for sample loading  | L-20, L8-20   |
| ■ 50 µl for droplet transfer  | L-50, L8-50   |
| ■ 8-channel, 200 µl for oil   | L8-200  |
| Filtered  | GP-L10F   |
|   | GP-L200F  |
| Pierceable foil plate seals for PX1 Plate Sealer  | 1814040   |
| Droplet reader oil for the QX600 Droplet Reader and QX200 Droplet Reader                  | 1863004   |
| You can use an empty droplet reader oil bottle to collect the waste from droplet reading. | N/A   |
|   | Cear well/clear shell semi-skirted plates  Microfluidic cartridge with gasket for the AutoDG  Pipet tips for the AutoDG  Pipet tip waste bins for the AutoDG  Automated droplet generation oils for the AutoDG:  Automated Droplet Generation Oil for Probes  Automated Droplet Generation Oil for EvaGreen®  Droplet generation oil for the QX200 Droplet Generator:  Droplet Generation Oil for Probes, 10 x 7 ml  Droplet Generation Oil for EvaGreen®, 2 x 7 ml  Droplet Generation Oil for EvaGreen®, 10 x 7 ml  20 µl for sample loading  50 µl for droplet transfer  8-channel, 200 µl for oil  Filtered  Pierceable foil plate seals for PX1 Plate Sealer  Droplet reader oil for the QX600 Droplet Reader and QX200 Droplet Reader |

# ddPCR Supermixes

Table 52. ddPCR supermixes

| Supermix   | Description  | Catalog number                           |  |
|--|--|--|--|
| ddPCR<br>EvaGreen®<br>Supermix                       | 2x supermix, for use in nucleic acid sample preparation with the QX600 and QX200 ddPCR systems   | 1864033<br>1864034<br>1864035<br>1864036 | 2ml (2 x 1ml)<br>5ml (5 x 1ml)<br>25ml (25 x 1ml)<br>50ml (50 x 1ml) |
| ddPCR Supermix<br>for Probes (no<br>dUTP)            | 2x supermix, for use in nucleic acid sample preparation with the QX600 and QX200 ddPCR systems   | 1863023<br>1863024<br>1863025            | 2ml (2 x 1ml)<br>5ml (5 x 1ml)<br>25ml (25 x 1)                      |
| ddPCR Supermix<br>for Probes                         | 2x supermix, for use in sample preparation with the QX600 and QX200 ddPCR systems  | 1863026<br>1863010<br>1863027<br>1863028 | 2ml (2 x 1ml)<br>5ml (5 x 1ml)<br>25ml (25 x 1ml)<br>50ml (50 x 1ml) |
| ddPCR Multiplex<br>Supermix                          | 4x supermix especially suited for probe-<br>based detection of multiple targets in DNA<br>samples using the QX600 and QX200<br>ddPCR systems | 12005909<br>12005910<br>12005911         | 1.2ml (2 x 0.6ml)<br>3 ml (5 x 0.6 ml)<br>12.5 ml (5 x 2.5 ml),      |
| ddPCR Supermix<br>for Residual DNA<br>Quantification | 2x supermix, for use in residual DNA detection with the QX600 and QX200 ddPCR systems  | 1864037<br>1864038<br>1864039<br>1864040 | 2ml (2 x 1ml)<br>5ml (5 x 1ml)<br>25ml (25 x 1ml)<br>50ml (50 x 1ml) |
| One-step RT-<br>ddPCR Advanced<br>Kit for Probes     | 200 or 500x 20 µl reaction kit, for absolute quantification of target RNA in a one-step format with the QX600 and QX200 ddPCR systems        | 1864021<br>1864022                       | 2ml (2 x 1ml)<br>5ml (5 x 1ml)                                       |

### **Buffer Controls**

Table 53. Buffer controls

| Buffer                                 | Catalog<br>Number |
|--|-------------------|
| ddPCR Buffer Control Kit for Probes    | 1863052           |
| QX200 Buffer Control Kit for EvaGreen® | 1864052           |

### ddPCR Kits

Table 54. ddPCR Kits

| Product   | Description  | Catalog<br>number |
|---|--|-------------------|
| ddPCR Mutation  | n Screening Kits   |                   |
| ddPCR KRAS G1   | 2/G13 Screening Kit  | 1863506           |
| ddPCR KRAS Q6   | 61 Screening Kit   | 12001626          |
| ddPCR BRAF V6   | 00 Screening Kit   | 12001037          |
| ddPCR NRAS Q6   | 61 Screening Kit   | 12001006          |
| ddPCR NRAS G1   | 12 Screening Kit   | 12001094          |
| ddPCR NRAS G1   | 12/G13 Screening Kit   | 12001627          |
| ddPCR EGFR Ex   | on 19 Deletions Screening Kit  | 12002392          |
|   | x multiplex assay and 2x ddPCR supermix for probes (no dUTP).  |                   |
|   | sidual DNA Quantification Kit  | 17000031          |
|   | sidual DNA Quantification Kit  | 17000032          |
|   |  |                   |
| 200x 20 μl reactions, includes 20x CHO or E.coli RDQ assay and 2x ddPCR |  |                   |
| Supermix for Re   | sidual DNA Quantification.   |                   |
| ddPCR Copy Nu   | ımber Determination Kits   |                   |
| ddPCR SMN1 C  | copy Number Determination Kit  | 1863500           |
|   | copy Number Determination Kit  | 1863503           |
| •   | ions, includes assay at 20x concentration, 2x ddPCR Supermix for P) and positive controls                          |                   |
| ddPCR Library (   | Quantification Kit   |                   |
| ddPCR Library 0   | Quantification Kit for Illumina TruSeq   | 1863040           |
| •   | ions, includes 1 vial of primers and probes at 20x concentration, 2x ix for Probes (no dUTP) and positive controls |                   |

# ddPCR Assays

Table 55. ddPCR Assays

| Product                            | Catalog nu | Catalog number |  |
|------------------------------------|------------|----------------|--|
| 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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