

# NormqPCR

March 24, 2012

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Bladder

*Data set of Andersen et al (2004)*

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## Description

This data set 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(Bladder)
```

## Format

A qPCRBatch object which contains an expression matrix with 28 observations on the following 14 variables which stand for expression data potential reference/housekeeping genes

Grade Grade of bladder cancer.

Sample.no. sample number.

ATP5B ATP synthase, H<sup>+</sup> 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.

RPS13 Ribosomal protein S13.

RPS23 Ribosomal protein S23.

GAPD Glyceraldehyde-3-phosphate dehydrogenase.

UBC Ubiquitin C.

FLJ20030 Hypothetical protein FLJ20030.

It also contains a "group" column in the pData slot, which gives information on the different sample classes, necessary for the NormFinder algorithm

**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>

**Examples**

```
data(Bladder)
head(exprs(Bladder.qPCRBatch))
pData(Bladder.qPCRBatch)
```

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BladderRepro

*Data set of Andersen et al (2004)*

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**Description**

This data set 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 28 observations on the following 14 variables which stand for expression data potential reference/housekeeping genes

Grade Grade of bladder cancer.

Sample.no. sample number.

CD14 CD14 molecule.

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.

It also contains a "group" column in the pData slot, which gives information on the different sample classes, necessary for the NormFinder algorithm

## 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](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>

## Examples

```
data(BladderRepro)
head(exprs(BladderRepro.qPCRBatch))
pData(BladderRepro.qPCRBatch)
```

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Colon

*Data set of Andersen et al (2004)*

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## Description

This data set 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 40 observations on the following 13 variables

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.

It also contains a "group" column in the pData slot, which gives information on the different sample classes, necessary for the NormFinder algorithm

### 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>

### Examples

```
data(Colon)
head(exprs(Colon.qPCRBatch))
pData(Colon.qPCRBatch)
```

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NormqPCR-package    *Functions for normalisation of RT-qPCR data.*

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### Description

Functions for normalisation of real-time quantitative PCR data.

### Details

Package:	NormqPCR
Type:	Package
Version:	0.99.3
Date:	2011-07-15
Depends:	R(>= 2.10.0), stats, RColorBrewer, Biobase, methods, ReadqPCR
License:	LGPL-3
LazyLoad:	yes
LazyData:	yes

```
require(NormqPCR)
```

**Author(s)**

Matthias Kohl, James Perkins

Maintainer: Matthias Kohl <Matthias.Kohl@stamats.de>

**References**

Jo Vandesompele, Katleen De Preter, Filip Pattyn et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology* 2002. 3(7):research0034.1-0034.11. <http://genomebiology.com/2002/3/7/research/0034/>

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>

Kenneth Livak, Thomase Schmittgen (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2<sup>ddCt</sup> Method. *Methods* 25, 402-408, 2001 <http://www.ncbi.nlm.nih.gov/pubmed/11846609>

**Examples**

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

## End(Not run)
```

---

combineTechReps      *Combines Technical Replicates*

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**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 <code>_TechRep.n</code> suffix.
...	Extra arguments, detailed below
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 <jperkins@biochem.ucl.ac.uk>

**Examples**

```
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))
```

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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")
```

**Arguments**

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

**References**

Kenneth Livak, Thomase Schmittgen (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2<sup>ΔΔCt</sup> Method. *Methods* 25, 402-408, 2001 <http://www.ncbi.nlm.nih.gov/pubmed/11846609>

**See Also**

selectHKs, deltaDeltaCt

**Examples**

```
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 <- deltaCt(qPCRBatch = qPCRBatch.taqman, hkgs = hkgs, calc="arith")
head(exprs(qPCRBatch.norm))
```

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deltaDeltaCt	<i>Perform normalization and differential expression with given house-keeping gene</i>
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**Description**

Normalise qPCR eset using housekeeping genes as control, then perform differential expression analysis using the delta delta Ct method. Differs from the deltaDeltaAvgCt method in that the hkg values are subtracted and the sd is calculated on the differences (i.e. paired). Suitable when housekeeping genes are from same wells/sample as the other detectors

**Usage**

```
deltaDeltaCt(qPCRBatch, ...)

## S4 method for signature 'qPCRBatch'
deltaDeltaCt(qPCRBatch, maxNACase=0, maxNAControl=0, hkgs, 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.

<code>maxNAControl</code>	Maximum number of NA values allowed before a detector's reading is discarded for samples designated as control.
<code>hkgs</code>	String containing the name of the housekeeping gene which will be used to normalise the rest of the genes.
<code>contrastM</code>	A binary matrix which designates case and control samples.
<code>case</code>	The name of the column in <code>contrastM</code> that corresponds to the case samples.
<code>control</code>	The name of the column in <code>contrastM</code> that corresponds to the control samples.
<code>paired</code>	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.
<code>hkgCalc</code>	String - either "arith" or "geom", details how the different housekeeper genes should be combined - either by using the arithmetic or geometric mean.
<code>statCalc</code>	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.

### Value

matrix with columns containing the detector ids,  $2^{\Delta\Delta Ct}$  values for the sample of interest and the calibrator sample, alongside their respective standard deviations, the  $2^{\Delta\Delta Ct}$  values and the minimum and maximum values (ie the values that are 1 sd away)

### Author(s)

James Perkins <jperkins@biochem.ucl.ac.uk>

### References

Kenneth Livak, Thomase Schmittgen (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the  $2^{\Delta\Delta Ct}$  Method. *Methods* 25, 402-408, 2001 <http://www.ncbi.nlm.nih.gov/pubmed/11846609>

### See Also

`selectHKs`, `deltaCt`

### Examples

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

ddCt.taqman <- deltaDeltaCt(qPCRBatch = qPCRBatch.taqman, maxNACase=1, maxNAControl=1,
head(ddCt.taqman)
```



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geNorm

*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

Jo Vandesompele, Katleen De Preter, Filip Pattyn et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology* 2002. 3(7):research0034.1-0034.11. <http://genomebiology.com/2002/3/7/research/0034/>

## Examples

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

---

geomMean

*Geometric Mean*

---

## Description

Computation of the geometric mean.

## Usage

```
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  $\text{prod}(x)^{(1/\text{length}(x))}$ .

## Value

geometric mean

## Note

A first version of this function appeared in package SLqPCR.

## Author(s)

Matthias Kohl <Matthias.Kohl@stamats.de>

## Examples

```
x <- rlnorm(100)
geomMean(x)
```

---

makeAllNAs	<i>Make all Ct values NA</i>
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---

**Description**

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

**Usage**

```
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 <code>limma</code> . Columns represent the different sample 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 <jperkins@biochem.ucl.ac.uk>

**Examples**

```
# 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",]

```

---

replaceAboveCutOff *Replace Ct values with new value*

---

## Description

Replace Ct 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 <jperkins@biochem.ucl.ac.uk>

## 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 =
exprs(qPCRBatch.taqman.replaced) ["Ccl20.Rn00570287_m1",]

```

---

replaceNAs	<i>Replace NAs with a given value</i>
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---

### 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 <jperkins@biochem.ucl.ac.uk>

### Examples

```
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

```
selectHKs(qPCRBatch, ...)

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

### Arguments

qPCRBatch	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.

### 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**

Jo Vandesompele, Katleen De Preter, Filip Pattyn et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology* 2002. 3(7):research0034.1-0034.11. <http://genomebiology.com/2002/3/7/research/0034/>

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>

**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)
```

---

stabMeasureM

*Gene expression stability value M*

---

**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**

```
stabMeasureM(x, log = TRUE, na.rm = 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

Jo Vandesompele, Katleen De Preter, Filip Pattyn et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biology* 2002. 3(7):research0034.1-0034.11. <http://genomebiology.com/2002/3/7/research/0034/>

## See Also

selectHKs

## 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)
```

---

stabMeasureRho

*Gene expression stability value rho*

---

## 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

```
stabMeasureRho(x, ...)

## S4 method for signature 'x'
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

rho	stability measure rho of Andersen et al. (2004)
d	used by <code>selectHKs</code>
v	used by <code>selectHKs</code>

**Author(s)**

Matthias Kohl <Matthias.Kohl@stamats.de>

**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>

**See Also**

`selectHKs`

**Examples**

```
data(Colon)
group <- pData(Colon.qPCRBatch)[,"Group"]
res.Colon <- stabMeasureRho(Colon.qPCRBatch, group = group,
                           log = FALSE)
sort(res.Colon) # cf. Table 3 in Andersen et al (2004)
```

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