RUN THE APPLICATION

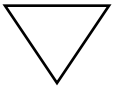
python qPCR.py
This program will save final and intermediate results in a specified file and error messages in **qPCR.log**.
This program requires the file **aParameters.in** to be present in the same directory.
The input is case sensitive.

INPUT FILES

Mean Ct of technical replicates

$$\overline{Ct}_{jkl} = \frac{\sum_{i=1}^{n} Ct_{ijkl}}{n}$$

for a given gene j/sample k/plate l ❶.

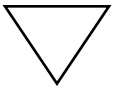


Relative quantity

$$\Delta Ct_{jkl} = Ct_{refererecence,jl} - \overline{Ct}_{jkl}$$

$$RQ_{jkl} = E_j^{\Delta Ct_{jkl}}$$

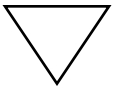
❹ Delta Ct for a given gene j/sample k. plate l. $Ct_{reference}$ can either be the mean or the minimum Ct for the all plate ❷. E= primer efficiency for a given gene j ❸.



Normalized relative quantity

$$NF_{kl} = \sqrt[f]{\prod_{p=1}^{f} RQ_{pkl}}$$

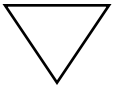
$$NRQ_{jkl} = \frac{RQ_{jkl}}{NF_{kl}}$$



Calibrated normalized relative quantity for inter-plates effect

$$CF_l = \sqrt[f]{\prod_{m=1}^{c} NRQ_{ml}}$$ ❺ $$CF_l = \sqrt[f]{\prod_{m=1}^{c} RQ_{ml}}$$ ❻

$$CNRQ_{jkl} = \frac{NRQ_{jkl}}{CF_l}$$



Calibrated expression

$$CExpr_{jk} = \frac{CNRQ_{jkl}}{\min(CNRQ)}$$

Input file

Tab delimited file. It should begin with primer efficiency values for each gene ❸ (float value between 0 and 100) then the line “gene <tab> individual name <tab> species/ tissue name <tab> Ct”. If a line starts with # symbol, it will be ignored. The compund species/tissue name is composed of the one letter species abbreviation and the full tissue name (e.g. mLiver is Macaque liver).
For instance:
Gene1 <tab> 95.6
Gene2 <tab> 96.6
Gene3 <tab> 98.6
Gene4 <tab> 99.5
gene <tab> individual name <tab> species/tissue name <tab> Ct
Gene1 <tab> individual1 <tab> mLiver <tab> 23.4
Gene2 <tab> individual1 <tab> mLiver <tab> 23.8
Gene3 <tab> individual1 <tab> mLiver <tab> 24.4
Gene3 <tab> individual1 <tab> mLiver <tab> 26.6
...
Gene1 <tab> interPlate1 <tab> interPlate cells <tab> 26.6
Gene2 <tab> interPlate1 <tab> interPlate cells <tab> 26.8
Gene3 <tab> interPlate1 <tab> interPlate cells <tab> 27.6
Gene4 <tab> interPlate1 <tab> interPlate cells <tab> 26.7

aParameters.in

This is the parameterization file.

PLATES = ../data/plate1.txt,../data/plate2.txt
OUTPUT FILE NAME = example.out
CONTROLS = control1,control2
STDV = -1
INTERRUNS = Yes
INTERRUNS (TISSUE,INDIVIDUAL) = cells,IMR32
INTERRUNS (GENES) = GOI1,GOI2
INTERRUNS (CONTROLS) = control3,control4
EXPRESSION REFERENCE = 0
FULL SPECIES NAMES = m:Macaque,c:Chimpanzee,h:Human
REPLICATES = Yes

PLATES
Input files, comma separated of plate file names (including path to files)

OUTPUT FILE NAME
output file (including path to the file) will contain all intermediate results and final calibrated results

CONTROLS

Comma separated list of control genes used in the analysis (all samples are suppose to have all the control genes listed) ❹.

STDV

Threshold for standard deviation of technical replicates ❶. If the observed standard deviation is greater than STDV then gene/sample/individual mean Ct across technical replicates will be ignored. If gene/sample/individual is ignored and it is a control genes then all the genes using this specific control gene/sample/individual will be eliminated from the analysis. Specify -1 to ignore this threshold.

FULL SPECIES NAMES

Comma separated list of species abbreviations and full names. This will be used for For instance, given m:Macaque, mLiver label is for Macaque liver.

REPLICATES

if Yes, then in case a gene/sample/individual is repeated across plate, the average of all the replicates will be reported at the end, otherwise all the calibrated expression values will be reported.

EXPRESSION REFERENCE

Should be 0 or 1. It will determine how to calculate $Ct_{reference}$ ❷.0= mean of all Cts for a plate will be used for the delta-delta Ct calculation. 1= minimum of all Cts for a plate will be used for the delta-delta Ct calculation.

INTERRUNS

If Yes, then an inter-plates calibration will be performed. If STDV is specified (not -1) and one of the genes of the inter-plates calibration fails then the whole plate will be ignored.

INTERRUNS (TISSUE,INDIVIDUAL)

Tssue and individual names for the inter-plates calibration. They should be unique and different from the tissue and individual of the data you want to analyze. The program recognizes the inter-plates calibrator with these two labels.

INTERRUNS (GENES) ❺

Comma delimited list of genes for inter-plates calibration. It should always be present.

INTERRUNS (CONTROLS) ❻

Comma delimited list of control genes. If empty then the plate will be calibrated with the geometric mean of the genes specified with INTERRUNS (GENES). Otherwise, it will perform the method described by Hellemans et al. (2007) that is normalizing

INTERRUNS (GENES) with INTERRUNS (CONTROLS) and use the geometric mean of this ratio as the inter-plates calibrator.

REFERENCES

Hellemans, J. *et al.* (2007) qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol.* **8**(2):R19.