

dpcReport: web server and software suite for unified analysis of digital PCRs and digital assays

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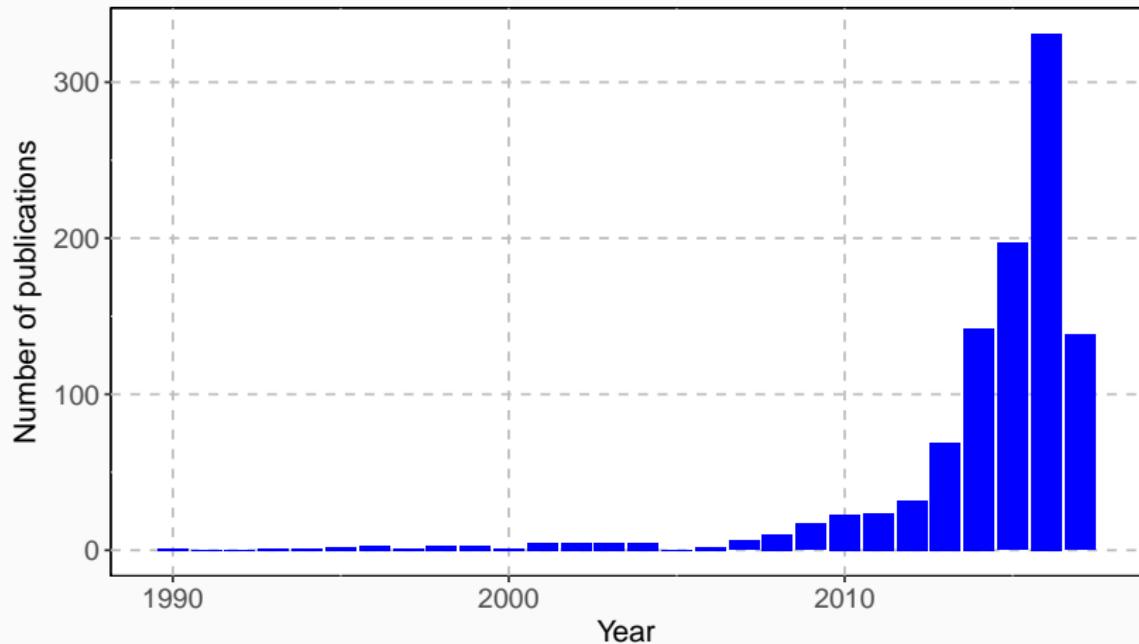
⁵Brandenburg University of Technology Cottbus-Senftenberg, Institute of Biotechnology

Outline

1. dPCR software.
2. Aim.
3. dpcReport framework.
4. Reproducibility.
5. Conclusions.

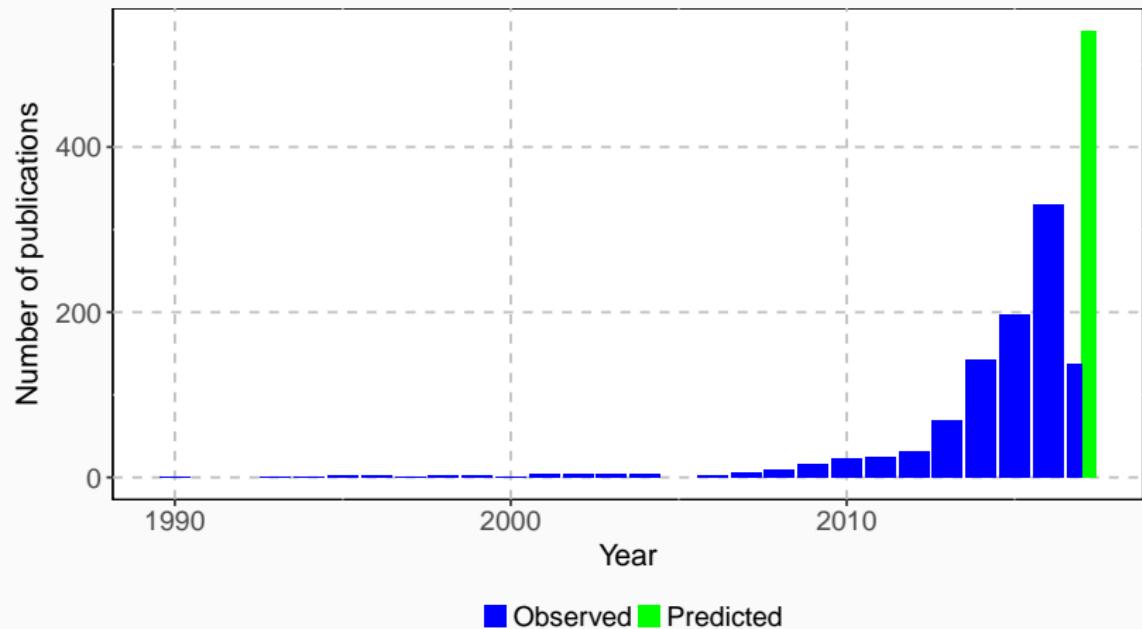
dPCR software

dPCR-related publications



Number of publications with words "digital PCR" or "dPCR" in the title/abstract.

dPCR-related publications



541 expected publications in 2017.

Vendor-provided dPCR software

Graphical user interfaces:

- QuantaSoft™ (Bio-Rad),
- OpenArray® Digital PCR Software (Thermo Fisher),
- Digital PCR Analysis software (Fluidigm).

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Closed-source software tied only to the vendor-specific data format.

Other dPCR software

Scripts and smaller tools tied to very specific task:

- **Mathematica** (Strain et al., 2013),
- **MS EXCEL** (Dobnik et al., 2015),
- **R** (Dreo et al., 2014; Trypsteen et al., 2015; Dorazio and Hunter, 2015; Vynck et al., 2016).

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- **R** (Dreo et al., 2014; Trypsteen et al., 2015; Dorazio and Hunter, 2015; Vynck et al., 2016).

R: a software environment and a programming language (R Core Team, 2016), extensively used in bioinformatics and biostatistics.

Other dPCR software

Web servers:

- uCount,
- definetherain (Jones et al., 2014),
- ddpcr (Attali et al., 2016) (also the **R** package).

Other dPCR software

Problems addressed:

- elimination of the rain,
- validity of Poisson assumption,
- better estimation of λ (mean number of template molecules per partition) and concentration.

Aim

Aim

Existing software	Desired software
closed-source (vendors), open-source (scientific)	open-source
partially reproducible	fully reproducible
tied to a specific dPCR platform	multi-platform
solving a specific problem	focused on common tasks
scattered	integrated

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dpcReport framework

dpcReport framework

dpcReport: a web server for dPCR data focused on the estimation of a template concentration in a sample.

dpcReport framework

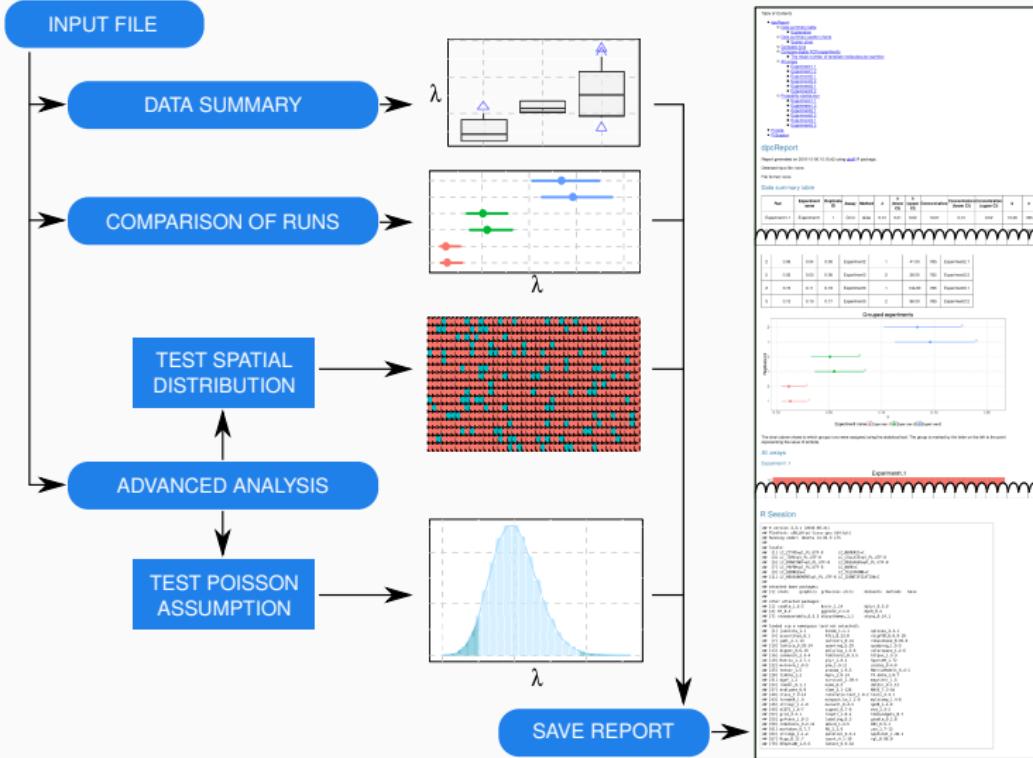
dpcReport: a web server for dPCR data focused on the estimation of a template concentration in a sample.

$$c = \lambda \times v \quad (1)$$

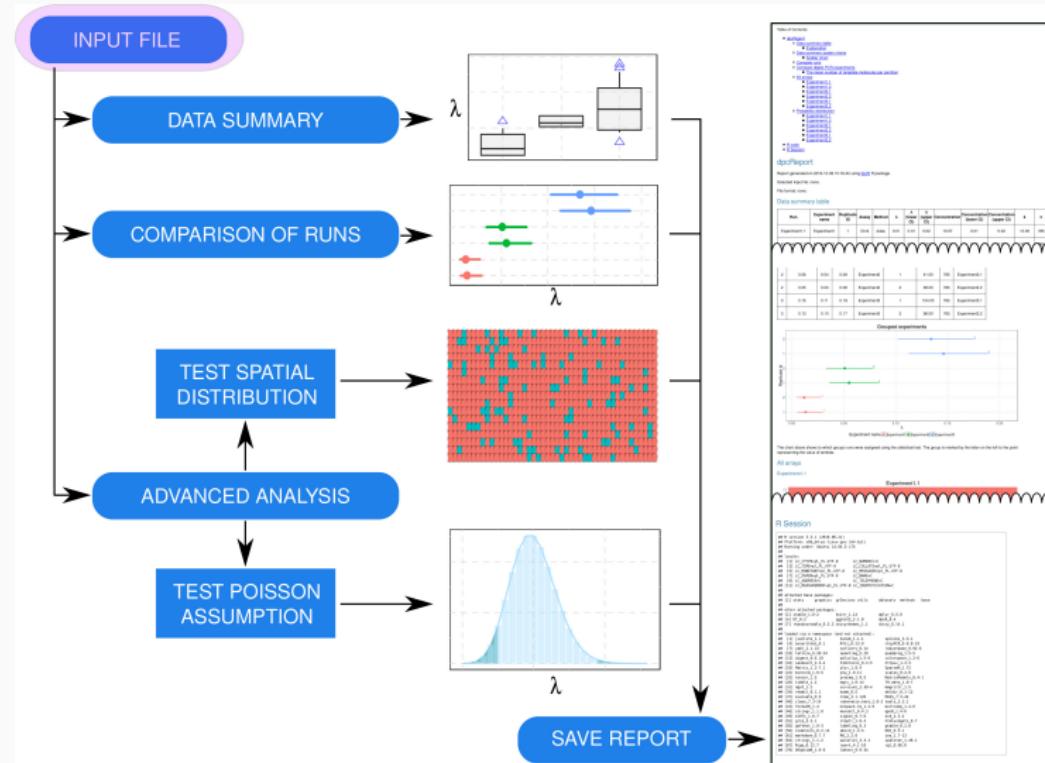
λ : mean number of template molecules per partition.

v : volume of a partition.

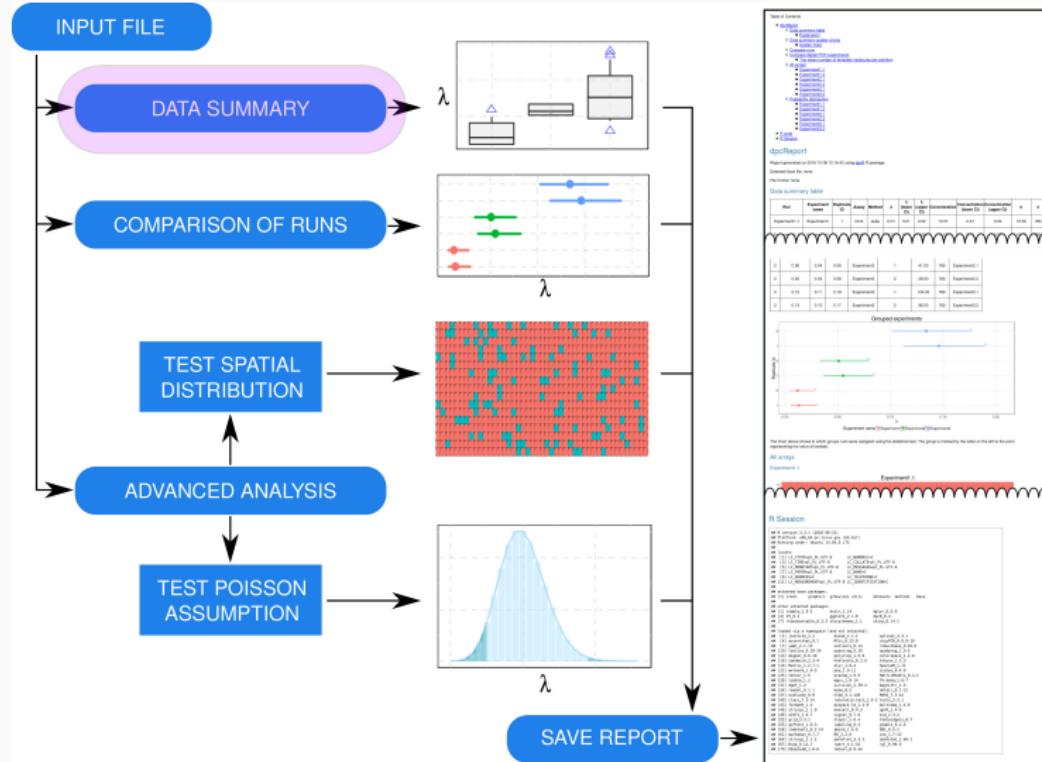
dpcReport framework



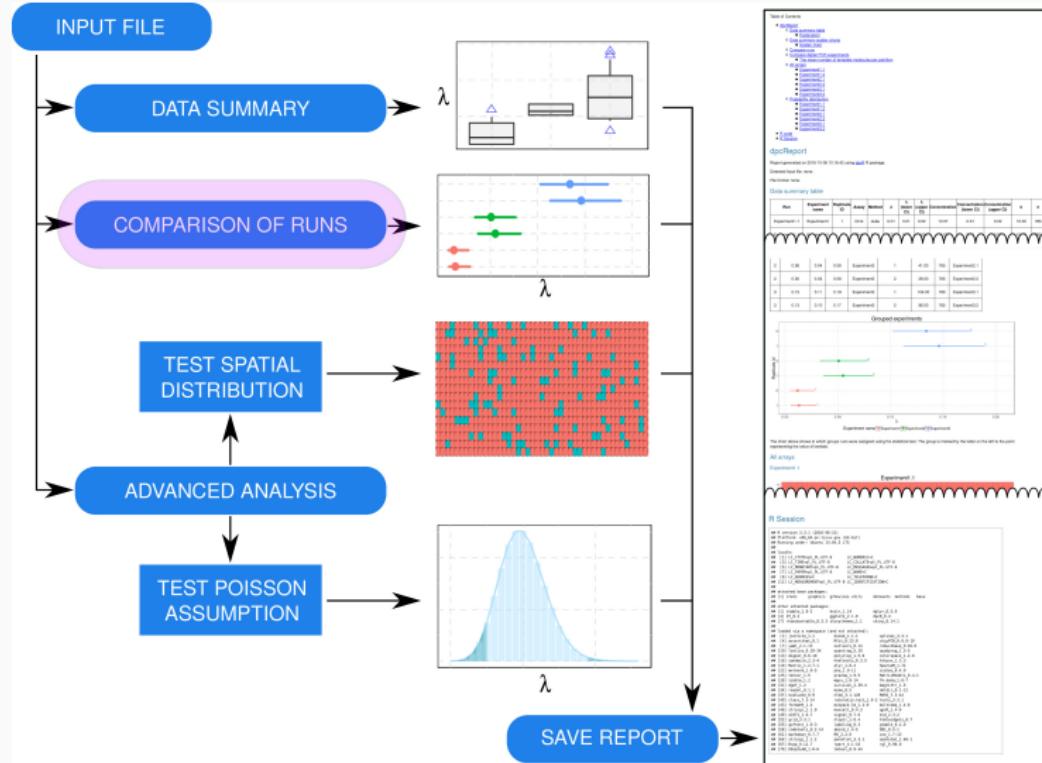
dpcReport framework



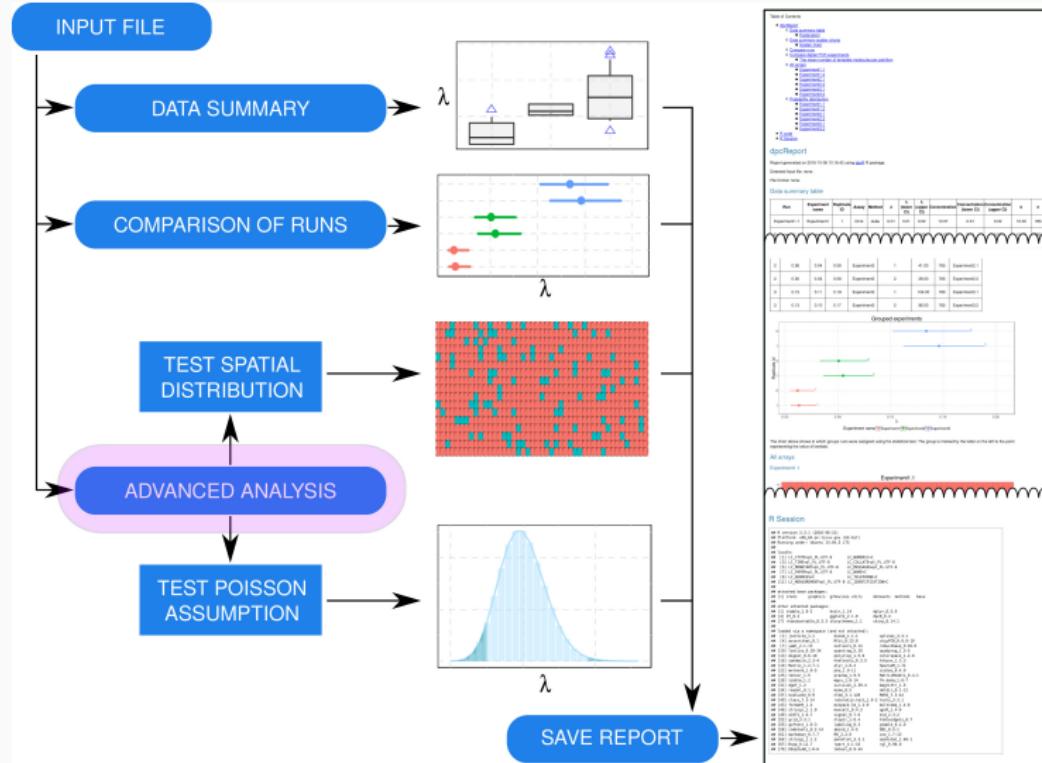
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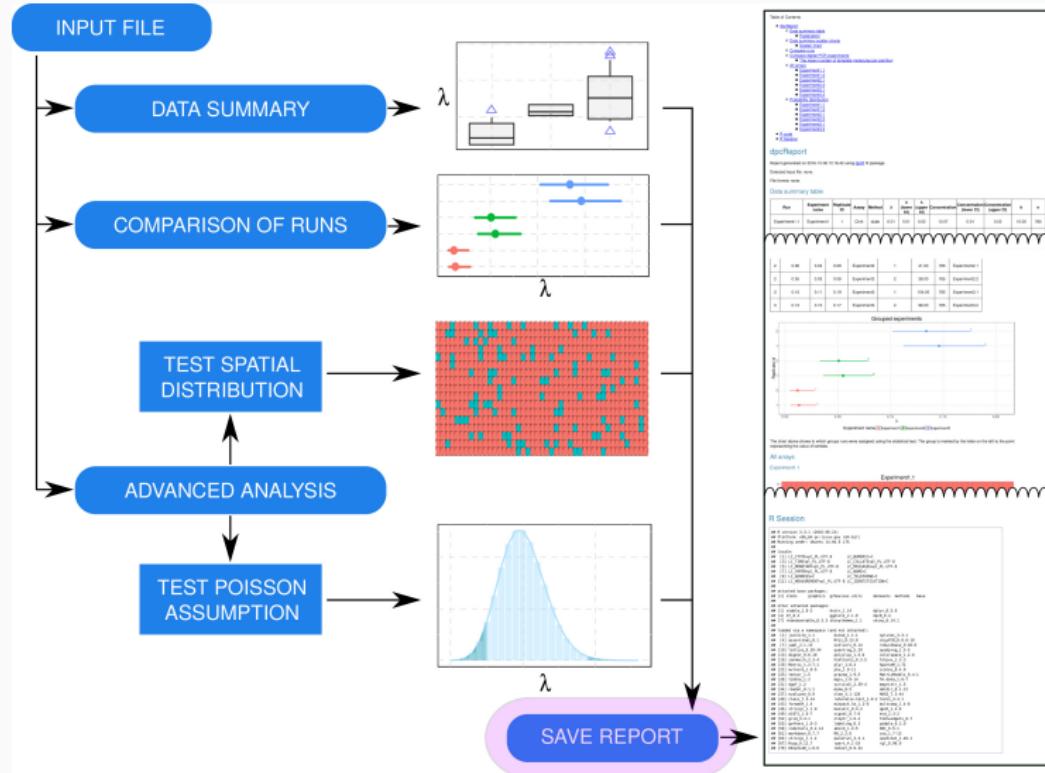
dpcReport framework



dpcReport framework



dpcReport framework



Input file

There are no universal format for dPCR data. Each system provides output in a different format. File formats differ between systems provided by the same vendor (QX100 vs QX200).

dpcReport supports analysis of data in following file formats:

- QX100 (Bio-Rad),
- QX200 (Bio-Rad),
- BioMark (Fluidigm).
- raw amplitude data.
- REDF.

REDF

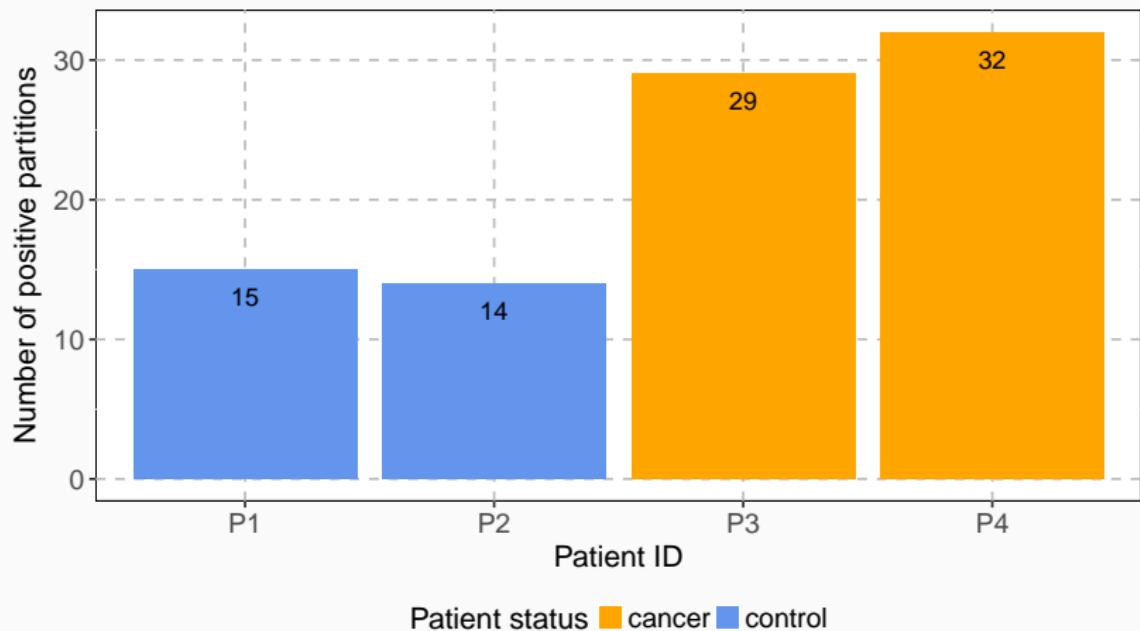
REDF (Raw Exchange Digital PCR format): universal and minimal format for dPCR data analysis.

experiment	replicate	assay	k	n	v	uv	threshold	panel_id
Experiment1	1	Chr4	11	765	1	0	1	1
Experiment1	2	MYC	9	765	1	0	1	2
Experiment2	1	Chr4	39	765	1	0	1	3
Experiment2	2	MYC	42	765	1	0	1	4
Experiment3	1	Chr4	92	765	1	0	1	5
Experiment3	2	MYC	85	765	1	0	1	6

Documentation: http://michbur.github.io/dpcR_manual/.

Comparison of multiple runs

dPCR was used in absolute quantification of levels of biomarker X in 4 patients.



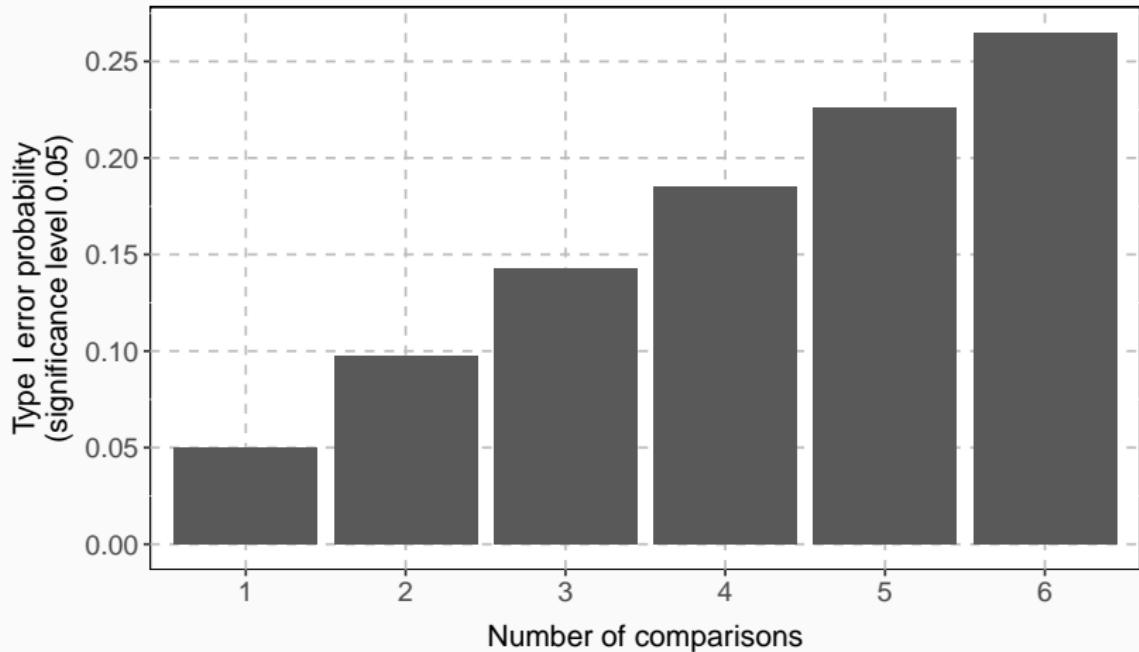
The number of partitions is constant and equal to 765.

Comparison of multiple runs

Are concentrations of biomarker X significantly different between patients?

k_1	k_2	p-value
15	14	1.0000
15	29	0.0488
15	32	0.0186
29	15	0.0488
32	15	0.0186
32	29	0.7982

Multiple comparison



Multiple comparison problem: the more comparisons, the higher chance of the type I error (a rejection of a true null hypothesis).

Comparison of multiple runs

Solution: False Discovery Rate (Benjamini and Hochberg, 1995)
(correction for multiple comparisons) used by
dpcReport (Burdukiewicz et al., 2016).

k_1	k_2	p-value
15	14	1.0000
15	29	0.0732
15	32	0.0559
29	15	0.0631
32	15	0.0559
32	29	0.9578

Comparison of multiple runs

Solution: False Discovery Rate (Benjamini and Hochberg, 1995) (correction for multiple comparisons) used by dpcReport (Burdukiewicz et al., 2016).

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15	14	1.0000
15	29	0.0732
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32	15	0.0559
32	29	0.9578

Comparison of multiple runs is one of the most common and crucial tasks in dPCR, but no software does that.

Comparison of multiple runs

Multiple comparison problem affects also confidence intervals when they are used for testing.

Aim of the study: how often the confidence interval of measured sample concentration covers the known sample concentration?

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- Data: three replicates of five dilutions of the reference gene measured using QX100 machine.

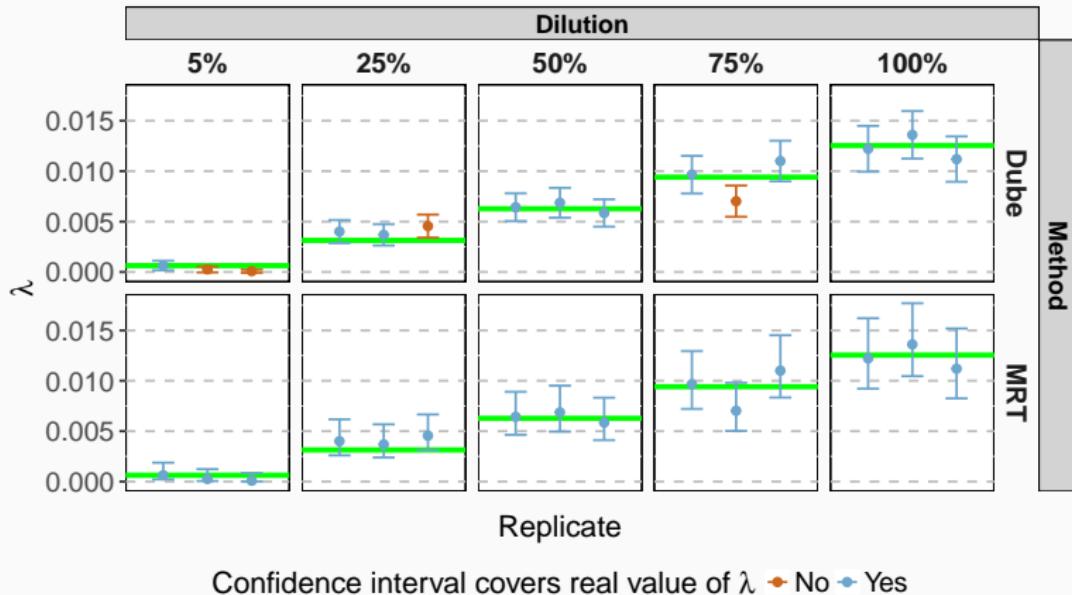
Comparison of multiple runs

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Aim of the study: how often the confidence interval of measured sample concentration covers the known sample concentration?

- Data: three replicates of five dilutions of the reference gene measured using QX100 machine.
- Methods: Bhat's confidence intervals (Bhat et al., 2009) and MRT confidence intervals (Burdukiewicz et al., 2016).

Comparison of multiple runs



Burdukiewicz et al. (2016)

Reproducibility

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Scientific software must support reproducibility, otherwise it is not scientific.

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Reports generated with dpcReport contain enough information to allow the full reproduction of the conducted analysis.

Reports

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 - [Explanation](#)
 - [Data summary scatter charts](#)
 - [Scatter chart](#)
 - [Compare runs](#)
 - [Compare digital PCR experiments](#)
 - [The mean number of template molecules per partition](#)
- [R code](#)
- [R Session](#)

dpcReport

Report generated on 2017-03-27 07:12:26 using [dpcR](#) R package.

Detected input file: 20130918_Dilution_log10.csv.

md5 checksum of the input file: 6bf3306199dd7af439d1c8acd08e23c1

The input was modified manually in dpcReport application.

File format: QX100.

Data summary table

Run	Experiment name	Replicate ID	Assay	Method	λ	λ (lower CI)	λ (upper CI)	Concentration	Concentration (lower CI)	Concentration (upper CI)	k	n
ABS1.A09.gDNA + P 10 ⁴	ABS1	A09.gDNA + P 10 ⁴	ileS	dube	0.07	0.07	0.08	970.44	0.08	0.09	936	13346
ABS1.A09.gDNA + P 10 ⁴	ABS1	A09.gDNA + P 10 ⁴	ileS	bhat	0.07	0.07	0.08	970.44	0.08	0.09	936	13346
ABS1.B09.gDNA		B09.gDNA										

Input file name

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Input file checksum

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A checksum allows detection of different files with the same name.

Manual alterations inside dpcReport

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ABS1.A09.gDNA + P 10^4	ABS1	A09.gDNA + P 10^4	ileS	dube	0.07	0.07	0.08	970.44	0.08	0.09	936	13346
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ABS1.B09.gDNA	...	B09.gDNA

R Session

dpcReport is based on the **R** package *dpcR*. All functionalities of dpcReport, including table and figure generation, are affected by changes in **R** and *dpcR*.

R Session

```
## R version 3.3.3 (2017-03-06)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 14.04.5 LTS
##
## locale:
## [1] LC_CTYPE=pl_PL.UTF-8      LC_NUMERIC=C
## [3] LC_TIME=pl_PL.UTF-8      LC_COLLATE=pl_PL.UTF-8
## [5] LC_MONETARY=pl_PL.UTF-8   LC_MESSAGES=pl_PL.UTF-8
## [7] LC_PAPER=pl_PL.UTF-8     LC_NAME=C
## [9] LC_ADDRESS=C              LC_TELEPHONE=C
## [11] LC_MEASUREMENT=pl_PL.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats      graphics    grDevices   utils      datasets   methods    base
##
## other attached packages:
## [1] xtable_1.8-2       knitr_1.15.1      digest_0.6.12
## [4] dplyr_0.5.0        DT_0.2          ggplot2_2.2.1
## [7] rhandsontable_0.3.4 shinythemes_1.1.1 shiny_1.0.0
## [10] dpcR_0.4
##
## loaded via a namespace (and not attached):
## [1] jsonlite_1.2        binom_1.1-1      splines_3.3.3
## [4] assertthat_0.1       highr_0.6        Rfit_0.23.0
## [7] chipPCR_0.0.8-11    yaml_2.1.14      outliers_0.14
## [10] robustbase_0.92-7   lattice_0.20-34  quantreg_5.29
## [13] quadprog_1.5-5      polyclip_1.5-6   colorspace_1.3-1
## [16] sandwich_2.3-4      htmltools_0.3.5  httpuv_1.3.3
## [19] Matrix_1.2-8        plyr_1.8.4       SparseM_1.74
## [22] mvtnorm_1.0-5       ptw_1.9-11      scales_0.4.1
## [25] tensor_1.5          pracma_1.9.5     MatrixModels_0.4-1
## [28] tibble_1.2           mgcv_1.8-16     TH.data_1.0-7
## [31] dgof_1.2            lazyeval_0.2.0   survival_2.40-1
## [34] magrittr_1.5          readxl_0.1.1     mime_0.5
## [37] deldir_0.1-12       evaluate_0.10   nlme_3.1-131
```

Reproducibility of the workflow

An analysis conducted in a GUI-based software, as *dpcReport*, is challenging to reproduce.

Reproducibility of the workflow

Choose elements of the report

- Data summary table
- Data summary scatter charts
- Compare runs
- Visualise and analyze individually each array
- Visualise and analyze probability distribution of each run
- R code used in the report generation

Be patient. The generation of the report may take few minutes.

 Save report

 Save input data (.csv)

dpcReport exports all steps of the analysis, including parameters adjusted manually by the user, in form of the **R** code that recreates the whole workflow.

Reproducibility of the workflow

R code

The R code below may be used to recreate reported results.

```
# Load packages
library(dpcR)
# if you do not have dpcR package, install it from GitHub:
# devtools::install_github("michbur/dpcR")
library(ggplot2) # ggplot2 library for nice plots
# Define theme for plots
cool_theme <- theme(plot.background=element_rect(fill = "transparent", colour = "transparent"),
panel.grid.major = element_line(colour="lightgrey", linetype = "dashed"), panel.background =
element_rect(fill = "white", colour = "black"), legend.background = element_rect(fill="NA"),
legend.position = "bottom", axis.text = element_text(size = 14), axis.title.x = element_text(size=17,
vjust = -0.1), axis.title.y = element_text(size = 17, vjust = 1), strip.text = element_text(size = 17,
face = "bold"), strip.background = element_rect(fill = "#9ecae1", colour = "black"), legend.text =
element_text(size=14), legend.title = element_text(size = 17), plot.title = element_text(size = 22),
legend.key = element_rect(fill = "white", colour = "black", linetype = "dashed", size = 0.5))
# Read and adjust data
# The input file is assumed to be in the current R working directory
input_data <- read_dpcr("20130918 Dilution_log10.csv", format = "QX100")

#####
# Print only table from summary.dpcr function
summary(input_data, print = FALSE)[["summary"]]

#####
# Prepare data for plots
plot_data <- summary(input_data, print = FALSE)[["summary"]]
plot_data <- plot_data[plot_data[["method"]] == "dube", ]
ggplot(plot_data, aes(x = experiment, y = lambda, ymax = lambda.up, ymin = lambda.low)) + geom_point(size =
4, alpha = 0.6, shape = 2, colour = "blue") + cool_theme + geom_boxplot(outlier.colour = NA, fill =
adjustcolor("lightgrey", alpha.f = 0.25), shape = 15) + ggtile(paste0("Experiment boxplot\nCI method: ",
```

Conclusions

Summary

dpcReport is an open-source, integrated software for the reproducible analysis of dPCR data.

Getting started

Web server: <http://tinyurl.com/dpcReport2>.

The screenshot shows the dpcReport web application. At the top, there is a blue header bar with white text containing the following navigation items: 'dpcReport' (which is bolded and blue), 'Input file', 'Data summary', 'Comparison of runs', 'Advanced analysis', 'Save report', and 'About'. Below the header, the main content area has a light gray background. It features a large blue header section with the text 'Welcome to dpcReport' in white. Below this, a smaller text says 'For in-depth description of the dpcReport, please refer to the **About** panel.' Underneath, there is a section titled 'Upload data' in blue. A sub-instruction 'Upload your data using the button below or analyze the preloaded data.' follows. A note 'Accepted data formats:' is present, followed by a bulleted list of supported file types. Another note 'See also [exemplary data files](#).' is shown, along with a list of accepted file formats. On the right side of the main content area, there is a vertical sidebar with the title 'dpcReport' at the top, followed by several sections with titles like 'Data summary', 'Comparison of runs', 'Advanced analysis', 'Save report', and 'About'.

dpcReport

Input file

Data summary

Comparison of runs

Advanced analysis

Save report

About

Welcome to dpcReport

For in-depth description of the dpcReport, please refer to the **About** panel.

Upload data

Upload your data using the button below or analyze the preloaded data.

Accepted data formats:

- raw data - comma-separated .csv file (for array digital PCR and droplet digital PCR).
- QX100 - data from QX100 Droplet Digital PCR System (Life Technologies).
- BioMark (Detailed Table Results) - BioMark (Fluidigm).
- BioMark (Summary Table Results) - BioMark (Fluidigm).
- amplification data: compressed (.zip) directory with amplification data from QX series.

See also [exemplary data files](#).

Accepted file formats:

- .CSV,
- .XLSX,
- .XLS,

Getting started

Local instance: <https://github.com/michbur/dpcR>.

```
install.packages("dpcR")
library(dpcR)
dpcReport()
```

Acknowledgements and funding

Collaborators:

- Jim Hugget and Alexandra Whale (LGC).
- Boris Fehse (University of Hamburg).
- Mario Menschikowski (Technical University of Dresden).
- Stefan Rödiger (Brandenburg Technical University).

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- KNOW Consortium Wrocław Center for Biotechnology,
- National Science Center (2015/17/N/NZ2/01845),
- COST action "Harmonising standardisation strategies to increase efficiency and competitiveness of European life-science research".

Local instance: <https://github.com/michbur/dpcR>.

Web server: <http://tinyurl.com/dpcReport2>.

Slides: <http://tinyurl.com/dpcReport-Freising>.

dpcReport and dpcR are part of pcRuniveRsum:

<http://michbur.github.io/pcRuniveRsum/> - everything for PCR in **R**.

References

- Attali, D., Bidshahri, R., Haynes, C., and Bryan, J. (2016). Ddpcr: An R package and web application for analysis of droplet digital PCR data. *F1000Research*, 5:1411.
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References II

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