dpcR: web server and R package for analysis of digital PCR experiments

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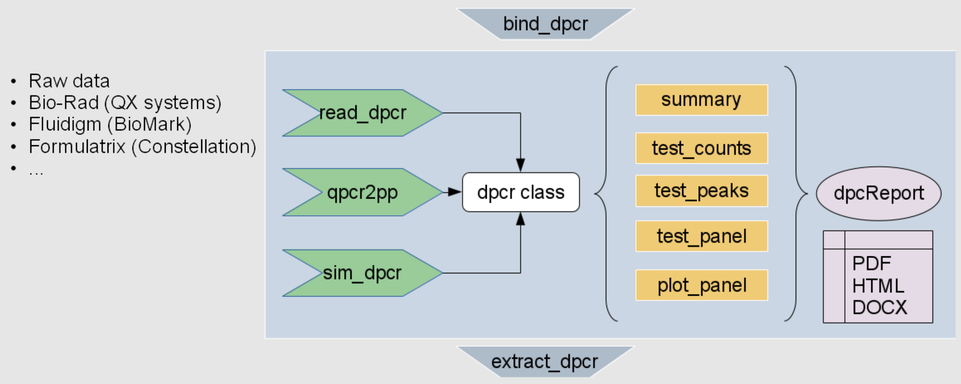
Introduction

dPCR reaction consists of multiple amplifications occurring in numerous small partitions. The result of dPCR is a binary vector describing states of partitions (positive in case of detected amplification, negative otherwise). This data is further used to estimate the main parameter, λ, which may be interpreted as the mean number of template molecules per partition.

We created *dpcR*, an open source tool for reproducible analysis of dPCR data, fully compatible with dMIQE requirements.

dpcR workflow



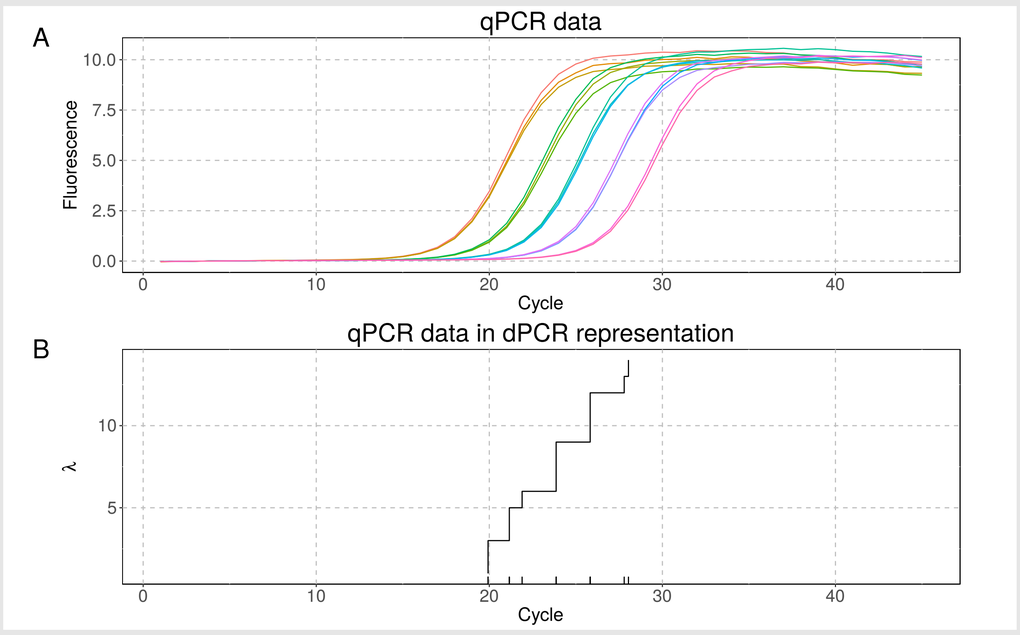
The workflow diagram shows main functions available at the each step of a dPCR data analysis.

Data import

Import functions limit availability of the package by determining which datasets can be easily processed using the provided framework. Since the RDML format for dPCR is not yet established, we wrote function *read_dpcr* streamlining data import from several systems produced by Bio-Rad, Fluidigm and Formulatrix.

To cover experimental or not yet included systems, we created a "raw data" format (see Supplementary Files for description). The user can manually arrange his data in this format and import it to the *dpcR* package. Such input files can be created in a spreadsheet program or a text editor.

Integration of qPCR data



The dPCR methodology may be used to analyze qPCR data (Mojtahedi et al., 2014). Quantification points (Cq) are computed using the real-time measurements of several amplification curves (A). Next, the Cq values are binarized and treated as the status of partitions effectively converting multiple qPCR experiments into a dPCR (B). This functionality is supported by the *qpcr2pp* function.

Case study: analysis of dPCR data

We present the functionalities of *dpcR* using the exemplary data set consisting of three experiments.

Experiment	Assay	Positive partitions	Total partitions
A	Chr4	10	765
A	MYC	9	765
B	Chr4	41	765
B	MYC	38	765
C	Chr4	104	765
C	MYC	96	765

Case study 1: calculation of the uncertainty

To determine the uncertainty of the estimated λ *dpcR* employs two previously published peer-reviewed methods Dube et al. (2008); Bhat et al. (2009).

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Availability and funding

dpcReport web server:
`www.smorfland.uni.wroc.pl/dpcReport`
dpcR download:
`http://cran.r-project.org/package=dpcR`
This research was partially funded by KNOW Consortium.

Bibliography

Bhat, S., Herrmann, J., Armishaw, P., Corbisier, P., and Emslie, K. R. (2009). Single molecule detection in nanofluidic digital array enables accurate measurement of DNA copy number. *Analytical and Bioanalytical Chemistry*, 394(2):457–467.

Dube, S., Qin, J., and Ramakrishnan, R. (2008). Mathematical analysis of copy number variation in a DNA sample using digital PCR on a nanofluidic device. *PloS one*, 3(8):e2876.

Mojtahedi, M., Fouquierd'Hérouël, A., and Huang, S. (2014). Direct elicitation of template concentration from quantification cycle (Cq) distributions in digital PCR. *Nucleic Acids Research*, 42(16):e126–e126.