

genorm^{PLUS}

qbase^{PLUS}



Easy . Fast . Reliable .

Subject

Summary

Detailed information

Step 1	Create e new empty experiment	p. 2
Step 2	Import run data	p. 2
Step 3	Select reference targets normalization strategy	p. 3
Step 4	Select candidate reference genes	p. 3
Step 5	Start genorm ^{PLUS} analysis	p. 3
Step 6	Interpret genorm ^{PLUS} results	p. 4

References

Subject

qbase^{PLUS} comes with genorm^{PLUS} functionality as a much improved version of the classic geNorm method. This chapter describes how genorm^{PLUS} enables evaluation of expression stability of candidate reference genes.

Summary

geNorm analysis enables the selection of the optimal set of reference genes from a series of tested candidate reference genes [Vandesompele et al., *Genome Biology*, 2002]. The qbase^{PLUS} implementation of geNorm (named geNorm^{PLUS}) provides five great benefits compared to its predecessor in Microsoft Excel:

- Calculation of relative quantities and geNorm analysis are combined in a single program to speed up analysis. Previously, many manual pre-calculations were needed.
- Experiments with missing data are automatically processed in a way that has the lowest impact on the overall analysis through intelligent retention of as many data points as possible.
- Whereas geNorm Excel could not distinguish the two most stably expressed genes, genorm^{PLUS} now allows ranking of candidate reference genes up to the single most stable gene.
- Interpretation of a geNorm result is not always straightforward and requires a certain level of expertise. To accommodate this, genorm^{PLUS} now includes an expert interpretation with recommendations on the number and nature of genes to be used for optimal normalization as well as information on the suitability of the selected genes.
- genorm^{PLUS} is at least 20 times faster than geNorm Excel

Detailed information

If your final experiment contains many samples, it is highly recommended to perform a geNorm pilot experiment. Such a pilot experiment involves the analysis of a set of candidate reference genes (preferentially more than 8, coming from different pathways and belonging to different functional classes) on a representative set of samples (typically 10 independent samples). Use the sample maximization approach [Hellemans et al., *Genome Biology*, 2007] to avoid unwanted technical variation that may interfere with proper geNorm analysis.

It is important that the samples are representative for the final experiment; if one works with treated and untreated samples, or with different biological subgroups, an equal number of samples from these subgroups should be studied.

Step 1 - Create a new empty experiment

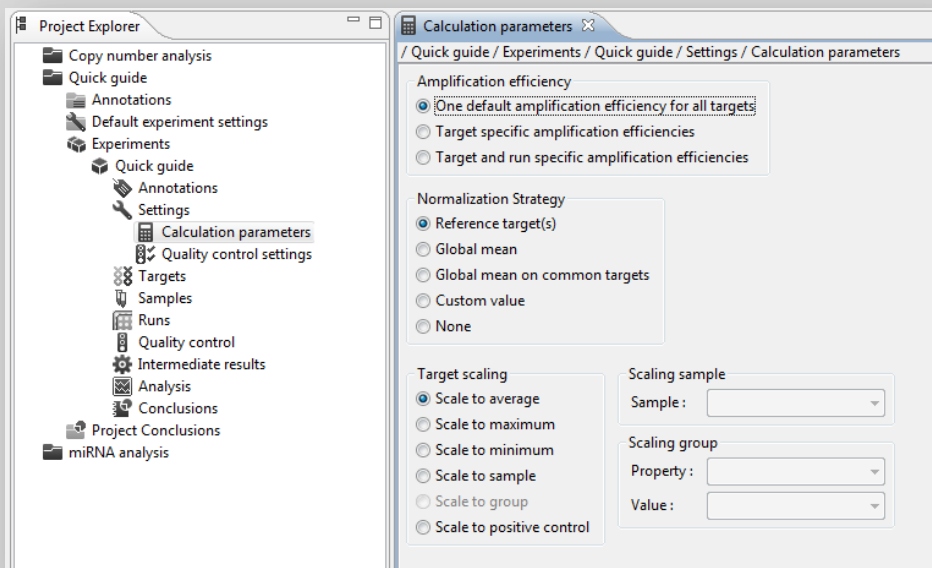
In qbase^{PLUS} qPCR run data are organized in experiments. To start data analysis, a new empty experiment needs to be created. Right click on the project (📁) in the qbase^{PLUS} project explorer tree in which you want to start a new experiment and click *New experiment*. If needed, create a new project first in a similar way (*New > Project*). An experiment name and description can be provided in the *Experiment properties* window.

Step 2 - Import run data

qbase^{PLUS} experiments contain data from one or more runs. Run data can be imported by clicking the downward pointing arrow (⌵) in the toolbar followed by the selection of the *Import Run* option.

More information can be found in the qbase^{PLUS} quick guide and the chapter on data import (<http://www.biogazelle.com/support/qbaseplus/manual>).

▼ Figure 1 - Reference targets normalization strategy



Step 3 - Select reference targets normalization strategy

As of version 2.0, qbase^{PLUS} supports a range of different normalization procedures to suit the specific needs of different types of experiments [premium license only]. For a geNorm pilot experiment it is required to apply the multiple reference target normalization approach.

Open the Settings node in the Project explorer, double click on Calculation Parameters, and select the option Reference target(s) (Figure 1).

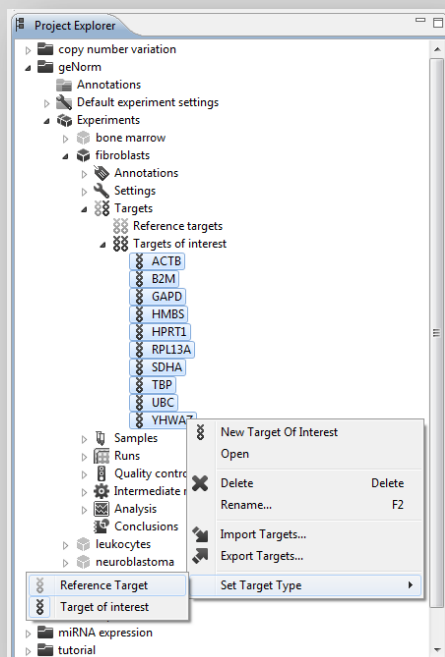
Step 4 - Select candidate reference genes

Mark all candidate reference genes to be evaluated by genorm^{PLUS} as reference targets: select all genes to be tested from the Targets of interest node and then use the Set target type option from the context menu to change the target type into Reference target (Figure 2).

Step 5 - Start genorm^{PLUS} analysis

Open the Analysis node in the Project explorer and start an analysis by double clicking geNorm.

Figure 2 – select candidate reference genes



Step 6 - Interpret genom^{PLUS} results

The geNorm window consists of three tabbed views (bottom): geNorm M, geNorm V and Interpretation.

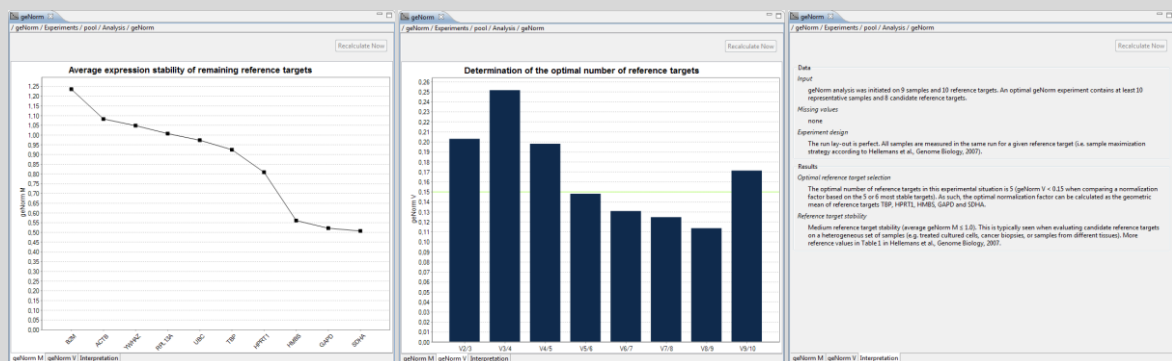
The first view, geNorm M (Figure 3 – left), shows a ranking of candidate genes according to their stability (expressed in geNorm M values) from most unstable genes at the left (high M value) to the best reference genes at the right (low M value). Note that genom^{PLUS} allows ranking of candidate reference genes up to the single most stable gene, whereas its Excel predecessor could not make a distinction between the 2 most stably expressed candidate reference genes.

The second view, geNorm V (Figure 3 – middle), shows a bar chart that helps determining the optimal number of reference genes to be used in subsequent analyses. A $V_{n/n+1}$ value is shown for every comparison between two consecutive numbers (n and n+1) of candidate reference genes. As a general guideline it is stated that the benefit of using an extra (n+1)th reference gene is limited as soon as the $V_{n/n+1}$ value drops below the 0.15 threshold, indicated with a horizontal green line.

The third view, Interpretation (Figure 3 – right), contains a summary and interpretation of the genom^{PLUS} results. The first section contains an evaluation of the input data with respect to the numbers of samples and candidate reference genes included, possible missing data and appropriate experimental design. While the old geNorm algorithm only worked if there were no missing data, genom^{PLUS} will automatically exclude samples or targets from the analysis until the dataset is complete, using an intelligent approach that minimizes the effect of data exclusion.

The second section will summarize the automatic interpretation of the genom^{PLUS} results, telling you exactly which reference genes to use in follow up studies without having to fully understand the geNorm M and geNorm V graphs. In addition, the stability of the selected reference genes will be compared against empirically determined reference values for acceptable geNorm M values [Hellemans et al., *Genome Biology*, 2007].

Figure 3 - genom^{PLUS} results



References

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F.

Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 2002 Jun 18;3(7):RESEARCH0034

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