SOP#: 003	Date Issued: 8/03/2021	Date Revised: 7/08/2021
TITLE:	**QX200™ Droplet Digital PCR system**	
SCOPE:	Research Personnel	
RESPONSIBILITY:	BORC staff	
PURPOSE:	To outline the proper procedures for use and maintenance of the **QX200™ Droplet Digital PCR system**.	

1 PURPOSE

This SOP explains how to apply QX200™ Droplet Digital PCR system in biomedical research such as gene expression, genotyping, absolute copy quantification etc.

2 RESPONSIBILITY

It is the responsibility of the BORC staff to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

3 BEFORE CONDUCTING YOUR EXPERIMENT

4 MAINTENANCE

4.1 Sample Preparation

Prepare the PCR reaction by combining 2x PCR supermix, 20x primers and probe, and DNA sample. Mix by vortexing in short pulses; centrifuge briefly.

- Thaw and equilibrate reaction components to room temperature. If the sample is prone to thermal degradation, prepare the reaction mix on ice, but equilibrate the reaction mix to room temperature (~3 min) before loading into the DG8™ cartridge for droplet formation
- Use one of the PCR supermixes recommended in Table 1.2, as these contain reagents required for droplet generation. Follow instructions in the product inserts to prepare the samples for droplet generation
- Vortex the supermixes thoroughly to ensure homogeneity, as a concentration gradient may form during –20°C storage. Alternatively, pipet up and down >5 times to mix. Centrifuge briefly to collect contents at the bottom of the tube before dispensing
- Assemble reaction mixtures in vials or in 96-well PCR plates. The advantage of using a PCR plate is that samples can be loaded into the DG8 cartridge using an 8-channel pipet

4.2 Operation of the QX200™ Droplet Generator

The QX200 droplet generator prepares droplets for up to eight samples at a time. Droplet generation takes ~2 minutes for each set of eight samples (~30 minutes for a 96-well plate). All 8 sample wells in the DG8 droplet generator cartridge must contain sample (or 1x buffer control), and all 8 oil wells must contain droplet generation oil. Do not load sample or oil into the DG8 cartridge unless it is inserted in the holder

4.2.1. Insert the DG8 cartridge into the holder with the notch in the cartridge at the upper left of the holder:

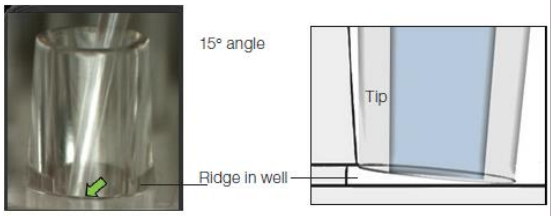
a. Open the cartridge holder by pressing the latches in the middle.

b. Slide the DG8 cartridge into the right half of the holder, then drop it down.

c. Press the halves of the holder together to snap it closed.

Air bubbles can cover the bottom of the well and result in 2,500–7,000 fewer droplets and poor data quality. They are difficult to see. To avoid creating air bubbles, use the following pipetting technique, which also ensures samples wet the bottoms of the wells so they are wicked into the microchannels (necessary for proper droplet generation).

- Use only 20 µl aerosol-barrier (filtered) Rainin pipet tips; do not use 200 µl pipet tips (see Table 1.2)
- Gently slide the pipet tip down the side of the well at a ~15° angle until it passes over the ridge near the bottom. Holding the angle, ground the pipet tip against the bottom edge of the sample well while slowly dispensing a small portion of the sample; do not pipet directly onto the side (wall) of the well
- After dispensing about half the sample, slowly draw the tip up the wall while dispensing the rest of the sample; do not push the pipet plunger past the first stop



Transfering sample to the sample wells (middle row) of the DG8 cartridge. Hold the pipet tip at a 15° angle and at the bottom of the well (middle and right panels); do not dispense sample onto the wall or side of the well.

4.2.2. Transfer 20 µl of each prepared sample to the sample wells (middle row) of the DG8 cartridge.

4.2.3. Dispense the droplet generation (DG) oil in the reagent trough (see Table 2.1 for volumes required; see Table 1.2 for PCR supermix and DG oil compatibility).

4.2.4. Using a multichannel pipet, fill each oil well (bottom row) with 70 µl DG oil from the reagent trough.

4.2.5. Hook the gasket over the cartridge holder using the holes on both sides. The gasket must be securely hooked on both ends of the holder; otherwise, pressure sufficient for droplet generation will not be achieved.

4.2.6. Open the QX200 droplet generator by pressing the button on the green top and place the cartridge holder into the instrument. When the holder is in the correct position, both the power (left light) and holder (middle light) indicator lights are green (see Table 2.2)

4.2.7. Press the button on the top again to close the door. This initiates droplet generation: a manifold positions itself over the outlet wells, drawing oil and sample through the microfluidic channels, where droplets are created. Droplets flow to the droplet well, where they accumulate. The droplet indicator light (at right) flashes green after 10 sec to indicate droplet generation is in progress.

4.2. 8. When droplet generation is complete, all three indicator lights are solid green. Open the door by pressing the button, and remove the holder (with DG8 cartridge still in place) from the unit. Remove the disposable gasket from the holder and discard it. The top wells of the cartridge contain droplets, and the middle and lower wells are nearly empty with a small amount of residual oil.

4.3 Preparation for PCR

4.3.1. Transfer droplets

Pipet 40 µl of the contents of the top wells (the droplets) into a single column of a 96-well PCR plate.

Use the following pipetting techniques to avoid shearing or coalescing the droplets:

To aspirate droplets from the DG8 cartridge:

- Use an 8-channel manual L-50 pipet with 200 µl tips (not wide- or narrow-bore)
- Place the cartridge holder on a flat surface and position the pipet tips in each of the 8 top wells at a ~30–45° angle, vertical into the junction where the side wall meets the bottom of the well. Do not position the pipet tip in a vertical orientation (90°) or against any flat surface of the well; do not allow the tips to be flat against the bottoms of the wells
- Slowly draw 40 µl of droplets into the pipet tip (should take ~5 sec, and ~5 µl air is expected); do not aspirate >40 µl, as this causes air to percolate through the droplets
- Pipet slowly. Apply a stable resistive force to the plunger to draw and aspirate droplets smoothly into and out of pipet tips

To dispense droplets into the 96-well plate, position the pipet tip along the side of the well — near, but not at, the bottom of the well — and slowly dispense the droplets (~5 sec).

To prevent evaporation and contamination with particulates, cover the plate (for example, with 8-cap strips or the lid from a pipet tip box) as you work.

Aspirating droplets from the DG8 cartridge.

Dispensing droplets into a 96-well PCR plate.

4.3.2 Seal the PCR plate

Seal the PCR plate with foil immediately after transferring droplets to avoid evaporation. Use pierceable foil plate seals that are compatible with the PX1™ PCR plate sealer and the needles in the QX200 droplet reader (for example, catalog #181-4040). Follow the instructions in the PX1 PCR Plate Sealer Instruction Manual (bulletin 10023997).

a. Set the plate sealer temperature to 180°C and time to 5 sec.

b. Touch the arrow to open the PX1 tray door. Position the support block on the tray with the 96-well side facing up. Place the 96-well plate onto the support block and ensure that all plate wells are aligned with the support block.

c. Cover the 96-well plate with one sheet of pierceable foil seal. (The yellow label on the Bio-Rad heat seal bag identifies the sealing surface.) Do not attempt to place the frame over the foil-covered plate. The frame is only for use with other seals.

d. Once the 96-well plate is secured on the support block and covered with the pierceable foil seal, touch the seal button. The tray will close and heat sealing will initiate.

e. When heat sealing is complete, the PX1 door opens automatically. Remove the plate from the block for thermal cycling. Remove the block from the PX1.

f. Check that all the wells in the plate are sealed; the depressions of the wells should be visible on the foil. Once sealed, the plate is ready for thermal cycling.

4.3.3 Once droplets are removed, press the latches on the DG8 cartridge holder to open it. Remove the empty DG8 cartridge and discard it.

4.4 Run PCR

Begin thermal cycling (PCR) within 30 min of sealing the plate, or store the plate at 4°C for up to 4 hr prior to thermal cycling. Refer to the supermix product inserts for cycling conditions.

4.5 Read plate in QX200 droplet reader

When PCR amplification is complete, remove the 96-well plate from the thermal cycler and read the droplets using the QX200 droplet reader (follow the instructions in the QX200 Droplet Reader Instruction Manual, bulletin 10031906).

4.5.1 . Power on the QX200 droplet reader using the switch at the back. Allow it to warm up for 30 min, then switch on the PC and launch QuantaSoft™ software.

4.5.2. Check the indicator lights on the front of the droplet reader (Table 2.1). The first two lights at left should be solid green, indicating power is on, there is sufficient oil in the designated oil reservoir, and there is <700 ml in the waste bottle. If the lights are flashing amber, the run cannot be started; clean out the waste bottle or replace the oil (see Section 4.2.2)

4.5.3. Place the 96-well PCR plate into the plate holder:

a. Place the 96-well PCR plate containing the amplified droplets into the base of the plate holder. Well A1 of the PCR plate must be in the top left position.

b. Move the release tabs of the top of the plate holder into the “up” position and place the top on the PCR plate. Firmly press both release tabs down to secure the PCR plate in the holder.

4.5.4 Press the button on the green lid to open the droplet reader. Load the plate holder into the droplet reader, and press the button on the lid again to close the cover. Confirm the first three indicator lights are green (Table 2.1).

4.5.5. In QuantaSoft software, click **Setup** in the left navigation bar to define your experiment .

- To open saved details (settings and data) from another experiment, click Plate > Load and select the file
- To open a saved template for a plate map (settings only, no data), click Template > Load and select the file
- To create a new template, click Template > New

- To overwrite the setup information for a plate that is open (experiment type and name, sample name, etc.), click Template > Load. In the Load template window, click Overwrite.

4.5.6 Use the well editor to adjust the settings

Enter the file name, then use the well editor and experiment editor to adjust the settings for your experiment.

1) To open the well editor, double-click on the well(s) you wish to edit. Selected wells are highlighted in gray, and the well editor appears across the top of the interface.
   - To select multiple wells, hold Ctrl and select the wells
   - To select wells in a continuous series (horizontal or vertical), hold Shift and select the first and last wells
   - To select all wells in the plate, double-click in the top left corner of the plate
   - To select a row or column, double-click the letter or number for that row or column
2) In the Sample panel, enter the sample Name and select the Experiment from the drop-down menu.
   - All saved experiments appear in the drop-down menu, along with the option to add experiment...
   - The sample name is case-sensitive; only wells with identical sample names can be treated as merged wells during data analysis
   - To create or edit an experiment, use the experiment editor (see Section 3.1.2)
3) Select the Supermix from the drop-down menu (required; selection cannot be changed after data collection).
4) Define Target 1 (channel 1, the FAM channel) and Target 2 (channel 2, the VIC or HEX channel). Assign each assay a Name and sample Type. Settings appear in the Applied Well Settings box as you enter them. When you are done, click Apply or OK to save the information. The settings appear in the well in the plate map.

4.5.7 Using the Experiment Editor to adjust the settings

Use the experiment editor to define the experiment type. To open the experiment editor, select Experiment > add experiment... in the well editor or select New or Edit (double-click on an experiment name) in the Experiments window under Setup. Four types of experiments are possible: Absolute Quantification (ABS), Rare Event Detection (RED), Copy Number Variation (CNV), and Gene Expression (GEX). A default list of experiments is supplied at installation, but you can create and save custom experiments; upgrade installations will preserve current experiment lists.

Settings are summarized in the Applied Well Settings box as you enter them. Click Apply or OK to save the experiment information. The settings appear in the well in the plate map.

If the Sample Name, Experiment, Target Name, Target Type, and Supermix all match across multiple wells (all are case-sensitive), the software can identify these as “merged” and provide an option to view merged data during analysis

4.5.8 Using the Advanced Options

Click Options in the Setup window to see all advanced options for data collection and analysis.

4.5.9. Run

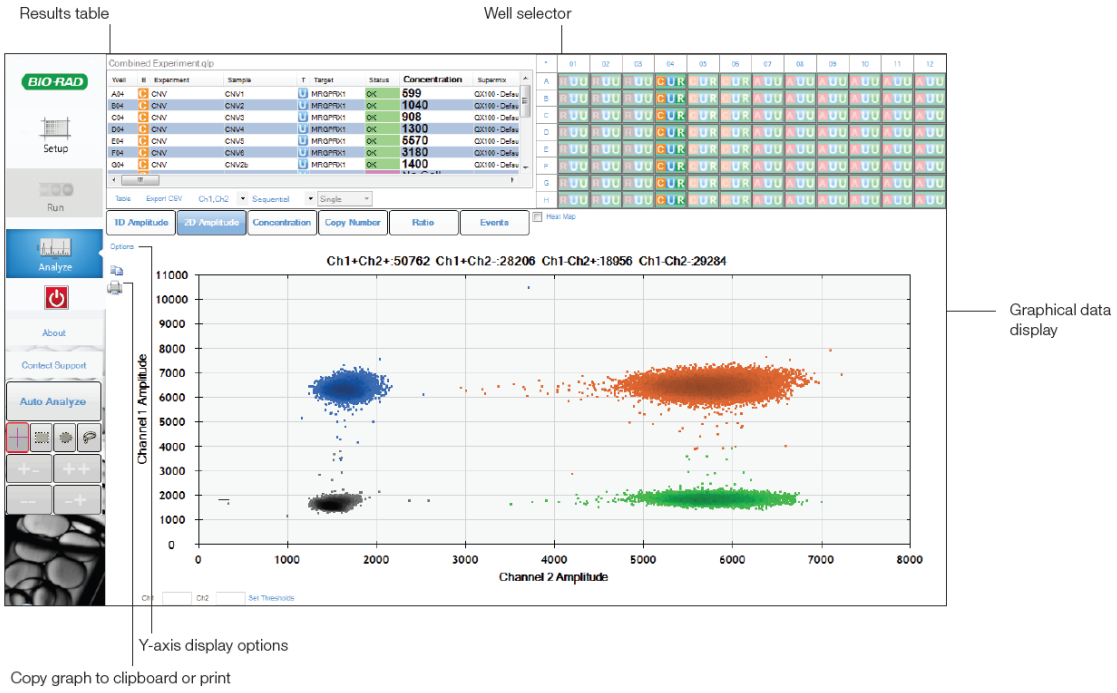
1. Click Run in the left navigation bar to start the run. The run indicator light (far right) flashes green to indicate droplet reading is in progress.
2. In the Run Options window, select the detection chemistry:
   - If a probe supermix is selected in the well editor, the probe dye sets appear. Select FAM/HEX or FAM/VIC
   - If an EvaGreen supermix is selected, the EvaGreen dye set appears; the screen confirms the number of EvaGreen wells configured on the plate
3. Up to 1 minute later, a green circle appears next to the abort button and flashes periodically to indicate the run is in progress. Active and analyzed wells are also highlighted in green in the plate map.
4. As each well is analyzed, the data appear across the top navigation area.
5. When droplet reading is complete, all four indicator lights are solid green. Open the door and remove the plate holder from the unit. Remove the 96-well PCR plate from the holder and discard it.

4.6 Data Analysis

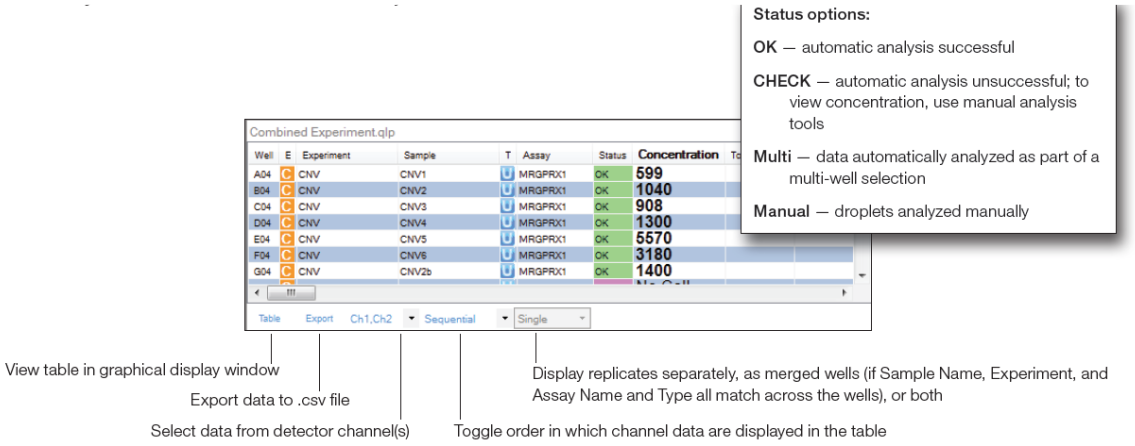
In the Setup window, load a plate (*filename*.qlp), then click Analyze to open and analyze the data. The data analysis interface is separated into three windows:

- Results table — summarizes results for wells selected in the well selector
- Well selector — enables selection of wells for targeted analysis
- Processed data/graphical display — allows visualization of graphical data from selected wells

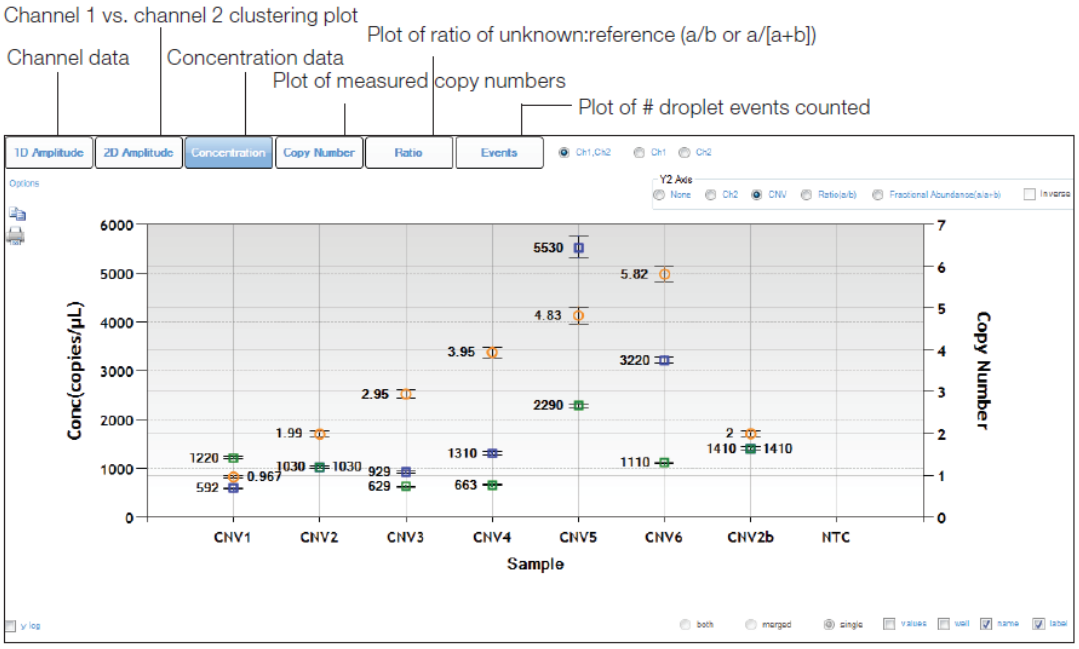
Data analysis interface. Data from a CNV analysis are shown.



Results table options. Data from a CNV analysis are shown.



Graphical data display options. A concentration plot from a CNV analysis is shown, with display options across the top.



4.6.1 Viewing Channel Data (1D Amplitude)

Click 1D Amplitude to visualize the data collected from each channel of selected wells. Use the radio buttons to select the channels to be displayed. This tab also provides options for adjusting the thresholds used in assigning positives and negatives for each channel.

When viewing a single well, change the threshold using one of the following options:

- Use the single-well threshold tool . The assigned threshold appears as a horizontal pink line

-Or-

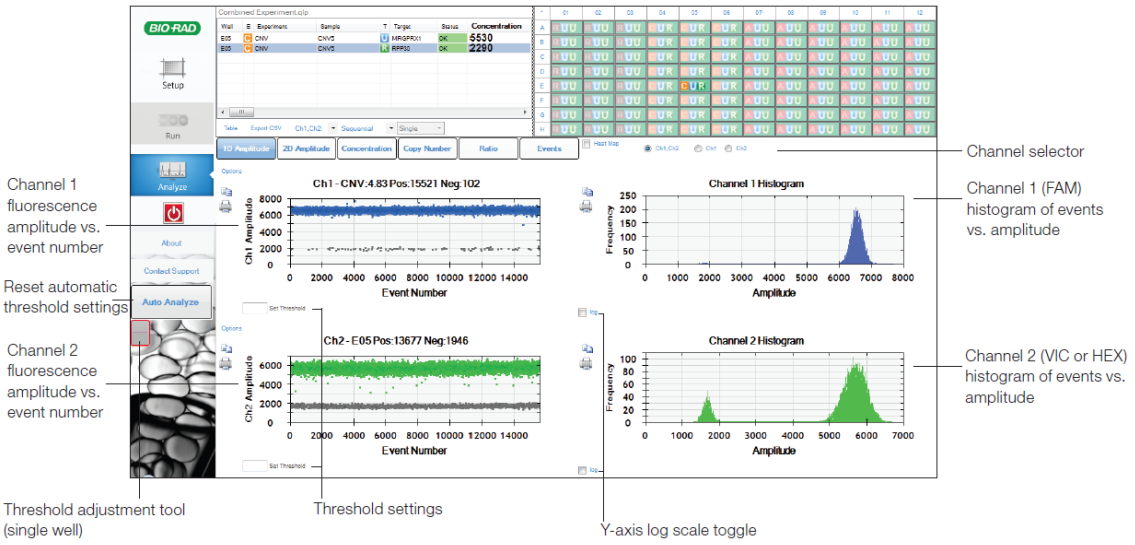
- Enter threshold values in the Set Threshold field

When viewing multiple wells, change the thresholds as follows:

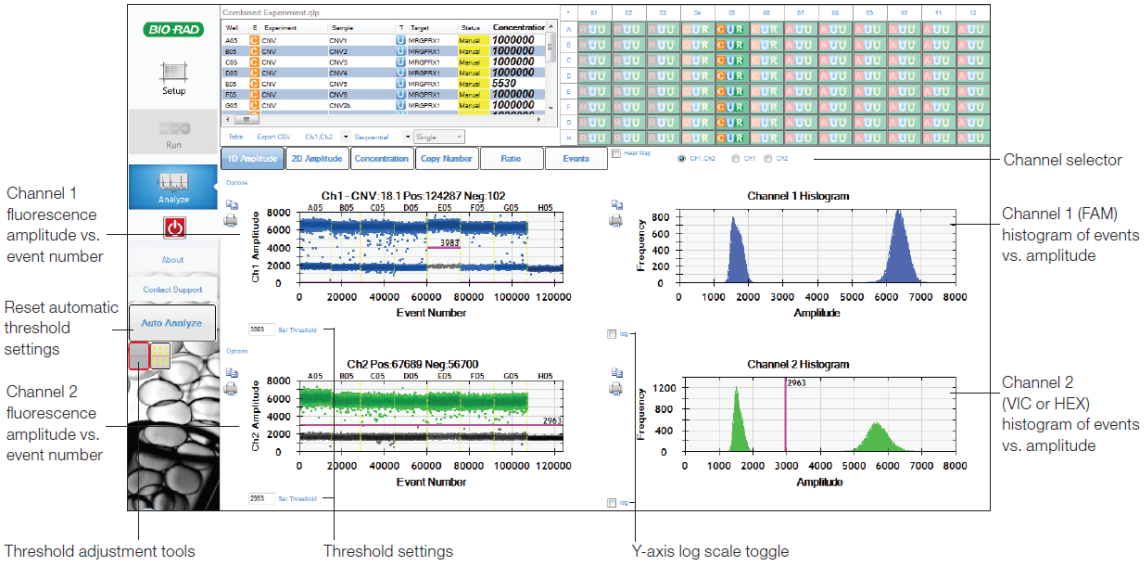
- Use the single-well threshold tool to change the threshold in a single well. Vertical yellow lines in the processed data plots show where droplet data from each well start and end, and the assigned threshold appears as a horizontal pink line
- Use the multi-well threshold tool to change the threshold in all the wells (appears as a pink line in the plots)

- To manually set threshold values for single or multiple wells, enter the values in the Set Threshold field below the plot and click Set Threshold or Enter.

Click Auto Analyze to revert to automatic threshold settings and calculations. Threshold adjustments can also be made in the 2D Amplitude clustering plots.



Viewing channel data for a single well. Processed data from both channels of a single well are shown. In channel 1, the single-well threshold tool is enabled (the threshold is shown by the pink line and the value in the Set Threshold field).



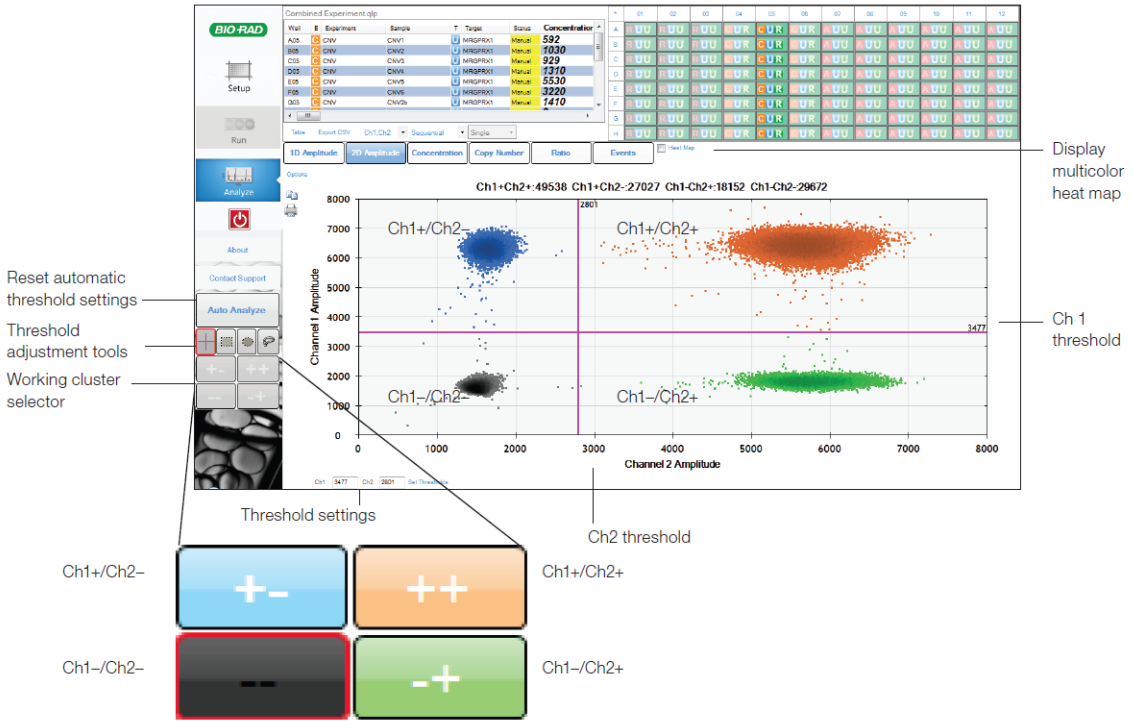
Viewing channel data for multiple wells. Processed data from both channels of multiple wells are shown. In channel 1, the single-well threshold tool is enabled (the threshold is indicated by the pink line and the value in the Set Threshold field; the status of that well in the results also shows Manual). In Channel 2, the multiple threshold tool is enabled.

4.6.2 Viewing Clustering Plots (2D Amplitude)

Click 2D Amplitude to view the channel 1 vs. channel 2 clustering plot and enable options for manually or automatically adjusting the thresholds used in assigning positives and negatives for each detection channel.

- To reset automatic thresholds for positives and negatives, click Auto Analyze
- To manually assign thresholds:
  - Use the thresholding crosshair to assign classification regions for the whole plot (the clustering tool is disabled when viewing the plot in heatmap mode)
  - Use the ellipse, rectangle, or lasso threshold adjustment tool to classify a region of the plot. Click the tool, then click the region type in the working cluster selector. Use the tool to select the region within the plot

Viewing clustering plots. Threshold adjustment options available in the clustering plot are shown. Threshold values are indicated by the pink lines in the plot.



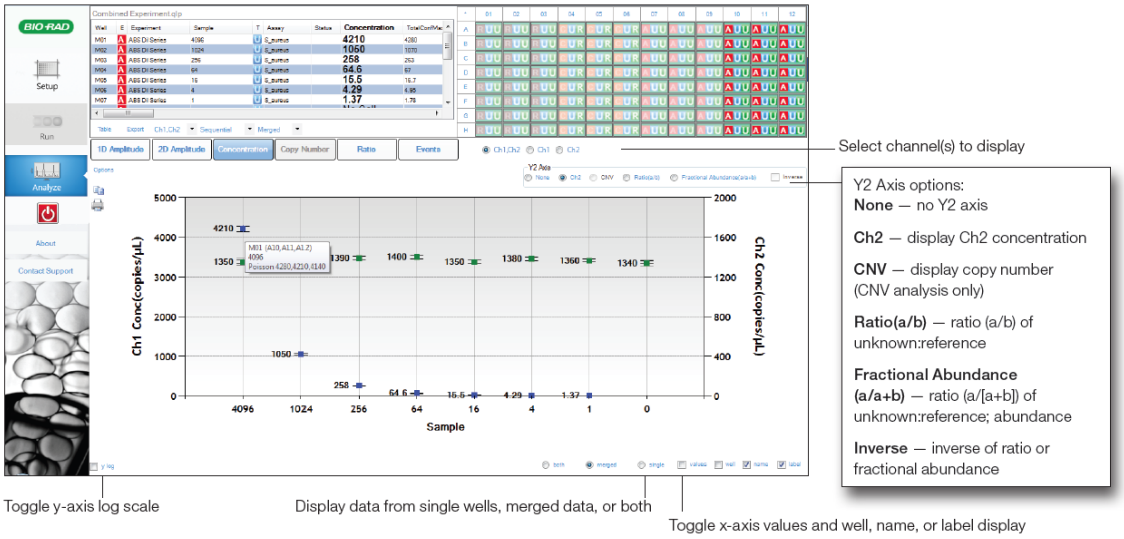
4.6.3 Viewing Concentration Data (Concentration)

Concentration data for each target appear in the wells in the plate map and are tabulated in the results table. Click Concentration to visualize data in concentration plots. Use the radio buttons to select the channels displayed. Error bars reflect total error or Poisson 95% confidence limits. These data can be exported for analysis in other spreadsheet applications (for example, Microsoft Excel).

The Copies/µL Well column displays the total amount of starting material in the ddPCR sample. The values shown reflect the product of the concentration (in copies per µl) multiplied by the 20 µl ddPCR reaction input used to make droplets. This value does not appear for merged well data.

This column is also exported in the *.csv file. The original concentration column and calculations remain unchanged.

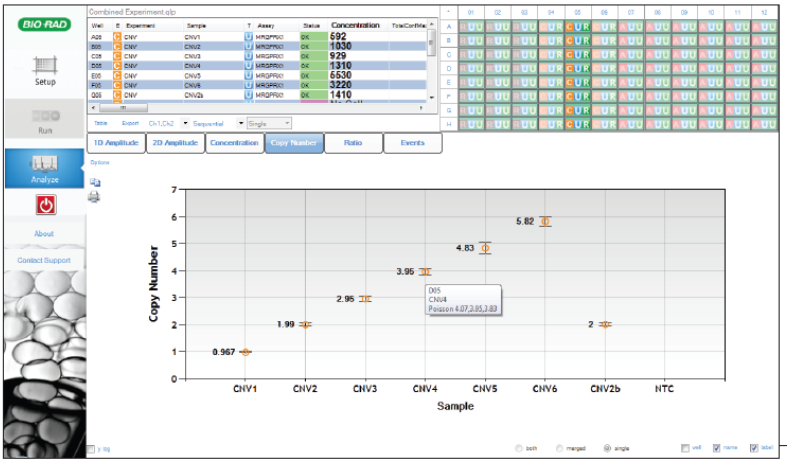
Viewing concentration data. Data from absolute quantification are shown. Hover over data points to reveal well identity, concentration, and Poisson confidence limits. Solid data points (shown) indicate merged data; open data points (not shown) indicate data from single wells.



4.6.4 Viewing Copy Number Data (Copy Number)

Click Copy Number to view copy number for selected wells/samples.

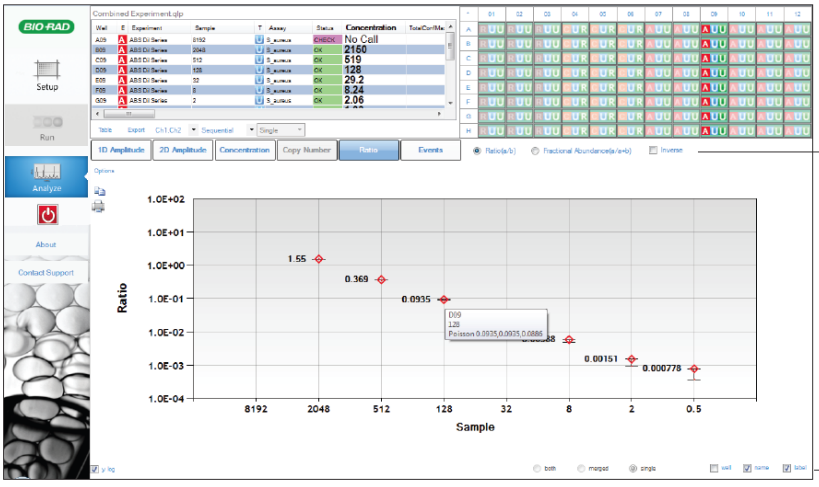
Viewing copy number data. Hover over data points to reveal well identity, concentration, and Poisson confidence limits. Solid data points (not shown) indicate merged data; open data points (shown) indicate data from single wells.



4.6.5 Viewing Ratio Data (Ratio)

Click Ratio to view ratio data for selected wells/samples. Use the radio buttons to select a plot of the Ratio (unkown:reference) or Fractional Abundance (% of sample); select Inverse to apply the inverse of either.

Viewing ratio data. Data from absolute quantification are shown. Hover over data points to reveal well identity, concentration, and Poisson confidence limits. Select y log to convert the y-axis to logarithmic scale (shown). Solid data points (not shown) indicate merged data; open data points (shown) indicate data from single wells.



4.6.6 Viewing Events

Click Events to view the number of droplet events counted for selected wells/samples. Use the radio buttons to select the channels displayed. View positive, negative, or total droplet counts, or any combination of these.

5 Maintenance

5.1 General Maintenance Procedures

Surfaces of the instrument may require general cleaning. Use deionized/distilled water for general wipe down with a slightly dampened cloth. For decontamination, 10% bleach followed by 70% ethanol and/or deionized/ distilled water may be used. Do not use acetone or tap water.

Inspect equipment regularly for damaged external components or wiring. Do not use if damaged.

Apply standard MSDS (Material Safety Data Sheet) and OSHA practices when handling and disposing of generated waste.

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. These nonflammable fluids are inert and have low environmental impact and low toxicity. Collect waste in a polyethylene container and discard within one month.

Droplets made with Bio-Rad master mix have antimicrobial properties, but microbial growth is possible. The waste profile should contain the following: fluorinated hydrocarbons, water, fluorescent dye (from probes), protein, and nucleic acids. Do not replace detachable power cord with an uncertified or an inadequately rated cord.

5.2. Replacing Droplet Reader Oil and Removing Waste

Replace the droplet reader oil and empty the waste receptacle as needed. Use the handle built into the side compartment to slide the carriage out:

- Use empty oil supply bottles as new waste bottles. Add 50 ml 10% bleach to the waste bottle to prevent microbial growth, and place a label on the waste bottle at this time
- Place the new bottle of oil in the oil position and screw the cap into place. In QuantaSoft™ software, under Instrument Routines in the Setup window, click Prime to fill the lines with oil before the system is run

- If the instrument has been unused for longer than a week, prime the system before running a plate

6 REFERENCES

Refer to the manufacturer's manual for additional information.

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