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

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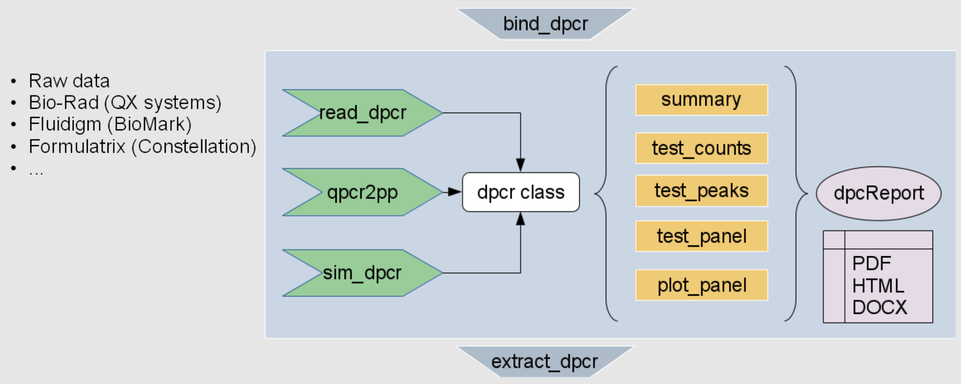
Introduction

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



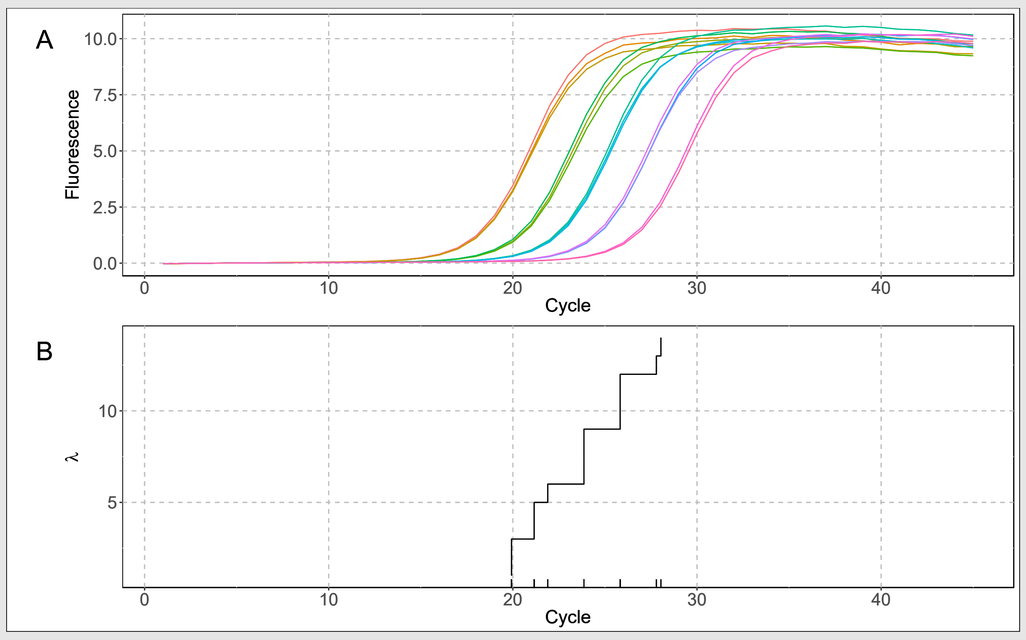
The workflow diagram shows main functions available at the each step of a dPCR data analysis.

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 (**?**). 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.

Calculation of the uncertainty

To determine the uncertainty of the estimated λ we employ two previously published peer-reviewed methods The first **?** uses the normal approximation to compute the confidence intervals for binomially distributed $\frac{k}{n}$.
The other method **?** is based on the uncertainty of the measurement and includes also the uncertainty caused by the variation of volume. The exact formula for the uncertainty of the λ value, u_λ, is:

$$u_\lambda = \sqrt{\frac{k}{n^2(1-\frac{k}{n})}} \tag{1}$$

and for the uncertainty of the concentration, u_c, of the template molecules:

$$u_c = C\sqrt{\left(\frac{1}{\log 1-\frac{k}{n}}\sqrt{\frac{k}{n(n-k)}}\right)^2 + \left(\frac{u_V}{V}\right)^2} \tag{2}$$

where u_V is uncertainty of the volume. Both methods are implemented in the *summary* function.

Availability and funding

signal.hsmm web server:
`www.smorfland.uni.wroc.pl/signalhsmm`
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Bibliography