

Package ‘qPCR.CFX.process’

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Type Package

Title Real-time qPCR data processing

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Author Dimitris Kokoretsis

Maintainer Dimitris Kokoretsis <dkokoret@gmail.com>

Description Calculates relative quantity of gene targets from real-time qPCR data (Cq values), exported from the Bio-Rad CFX Connect real-time PCR software.

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Encoding UTF-8

LazyData true

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ggplot2,
ggthemes

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R topics documented:

qPCR_analysis_expression	1
qPCR_analysis_ntc	2
qPCR_analysis_std_curve	3
qPCR_analysis_unk_rxns	3
qPCR_analysis_wrap	4
Index	5

qPCR_analysis_expression

Real-time qPCR relative quantity calculation.

Description

Calculates the relative quantity of target genes between one control sample and one or more test samples. Based on one or multiple reference genes.

Usage

```
qPCR_analysis_expression(unkdata, refgene, efficiencies = NULL, control = NULL)
```

Arguments

unkdata	A <code>data.frame</code> containing the technical replicate means of the unknown reactions. Result from <code>qPCR_analysis_unk_rxns</code> function.
refgene	Character vector with the name(s) of the reference gene(s).
efficiencies	A <code>data.frame</code> containing the efficiencies of each primer pair. The <code>\$efficiencies</code> <code>data.table</code> resulting from the <code>qPCR_analysis_stdcurve</code> function. Defaults to <code>NULL</code> , which assumes all primer pairs' efficiency being equal to 100%.
control	Character. The name of the control sample, against which all other samples will be compared. Defaults to the 1st sample alphabetically.

Details

Applies the Pfaffl calculation method, accounting for different primer efficiencies (Pfaffl, 2001). The common-base calculation method described by Ganger et al. 2017 is followed, which gives identical results to the Pfaffl method. If efficiencies are not entered (`NULL`), it defaults to the delta-delta-Ct method (Livak & Schmittgen, 2001).

Value

A `data.table` with the calculated expression of each sample, biological replicate and target gene.

qPCR_analysis_ntc

Real-time qPCR non-template control processing.

Description

Parses primary qPCR data (Cq values) and summarizes results for non-template controls. Compatible with Cq data exported from Bio-Rad CFX Connect real-time PCR machine.

Usage

```
qPCR_analysis_ntc(d)
```

Arguments

`d` `data.frame`, Cq value data exported from Bio-Rad CFX Connect real-time PCR machine.

Value

A `data.table` with primer targets and Cq values, each row being a non-template control reaction.

qPCR_analysis_std_curve

Real-time qPCR standard curve and primer efficiency calculation.

Description

Parses primary qPCR data (Cq values) and calculates primer efficiencies based on standard curve serial dilutions. Compatible with Cq data exported from Bio-Rad CFX Connect real-time PCR machine.

Usage

```
qPCR_analysis_std_curve(d, plot = TRUE)
```

Arguments

`d` `data.frame`, Cq value data exported from Bio-Rad CFX Connect real-time PCR machine.

`plot` Logical. If TRUE, also draws and returns the standard curves on a scatter plot with trend lines. Defaults to TRUE.

Details

Standard curve slopes and resulting efficiencies are calculated by fitting the Cq values (y) against the log(starting quantity) (x) on a linear model (function `lm(y~x)`).

Value

A list of 3 elements:

- `$data`: A `data.table` with the Cq values against log(Starting quantity) for each target gene.
- `$efficiencies`: A `data.table` with the calculated efficiencies for each target gene.
- `$plot`: The scatter plot with the drawn standard curves, if requested. Points are technical means, error bars are technical standard deviations.

qPCR_analysis_unk_rxns

Real-time qPCR unknown sample reaction processing.

Description

Parses primary qPCR data (Cq values) and calculates technical means for each sample, biological replicate and gene. Compatible with Cq data exported from Bio-Rad CFX Connect real-time PCR machine.

Usage

```
qPCR_analysis_unk_rxns(d)
```

Arguments

d	data.frame, Cq value data exported from Bio-Rad CFX Connect real-time PCR machine.
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Value

A data.table with Cq values for each sample, biological replicate and gene.

qPCR_analysis_wrap

Real-time qPCR result processing.

Description

Performs the whole processing of real-time qPCR data (Cq values), including non-template control summary, standard curve analysis and expression analysis. Compatible with Cq data exported from Bio-Rad CFX Connect real-time PCR machine.

Usage

```
qPCR_analysis_wrap(
  d,
  refgene,
  control = NULL,
  std.curve = TRUE,
  std.curve.plot = TRUE
)
```

Arguments

d	data.frame, Cq value data exported from Bio-Rad CFX Connect real-time PCR machine.
refgene	Character vector with the name(s) of the reference gene(s).
control	Character. The name of the control sample, against which all other samples will be compared. Defaults to the 1st sample alphabetically.

<code>std.curve</code>	Logical. Determines whether or not to calculate efficiencies from standard curves or not. If FALSE, standard curve reactions will be ignored and efficiencies of 100% will be assumed. Defaults to TRUE.
<code>std.curve.plot</code>	Logical. Determines whether or not to plot standard curve data in a scatter plot with trend lines. Defaults to TRUE.

Value

A list of 4 elements:

- `$NTC`: Results from `qPCR_analysis_ntc` function.
- `$std.curve`: Results from `qPCR_analysis_std_curve` function.
- `$unk.rxn`: Results from `qPCR_analysis_unk_rxns` function.
- `$expression`: Results from `qPCR_analysis_expression` function.

Index

qPCR_analysis_expression, [1](#)
qPCR_analysis_ntc, [2](#)
qPCR_analysis_std_curve, [3](#)
qPCR_analysis_unk_rxns, [3](#)
qPCR_analysis_wrap, [4](#)