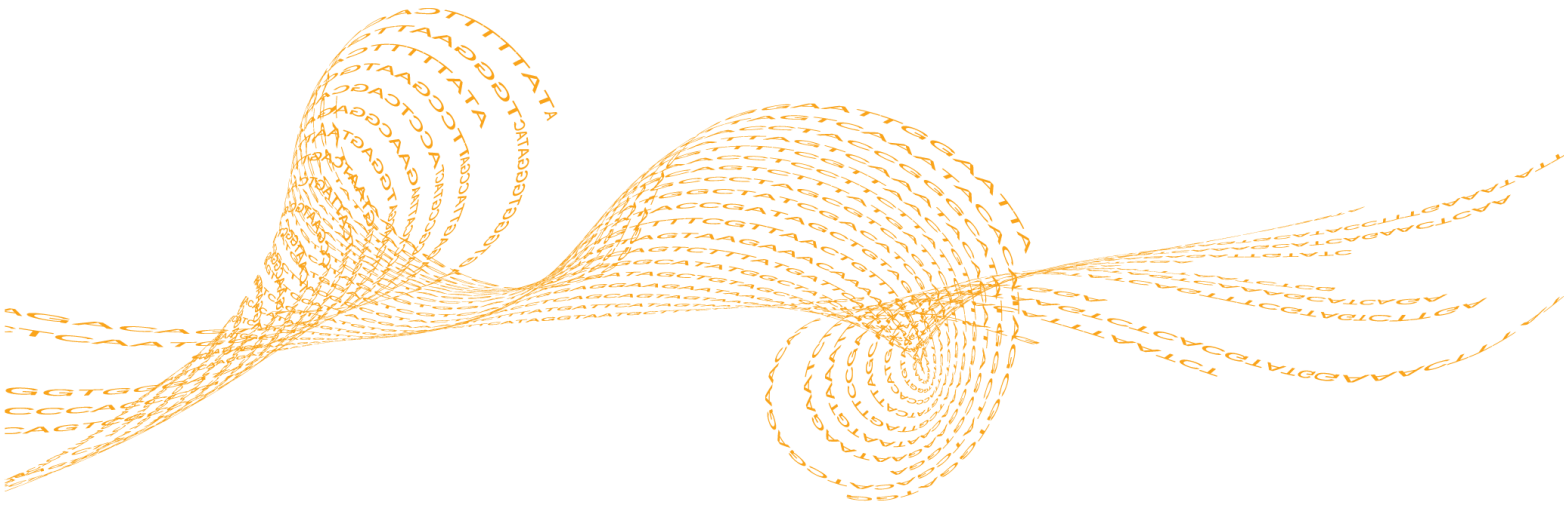
EcoStudy Software

User Guide

FOR RESEARCH USE ONLY



ILLUMINA PROPRIETARY
Part # 15030781 Rev A
February 2012

illumina®

What is EcoStudy?

The EcoStudy software enables you to combine data from multiple Eco experiments into a study for analysis. Currently, only Standard Curve and Relative Quantification experiments are supported.

NOTE
Illumina does not recommend combining experiments from multiple instruments because differences in raw fluorescence values between instruments might increase the systematic error in your measurements.

Requirements for a Standard Curve Study

To ensure successful analysis, a Standard Curve study must contain:

- One or more experiments of the same type, with the same thermal profile (except for melt profile) and number of thermal cycles
- One mother plate (if the study includes more than one experiment)
- At least two standards with different values
- One or more plate control wells in each plate

Requirements for a Relative Quantification Study

To ensure successful analysis, a Relative Quantification study must contain:

- One or more experiments of the same type, with the same thermal profile (except for melt profile) and number of thermal cycles
- One mother plate (if the study includes more than one experiment)
- One or more plate control wells in each plate
- One reference sample against which the target (non-reference) samples will be analyzed
- At least one target (non-reference) assay

Setting Up a Study

Setting up a study is a three-part process, described in detail in the following sections of this document.

- First, you must add one or more experiments to a study. The experiments must be of the same type (Standard Curve or Relative Quantification), have the same thermal profile (except for melt profile), and have the same number of thermal cycles.
- Next, for studies containing more than one experiment, you designate one experiment as the mother plate. The mother plate is the plate against which the other experiments in the study will be compared. EcoStudy automatically selects one experiment to serve as the mother plate. However, you can change this setting to designate any other experiment as the mother plate.
- Finally, you designate at least one well on the mother plate to serve as a plate control. Once assigned on the mother plate, EcoStudy automatically identifies wells containing the same assays, reporter dyes, and sample name on the other plates in your study. During analysis, the EcoStudy software uses the Cq data from the plate control wells identified on each plate as a basis for comparing values across plates.

Adding an Experiment

When you start the EcoStudy software, it opens to a new (blank) study. To analyze data from multiple experiments in the study, you must add two or more Eco experiments of the same type (Standard Curve or Relative Quantification), with the same thermal profile (except the melt profile) and number of thermal cycles.

1 Select **File** | **Add Experiment**.

TIP
You can also add experiments by dragging-and-dropping experiments onto the EcoStudy software or by right-clicking the Experiment list on the Amplification Plot, Melt Curve, or Results tab and selecting **Add Experiment**.

2 Navigate to the *.ecod experiment data files you want to include in your study and click **Open**.

TIP
If the experiment files are saved in the same folder, you can Ctrl- or Shift-click to select multiple files for inclusion in your study at once.

3 Repeat this procedure until all desired experiments are added to your study.

NOTE
EcoStudy automatically selects one experiment to serve as the *mother plate*. The mother plate is the plate against which the other experiments in the study will be compared. You can change this setting to designate any other experiment as the mother plate. For more information, see *Designating the Mother Plate* on page 5.

NOTE
If one or more of the experiments you selected is of a different type, has a different thermal profile, or has a different number of thermal cycles than the first experiment added to the study, an error message opens for each unsupported experiment. Click **OK** through each error message. EcoStudy will add any remaining supported experiments to your study.

Designating the Mother Plate

Every study must have one experiment designated as the *mother plate*. The mother plate is the plate against which the other experiments in the study will be compared. The other experiments in the study are referred to as *daughter plates*.

1 Click the **Plate Layout** tab.

2 From the Experiments drop-down menu, select the experiment you want to use as the mother plate for your study.
- For Standard Curve studies, the mother plate must have at least two wells with the role Standard, but with different quantities.
- For Relative Quantification studies, any plate can be used as the mother plate. However, the mother plate must have at least one well that meets the criteria to serve as a plate control. For more information on plate control requirements, see *Designating Plate Control Wells in your Study* on page 5.

3 Click the **Mother Plate** check box.
The previously designated mother plate is demoted to a be a daughter plate.

NOTE
If you had a plate control well designated on the previous mother plate, it is cleared and a new plate control will need to be assigned on the new mother plate. For more information, see *Designating Plate Control Wells in your Study* on page 5.

Designating Plate Control Wells in your Study

At least one well on the mother plate must be designated as the *plate control*. Once assigned on the mother plate, EcoStudy automatically identifies wells containing the same assays, reporter dyes, and sample name on the other plates in your study. During analysis, the EcoStudy software uses the Cq data from the plate control wells identified on each plate as a basis for comparing values across plates.

1 Click the **Plate Layout** tab.

2 From the Experiments drop-down menu, select the experiment designated as the mother plate for your study.

3 Right-click the desired well on the plate layout diagram and select **Assign as Plate Control**.
- For Standard Curve studies, the well you designate as the plate control must have an assay with the role **Standard**, **Unknown**, or **Positive**, as well as have a sample assigned. Additionally, the plate control well must not be excluded from the study.
- For Relative Quantification studies, the well you designate as the plate control must have an assay with the role **Unknown** or **Positive**, as well as have a sample assigned. Additionally, the plate control well must not be excluded from the study.

Wells containing the same assays, reporter dyes, sample name, and assay names are labeled **Plate Control** on the plate layout diagram for every experiment in your study.
On the other tabs in the EcoStudy software, plate control wells are indicated by the C icon in the Well Table.

Specifying Analysis Settings for your Study

As you change settings in your study, including adding experiments and changing the mother plate and plate control designations, the EcoStudy software automatically re-analyzes your data using the default baseline, threshold, and Cq variation values. If you want to use values other than the default settings, you can specify so in the Analysis Settings dialog box, as described in the following sections.

Analyzing a Standard Curve Study

1 Click the **Analysis Settings** button.

2 On the Cq Settings tab, for each assay, do the following:

a Determine whether you want to specify the baseline start and end values or allow the EcoStudy software to auto-calculate those values.

If you specify the baseline **Start** and **End** values yourself, you can use any numeric value between 1 and the total number of cycles in your study. The baseline **End** value must be equal to or higher than the baseline **Start** value.

b Determine whether you want to specify the threshold or allow the EcoStudy software to auto-calculate the value.

If you specify the Threshold value yourself, you can use any numeric value above 0 (zero).

NOTE
For more information on the auto-baseline and auto-threshold algorithms, see the Eco Real-Time PCR System User Guide.

c Specify the desired Cq Variation value.

If a plate control well in your study has a Cq Variation value above the one you specify, it is indicated by a icon in the Well Table, so you can exclude that well from the analysis, if desired. To exclude the well, right-click it in the Well Table and select **Exclude**.

3 Click **OK**.

EcoStudy re-analyzes your data using the new analysis settings.

Analyzing a Relative Quantification Study

1 Click the **Analysis Settings** button.

2 On the RQ Settings tab, do the following:

a From the **RQ Analysis Method** drop-down menu, specify the desired analysis method: **Singleplex** or **Multiplex**.

— If your study includes only singleplexed wells, the drop-down menu only includes that one option.

— If your study includes both singleplexed and multiplexed wells, both options are available. Only the wells matching your selection will be analyzed. The other wells will be omitted from the analysis.

b From the **RQ Algorithm** drop-down menu, specify the desired normalization method: **ΔΔCq** or **Efficiency Correction**.

The ΔΔCq method is supported only if you have a single reference gene and the amplification efficiencies are equal and at 100%. If your study does not meet these requirements, you must use the Efficiency Correction method. For

more information on these methods, see the Eco Real-Time PCR System User Guide.

c From the **Reference Sample** drop-down menu, select one sample to be used as the reference sample.

d For each assay, specify:

- **PCR Efficiency %**: can be any numeric value between 0-200%
- **Error %**: can be any numeric value greater than or equal to 0 (zero)

e Select the **Reference Assay** check box for every assay you want to use as a reference assay.

You must leave at least one assay unchecked to be used as the target assay.

3 On the Cq Settings tab, for each assay, do the following:

a Determine whether you want to specify the baseline start and end values or allow the EcoStudy software to auto-calculate those values.

If you specify the baseline **Start** and **End** values yourself, you can use any numeric value between 1 and the total number of cycles in your study. The baseline **End** value must be equal to or higher than the baseline **Start** value.

b Determine whether you want to specify the threshold or allow the EcoStudy software to auto-calculate the value.

If you specify the Threshold value yourself, you can use any numeric value above 0 (zero).

NOTE
For more information on the auto-baseline and auto-threshold algorithms, see the Eco Real-Time PCR System User Guide.

c Specify the desired Cq Variation value.

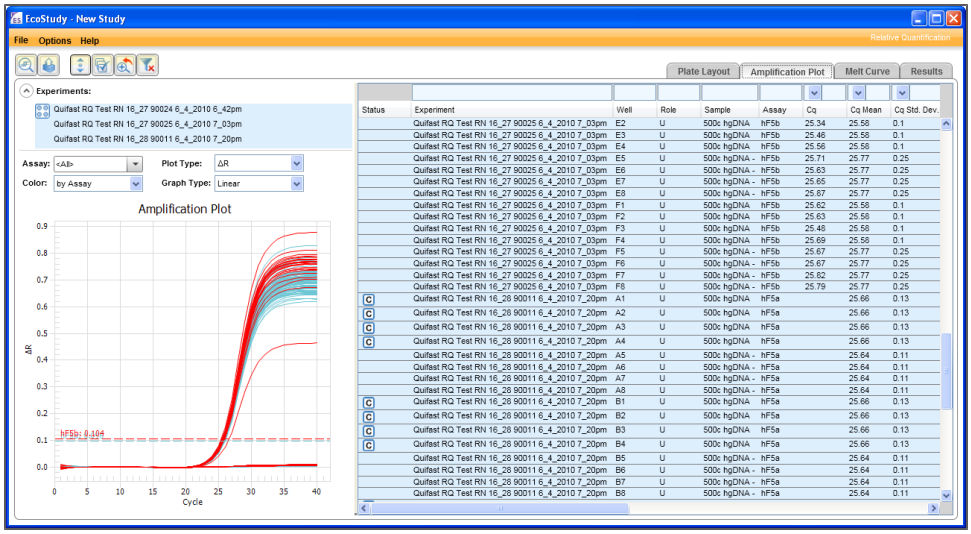
If a plate control well in your study has a Cq Variation value above the one you specify, it is indicated by a  icon in the Well Table, so you can exclude that well from the analysis, if desired. To exclude the well, right-click it in the Well Table and select **Exclude**.

4 Click **OK**.

EcoStudy re-analyzes your data using the new analysis settings.

Reviewing the Data in your Study

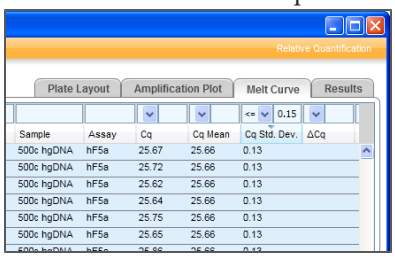
The data in your study can be viewed graphically or in a table on the Amplification Plot, Melt Curve, and Results tabs in the EcoStudy software. The example here shows the Amplification Plot tab for a Relative Quantification study containing three experiments.



Well Table

The Well Table appears on the Amplification Plot, Melt Curve, and Results tabs. The columns in the Well Table vary depending on your type of study. Using the Well Table settings, you can control what data are included in the Amplification Plot, Melt Curve, and Results graphs.

- Select specific rows in the Well Table to display the data in the graph.
  By default, all rows are selected (highlighted in yellow) and so data for all wells in your study are shown in the graph.
- Hover over a row in the Well Table to highlight it in the graph.
- Specify filter criteria in the empty fields above each column to find data more easily.
  In this example, the Well Table is filtered to show only wells with a Cq Standard Deviation less than or equal to 0.15.

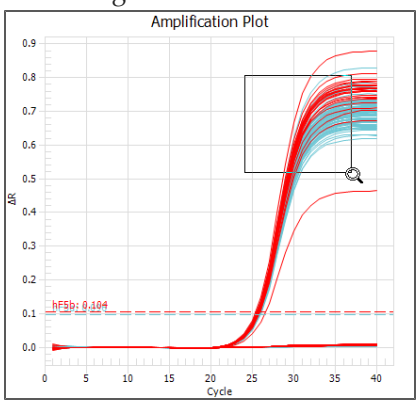


  To clear the filter and return to the full data set, click the **Clear Filter** button.
- Click a column heading to sort the Well Table by the data in that column.
- Select the **Disable Auto Scroll** button to stop the Well Table from scrolling vertically as you highlight data in the graph.

Amplification Plot

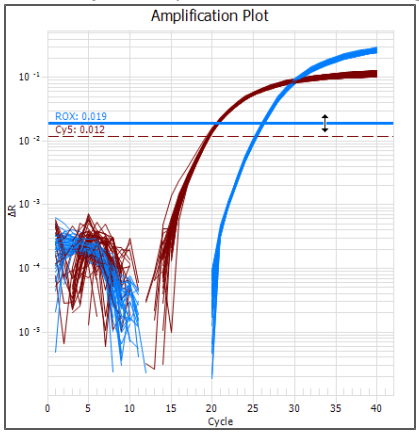
The Amplification Plot displays the normalized or baseline-subtracted fluorescence signal for a well at each cycle. Using the Amplification Plot settings, you can control how data are displayed in the Well Table and graph.

- Hover over a line in the Amplification Plot to highlight it in the Well Table.
- From the **Experiments** list, select one or more experiments to limit what data appear in the graph and Well Table.
- From the **Assay** drop-down menu, select one or more assays to limit what data appear in the graph and Well Table.
- From the **Plot Type** drop-down menu, specify whether you want to show normalized (R) or baseline-subtracted (ΔR) data in the graph.
- From the **Graph Type** drop-down menu, specify whether you want to show the data using a logarithmic or linear scale.
- Click-drag the mouse over an area of the graph to zoom in on that area.



To clear the zoom and return to the full view of the graph, click the **Undo Zoom** button.

- Drag the threshold line for an assay up or down on the graph to change the analysis settings without opening the Analysis Settings dialog box. The threshold can only be adjusted when viewing the data on a log scale.

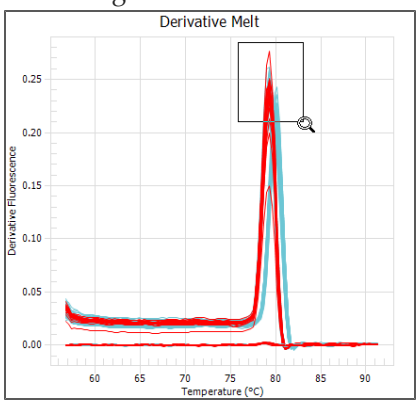


- If you turned off the Auto Baseline option in the Analysis Settings dialog box, you can drag the baseline start and end point indicators for an assay left or right on the graph to change the baseline settings.

Melt Curve

The Melt Curve graph displays the fluorescence signal or derivative fluorescence signal for a well at every 0.3° C (0.54° F) of melting. Using the Melt Curve settings, you can control how data are displayed in the Well Table and graph.

- Hover over a line in the Melt Curve graph to highlight it in the Well Table.
- From the **Experiments** list, select one or more experiments to limit what data appear in the graph and Well Table.
- From the **Assay** drop-down menu, select one or more assays to limit what data appear in the graph and Well Table.
- From the **Plot Type** drop-down menu, specify whether you want to show Raw or Derivative Melt data in th graph.
- Click-drag the mouse over an area of the graph to zoom in on that area.



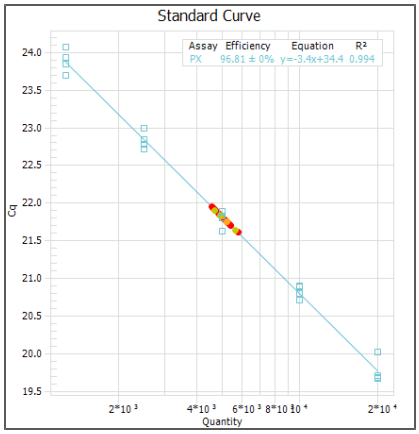
To clear the zoom and return to the full view of the graph, click the **Undo Zoom** button.

Results Tab

The Results graph displays the results of your study in one of the following formats, depending on the type of study you ran: Standard Curve or Relative Quantification.

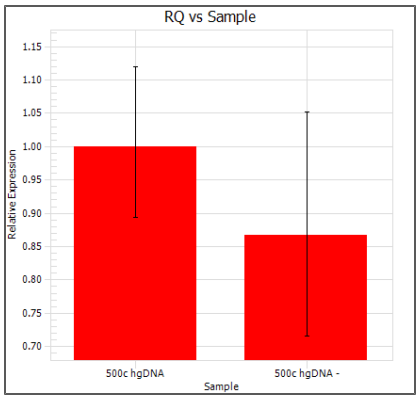
Standard Curve

Standard Curve studies generate a standard curve line graph. The slope, PCR efficiency and standard error percentage, and R^2 of that curve appear in the graph's legend. The standard curve is always shown, though you can decide which data points to include in the graph by selecting the appropriate rows in the Well Table.



Relative Quantification

Relative Quantification studies generate a bar graph that includes error bars. The displayed histogram can be toggled to group data by assay or by sample.



Exporting Study Data to a Report or Presentation

You can export the data from your study in a variety of formats, including comma- and tab-delimited text files, Microsoft Excel spreadsheet, and Microsoft PowerPoint.

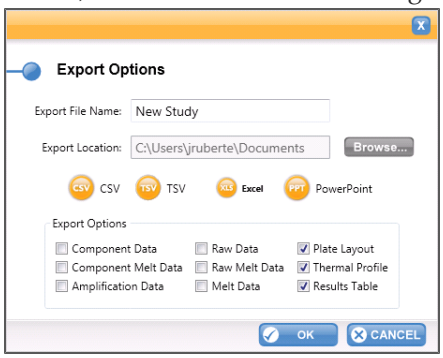
1 Click the **Export** button.

2 Type a name for the exported file.
The Export File Name field is pre-populated with the name of the study or, if you have not yet named the study, with the name **New Study**.

3 Click **Browse** and navigate to the folder where you want the exported data saved.

4 Select the format in which you want your data exported.

5 In the Export Options area, select the check box for each type of data you want exported.
The options available here vary depending on the type of study you ran and the file format you chose to output. If a type of data is not available, it appears grayed out in the Export Options area. In this example, the Plate Layout, Thermal Profile, and Results Table are being exported to Excel.



6 Click **OK**.
Your exported data opens in the appropriate software application for the file format you chose to export.

Technical Assistance

For technical assistance, go to http://www.illumina.com/qpcr.

MSDSs

Material safety data sheets (MSDSs) are available on the Illumina website at http://www.illumina.com/msds.

Product Documentation

If you require additional product documentation, you can obtain PDFs from the Illumina website. Go to http://www.illumina.com/support/documentation.ilmn.

When you click on a link, you will be asked to log in to My Illumina. After you log in, you can view or save the PDF. To register for a My Illumina account, please visit https://my.illumina.com/Account/Register.

Illumina
Headquartered in San Diego, California, U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North America)
techsupport@illumina.com
www.illumina.com