# Package 'ruijter'

April 23, 2024

**Type** Package

**Title** Technical Data Sets by Ruijter et al. (2013)

**Version** 0.1.3

**Description** The real-time quantitative polymerase chain reaction (qPCR) technical data sets by Ruijter et al. (2013) <doi:10.1016/j.ymeth.2012.08.011>: (i) the four-point 10-fold dilution series; (ii) 380 replicates; and (iii) the competimer data set. These three data sets can be used to benchmark qPCR methods. Original data set is available at <https://medischebiologie.nl/wp-content/uploads/2019/02/qpcrdatamethods.zip>. This package fixes incorrect annotations in the original data sets.

**License** CC BY 4.0

**Encoding** UTF-8

**LazyData** true

**RoxygenNote** 7.3.1

**Depends** R (>= 2.10)

**URL** https://github.com/ramiromagno/ruijter, https://rmagno.eu/ruijter/

**BugReports** https://github.com/ramiromagno/ruijter/issues

**Imports** tibble

**NeedsCompilation** no

**Author** Ramiro Magno [aut, cre] (<https://orcid.org/0000-0001-5226-3441>), Pattern Institute [cph, fnd]

**Maintainer** Ramiro Magno <rmagno@pattern.institute>

**Repository** CRAN

**Date/Publication** 2024-04-22 23:10:03 UTC

## R topics documented:

ds_380 . . . . . 2
ds_94_4 . . . . . 3
ds_competimer . . . . . 4

**Index** **6**

---

`ds_380` *380-replicates set*

---

## Description

The 380-replicates set is a dilution consisting of 150,000 molecules (see the Admonition section below) of the MYCN oligonucleotide was created in 10 ng/µl yeast tRNA carrier. qPCR amplifications were performed in 380 replicated 8 µl reactions and quadruplicated reactions of the NTC sample were performed on the same 384-well plate.

qPCR was done on a CFX 384 instrument (Bio-Rad). A 384-well qPCR plate was prepared using a 96-well head pipetting robot (Tecan Freedom Evo 150). qPCR amplifications were performed in 8 µl containing:

- 4 µl iQ SYBR Green Supermix (Bio-Rad);
- 0.4 µl forward and 0.4 µl reverse primer (5 µM each);
- 0.2 µl nuclease-free water;
- 3 µl of standard oligonucleotide.

All reactions were performed in 384-well plates (Hard-Shell 384-well microplates and Microseal B clear using adhesive seals (Bio-Rad).

The cycling conditions were comprised of 3 min polymerase activation at 95 °C and 45 cycles of 15 s at 95 °C and 30 s at 60 °C followed by a dissociation curve analysis from 60 to 95 °C.

**Admonition:**

The concentration information for the curves in this data set was seemingly incorrectly indicated in the original publication (confirmed by email with the senior author Prof. Jo Vandesompele). Instead of the 15,000 copies reported in the original paper, the number of copies was most likely 150,000 copies (10x the value indicated).

## Usage

```
ds_380
```

## Format

`ds_380`**:**

A data frame with 17,280 rows and 9 columns:

`well` Well identifier.

`replicate` Replicate identifier.

`dye` In all reactions the SYBR Green I master mix (Roche) was used, so the value is always `"SYBR"`.

`target` Target identifier: MYCN.

`sample_type` Sample type: `"ntc"` (no template control) or `"std"` (standard).

`copies` Standard copy number: either `0` (for NTCs), or `150000` for standards.

`dilution` Dilution factor (the reciprocal of `copies`). Higher number means greater dilution.

`cycle` PCR cycle.

`fluor` Raw fluorescence values.

## Source

https://medischebiologie.nl/wp-content/uploads/2019/02/qpcrdatamethods.zip

---

`ds_94_4` *94-replicates-4-dilutions set*

---

## Description

The 94-replicates-4-dilutions set is a dilution series consisting of four 10-fold serial dilution points, starting from 15,000 molecules down to 15 molecules, created using 10 ng/µl yeast tRNA as carrier (Roche). The same dilution of the carrier was used to create the NTC sample.

qPCR was done on a CFX 384 instrument (Bio-Rad). A 384-well qPCR plate was prepared using a 96-well head pipetting robot (Tecan Freedom Evo 150). qPCR amplifications were performed in 8 µl containing:

- 4 µl iQ SYBR Green Supermix (Bio-Rad);
- 0.4 µl forward and 0.4 µl reverse primer (5 µM each);
- 0.2 µl nuclease-free water;
- 3 µl of standard oligonucleotide.

A total of 94 replicated reactions were dispensed for each of 4 dilution points. In addition, the NTC reaction was analyzed in 8 replicates.

All reactions were performed in 384-well plates (Hard-Shell 384-well microplates and Microseal B clear using adhesive seals (Bio-Rad).

The cycling conditions were comprised of 3 min polymerase activation at 95 °C and 45 cycles of 15 s at 95 °C and 30 s at 60 °C followed by a dissociation curve analysis from 60 to 95 °C.

**Admonition:**

The concentration information for the curves in this data set was seemingly incorrectly indicated (confirmed by email with the senior author Prof. Jo Vandesompele). The annotation provided in the supplementary material that accompanies the original publication was reversed. So, to correct for this, the annotation of the number of copies in `ds_94_4` has been changed:

- 15 became 15,000
- 150 became 1,500
- 1,500 became 150
- 15,000 became 15

## Usage

```
ds_94_4
```

## Format

`ds_94_4`:

A data frame with 17,280 rows and 9 columns:

`well` Well identifier.

`replicate` Replicate identifier.

`dye` In all reactions the SYBR Green I master mix (Roche) was used, so the value is always `"SYBR"`.

`target` Target identifier: MYCN.

`sample_type` Sample type: `"ntc"` (no template control) or `"std"` (standard).

`copies` Standard copy number: either `0` (for NTCs), or `15`, `150`, `1500` or `15000` for standards.

`dilution` Dilution factor (the reciprocal of `copies`). Higher number means greater dilution.

`cycle` PCR cycle.

`fluor` Raw fluorescence values.

## Source

https://medischebiologie.nl/wp-content/uploads/2019/02/qpcrdatamethods.zip

---

# ds_competimer *Competimer set*

---

## Description

Competitive primers were synthesized on basis of identical sequence and blocked by amination at the 3' end to allow annealing, but avoid elongation during the PCR process.

A six-point 4-fold serial dilution series made from reference human genomic DNA (Roche), starting from 64 ng/µl down to 0.0625 ng/µl, was created in 10 ng/µl yeast tRNA as carrier. The same dilution of the carrier was used to create a NTC sample.

qPCR amplifications were performed in 7.5 µl total reaction volume containing:

- 3.75 µl 2x custom made qPCR SYBR green I Mastermix (Eurogentec)
- 0.375 µl forward primer (5 µM) and 0.375 µl reverse primer (5 µM)
- 1 µl of a mixture of nuclease-free water and equal amounts of both forward and reverse competitive (aminated) primers
- 2 µl diluted standard

A total of 7 competitive mixes were prepared for each dilution point, containing 0%, 5%, 10%, 20%, 30%, 40%, and 50% (of the total amount of primer) competitive (aminated) forward and reverse primers. Each reaction was run in triplicate.

The qPCR cycling was performed on the LightCycler480 (Roche) using white LightCycler480 384-multiwell plates with Light-Cycler480 sealing foils (Roche).

The cycling conditions were com prised of 10 min polymerase activation at 95 °C, and 45 cycles of 15 s at 95 °C, 30 s at 60 °C, followed by a dissociation curve analysis from 60 to 95 °C.

## Usage

```
ds_competimer
```

## Format

`ds_competimer`:

A data frame with 6,615 rows and 10 columns:

`well` Well identifier.

`replicate` Replicate identifier.

`dye` In all reactions the SYBR Green I master mix (Roche) was used, so the value is always `"SYBR"`.

`pct` Percentage of competitive (aminated) primers in the mix.

`conc` Concentration of reference human genomic DNA (Roche): from 64 ng/µl down to 0.0625 ng/µl.

`target` Target identifier: AluSx.

`sample_type` Sample type: `"ntc"` (no template control) or `"std"` (standard).

`dilution` Dilution factor (the reciprocal of `conc`, i.e. `64 / conc`). Higher number means greater dilution.

`cycle` PCR cycle.

`fluor` Raw fluorescence values.

## Source

https://medischebiologie.nl/wp-content/uploads/2019/02/qpcrdatamethods.zip

# Index

∗ **datasets**
ds_380, 2
ds_94_4, 3
ds_competimer, 4

ds_380, 2
ds_94_4, 3
ds_competimer, 4