

USER GUIDE

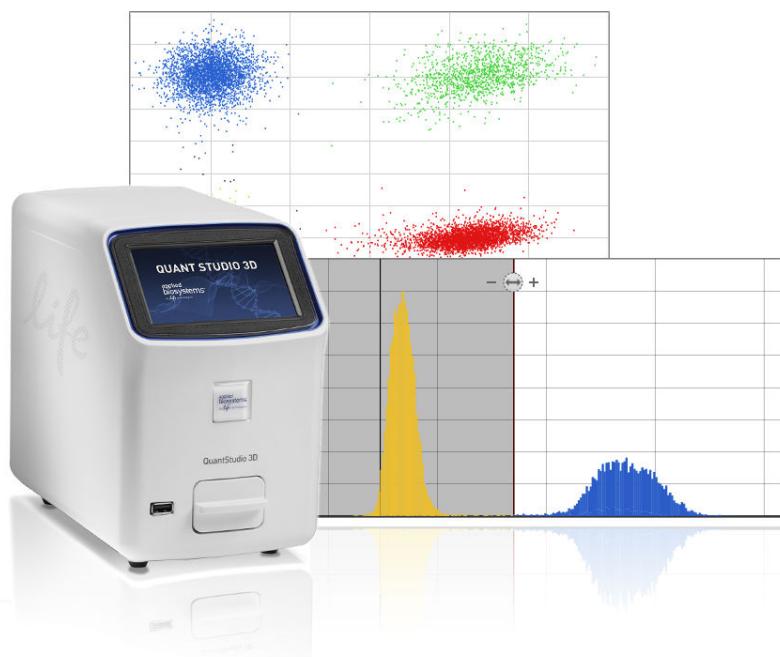
applied
biosystems®
by *life* technologies™

QuantStudio™ 3D AnalysisSuite™ Software

for use with QuantStudio™ 3D Digital PCR System

Publication Number MAN0008161

Revision 1.0



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About This Guide

Revision history

Revision	Date	Description
1.0	July 2013	Initial version.

Purpose

This guide provides step-by-step instructions for using the QuantStudio™ 3D AnalysisSuite™ Software to analyze digital PCR experiment data generated by the QuantStudio™ 3D Digital PCR System and describes how to troubleshoot the data.

Prerequisites

This guide assumes that you have access to and are familiar with the procedures provided in the *QuantStudio™ 3D Digital PCR System User Guide* (see “Related documentation” on page 60).

This guide also assumes that you have a general understanding of:

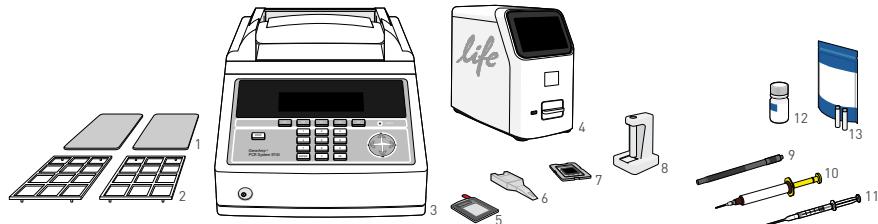
- Data storage, file transfers, and copying and pasting.
- The Microsoft® Windows® operating system, the Internet, and Internet-based browsers.

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About the QuantStudio™ 3D Digital PCR System

The QuantStudio™ 3D Digital PCR System uses fluorescent-labeled, probe-based quantitative polymerase chain reaction (PCR) reagents to provide quantitative and qualitative research detection of target nucleic acid sequences (targets) using post-PCR (endpoint) analysis. The QuantStudio™ 3D Instrument performs imaging and primary analysis of QuantStudio™ 3D Digital PCR 20K Chips that have been loaded with TaqMan® assays and thermal cycled using a Dual Flat Block GeneAmp® PCR System 9700. Secondary analysis and post-processing is performed by the QuantStudio™ 3D AnalysisSuite™ Software to yield relative or absolute quantification results from the raw imaging data.

The following figure lists the components of the QuantStudio™ 3D System.



① QuantStudio™ 3D Digital PCR Thermal Pads	⑧ UV-Curing Stylus Stand
② QuantStudio™ 3D Digital PCR Chip Carriers	⑨ UV-Curing Stylus for Chip Sealant
③ Dual Flat Block GeneAmp® PCR System 9700	⑩ UV-Activated Chip Sealant Syringe
④ QuantStudio™ 3D Digital PCR Instrument	⑪ QuantStudio™ 12K Flex OpenArray® Immersion Fluid
⑤ QuantStudio™ 3D Digital PCR Chip Case Lid	⑫ QuantStudio™ 3D Digital PCR Master Mix
⑥ QuantStudio™ 3D Digital PCR Sample Loading Blade	⑬ TaqMan® Assays
⑦ QuantStudio™ 3D Digital PCR 20K Chip	⑭ (Not shown) QuantStudio™ 3D Tilt Base for Dual Flat Block GeneAmp® PCR System 9700
	⑮ (Not shown) QuantStudio™ 3D AnalysisSuite™ Software

About digital PCR experiments

What is a digital PCR experiment?

Digital PCR (dPCR) is a molecular biology technique used to quantify the number of starting copies of a target nucleic acid sequence in a genomic or complementary DNA sample without the use of a standard. Digital PCR analysis requires that at least some PCR reactions within the sample replicate group have zero copies (individual reactions will contain either zero, one, or a few target molecules). Amplification is detected in reactions receiving at least one molecule and classified as positive while no amplification is detectable in reactions not receiving target and is conversely classified as negative. Following the PCR, the total number of negative reactions is fit to a Poisson distribution to estimate the absolute copies of template molecules present in the sample volume.

For concentrated samples, dilution to the single-molecule limit may be required. For detection of rare targets (mutant sequences, pathogens, transgene content) dilution is generally not required.

Applications of digital PCR include, but are not limited to, quantification of low-level pathogens, detection of rare sequences, gene expression in single cells, and low-fold copy number discrimination of genes/targets.

Elements of dPCR experiments

Digital PCR experiments include:

- **Sample** – The genomic or complementary DNA sample that contains an unknown number of copies of the target nucleic acid sequence. In a digital PCR experiment samples are diluted down to a limiting quantity, such that on average, individual PCR reactions contain a single target molecule.
- **QuantStudio™ 3D Digital PCR Master Mix** – An optimized mixture of dNTPs, salt, buffer, DNA polymerase, and ROX™ passive reference dye designed for use with TaqMan® assays and the QuantStudio™ 3D Digital PCR System.
- **TaqMan® assay** – A mixture that includes forward and reverse primers and one or more specific fluorescent dye-labeled probes for the target nucleic acid sequence.
- **(Optional) Technical replicates** – Chips that contain identical sample/assay/reaction mix combinations and volumes. Each QuantStudio™ 3D Digital PCR 20K Chip contains up to 20,000 reaction wells (resulting from the loading of a single PCR reaction/sample).
- **(Optional) Negative controls (NTCs)** – Samples that should not amplify.
 - **No template controls** – Samples that contain water or buffer instead of template.
 - **No target controls** – Samples that contain genetic material but not the target template.

IMPORTANT! You can identify a negative control in the QuantStudio™ 3D AnalysisSuite™ Software by naming a Sample "NTC" or something similar. The Software 'treats' the NTC-named samples in the same way as the other samples. You need to look out for any amplification in the NTC-named samples as the software does not produce a flag if amplification occurs in those reaction wells.

Digital PCR experiment setup

In a digital PCR experiment performed on the QuantStudio™ 3D Digital PCR System, dilutions of each gDNA or cDNA sample are loaded into the wells of a QuantStudio™ 3D Digital PCR 20K Chip that contains QuantStudio™ 3D Digital PCR Master Mix and TaqMan® assay appropriate for your experiment. If the target concentration is high, samples are diluted down to a limiting quantity prior to assembly of the reaction mix in the Digital PCR 20K Chip, such that a portion of the individual PCR reactions receive no target molecules.

About the QuantStudio™ 3D AnalysisSuite™ Software

The QuantStudio™ 3D AnalysisSuite™ Software performs statistical analysis of digital PCR experiments and can be used to detect and measure the absolute or relative number of target nucleic acid sequences in a variety of biological samples.

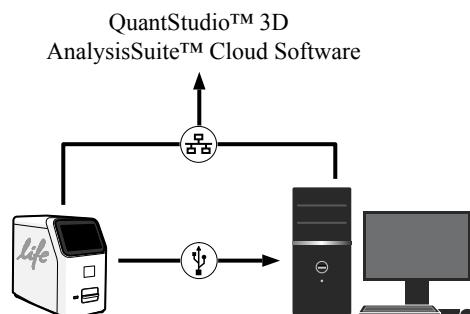
Compatible instruments

AnalysisSuite™ Software can be used to analyze the results of digital PCR experiments run on the QuantStudio™ 3D Instrument that have been exported as experiment data (.eds) files.

Note: Refer to the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720) for detailed information on the instrument operational and data workflow.

QuantStudio™ 3D System data flow

For each imaged Digital PCR 20K Chip, the QuantStudio™ 3D Instrument generates and saves a single experiment (.eds) file that contains the processed imaging data and the results from the primary analysis. Depending on the chosen data destination, the instrument transfers the saved imaging data directly to the cloud for immediate access by the QuantStudio™ 3D AnalysisSuite™ Software, or to a network file server or local USB drive for later manual import.



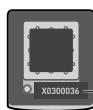
About the data files

The instrument names each experiment file uniquely by combining the ID of the chip and the date/time of the run according to the following formula:

BO498N_130531_195817.eds

① ② ③

① **Chip ID** - The label applied to the QuantStudio™ 3D Digital PCR Chip Case Lid that uniquely identifies the Digital PCR 20K Chip to which it is applied.



Chip ID

② **Date stamp** - The date at which the Digital PCR 20K Chip was run, expressed in the "yyymmdd" format where *yy* is the last two digits of the year, *mm* is the numerical order of the month, and *dd* is the day of the run.

③ **Time stamp** - The time at which the Digital PCR 20K Chip was run, expressed in the "hhmmss" format where *hh* is the hour (in 24-hour format), *mm* is the minutes, and *ss* is the seconds.

IMPORTANT! If the instrument cannot read the ID of a loaded chip, then it uses the instrument name instead to name the file.

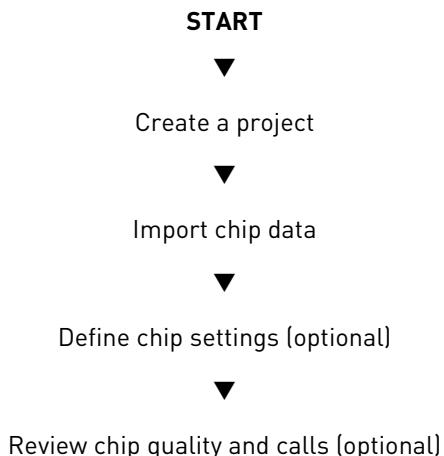
Note: In the example shown above, the file name indicates that the file contains data for chip *BO498N* which was run at 7:58 pm on May 31, 2013.

About the secondary analysis

The Absolute Quantification and Relative Quantification modules of the AnalysisSuite™ Software generate calls for the reaction wells on each chip that contain viable PCR products. Using the call data, the software calculates copy number values for the sample present on the chip and generates confidence intervals. The copy number values are generated according to a Poisson maximum-likelihood algorithm (Fazekas de St. Groth, S, 1982). When combining replicates and dilution series, the software calculates a single quantification result and generates a confidence interval for that result.

General analysis workflow

The following figure shows the general workflow for analyzing digital PCR experiments using the AnalysisSuite™ Software. Both Absolute Quantification (single reporter) and Relative Quantification (dual reporter) experiments follow the same general analysis procedure.



▼
Review chip results and project results

▼
Export project data

▼
FINISH

Access the software from a cloud account

When deployed in cloud configuration, the AnalysisSuite™ Software is accessed as an internet application that is optimized for use with the Microsoft® Windows® operating system running the Google® Chrome™ web browser v4.0 or later.

To access the software from a cloud account:

1. Confirm that your computer has an internet connection.

Note: If your computer does not have an internet connection, contact a Life Technologies Sales Representative (see “Obtaining support” on page 60) for information on available AnalysisSuite™ Software deployment options.

2. Open a browser window and go to <https://dhap.apps.invitrogen.com/quantstudio3d>.

Note: Using your browser settings, confirm that cookies and Javascript are turned on for the website to function correctly. If you cannot access the website, then contact Life Technologies Customer Support.

3. Select to **Launch QuantStudio™ 3D AnalysisSuite™ Cloud Software**.

Note: You can also access the software from the Software Downloads page (see “Obtaining support” on page 60).

4. Sign into the software using your Life Technologies user name and password, or follow the instructions to create a new user account.

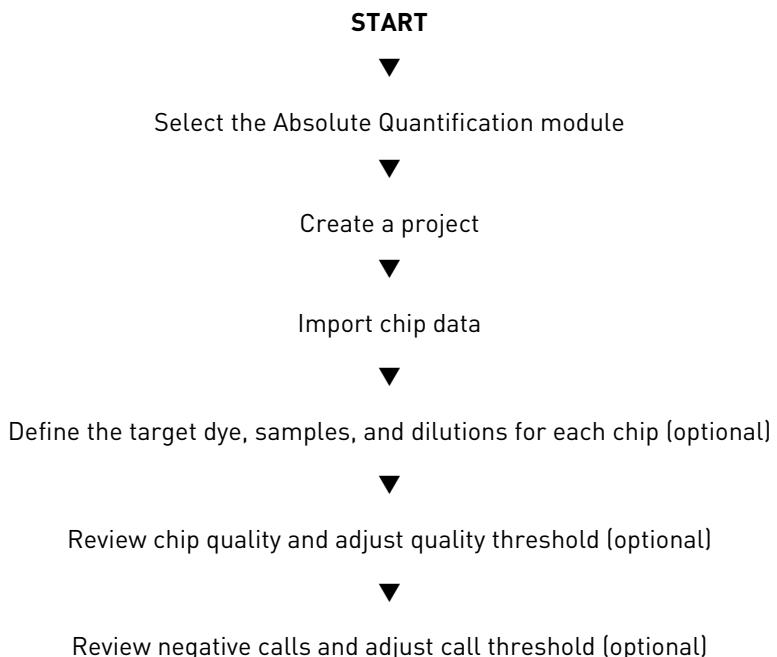
Note: If you cannot sign in to the software, contact Life Technologies Customer Support.

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Absolute Quantification analysis workflow

The following figure shows the workflow for analyzing single reporter digital PCR experiments using the Absolute Quantification module of the AnalysisSuite™ Software.



▼
Review calculated quantities and adjust analysis settings (optional)

▼
Export project data

▼
FINISH

Note: If you cannot view the results of an open project, then contact Life Technologies Technical Support (see “Obtaining support” on page 60).

Select an analysis module

1. Log into the AnalysisSuite™ Software using your Life Technologies user name and password (see “Access the software from a cloud account” on page 11).
2. Select an analysis module from the QuantStudio™ 3D AnalysisSuite™ Software homepage. The software opens to the Project Listing screen.

Note: To select a different analysis module, click **QuantStudio™ 3D AnalysisSuite™** at the top of any screen in the software.

Create a project

1. In the Project Listing screen, click **Create project**.
2. Enter a unique project name up to 100-characters in length.
The project name:
 - Should be descriptive and easy to remember.
 - Cannot contain the following characters:
% * ? | ; : , ! @ # \$ () < > / \ " ' ` ~ [] { } = & ^ -
 - Cannot end with a period (.).
3. Click **OK**. The software saves the project.
4. In the Import Data tab, import data into the project as needed.

Import chip data

A project can include experiment data from up to 100 chips. You can import chips from your Life Technologies cloud account or from your computer.

Note: Refer to the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720) for information on transferring data files from the QuantStudio™ 3D

Digital PCR Instrument to another location (cloud storage service, network file server, or USB drive).

Import data

Select the chip(s) to import.

Available to import from cloud account:

<input type="checkbox"/>	Name
<input checked="" type="checkbox"/>	1A_X210196L_130508_162210.eds Run Date: May 08, 2013 09:22 AM

Delete chip(s)

Show filters

Delete chip(s)

Data in the project:

<input type="checkbox"/>	Name
<input checked="" type="checkbox"/>	6C_X210017C_130508_181054.eds Run Date: May 08, 2013 11:10 AM
<input type="checkbox"/>	5C_X2102409_130508_180829.eds Run Date: May 08, 2013 11:08 AM

Import from cloud account

If you transferred completed experiment (.eds) files from chips run on a QuantStudio™ 3D Instrument to your Life Technologies cloud account, they are listed in the Import Data tab and are available for import into the current project.

In the Import Data tab, select the chip(s) to import from your Life Technologies cloud account:

- **Individual chip** – Select a row in the table, then click (Import into project).
- **Multiple chips** – Click-drag or press **Shift** to select continuous rows, or press **Ctrl** to select discontinuous rows, then click **Import chip(s)**.

The selected chip(s) are added to the project list on the right. You can select to delete any chip before continuing with project setup.

Note: If a transferred experiment file is not visible in the Import Data tab, then contact Life Technologies Technical Support (see “Obtaining support” on page 60).

Import from local source

To import chip data from your computer:

1. In the Import Data tab, click **Import from local source**.
2. Navigate to and select one or more experiment (.eds) files to import, then click **Open**.

Note: Click-drag or press **Shift** to select continuous files, or press **Ctrl** to select discontinuous files.

The selected files are added to the project list on the right. You can select to delete any file before continuing with project setup.

Define chip settings

The Define Chips tab is automatically populated with the default values from chips imported into a project. You can edit the default chip settings applied to the chips in a project, select the default chip settings to use at chip import, import new chip settings from an existing experiment (.eds) file, and apply the same chip settings to multiple chips in the project.

Note: During analysis, chips with the same sample, target, and dilution settings are considered technical replicates, while chips with the same sample and target but different dilution settings are considered a dilution series.

- To edit default chip settings:
 - Click a field in the table and enter a value.

Note: Optionally, enter an editable comment for a chip.
 - As needed, use the options below the table to continue to set up (add or edit) chip settings and select the default chip settings to use at chip import.
- To assign the same chip settings to multiple chips:
 - a. Select the top checkbox to select all rows, or select individual checkboxes. Click-drag or press **Shift** to select continuous rows. Press **Ctrl** to select discontinuous rows.
 - b. Click **Assign settings to multiple chips**, select the chip settings to use, then click **Save**.
- To import new chip settings into the project:
 - a. Click **Import settings**.
 - b. Navigate to and select an existing experiment (.eds) file, then click **Open**.

Set up targets

After you create a project, you can use the Define Chips tab to edit existing targets, add new targets, and select the default targets for the project.

IMPORTANT! If a dye is not used in a chip, do not define target settings for that dye. By default for Absolute Quantification experiments, the software defines a single target using the FAM™ reporter dye.

- To edit existing targets:
 - In the chip settings table, click a field in the **Target (FAM)** or optionally the **Target (VIC)** column, then:
 - Enter a new target name (do not use spaces). A new target is added to the target details table and is available for selection in the chip settings table.
 - Select a new target from the drop-down menu.
 - Below the chip settings table, click **Show details** next to a Target type. Optionally, edit the target settings.
- Note:** Changes to target settings are automatically shown in the chip settings table.
- To add and define new targets:
 - a. Below the chip settings table, click **Add** next to a Target type. A new target is added to the target details table and is available for selection in the chip settings table.
 - b. (Optional) Edit the target **Name**.
 - c. (Optional) Click **Delete** to remove a setting.

Note: You cannot delete settings that are currently assigned to one or more chips.

- d. (Optional) Select a default target.
 - To select a default target:
 - a. Below the chip settings table, click **Show details** next to a Target type.
 - b. Select the **Default value** option for the target you wish to apply to each new chip upon import into the project. This target will be shown by default in the chip settings table. Click **Clear** to remove your selection.

Set up samples

After you create a project, you can use the Define Chips tab to edit existing samples, add new samples, and select the default samples for the project.

- To edit existing samples:
 - In the chip settings table, click a field in the **Sample** column, then:
 - Enter a new sample name (do not use spaces). A new sample is added to the sample details table and is available for selection in the chip settings table.
 - Select a new sample from the drop-down menu.
 - Below the chip settings table, click **Show details** next to the Sample type. Optionally, edit the sample settings.
- Note:** Changes to sample settings are automatically shown in the chip settings table.
- To add and define new samples:
 - a. Below the chip settings table, click **Add** next to the Sample type. A new sample is added to the sample details table and is available for selection in the chip settings table.
 - b. (Optional) Edit the sample **Name**.
 - c. (Optional) Click **Delete** to remove a setting.

Note: You cannot delete settings that are currently assigned to one or more chips.
- d. (Optional) Select a default sample.
- To select a default sample:
 - a. Below the chip settings table, click **Show details** next to the Sample type.
 - b. Select the **Default value** option for the sample you wish to apply to each new chip upon import into the project. This sample will be shown by default in the chip settings table. Click **Clear** to remove your selection.

Set up dilutions

After you create a project, you can use the Define Chips tab to edit existing dilutions, add new dilutions, and select the default dilutions for project.

- To edit existing dilutions:
 - In the chip settings table, click a field in the **Dilution** column, then:
 - Enter a new dilution. A new dilution is added to the dilution details table and is available for selection in the chip settings table.
- **Note:** You can enter the dilution as numeric values (for example, 0.01), alphanumeric values (for example, 1 to 100), or in scientific notation (for example, 1E-2).



- Select a new dilution from the drop-down menu.
- Below the chip settings table, click **Show details** next to the Dilution type. Optionally, edit the dilution settings.

Note: Changes to dilution settings are automatically shown in the chip settings table.

- To add and define new dilutions:
 - Below the chip settings table, click **Add** next to the Dilution type. The software computes the next dilution based on the default dilution factor of 10. This new dilution is added to the dilution details table and is available for selection in the chip settings table.
 - (Optional) Edit the dilution value shown in the **Name** column.
 - (Optional) Click **Delete** to remove a setting.
 - (Optional) Select a default dilution.
- To select a default dilution:
 - Below the chip settings table, click **Show details** next to the Dilution type.
 - Select the **Default value** option for the dilution you wish to apply to each new chip upon import into the project. This dilution will be shown by default in the chip settings table. Click **Clear** to remove your selection.

Import chip setup

You can import existing chip setup information from a saved comma-separated (.csv) file instead of entering chip settings into the Define Chips tab.

1. Select the **Define Chips** tab of an open project.

2. Click **Upload setup**, then navigate to and select the chip setup (.csv) file you wish to import in to the project.

Note: Make sure to select the correct chip setup type for your analysis module.

3. Click **Open** to add the setup information to the project.
4. (Optional) Continue to define the chip settings.

View the data quality for each chip

You can use the Review Quality tab to perform a quality check on the digital PCR data from each chip in a project and conduct further analysis, if necessary. The data views in the Review Quality tab:

- Visualize the spatial distribution of data across the chips.
- Provide an overview of the observed dye intensities for reaction wells (data points) that exceed the user-defined quality threshold (see “About chip quality” on page 51).
- Are color-coded by the assigned call by default.

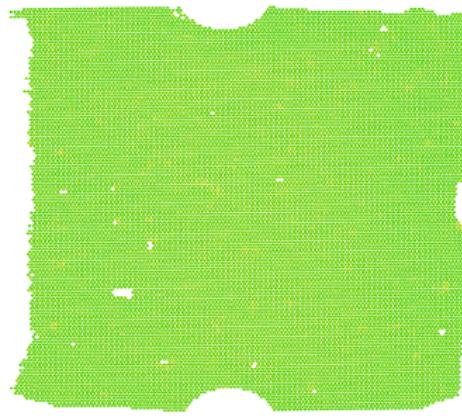
Note: The information displayed and the activities you can perform in the Review Quality tab will vary based on your experiment type.

Review chip quality

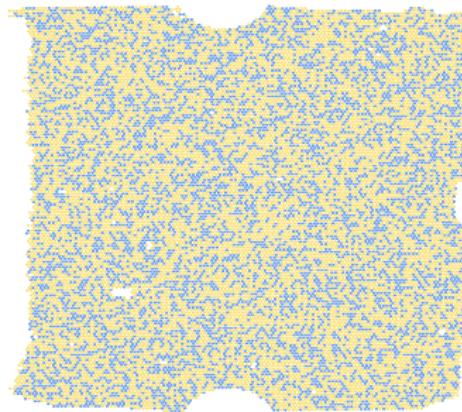
1. Select the **Review Quality** tab of an open project.
2. Select a chip to view:
 - Select a row in the Table view tab.
 - Navigate to and select an image in the Chip view thumbnails tab. Move the pointer over an image to view the experiment name.

Note: Use the flag indicators to determine which chips to view. Life Technologies recommends performing a quality check of the data for chips that display a  or  flag (see “About data quality flags” on page 51).

3. Select the data point color options:
 - **Color by quality** – Displays each data point by quality, on a continuous color scale from red (low quality) to green (high quality). You can use this option to include or exclude reaction wells from the results. For example:



- **Color by calls** (default) – Displays each data point by the assigned call, based on the target (dye) signal detected in a reaction well. You can use this option to verify the uniformity of calls across a chip. For example:



Note: Data points that do not meet the default quality threshold are automatically filtered out (removed) by the software and displayed in white. You can lower the quality threshold to include these reaction wells in the results. Each chip fiducial (area of attachment of the chip to the chip base) is also filtered out by the software and displayed in a white semi-circle at the chip edge(s).

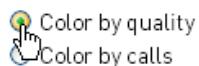
4. Review the data points in each view and adjust the quality threshold or omit chips from the results as needed.

Set the quality threshold

In the Review Quality tab, select a chip to view and adjust the quality threshold until you have an acceptable balance of data quantity and quality.

Note: Life Technologies has validated the system using the default quality threshold of 0.5. Increasing the quality threshold may not necessarily improve your results. We recommend adjusting the quality threshold if you observe obvious artifacts (such as debris, bubbles, or excess PCR reaction) which produce lower quality data points that can be excluded from analysis by increasing the quality threshold. See Appendix C, “Troubleshooting” for more information.

1. Select the **Color by quality** data point color option.



2. Drag the slider (below the Chip view) to the desired quality threshold, in 0.02 increments from 0 to 1. A higher quality threshold will filter out (remove) more data points from the results.



The software automatically reanalyzes the chip results and updates the following:

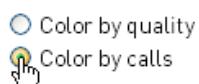
- Color and number of data points in each view that are above the quality threshold.
- Quality Threshold value shown in the Table view tab.
- Flag indicator, if applicable. For example, a or flag indicates that a user has modified the analysis results originally generated by the instrument software.

After you review the chip level results, you can continue to adjust the quality threshold, review the project level results, or omit the chip results from the project.

Set the call threshold

In the Review Quality tab, select a chip to view and adjust the fluorescence (call) threshold in the Histogram view to improve the call assignment accuracy. The software automatically sets the call threshold for chips in an Absolute Quantification project based on the current chip quality settings. The Histogram view provides an overview of the observed dye intensities (fluorescence) for data selected by the quality threshold.

1. Select the **Color by calls** data point color option.

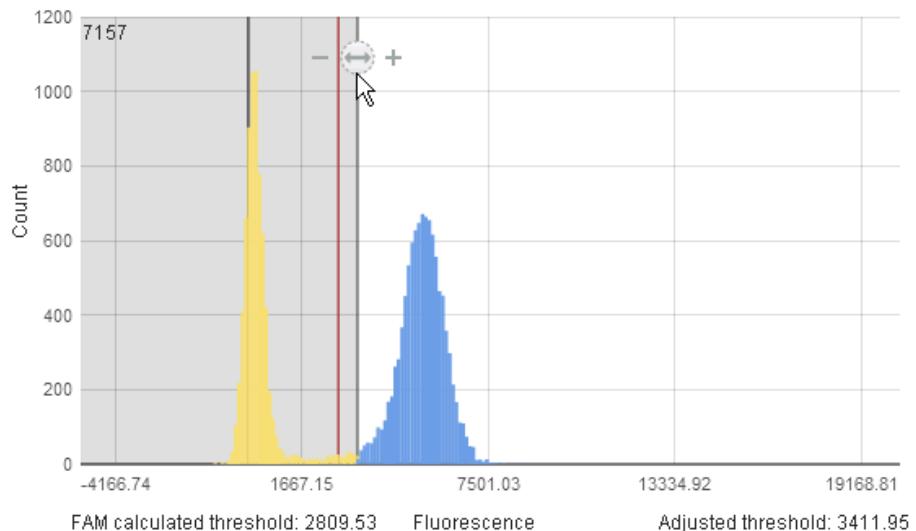


2. In the Histogram view:

- a. (Optional) Click , then drag the slider to the desired y-axis scale to show in the plot. Click anywhere in the Count dialog to close the dialog.

- b. Drag the grey call slider to the desired call threshold.

The red vertical line represents the call threshold calculated by the software. Data points to the left of the call threshold represent unamplified wells (negative calls). The black vertical line represent the origin, which does not affect call assignments.



The software automatically reanalyzes the chip results and updates the following:

- Adjusted threshold value shown below the histogram.

Note: This threshold remains unchanged if less than 5000 data points exceed the quality threshold.

- Number of negative wells shown in the See Results tab for each replicate.
- Flag indicator, if applicable. For example, a or flag indicates that a user has modified the analysis results originally generated by the instrument software.

After you review the chip level results, you can continue to adjust the call threshold, review the project level results, or omit the chip results from the project.

Omit chip results

If you determine that the data quality for a chip is unacceptable after reviewing the data in the Review Quality tab, you can omit the chip results from the project.

Note: Omitting a chip does not delete the chip from the project. You can select to include data from an omitted chip in the project results at any time.

- To omit a chip from the results:
 - Select a row in the Table view tab, then select the **Omit** checkbox.
 - Select an image in the Chip view thumbnails tab, then click **Omit this chip from results**.

The software automatically removes the chip data from the project results and disables the data views for the selected chip.

- To include an omitted chip in the results:
 - Select a row in the Table View tab, then deselect the **Omit** checkbox.
 - Select an image in the Chip view thumbnails tab, then click **Include this chip in results**.

The software automatically includes the chip data in the project results and enables the data views for the selected chip.

View the analysis results for a project

To view the analysis results for all of the chips (experiments) in a project:

1. Select the **See Results** tab of an open project.
2. If needed, select the **Results** tab if it is not already shown by default.

About the Absolute Quantification results

By default, the Results tab for Absolute Quantification projects displays the aggregate results for each unique sample and target combination, which may include replicates and/or multiple dilutions.

- **Bar graph** – By default, displays the data group (Sample-Target) on the X-axis and sample quantity (Copies/ μ L) on the Y-axis. Error bars represent the theoretical confidence interval based on the Poisson distribution.

Note: You can use the available data display and table display settings to change the default data group shown in the graph (see “Review the Absolute Quantification results” on page 22).

- **Results table** – Includes the following:

Column	Description
Color	Color assigned to the data group
Target	Target name
Sample	Sample name
Copies/ μ L	Quantity of sample in copies/ μ L
CI Copies/ μ L	Lower and upper confidence interval for quantity of sample in copies/ μ L
Precision	Calculated precision (%) for the data group, defined as size of the confidence interval for distinguishing between two sample concentrations at a given confidence level Note: You can improve (lower) the calculated precision by combining multiple chips into one “virtual” chip. To do this, apply the same sample name to all chips of interest.
Chips	Number of chips containing the data group
Comment	Chip level comment, if entered on the Define Chips tab or as generated by the software if set precision is exceeded

Note: The calculated sample quantity (Copies/ μ L) represents the concentration of the sample in the PCR reaction mix, not the concentration of the original sample before dilution (stock concentration).

Review the Absolute Quantification results

Review the calculated quantities shown in the Results tab for an Absolute Quantification project, and use the display settings to show and group the data as needed.

- In the bar graph, you can:
 - Move the pointer over a bar in the graph to view a summary of the results for that data group.

- Adjust the data display and analysis settings (see “View the Absolute Quantification analysis and display settings” on page 24).
- View the graph as an image file.

- In the results table, you can:
 - Use the checkboxes to show or hide data in the bar graph. Click-drag or press **Shift** to select continuous rows, or press **Ctrl** to select discontinuous rows. Use the top checkbox to select or deselect all rows.
 - Select a color square in the Color column to replace the color associated with the data group.
 - Use the table display settings to show, sort, or group the data (see “Change the display of table data” on page 49).

View the analysis results for each chip

To view the analysis results for each chip in a project:

1. Select the **See Results** tab of an open project.
2. If needed, select the **Replicates** tab if it is not already shown by default.

About the Absolute Quantification replicate results

For Absolute Quantification experiments, the Replicates tab reports the results for each chip in the project. Results can be grouped by replicate (chips assigned with the same sample, target, and dilution) to help identify any outliers.

The Replicates table includes the following:

Column	Description
Sample	Sample name
Target	Target name
Dilution	Sample dilution factor entered for the chip
Chip	Chip assigned with the same sample, target, and dilution
Copies/Rxn	Quantity of sample in copies/reaction well
CI Copies/Rxn	Lower and upper confidence interval for quantity of sample in copies/reaction well
Copies/µL	Quantity of sample in copies/µL
CI Copies/µL	Lower and upper confidence interval for quantity of sample in copies/µL
# of Neg	Total number of negative calls in the chip, as determined from the Review Quality histogram Note: Negative call means the software determines that a well does not contain any copy of the target labeled with the assigned target dye.

Column	Description
# qualified by QT	Total number of reaction wells in the chip that exceed the selected quality threshold
# of Filled	Total number of filled reaction wells in the chip
Run Date	Date and time the chip run was completed on the instrument
Flag	Quality flag for the chip, if present
Comment	Chip level comment, if entered on the Define Chips tab

Note: The calculated sample quantity (Copies/Rxn and Copies/ μ L) represents the concentration of the sample loaded into the chip, not the concentration of the original sample before dilution (stock concentration).

Review the Absolute Quantification replicate results

Review the calculated quantities for each chip shown in the Replicates tab for an Absolute Quantification project, and omit any outliers.

- Select the results to display in the table (see “Change the display of table data” on page 49):
 - Drag a column heading above the table to group the table data by the selected attribute. To create a hierarchical grouping, repeat this process with additional columns.
 - Adjust additional table display settings to show or sort the table data.
- Select an experiment (.eds) file in the **Chip** column to review the chip quality and omit the chip from the results, if necessary.

View the Absolute Quantification analysis and display settings

The See Results tab also contains the data analysis and display settings for the project, which you can select in the settings view at the top of the tab.



1. Click **Show settings** to open the settings view.
2. Select **Color by** to assign the bar graph with one of the default color categories (**Sample** or **Target**), or by the **User defined** color selected in the Results table. The software automatically updates the data display in the bar graph, and in the **Color** column of the Results table.
3. Select the data analysis settings:
 - **Confidence level (%)** – Select the confidence level to use for the project, as shown in the bar graph and Results table. The default value is 95%.
 - **Desired precision (%)** – Select the desired precision for the software to use to generate recommended actions for the project. The default value is 10%.

Note: If the calculated value shown in the Precision column of the Results table exceeds the desired precision entered here, the software may provide a suggestion for further dilutions of the PCR reaction mix to achieve the desired precision (see the Comments column for more information).

The software automatically updates the data display in both the bar graph and Results table.

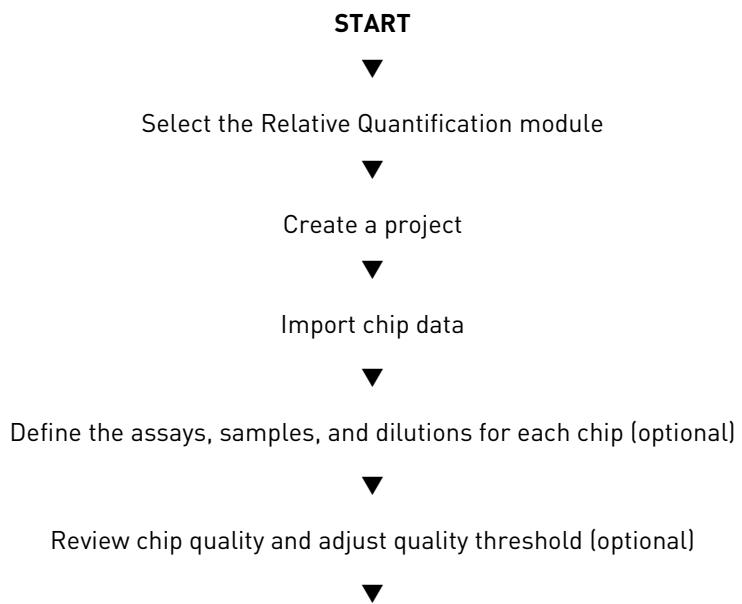
4. Click **Hide settings** to close the settings view.

Analyze Relative Quantification Experiments

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Relative Quantification analysis workflow

The following figure shows the workflow for analyzing dual reporter digital PCR experiments using the Relative Quantification module of the AnalysisSuite™ Software.



Review data point calls and manually assign calls (optional)



Review calculated ratios and quantities and adjust analysis settings (optional)



Export project data



FINISH

Note: If you cannot view the results of an open project, then contact Life Technologies Technical Support (see “Obtaining support” on page 60).

Select an analysis module

1. Log into the AnalysisSuite™ Software using your Life Technologies user name and password (see “Access the software from a cloud account” on page 11).
2. Select an analysis module from the QuantStudio™ 3D AnalysisSuite™ Software homepage. The software opens to the Project Listing screen.

Note: To select a different analysis module, click **QuantStudio™ 3D AnalysisSuite™** at the top of any screen in the software.

Create a project

1. In the Project Listing screen, click **Create project**.
2. Enter a unique project name up to 100-characters in length.
The project name:
 - Should be descriptive and easy to remember.
 - Cannot contain the following characters:
% * ? | ; : , ! @ # \$ () < > / \ " ' ` ~ [] { } = & ^ -
 - Cannot end with a period (.).
3. Click **OK**. The software saves the project.
4. In the Import Data tab, import data into the project as needed.

Import chip data

A project can include experiment data from up to 100 chips. You can import chips from your Life Technologies cloud account or from your computer.

Note: Refer to the *QuantStudio™ 3D Digital PCR System User Guide* (Pub. no. MAN0007720) for information on transferring data files from the QuantStudio™ 3D Digital PCR Instrument to another location (cloud storage service, network file server, or USB drive).

Import data

Select the chip(s) to import.

Available to import from cloud account:

	Name
<input type="checkbox"/>	1A_X210196L_130508_162210.eds
<input checked="" type="checkbox"/>	Run Date: May 08, 2013 09:22 AM

Delete chip(s)

Show filters

Data in the project:

	Name
<input checked="" type="checkbox"/>	6C_X210017C_130508_181054.eds
<input checked="" type="checkbox"/>	Run Date: May 08, 2013 11:10 AM
<input type="checkbox"/>	5C_X2102409_130508_180829.eds
<input type="checkbox"/>	Run Date: May 08, 2013 11:08 AM

Delete chip(s)

Import from cloud account

If you transferred completed experiment (.eds) files from chips run on a QuantStudio™ 3D Instrument to your Life Technologies cloud account, they are listed in the Import Data tab and are available for import into the current project.

In the Import Data tab, select the chip(s) to import from your Life Technologies cloud account:

- **Individual chip** – Select a row in the table, then click  (Import into project).
- **Multiple chips** – Click-drag or press **Shift** to select continuous rows, or press **Ctrl** to select discontinuous rows, then click **Import chip(s)**.

The selected chip(s) are added to the project list on the right. You can select to delete  any chip before continuing with project setup.

Note: If a transferred experiment file is not visible in the Import Data tab, then contact Life Technologies Technical Support (see “Obtaining support” on page 60).

Import from local source

To import chip data from your computer:

1. In the Import Data tab, click **Import from local source**.
2. Navigate to and select one or more experiment (.eds) files to import, then click **Open**.

Note: Click-drag or press **Shift** to select continuous files, or press **Ctrl** to select discontinuous files.

The selected files are added to the project list on the right. You can select to delete  any file before continuing with project setup.

Define chip settings

The Define Chips tab is automatically populated with the default values from chips imported into a project. You can edit the default chip settings applied to the chips in a project, select the default chip settings to use at chip import, import new chip settings

from an existing experiment (.eds) file, and apply the same chip settings to multiple chips in the project.

Note: During analysis, chips with the same sample, target, and dilution settings are considered technical replicates, while chips with the same sample and target but different dilution settings are considered a dilution series.

- To edit default chip settings:
 - Click a field in the table and enter a value.
Note: Optionally, enter an editable comment for a chip.
 - As needed, use the options below the table to continue to set up (add or edit) chip settings and select the default chip settings to use at chip import.
- To assign the same chip settings to multiple chips:
 - a. Select the top checkbox to select all rows, or select individual checkboxes. Click-drag or press **Shift** to select continuous rows. Press **Ctrl** to select discontinuous rows.
 - b. Click **Assign settings to multiple chips**, select the chip settings to use, then click **Save**.
- To import new chip settings into the project:
 - a. Click **Import settings**.
 - b. Navigate to and select an existing experiment (.eds) file, then click **Open**.

Set up assays

After you create a project, you can use the Define Chips tab to edit existing assays, add new assays, and select the default assays for the project.

The default assay in the software uses the following settings:

Assay	Target Dye
assay(default)	FAM

Note: The software automatically assigns FAM™ dye as the Target Dye to each new chip that is imported into a Relative Quantification project. The software uses the Target Dye as the numerator in reported ratio results.

- To edit existing assays:
 - In the chip settings table, click a field in the **Assay** column, then:
 - Enter a new assay name (do not use spaces) and optionally select a new Target Dye. A new assay is added to the assay details table and is available for selection in the chip settings table.
 - Select a new assay from the drop-down menu. The Target Dye defined for the selected assay is automatically shown.
 - Below the chip settings table, click **Show details** next to the Assay type. Optionally, edit the assay settings.

Note: Changes to assay settings are automatically shown in the chip settings table.

- To add and define new assays:
 - Below the chip settings table, click **Add** next to an Assay type. A new assay is added to the assay details table and is available for selection in the chip settings table.
 - (Optional) Edit the assay settings:
 - Enter a new **Assay** name (do not use spaces).
 - Select a **Target Dye** from the drop-down menu.
 - Enter a **Description** for the assay.
 - (Optional) Click **Delete** to remove a setting.

Note: You cannot delete settings that are currently assigned to one or more chips.

 - (Optional) Select a default assay.
- To select a default assay:
 - Below the chip settings table, click **Show details** next to the Assay type.
 - Select the **Default value** option for the assay you wish to apply to each new chip upon import into the project. This assay will be shown by default in the chip settings table. Click **Clear** to remove your selection.

Set up samples

After you create a project, you can use the Define Chips tab to edit existing samples, add new samples, and select the default samples for the project.

- To edit existing samples:
 - In the chip settings table, click a field in the **Sample** column, then:
 - Enter a new sample name (do not use spaces). A new sample is added to the sample details table and is available for selection in the chip settings table.
 - Select a new sample from the drop-down menu.
 - Below the chip settings table, click **Show details** next to the Sample type. Optionally, edit the sample settings.

Note: Changes to sample settings are automatically shown in the chip settings table.
- To add and define new samples:
 - Below the chip settings table, click **Add** next to the Sample type. A new sample is added to the sample details table and is available for selection in the chip settings table.
 - (Optional) Edit the sample **Name**.
 - (Optional) Click **Delete** to remove a setting.

Note: You cannot delete settings that are currently assigned to one or more chips.

 - (Optional) Select a default sample.

- To select a default sample:
 - a. Below the chip settings table, click **Show details** next to the Sample type.
 - b. Select the **Default value** option for the sample you wish to apply to each new chip upon import into the project. This sample will be shown by default in the chip settings table. Click **Clear** to remove your selection.

Set up dilutions

After you create a project, you can use the Define Chips tab to edit existing dilutions, add new dilutions, and select the default dilutions for project.

- To edit existing dilutions:
 - In the chip settings table, click a field in the **Dilution** column, then:
 - Enter a new dilution. A new dilution is added to the dilution details table and is available for selection in the chip settings table.
- Note:** You can enter the dilution as numeric values (for example, 0.01), alphanumeric values (for example, 1 to 100), or in scientific notation (for example, 1E-2).



- Select a new dilution from the drop-down menu.
- Below the chip settings table, click **Show details** next to the Dilution type. Optionally, edit the dilution settings.

Note: Changes to dilution settings are automatically shown in the chip settings table.

- To add and define new dilutions:
 - a. Below the chip settings table, click **Add** next to the Dilution type. The software computes the next dilution based on the default dilution factor of 10. This new dilution is added to the dilution details table and is available for selection in the chip settings table.
 - b. (Optional) Edit the dilution value shown in the **Name** column.
 - c. (Optional) Click **Delete** to remove a setting.
- Note:** You cannot delete settings that are currently assigned to one or more chips.
- (Optional) Select a default dilution.
- To select a default dilution:
 - a. Below the chip settings table, click **Show details** next to the Dilution type.

b. Select the **Default value** option for the dilution you wish to apply to each new chip upon import into the project. This dilution will be shown by default in the chip settings table. Click **Clear** to remove your selection.

Import chip setup

You can import existing chip setup information from a saved comma-separated (.csv) file instead of entering chip settings into the Define Chips tab.

1. Select the **Define Chips** tab of an open project.
2. Click **Upload setup**, then navigate to and select the chip setup (.csv) file you wish to import in to the project.
Note: Make sure to select the correct chip setup type for your analysis module.
3. Click **Open** to add the setup information to the project.
4. (Optional) Continue to define the chip settings.

View the data quality for each chip

You can use the Review Quality tab to perform a quality check on the digital PCR data from each chip in a project and conduct further analysis, if necessary. The data views in the Review Quality tab:

- Visualize the spatial distribution of data across the chips.
- Provide an overview of the observed dye intensities for reaction wells (data points) that exceed the user-defined quality threshold (see “About chip quality” on page 51).
- Are color-coded by the assigned call by default.

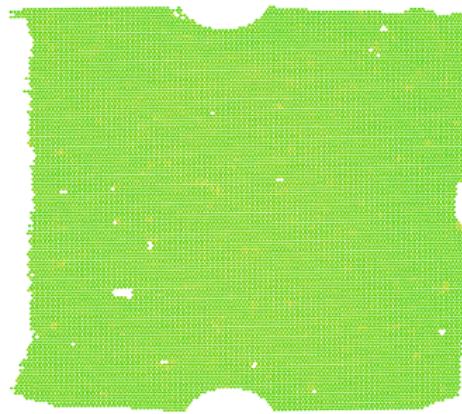
Note: The information displayed and the activities you can perform in the Review Quality tab will vary based on your experiment type.

Review chip quality

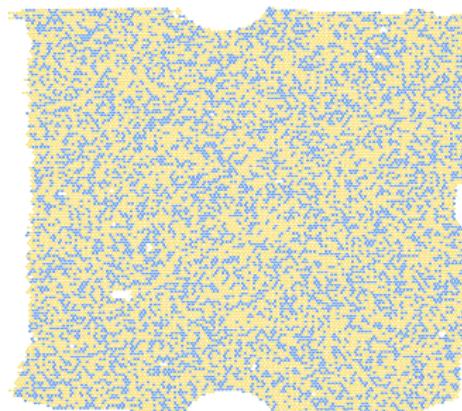
1. Select the **Review Quality** tab of an open project.
2. Select a chip to view:
 - Select a row in the Table view tab.
 - Navigate to and select an image in the Chip view thumbnails tab. Move the pointer over an image to view the experiment name.

Note: Use the flag indicators to determine which chips to view. Life Technologies recommends performing a quality check of the data for chips that display a  or  flag (see “About data quality flags” on page 51).

3. Select the data point color options:
 - **Color by quality** – Displays each data point by quality, on a continuous color scale from red (low quality) to green (high quality). You can use this option to include or exclude reaction wells from the results. For example:



- **Color by calls** (default) – Displays each data point by the assigned call, based on the target (dye) signal detected in a reaction well. You can use this option to verify the uniformity of calls across a chip. For example:



Note: Data points that do not meet the default quality threshold are automatically filtered out (removed) by the software and displayed in white. You can lower the quality threshold to include these reaction wells in the results. Each chip fiducial (area of attachment of the chip to the chip base) is also filtered out by the software and displayed in a white semi-circle at the chip edge(s).

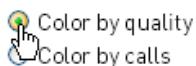
4. Review the data points in each view and adjust the quality threshold or omit chips from the results as needed.

Set the quality threshold

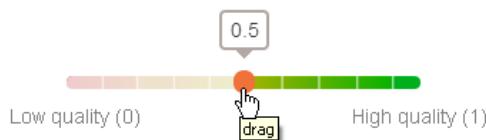
In the Review Quality tab, select a chip to view and adjust the quality threshold until you have an acceptable balance of data quantity and quality.

Note: Life Technologies has validated the system using the default quality threshold of 0.5. Increasing the quality threshold may not necessarily improve your results. We recommend adjusting the quality threshold if you observe obvious artifacts (such as debris, bubbles, or excess PCR reaction) which produce lower quality data points that can be excluded from analysis by increasing the quality threshold. See Appendix C, “Troubleshooting” for more information.

1. Select the **Color by quality** data point color option.



2. Drag the slider (below the Chip view) to the desired quality threshold, in 0.02 increments from 0 to 1. A higher quality threshold will filter out (remove) more data points from the results.



The software automatically reanalyzes the chip results and updates the following:

- Color and number of data points in each view that are above the quality threshold.
- Quality Threshold value shown in the Table view tab.
- Flag indicator, if applicable. For example, a or flag indicates that a user has modified the analysis results originally generated by the instrument software.

After you review the chip level results, you can continue to adjust the quality threshold, review the project level results, or omit the chip results from the project.

Omit chip results

If you determine that the data quality for a chip is unacceptable after reviewing the data in the Review Quality tab, you can omit the chip results from the project.

Note: Omitting a chip does not delete the chip from the project. You can select to include data from an omitted chip in the project results at any time.

- To omit a chip from the results:
 - Select a row in the Table view tab, then select the **Omit** checkbox.
 - Select an image in the Chip view thumbnails tab, then click **Omit this chip from results**.

The software automatically removes the chip data from the project results and disables the data views for the selected chip.

- To include an omitted chip in the results:
 - Select a row in the Table View tab, then deselect the **Omit** checkbox.
 - Select an image in the Chip view thumbnails tab, then click **Include this chip in results**.

The software automatically includes the chip data in the project results and enables the data views for the selected chip.

View the Relative Quantification calls

For Relative Quantification experiments, the software automatically assigns a call to each data point in a chip based on single dye thresholds that define each call type. You can use the data viewing tools in the Relative Quantification module to review

the automatic call assignments for each chip in a project and manually assign calls as needed.

Note: Changes to the quality threshold will not override any manual call assignments.

About the Relative Quantification calls

The Review Calls tab displays the call assigned to each data point in the selected chip(s) of a Relative Quantification project.

- **Scatter plot** – Displays the signal from the FAM™ reporter dye on the Y-axis against the signal from the VIC® reporter dye on the X-axis. By default, all data points are shown in the plot. The data points in the plot are color-coded according to the following call types:

Call (Color)	Description
FAM (blue)	Data points with only FAM™ reporter dye signal, generally located closer to the Y-axis of the plot, away from the origin
VIC (red)	Data points with only VIC® reporter dye signal, generally located closer to the X-axis of the plot, away from the origin
FAM + VIC (green)	Data points with both FAM™ and VIC® reporter dye signal, generally located midway between the FAM and VIC data point clusters in the plot, away from the origin

Call (Color)	Description
No Amp (yellow)	Data points with no signal, generally located near the origin of the plot
Undetermined (dark grey)	Data points with unresolved signal, manual call, or originally below the default quality threshold then included at a lower quality threshold, generally located anywhere on the plot

- **Results table** – Includes the following:

Column	Description
Show	When selected, shows data points in the scatter plot for the associated chip
Assay	Assay name
Sample	Sample name
Dilution	Sample dilution factor entered for the chip
Chips	Chip containing the data group
Omit	When selected, omits the call results for the associated chip

Review the Relative Quantification calls

Use the data viewing tools present in the Review Calls tab of a Relative Quantification project to verify the automatic call assignments and edit calls as needed.

- In the scatter plot:
 - Change the plot display settings as needed:
 - **To move within the plot display** – Click  , click  , then drag  to reposition the plot. Click  to return to the original zoom level.

- **To change the plot display** – Click  , then drag a slider to the desired X-axis, Y-axis, or data point size to show in the plot. Click anywhere in the Adjust Axis dialog to close the dialog.

IMPORTANT! The software automatically scales the plot to display all data points in the selected chip(s). To easily view particular data points in the plot, increase the data point size as needed.

- Review the call for each data point and edit as needed (see “Edit calls” on page 37).
- In the results table:
 - Use the table display settings to show, sort, or group the data (see “Change the display of table data” on page 49).
 - Use the Show checkboxes to show or hide data in the scatter plot. Click and drag or press **Shift** to select continuous rows, or press **Ctrl** to select discontinuous rows. Use the top checkbox to select or deselect all rows.
 - Omit the call results for a chip (see “Omit chip data” on page 39).

Edit calls

In the Review Calls tab for Relative Quantification projects, select one or more chips to view and confirm that data points cluster as expected. Review the call for each data point and edit as needed.

IMPORTANT! Due to the high number of data points present in a chip, there may be some overlap in the display of individual data points. Make sure to verify the assigned call for each data point.

1. Use the results table to show or hide the data from one or more chips in the scatter plot, and adjust plot settings as needed (see “Review the Relative Quantification calls” on page 36).
2. Use the following methods to review each cluster in the scatter plot and select data points for editing:
 - **Data points in a cluster** – This is an ideal method for reviewing data points from a single chip.
 - a. Click a call type (FAM, VIC, and so on) to bring that data point cluster to the foreground.



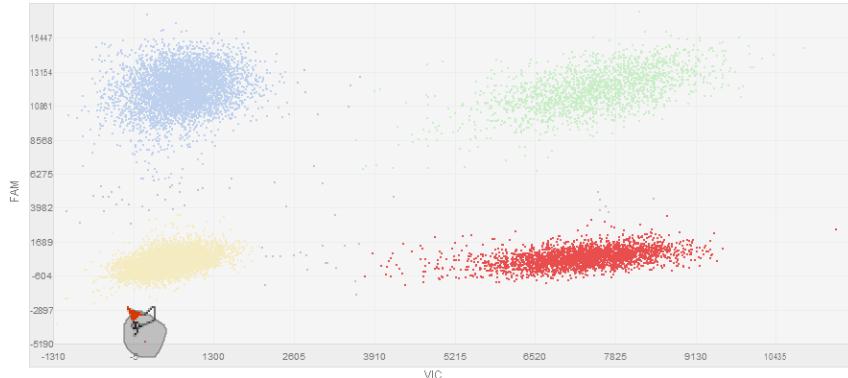
Note: By default, all data points are shown in the plot. You can only edit data points that are in the foreground.

- b. Confirm that the data points are shown in the expected location in the plot (see “About the Relative Quantification calls” on page 35).

Note: For example, normally the data points for the VIC call type should cluster closer to the X-axis of the plot, away from the origin. The presence of data points near the origin among the VIC data points may

indicate that samples failed to amplify. See Appendix C, “Troubleshooting” for more information.

- c. Click  (selection tool), click-drag around one or more data points in the foreground that you wish to edit, then release the mouse button.

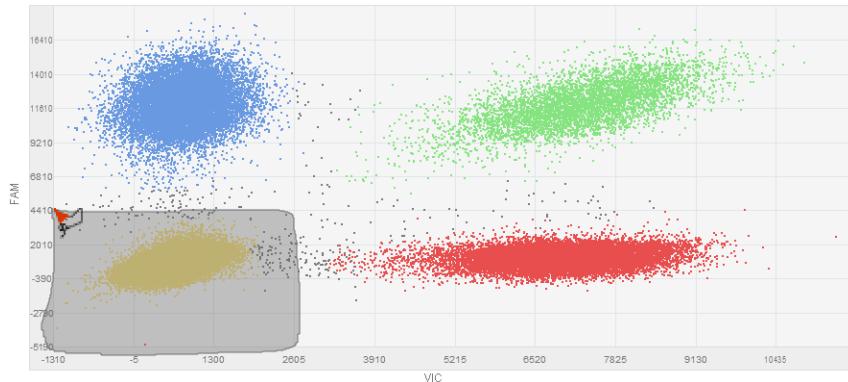


- d. Go to step 3 to manually assign the expected call type to all points in the selected foreground region.
- e. Repeat this process for each call type.
- All data points in a region – This is an ideal method for reviewing data points from multiple chips.

- a. Click All to bring all data points in the plot to the foreground.



- b. Click  (selection tool), click-drag around a region in the plot that you wish to edit, then release the mouse button.



- c. Go to step 3 to manually assign the expected call type to all points in that region.

- 3. Manually assign a call:

- Click a call type.



- Right-click in the plot and select a call type from the drop-down menu.



The software automatically reassigns the new call type to your selection.

Note: Adjusting the quality threshold will not override any manual call assignments. However, after you adjust the quality threshold we recommend that you review the call for each data point to verify if there are any new undetermined calls in the plot that are hidden from view by other data points.

4. If required, omit the chip data from the results (see “Omit chip data” on page 39).

Omit chip data

If you determine that the calls for a chip in a Relative Quantification project are unacceptable after reviewing the data in the Review Calls tab, you can omit the chip data from the project results.

Note: Omitting chip data does not delete the chip from the project. You can select to include omitted chip data in the project results at any time.

- **Omit chip data** – In the data table, select the **Show** checkbox to show the calls for a chip in the scatter plot, then select the **Omit** checkbox for the selected chip. The software automatically removes the chip data from the project results and disables the data views for the selected chip.
- **Include omitted chip data** – In the data table, select the **Show** checkbox to show the calls for a chip in the scatter plot, then deselect the **Omit** checkbox for the selected chip. The software automatically includes the chip data in the project results and enables the data views for the selected chip.

View the analysis results for a project

To view the analysis results for all of the chips (experiments) in a project:

1. Select the **See Results** tab of an open project.
2. If needed, select the **Results** tab if it is not already shown by default.

About the Relative Quantification results

For Relative Quantification experiments, the Results tab displays the aggregate results for each unique sample and assay combination, which may include replicates and/or multiple dilutions.

- **Bar graph** – Displays the data group (Sample-Assay) on the X-axis and dye signal ratio (Target/Total) on the Y-axis.

Note: You can use the available data display and table display settings to change the default data group shown in the graph (see “Review the Relative Quantification results” on page 40).

- **Results table** – Includes the following:

Column	Description
Color	Color assigned to the data group
Assay	Assay name
Sample	Sample name
Target/Total	Calculated ratio of target dye signal over total dye (FAM™ + VIC®) signal from the target gene(s)
CI Target/Total	Upper and lower confidence interval for Target/Total dye signal ratio
Copies/µL (FAM and VIC)	Quantity of sample in copies/µL for each dye
CI Copies/µL (FAM and VIC)	Upper and lower confidence interval for quantity of sample in copies/µL for each dye
Precision (FAM and VIC)	Calculated precision (%) of each dye for the data group, defined as size of the confidence interval for distinguishing between two sample concentrations at a given confidence level Note: You can improve (lower) the calculated precision by combining multiple chips into one “virtual” chip. To do this, apply the same sample name to all chips of interest.
Chips	Number of chips containing the data group
Comment	Chip level comment, if entered on the Define Chips tab

Note: The calculated sample quantity (Copies/µL) represents the concentration of the sample in the PCR reaction mix, not the concentration of the original sample before dilution (stock concentration).

Review the Relative Quantification results

Review the calculated ratios and quantities shown in the Results tab for a Relative Quantification project, and use the display settings to show and group the data as needed.

- In the bar graph:
 - Move the pointer over a bar in the graph to view a summary of the results for that data group.
 - Adjust the data display and analysis settings (see “View the Relative Quantification analysis and display settings” on page 42).
 - View the graph as an image file.
- In the results table, you can:
 - Use the checkboxes to show or hide data in the bar graph. Click-drag or press **Shift** to select continuous rows, or press **Ctrl** to select discontinuous rows. Use the top checkbox to select or deselect all rows.
 - Select a color square in the Color column to replace the color associated with the data group.

- Use the table display settings to show, sort, or group the data (see “Change the display of table data” on page 49).

View the analysis results for each chip

To view the analysis results for each chip in a project:

1. Select the **See Results** tab of an open project.
2. If needed, select the **Replicates** tab if it is not already shown by default.

About the Relative Quantification replicate results

For Relative Quantification experiments, the Replicates tab reports the results for each chip in the project. Results can be grouped by replicate (chips assigned with the same sample, assay, and dilution) to help identify any outliers.

The Replicates table includes the following:

Column	Description
Assay	Assay name
Sample	Sample name
Dilution	Sample dilution factor entered for the chip
Chip	Chip assigned with the same sample, assay, and dilution
Target/Total	Calculated ratio of target dye signal over total dye (FAM™ + VIC®) signal from the target gene(s)
CI Target/Total	Lower and upper confidence interval for Target/Total dye signal ratio
Copies/Rxn (FAM and VIC)	Quantity of sample in copies/reaction well, for each dye
CI Copies/Rxn (FAM and VIC)	Lower and upper confidence interval for quantity of sample in copies/reaction well, for each dye
Copies/µL (FAM and VIC)	Quantity of sample in copies/µL, for each dye
CI Copies/µL (FAM and VIC)	Lower and upper confidence interval for quantity of sample in copies/µL, for each dye
# of Neg (FAM and VIC)	Total number of negative calls for each dye in the chip, as determined from the Review Calls scatter plot Note: Negative call means the software determines that a well does not contain any copy of the target labeled with the assigned target dye.

Column	Description
# of Undetermined	Total number of undetermined calls in the chip, as determined from the Review Calls scatter plot Note: Undetermined calls are those data points with unresolved signal, manual call, or originally below the default quality threshold then included at a lower quality threshold, generally located anywhere on the plot.
# qualified by QT	Total number of reaction wells in the chip that exceed the selected quality threshold
# of Filled	Total number of filled reaction wells in the chip
Run Date	Date and time the chip run was completed on the instrument
Flag	Quality flag for the chip, if present
Comment	Chip level comment, if entered on the Define Chips tab

Note: The calculated sample quantity (Copies/Rxn and Copies/ μ L) represents the concentration of the sample loaded into the chip, not the concentration of the original sample before dilution (stock concentration).

Review the Relative Quantification replicate results

Review the calculated ratios and quantities and the assigned calls shown in the Replicates tab for an Relative Quantification project, and omit any outliers.

- Select the results to display in the table (see “Change the display of table data” on page 49):
 - Drag a column heading above the table to group the table data by the selected attribute. To create a hierarchical grouping, repeat this process with additional columns.
 - Adjust additional table display settings to show or sort the table data.
- Select an experiment (.eds) file in the **Chip** column to review the chip quality and omit the chip from the results, if necessary.

View the Relative Quantification analysis and display settings

The See Results tab also contains the data analysis and display settings for the project, which you can select in the settings view at the top of the tab.



1. Click **Show settings** to open the settings view.
2. Select **Color by** to assign the bar graph with one of the default color categories (**Sample** or **Assay**), or by the **User defined** color selected in the Results table.

The software automatically updates the data display in the bar graph, and in the Color column of the Results table.

3. Select the data analysis settings:

- **Confidence level (%)** – Select the confidence level to use for the project, as shown in the bar graph and Results table. The default value is 95%.
- **Desired precision (%)** – Select the desired precision for the software to use to generate recommended actions for the project. The default value is 10%.

Note: If the calculated value shown in the Precision column of the Results table exceeds the desired precision entered here, the software may provide a suggestion for further dilutions of the PCR reaction mix to achieve the desired precision (see the Comments column for more information).

The software automatically updates the data display in both the bar graph and Results table.

4. Click **Hide settings** to close the settings view.

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■ Results plot	45

Export data

Export chip setup

You can export the setup information for each chip in a project into a comma-separated (.csv) file, for use with other projects and that you can open in a spreadsheet program such as Microsoft® Excel®.

1. Select the **Define Chips** tab of an open project.

2. Click **Export settings**.

The software automatically exports all of the chip settings in the project to a comma-separated (.csv) file using the default file name (<project name>_Setup) and browser downloads location.

Export project level results

The Export tab contains an overview of the project data that you can export and open in a spreadsheet program such as Microsoft® Excel®.

1. Select the **Export** tab of an open project.

2. Review the information shown in each section:

Section	Description
Summary	Displays the setup properties and analysis settings for the project Note: The software automatically appends this summary to the top of each exported file.
Results	Displays the results for all chips that are assigned to the same sample group, which may include replicates and/or multiple dilutions
Replicates	Displays the results for all chips that are assigned to the same sample group and dilution
Digital calls	Displays the digital calls for each chip in the project

3. Adjust the table display settings as needed.

Note: While you can adjust the display of data in each table for review purposes, the software will export all data using the default table display settings.

4. Click Export.

The software automatically exports the project data to a comma-separated (.csv) file using the default file name (<project name>_export) and browser downloads location.

Export projects

Transfer one or more projects out to another work station running AnalysisSuite™ Software or into files that you can archive or pass to another user.

1. Select an analysis module from the QuantStudio™ 3D AnalysisSuite™ Software homepage.

2. In the Project Listing screen, use the checkboxes to select one or more projects to transfer out of the software.

Note: Click-drag or press **Shift** to select continuous rows, or press **Ctrl** to select discontinuous rows. Use the top checkbox to select or deselect all rows.

3. Click **Export project(s)**.

The software automatically exports each project to a .las file using the project name and browser downloads location.

Results plot

Save the Results plot

You can save the plot shown in the See Results tab as a Portable Network Graphic (PNG) file.

1. Select the **See Results** tab of an open project.

2. Adjust the plot display settings as needed.

3. Click **View as image**, then click **Save As** in the image dialog box.

The software automatically saves the image as a PNG file using the default file name (Result_Plot_<project name>.png) and browser downloads location.

4. Close the image dialog box.

Print the Results plot

You can print an image of the plot shown in the See Results tab.

1. Select the **See Results** tab of an open project.

2. Adjust the plot display settings as needed.

3. Click **View as image**, then click **Print** in the image dialog box.

The software automatically opens the image in a new Untitled tab.

4. In the Print dialog box, select the printer and print options, then click **Print**.

Note: Make sure to close the Untitled tab when the print is complete.

5. Close the image dialog box.



Manage Projects, Properties, and Display Settings

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Manage projects

You can create new projects and manage existing projects in the software from the Project Listing screen, which shows all of the projects that are present in a selected analysis module.

View the list of projects

To view the Project Listing screen, first log into the software then:

- Select an analysis module from the QuantStudio™ 3D AnalysisSuite™ Software homepage.
- Click  (Home) at the top of any screen in an open project.

Note: To select a different analysis module, click **QuantStudio™ 3D AnalysisSuite™** at the top of any screen in the software.

Create a project

1. In the Project Listing screen, click **Create project**.

2. Enter a unique project name up to 100-characters in length.

The project name:

- Should be descriptive and easy to remember.
- Cannot contain the following characters:
% * ? | ; : , ! @ # \$ () < > / \ " ' ` ~ [] { } = & ^ -
- Cannot end with a period (.)

3. Click **OK**. The software saves the project.

4. In the Import Data tab, import data into the project as needed.

Open a project

1. In the Project Listing screen, navigate to and select a project name in the Projects table to open it.

Note: By default, a project opens to the Project Dashboard tab. Click the **Import Chips** link to import chips to an empty project.



2. Manage the project properties as needed.

Copy a project

You can create a copy of an existing project for use with the same Life Technologies cloud account.

1. Open the project to be copied (see “Open a project” on page 46).
Note: Make sure to note down the original name for later use.
2. Rename the project (see “Rename a project” on page 48).
3. Export the renamed project from the software (see “Export projects” on page 45).
4. Rename the open project using the original project name noted in step 1.
Note: Project names in the software must be unique.
5. Import the renamed project into the software (see “Import a project” on page 47).
The software shows both project names (the original and the copy) in the Project Listing screen for the logged in user.

Import a project

You can use the Project Listing screen to import projects from another location or under a different name. Only files of type .las can be transferred to the AnalysisSuite™ Software.

1. In the Project Listing screen, click **Import project**, then navigate to and select the project (.las) file you wish to import into the software.

IMPORTANT! Make sure the project you select to import has a unique project name (see “Rename a project” on page 48).
2. Click **Open** to add the project to the Projects table.

Manage project properties

View the project summary

The Project Dashboard tab contains a summary of the latest information about a project. You can review the project level notifications and results, and navigate to detailed setup and results screens from this tab.

1. Select the **Project Dashboard** tab of an open project, if not already selected.
Note: By default, a project opens to the Project Dashboard tab. Click the **Import Chips** link to import chips to an empty project.
2. View a summary of the latest information about the project:
 - **Notifications** – Displays the data flags and comments for the project. From here, you can add new comments or reply to existing comments.
 - **Results to date** – Plots the analysis results for all chips in the project. Click **See details** to navigate to the See Results tab and review the results in more detail.



- **Description** – Optionally, click to enter a description for the project if none is displayed in this field. The project description is included in the export of project data.

- **Targets or Assays, Samples, Dilutions, and Chips** – Displays the chips and chip settings associated with the project. Click **See details** next to a setting to navigate to the Define Chips tab and review the settings in more detail.

3. Optionally, edit the following project properties from this tab:
 - Add a comment, or read and reply to comments in the project.
 - Enter a description for the project.

Rename a project

You can rename a project at any time (for example, if you wish to create a copy of an existing project).

1. Click **Edit project name** from any screen of an open project.
2. Enter a unique project name up to 100-characters in length.
The project name:
 - Should be descriptive and easy to remember.
 - Cannot contain the following characters:
% * ? | ; : , ! @ # \$ () < > / \ " ' ` ~ [] { } = & ^ -
 - Cannot end with a period (.).
3. Press **Enter**.

Add comments to a project

The Add Comments dialog box allows you to enter detailed information about the project (for example, observations about the data, reasons why you made specific decisions, and so on). You can add comments to a project at any time. You may prefer to enter comments after viewing and analyzing the data.

1. Click **Add comment** in the Notifications section of the Project Dashboard tab, or at the top of any other screen in an open project.
2. Enter up to 255 characters to associate with the project, then click **POST**.

Note: After you click **POST**, the comment is time stamped and permanently recorded in the project, and it cannot be modified or removed.

3. (Optional) In the Notifications section of the Project Dashboard tab, click **Read/Reply** and repeat step 2 to respond to a posted comment.

Note: You can also add an editable comment to any chip in the project from the Define Chips tab.



Manage project display settings

Apply a filter

You can filter the chip data shown in the Import Data and Review Quality tabs as follows (for example, to show fewer chips or to view specific chip results):

- **Filter the chips to import** – In the Import Data tab, click **Show Filters**, then:
 - **Show by import state** – Select **Chips not imported** yet to view the remaining chips available for import into the project. Select **All Chips** to remove the filter criteria.
 - **Filter by run date** –
 - Select the **Run in the past** checkbox, then enter the run date settings for the chips to import. Deselect the checkbox to remove the filter criteria.
 - Select the **Date range of run** checkbox, then click  to select a data range, or enter a data range for the chips to import. Deselect the checkbox to remove the filter criteria.

The software automatically updates the chip data shown in the Import Data tab according to your filter criteria.

- **Filter the chip quality data** – In the Review Quality tab, click **Show Filters**, then:
 - **Search for a specific chip** – Enter all or part of the chip name you wish to view, then click **Go**. Click **Clear** to remove the filter criteria.
 - **Sort the chip results** – Select to sort by **Flagged chips** or **Recently ran chips**.

The software automatically updates the chip data shown in the Review Quality tab according to your filter criteria.

Change the display of table data

You can use any of the following settings to change the display of table data in an open project, if available:

- **Show/hide columns** – Click  to the right of a column heading, then select the contents to show or hide in the table from the **Columns** drop-down menu.
- **Sort column entries** – To sort column content:
 - Click once on a column heading to sort the rows in the table according to the content in the column. You can change the sorting order with a second click. A third click will clear the sorting order.
 - Click  to the right of a column heading, then select to **Sort Ascending** or **Sort Descending** from the drop-down menu.
- **Change column order** – Click a column heading and drag the column to the desired position.
- **Group column entries** – Drag a column heading above the table to group the table data by the selected attribute. To create a hierarchical grouping, repeat this process with additional columns, then:
 - Click  to expand all entries for a group and click  to collapse the entries.
 - Click  to sort the table data according to the selected group. You can change the sorting order with a second click. A third click will clear the sorting order.



- Click  to remove the group.
- **Adjust the viewable area of a table section** – Click-drag the split line to adjust the viewable area of each table section.



About the Software Quality Metrics

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About chip quality

The software assesses whether the data on a chip is reliable based upon loading, signal, and noise characteristics and displays quality indicators for each chip in a project:

- **Quality threshold** – A measure of the individual well quality for a chip, on a continuous scale from 0 (low quality) to 1 (high quality). The default quality threshold is 0.5. You can adjust this threshold to filter the wells (data points) to include or exclude from analysis.
- **Quality flag** – A measure of the overall chip quality, based on well quality thresholds and other data analysis characteristics (see “About data quality flags” on page 51). Use to determine which chips to review.

About data quality flags

Data quality flags generated and displayed by the software are a measure of the overall chip quality, based on well quality thresholds and other data analysis characteristics.

- No flag – Review of the analysis results is not required. A flag is not shown in the software (or it is shown as  (green) on the instrument touchscreen) if the data meets all quality thresholds.
-  (yellow) – Review of the analysis results is suggested. The flag is shown if the:
 - Instrument cannot clearly identify the population of unamplified wells.
 - Distribution of unamplified wells on the chip is not uniform.
 - Sample concentration is outside the optimal range (200-2000 copies/µL) currently defined by the Life Technologies chip quality metrics.
-  (red) – Review of the analysis results is strongly suggested. This flag is shown if the total number of filled wells is below the default threshold of 5000 or the percentage of low quality wells is above the default threshold of 5%.
-  (yellow) or  (red) – Indicates that a user has modified the analysis results originally generated by the instrument software.

Note: To revert an edited flag to its default state, delete the associated chip from the project, then import it back into the project.

Note: For single reporter experiments, the software displays a quality flag if it is associated with the selected target dye. For dual reporter experiments, the software displays the poorest quality flag associated with the target dyes.



Troubleshooting

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Troubleshooting quality flags in the software

The following table summarizes some of the more common problems that can trigger a data quality flag as viewed in the AnalysisSuite™ Software. See Appendix B, “About the Software Quality Metrics” for more information about the data quality assessment process used by the software.

Observation	Possible Cause	Action
Chip displays ! flag.	The total number of filled wells is below the default threshold of 5000 or the percentage of low quality wells is above the default threshold of 5%.	Look for any visible chip failures (for example, leaking or a large bubble, liquid on the chip lid, cracked chip, and so on): <ul style="list-style-type: none">• If present – Discard the chip and run the sample again.• If not present – Re-image the chip and import the new experiment file into the software. If the chip still displays a ! flag, discard the chip and run the sample again.
Chip displays ! flag.	The sample concentration is outside of the optimal range (200-2000 copies/µL) currently defined by the Life Technologies chip quality metrics.	Run the sample again at a higher dilution. If the chip still displays a ! flag, then troubleshoot the chip image in the software (see “Troubleshooting chip images using the Chip View” on page 54). Note: Refer to the dilution recommendation shown in the Comment column of the Results tab.

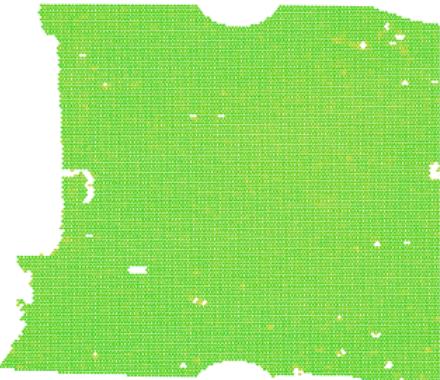
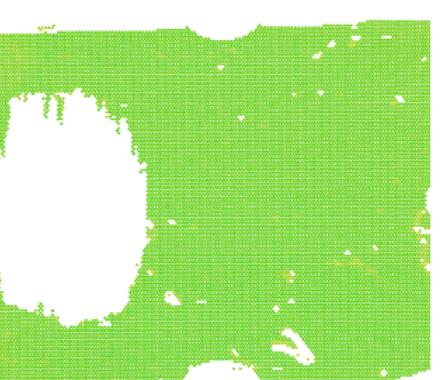
Observation	Possible Cause	Action
Chip displays  flag. <i>(continued)</i>	The instrument cannot clearly identify the population of unamplified wells.	<p>Review the data in the Review Quality tab of the Absolute Quantification module. In the Histogram view, if you observe:</p> <ul style="list-style-type: none"> • A single peak (mono-modal) – Confirm that the call threshold is on the appropriate side of the peak based on the chip settings (for example, NTC or low dilution). Manually adjust the call threshold to improve the call assignment accuracy, if necessary. • Two peaks (bi-modal) with poor separation or more than two peaks – <ul style="list-style-type: none"> – Increase the quality threshold to create a more bi-modal distribution, if possible. Make sure to balance the number of data points included in the analysis against the desired data quality and call separation. – Manually adjust the call threshold to improve the call assignment accuracy. If this is not possible, omit the chip from the results. <p>Note: If you set up both target dyes for the chip, review the data in the scatter plot view of the Relative Quantification module and verify that calls are clearly separated in the plot.</p>
	The distribution of unamplified wells on the chip is not uniform.	<p>Review the data in the Chip view of the Review Quality tab. Select the Color by calls data point color option, then:</p> <ol style="list-style-type: none"> 1. Verify that calls are randomly distributed across the chip. 2. Increase the quality threshold to adjust the negative call distribution across the chip, if possible. Make sure to balance the number of data points included in the analysis against the desired data quality and call distribution. 3. (Optional) If calls are still not randomly distributed across the chip, omit the chip from the results.

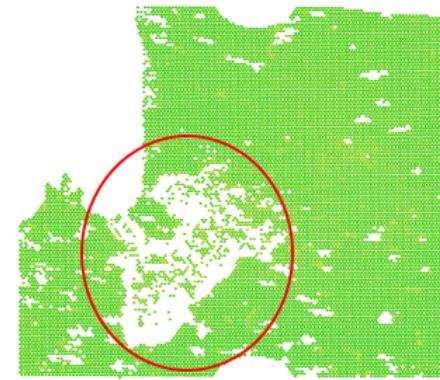
Troubleshooting chip images using the Chip View

The following table summarizes some of the more common problems that can affect imaging of Digital PCR 20K Chips.

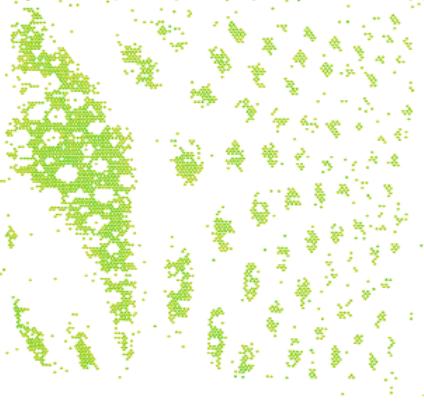
Note: In the examples shown below, the conditions triggered a data flag in the Digital PCR 20K Chip and the conditions were diagnosed using the Chip View of the AnalysisSuite™ Software.



Observation	Possible Cause	Action
<p>Chip view</p> 	<p>Dust or other debris are present on the Chip Sealant during imaging.</p>	<p>No action required. The AnalysisSuite™ Software can compensate for small quantities of dust and debris on the Digital PCR 20K Chip.</p>
<p>Chip view</p> 	<p>A bubble was present in the Sample Loading Blade when it was used to apply the PCR reaction to the Digital PCR 20K Chip.</p>	<p>If possible, use the AnalysisSuite™ Software to filter the low quality data points, or discard the chip and run the sample again.</p> <p>When loading the Sample Loading Blades:</p> <ul style="list-style-type: none"> • If you are using a manual pipette, pipette to the first stop. • If you are using an electronic pipette, decrease your pipetting speed. • If a bubble does form in the Sample Loading Blade, gently tap it to remove bubble before loading.

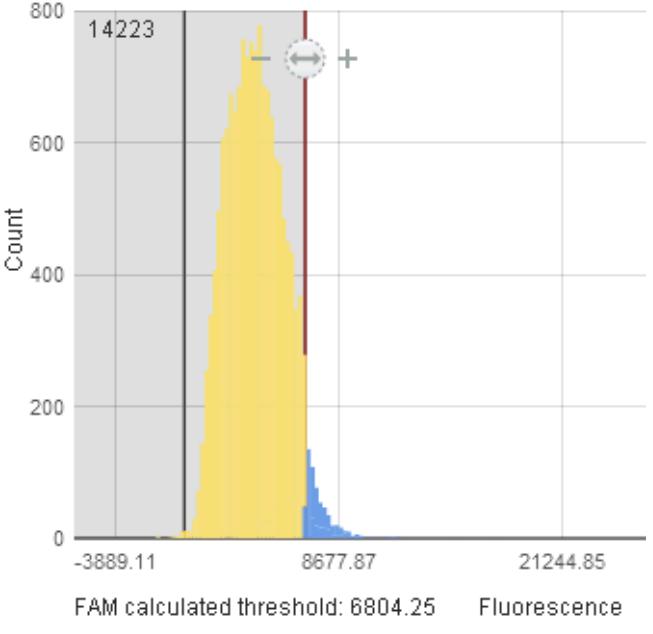
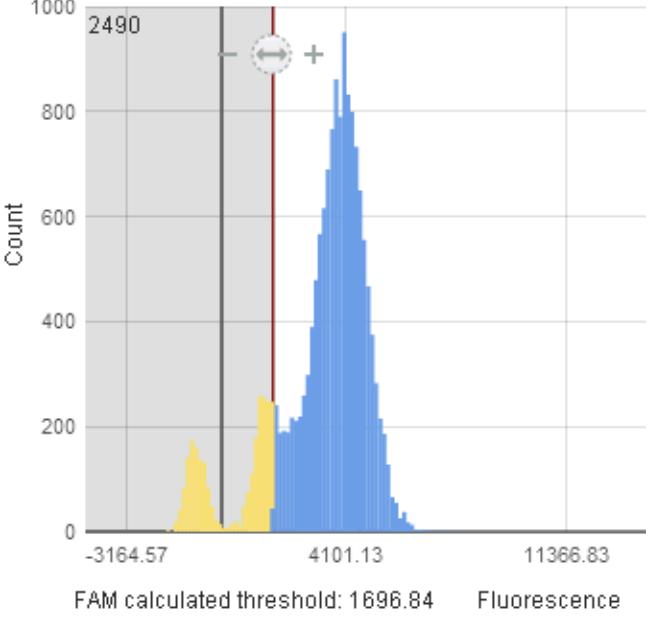
Observation	Possible Cause	Action
<p>Chip view</p> 	<ul style="list-style-type: none"> Excess PCR reaction was present on the Digital PCR 20K Chip after loading it with the Sample Loading Blade. The Sample Loading Blade was drawn across the chip too quickly or at an angle shallower than 70-80°. 	<p>If possible, use the AnalysisSuite™ Software to filter the low quality data points, or discard the chip and run the sample again.</p> <p>To prevent leaving excess PCR reaction on the Digital PCR 20K Chips:</p> <ul style="list-style-type: none"> Confirm that the heated block used to load Digital PCR 20K Chips is set to $40\pm1^\circ\text{C}$. Make sure to wait 20 seconds after loading a Digital PCR 20K Chip before pre-wetting it with Immersion Fluid. Make sure to install the Tilt Base to the GeneAmp® PCR System 9700. You must thermal cycle the Digital PCR 20K Chips at a 11° angle. Make sure to draw the Sample Loading Blade across the chip slowly (>10 seconds) and at a $70\text{-}80^\circ$ angle.
<p>Chip view</p> 		



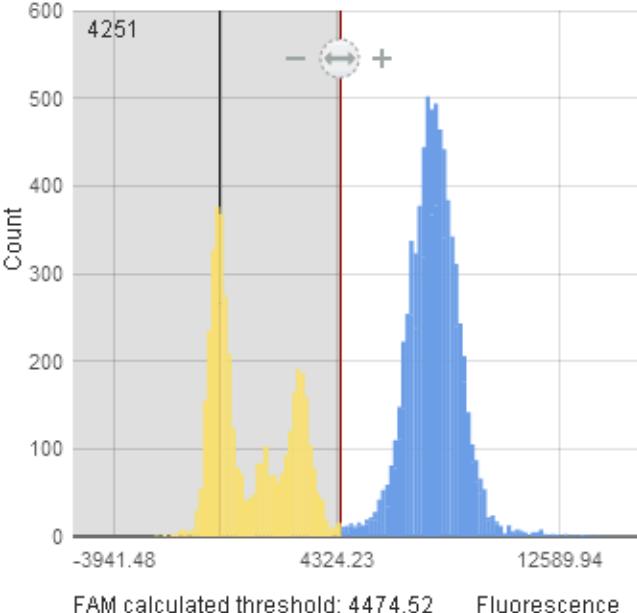
Observation	Possible Cause	Action
<p>Chip view</p> 	<ul style="list-style-type: none">The Digital PCR 20K Chip leaked during thermal cycling or imaging.A large bubble was present in the chip (insufficient Immersion Fluid).Immersion Fluid was not applied to the chip immediately after loading (evaporation of the PCR reaction).Excess Immersion Fluid is present on the Chip Case Lid.	<p>If present, remove excess Immersion Fluid from the chip lid and run the chip again.</p> <p>If possible, use the AnalysisSuite™ Software to filter the low quality data points.</p> <p>Make sure to apply Immersion Fluid to each chip immediately after loading it with PCR reaction.</p> <p>To minimize leakage, when sealing each Digital PCR 20K Chip:</p> <ul style="list-style-type: none">Wear correctly fitted gloves to prevent the glove material from snagging during lid application.Make sure that the Chip Case Lid is correctly aligned to the Chip Case.Firmly press all four corners when applying the Chip Case Lid.Cure the Chip Sealant under ultraviolet light for at least 30 seconds.
<p>Chip view</p> 		

Troubleshooting analysis results

The following table summarizes some of the more common problems that can affect the analysis results for a project as viewed in the AnalysisSuite™ Software. In some of the examples shown below, the conditions triggered a data quality flag.

Observation	Possible Cause	Action
Mono-modal (single) peak observed in Histogram view of an Absolute Quantification project. 	PCR reaction does not contain template (is a negative control). PCR reaction is missing template. Concentration of sample is too high.	No further action required. Discard the chip and run the sample again. Discard the chip and run the sample again at a higher dilution.
Poor peak separation observed in Histogram view of an Absolute Quantification project. 	Acceptable performance for the selected assay. Excess PCR reaction present on the chip after loading. Low efficiency assay.	No further action required. Discard the chip and run the sample again. Adjust thermal cycling conditions or use a different assay.



Observation	Possible Cause	Action
<p>Multi-modal (more than two) peaks observed in Histogram view of an Absolute Quantification project.</p>  <p>Count</p> <p>4251</p> <p>400</p> <p>300</p> <p>200</p> <p>100</p> <p>0</p> <p>-3941.48 4324.23 12589.94</p> <p>FAM calculated threshold: 4474.52 Fluorescence</p>	<p>Low efficiency assay.</p>	<ul style="list-style-type: none"> • Increase the quality threshold to create a more bi-modal distribution, if possible. Make sure to balance the number of data points included in the analysis against the desired data quality and call separation. If this is not possible, omit the chip from the results. • Adjust thermal cycling conditions or use a different assay.
<p>The See Results tab does not display numerical results after combining multiple chips with the same data group (sample and target or assay combination).</p>	<p>The software cannot calculate numerical results for one or more of the chips in the data group. For example, the sample concentration is either too high or too low.</p>	<p>Omit the chip(s) that do not contain numerical results from the project.</p>
<p>The calculated call threshold does not update in the Review Quality tab after increasing the quality threshold.</p>	<p>The number of data points is less than the minimum threshold of 5000.</p>	<p>Decrease the quality threshold to include more than 5000 data points.</p>

Documentation and Support

Related documentation

Document	Publication number	Description
<i>QuantStudio™ 3D Digital PCR System User Guide</i>	MAN0007720	Describes the QuantStudio™ 3D Digital PCR System hardware and software and provides information on preparing, maintaining, and troubleshooting the system.
<i>QuantStudio™ 3D Digital PCR System Quick Reference</i>	MAN0008159	Describes the QuantStudio™ 3D Digital PCR System hardware and software and provides brief, step-by-step procedures for common tasks.

Portable document format (PDF) versions of this guide and related documentation are available at www.lifetechnologies.com/quantstudio3D.

Note: To open the user documentation, use the Adobe® Reader® software available from www.adobe.com

Note: For additional documentation or if you cannot access the user documentation, see “Obtaining support” on page 60.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

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