Software Quick-Start GuideAugust 2018Instructions for Q-Rex Gene Expression Plug-inversion 2.0

Adaptions for using the comparative C_T method (2- $\Delta\Delta C_q$)

In the Q-Rex Gene Expression update to version 2.0, the relative quantitation calculation is based on the more generalized mathematical model suggested by Pfaffl*. This has the following advantages for the user:

- Normalization to more than one reference gene
- Adjustment of the real-time PCR efficiency

In case of 100% reaction efficiency for all tested targets and one reference gene, the relative quantity (RQ) results with version 2.0 are the same as with comparative C_T method ($2^{-\Delta\Delta Cq}$) used in version 1.0. In fact, the $2^{-\Delta\Delta Cq}$ method is simply a special case of the Pfaffl method, in which the efficiency of the target and reference gene is 2 (100%).

This guide provides step-by-step instructions for the setup with Q-Rex Gene Expression Plug-in version 2.0 to ensure the RQ values are the same as with version 1.0.

Procedure

- 1. Open your finished experiment.
- 2. Make sure to define only one target as a reference gene (RG) in the sample layout step of the Step Marker. If you are working with SYBR Green, add and define new targets and assign them accordingly to the samples.

arge	et	Acquisition	Туре		
ef ge	ene	Green	RG	*	
targe	t 1	Yellow	Test	*	
targe	t 2	Orange	Test	×	

* Pfaffl, M.W. (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45.



Sample to Insight

3. Add gene-expression analysis to the experiment by clicking the menu icon in the "Analysis" step of the Step Marker and selecting the gene expression analysis from the list.

Run		
Analysis	Add analysis	Absolute Quantification
Report/Export	Remove analysis	Basic
		Gene Expression

4. Before you can get gene expression results, you need to define the efficiency and calibrator following the yellow highlighted fields. Check the "Use efficiency of 100% for all targets" box in the "Gene expression" panel.

Before efficiency defined	After efficincy defined
Analysis Tube Selector	Analysis Tube Selector
larget refigene	Target ref gene
Gene expression parameters are invalid.	Filter data
Filter data	Normalization
Normalization	Cq calculation
Cq calculation	Threshold 0,0224
Threshold 0,0224	Threshold start cycle 1
Threshold start cycle	Calculate auto threshold
Calculate auto threshold	
Gene expression	Gene expression
Use efficiency of 100% for all targets	Use efficiency of 100% for all targets
Define efficiency per target	Define efficiency per target
Calibrator sample	Calibrator sample
- none selected -	calibrator
Reference gene	Reference gene
Target	✓ ref.gene
✓ ret gene	
Crosstalk compensation	Crosstalk compensation
Copy settings to	Copy settings to

5. Change to the "Sample result" view.

Notice that, in version 2.0 of the Q-Rex Gene Expression Plug-in, the results are grouped at acquisition and are ordered one after the other.

The RQ values are the same as in the previous version. However, the RQ min/max in the previous version was the standard deviation (SD) and in version 2.0 is the standard error (SE).

Note: Make sure that the technical replicates are have exactly the same name, otherwise you will not see RQ results or the relative quantity plot.

Exporting $\Delta\Delta Cq$ values

You can export $\Delta\Delta Cq$ values with Q-Rex Software version 1.1.

- 1. To export $\Delta\Delta$ Cq values, switch to the "Report/export" step in the Step Marker.
- 2. Click the "Export" tab.
- 3. Select the "Relative expression $(2^{-\Delta\Delta Cq})$ " export format.
- 4. Click "Export".
- 5. Enter a file name for the export and the location where it should be saved.

The Q-Rex Software uses the export format as a default file name.

6. Click "OK" to save the export file.



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