QuantaSoft™ Analysis Pro Software

Instruction Manual

Version 1.0

BIO-RAD

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1 QuantaSoft Analysis Pro Introduction

The Bio-Rad QuantaSoft™ Analysis Pro (QuantaSoft AP) Software is a complementary analysis tool that enables data analysis of multiplex experiments and advanced applications and assay strategies for Droplet Digital PCR (ddPCR). QuantaSoft AP is for use with data files generated on the QX100 or QX200 Droplet Reader using QuantaSoft Standard Edition Software versions 1.4 and later.

Benefits of using QuantaSoft AP include:

- Analyze multiple targets within a well using amplitude multiplexing and probe mixing strategies
- Detect genome edit and non-wild type events with a “drop-off assay” analysis option
- Visualize and export data with improved flexibility
- Customize an experiment using an advanced droplet classification method for cutting edge research with ddPCR technology

2 QuantaSoft Analysis Pro Installation

QuantaSoft™ Analysis Pro (AP) software is available to download and install from www.bio-rad.com. There is no installation disc provided for the software. QuantaSoft Standard Edition is still required to operate the QX100 or QX200 Droplet Reader and collect data.

Table 1. Minimum system requirements.

System	Minimum
CPU	17 Quad Core
Processor speed	2.40 GHz
Installed RAM	8 GB RAM
Hard drive	500 GB, 7,200 rpm
Video graphics	1,696 MB
Dedicated	64 MB
Operating system	Windows 7 or Windows 10, 64 bit

QuantaSoft Analysis Pro will only run at a resolution of 1,920 x 1,080 and 100% font.

Installation

1. Go to **http://www.bio-rad.com/en-us/product/qx200-droplet-digital-pcr-system** and click on the Download tab. Download QQuantasoftAP_1.0.596_Setup.exe (QuantaSoft Analysis Pro Installer).
2. From your download folder, double click the file to begin installation.
3. The QuantaSoft Analysis Pro InstallShield® Wizard will pop up and guide you through the installation process.
4. Follow the onscreen instructions and click Finish to complete installation.
5. To access the End-User License Agreement (EULA), open QuantaSoft AP, click the Help icon, and click the EULA link.

Note: Optional: If Microsoft Visual C++ 2015 Update 1 Redistribution Package (X64) has not been installed on your computer yet, you will be prompted to install it. Optional: If .NET Framework 4.5.2 has not been installed on your computer yet, you will be prompted to install it.

3 Using QuantaSoft Analysis Pro

QuantaSoft™ Analysis Pro (AP) software opens and analyzes files generated by QuantaSoft Software v1.4 and later. This software is for analysis only; it cannot be used to run the QX100 or QX200 Droplet Reader. Additionally, QuantaSoft AP cannot open .qlb, .qlt, or any files from QuantaSoft v1.7 Regulatory Edition.

QuantaSoft AP can open the following files:

- **Results (*.qlp)** — user-defined plate information and collected data from a run completed on the QX100 or QX200 Droplet Reader
- **Results (*.ddpcr)** — user files that were created by QuantaSoft AP
- **Plate Setup File (*.ddplt)** — plate setup file that is created in QuantaSoft AP and can be saved for expedited setup of additional plates

How to Open a File

Opening QuantaSoft AP and Files

1. QuantaSoft AP can be opened by double clicking the shortcut on the desktop or through the start menu.
2. QuantaSoft AP can open .qlp files generated in QuantaSoft v 1.4 or later, .ddpcr and .ddplt files.
   a. .ddPCR files can be opened by double clicking a file or by dragging and dropping it into QuantaSoft Analysis Pro window.
   b. .ddplt files can be opened by clicking on Plate Editor Tools in the menu bar and selecting Import Plate Setup.
   c. .qlp files can be opened by dragging and dropping into the analysis pro window or by clicking the **Browse** to find and open the file.

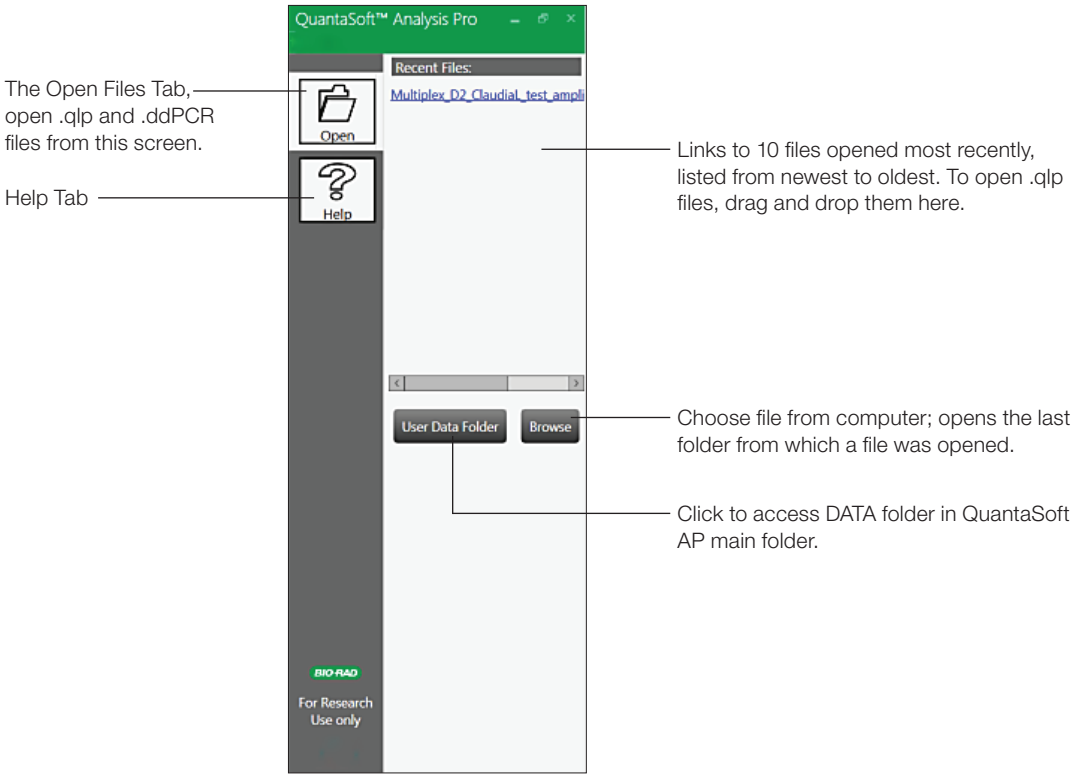


Fig. 1. Control panel of QuantaSoft Analysis Pro, Open Files Tab.

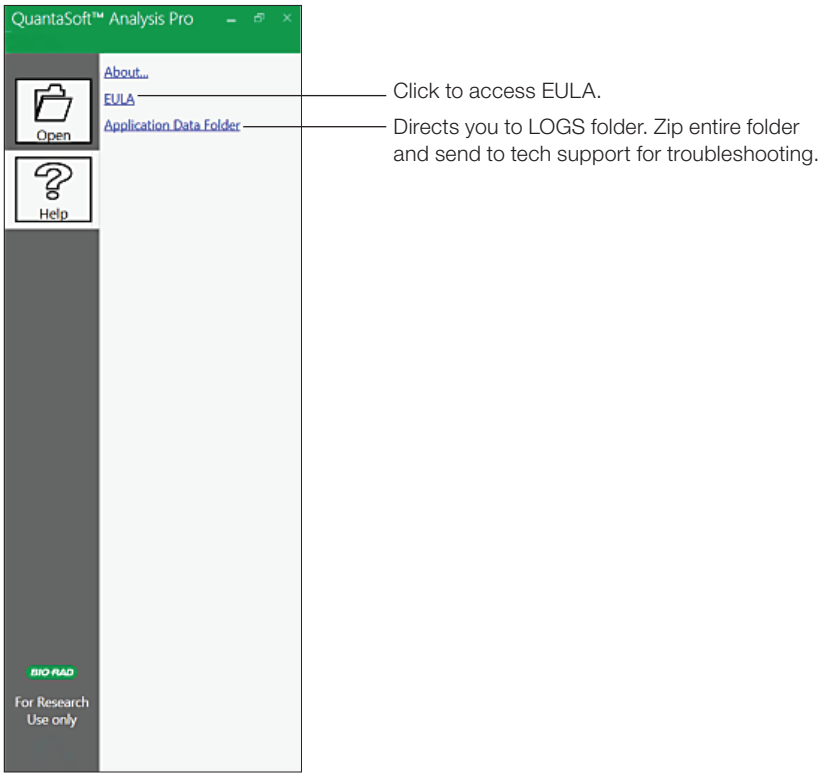


Fig. 2. Control panel of QuantaSoft Analysis Pro, Help Tab.

Overview of Screens and Buttons

Results files will open to the Dashboard tab. You can navigate to any of the software analysis tabs, which are discussed in detail in this section.

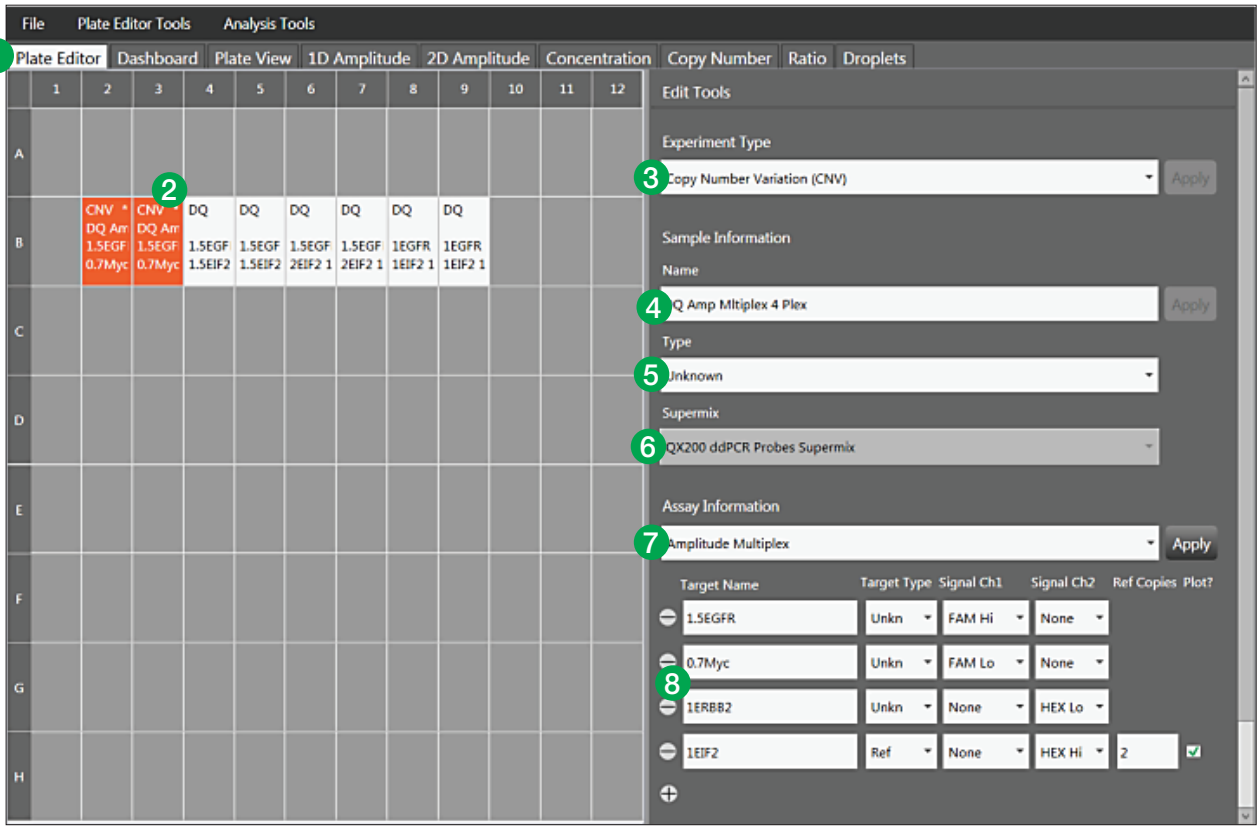


Fig. 3. Plate Editor Tab – In this screen you will configure information for each plate, selecting parameters that are required for all analysis modes. All information entered here is carried through each tab.

Legend Overview

1. Click different tabs to access plots, tables and analyses for the plate.
2. Select an individual or multiple wells to lay out experiment.
3. **Experiment Type** — Select from dropdown list to pick experiment type.
4. **Name** — Type a name for the sample selected.
5. **Type** — Select sample type: unknown, NTC, positive control, or negative control.
6. The Supermix is selected during plate setup prior to running the QX100 or QX200 in QuantaSoft Software and cannot be changed in QuantaSoft Analysis Pro.
7. **Assay Information** — Select the type of assay being run: Simplex/Duplex, Amplitude Multiplex, Probe Mix Triplex, or Advanced Classification Mode.
8. Name your targets, select either unknown or reference, and identify dye for each detection channel. If Copy Number Variation is selected under Experiment Type you will have the option to input the Copy Number for the reference target.

Note: Plate information saved in the .qlp file will be automatically imported into the relevant Edit Tools fields.

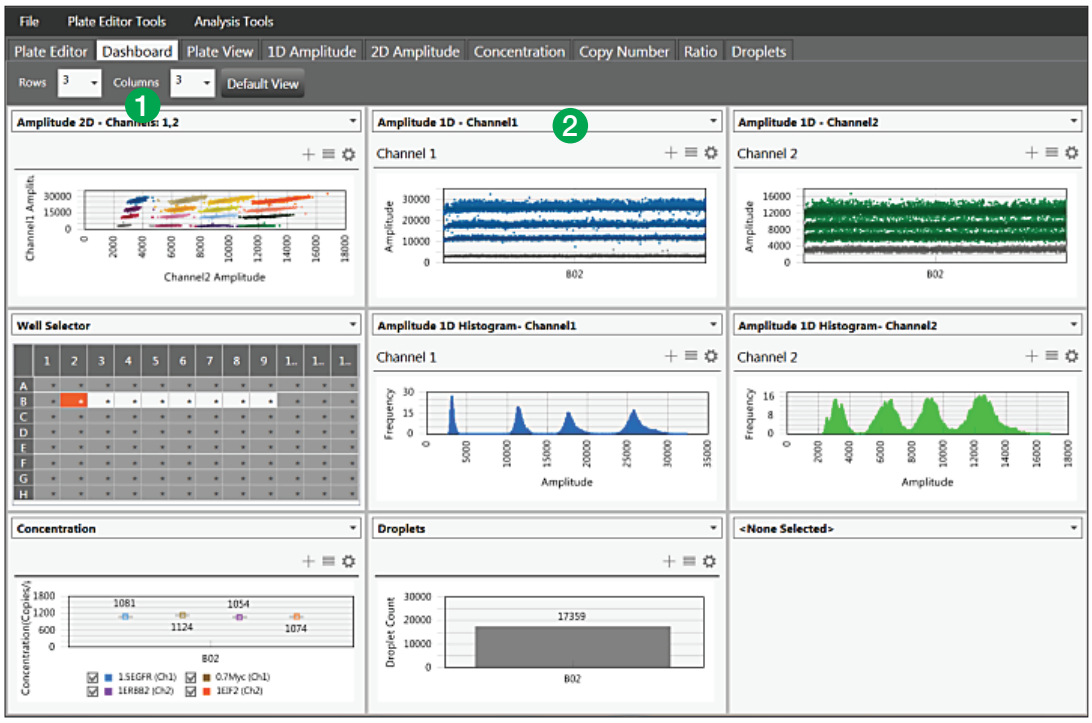


Fig. 4. Dashboard tab.

Legend Overview

A single screen view with a summary of all analyses. Within this tab you can select which experiment results should be summarized for the selected experiment type. Data on this screen will update with changes made in other analysis tabs.

1. Customize dashboard by determining how many windows you want displayed on the screen.
2. Use the drop down menu in each block to select which plots, tables, and options are displayed.

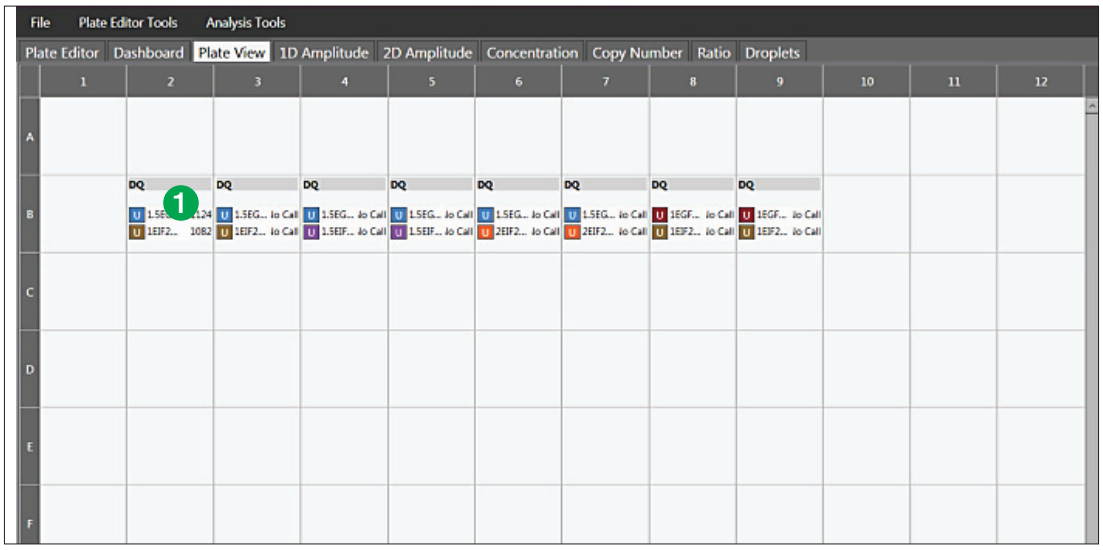


Fig. 5. Plate View tab.

Legend Overview

A single screen view that shows results in a plate layout.

1. Identifies the experiment type, sample name, target name, target type, and concentration of each target by well in a plate layout.

Fig. 6. 1D Amplitude tab.

Legend Overview

A single screen to view 1D fluorescence amplitude plots and data results for the plate.

1. Graph Tools
   - Threshold
     - View Mode — prevents changing of thresholds
     - Threshold Single Well — establish threshold for positive and negative droplets in either Channel 1 or Channel 2 by individual well
     - Threshold Multiple Wells — establish threshold for positive and negative droplets in either Channel 1 or Channel 2 by multiple wells
   - Graph Views — toggle checkboxes to display or hide histograms and heat maps
   - Show
     - Both — all selected wells will be plotted for Merged and Individual Wells
     - Merged Wells — merges data from all selected wells into a single data point
     - Individual Wells — plots each well as a separate data point in any data plot
   - Auto Analyze — sets automatic thresholds in the well or group of wells selected, overriding any previously set thresholds. Auto analysis is only available for simplex/duplex experiments; the button will not be active in other analysis modes.
2. 1-D Fluorescence Amplitude Plots — shows all accepted droplets for a selected well or wells on the plate in both Channel 1 (FAM/EvaGreen) and Channel 2 (HEX/VIC). Once thresholds are established, negative droplets are displayed in grey, while positive droplets in Channel 1 are blue and positive droplets in Channel 2 are green.
3. 1-D Fluorescence Amplitude Histograms — displays frequency of droplets at specific fluorescence amplitudes for each well or group of wells selected. The Channel 1 (FAM/EvaGreen) histogram is shown in blue and Channel 2 (HEX/VIC) histogram in green.
4. Well selector — click on one well or select multiple wells to analyze in this tab.
5. Data Table — results for a selected well or group of wells, displayed by well number and sortable by any column by clicking on its header. See Appendix for a complete list of data table column definitions.

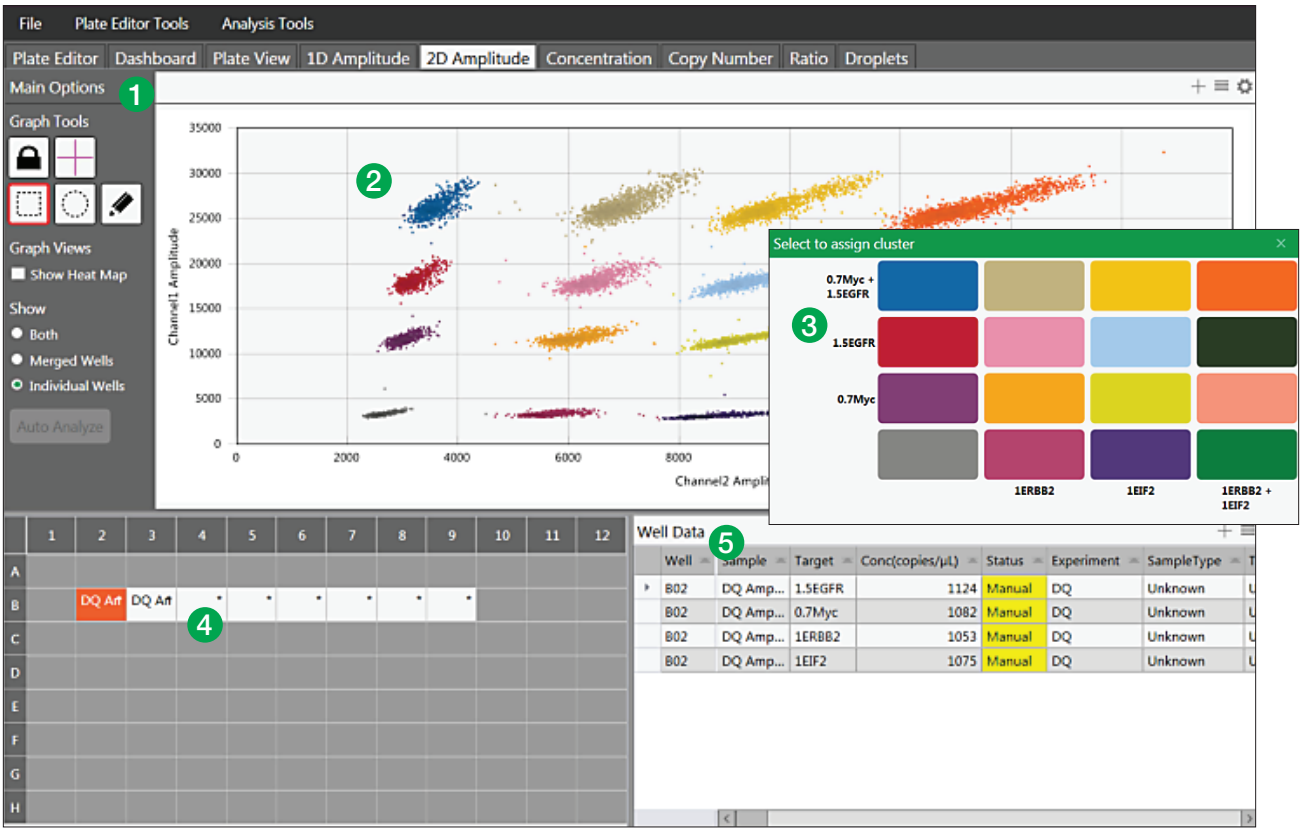


Fig. 7. 2D Amplitude tab.

Legend Overview

A single screen to view 2D fluorescence amplitude plots and data results for the plate.

1. Graph Tools
   - Threshold
     - View Mode — prevents changing of thresholds
     - Line mode — establishes thresholds across both Channel 1 and Channel 2, dividing droplets into distinct clusters separated by different colors. Click anywhere on the plot to apply "best fit" threshold lines to the data. Adjust the threshold values by dragging the lines horizontally or vertically in the plot area, by entering a new threshold value in the boxes at the end of each threshold line, or by clicking the up and down arrows to incrementally adjust the numbers.
     - Cluster mode — establishes thresholds across both Channel 1 and Channel 2, dividing droplets into distinct clusters separated by different colors. Click any of the three Threshold Cluster Mode buttons (square, circle or free form). Draw the shape chosen around the cluster of droplets to classify them.
   - Graph Views — toggle checkbox to display or hide heat map in amplitude events chart
   - Show
     - Both — all selected wells will be plotted for Merged and Individual Wells
     - Merged Wells — merges data from all selected wells into a single data point
     - Individual Wells — plots each well as a separate data point in any data plot
2. 2-D amplitude events chart — graphical depiction of the amplitude of all qualified droplets per well or group of wells in channel 1 or channel 2.
3. Cluster Threshold Mode Pop-up (inset) — when using the cluster mode tools to draw a shape around a cluster of droplets, the pop-up tool will appear to help you select the correct target combination when you release the mouse button. 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.
4. Well selector — select one or multiple wells to analyze in this tab.
5. Data Table — results for a selected well or group of wells, displayed by well number and sortable by any column by clicking on its header. See Appendix for a complete list of data table column definitions.

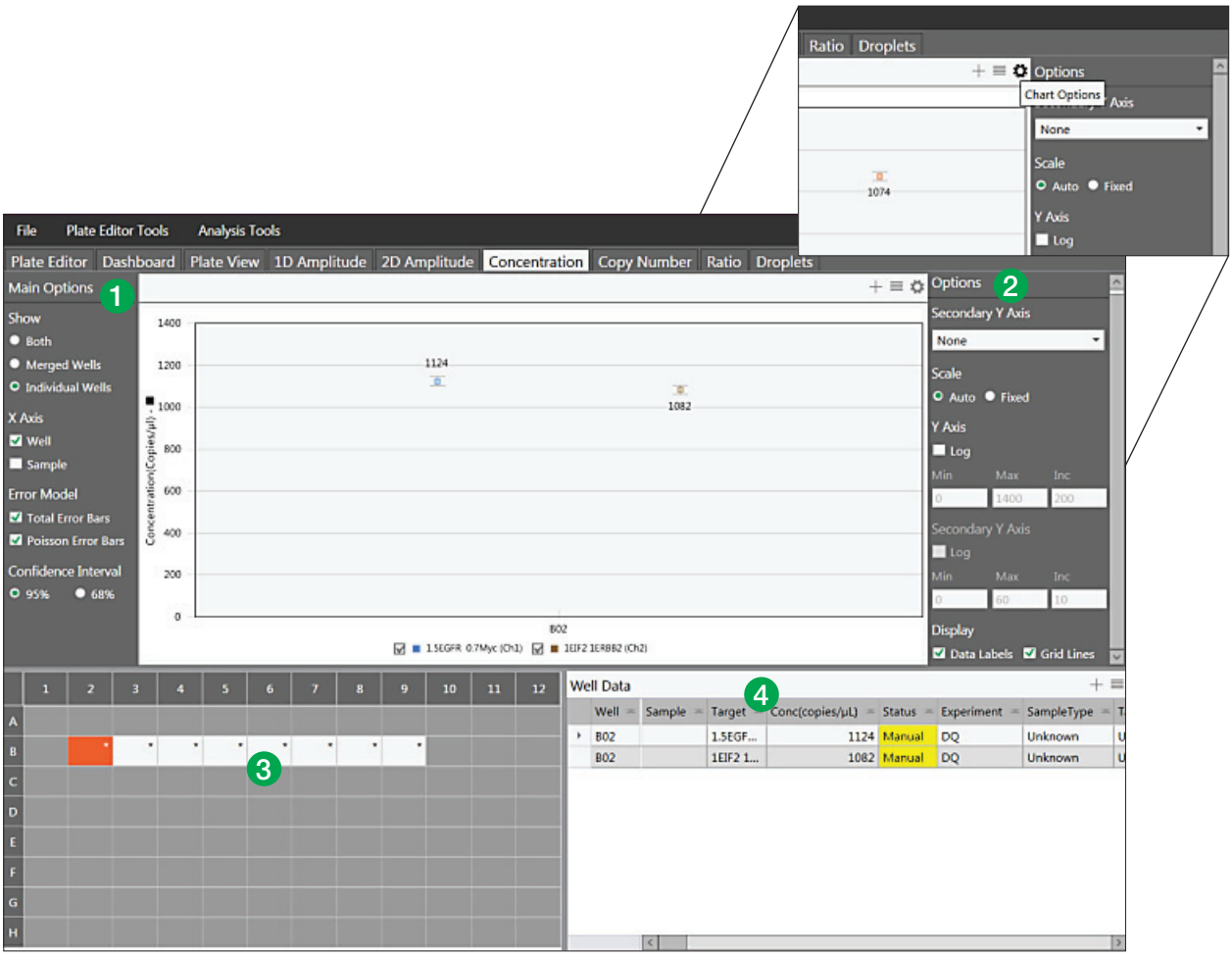


Fig. 8. Concentration tab. Inset: To expand the options menu of any plot or chart, click on the gear icon at the top right of the chart. Click on the icon with three horizontal lines to bring up the chart menu, which is also available by right clicking on the plot area. The plot image can be saved, exported or printed from this menu. Click on the + icon to expand any plot area to full screen.

Legend Overview

This tab contains the concentration plot for individual or multiple wells and allows you to adjust plot parameters.

1. Main Options
   - Show
     - Both — all selected wells, both merged and individual, will be plotted
     - Merged Wells — merges data from all selected wells into a single data point on the plot
     - Individual Wells — plots each well as a separate data point
   - X Axis — choose how the x axis labels are displayed and plotted, by sample name or by well number
   - Error Model — select Total Error Bars, Poisson Error Bars, or both
   - Confidence Interval — select either 95% or 68%; 95% is the default
2. Options – click the gear icon to bring up chart options menu, see inset above.
   - Secondary Y-Axis — enable an optional secondary axis to plot channel 2 concentration, ratio, copy number, or fractional abundance
   - Scale — choose auto or fixed
   - Log — change the axis to a logarithmic scale
   - Range — set minimum, maximum, and increment for y-axis and secondary y-axis (if chosen)
   - Display — toggle checkbox to choose whether to display Data Labels and Grid Lines
3. Well selector — select one or multiple wells to analyze in this tab
4. Data Table — results for a selected well or group of wells, displayed by well number and sortable by any column by clicking on its header. See Appendix for a complete list of data table column definitions.

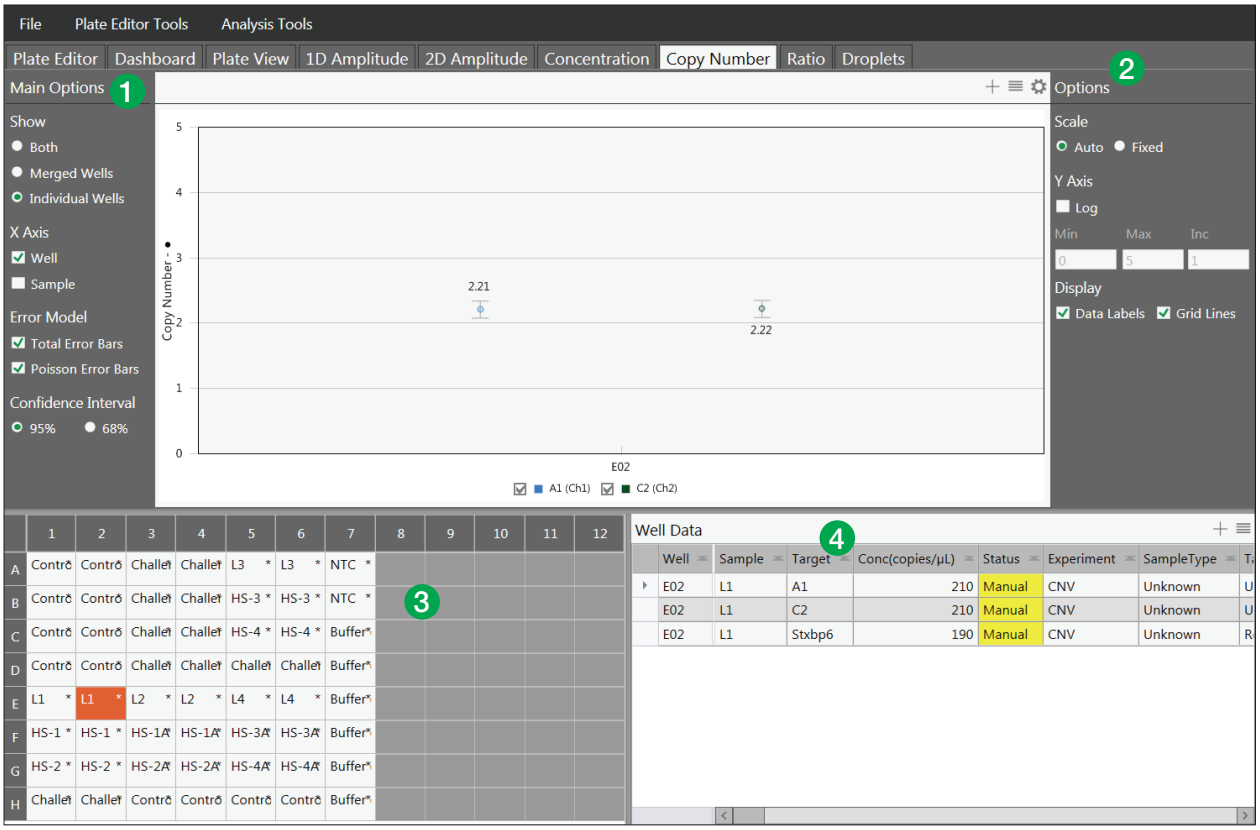


Fig. 9. Copy Number tab.

Legend Overview

This tab shows the copy number plot for individual or multiple wells and allows you to adjust plot parameters.

1. Main Options
   - Show
     - Both — all selected wells, both merged and individual, will be plotted
     - Merged Wells — merges data from all selected wells into a single data point on the plot
     - Individual Wells — plots each well as a separate data point
   - X Axis — choose how the x axis labels are displayed and plotted, by sample name or by well number
   - Error Model — select Total Error Bars, Poisson Error Bars, or both
   - Confidence Interval — select either 95% or 68%; 95% is the default
2. Options – click the gear icon to bring up chart options menu.
   - Scale — choose auto or fixed
   - Log — change the axis to a logarithmic scale
   - Range — set minimum, maximum, and increment for y-axis and secondary y-axis (if chosen)
   - Display — toggle checkbox to choose whether to display Data Labels and Grid Lines
3. Well selector — select one or multiple wells to analyze in this well
4. Data Table — results for a selected well or group of wells, displayed by well number and sortable by any column by clicking on its header. See Appendix for a complete list of data table column definitions.

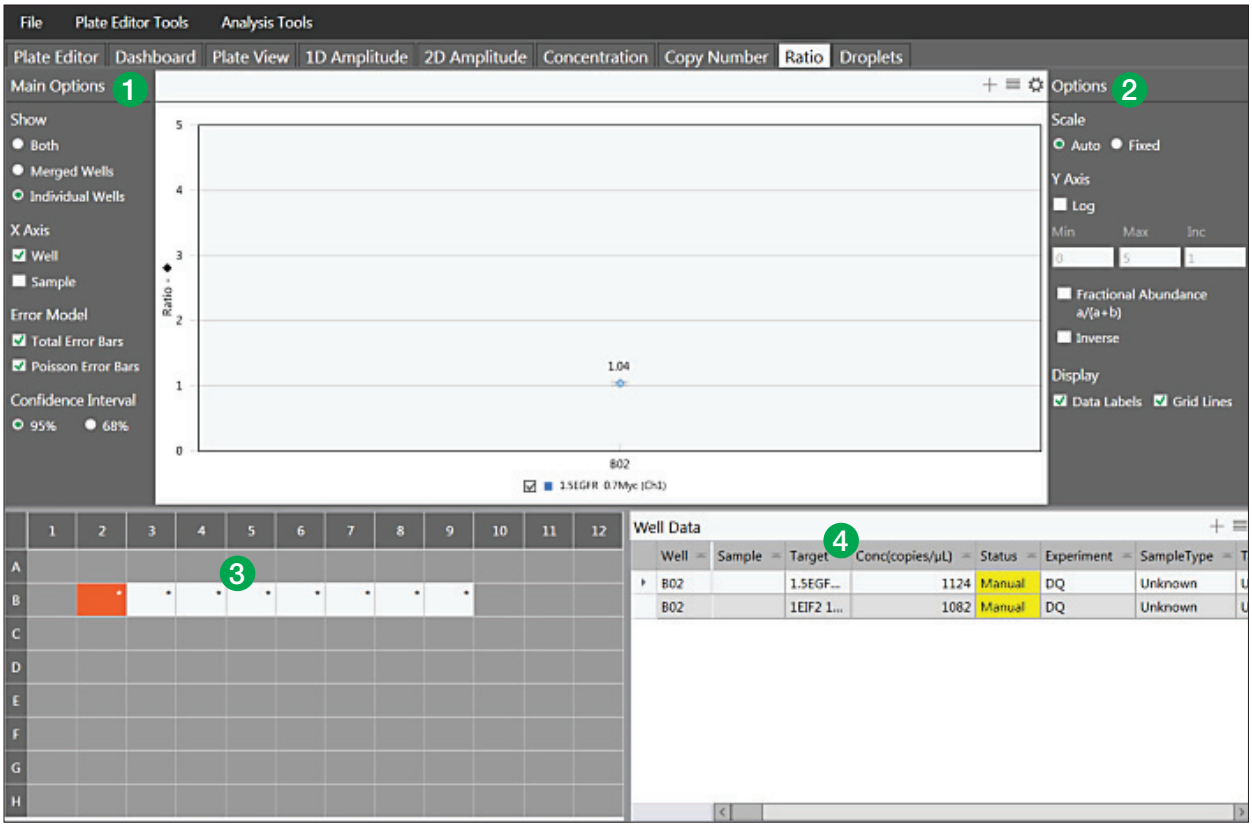


Fig. 10. Ratio tab.

Legend Overview

This tab shows the ratio plot for individual or multiple wells and allows you to adjust plot parameters.

1. Main Options
   - Show
     - Both — all selected wells, both merged and individual, will be plotted
     - Merged Wells — merges data from all selected wells into a single data point on the plot
     - Individual Wells — plots each well as a separate data point
   - X Axis — choose how the x axis labels are displayed and plotted, by sample name or by well number
   - Error Model — select Total Error Bars, Poisson Error Bars, or both
   - Confidence Interval — select either 95% or 68%; 95% is the default
2. Options – click the gear icon to bring up chart options menu
   - Scale — choose auto or fixed
   - Log — change the axis to a logarithmic scale
   - Range — set minimum, maximum, and increment for y-axis
   - Fractional Abundance a/(a + b) — toggle checkbox to plot each unknown as a percent of the reference
   - Inverse — toggle checkbox to plot the reference as a percent of each unknown (the inverse fractional abundance)
   - Display — toggle checkbox to choose whether to display Data Labels and Grid Lines
3. Well selector — select one or multiple wells to analyze in this tab
4. Data Table — results for a selected well or group of wells, displayed by well number and sortable by any column by clicking on its header. See Appendix for a complete list of data table column definitions

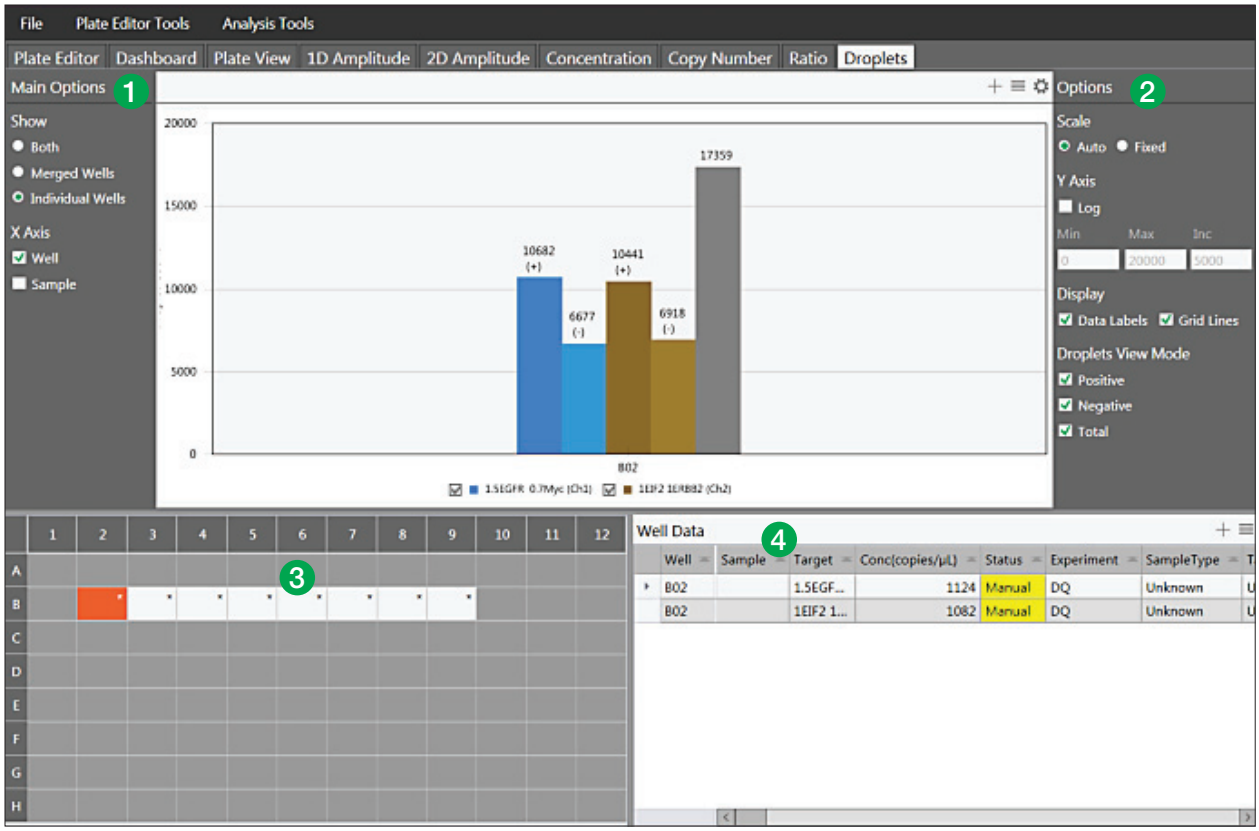


Fig. 11. Droplets tab.

Legend Overview

This tab shows the droplets plot for one or more wells selected. Total droplets are shown by default for each well.

1. Main Options
   - Show
     - Both — all selected wells, both merged and individual, will be plotted
     - Merged Wells — merges data from all selected wells into a single data point on the plot
     - Individual Wells — plots each well as a separate data point
   - X Axis — select well name, sample name, or both
2. Options – click the gear icon to bring up chart options menu
   - Scale — choose auto or fixed
   - Log — change the axis to a logarithmic scale
   - Range — set minimum, maximum, and increment for y-axis
   - Display — toggle checkboxes to display Data Labels and Grid Lines
   - Droplets View Mode — toggle to choose any or all: Positive, Negative, Total
3. Well selector — select one or multiple wells to analyze in this tab
4. Data Table — results for a selected well or group of wells, displayed by well number and sortable by any column by clicking on its header. See Appendix for a complete list of data table column definitions

General Overview

Keyboard Shortcuts

Keyboard shortcuts in QuantaSoft Analysis Pro are only usable in the Plate Editor Tab. After clicking on a tab: left and right arrows can be used to scroll through the tabs. In the Plate Editor Tab, after selecting a well: up, down, left, and right arrow keys can be used to scroll through the plate to select a well.

Table 2. Keyboard shortcuts.

Keys	Function
Control + C	Copy contents of a well
Control + V	Paste copied well information
Control + Z	Undo last action

Rounding in QuantaSoft Analysis Pro

QuantaSoft Analysis Pro introduces a different set of rounding rules from the standard QuantaSoft software. Additional significant figures may be displayed in results.

- When the concentration call is >1,000, QuantaSoft displays 3 significant figures, while QuantaSoft AP rounds to the whole integer
- When the concentration call is <1, QuantaSoft displays two figures to the right of the decimal, while QuantaSoft AP displays three figures to the right of the decimal.

Table 3. Examples of rounding in QuantaSoft as compared to QuantaSoft AP.

QuantaSoft	QuantaSoft AP
3720.0	3723.0
4220.0	4218.0
0.67	0.675
1.12	1.12

Experiment Types

QuantaSoft Analysis Pro has five different experiment analysis types, Direct Quantification, Copy Number Variation, Mutation Detection, Drop Off, and Gene Expression. Selecting an experiment type enables different assay options and analysis tools.

Table 4. Experiment types.

Direct Quantification (DQ)	Uses absolute quantification to determine the concentration (copies/µl) of target DNA copies in a sample and is the default experiment type.*
Copy Number Variation (CNV)	Determines concentration as with DQ and calculates the copy number of an unknown target relative to a known reference or references within the same well
Mutation Detection (MUT)	Determines concentration as with DQ and calculates the fractional abundance of an unknown mutant present at a low frequency in a wild-type background
Drop Off (DOF)	Determines absolute quantification of targets for assays designed to detect non-wild type sequences, such as indels and genome edits. The experiment type 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.
Gene Expression (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.

* QuantaSoft AP Software measures the number of positive and negative droplets for each target in a well. Droplets are assigned as positive or negative by thresholding based on their fluorescence amplitude. The software calculates the starting concentration of each target DNA molecule by modeling as a Poisson distribution; the formula used for Poisson modeling is: Copies per droplet = -ln(1-p) where p= fraction of positive droplets. Concentrations are provided in units of copies per microliter of input sample with 95% confidence intervals. For additional information, please see the ddPCR Applications Guide (bulletin #6407).

The experiment types created by QuantaSoft Standard Edition Software versions 1.4 or later will automatically be mapped (but can be changed) into QuantaSoft Analysis Pro as follows:

ABS **–>** DQ

RED **–>** DQ

CNV **–>** CNV

GEX **–>** GEX

Assay Options

QuantaSoft Analysis Pro offers four assay configurations for varying levels and strategies of multiplexing targets within a well.

Table 5. Assay information.

Simplex/duplex	Experiment 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 and is available for DQ, CNV, MUT and GEX experiment types.
Amplitude multiplex	Method to increase multiplexing up to four targets per well, with one or two targets detected per channel. Available for DQ, CNV and GEX experiment types.
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. Available for DQ, CNV and GEX experiment types.
Advanced classification method	Method to increase multiplexing rate up to 20 targets per well in any combination of targets detected per channel and requires manual classification of droplets. Available for DQ experiment type only
Basic Drop-Off	Exactly two targets per well, with the reference detected in both channels and the unknown detected in either one of the channels. Click on the Learn More link under Assay Details for setup help. Available for DOF experiment type only.

Experiment Examples

Direct Quantification of Four (4) Targets in a Single Well by Amplitude Multiplexing

1. Open a file and go to the Plate Editor tab.
2. Select well(s) and set Experiment Type to Direct Quantification (DQ) at the top of the Edit Tools window to the right.

   Note: the default experiment type is DQ for .qlp files with ABS as the experiment configured in QuantaSoft 1.4 or later.
3. Click Apply to the right of the Experiment Type box in order to activate the experiment options in sections below.
4. Enter or modify sample name and type; the default sample type is Unknown. The supermix was assigned at the time of data collection and is not editable during data analysis. Click Apply to the right of the Sample Name.
5. Under Assay Information, select Amplitude Multiplex from the drop down menu and click Apply to the right.

   A maximum of 4 targets can be added by using the + icon at the bottom of the target list. Targets can be removed from the well by clicking the icons to the left of each target.

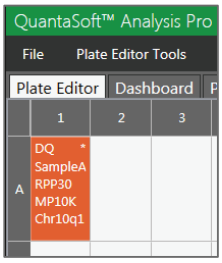


Fig. 12. Experiment type, sample name, and each target name are shown in the well once applied. The * in the top right corner of the well indicates additional targets have been assigned to the well than what is visible on the screen. View all target information in the Edit Tools side panel for a selected well or wells.

6. Enter target names and select the target type (unknown or reference) for each target in the well. Each target selected as a reference will enable ratio calculations of results. If no reference is selected, the target labeled with HEX/VIC channel 2 signal will be used as the default reference in calculating ratio values. If more than one reference is selected in the well, checkboxes appear to the right for using the reference in a geomean or single ratio calculation (Refer to second experiment example for using multiple references within a well).

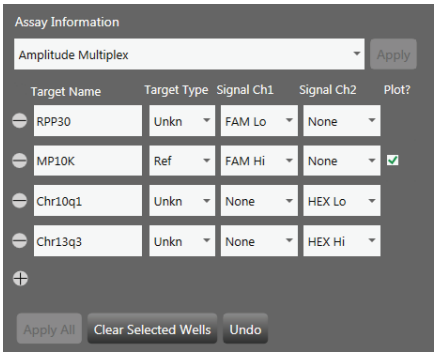


Fig. 13. Set Experiment Type to Direct Quantification (DQ) and enter target information.

7. Assign each target an expected signal in either channel 1 (FAM/EvaGreen) or channel 2 (HEX/VIC). 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 (for more on probe mixing, see Experiment Example number two).
8. Click Apply All at the bottom to confirm all well settings.

   Note: you can undo any well value that is applied by clicking the Undo button at the bottom, or by using Ctrl+Z on your keyboard (see page 15, on keyboard shortcuts). Wells can be copied and pasted onto other wells to expedite plate setup.
9. Once all wells are labeled, go to the 2D Amplitude tab to evaluate data and manually set thresholds. Automatic thresholding is not available for Amplitude Multiplexing experiments.

10. There are two options for setting the thresholds in the 2D plot. To use threshold lines, click the Threshold Line Mode button (pink crosshair) under Graph Tools to the left of the plot. Click anywhere on the plot to apply “best fit” threshold lines to the data. Adjust the threshold values by dragging the lines horizontally or vertically in the plot area, by entering a new threshold value in the boxes at the end of each threshold line, or by clicking the up and down arrows to incrementally adjust the numbers.

    If the data is not orthogonal and the threshold lines do not adequately divide the populations of droplets, use threshold clusters. Click any of the three Threshold Cluster Mode buttons (square, circle, or freehand). Draw the shape chosen around the cluster of droplets to be classified first. When you release the mouse button, a pop-up tool will appear 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.

11. The concentration results can be viewed in the data table below the plot area and in a concentration plot of the Concentration tab.

12. If one of your samples was identified as a Reference under Assay Information, the Ratio tab will provide a ratio of concentration for unknown versus the reference. For multiple references in a single well, see Experiment Example 2.

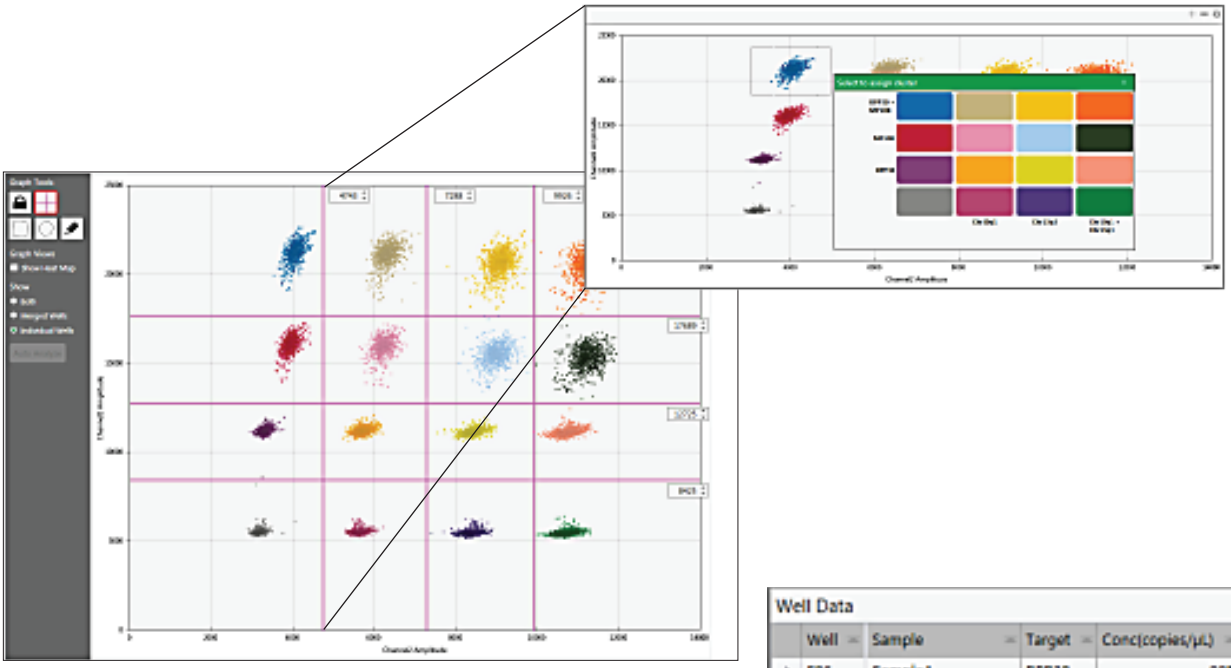


Fig. 14. Threshold with lines. Inset: Cluster with an automated pop-up tool to help you select the right target combination.

Well Data

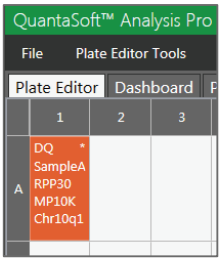
	Well	Sample	Target	Conc(copies/µL)
▸	F06	SampleA	RPP30	905
	F06	SampleA	MP10K	903
	F06	SampleA	Chr10q1	916
	F06	SampleA	Chr13q3	930

Fig. 15. Concentrations displayed in data table.

Copy Number Variation with Probe Mix Triplex (Two Targets and a Reference)

1. Open a file and go to the Plate Editor tab.
2. Select well(s) and set Experiment Type to Copy Number Variation (CNV) at the top of the Edit Tools window to the right.

   Note: the default experiment type is CNV for .qlp files with CNV as the experiment configured in QuantaSoft 1.4 or later.



3. Click Apply to the right of the Experiment Type box in order to activate the experiment options in sections below.
4. Enter or modify sample name and type; the default sample type is Unknown. The supermix was assigned at the time of data collection and is not editable during data analysis. Click Apply to the right of the Sample Name.
5. Under Assay Information, select Probe Mix Triplex from the drop down menu and click Apply to the right. Only 3 targets will display and no additional targets can be added.

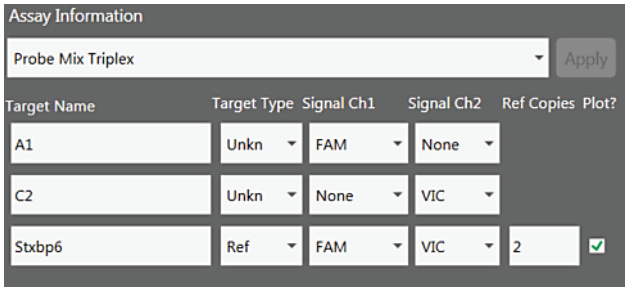


Fig. 16. Enter target information.

6. Enter target names and select the target type (unknown or reference) for each target in the well. Each target selected as a reference will enable ratio calculations of results. If no reference is selected, the target labeled with HEX/VIC channel 2 signal will be used as the default reference in calculating ratio values. If more than one reference is selected in the well, checkboxes appear to the right for using the reference in a geomean or single ratio calculation.
7. Assign the first and second target an expected signal in either channel 1 (FAM/EvaGreen) or channel 2 (HEX/VIC), the third target should be assigned the signals from both channel 1 and channel 2. In this example the third target (channel 1 and channel 2 combined) is the reference. Assign the reference its correct Copy Number.
8. Click Apply All at the bottom to confirm all well settings.

   Note: you can undo any well value that is applied by clicking the Undo button at the bottom, or by using Ctrl+Z on your keyboard (see Chapter 3 on keyboard shortcuts). Wells can be copied and pasted onto other wells to expedite plate setup.
9. Once all wells are labeled, go to the 2D Amplitude tab to evaluate data and manually set thresholds. Automatic thresholding is not available for Probe Mix Triplex experiments.
10. There is only one option for setting the thresholds for CNV experiments in the 2D plot. Click any of the three Threshold Cluster Mode buttons (square, circle, or freehand). Draw the shape chosen around the cluster of droplets to be classified first. When you release the mouse button, a pop-up tool will appear 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.

11. The concentration results can be viewed in the data table below the plot area and in a concentration plot of the Concentration tab.
12. The copy number results can be viewed in the data table below the plot area and in a copy number plot of the Copy Number Tab.

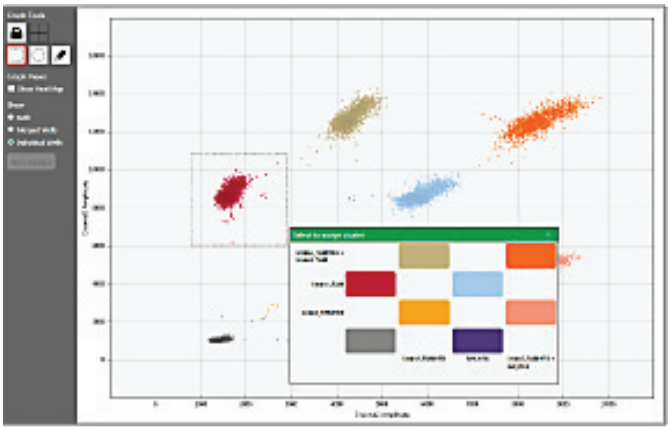


Fig. 17. Cluster with an automated pop-up tool to help you select the right target combination.

Well Data

Well	Sample	Target	Conc(copies/µL)
E05	Raji	Unkn1_FAM	730
E05	Raji	Ref_HEX	707
E05	Raji	Unkn2_FAMHEX	685

Fig. 18. Concentrations displayed in data table.

Genome Edit Detection using Drop-Off Assays

1. Open a file and go to the Plate Editor tab.
2. Select well(s) and set Experiment Type to Drop Off (DOF) at the top of the Edit Tools window to the right.

   Note: the default experiment type is DQ for .qlp files with ABS as the experiment configured in QuantaSoft 1.4 or later.
3. Click Apply to the right of the Experiment Type box in order to activate the experiment options in sections below.
4. Enter or modify sample name and type; the default sample type is Unknown. The supermix was assigned at the time of data collection and is not editable during data analysis. Click Apply to the right of the Sample Name.
5. Assay Information will automatically assign Basic Drop-Off. In this mode only two targets are available.

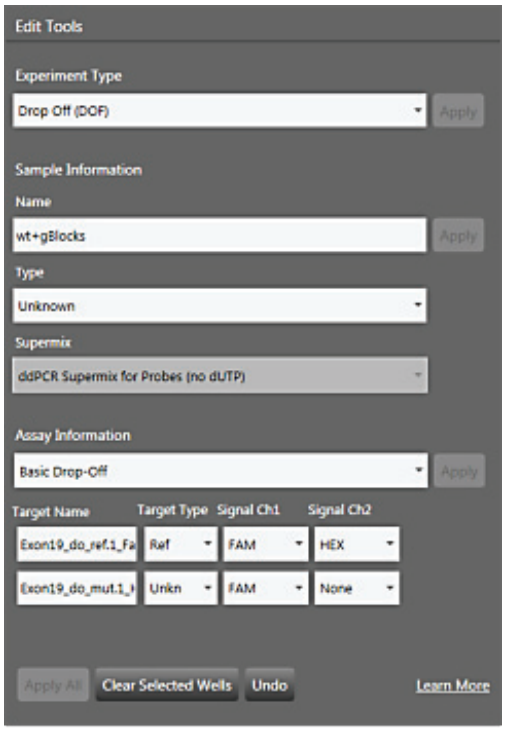


Fig. 19. Set Experiment Type to Drop Off (DOF) and enter target information. New users can click Learn More in the lower right-hand corner for contextual help.

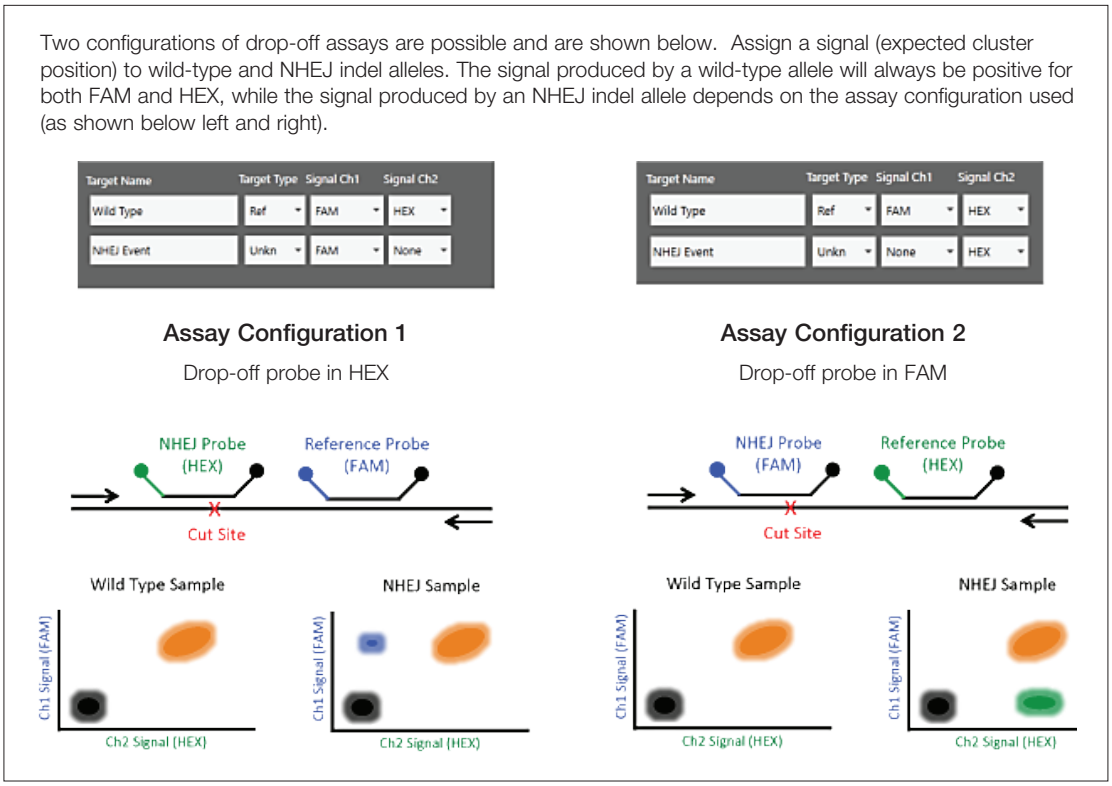


Fig. 20. Drop-Off Experiment setup for NHEJ quantification. This information is available by clicking on the "learn more" link at the bottom right in the Edit Tools window.

6. Enter target names and select the target type (unknown or reference) for each target in the well. For this mode you should have one reference and one unknown. Each target selected as a reference will enable ratio calculations of results.
7. Assign the reference signals in both channel 1 (FAM/EvaGreen) and channel 2 (HEX/VIC) and assign the unknown a signal in either channel 1 or channel 2, depending on the signal of the NHEJ probe.
8. Click Apply All at the bottom to confirm all well settings.

   Note: you can undo any well value that is applied by clicking the Undo button at the bottom, or by using Ctrl+Z on your keyboard (see Chapter 3 on keyboard shortcuts). Wells can be copied and pasted onto other wells to expedite plate setup.
9. Once all wells are labeled, go to the 2D Amplitude tab to evaluate data and manually set thresholds. Automatic thresholding is not available for Drop Off experiments.
10. There is only one option for setting the thresholds for Drop Off experiments in the 2D plot. Click any of the three Threshold Cluster Mode buttons (square, circle, or freehand). Draw the shape chosen around the cluster of droplets to be classified first. When you release the mouse button, a pop-up tool will appear 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.
11. The concentration results can be viewed in the data table below the plot area and in a concentration plot of the Concentration tab.

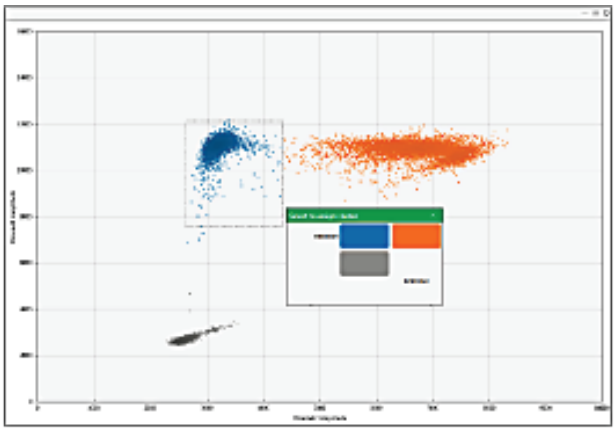


Fig. 21. Cluster with an automated pop up tool to help you select the right target combination.

Well Data

	Well	Sample	Target	Conc(copies/µL)	Experiment
▸	F04	wt only	Exon19_do_ref.1_Fam	876	DOF
	F04	wt only	Exon19_do_mut.1_Hex	0	DOF
	G04	wt+gBlocks	Exon19_do_ref.1_Fam	887	DOF
	G04	wt+gBlocks	Exon19_do_mut.1_Hex	1554	DOF

Fig. 22. Concentrations displayed in data table.

Advanced Classification Method

The Advanced Classification Method is intended for experienced users of Droplet Digital PCR. This analysis mode allows complete control of droplet identification and does not force or preassign any clusters. With that capability higher degrees of multiplexing can be accomplished through radial dye mixing. This capability comes with a draw back, multiple occupancy droplets become more difficult to identify so achieving a complete count of all targets might not be possible. The use of this mode for novice users is discouraged. This section will detail an example of utilizing this mode to accomplish multiplexing beyond what is offered through other assay modes.

1. Open a file and go to the Plate Editor tab.
2. Select well(s) and set Experiment Type to Direct Quantification (DQ) at the top of the Edit Tools window to the right.

   Note: the default experiment type is DQ for .qlp files with ABS as the experiment configured in QuantaSoft 1.4 or later.

3. Click Apply to the right of the Experiment Type box in order to activate the experiment options in sections below.
4. Enter or modify sample name and type; the default sample type is Unknown. The supermix was assigned at the time of data collection and is not editable during data analysis. Click Apply to the right of the Sample Name.
5. Under Assay Information, select Advanced Classification Method from the drop down menu and click Apply to the right. A maximum of 10 targets can be added by using the + icon at the bottom of the target list. Targets can be removed from the well by clicking the icons to the left of each target.
6. Enter target names and select the target type (unknown or reference) for each target in the well. Each target selected as a reference will enable ratio calculations of results. If no reference is selected, the target labeled with HEX/VIC channel 2 signal will be used as the default reference in calculating ratio values. If more than one reference is selected in the well, checkboxes appear to the right for using the reference in a geomean or single ratio calculation

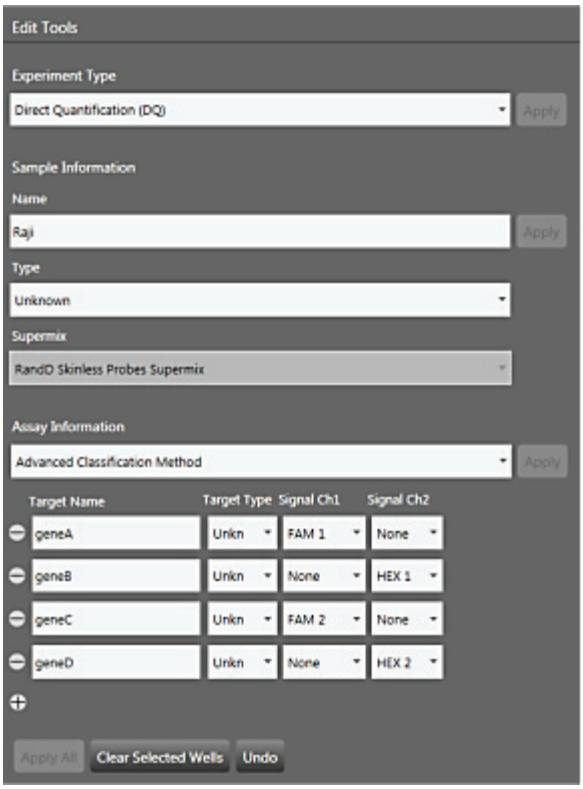


Fig. 23. Set Experiment Type to Direct Quantification (DQ). Select Advanced Classification Method under Assay Information.

7. Assign the first target an expected signal in either channel 1 (FAM/EvaGreen) or channel 2 (HEX/VIC). Assign your last target a signal in either channel 1 or channel 2, which was not selected for your first target. Each target in between will be assigned a signal in either channel 1 or channel 2 depending on mixture ratio of the target. Fig. 23 shows examples of potential signal values
8. Click Apply All at the bottom to confirm all well settings.

   Note: you can undo any well value that is applied by clicking the Undo button at the bottom, or by using Ctrl+Z on your keyboard (see Chapter 3 on keyboard shortcuts). Wells can be copied and pasted onto other wells to expedite plate setup.

9. Click the 2D Amplitude tab. All droplets are unclassified by default and will appear red. There is only one option for setting the thresholds for Advanced Classification experiments in the 2D plot. Use one of the Threshold Cluster Mode Tools (box, circle or freehand) to assign droplet clusters to Targets.

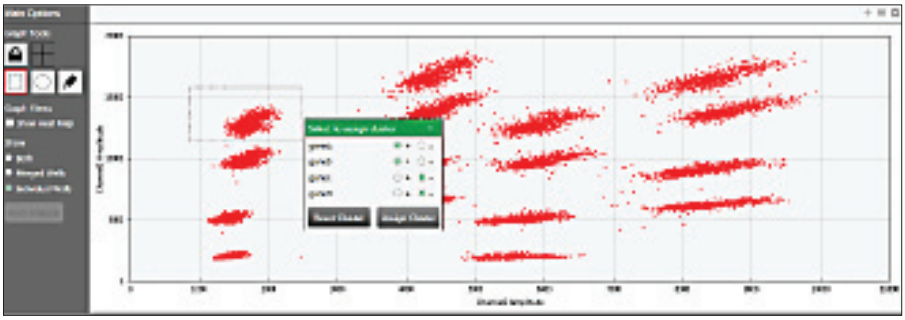
   Upon selection of droplet groups, a cluster assignment box will appear. Identify the composition of the cluster i.e. if the cluster is composed of positive droplets for Target-2, select the positive radial button for Target-2 and the negative radial buttons for the rest.

   The negative cluster should be described as negative for all the target genes and must be assigned to calculate concentrations.

   There must be at least one positive cluster for each target as well as the negative cluster assigned for calculations to take place. Unassigned droplets will be set aside for calculation purposes. If unsure of droplet/cluster composition, leave it unassigned (red).

10. The concentration results can be viewed in the data table below the plot area and in a concentration plot of the Concentration tab.

11. If one of your samples was identified as a Reference under Assay Information, the Ratio tab will provide a ratio of concentration for unknown versus the reference.



Well Data

Well	Sample	Target	Conc(copies/µL)	Status
C05	Raji	geneA	798	Manual
C05	Raji	geneB	733	Manual
C05	Raji	geneC	674	Manual
C05	Raji	geneD	699	Manual

Fig. 24. Concentrations displayed in data table.

Appendix

Data Table Column Definitions

The Data Table appears in the lower right hand corner of every tab except for the Plate Editor, Dashboard, and Plate View tabs. This table displays 65 fields that track all the data generated for each target in a well as clusters are identified through thresholding or manual clustering tools. Each of these fields is defined here.

Table 6. Data table column definitions.

Column Name	Description
Well	The Well Location in the Plate the sample is from
Sample	The sample name used to identify the well in the Plate Editor tab
Target	Target Name from Plate Editor Tab
Conc(copies/µl)	Concentration of the target recorded as copies/µl
Status	Notifies if clusters were identified or not and if they are the method employed (manual or auto analyze)
Experiment	Experiment Type from Plate Editor tab
SampleType	Type from Plate Editor Type
TargetType	Target Type assigned to target in the Plate Editor tab
Supermix	Supermix from Plate Editor tab
DyeName(s)	Dye assigned to Ch1 and Ch2 to a target in the Plate Editor tab
Copies/20µlWell	Concentration of the target normalized to a volume of 20 µl
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 created
Positives	Number of droplets that contain the target
Negatives	Number of droplets that do not contain the target
Ch1+Ch2+	Number of droplets that contain both Ch1 and Ch2 targets
Ch1+Ch2-	Number of droplets that contain just the Ch1 target
Ch1-Ch2+	Number of droplets that contain just the Ch2 target
Ch1-Ch2-	Number of droplets that contain neither Ch1 or Ch2 targets
Linkage	Shows number of copies of a target that are present in overabundance vs. the expected value in copies per µl
CNV	Copy number calculated for the target
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

continues

Column Name	Description
PoissonCNVMin	Minimum copy number normalized for the low error bar of the droplet Poisson distribution for the 95% Confidence Interval
ReferenceCopies	Copy number identified for the reference target in the Plate Editor tab
UnknownCopies	This field is currently unused in QuantaSoft Analysis Pro
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
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
Fractional Abundance	Calculation of fractional abundance of this unknown target vs. the reference target
TotalFractional AbundanceMax	For merged wells the high error bar for the fractional abundance of the combined wells at a 95% Confidence Interval
TotalFractional AbundanceMin	For merged wells the low error bar for the fractional abundance of the combined wells at a 95% Confidence Interval
PoissonFractional AbundanceMax	Maximum fractional abundance normalized for the high error bar of the droplet Poisson distribution for the 95% Confidence Interval
PoissonFractional AbundanceMin	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	Comments cannot be added in QuantSoft Analysis Pro but will carry over comments from .qlp files
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
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
TotalFractional AbundanceMax68	For merged wells the high error bar for the fractional abundance of the combined wells at a 68% Confidence Interval
TotalFractional AbundanceMin68	For merged wells the low error bar for the fractional abundance of the combined wells at a 68% Confidence Interval
PoissonFractional AbundanceMax68	Maximum fractional abundance normalized for the high error bar of the droplet Poisson distribution for the 68% Confidence Interval
PoissonFractionald AbundanceMin68	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

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5. Audit Rights. Bio-Rad or a Bio-Rad agent may examine End User's System, desktop computers, laptops, servers, books and records for the purposes of verifying End User's compliance with the terms of this Agreement, upon reasonable notice and during normal business hours at End User's place of business. End User shall have the right to request that the agent acting on behalf of Bio-Rad sign an appropriate non-disclosure agreement with respect to any End User confidential information that may be viewed during an audit.
6. Relationship of the Parties. Nothing contained in this Agreement creates a joint venture, partnership, agency or similar endeavor between the parties. Each party is acting solely as an independent contractor and neither party has any power or authority to direct or indirectly bind or act on behalf of the other.
7. Agreement Construction. This Agreement (including accompanying purchase orders, if any) sets forth the entire agreement between the parties and supersedes all prior agreements, understandings and discussions regarding the subject matter hereof. No purported amendment or modification of any provision of this Agreement will be binding unless set forth in a written document agreed upon by the parties. No waiver by either party of any default of the other party will be held to be a waiver of any other or subsequent default. No waiver shall be effective unless it is in writing and is signed by the party against which it is asserted. If a provision contained or referred to in this Agreement is determined to be legally invalid or unenforceable, that provision will be ineffective to the extent of the invalidity or unenforceability without affecting the remaining provisions of the Agreement, which will continue to be valid and enforceable to the fullest extent permitted by law.

Bio-Rad
Laboratories, Inc.

Life Science
Group

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