

Package ‘qPCR.CFX.process’

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Type Package

Title Real-time qPCR data processing

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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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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 data.frame containing the technical replicate means of the unknown reactions. Result from qPCR_analysis_unk_rxns function.
refgene	Character vector with the name(s) of the reference gene(s).
efficiencies	A data.frame containing the efficiencies of each primer pair. The \$efficiencies data.table resulting from the qPCR_analysis_stdcurve function. Defaults to NULL, 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 gene of interest. Contains the following fields:

- Sample: Name of sample template.
- Biol.rep: Biological replicate number.
- Target: Name of gene of interest.
- Cq.tech.mean: Arithmetic average of Cq between technical replicates, for each gene of interest, sample and biological replicate.
- [reference.gene].Cq.tech.mean: Arithmetic average of Cq between technical replicates, for each reference gene, sample and biological replicate. Field is repeated for each reference gene, with the [reference.gene] part being the gene's name.
- [reference.gene].amplification.base: Amplification base of each reference gene, as provided by standard curve calculations. If not provided, amplification base 2 will be assumed. Field is repeated for each reference gene, with the [reference.gene] part being the gene's name.

- `[reference.gene].Cq.weighed`: Weighed Cq for each reference gene and biological replicate. Calculated as follows: `[reference.gene].Cq.tech.mean * log2([reference.gene].amplification.base)`. Field is repeated for each reference gene, with the `[reference.gene]` part being the gene's name.
- `Ref.Cq.weighed.mean`: Arithmetic mean of weighed Cq between all reference genes, for each sample and biological replicate.
- `G0I.amplification.base`: Amplification base of each gene of interest, as provided by standard curve calculations. If not provided, amplification base 2 will be assumed.
- `G0I.Cq.weighed`: Weighed Cq for each gene of interest and biological replicate. Calculated as follows: `Cq.tech.mean * log2(G0I.amplification.base)`.
- `DCq.weighed`: Difference between `Ref.Cq.weighed.mean` and `G0I.Cq.weighed`.
- `control.DCq.weighed`: Average `DCq.weighed` of designated control sample, for each gene of interest.
- `log2.fold.change`: Difference between `DCq.weighed` and `control.DCq.weighed`. Also equal to base-2 logarithm of fold-change.
- `fold.change`: Fold-change of quantity in relation to control sample. Calculated as $2^{(\log_2 \text{fold.change})}$.

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.
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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.

Value

A `data.table` with Cq values for each sample, biological replicate and gene. Contains the following fields:

- `Sample`: Name of sample template.
- `Biol.rep`: Biological replicate number.
- `Target`: Name of target gene.
- `Cq.tech.mean`: Arithmetic mean between technical replicates.
- `Cq.tech.sd`: Standard deviation between technical replicates.

qPCR_analysis_wrap	<i>Real-time qPCR result processing.</i>
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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.

`std.curve` 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`.

`std.curve.plot` 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.

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