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

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Outline

dPCR software

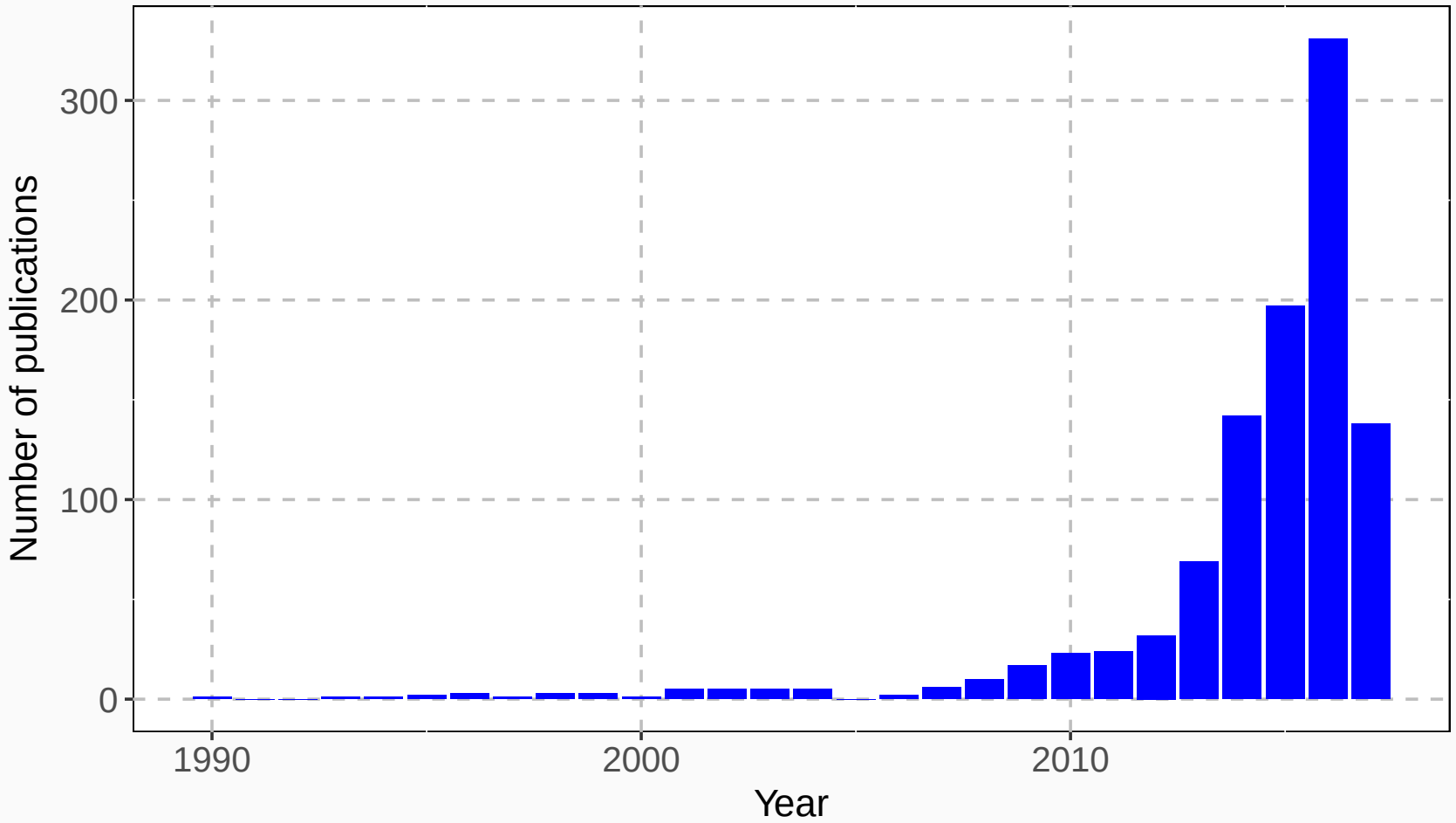
Aim

Methods

Reproducibility

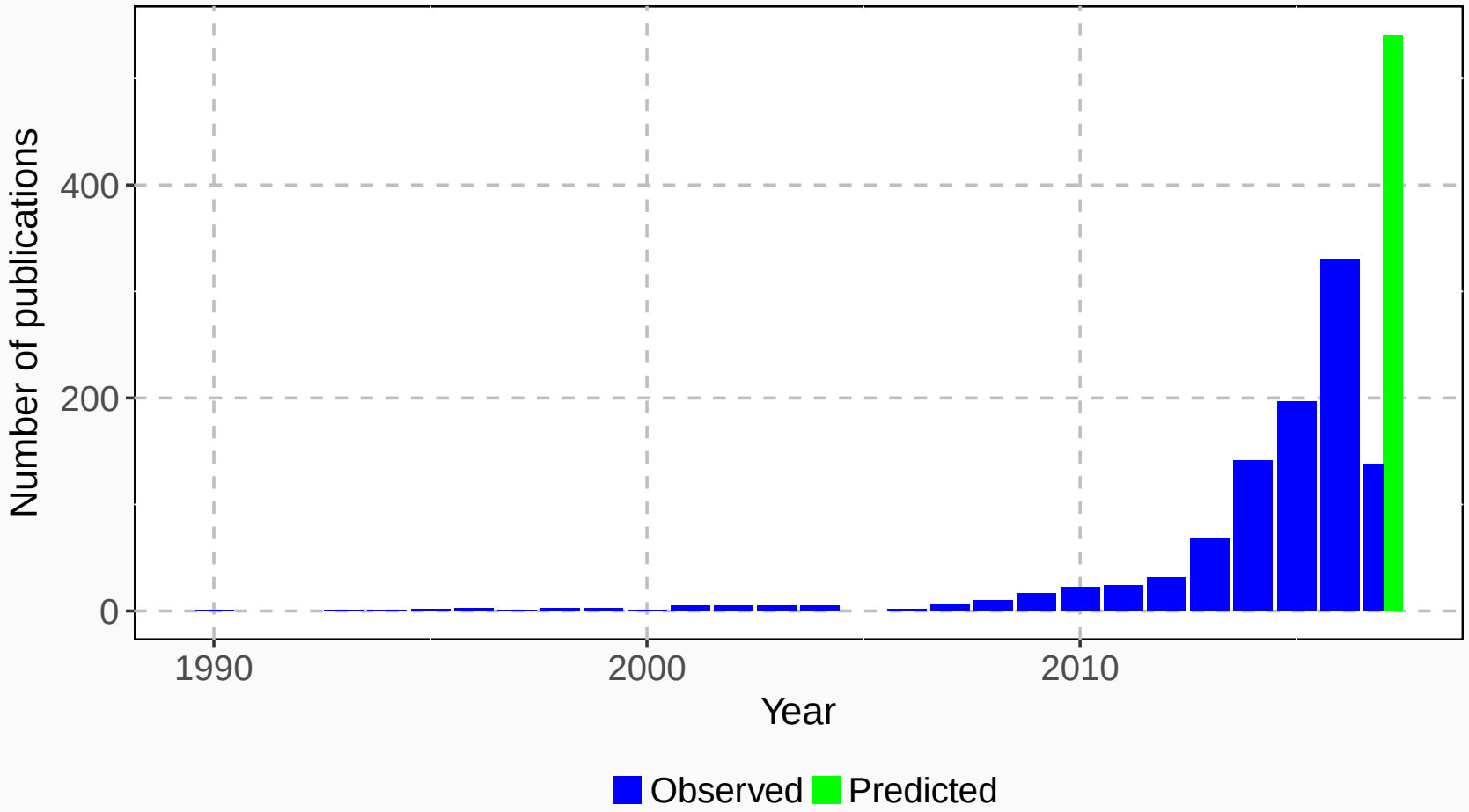
dPCR software

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™ (Biorad),
- 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).

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

Other dPCR software

Web servers (limited to published web servers):

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

Aim

Aim

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

Aim

Create framework for dPCR data analysis:

Aim

Create framework for dPCR data analysis:

- tailored for the most common tasks,

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Create framework for dPCR data analysis:

- tailored for the most common tasks,
- universal,

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Methods

Multiple comparison

Uniformly most powerful test

Multiple comparison

Confidence intervals

Array quality control

Reproducibility

Reproducibility

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

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

Reports

Table of Contents

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										

Date and time

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Input file name

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Input file checksum

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ABS1.B09.gDNA		B09.gDNA										

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

Manual alterations inside dpcReport

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ABS1.B09.gDNA		B09.gDNA										

R Session

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 more challenging to reproduce.

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) + 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`

Acknowledgements and funding

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- Jim Hugget and Alexandra Whale.
- Stefan Rödiger.

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