Software Quick-Start Guide August 2018

Instructions for Q-Rex Gene Expression Plug-in version 2.0

Adaptions for using the comparative C_T method ($2^{-\Delta\Delta Cq}$)

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

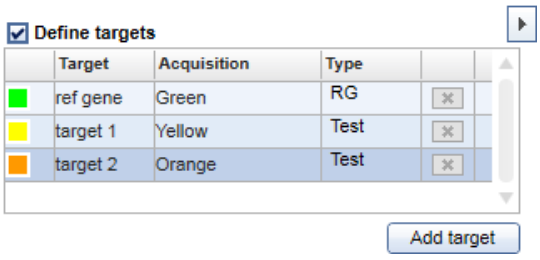
In case of 100% reaction efficiency for all tested targets and one reference gene, the relative quantity (RQ) results with version 2.0 are the same as with comparative C_T method ($2^{-\Delta\Delta Cq}$) used in version 1.0. In fact, the $2^{-\Delta\Delta Cq}$ method is simply a special case of the Pfaffl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

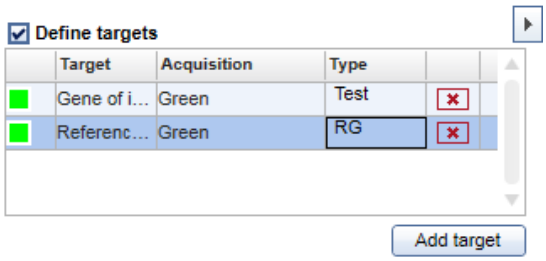
Procedure

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.

Probe

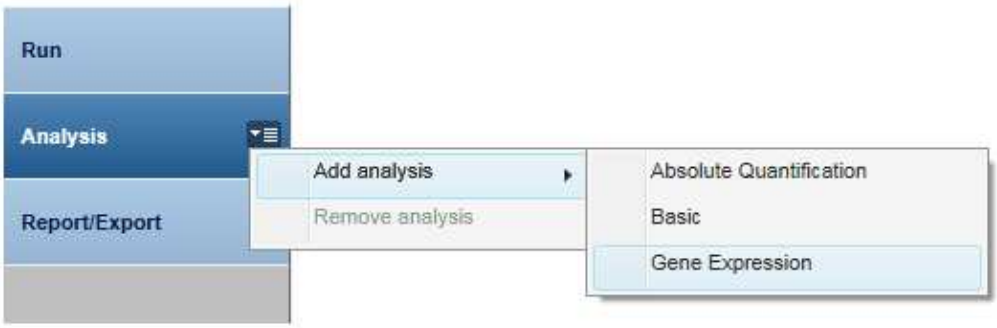


SYBR Green

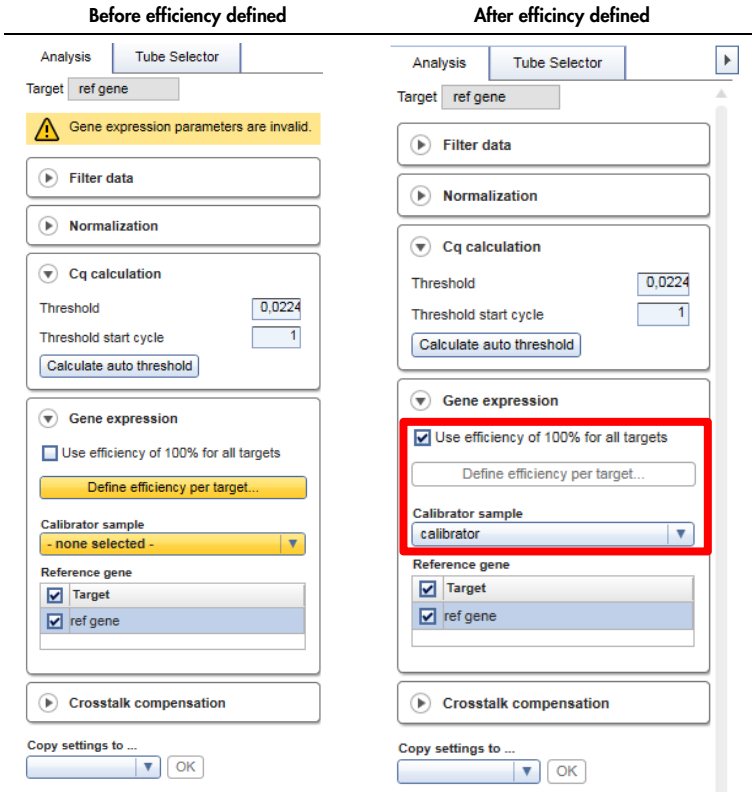


* Pfaffl, M.W. (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. **29**, e45.

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.



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.



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).

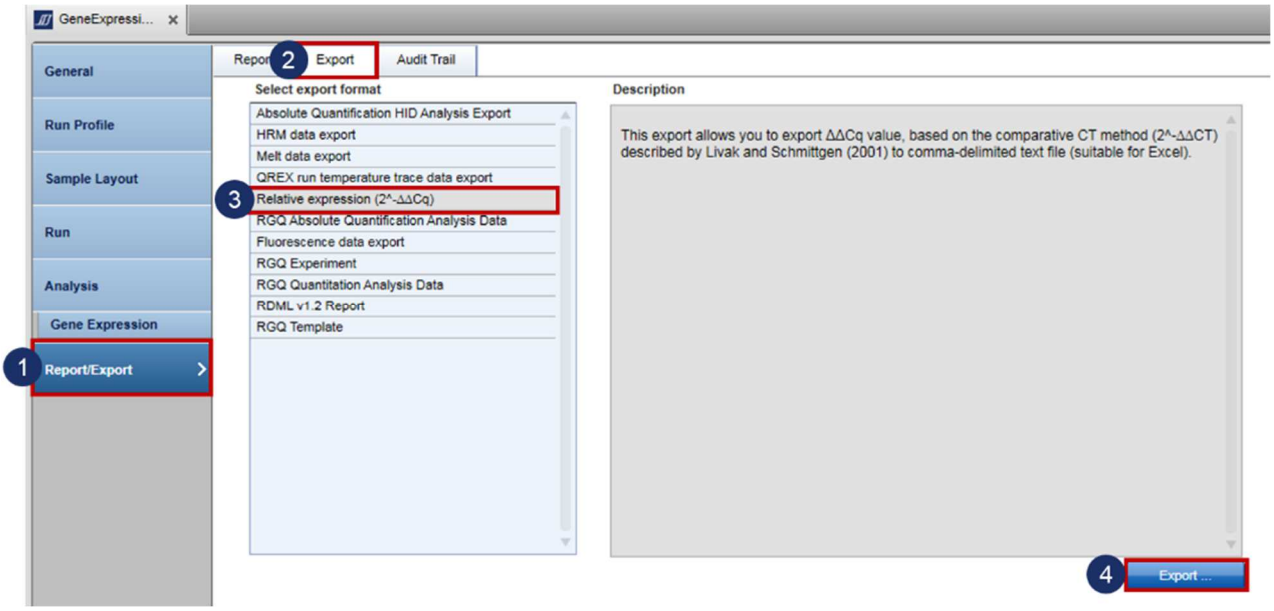
   Note: Make sure that the technical replicates are have exactly the same name, otherwise you will not see RQ results or the relative quantity plot.

Exporting ΔΔCq values

You can export ΔΔCq values with Q-Rex Software version 1.1.

1. To export ΔΔCq values, switch to the “Report/export” step in the Step Marker.
2. Click the “Export” tab.
3. Select the “Relative expression (2^-ΔΔCq)” 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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