chipPCR: an R Package to Pre-Process Amplification Curve Data

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ABSTRACT

Motivation: The quantitative real-time polymerase chain reaction (qPCR) and quantitative isothermal amplification (qIA) are standard methods for quantification of nucleic acids. Numerous real-time read-out technologies with different technical foundations have been developed. Despite the continuous interest in amplification based techniques, there are only few tools for amplification data pre-processing. Especially, during development of new instruments a transparent tool for precise control of raw data is indispensable.

Results: *chipPCR* is an **R** package for pre-processing and quality analysis of amplification curve data. The package takes advantage of **R**'s *S4* object model and offers an extensible environment. It contains tools for the raw data exploration: normalization, baselining, imputation of missing values, smoothing amplification curves and detecting the start and end of an amplification curve. Capabilities of the software are enhanced by implementation of algorithms yet not present in **R**, as a 5-point stencil for derivative interpolation. Simulation tools, statistical tests, plots for data quality management, amplification efficiency/quantification cycle calculation, and 22 data sets from various qPCR and qIA experiments are also part of the package. The core functionalities of *chipPCR* are integrated in GUIs (web-based and standalone *shiny* applications) streamlining analysis and report generation.

Availability: Stable: http://cran.r-project.org/web/packages/chipPCR
Source code: https://github.com/michbur/chipPCR
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Supplementary: Supplementary data are available at Bioinformatics online.

1 INTRODUCTION

Quantitative polymerase chain reaction (qPCR) and quantitative isothermal amplification (qIA) are standard methods to amplify nucleic acids. These methods are used in real-time monitoring technologies, such as our previously reported VideoScan technology, microfluidic systems, point-of-care devices, and qPCR cyclers. Real-time technologies enable the quantification of nucleic acids by calculation of specific curve parameters like the quantification point (Cq) and the amplification efficiency (AE) (Rödiger *et al.*, 2013a, 2014; Pabinger *et al.*, 2014). The fundamental steps of amplification

curve analysis are: (1) raw data read-in, (2) pre-processing (e.g., noise reduction), (3) amplification curve processing (e.g., Cq calculation), (4) post-processing, (5) data export/report generation. **R** belongs to the most used bioinformatic s tools and is a rapid adopter for various technologies like digital PCR, NanoString nCounter Platform, and qPCR (Waggott *et al.*, 2012; Pabinger *et al.*, 2014). Most qPCR **R** packages focus on the read-in and (post)-processing of data from commercial qPCR systems. **R** packages for the steps 1. and 3.–5. are available (Pabinger *et al.*, 2014; Perkins *et al.*, 2012; McCall *et al.*, 2014; Gehlenborg *et al.*, 2013).

However, there is no **R** package for pre-processing and quality analysis of amplification curve data. Pre-processing is in most commercial cyclers a "black box", which sets severe limits to reproducible research (Leeper, 2014). Developmental qPCR and qIA technologies depend on tools to pre-process the raw data. Pre-processing algorithms remove stochastic errors and artefacts (Suppl. Sect. 2). Pre-processing addresses raw data inspection, raw data transformation in a format for successive analysis steps (e.g., smoothing, imputation), data reduction (e.g., removal of invalid sets) and data quality management. Misinterpretations are more likely if "arbitrary" corrections are performed. A manual alteration is in contradiction to reproducible research. The *chipPCR* package ("Lab-on-a-**Chip**" & **PCR**) was developed to fill this gap and to automatize pre-processing, data analysis/visualization and to offer a quality control for the statistical data analysis of qPCR and qIA experiments. **R** offers sophisticated statistical tools and allows reproducing analysis on different platforms as well as adopting to changing experimental setups. Moreover, it is desirable to set up workflows in an open environment, which enables downstream analyses and which offers powerful tools for data visualizations and automatic report generation. The target audience encompasses developers and users who process raw data of commercial systems.

2 IMPLEMENTATION

chipPCR package was implemented in the **R** software environment as described elsewhere (R Core Team, 2014; Rödiger *et al.*, 2012). *chipPCR* is a relative of the *MBmca* (Rödiger *et al.*, 2013b), the *RDML* (Blagodatskikh *et al.*, 2014), and the *dpcR* (Pabinger *et al.*, 2014) packages but focusses on pre-processing of amplification curves. The package contains pre-processor functions (smoothing, imputation, background correction, normalization), a function for single-blinded randomized rating, quality analysis summary functions, a function to calculate the amplification efficiency, functions for

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amplification curve simulation and report generation (Suppl. Sect. 3). The supplemental material (packages vignette), uses Donald Knuth's literate programming principle (Knuth, 1984) to present the source code in a convenient way. *chipPCR*'s naming convention is *period.separated* (Bååth, 2012). We use **R**'s object model *S4* class system (see *methods* package) to separate between interface and implementation. It requires a higher effort than *S3* classes, but assures better control on the object structure and the method dispatch. As a perquisite for high-throughput technologies, we avoided loops in the core structures and left options for partially parallel computing usage (*smoother* function) to keep the code fast. *chipPCR* includes a set of classes for plotting. The output of our custom made plots is minimalistic, but many parameters can be adjusted directly or by the ellipse parameter.

Our goal is to make our software available also for researchers not fluent in **R**. We implemented core functionality of our package in various GUI-technologies available in **R** (Rödiger *et al.*, 2012; RStudio and Inc., 2014). Some of them are available as the web-based services. It is possible to run the GUI-applications as service on a server without installing **R** (e.g., http://michbur.shinyapps.io/MFIaggr_gui/), on the local desktop (e.g., Fig. S2, S6), or as deployed from an external source for a local **R** installation. The functions *AmpSim*, *th.cyc*, *bg.max* and *amptester* are part of online GUIs. We hope to build monolithic systems to parse, pre-process and analyze amplification curve data in a combined work-flow.

We have chosen not to rely on specialized parser but use native **R** workspaces, and dedicated **R** packages as default data format for import and export as described elsewhere (Perkins *et al.*, 2012; R Core Team, 2014). *chipPCR* presents *S4* objects with tailored summary and plot methods. Data sets are an essential element of reproducible research (Leeper, 2014). Our package contains 22 data sets from commercial and experimental cyclers along with the experimental settings (e.g., helicase dependent amplification (HDA)) (Suppl. Sect. 10).

3 EXAMPLE: QUALITY ANALYSIS

MFIaggr is a versatile analytical and graphical tool for fast multiple comparison of the cycle dependent signal dispersion and distribution (Fig. 1). The continuous explanatory variable x (cycle number) is used to describe its relationships to n continuous predictor variables y_i (fluorescence values), where $i \in \{1, ..., n\}$. Use cases include the comparison of independent reaction vessels or the analysis of replicate experiments (Suppl. Sect. 6). In particular, this function might be useful for quality management during the development of high-throughput technologies. An analysis via the *shiny MFIaggr.gui* app is shown in Fig. S6.

4 RESULTS AND CONCLUSIONS

There is an ongoing development for qPCR and qIA technologies. *chipPCR* is the first **R** package for the pre-processing and raw data quality analysis of amplification curve data of such systems. Though, *chipPCR* primarily targets pre-processing we also implemented standard methods to process amplification curve data. Functions of *chipPCR* are embeddable in customized routines with other packages (see Suppl.). For example, the packages *dpcR* and *MBmca* depend on *chipPCR* technology. *chipPCR* is build from smaller blocks. We claim that the modular structure of *chipPCR* package allows user to perform flexible data analysis adjusted to their needs. Users can do estimations by hand. For example for quantification cycle (second derivative maximum, SDM) estimation, solely the *chipPCR* functions *inder* and *smoother* are needed. *smoother* will be a method of smoothing in *inder* and by putting data in the *bg* object with summary method *summary-der*
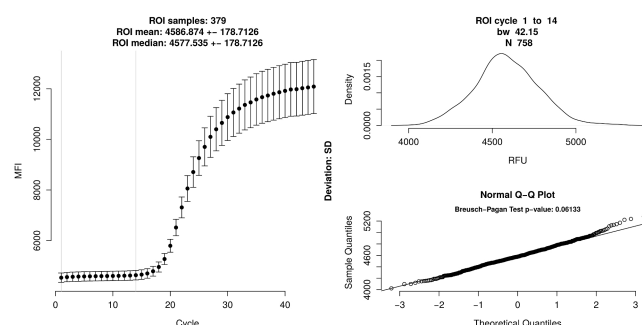

Fig. 1. Amplification curve analysis of 379 replicates. Cycles 1 to 14 were selected as region of interest (ROI) for *MFIaggr* to analyzes the cycle-dependent variance (left panel) and gives a density plot (right upper panel) and quantile-quantile analysis (right lower panel). The plots indicates that the data of the background range are normal distributed.

the user obtains the SDM. Thanks to the GUI it should be easy even for an users without any **R** experience omitting the biggest limitation of all **R** packages related to qPCR and qIA.

REFERENCES

Bååth, R. (2012). The State of Naming Conventions in R. *The R Journal*, **4**(2), 74–75.

Blagodatskikh, K. A., Roediger, S., and Burdukiewicz, M. (2014). Importing real-time thermo cycler (qPCR) data from RDML format files.

Gehlenborg, N., Noble, M. S., Getz, G., Chin, L., and Park, P. J. (2013). Nozzle: a report generation toolkit for data analysis pipelines. *Bioinformatics*, **29**(8), 1089–1091.

Knuth, D. E. (1984). Literate Programming. *The Computer Journal*, **27**(2), 97–111.

Leeper, T. J. (2014). Archiving Reproducible Research and Dataverse with R. *The R Journal*, **6**(1), NN–NN.

McCall, M. N., McMurray, H. R., Land, H., and Almudevar, A. (2014). On non-detects in qPCR data. *Bioinformatics (Oxford, England)*.

Pabinger, S., Rödiger, S., Kriegner, K., Vierlinger, K., and Weinhäusel, A. (2014). A survey of tools for the analysis of quantitative PCR (qPCR) data. *Biomolecular Detection and Quantification*.

Perkins, J. R., Dawes, J. M., McMahon, S. B., Bennett, D. L., Orengo, C., and Kohl, M. (2012). ReadqPCR and NormqPCR: R packages for the reading, quality checking and normalisation of RT-qPCR quantification cycle (Cq) data. *BMC Genomics*, **13**(1), 296. PMID: 22748112.

R Core Team (2014). R: A Language and Environment for Statistical Computing.

Rödiger, S., Friedrichsmeier, T., Kapat, P., and Michalke, M. (2012). RKWard: A Comprehensive Graphical User Interface and Integrated Development Environment for Statistical Analysis with R. *Journal of Statistical Software*, **49**(9), 1–34.

Rödiger, S., Schierack, P., Böhm, A., Nitschke, J., Berger, I., Frömmel, U., Schmidt, C., Ruhland, M., Schimke, I., Roggenbuck, D., Lehmann, W., and Schröder, C. (2013a). A highly versatile microscope imaging technology platform for the multiplex real-time detection of biomolecules and autoimmune antibodies. *Advances in Biochemical Engineering/Biotechnology*, **133**, 35–74.

Rödiger, S., Böhm, A., and Schimke, I. (2013b). Surface Melting Curve Analysis with R. *The R Journal*, **5**(2), 37–53.

Rödiger, S., Liebsch, C., Schmidt, C., Lehmann, W., Resch-Genger, U., Schedler, U., and Schierack, P. (2014). Nucleic acid detection based on the use of microbeads: a review. *Microchimica Acta*, **181**(11-12), 1151–1168.

RStudio and Inc. (2014). shiny: Web Application Framework for R. R package version 0.10.1.

Waggott, D., Chu, K., Yin, S., Wouters, B. G., Liu, F.-F., and Boutros, P. C. (2012). NanoStringNorm: An Extensible R Package For the Pre-Processing of NanoString mRNA and miRNA Data. *Bioinformatics*, page bts188.