

About “qPCR data with Methods paper”

The collection of qPCR data that you can download from this site consists of the data that were used in the comparison of PCR curve analysis methods carried out by the original developers of these analysis methods. The purpose of this comparison is to help the user of these curve analysis methods in the choice of the appropriate method for his/her application. Secondly we like to provide a benchmark for developers of qPCR data analysis programs. The raw fluorescence in this collection can be used to test the developed algorithms. We noticed that in the past our methods were not always applied in the correct and currently recommended way. To avoid such unjustified use, also the results of our analysis of these data can be found in this data collection. The results of the comparison of the included curve analysis methods, and the details on how bias, resolution and precision were evaluated, can be found in our contribution to the Transcriptional Biomarker issue of Methods that will be released in early 2013. For the reference to the online publication: see below.

References

Paper in the Transcriptional Biomarker issue of Methods:

- [Ruijter et al.](#) Evaluation of qPCR curve analysis methods for reliable biomarker discovery: Bias, resolution, precision, and implications. *Methods*, Volume 59, Issue 1, January 2013, Pages 32-46

The biomarker dataset used in this study was originally published in:

- [Vermeulen et al.](#) Predicting outcomes for children with neuroblastoma using a multigene-expression signature: a retrospective SIOPEN/COG/GPOH study. *Lancet Oncology* 10: 663-671, 2009

The individual curve analysis methods: original papers, online implementations, stand-alone programs and websites.

- **5PSM:** [Spiess et al. \(2008\)](#) Highly accurate sigmoidal fitting of real-time PCR data by introducing a parameter for asymmetry. *BMC Bioinformatics* 9: 221, 2008. **Website:** [qpcR software](#).
- **Cy0:** [Guescini et al. \(2008\)](#) A new real-time PCR method to overcome significant quantitative inaccuracy due to slight amplification inhibition. *BMC Bioinformatics* 9: 326, 2008. **Website:** [Cy0 method](#).
- **FPK-PCR:** [Lievens et al. \(2011\)](#) Enhanced analysis of real-time PCR data by using a variable efficiency model: FPK-PCR. *Nucleic Acids Research* 40: e10, 2011.
- **LinRegPCR:** [Ruijter et al. \(2009\)](#) Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Research* 37: e45, 2009. **Program download:** [LinRegPCR](#). **Website:** [LinRegPCR](#)
- **LRE qPCR:** [Rutledge. \(2011\)](#) A Java program for LRE-based real-time qPCR that enables large-scale absolute quantification. *PLoS One*: e17636, 2011. **Program download:** [LRE Analyzer program](#).
- **MAK2:** [Boggy and Woolf. \(2010\)](#) A mechanistic model of PCR for accurate quantification of quantitative PCR data. *PLoS One*: e12355, 2010. **Website:** [DNA Software](#)

- **PCR Miner:** [Zhao and Fernald. \(2005\)](#) Comprehensive algorithm for quantitative real-time polymerase chain reaction. J Comput Biol 12: 1047-1064, 2005. **Website:** [PCR Miner](#).

The web-based [CAmpER](#) software, developed by Jochen Blom, implements two curve analysis algorithms:

- **DART:** [Peirson et al. \(2003\)](#) Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis. Nucleic Acids Research: e73, 2003.
- **FPLM:** [Tichopad et al. \(2003\)](#) Standardized determination of real-time PCR efficiency from a single reaction set-up. Nucleic Acids Research: e122, 2003.

Version History

qPCRDataMethods version 1.1 released September 2012

Added the analysis template for concentration series to the data

Analysis template:

Excel file with:

- calculation of performance indicators
- F0 data per curve analysis method
- Cq values per curve analysis method

qPCRDataMethods version 1.0 released September 2012

First release with online publication of the Methods paper

Raw fluorescece data:

All raw data are in Excel in a "one reaction per row - one cycle per column" format

Biomarker dataset:

- 64 genes (in 4 Excel files)
- 366 tissue samples
- dilution series in triplicate
- no template control

Technical dataset:

- 380_replicates

- 94_replicates_4_dilutions
- competitors

Analysis results:

Biomarker dataset:

- 1 Excel file with results per analysis method
- 1 sheet per observed parameter

Technical dataset:

- 1 Excel file per dataset
- 1 sheet with results per analysis method