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.

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, stan- dards, 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 Quantifica- tion calculations

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

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.

New Experiment				_	-	User:	System Admi	'n			(
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- 4. Enter Sample information
 - a. In *Sample Editor > Step 2: Select Samples*, select the wells that contain the same DNA samples.

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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.

Step 3: Edit Rei Quar	nt Properties
Sample Name	
-Sample Type	
Unknown	Negative Control
O Positive Control/0	Calibrator
C Standard Conce	ntration Auto Std Curve

- 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)

Step 3: Edit Rel Quant F	Properties
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6. In *Sample Editor > Step 2: Select Samples*, select all the wells and set replicates by clicking **Auto Replicate**.

Step 3: Edit Rel Quant Properties
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7. Click **Analysis**.

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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 $\Delta\Delta$ CT-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.*

Instrument:						
	Virtual LightCycler 480 96 System II / Not	Connected		Database:	My Computer (Traceable)	Barba
Window:	Demo Abs Quant with SYBR Green I		•	User:	System Admin	
Window: Experiment Subset Editor Analysis Report Sum.	Demo Abs Guant with SYBR Green I Analyses Overview -Create New Analysis Abb Quant/And Derivative Max Advanced Relative Quantification Color Compensation Tradpoint Genotyping Gene Scanning Melt Curve Genotyping Th Calling]		User:	System Admin	
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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**.

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	22/22	Sample 2	Target	reference					
	A3/A3	Sample 3	Target	reference					1
	24/24	Sample 4	Target	reference					
	AS/AS	Sample 5	Target	reference					
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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 doubleclick on the corresponding **Target Name**.

Experiment	Analyses Advanced	Relative Quantification Dual Color UPL, Progra	for Dual Color UPL	nces: In-Run, Abs Qu	ant Types Aks Quant/2nd Derivative	Ð
Subset Editor	Re	esuits	Manual Pairing		Target Name	62
Cample	Target Name	Filter Combination	Standards/Efficiency	Efficiency Value		
Editor	rererence	965-510 965-510	Efficiency	2.00		동물
Analysis						
Report						
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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**.

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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.