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

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dPCR software

Aim

Reproducibility

dPCR software

Words, words

Create framework for dPCR data analysis:

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• tailored for the most common tasks,

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- tailored for the most common tasks,
- unified,

Create framework for dPCR data analysis:

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- unified,
- reproducible.

Reproducibility

Reproducibility

Scientific software supports reproducibility, otherwise it is not scientific.

A report must contain enough information to allow the full reproduction of the conducted analysis.

Reports

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- dpcReport
 - Data summary table
 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.

	Run	Experiment name	Replicate ID	Assay	Method	λ	λ (lower Cl)	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
Г	ABS1.B09.gDNA		B09.gDNA							0.44		****	44000

Date and time

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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
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					0.40	4474.07		0.40	****	44000

Input file name

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	Run	Experiment name	Replicate ID	Assay	Method	λ	λ (lower Cl)	λ (upper CI)	Concentration	Concentration (lower CI)	Concentration (upper CI)	k	n
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ľ	ABS1.B09.gDNA		B09.gDNA								0.40	****	*****

Input file checksum



Run	Experiment name	Replicate ID	Assay	Method	λ	λ (lower Cl)	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	1004	B09.gDNA				0.00	0.40	4474.07	0.44	0.40	4440	44000	

Changes in case of the manual alteration of the input file.

Manual alterations inside dpcReport

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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.

	Run	Experiment name	Replicate ID	Assay	Method	λ	λ (lower Cl)	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
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ľ	ABS1.B09.gDNA		B09.gDNA								0.40	****	*****

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:
                graphics grDevices utils
## [1] stats
                                               datasets methods
                                                                   base
##
## other attached packages:
   [1] xtable 1.8-2
                            knitr 1.15.1
                                                digest 0.6.12
## [4] dplvr 0.5.0
                            DT 0.2
                                                gaplot2 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] daof 1.2
                             lazveval 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 more challenging to reproduce.

dpcReport exports all steps of the analysis, including parameters adjusted manually by the user, in form of the ${\bf R}$ 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"),</pre>
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.
viust = -0.1), axis.title.v = element text(size = 17, viust = 1), strip.text = element text(size = 17,
face = "bold"), strip.background = element rect(fill = "#9ecael", 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"]]</pre>
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) + ggtitle(paste0("Experiment boxplot\nCI method: ".
```

Summary

dpcReport is the integrted environment for the analysis of dPCR data.

Availability

Web server: www.smorfland.uni.wroc.pl/shiny/dpcReport
R package (including your local instance of dpcReport):
www.github.com/michbur/dpcR

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- Małgorzata Kotulska.
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- Stefan Rödiger,
- biogram package
 (https://cran.r-project.org/package=biogram):
 - Piotr Sobczyk,
 - · Chris Lauber,
- AmyLoad database (comprec-lin.iiar.pwr.edu.pl/amyload):
 - Paweł Woźniak,

References I