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# PCR efficiency correction

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## Subject

PCR efficiency calculation using a standard serial dilution series and efficiency correction using the universal quantification model employed in qbase$^{PLUS}$.

## Summary

In the Calculation parameters window, the user can choose between default amplification efficiency for all targets, and an individual PCR efficiency for each target. If the experiment contains a standard dilution series for each target, the E value (base of the exponential function, e.g. 2 for 100% PCR efficiency) will be automatically computed by qbase$^{PLUS}$. The user can overwrite this calculated value. If there is no standard dilution series, then the user needs to enter the E value manually.

## Note

Various algorithms are proposed in the literature to estimate the PCR efficiency based on a single amplification curve. Unfortunately, many of these methods do not provide a precise nor accurate estimate of the efficiency. One should therefore be cautious in using these single reaction PCR efficiency values; it is generally recommended to calculate the mean efficiency of all reaction wells in which the same target is amplified (more information in Karlen et al., BMC Bioinformatics, 2007).

## Detailed information

qbase$^{PLUS}$ employs a universal and flexible quantification model (Hellemans et al., Genome Biology, 2007). This means that the user has full control over how calculations are performed: PCR efficiency correction or not, use of standard dilution series or not, one or multiple reference genes for normalization, etc. Based on a few user settings, qbase$^{PLUS}$ automatically understands how calculations should be performed.

# Step-by-step

## Step 1 - Set amplification efficiency type

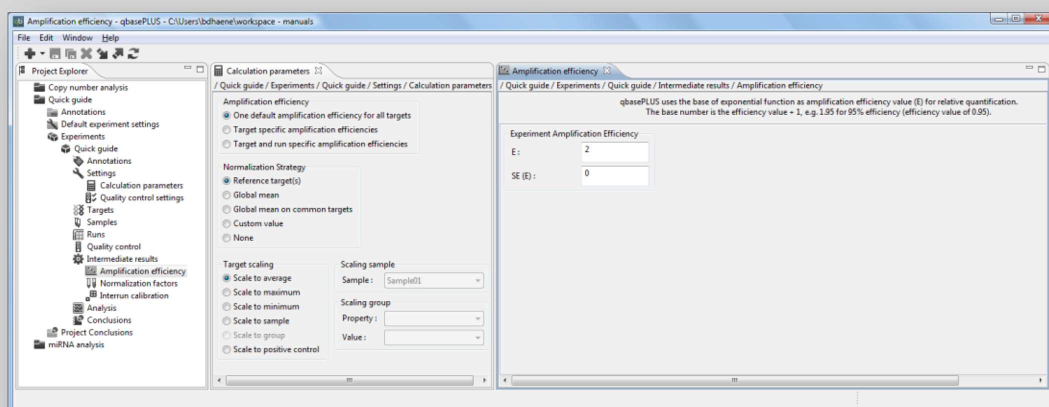
Select Calculation parameters in the Project explorer. A tab will appear in the Main window, where you can define the PCR amplification efficiency to be used for the calculations: a default amplification efficiency for all targets, a target specific amplification efficiency, or a target and run specific amplification efficiency.

## Step 2 - Use one default amplification efficiency

If a default value is selected for all targets, the user can define this E value in the Amplification efficiency window (available from the Intermediate results section). qbase$^{PLUS}$ uses the base of the exponential function (E value) as the amplification efficiency value for relative quantification (see formula 5 in Hellemans et al., Genome Biology, 2007). This base is the efficiency value + 1, e.g. an E value of 1.95 for 95% efficiency (efficiency value of 0.95).

If you want to use the delta-delta-Cq quantification model as originally described by Livak and Schmittgen, (Methods, 2001), then you should enter 2 as E value (with an error of 0, SE(E) = 0) (Figure 1). Doing so, you assume that all targets amplify with the same (optimal) PCR efficiency of 100%.

Figure 1 - One default amplification efficiency for all targets



## Step 3 - Use target specific amplification efficiencies

The gold standard method for PCR efficiency estimation is a serial dilution of nucleic acid template that is as similar to the samples under investigation as possible (e.g. a mixture of cDNA from a representative set of your samples). qbase$^{PLUS}$ offers the possibility to make use of target specific amplification efficiencies in case such serial dilutions are included for the targets in the experiment. Alternatively, users can manually enter previously determined target specific amplification efficiencies in the Amplification efficiency window. In our own experiments, we aim for E values in the range of 1.90 – 2.10 (PCR efficiency between 90 and 110%) with standard errors typically below 0.01 (1%).

The E value can be calculated from the slope of a serial dilution as follows: $E = 10^{(-1/slope)}$ (with an E of 2 being perfect, indicating 100% efficiency).

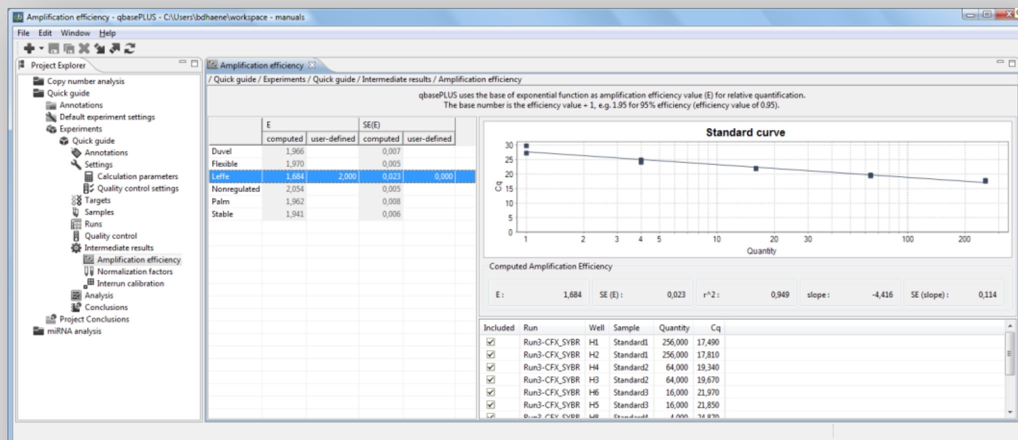
The Amplification efficiency window can be opened by double clicking 'Amplification efficiency' in the Project Explorer (Figure 2). Here, the user also has the opportunity to manually enter an efficiency value for each target by providing a 'user-defined' E-value. The user-defined value overrules the computed E-value.

By unticking the box in front of an outlier well, certain dilution points can be excluded from the calculation of the efficiency value. By hovering over a data point in the standard curve, the identity of an outlier reaction is easily determined.

## Step 4 - Make amplification efficiencies run specific

If a target is measured in different runs, and a serial dilution for that target is present in each run, qbase$^{PLUS}$ offers the possibility to use a run-specific target amplification efficiency. Hence, each Cq value will be converted to a relative quantity (RQ) using the efficiency measured in that particular run.

Figure 2 – Target specific amplification efficiency



## References


Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J.

qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol. 2007;8(2):R19.

Livak KJ, Schmittgen TD.

Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 2001;25(4):402-8

Pfafll MW.

A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 2001 May 1;29(9):e45. 2001

Karlen Y, McNair A, Perseguers S, Mazza C, Mermod N.

Statistical significance of quantitative PCR. BMC Bioinformatics. 2007 Apr 20;8:131.