Relative Quantification Analysis

Relative Quantification compares the levels of two different target sequences in a single sample (e.g., target gene of interest (GOI) and another gene) and expresses the final result as a ratio of these targets. For comparison purposes the second gene is a reference gene that is found in constant copy numbers under all test conditions. The reference is used for normalization of sample-to-sample differences.

The ratio of the same two sequences can be compared to a standard sample called a "calibrator." The "calibrator" is typically a positive sample with a stable ratio of target-to-reference and is used to normalize all samples within one run, but in addition provides a constant calibration point between several LightCycler ® 480 System runs.

Important: *For more information, please consult Chapter D, Section 4.3 (pages 179-205) of the LightCycler® 480 Operator's Manual.*

Performing a Relative Quantification Analysis

1. Open the experiment for analysis in the main window.

   Note: *If the experiment just completed, the run will remain open and ready for analysis. Previously created and performed experiments are located in the <***Experiments***> folder of the Navigator.*

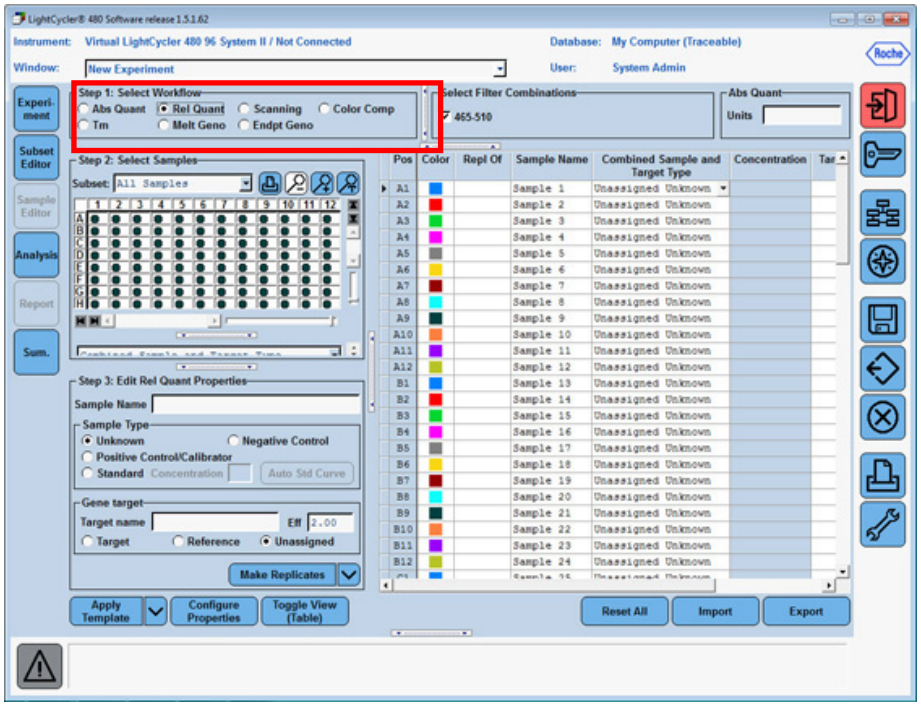
2. If the sample information was entered during run setup, then proceed to step 5. Otherwise, click the **Sample Editor** icon.

   Important: *Relative Quantification Analysis uses the following identifiers for calculation, please note there are mandatory identifiers required:*

Property	Description	Valid Values
Sample Name	Name of material of interest. If multiple targets/references are used, the sample name is used to identify groups for pairing.	Alphanumeric value (≤ 25 characters) Default value is "Sample ###", where ### is a serial number
Target Name	Name of the gene target *The term "target" in this field is different to the sample type "Target".* *e.g.,* "Gene1" for all samples, standards, calibrators, and negatives probing gene1	Alphanumeric value (≤ 25 characters) Default value is blank
Sample Type (mandatory)	Type of sample	▶ Unknown ▶ Positive Control/Calibrator ▶ Negative Control ▶ Standard
Target Type (mandatory)	Type of target	▶ Target ▶ Reference ▶ Unassigned *Unassigned is excluded from all Relative Quantification calculations*

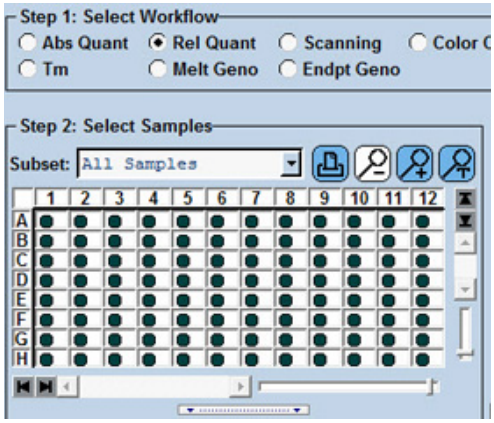
Note: *If you are performing an experiment using different filter combinations, make sure you select the appropriate filter combination for entering the sample information.*

3. In *Sample Editor* > *Step 1: Select Workflow*, select **Rel Quant** workflow.



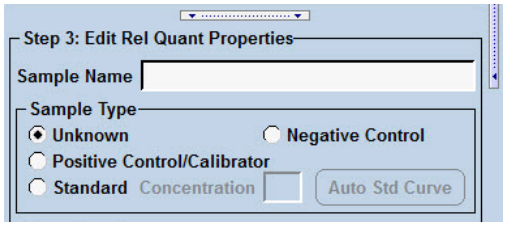
4. Enter Sample information

   a. In *Sample Editor* > *Step 2: Select Samples*, select the wells that contain the same DNA samples.



   b. In *Sample Editor* > *Step 3: Edit Rel Quant Properties*, enter the appropriate sample name and sample type information for the selected wells.

      Note: *For more information, please consult Chapter D, Section 4.3.3 (page 183) of the LightCycler® 480 Operator's Manual.*

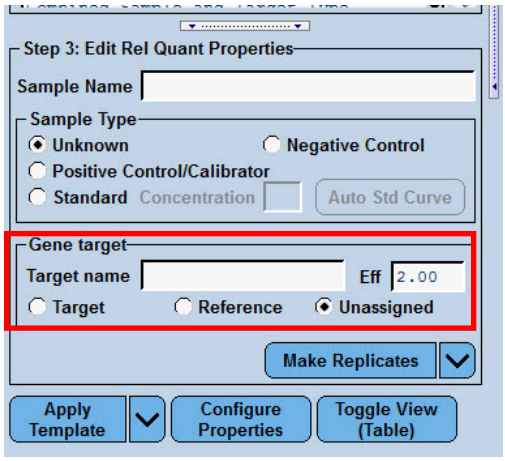


5. Enter **Gene target** information.

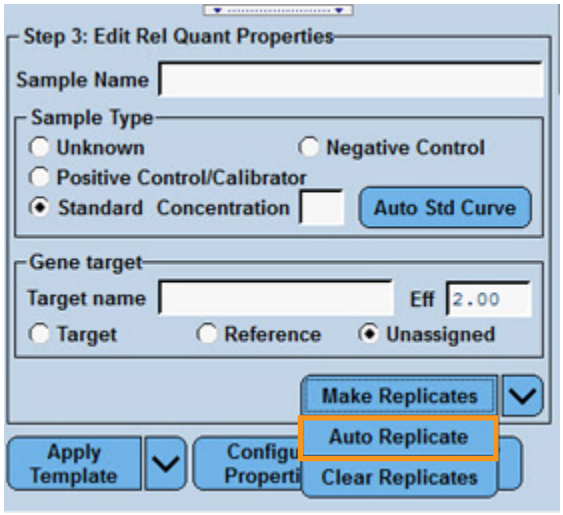
   a. In *Sample Editor* > *Step 2: Select Samples*, select the wells that contain the same gene target.

   b. In *Sample Editor* > *Step 3: Edit Rel Quant Properties,* enter the appropriate target name and select target type (target or reference) information for the selected wells

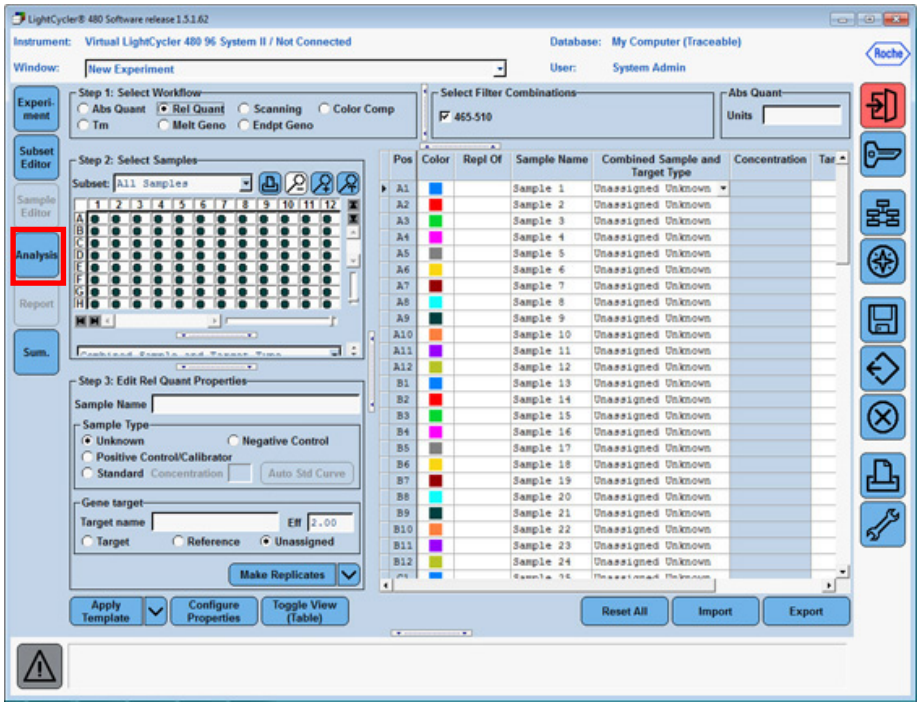
   Note: *Target is the gene of interest and Reference is the housekeeping gene (e.g., actin)*



6. In *Sample Editor* > *Step 2: Select Samples*, select all the wells and set replicates by clicking **Auto Replicate**.



7. Click **Analysis**.

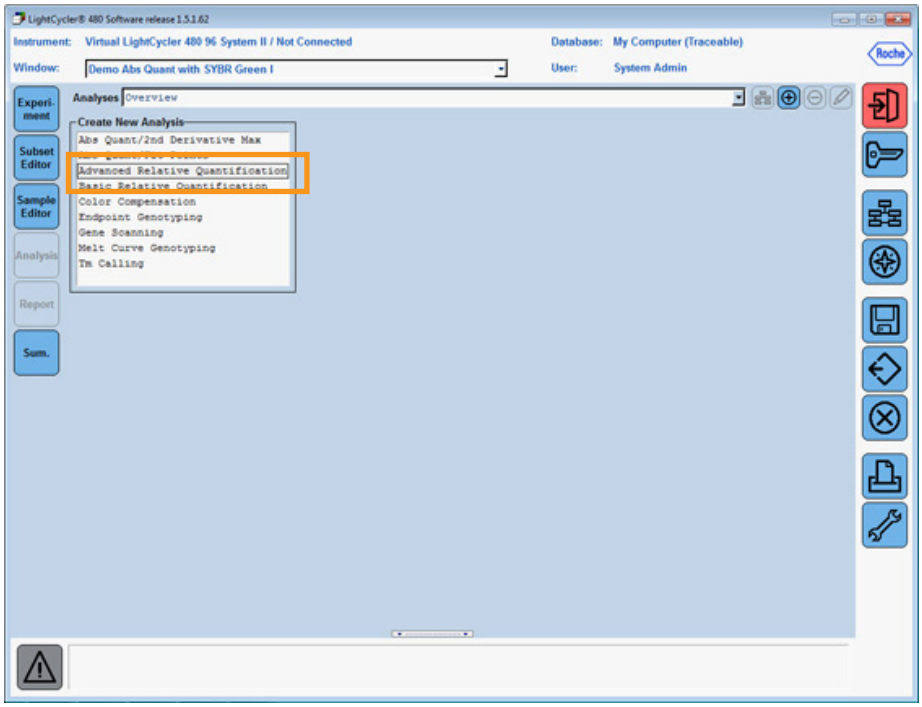


8. Select **Advanced Relative Quantification**.

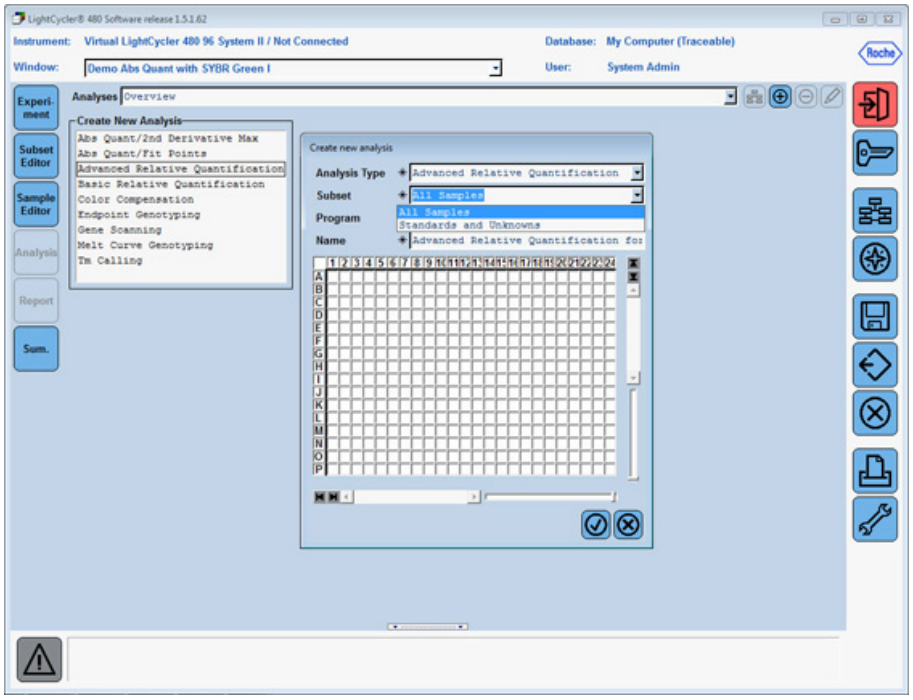
Important: *Two algorithms are available for the Relative Quantification Analysis*

- Basic Relative Quantification
  - automated, easy-to-use mode
  - based upon the ΔΔCT-method
- Advanced Relative Quantification
  - flexible mode, sophisticated software algorithms
  - E-Method (Efficiency Method)

For more information, please consult Chapter D, Section 4.2.3 (pages 179-205) of the LightCycler® 480 Operator's Manual.

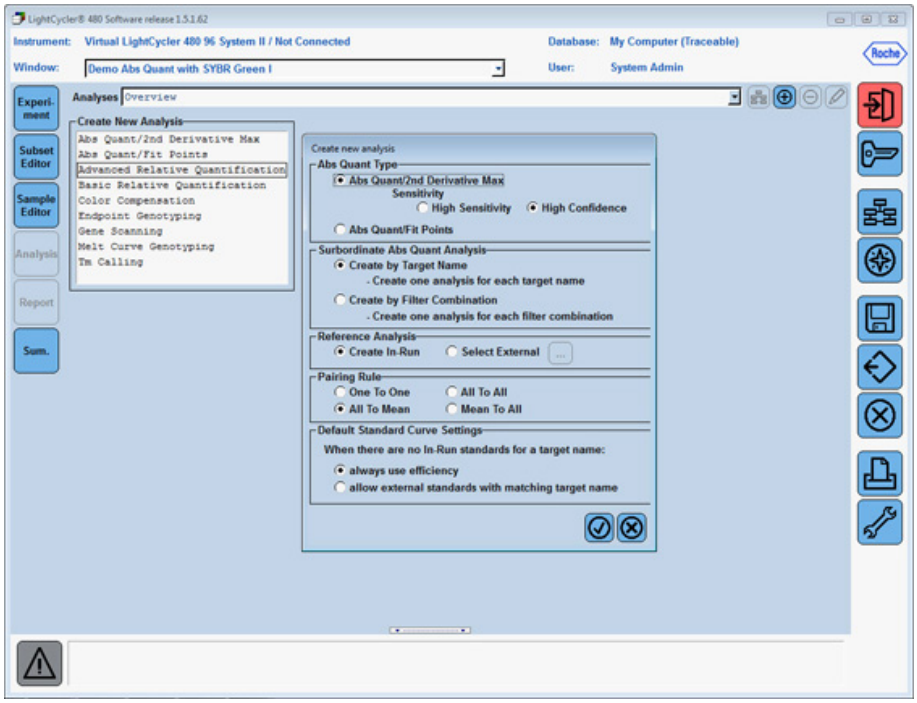


9. Select all samples or subset to be analyzed and click  .



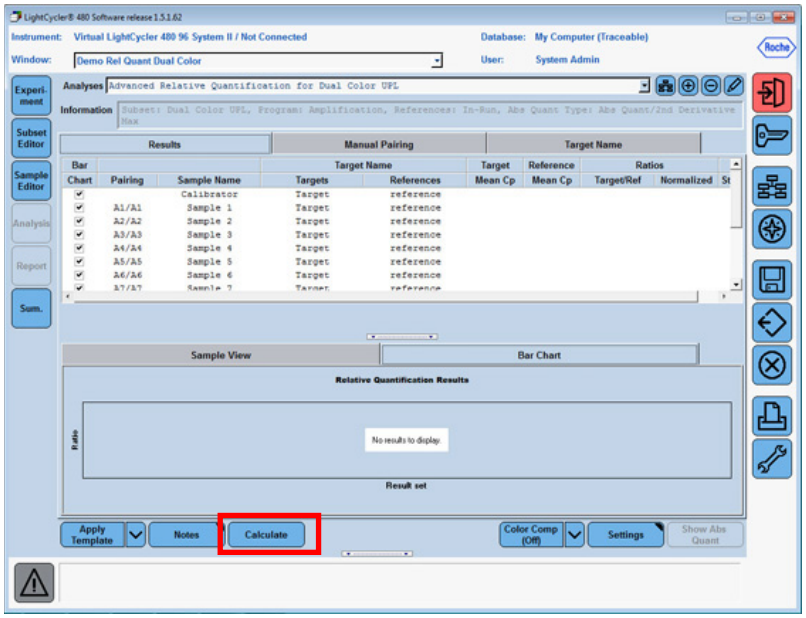
10. Leave defaults and click  .

Note: *For more information about this dialog box, please consult Chapter D, Section 4.3.5 (pages 190-192) of the LightCycler® 480 Operator's Manual.*



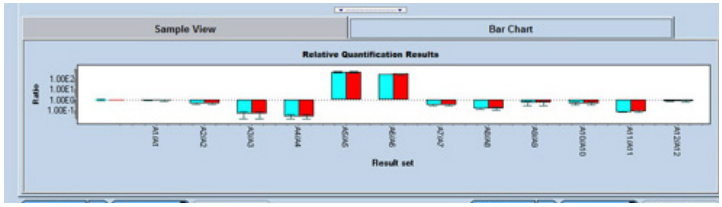
11. The software automatically pairs the target to the corresponding reference.

    a. To obtain results, click **Calculate**.



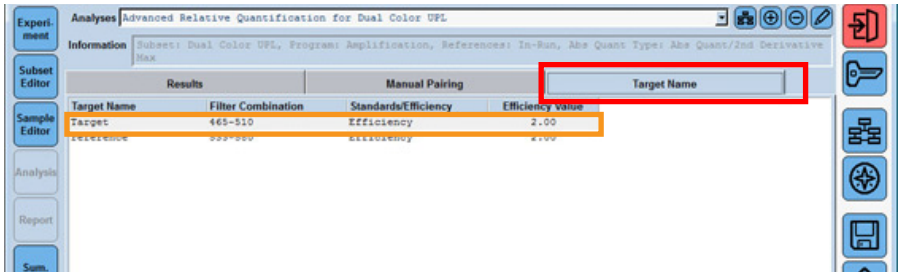
Note: *If a calibrator sample was used, the normalized ratio is the fold difference between samples and calibrator, represented as a red bar in the bar chart.*

*If a calibrator sample was **not** used, then the Target/Ref Ratio column will provide results, comparing only target to reference amounts within a single sample, represented as a blue bar in the bar chart.*

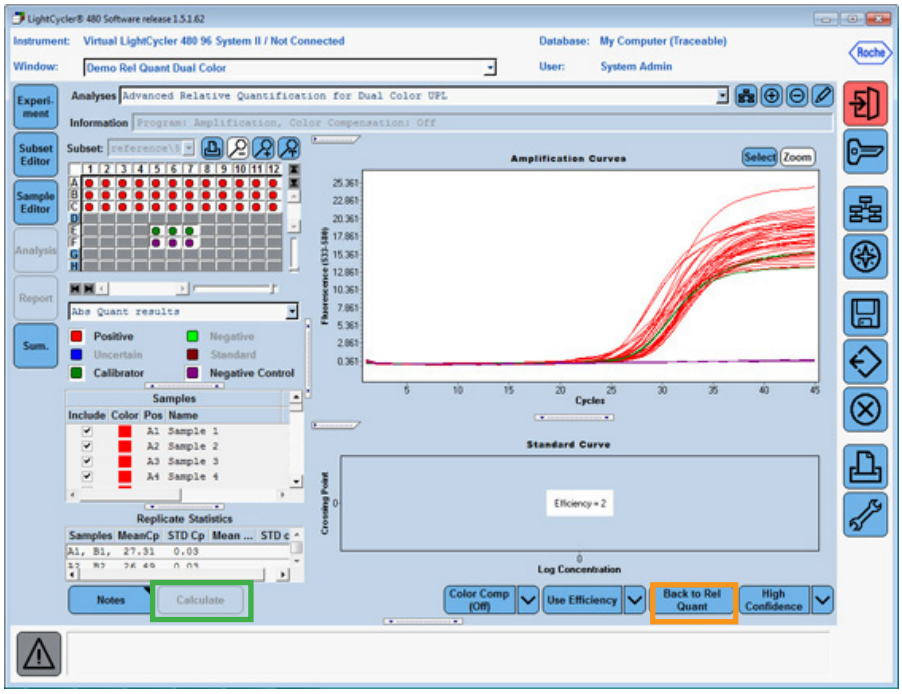


Note: *For more information, please consult Chapter D, Section 4.3.7 (pages 198-199) of the LightCycler® 480 Operator's Manual.*

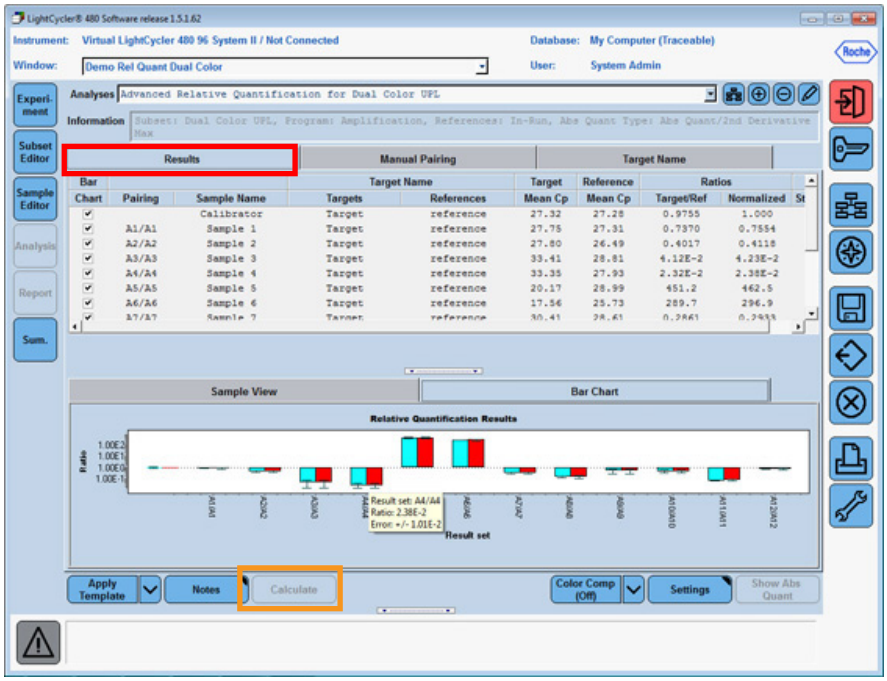
12. To view amplification curves from the analysis or edit sample settings, click the **Target Name** tab and double-click on the corresponding **Target Name**.



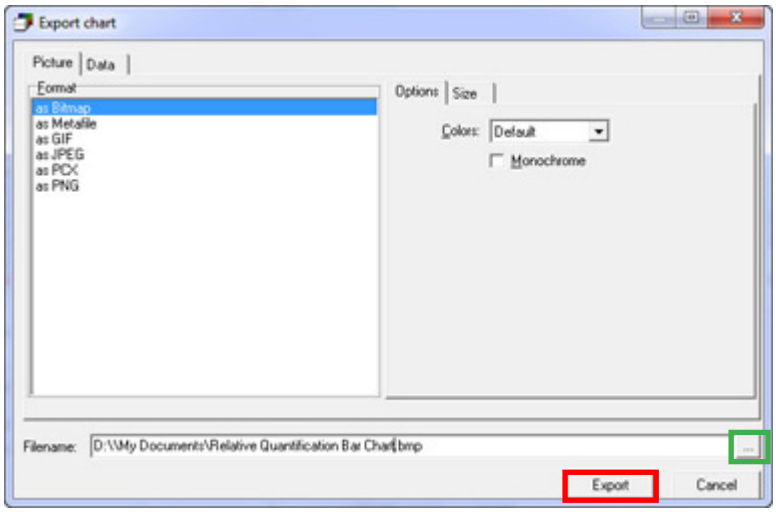
a. Once sample editing is complete, click **Calculate**, then click the **Back to Rel Quant** icon.



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



13. To export individual data figures or tables, right-click within the information section you want to export, then choose the external format to use. Click the **browse** (**...**) button to browse to the location to save the file and assign a name to your export file. Click **Export**.



14. Export the entire experiment through the Navigator using the *Export* button. Map to the appropriate drive/ folder for saving. The complete file experiment can be imported into another computer with the same release of LightCycler® 480 software.