Package ‘NormqPCR’

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Description Functions for the selection of optimal reference genes and the normalisation of real-time quantitative PCR data.
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R topics documented:

NormqPCR-package ................................................................. 2
Bladder ................................................................................. 3
BladderRepro .......................................................................... 5
NormqPCR-package

Functions for normalisation of RT-qPCR data.

Description

Functions for normalisation of real-time quantitative PCR data.

Details

Package: NormqPCR
Type: Package
Version: 1.7.1
Date: 2014-08-13
Depends: R(>= 2.14.0), stats, RColorBrewer, Biobase, methods, ReadqPCR, qpcR
Imports: ReadqPCR
biocViews: MicrotitrePlateAssay, GeneExpression, qPCR
License: LGPL-3
LazyLoad: yes
LazyData: yes

require(NormqPCR)

Author(s)

Matthias Kohl, James Perkins, Nor Izayu Abdul Rahman
Maintainer: James Perkins <jimrperkins@gmail.com>
References


Claus Lindbjerg Andersen, Jens Ledet Jensen and Torben Falck Orntoft (2004). Normalization of Real-Time Quantitative Reverse Transcription-PCR Data: A Model-Based Variance Estimation Approach to Identify Genes Suited for Normalization, Applied to Bladder and Colon Cancer Data Sets. *CANCER RESEARCH* 64, 5245-5250, August 1, 2004. [http://cancerres.aacrjournals.org/cgi/content/full/64/15/5245](http://cancerres.aacrjournals.org/cgi/content/full/64/15/5245)


Examples

```r
# some examples are given in the vignette
# Not run:
library(NormqPCR)
vignette("NormqPCR")

# End(Not run)
```

---

**Bladder**

*Bladder dataset of Andersen et al (2004)*

---

**Description**

This dataset was used in Andersen et al (2004) to demonstrate normalization of real-time quantitative RT-PCR data by geometric averaging of housekeeping genes.

**Usage**

```r
data(Bladder)
```

**Format**

A qPCRBatch object which contains an expression matrix with the expression of 14 genes measured in 28 samples. The sample information is saved in the phenoData slot with variables

- Sample.no.: sample number.
- Grade: Grade of bladder cancer.
The following information on the measured genes is saved in the variables Symbol and Gene.name of the featureData slot.

**ATP5B** ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide.  
**HSPCB** Heat shock 90-kDa protein 1, beta.  
**S100A6** S100 calcium-binding protein A6 (calcylin).  
**FLOT2** Flotillin 2.  
**TEGT** Testis enhanced gene transcript (BAX inhibitor 1).  
**UBB** Ubiquitin B.  
**TPT1** Tumor protein, translationally controlled 1.  
**CFL1** Cofilin 1 (non-muscle).  
**ACTB** Actin, beta.  
**RPS23** Ribosomal protein S23.  
**GAPD** Glyceraldehyde-3-phosphate dehydrogenase.  
**UBC** Ubiquitin C.  
**FLJ20030** Hypothetical protein FLJ20030.

For a detailed annotation see Table 1 in Anderson et al. (2004).

**Details**

The genes included in this data set were selected by screening 99 bladder sample expression profiles.

**Source**

The data set was obtained from [http://www.mdl.dk/Publications_sup1.htm](http://www.mdl.dk/Publications_sup1.htm)

**References**

Claus Lindbjerg Andersen, Jens Ledet Jensen and Torben Falck Orntoft (2004). Normalization of Real-Time Quantitative Reverse Transcription-PCR Data: A Model-Based Variance Estimation Approach to Identify Genes Suited for Normalization, Applied to Bladder and Colon Cancer Data Sets. CANCER RESEARCH 64, 5245-5250, August 1, 2004. [http://cancerres.aacrjournals.org/cgi/content/full/64/15/5245](http://cancerres.aacrjournals.org/cgi/content/full/64/15/5245)

**Examples**

```r
data(Bladder)
Bladder
head(exprs(Bladder))
pData(Bladder)
fData(Bladder)
```
Description

This dataset was used in Andersen et al (2004) to demonstrate normalization of real-time quantitative RT-PCR data by geometric averaging of housekeeping genes.

Usage

data(BladderRepro)

Format

A qPCRBatch object which contains an expression matrix with the expression of 8 genes measured in 26 samples. The sample information is saved in the phenoData slot with variables

Sample.no. sample number.
Grade Grade of bladder cancer.

The following information on the measured genes is saved in the variables Symbol and Gene.name of the featureData slot.

CD14 CD14 antigen.
FCN1 Ficolin (collagen/fibrinogen domain containing) 1.
CCNG2 Cyclin G2.
NPAS2 Neuronal PAS domain protein 2.
UBC Ubiquitin C.
CFL1 Cofilin 1 (non-muscle).
ACTB Actin, beta.
GAPD Glyceraldehyde-3-phosphate dehydrogenase.

For a detailed annotation see Table 1 and Supplementary table 1 in Anderson et al. (2004).

Details

This data set was used to check the reproducibility of the results obtained in Andersen et al (2004).

Source

The data set was obtained from http://www.mdl.dk/Publications_sup1.htm

References

Examples

data(BladderRepro)
BladderRepro
head(exprs(BladderRepro))
pData(BladderRepro)
fData(BladderRepro)


Description

This dataset was used in Andersen et al (2004) to demonstrate normalization of real-time quantitative RT-PCR data by geometric averaging of housekeeping genes.

Usage

data(Colon)

Format

A qPCRBatch object which contains an expression matrix with the expression of 13 genes measured in 40 samples. The sample information is saved in the phenoData slot with variables

Sample.no. sample number.
Classification Classification of colon cancer.

The following information on the measured genes is saved in the variables Symbol and Gene.name of the featureData slot.

UBC Ubiquitin C.
UBB Ubiquitin B.
SUI1 Putative translation initiation factor.
NACA Nascent-polypeptide-associated complex alpha polypeptide.
FLJ20030 Hypothetical protein FLJ20030.
CFL1 Cofilin 1 (non-muscle).
ACTB Actin, beta.
CLTC Clathrin, heavy polypeptide (Hc).
RPS13 Ribosomal protein S13.
RPS23 Ribosomal protein S23.
GAPD Glyceraldehyde-3-phosphate dehydrogenase.
TPT1 Tumor protein, translationally controlled 1.
TUBA6 Tubulin alpha 6.

For a detailed annotation see Table 1 in Anderson et al. (2004).
**combineTechReps**  

**Details**  
The genes included in this data set were selected by screening 161 colon sample expression profiles.

**Source**  
The data set was obtained from [http://www.mdl.dk/Publications_sup1.htm](http://www.mdl.dk/Publications_sup1.htm)

**References**  
Claus Lindbjerg Andersen, Jens Ledet Jensen and Torben Falck Orntoft (2004). Normalization of Real-Time Quantitative Reverse Transcription-PCR Data: A Model-Based Variance Estimation Approach to Identify Genes Suited for Normalization, Applied to Bladder and Colon Cancer Data Sets. CANCER RESEARCH 64, 5245-5250, August 1, 2004. [http://cancerres.aacrjournals.org/cgi/content/full/64/15/5245](http://cancerres.aacrjournals.org/cgi/content/full/64/15/5245)

**Examples**
```
data(Colon)  
Colon  
head(exprs(Colon))  
pData(Colon)  
fData(Colon)
```

---

**Description**  
Takes expression set of qPCR values containing technical replicates and combines them.

**Usage**
```
combineTechReps(qPCRBatch, ...)  
```

```
## S4 method for signature 'qPCRBatch'
combineTechReps(qPCRBatch, calc="arith")
```

**Arguments**
- **qPCRBatch**: Expression set containing qPCR data, read in by a ReadqPCR function and containing technical reps, denoted by _TechRep.n suffix.
- **calc**: use median, arithmetic or geometric mean for combining the values

**Details**  
Takes exprs of qPCR values containing technical replicates and combines them using a specified centrality measure.
Value

qPCRBatch with same number of samples, but with less features, since all technical replicates are replaced with a single value of their means.

Author(s)

James Perkins <jimrperkins@gmail.com>

References


Examples

```r
path <- system.file("exData", package = "NormqPCR")
qPCR.example.techReps <- file.path(path, "qPCR.techReps.txt")
qPCRBatch.qPCR.techReps <- read.qPCR(qPCR.example.techReps)
rownames(exprs(qPCRBatch.qPCR.techReps))
combinedTechReps <- combineTechReps(qPCRBatch.qPCR.techReps)
rownames(exprs(combinedTechReps))
```

---

**combineTechRepsWithSD**  
*Combines Technical Replicates*

Description

Takes expression set of qPCR values containing technical replicates and combines them. In addition the appropriate standard deviation (SD) is computed.

Usage

```r
combineTechRepsWithSD(qPCRBatch, ...)
```

## S4 method for signature 'qPCRBatch'

```r
combineTechRepsWithSD(qPCRBatch, calc="arith")
```

Arguments

- `qPCRBatch`  
  Expression set containing qPCR data, read in by a ReadqPCR function and containing technical reps, denoted by _TechRep.n suffix.

- `...`  
  Extra arguments, detailed below

- `calc`  
  use median, arithmetic or geometric mean for combining the values
**ComputeNRQs**

**Details**

This function computes normalized relative quantities (NRQs) for a `qPCRBatch`.

Takes `exprs` of qPCR values containing technical replicates and combines them using a specified centrality measure.

The arithmetic mean (calc="arith") is combined with the classical standard deviation. In case of the geometric mean (calc="geom") the classical standard deviation of the log-values is exponentiated. The median (calc="median") is calculated in connection with the MAD.

**Value**

A `qPCRBatch` with same number of samples, but with less features, since all technical replicates are replaced with a single value of their means. In addition the slot `assayData` includes a matrix with SD values which can be accessed via `se.exprs`.

**Author(s)**

Matthias Kohl <Matthias.Kohl@stamats.de>

**References**


**See Also**

`combineTechReps`

**Examples**

```r
path <- system.file("exData", package = "NormqPCR")
quPCR.example.techReps <- file.path(path, "qPCR.techReps.txt")
quPCRBatch.qPCR.techReps <- read.qPCR(qPCR.example.techReps)
rownames(exprs(qPCRBatch.qPCR.techReps))
combinedTechReps <- combineTechRepsWithSD(qPCRBatch.qPCR.techReps)
rownames(exprs(combinedTechReps))
exprs(combinedTechReps)
se.exprs(combinedTechReps)
```
Usage
ComputeNRQs(qPCRBatch, ...)  
## S4 method for signature 'qPCRBatch'
ComputeNRQs(qPCRBatch, hkgs)

Arguments
qPCRBatch an object of class qPCRBatch.
hkgs Names of reference/housekeeping genes.
... other parameters to be passed to downstream methods.

Details
Allows the user to normalized relative quantities as defined in Hellemanns et al. (2007).

Value
Object of class "qPCRBatch".

Author(s)
Nor Izayu Abdul Rahman, Matthias Kohl <Matthias.Kohl@stamats.de>

References
Jan Hellemans, Geert Mortier, Anne De Paepe, Frank Speleman and Jo Vandesompele (2007). qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biology, 8:R19

See Also
qPCRBatch-class

Examples
## Example data
path <- system.file("exData", package = "ReadqPCR")
qPCR.example <- file.path(path, "qPCR.example.txt")
Cq.data <- read.qPCR(qPCR.example)

## combine techincal replicates
Cq.data1 <- combineTechRepsWithSD(Cq.data)

## add efficiencies
Effs <- file.path(path, "Efficiencies.txt")
Cq.effs <- read.table(file = Effs, row.names = 1, header = TRUE)
CqValues <- function(object, ...) {
  # S4 method for signature 'CyclesSet'
  if (is(object, 'CyclesSet')) {
    # CqValues(object, Effmethod = "expfit", group = NULL,
    # model = l5, check = "uni2", checkPAR = parKOD(),
    # remove = "none", exclude = NULL, type = "cpD2",
    # labels = NULL, norm = FALSE, baseline = NULL,
    # basefac = 1, smooth = NULL,
    # smoothPAR = list(span = 0.1),
    # factor = 1, opt = FALSE,
    # optPAR = list(sig.level = 0.05, crit = "ftest"),
    # plot = FALSE, verbose = FALSE, ...)
    # CqValues(object, Effmethod = "expfit", group = NULL,
    # model = l5, check = "uni2", checkPAR = parKOD(),
    # remove = "none", exclude = NULL, type = "cpD2",
    # labels = NULL, norm = FALSE, baseline = NULL,
    # basefac = 1, smooth = NULL,
    # smoothPAR = list(span = 0.1),
    # factor = 1, opt = FALSE,
    # optPAR = list(sig.level = 0.05, crit = "ftest"),
    # plot = FALSE, verbose = FALSE, ...)
    return(CqValues(object, Effmethod = "expfit", group = NULL,
                    model = l5, check = "uni2", checkPAR = parKOD(),
                    remove = "none", exclude = NULL, type = "cpD2",
                    labels = NULL, norm = FALSE, baseline = NULL,
                    basefac = 1, smooth = NULL,
                    smoothPAR = list(span = 0.1),
                    factor = 1, opt = FALSE,
                    optPAR = list(sig.level = 0.05, crit = "ftest"),
                    plot = FALSE, verbose = FALSE, ...))
  }
  stop("CqValues can only be called on objects of class CyclesSet.")
}

CqValues <- function(object, Effmethod = "expfit", group = NULL,
                      model = l5, check = "uni2", checkPAR = parKOD(),
                      remove = "none", exclude = NULL, type = "cpD2",
                      labels = NULL, norm = FALSE, baseline = NULL,
                      basefac = 1, smooth = NULL,
                      smoothPAR = list(span = 0.1),
                      factor = 1, opt = FALSE,
                      optPAR = list(sig.level = 0.05, crit = "ftest"),
                      plot = FALSE, verbose = FALSE, ...)

Arguments

  object
    an object of class CyclesSet.

  Effmethod
    a character vector defining the methods for computing amplification efficiency.

  group
    a vector containing the grouping for possible replicates.

  model
    the model to be used for all runs. Default model is l5.

  check
    the method for kinetic outlier detection in KOD. Method "uni2" is set as default
    which is a test on sigmoidal structure.

  checkPAR
    parameters to be supplied to the check method. See parKOD.

  remove
    indicates which runs to be removed. Either none of them, those which failed to
    fit or from the outlier methods.

  exclude
    indicates samples to be excluded from calculation, either "" for samples with
    missing column names or a regular expression defining columns (samples); see
    'Details' and 'Examples' in modlist.
CqValues

- **type**: the point on the amplification curve which is used for efficiency estimation; see `efficiency`.
- **labels**: a vector containing labels which define replicate groups. See more details in `pcrbatch` and `ratiobatch`.
- **norm**: a logical value which determines whether the raw data should be normalized within \([0, 1]\) before model fitting or not.
- **baseline**: type of baseline subtraction. More details in `efficiency`.
- **basefac**: a factor when using averaged baseline cycles, such as 0.95.
- **smooth**: the curve smoothing method. See more details in `pcrbatch`.
- **smoothPAR**: parameters to be supplied to smoothing method in `smooth`.
- **factor**: a multiplication factor for the fluorescence response values.
- **opt**: a logical value which determines whether model selection should be applied to each model or not.
- **optPAR**: parameters to be supplied for model selection in `mselect`.
- **plot**: a logical value. If TRUE, the single runs are plotted from the internal `modlist` for diagnostics.
- **verbose**: a logical value. If TRUE, fitting and tagging results will be displayed in the console.
- **...**: other parameters to be passed to downstream methods.

**Details**

Allows the user to compute Cq value and amplification efficiency. In addition, all values generated during the computations are saved. This function has four choices of methods for computing amplification efficiency values which are the methods provided by package `qpcR`.

More details on technical replication and normalization is given in the vignette `NormqPCR`.

**Value**

Object of class "qPCRBatch".

**Author(s)**

Nor Izayu Abdul Rahman, Matthias Kohl <Matthias.Kohl@stamats.de>

**References**


**See Also**

`pcrbatch, CyclesSet-class, qPCRBatch-class`
deltaCt

Examples

```r
## Read in the raw qPCR data from file "LC480_Example.txt"
path <- system.file("exData", package = "ReadqPCR")
LC480.example <- file.path(path, "LC480_Example.txt")
cycData <- read.LC480(file = LC480.example)

## Read in the sample information data from file "LC480_Example_SampleInfo.txt".
LC480.SamInfo <- file.path(path, "LC480_Example_SampleInfo.txt")
samInfo <- read.LC480SampleInfo(LC480.SamInfo)

## Merge information
cycData1 <- merge(cycData, samInfo)

## Compute Cq values
## 1) use sigmoidal model
res1 <- CqValues(cycData1, Effmethod = "sigfit")
  effs(res1)
  se.effs(res1)

## 2) fit exponential model (default)
res2 <- CqValues(cycData1, Effmethod = "expfit")
  effs(res2)
  se.effs(res2)

## 3) use window of linearity
res3 <- CqValues(cycData1, Effmethod = "sliwin")
  effs(res3)
  se.effs(res3)

## 4) linear regression of efficiency
res4 <- CqValues(cycData1, Effmethod = "LRE")
  effs(res4)
  se.effs(res4)
```

deltaCt

Perform normalization with a given housekeeping gene

Description

Normalise qPCR eset using a given housekeeping gene as control, then perform differential expression analysis using the delta delta Ct method

Usage

deltaCt(qPCRBatch, ...)

# S4 method for signature 'qPCRBatch'
deltaCt(qPCRBatch, hkgs, combineHkgs=FALSE, calc="arith")
deltaCq(qPCRBatch, hkgs, combineHkgs=FALSE, calc="arith")

**Arguments**

- `qPCRBatch`: qPCR-specific expression set, containing qPCR data.
- `...`: Extra arguments, detailed below
- `hkgs`: String containing the name of the name of the housekeeping gene which will be used to normalise the rest of the genes.
- `combineHkgs`: Logical - if TRUE, then as long as more than one housekeeper given for argument hkgs, it will combine the housekeepers by finding the geometric mean. Housekeepers can be found using geNorm or NormFinder algorithms.
- `calc`: use arithmetic or geometric mean.

**Details**

Takes expression set of qPCR values and normalises them using a housekeeping gene. Returns a qPCRBatch with exprs set of the same dimensions but with the given hkg value subtracted.

**Value**

qPCRBatch with exprs set of the same dimensions but with the given hkg value subtracted.

**Author(s)**

James Perkins <jimrperkins@gmail.com>

**References**


**See Also**

selectHKs, deltaDeltaCq

**Examples**

```r
path <- system.file("exData", package = "NormqPCR")
taqman.example <- file.path(path, "example.txt")
qPCRBatch.taqman <- read.taqman(taqman.example)
hkgs<="Actb-Rn00667869_m1"
qPCRBatch.norm <- deltaCq(qPCRBatch = qPCRBatch.taqman, hkgs = hkgs, calc="arith")
head(exprs(qPCRBatch.norm))
```
**deltaDeltaCt**

Perform normalization and differential expression with given housekeeping gene

---

**Description**

Normalise qPCRBatch RT-qPCR data using housekeeping genes as control, then perform differential expression analysis using the delta delta Cq method.

**Usage**

```r
deltaDeltaCt(qPCRBatch, ...)  
## S4 method for signature 'qPCRBatch'  
deltaDeltaCt(qPCRBatch, maxNACase=0, maxNAControl=0, hkg$s, contrastM,  
    case, control, paired=TRUE, hkgCalc="arith", statCalc="arith")  
deltaDeltaCq(qPCRBatch, maxNACase=0, maxNAControl=0, hkg$s, contrastM,  
    case, control, paired=TRUE, hkgCalc="arith", statCalc="arith")
```

**Arguments**

- `qPCRBatch` qPCR-specific expression set, containing qPCR data.
- `...` Extra arguments, detailed below
- `maxNACase` Maximum number of NA values allowed before a detector’s reading is discarded for samples designated as case.
- `maxNAControl` Maximum number of NA values allowed before a detector’s reading is discarded for samples designated as control.
- `hkg$s` String containing the name of the name of the housekeeping gene which will be used to normalise the rest of the genes.
- `contrastM` A binary matrix which designates case and control samples.
- `case` The name of the column in `contrastM` that corresponds to the case samples.
- `control` The name of the column in `contrastM` that corresponds to the control samples.
- `paired` Logical - if TRUE the detectors and housekeepers in the same sample will be paired for calculating standard deviation, effectively meaning we will be calculating standard deviation of the differences. If FALSE, there will be no pairing, and standard deviation will be pooled between the detector and housekeepers.
- `hkgCalc` String - either "arith" or "geom", details how the different housekeeper genes should be combined - either by using the arithmetic or geometric mean.
- `statCalc` String - either "arith" or "geom", details how genes should be combined - either by using the arithmetic or geometric mean.

**Details**

Takes expression set of qPCR values and normalises them using different housekeeping genes. Returns separate sets of values for each housekeeping gene given.
geNorm

Value

matrix with columns containing the detector ids, 2^delta Cq values for the sample of interest and the callibrator sample, alongside their respective standard deviations, the 2^delta delta Cq values and the minimum and maximum values (ie the values that are 1 sd away)

Author(s)

James Perkins <jimrperkins@gmail.com>

References


See Also

selectHKs, deltaCq

Examples

```r
path <- system.file("exData", package = "NormqPCR")
taqman.example <- file.path(path, "example.txt")
qPCRBatch.taqman <- read.taqaqman(taqman.example)
hkg <- "Actb-Rn00667869_m1"

contM <- cbind(c(0,0,1,1,0,0,1,1),c(1,1,0,0,1,1,0,0))
colnames(contM) <- c("interestingPhenotype","wildTypePhenotype")
rownames(contM) <- sampleNames(qPCRBatch.taqman)

ddCq.taqman <- deltaDeltaCq(qPCRBatch = qPCRBatch.taqman, maxNACase = 1, maxNAControl = 1, hkg = hkg, contrastM = contM, case = "interestingPhenotype", control = "wildTypePhenotype", statCalc = "geom", hkgCalc = "arith")

head(ddCq.taqman)
```

Data set of Vandesompele et al (2002)

Description

This data set was used in Vandesompele et al (2002) to demonstrate normalization of real-time quantitative RT-PCR data by geometric averaging of housekeeping genes.
Usage
data(geNorm)

Format
A qPCRBatch object which contains an expression matrix with 85 observations on the following 10 variables which stand for expression data of ten potential reference/housekeeping genes

ACTB  actin, beta
B2M  beta-2-microglobulin
GAPD  glyceraldehyde-3-phosphate dehydrogenase
HMBS  hydroxymethylbilane synthase
HPRT1  hypoxanthine phosphoribosyltransferase 1
RPL13A  ribosomal protein L13a
SDHA  succinate dehydrogenase complex subunit A
TBP  TATA box binding protein
UBC  ubiquitin C
YWHAZ  tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide

Details
The row names of this data set indicate the various human tissues which were investigated.

BM  9 normal bone-marrow samples
POOL  9 normal human tissues from pooled organs (heart, brain, fetal brain, lung, trachea, kidney, mammary gland, small intestine and uterus)
FIB  20 short-term cultured normal fibroblast samples from different individuals
LEU  13 normal leukocyte samples
NB  34 neuroblastoma cell lines (independently prepared in different labs from different patients)

Source
The data set was obtained from http://genomebiology.com/content/supplementary/gb-2002-3-7-research0034-s1.txt

References

## geomMean

**Examples**

```r
data(geNorm)
str(exprs(geNorm.qPCRBatch))
sampleNames(geNorm.qPCRBatch)
```

---

**geomMean**  
**Geometric Mean**

### Description

Computation of the geometric mean.

### Usage

```r
geomMean(x, na.rm = TRUE)
```

### Arguments

- `x`  
  numeric vector of non-negative Reals

- `na.rm`  
  a logical value indicating whether NA values should be stripped before the computation proceeds.

### Details

The computation of the geometric mean is done via `prod(x)^((1/length(x)))`.

### Value

`geometric mean`

### Note

A first version of this function appeared in package SLqPCR.

### Author(s)

Matthias Kohl  
<Matthias.Kohl@stamats.de>

### References


### Examples

```r
x <- rlnorm(100)
geomMean(x)
```
### makeAllNAs

**Make all Cq values NA**

**Description**

Make all Cq values for a given detector NA when the number of NAs for that detector is above a given threshold.

**Usage**

```r
makeAllNAs(qPCRBatch, ...)  
```

```
## S4 method for signature 'qPCRBatch'
makeAllNAs(qPCRBatch, contrastM, sampleMaxM)
```

**Arguments**

- `qPCRBatch`: Expression set containing qPCR data.
- `...`: Extra arguments, detailed below
- `contrastM`: Contrast Matrix like that used in limma. Columns represent the different samples types, rows are the different samples, with a 1 or 0 in the matrix indicating which sample types the different samples belong to.
- `sampleMaxM`: Sample Max Matrix. Columns represent the different sample types. There is one value per column, which represents the max number of NAs allowed for that sample type.

**Details**

Make all NAs when number of NAs above a given threshold.

**Value**

`qPCRBatch` object with a new exprs slot, everything else equal.

**Author(s)**

James Perkins <jimrperkins@gmail.com>

**References**

Examples

```r
# read in the data
path <- system.file("exData", package = "NormqPCR")
taqman.example <- file.path(path, "example.txt")
qPCRBatch.taqman <- read.taqman(taqman.example)
exprs(qPCRBatch.taqman)["Ccl20.Rn00570287_m1",] # values before

# make contrastM
a <- c(0,0,1,1,0,0,1,1) # one for each sample type, with 1 representing
b <- c(1,1,0,0,1,1,0,0) # position of sample type in the samplenames vector
contM <- cbind(a,b)
colnames(contM) <- c("case","control") # then give the names of each sample type
rownames(contM) <- sampleNames(qPCRBatch.taqman) # and the rows of the matrix
contM

# make sampleMaxM
sMaxM <- t(as.matrix(c(3,3))) # now make the sample max matrix
colnames(sMaxM) <- c("case","control") # make sure these line up with samples
sMaxM

# function
qPCRBatch.taqman.replaced <- makeAllNAs(qPCRBatch.taqman, contM, sMaxM)
exprs(qPCRBatch.taqman.replaced)["Ccl20.Rn00570287_m1",]
```

---

`makeAllNewVal`  
`Make all Cq values NA`

Description

Make all Cq values for a given detector NA when the number of NAs for that detector is above a given threshold

Usage

`makeAllNewVal(qPCRBatch, ...)`

# S4 method for signature 'qPCRBatch'
`makeAllNewVal(qPCRBatch, contrastM, sampleMaxM, newVal)`

Arguments

- `qPCRBatch`: Expression set containing qPCR data.
- `...`: Extra arguments, detailed below
- `contrastM`: Contrast Matrix like that used in limma. Columns represent the different samples types, rows are the different samples, with a 1 or 0 in the matrix indicating which sample types the different samples belong to.
Sample Max Matrix. Columns represent the different sample types. There is one value per column, which represents the max number of NAs allowed for that sample type.

New value to give the values in the group where the NAs are above the threshold.

Details

Make all a given value when number of NAs above a given threshold, with different thresholds for the different sample classes, using sMaxM and contM to provide this information, as detailed below.

Value

qPCRBatch object with a new exprs slot, everything else equal

Author(s)

James Perkins <jimrperkins@gmail.com>

References


Examples

```r
# read in the data
path <- system.file("exData", package = "NormqPCR")
taqman.example <- file.path(path, "example.txt")
qPCRBatch.taqman <- read.taqman(taqman.example)
exprs(qPCRBatch.taqman)["Ccl20.Rn00570287_m1",] # values before

# make contrastM
a <- c(0,0,1,1,0,0,1,1) # one for each sample type, with 1 representing
b <- c(1,1,0,0,1,1,0,0) # position of sample type in the samplenames vector
contM <- cbind(a,b)
colnames(contM) <- c("case","control") # then give the names of each sample type
rownames(contM) <- sampleNames(qPCRBatch.taqman) # and the rows of the matrix
contM

# make sampleMaxM
sMaxM <- t(as.matrix(c(3,3))) # now make the sample max matrix
colnames(sMaxM) <- c("case","control") # make sure these line up with samples
sMaxM

# function
qPCRBatch.taqman.replaced <- makeAllNewVal(qPCRBatch.taqman, contM, sMaxM)
exprs(qPCRBatch.taqman.replaced)["Ccl20.Rn00570287_m1",]
```
Description

Replace Cq values above a given threshold with a new value

Usage

```
replaceAboveCutOff(qPCRBatch, ...)
```

```
## S4 method for signature 'qPCRBatch'
replaceAboveCutOff(qPCRBatch, newVal=NA, cutOff=38)
```

Arguments

- `qPCRBatch` Expression set containing qPCR data.
- `...` Extra arguments, detailed below
- `newVal` The new value with which to replace the values above the cutoff
- `cutOff` the minimal threshold above which the values will be replaced

Details

Replaces values in the exprs slot of the qPCRBatch object that are above a threshold value with a new number

Value

qPCRBatch object with a new exprs slot

Author(s)

James Perkins <jimrperkins@gmail.com>

References


Examples

```
path <- system.file("exData", package = "NormqPCR")
taqman.example <- file.path(path, "example.txt")
qPCRBatch.taqman <- read.taqman(taqman.example)
exprs(qPCRBatch.taqman)["Ccl20.Rn00570287_m1",]
qPCRBatch.taqman.replaced <- replaceAboveCutOff(qPCRBatch.taqman, newVal = NA, cutOff = 35)
exprs(qPCRBatch.taqman.replaced)["Ccl20.Rn00570287_m1",]
```
replaceNAs

Description

Replace NAs with a given value

Usage

replaceNAs(qPCRBatch, ...)## S4 method for signature 'qPCRBatch'
replaceNAs(qPCRBatch, newNA)

Arguments

qPCRBatch Expression set containing qPCR data.
... Extra arguments, detailed below
newNA The new value to replace the NAs with

Details

Replaces NA values in the exprs slot of the qPCRBatch object with a given number

Value

qPCRBatch object with a new exprs slot

Author(s)

James Perkins <jimrperkins@gmail.com>

References


Examples

```r
path <- system.file("exData", package = "NormqPCR")
taqman.example <- file.path(path, "example.txt")
qPCRBatch.taqman <- read.taqman(taqman.example)
qPCRBatch.taqman.replaced <- replaceNAs(qPCRBatch.taqman, newNA = 40)
exprs(qPCRBatch.taqman.replaced)["Ccl20.Rn00570287_m1",]
```
selectHKs

Selection of reference/housekeeping genes

Description
This function can be used to determine a set of reference/housekeeping (HK) genes for gene expression experiments.

Usage

```r
selectHKs(qPCRBatch, ...) 
```

```r
## S4 method for signature 'matrix'
selectHKs(qPCRBatch, group, method = "geNorm", minNrHKs = 2, log = TRUE, Symbols, trace = TRUE, na.rm = TRUE)
```

```r
## S4 method for signature 'qPCRBatch'
selectHKs(qPCRBatch, group, method = "geNorm", minNrHKs = 2, log = TRUE, Symbols, trace = TRUE, na.rm = TRUE)
```

Arguments

- `qPCRBatch`: matrix or qPCRBatch, containing the data (expression matrix) in the exprs slot
- `...`: Extra arguments, detailed below
- `group`: optional factor not used by all methods, hence may be missing
- `method`: method to compute most stable genes
- `minNrHKs`: minimum number of HK genes that should be considered
- `log`: logical: is data on log-scale
- `Symbols`: gene symbols
- `trace`: logical, print additional information
- `na.rm`: a logical value indicating whether NA values should be stripped before the computation proceeds.

Details
This function can be used to determine a set of reference/housekeeping (HK) genes for gene expression experiments. The default method "geNorm" was proposed by Vandesompele et al. (2002). Currently, the geNorm method by Vandesompele et al. (2002) and the NormFinder method of Andersen et al. (2004) are implemented.

Vandesompele et al. (2002) propose a cut-off value of 0.15 for the pairwise variation. Below this value the inclusion of an additional housekeeping gene is not required.
selectHKs

Value

If method = "geNorm" a list with the following components is returned

- **ranking** ranking of genes from best to worst where the two most stable genes cannot be ranked
- **variation** pairwise variation during stepwise selection
- **meanM** average expression stability M

If method = "NormFinder" a list with the following components is returned

- **ranking** ranking of genes from best to worst where the two most stable genes cannot be ranked
- **rho** stability measure rho of Andersen et al. (2004)

Author(s)

Matthias Kohl <Matthias.Kohl@stamats.de>

References


Examples

data(geNorm)
tissue <- as.factor(c(rep("BM", 9), rep("FIB", 20), rep("LEU", 13), rep("NB", 34), rep("POOL", 9)))
res.BM <- selectHKs(geNorm.qPCRBatch[, tissue == "BM"], method = "geNorm", Symbols = featureNames(geNorm.qPCRBatch), minNrHK = 2, log = FALSE)
Description

Computation of the gene expression stability value M for real-time quantitative RT-PCR data. For more details we refer to Vandesompele et al. (2002).

Usage

\[
\text{stabMeasureM}(x, \text{log} = \text{TRUE}, \text{na.rm} = \text{TRUE})
\]

Arguments

- **x**: matrix or data.frame containing real-time quantitative RT-PCR data
- **log**: logical: is data on log-scale
- **na.rm**: a logical value indicating whether NA values should be stripped before the computation proceeds.

Details

The gene expression stability value M is defined as the average pairwise normalization factor; i.e., one needs to specify data from at least two genes. For more details see Vandesompele et al. (2002). Note this dispatches on a transposed expression matrix, not a qPCRBatch object since it is only called from within the selectHKs method.

Value

numeric vector with gene expression stability values

Author(s)

Matthias Kohl <Matthias.Kohl@stamats.de>

References


See Also

selectHKs
Examples

```r
data(geNorm)
tissue <- as.factor(c(rep("BM", 9), rep("FIB", 20), rep("LEU", 13),
                    rep("NB", 34), rep("POOL", 9)))
res.BM <- selectHKs(geNorm.qPCRBatch[, tissue == "BM"], method = "geNorm",
                    Symbols = featureNames(geNorm.qPCRBatch), minNrHK = 2, log = FALSE)
```

Description

Computation of the gene expression stability value rho for real-time quantitative RT-PCR data. For more details we refer to Andersen et al. (2004).

Usage

```r
stabMeasureRho(x, ...)
```

### S4 method for signature 'x'

```r
stabMeasureRho(x, group, log = TRUE, na.rm = TRUE, returnAll = FALSE)
```

Arguments

- `x`: matrix containing real-time quantitative RT-PCR data, or qPCRBatch object
- `...`: Extra arguments, detailed below
- `group`: grouping factor, either a factor vector or a phenoData column called "Group"
- `log`: logical: is data on log-scale
- `na.rm`: a logical value indicating whether NA values should be stripped before the computation proceeds.
- `returnAll`: logical, return additional information.

Details

The gene expression stability value rho is computed. For more details see Andersen et al. (2004).

Value

numeric vector with gene expression stability values

If `returnAll` == `TRUE` a list with the following components is returned

- `d`: used by selectHKs
- `v`: used by selectHKs
Author(s)
Matthias Kohl <Matthias.Kohl@stamats.de>

References

See Also
selectHKs

Examples
data(Colon)
Class <- pData(Colon)[,"Classification"]
res.Colon <- stabMeasureRho(Colon, group = Class, log = FALSE)
data(Bladder)
Grade <- pData(Bladder)[,"Grade"]
res.Bladder <- stabMeasureRho(Bladder, group = Grade, log = FALSE)
Index

* classes
  ComputeNRQs, 9
  CqValues, 11

* datasets
  Bladder, 3
  BladderRepro, 5
  Colon, 6
  geNorm, 16

* data
  combineTechReps, 7
  combineTechRepsWithSD, 8
  deltaCt, 13
  deltaDeltaCt, 15
  geomMean, 18
  makeAllNAs, 19
  makeAllNewVal, 20
  replaceAboveCutOff, 22
  replaceNAs, 23
  selectHKs, 24
  stabMeasureM, 26
  stabMeasureRho, 27

* package
  NormqPCR-package, 2

Bladder, 3
BladderRepro, 5

Colon, 6
combineTechReps, 7, 9
combineTechReps,qPCRBatch-method
  (combineTechReps), 7
combineTechRepsWithSD, 8
combineTechRepsWithSD,qPCRBatch-method
  (combineTechRepsWithSD), 8
ComputeNRQs, 9
ComputeNRQs,qPCRBatch-method
  (ComputeNRQs), 9
ComputeNRQs-methods (ComputeNRQs), 9
CqValues, 11
CqValues,CyclesSet-method (CqValues), 11

CqValues-methods (CqValues), 11
CyclesSet, 11

deltaCq (deltaCt), 13
deltaCt, 13
deltaCt,qPCRBatch-method (deltaCt), 13
deltaCt-methods (deltaCt), 13
deltaDeltaCq (deltaDeltaCt), 15
deltaDeltaCq,qPCRBatch-method
  (deltaDeltaCt), 15
deltaDeltaCq-methods (deltaDeltaCt), 15
deltaDeltaCt, 15
deltaDeltaCt,qPCRBatch-method
  (deltaDeltaCt), 15
deltaDeltaCt-methods (deltaDeltaCt), 15

efficiency, 12

geNorm, 16
gemMean, 18

KOD, 11

makeAllNAs, 19
makeAllNAs,qPCRBatch-method
  (makeAllNAs), 19
makeAllNewVal, 20
makeAllNewVal,qPCRBatch-method
  (makeAllNewVal), 20
modlist, 11
mselect, 12

NormqPCR (NormqPCR-package), 2
NormqPCR-package, 2

parKOD, 11
pcrbatch, 11, 12

qPCRBatch, 10

ratiobatch, 12

29
replaceAboveCutOff, 22
replaceAboveCutOff, qPCRBatch-method
  (replaceAboveCutOff), 22
replaceNAs, 23
replaceNAs, qPCRBatch-method
  (replaceNAs), 23

selectHKs, 24
selectHKs, matrix-method (selectHKs), 24
selectHKs, qPCRBatch-method (selectHKs), 24
selectHKs-methods (selectHKs), 24
stabMeasureM, 26
stabMeasureRho, 27
stabMeasureRho, matrix-method
  (stabMeasureRho), 27
stabMeasureRho, qPCRBatch-method
  (stabMeasureRho), 27
stabMeasureRho, x-method
  (stabMeasureRho), 27
stabMeasureRho-methods
  (stabMeasureRho), 27