Supplement to: chipPCR: an R Package to Pre-Process Raw Data of Amplification Curves

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Contents

The previous vignette still applies and can be found online at github. For further reading please refer to the paper Rödiger, Burdukiewicz, and Schierack (2015) about the *chipPCR* package.

1 Summary

Background: Both the quantitative real-time polymerase chain reaction (qPCR) and isothermal amplification are standard methods used for the quantification of nucleic acids (DNA, RNA). Numerous real-time read-out technologies with different technical platforms have been developed so far. However, analysis of amplification curves consists of cascaded steps implemented similarly across all existing technological platforms. Despite the growing interest in amplification-based techniques, there are only few open source tools for pre-processing real-time amplification data. The availability of software for pre-processing raw amplification data is mandatory in different scenarios, for example during the development, optimization and improvement of the functionality of instruments.

Results and Conclusion: chipPCR is a versatile *R* package for pre-processing (e.g., imputation of missing values, smoothing) and quality analysis of raw data for amplification curves coming from conventional quantitative polymerase chain reactions (qPCR), and quantitative isothermal amplification (qIA) reactions. The package contains datasets, which were generated by helicase-dependent amplification (HDA) or polymerase chain reaction (PCR) under various temperature conditions and detection systems, such as hydrolysis probes and intercalating dyes. The structure of the packages is amenable for integration to Web-based and standalone *shiny* applications.

2 Availability, requirements and setting up a working environment

Convention: According to the MIQE guidelines ("Minimum Information for publication of Quantitative real-time PCR Experiments'' (Bustin et al. 2009) is the threshold cycle (Ct) referred to as quantification cycle (Cq). We use the expression Cq exclusively regardless of the amplification method and mathematical principle. The abbreviations MFI (mean fluorescence intensity), RFU (relative fluorescence intensity) and refMFI (referenced MFI) are arbitrary units of fluorescence intensity.

The software is available in an **R** environment or through web browser applications:

- Project name: chipPCR,
- Project homepage (development),
- Project homepage at CRAN,
- Operating System: Platform independent,
- Other requirement: R 3.1.0 or higher,
- License: GPL-3

We use **R**'s *S4* class system (see *methods* package) to separate the interface and the implementation because, unlike the **R**'s *S3* class system, it requires the explicit declaration of classes and the inheritance and relationship for each class or method. Therefore, the number and types of objects in slots in an instance of a class have to be established at the time of the class definition. Objects belonging to the class are validated against this definition and have to fulfill it at any time. *setGeneric* declares generic functions. *S4* methods are declared by calls to *setMethod* together with the name of generics and signatures of the arguments. Signatures are used for identification of classes of one or more arguments of the methods.

S4 classes therefore require a higher development effort than *S3* classes, but offer more stringent control over the contents of created objects. Additional information (e.g., results, parameters) can also be included. *S4* assures better control on the object structure and chosen method dispatch (Karatzoglou et al. 2004).

For high-throughput capability, we avoided loops in the core structures of the *chipPCR* package and partially used parallel computing (*smoother* function) to keep the code fast. This package supports the use of most popular R packages, thus providing a communication layer required for parallel computing: *parallel* and *snow*.

This vignette can be viewed from **R** using command: `vignette("chipPCR")`. All experimental details for the datasets are described in the *chipPCR* package manual and in the citations. To start an analysis it is required to choose a dataset (as shown below).

3   Acknowledgment

Authors' contributions: SR conceived the study, and participated in its design and coordination and wrote the manuscript. SR and MB developed the software. All authors read and approved the final version of the manuscript.

References

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