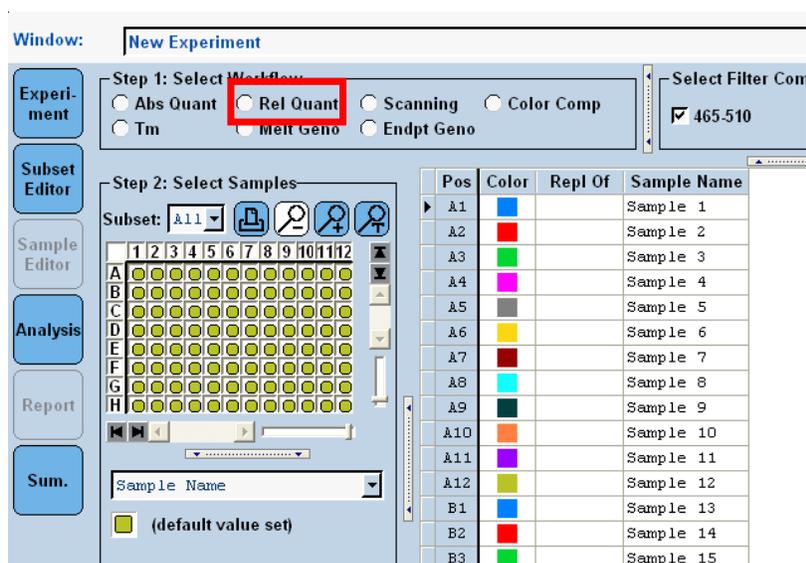


LightCycler® 480 Instrument Quick Guide

Relative Quantification

Performing a Relative Quantification Analysis:

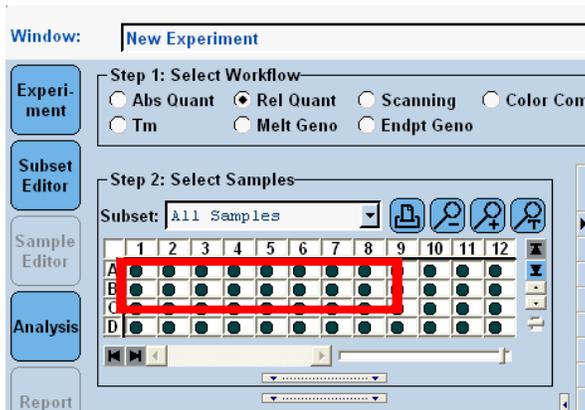
1. Open the experiment that you want to analyze in main window¹.
2. If sample information was entered during run setup then proceed to Step 5; otherwise click the **<Sample Editor>** object on the left of the active display.
3. In the **<Sample Editor>** select the Workflow **<Rel Quant>** under Step 1.



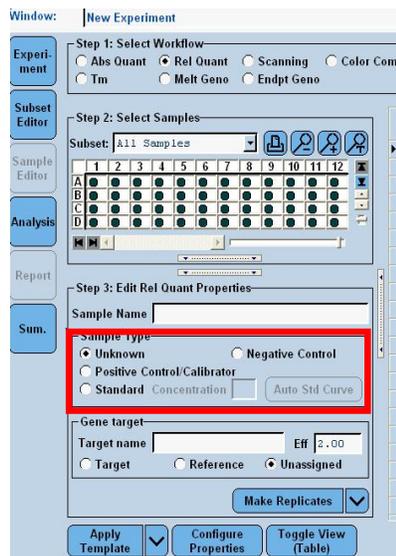
¹ If the experiment has just completed, the run will remain open and ready to analyze. Previously created and performed experiments will be located in the **<Experiments>** Folder of the Navigator.

Relative Quantification

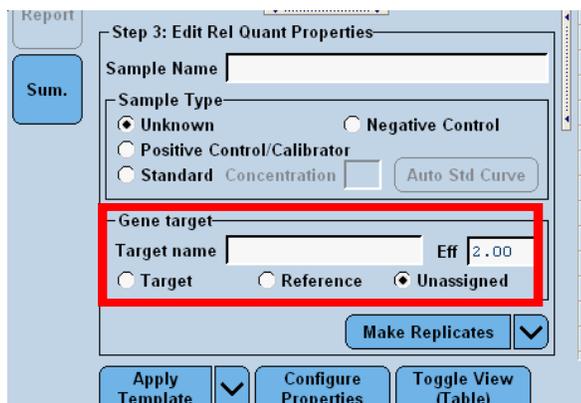
4. To enter the information for like samples:
 - a. Select control samples on the plate layout (these are the baseline or untreated samples).



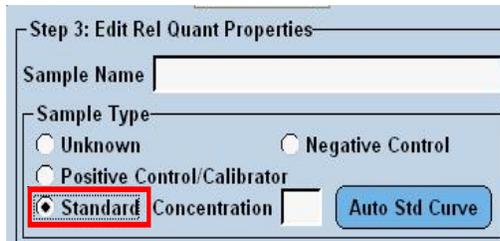
- b. Enter sample name and choose Sample Type <**Positive Control Calibrator**>.



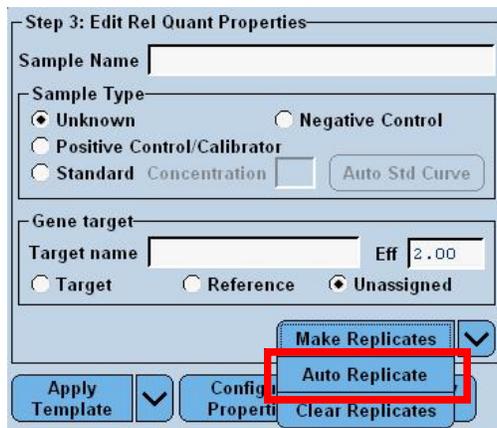
- c. Enter target name (e.g., name of the gene being amplified in the selected wells), and select gene target information related to the relative quantification analysis (identity gene target as “Target” or “Reference.” “Target” is the **gene of interest** and “Reference” is the **Housekeeping Gene** (e.g., actin).



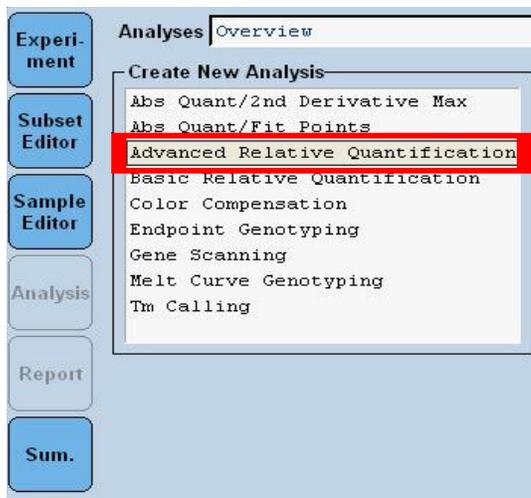
- d. For all other samples, enter sample name and choose Sample Type <Unknown or Negative Control> (refer to step f for setting up Standards).
- e. Enter gene name and define Target or Reference as described in step c.
- f. Click on wells with one standard concentration and enter sample names, concentration and click **Standard**. Repeat for other concentrations being used.



- g. Set replicates by clicking **Auto Replicate**.

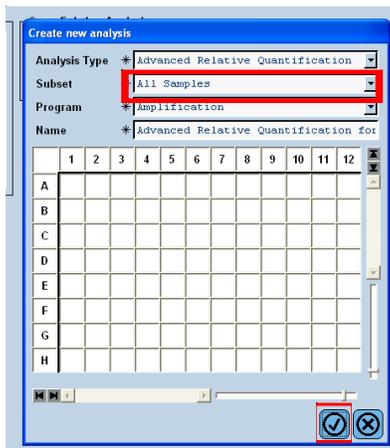


- 5. Click <Analysis>.
 - a. Select **Advanced Relative Quantification**.

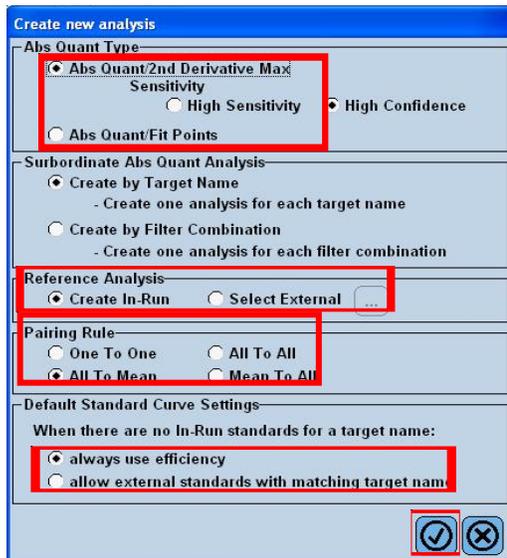


Relative Quantification

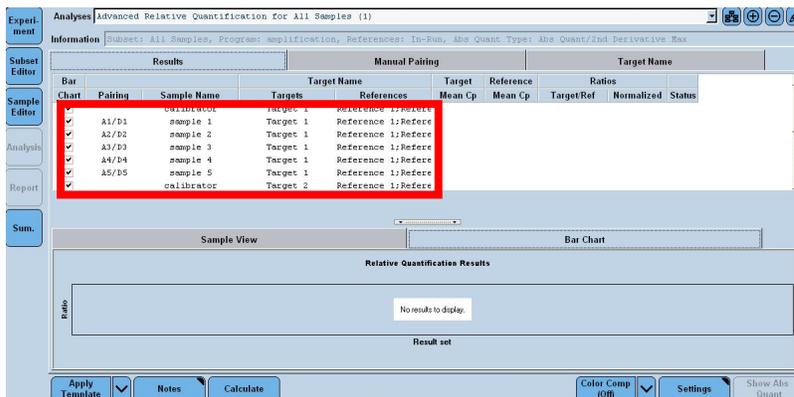
b. Select all samples or subset to be analyzed, and click the checkmark icon.



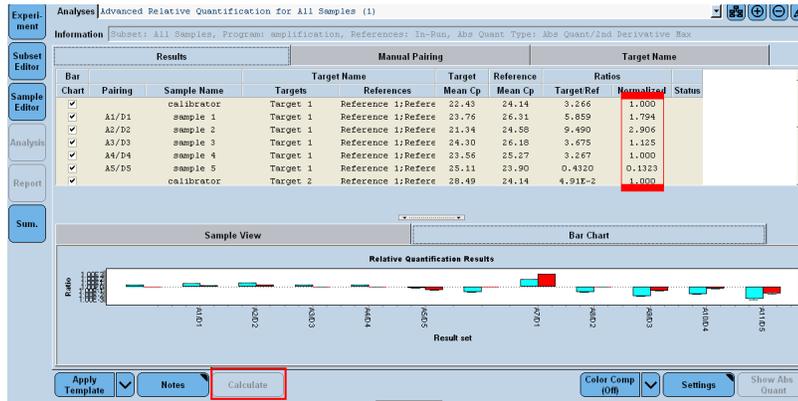
c. Select default settings and click the checkmark icon.



d. The software automatically matches the target to the corresponding reference.



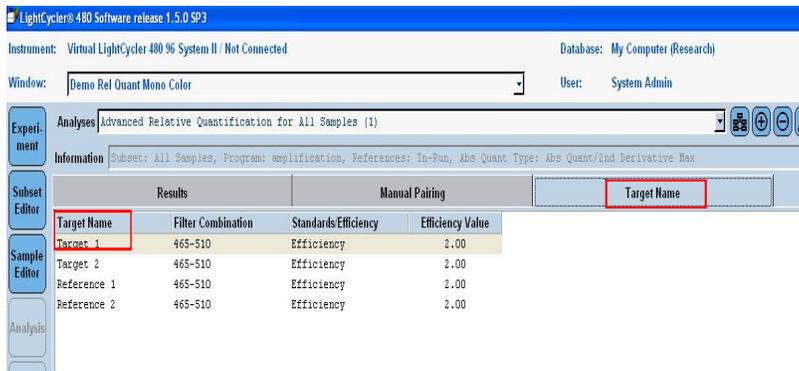
- e. To obtain results, click <Calculate>.
- If a calibrator sample was used, the column of interest is the normalized ratio which gives you the efficiency corrected normalized fold change of the sample over the calibrator control. A number of 2 in this column would mean a two-fold increase compared to the calibrator sample as the normal ratio.
 - If a calibrator sample was not used, then the Target/Ref Ratio column will give your results, comparing target to reference amounts within a single sample.



Note: All results are exportable to other programs. Export the graphics or data table by right-clicking the object and follow the export windows.

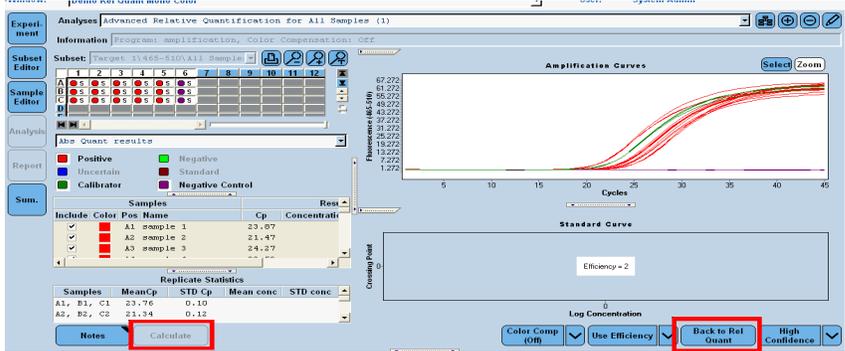
To export the Data, select the data by clicking on a row. Click <Ctrl> and <A> together to select all, click <Ctrl> and <C> together to copy and <Ctrl> and <V> together to paste into another presentation, spreadsheet or document.

- f. Click the **Target Name** tab, and double-click on the corresponding **Target Name** to view, edit settings, or remove samples from the analysis.

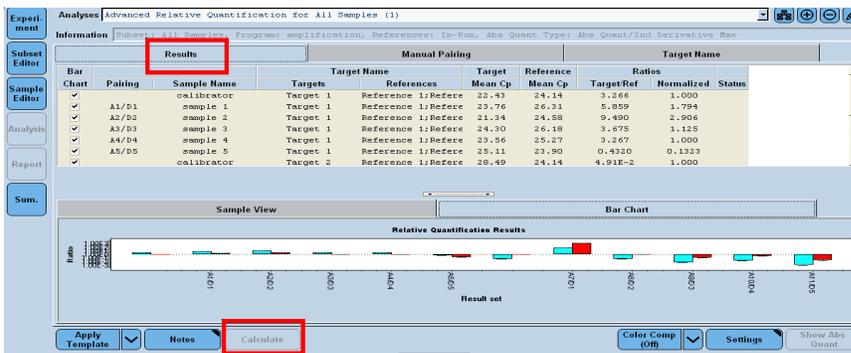


Relative Quantification

g. Once sample editing is done, click calculate, and then click **Back to Rel Quant** icon.



h. Click the Rel Quant **Results** tab, and then click **Calculate** to obtain updated results.



LightCycler® 480 Reagents and Disposables:

Product name	Cat. No.	Pack Size/Description
LightCycler® 480 SYBR Green I Master	04 707 516 001	5mL (5 x 1mL)
LightCycler® 480 Probes Master	04 707 494 001	5mL (5 x 1mL)
LightCycler® 480 Genotyping Master	04 707 524 001	Master Mix, 5x conc., 4 x 384 µl, ready-to-use hot start multiplex PCR reaction mix, containing a modified Taq DNA polymerase, reaction buffer, dNTP mix (with UTP instead of dTTP) and 15 mM MgCl ₂
LightCycler® 480 Control Kit	04 710 924 001	Kit for quantitative real-time PCR and genotyping control reactions using the LightCycler® 480 Instrument

Product name	Cat. No.	Pack Size/ Description
LightCycler® 480 Multiwell Plate 96	04 729 692 001	5 x 10 plates and sealing foils
LightCycler® 480 Multiwell Plate 384	04 729 749 001	5 x 10 plates and sealing foils
LightCycler® 480 Sealing Foil	04 729 757 001	50 foils

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