

Package ‘ddCt’

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Title The ddCt Algorithm for the Analysis of Quantitative Real-Time PCR (qRT-PCR)

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Description The Delta-Delta-Ct (ddCt) Algorithm is an approximation method to determine relative gene expression with quantitative real-time PCR (qRT-PCR) experiments. Compared to other approaches, it requires no standard curve for each primer-target pair, therefore reducing the working load and yet returning accurate enough results as long as the assumptions of the amplification efficiency hold. The ddCt package implements a pipeline to collect, analyse and visualize qRT-PCR results, for example those from TaqMan SDM software, mainly using the ddCt method. The pipeline can be either invoked by a script in command-line or through the API consisting of S4-Classes, methods and functions.

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biocViews GeneExpression, DifferentialExpression, MicrotitrePlateAssay, qPCR

License LGPL-3

Depends R (>= 2.3.0), Biobase (>= 1.10.0), RColorBrewer (>= 0.1-3), xtable, lattice, methods

Collate AllClasses.R AllGenerics.R AllFunctions.R AllMethods.R
AllGlobals.R absoluteQ.R ddCtSplit.R output.R visualization.R

Suggests RUnit

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barploterrbar	<i>Barplot with error bars.</i>
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Description

Barplot with error bars.

Usage

```
barploterrbar(y, y1, yh, barcol="orange", errcol="black", horiz=FALSE,
w=0.2, theCut=NULL, columnForDiffBars=TRUE, cex.axis =
par("cex.axis"), zeroForNA=TRUE, legend=FALSE, groups = NULL, order=FALSE, ...)
```

Arguments

y	Numeric vector.
y1	Numeric vector of same length as y.
yh	Numeric vector of same length as y.
barcol	Color of the bars.
errcol	Color of the error bars.
horiz	Logical. As in barplot .
w	Size of the error bar ticks.
theCut	The cut value
columnForDiffBars	Whether the matrix should be transposed (by default the rows are for diff bars)
zeroForNA	Draw 0 instead of NA
cex.axis	Axis font cex
legend	Should a legend be plotted ?
groups	a factor - if specified the bars are colored according to the group they belong to
order	plot sample values in descending order
...	Further arguments that get passed on to barplot .

Details

The function calls [barplot](#) with `y` and decorates it with error bars according to `yl` and `yh`.

Value

The function is called for its side effect, producing a plot.

Author(s)

Markus Ruschhaupt, Florian Hahne

See Also

[barplot](#)

Examples

```
y <- matrix(runif(80), ncol=5)
ym <- apply(y, 2, mean)
dy <- apply(y, 2, sd)*2/sqrt(nrow(y))
barploterrbar(ym, ym-dy, ym+dy, barcol="#0000c0", errcol="orange")
```

ddCtAbsolute

absolute quantification for Taqman data

Description

absolute quantification for Taqman data

Usage

```
ddCtAbsolute(raw.table, addData, type = "mean", ADD = -30.234, DIV = -1.6268, sampleInformation = NULL,
```

Arguments

<code>raw.table</code>	data frame. It must contain columns with the following names: 'Ct', 'Sample', 'Detector', 'Platenname'. The column 'Ct' must contain numeric values.
<code>addData</code>	add data
<code>type</code>	character of length 1. 'mean' or 'median' - which method should be used for the aggregation of the replicates
<code>ADD</code>	Add constant
<code>DIV</code>	Div constant
<code>sampleInformation</code>	if specified it must be an object of class <code>phenoData</code> with a column named 'Sample'.
<code>toZero</code>	boolean - if there is only one replication should the error be treated as zero ? (only if 'type' is mean)
<code>filename</code>	character of length 1. The name of the file the warnings should be stored in.

Value

An object of class `eSet`. The assayData has the following components: `exprs`, `error`, `Ct`, `Ct.error`, `Difference`, `number_NA`, `number`, `Plate`.

Author(s)

Markus Ruschhaupt <mailto:m.ruschhaupt@dkfz.de>

References

~put references to the literature/web site here ~

ddCtExpression-class *ddCt Expression*

Description

This class is a subclass of `ExpressionSet` and represents objects which are produced by the `ddCt` algorithm in the `ddCtExpression` method

Extends

Class `ExpressionSet`, directly. Class `eSet`, by class "`ExpressionSet`", distance 2. Class `VersionedBiobase`, by class "`ExpressionSet`", distance 3. Class `Versioned`, by class "`ExpressionSet`", distance 4.

Methods

Ct signature(object = "ddCtExpression"): returns the Ct value of this ddCtExpressionobject

CtErr signature(object = "ddCtExpression"): returns the error number of the Ct value of this ddCtExpressionobject

dCt signature(object = "ddCtExpression"): returns the dCt value of this ddCtExpressionobject

dCtErr signature(object = "ddCtExpression"): returns the error number of the dCt value of this ddCtExpressionobject

ddCt signature(object = "ddCtExpression"): returns the ddCt value of this ddCtExpressionobject

ddCtErr signature(object = "ddCtExpression"): returns the error number of the ddCt value of this ddCtExpressionobject

level signature(object = "ddCtExpression"): returns the levels in this ddCtExpressionobject

levelErr signature(object = "ddCtExpression"): returns the error number of the levels in this ddCtExpressionobject

numberCt signature(object = "ddCtExpression"): returns the Ct number of this ddCtExpressionobject

- numberNA** signature(object = "ddCtExpression"): returns the NA number of this ddCtExpression object
- elist** signature(object = "ddCtExpression"): returns a data frame which represents this expression object
- elistWrite** signature(object = "ddCtExpression", file = "character"): writes ddCtExpression object into a file

Author(s)

Rudolf Biczok <mailto:r.biczok@dkfz.de>

See Also

[SDMFrame](#): reader for SDM files [elist](#), [elistWrite](#): utility functions for ddCtExpression objects
[ddCtExpression](#): the method which invokes the ddCt algorithm

Examples

```
## read a SDM file
sampdat <- SDMFrame(system.file("extdata", "Experiment1.txt",
                               package="ddCt"))

## call ddCtExpression method to get a ddCt calculated expression
result <- ddCtExpression(sampdat,
                        calibrationSample="Sample1",
                        housekeepingGenes=c("Gene1", "Gene2"))

## use getter methods
ddCt(result)
ddCtErr(result)
```

ddCtExpression-methods

Apply the ddCt algorithm for a given data set

Description

Apply the ddCt algorithm for a given data set

Arguments

- | | |
|---------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| object | SDMFrame Data object which holds a data set containing columns with the following names: 'Ct', 'Sample', 'Detector', 'Platename'. The column 'Ct' must contain numeric values. |
| algorithm | character. Name of the calibration samples. |
| warningStream | character of length 1. The name of the file the warnings should be stored in. |

calibrationSample	character. Name of the calibration samples.
housekeepingGenes	character. Name of the housekeeping genes.
type	character of length 1. 'mean' or 'median'- which method should be used for the aggregation of the replicates
sampleInformation	if specified it must be an object of class phenoData with a column named 'Sample'.
toZero	boolean - if there is only one replication should the error be treated as zero ? (only if 'type' is mean)
efficiencies	n.V.
efficiencies.error	n.V.

Value

A an object of class [ddCtExpression](#).

usage

```
ddCtExpression(object, warningStream = "warning.output.txt", algorithm="ddCt" calibrationSample, housekeepingGenes, type="mean", sampleInformation=NULL, toZero=TRUE, efficiencies = NULL, efficiencies.error = NULL)
```

Methods

object = "InputFrame" An object of [InputFrame](#), constructed with the method [InputFrame](#)

Author(s)

Rudolf Biczok <<mailto:r.biczok@dkfz.de>>

References

Analysis of relative gene expression data using real-time quantitative PCR and the 2⁻(Delta -Delta C(T)) Method. KJ Livak and TD Schmittgen, Methods, Vol. 25, No. 4. (December 2001), pp. 402-408

See Also

[InputFrame](#): reader for SDM files [ddCtExpression](#): representation for ddCt calculated expressions

Examples

```
## read a SDM file
sampdat <- SDMFrame(system.file("extdata", "Experiment1.txt", package="ddCt"))

## call ddCtExpression method from class SDMFrame
## to get a ddCt calculated expression
result <- ddCtExpression(sampdat,
                          calibrationSample="Sample1",
                          housekeepingGenes=c("Gene1", "Gene2"))

result
```

elistWrite-methods *Write ddCtExpression object into data frame or files*

Description

ddCtExpression object contains a list of matrices as the results of [ddCt](#) method. `elist` combines these lists into one data frame, and `elistWrite` writes the data frame into file.

`summary` is a wrapper for the `elist` method

Usage

```
elist(object,...)
summary(object,...)
elistWrite(object,file,...)
```

Arguments

<code>object</code>	an ExpressionSet object.
<code>file</code>	output file.
<code>...</code>	additional arguments passed to <code>write.table</code> .

Details

`elist` is a wrapper to `as(object, "data.frame")` function.

Value

A data frame or output file.

Author(s)

Jitao David Zhang <jitao_david.zhang@roche.com>

Examples

```
## read a SDM file
sampdat <- SDMFrame(system.file("extdata", "Experiment1.txt", package="ddCt"))

## call ddCtExpression method from class SDMFrame
## to get a ddCt calculated expression
result <- ddCtExpression(sampdat,
                        calibrationSample="Sample1",
                        housekeepingGenes=c("Gene1", "Gene2"))

## call elist
elistResult <- elist(result)
elistResult
```

errBarchart-methods *Draw barchart of relative expression level with error-bars*

Description

Draw barchart (with error-bars) of relative expression level represented in [ddCtExpression](#) object. The barchart is implemented as grid plot by `lattice` package, where each panel represents one sample and the relative expression values of detectors (as well as their standard errors) are depicted as bars.

Detectors which are not determined are marked by grey ND.

Two types of figures are supported: either condition on samples (`by="Sample"`) or on detectors (`by="Detector"`).

Methods

object = "ddCtExpression", by="character" An object of `ddCtExpression`, constructed with the method [ddCtExpression](#)

errBarchartParameter-class *Class "errBarchartParameter"*

Description

Parameter object for `errBarchart`

Objects from the Class

Objects can be created by calls of the form `new("errBarchartParameter", ...)`. So far the object is only internally used, but in the near future it will be exported.

Slots

exprsUndeterminedLabel: Object of class "character", specifying the text label when the expression level is 'Undetermined'

Methods

exprsUndeterminedLabel signature(object = "errBarchartParameter"): getting the text label when the expression level is 'Undetermined'

show signature(object = "errBarchartParameter"): print method

Note

So far it is only internally used

Author(s)

Jitao David Zhang <jitao_david.zhang@roche.com>

Examples

```
## Internally used
## param <- new("errBarchartParameter")
## exprsUndeterminedLabel(param)
```

getDir

Auxillary functions for the executive scripts

Description

getDir creates a directory in case it does not exist and returns the directory name.

Usage

```
getDir(dir, ...)
```

Arguments

dir	Directory name
...	Other parameters passed to dir.create

Details

Auxillary functions

Value

getDir returns the directory name

Author(s)

Jitao David Zhang <jitao_david.zhang@roche.com>

Examples

```
getDir(tempdir())
```

InputFrame-class

Working with SDM files

Description

The class InputFrame provides core functionalities to read gene and sample information from SDM files and calculate them with a ddCt algorithm.

The function InputFrame reads the data given in the columns 'Detector', 'Sample' and 'Ct' of the specified SDM output files and stores them as a data.frame. An additional column including the respective filename is added.

Slots

coreData: Object of class "data.frame": Holds all the required data extracted from the SDM file

files: Object of class "character" contains the source SDM files

Methods

[,][,]\$ signature(x = "InputFrame"): primitive accessors. Returns an object of InputFrame-class with the subset data.

names signature(x = "InputFrame"): returns the column names in this SDM object

ddCtExpression signature(object = "InputFrame"): runs a ddCt algorithm with this SDM object and returns a object of class [ddCtExpression](#)

fileNames signature(object="InputFrame"): returns the source SDM file names.

detectorNames signature(object = "InputFrame"): returns the detector names in this SDM object

detectorNames<- signature(object = "InputFrame", value = "character"): replaces the detector names in this SDM object

sampleNames signature(object = "InputFrame"): returns the sample names in this SDM object

sampleNames<- signature(object = "InputFrame", value = "character"): replaces the sample names in this SDM object

uniqueDetectorNames signature(object = "InputFrame"): returns a vector of unique detector names in this SDM object

uniqueDetectorNames<- signature(object = "InputFrame", target = "missing", value = "character"): replaces all detector names given by the 'names' attribute in 'value' with new detector names

uniqueDetectorNames<- signature(object = "InputFrame", target = "character", value = "character"):
replaces all detector names given by 'target' with new detector names

uniqueSampleNames<- signature(object = "InputFrame", target = "missing", value = "character"):
replaces all sample names given by the 'names' attribute in 'value' with new sample names

uniqueSampleNames<- signature(object = "InputFrame", target = "character", value = "character"):
replaces all sample names given by 'target' with new sample names

uniqueSampleNames signature(object = "InputFrame"): returns a vector of unique sample names in this SDM object

removeSample signature(object = "InputFrame", sample="character"): removes the sample(s) specified from the InputFrame object

replaceDetector signature(object = "InputFrame", target="character", value="character"):
replace the detectors equal to the target with the value. Both target and value can be vectors of the same length, then the replace takes place iteratively.

replaceSample signature(object = "InputFrame", target="character", value="character"):
replace the samples equal to the target with the value. Both target and value can be vectors of the same length, then the replace takes place iteratively.

show signature(object="InputFrame"): pretty print of the InputFrame instance.

rightCensoring signature(object="InputFrame", threshold="numeric"): Right censoring the Ct value, which targets the data points above a certain value (threshold). High Ct values (higher than 40 or 45 by the rule of thumb) are often not accurate and may indicate too weak expression. The function performs the right censoring on the data and set the value above the threshold as NA (by default) or a given value. See the example.

coreData signature(object="InputFrame"): returns the data frame read from SDM file.

coreData<- signature(object="InputFrame"): replace the data frame read from SDM file.

Ct signature(object="InputFrame"): returns the Ct value of the SDM file.

Ct signature(object="InputFrame", value="numeric"): replace the Ct value in the object with the new values, and return the object.

Author(s)

Rudolf Biczok <mailto:r.biczok@dkfz.de>, Jitao David Zhang <mailto:jitao_david.zhang@roche.com>

See Also

[SDMFrame](#) function reads in data from SDM files. Data from SDM files is used to construct [ddCtExpression](#) objects to analyze differential expression.

Examples

```
## read a SDM file
sampdat <- SDMFrame(system.file("extdata", "Experiment1.txt",
                               package="ddCt"))

## you can also write
## sampdat <- new("SDMFrame",system.file("extdata", "Experiment1.txt",
##                                     package="ddCt"))
```

```

## use the getter methods
sampleNames(sampdat)

## or the overloaded primitive accessors
sampdat[1:3,"Sample"]

## see all unique samples
uniqueSampleNames(sampdat)

## replace all sample names Sample1 and Sample2 in sampdat
## with NewSample1 and NewSample2
uniqueSampleNames(sampdat,c("Sample1","Sample2")) <- c("NewSample1","NewSample2")
uniqueSampleNames(sampdat)

## or use this syntax to replace the gene names
uniqueDetectorNames(sampdat) <- c(Gene1="NewGene1", Gene2="NewGene2")
uniqueDetectorNames(sampdat)

## remove sample or detector
removeSample(sampdat, "Sample1")
removeDetector(sampdat, "Gene1")

## replace sample or detector
replaceSample(sampdat, "Sample1", "Sample0")
replaceDetector(sampdat, "Gene1", "PLCG1")

## right censoring the data
rightCensoring(sampdat, 35)
rightCensoring(sampdat, 35, 35)

```

InputReader-class *Class "InputReader"*

Description

Abstract factory for data input

Objects from the Class

A virtual Class: No objects may be created from it.

Slots

files: Input files

colmap: Column mapping

Author(s)

Rudolf Biczok and Jitao David Zhang

Examples

```
showClass("InputReader")
```

```
removeNTC-methods      Remove NTC samples
```

Description

NTC stands for Non-template controls. This method remove the NTC samples from the input object.

Methods

signature(object = "ddCtExpression") An object hat has been analyzed with the ddCt method
signature(object = "InputFrame") An input object

```
replaceVectorByEquality
      REPLACE ITEMS OF VECTOR BY EQUALITY
```

Description

The function replces (or updates) the items of a given vector by checking the equality with the target parameter. If found, the item will be replaced by the value parameter. The length of both target and value must be the same and could be longer than 1, in which case the replace will be iterated.

Usage

```
replaceVectorByEquality(vector, target, value)
```

Arguments

vector	A vector to be replaced. The items of the vector must be atom types, since the equality is checked by '=='.
target	targets to be replaced, could be either single or a vector
value	values to be replaced at the positions of targets, must be of the same length of target

Details

A warning will be prompted if any item in the target cannot be found

Value

A vector of the same length as the parameter vector

Author(s)

Jitao David Zhang

See Also

`==` for checking equality.

Examples

```
vector <- c("java", "perl", "python", "c#")
replaceVectorByEquality(vector, target="c#", value="c/c++")
replaceVectorByEquality(vector, target=c("c#", "perl"), value=c("c/c++", "R"))
```

SDMFrame

Read an SDM file

Description

Read an SDM file: Data Output File for SDS, Version 2.1

Usage

```
SDMFrame(file)
readSDM(file)
```

Arguments

`file` Character vector of filenames

Details

This function reads the data given in the columns 'Detector', 'Sample' and 'Ct' of the specified SDM output file(s) and stores them as a data.frame. An additional column including the respective filename is added.

This function is a wrapper for the SDMFrame constructor

Value

A object of class [SDMFrame](#)

Author(s)

Rudolf Biczok <mailto:r.biczok@dkfz.de>

Examples

```

## read a SDM file
sampdat <- SDMFrame(system.file("extdata", "Experiment1.txt",
                                package="ddCt"))

## you can also write
## sampdat <- new("SDMFrame",system.file("extdata", "Experiment1.txt",
##                                       package="ddCt"))

## or with
## sampdat <- readSDM(system.file("extdata", "Experiment1.txt",
##                                package="ddCt"))

## use the getter methods
sampleNames(sampdat)

## or the overloaded primitive accessors
sampdat[1:3,"Sample"]

## see all unique samples
uniqueSampleNames(sampdat)

## replace all sample names Sample1 and Sample2 in sampdat
## with NewSample1 and NewSample2
uniqueSampleNames(sampdat,c("Sample1","Sample2")) <- c("NewSample1","NewSample2")
uniqueSampleNames(sampdat)

## or use this syntax to replace the gene names
uniqueDetectorNames(sampdat) <- c(Gene1="NewGene1", Gene2="NewGene2")
uniqueDetectorNames(sampdat)

```

write.htmltable

Write a data frame into an html table within a html page

Description

Write a 'data.frame' into an html table within a html page

Usage

```
write.htmltable(x, filename, title = "", sortby = NULL, decreasing = TRUE, open = "wt")
```

Arguments

x	'data.frame'
filename	character. File name.
title	character. Title of html page
sortby	character. Name of column by which to sort the table rows

decreasing logical. Should the sort order be increasing or decreasing?
open character. This argument is passed onto 'file'

Value

The function is called for its side effect: writing a file

Author(s)

Wolfgang Huber

writeSimpleTabCsv *Write a data frame into a tab delimited file*

Description

Write a 'data.frame' into a tab delimited file (not quoted and no-row-name CSV file)

Usage

```
writeSimpleTabCsv(x, file, ...)
```

Arguments

x 'data.frame'
file character. File name.
... Additional arguments passed onto the function

Value

The function is called for its side effect: writing a file

Author(s)

Wolfgang Huber

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