Short introduction!

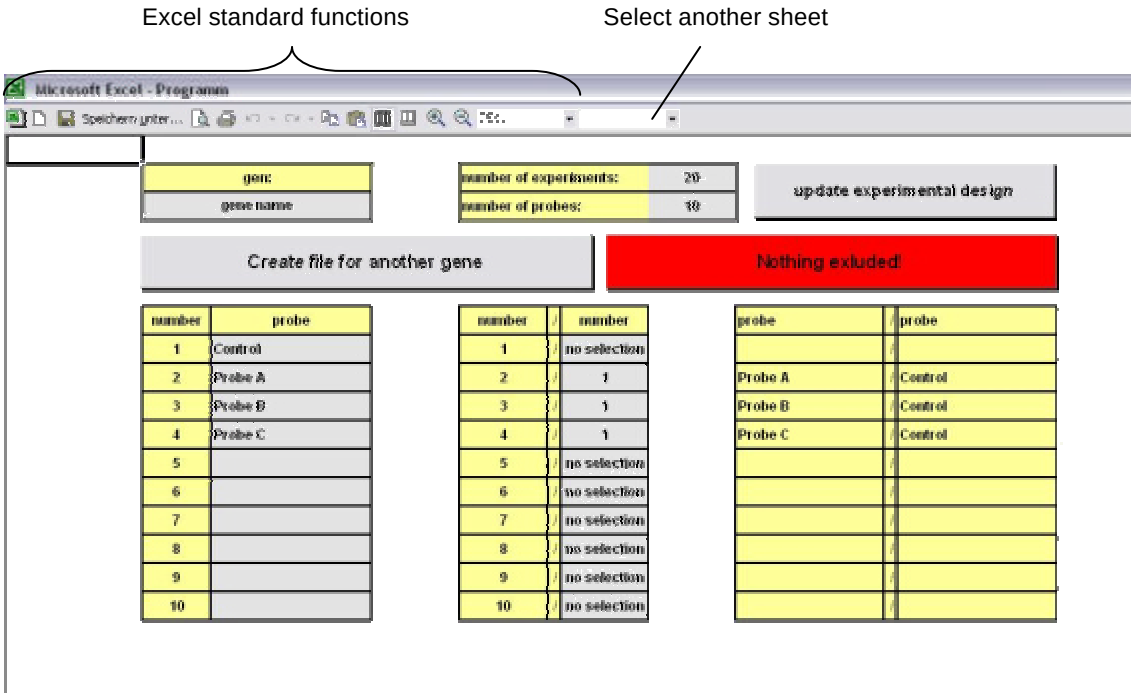
(A detailed one will follow as soon as possible!)

To guarantee a proper calculation it is important to mention some points:

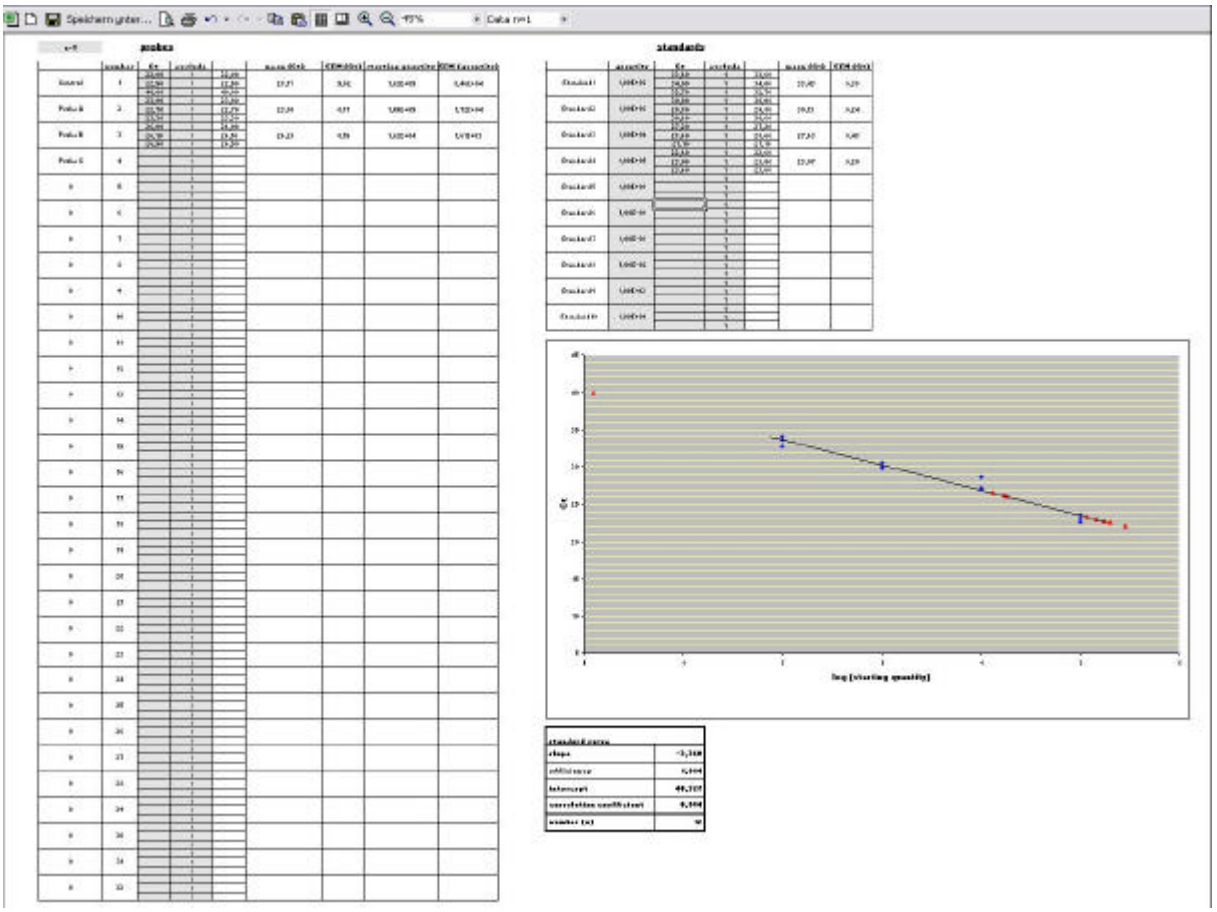
You have to allow the execution of macros, permanently or on request!
I tested the qCalculator already with Excel 97, 2000, 2002 and 2003 (OS: Windows 98, 2000 and XP). Of course I can give no warranties about probable risks, damages, bugs and other side effects of this program. You use the qCalculator on your own risk!

In the whole application only grey fields can be modified. All changes are considered immediately. Only if you want to change the layout, e.g. the number of probes and/or experiments you have to confirm your settings by pressing a button. You can change to the different analysis sheets of each file in the drop-down menu at the top (see screenshots above)!

In this application the results of each gene (target or internal standard) are saved in separate files. So please start with one of your target or internal standard genes.



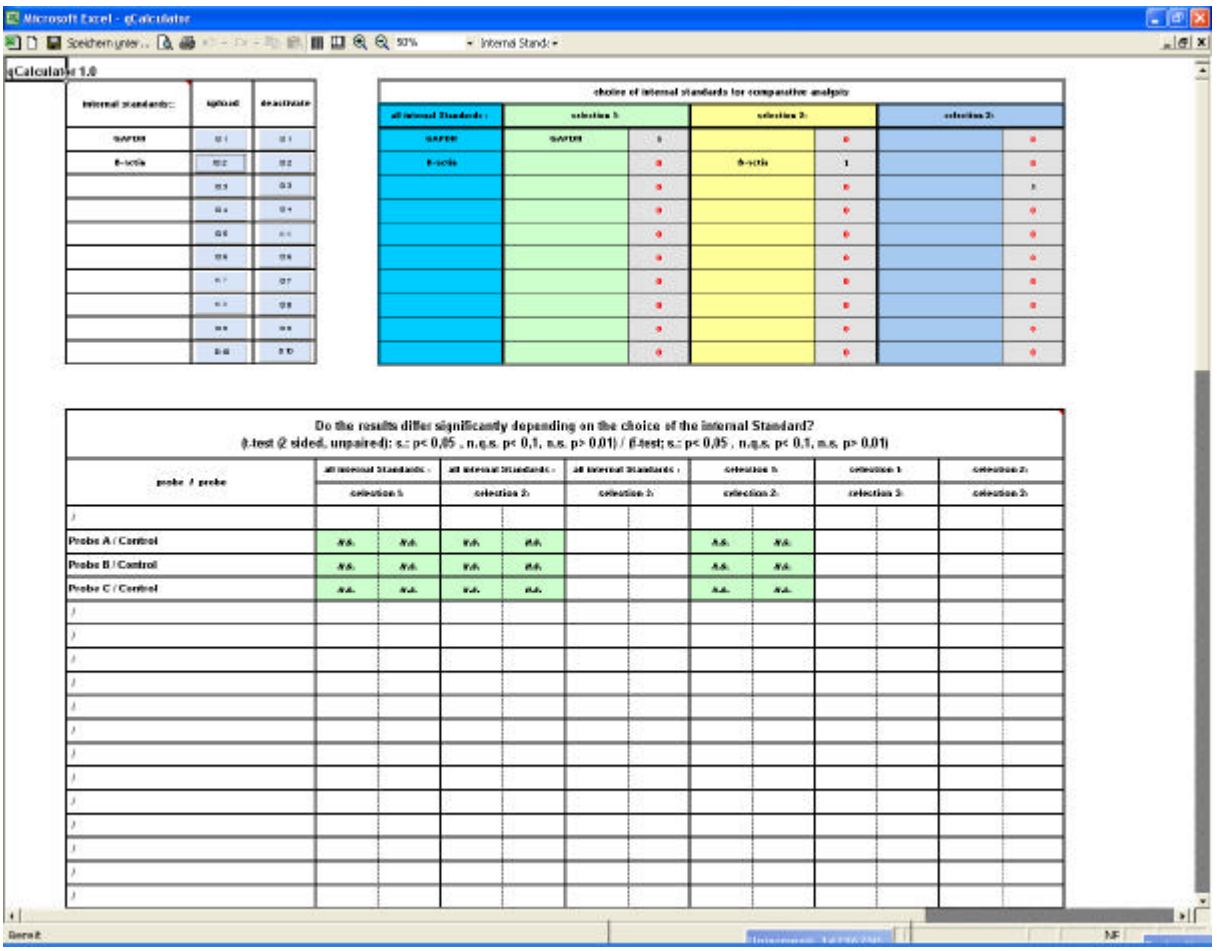
Open the qCalculator and fill-in the name of your first gene (instead of gene name). Then enter the names of your probes. After that you can select (right side) which samples you want to compare relatively. It is possible to change this selection at anytime. To improve clearness adjust the number of experiments and probes and press the button "update experimental design". It is of course possible to expand or reduce later. If you reduce, hidden data is excluded from calculations but not deleted. If you expand again, it will be included automatically.



After that switch to the data sheets and enter your data. The determined efficiencies from your standard curves were automatically collected and mentioned for the calculations (see efficiency sheet).

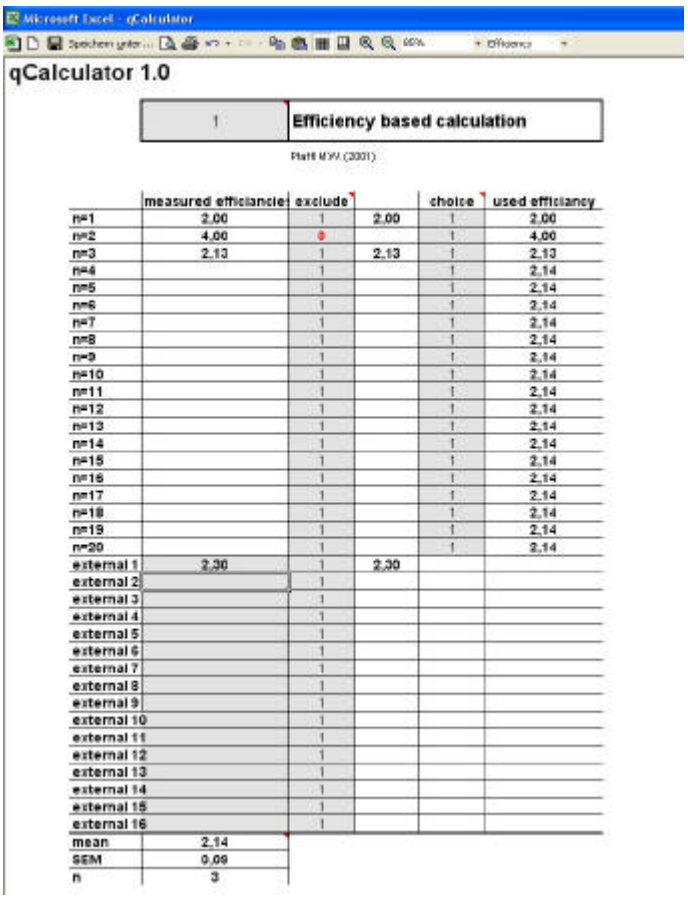
For the next gene please press the button “Create file for another gene” on the setup-sheet. Follow the instructions and save the new file in the same folder like the initial one!

Please fill in the data in the same manner as in the previous sheet. If you entered already the names of the samples, they will appear for an easier orientation.



After saving all files, go to the file of your target gene and select the “internal standard” sheet. After pressing one of the upload-buttons (B1-10), you can choose the file containing the data of your internal standard (e.g. housekeeper). Of course only files created with this program are suitable for this procedure. At the right side of these buttons you can select which internal standards you want to compare.

Your selections were statistically compared and the results were illustrated below. Of course you have to enter more than one experiment for these tests. (Please mention: If you exclude values at the result sheet, its not taken in account in the statistical analysis.)



On the “Efficiency sheet” you can manage the determined efficiencies and select the mathematical model of your calculations:

0) $ratio = 2^{-\Delta\Delta C_T}$

Livak,K.J. and T.D.Schmittgen. 2001 (Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25:402-408.)

or

1) $$ratio = \frac{E^{\Delta C_{T(\text{target})}}}{E^{\Delta C_{T(\text{internal standard})}}}$$

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

These results were displayed as log_2-values to obtain normal distributed values. In the case of multiple internal standards, the results were calculated separately on the basis of each of them. The arithmetic mean of these values are calculated for each experiment.

At the result-sheet you will find them separate for each selection of internal-standards. These results are illustrated as a bar charts at the results-sheet.
If you want to check your internal standards separately for each experiment, switch to the IS sheets.

Of course I did not mention all features in this very short introduction, but I hope that the qCalculator is mainly self-explanatory. For a little more aid, I added short comments to important fields (red corners) which will appear if your pointer gets in touch.

Sincerely
Ralf Gilsbach

Please report bugs or comments to: Ralf.Gilsbach@gmx.de

Ralf Gilsbach
Institut of Pharmacology & Toxicology
Reuterstr. 2b
53113 Bonn