

SLqPCR

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SLqPCR-package

Functions for analysis of real-time quantitative PCR data at SIRS-Lab GmbH

Description

Functions for analysis of real-time quantitative PCR data at SIRS-Lab GmbH

Details

Package: SLqPCR
Type: Package
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Depends: R(>= 2.4.0), stats, RColorBrewer
License: GPL (version 2 or later)

`require(SLqPCR)`

Author(s)

Dr. Matthias Kohl (SIRS-Lab GmbH) <http://www.sirs-lab.com>

Maintainer: Dr. Matthias Kohl <kohl@sirs-lab.com>

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/>

`SLqPCRdata`*SIRS-Lab inhouse qPCR data*

Description

This data is part of a SIRS-Lab inhouse real-time quantitative PCR experiment.

Usage

```
data(SLqPCRdata)
```

Format

A data frame with 16 observations on the following 4 variables.

Gene1 a numeric vector, average take-off values of gene 1

Gene2 a numeric vector, average take-off values of gene 2

HK1 a numeric vector, average take-off values of housekeeper 1

HK2 a numeric vector, average take-off values of housekeeper 2

Details

The row names of this data set indicate the probes which were investigated. The take-off values are mean values of three replicates.

Source

www.sirs-lab.com

References

www.sirs-lab.com

Examples

```
data(SLqPCRdata)
SLqPCRdata
```

`geneStabM`*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

```
geneStabM(relData, na.rm = FALSE)
```

Arguments

<code>relData</code>	matrix or data.frame containing real-time quantitative RT-PCR data
<code>na.rm</code>	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).

Value

numeric vector with gene expression stability values

Author(s)

Dr. Matthias Kohl (SIRS-Lab GmbH) <kohl@sirs-lab.com>

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/>

geomMean

Geometric Mean

Description

Computation of the geometric mean.

Usage

```
geomMean(x, na.rm = FALSE)
```

Arguments

<code>x</code>	numeric vector of non-negative Reals
<code>na.rm</code>	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

Author(s)

Dr. Matthias Kohl (SIRS-Lab GmbH) <kohl@sirs-lab.com>

`normPCR`*Normalization of real-time quantitative RT-PCR data*

Description

This function can be used to normalize real-time quantitative RT-PCR data.

Usage

```
normPCR(relData, HKs, method = "Vandesompele", na.rm = FALSE)
```

Arguments

<code>relData</code>	matrix or data.frame containing relative quantities (genes in columns)
<code>HKs</code>	integer, column numbers of housekeeping genes
<code>method</code>	method for the computation
<code>na.rm</code>	a logical value indicating whether NA values should be stripped before the computation proceeds.

Details

This function can be used to normalize real-time quantitative RT-PCR data. The default method "Vandesompele" was proposed by Vandesompele et al. (2002).

Currently, only the method by Vandesompele et al. (2002) is implemented.

Value

Normalized expression data

Author(s)

Dr. Matthias Kohl (SIRS-Lab GmbH) <kohl@sirs-lab.com>

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(SLqPCRdata)
relData <- apply(SLqPCRdata, 2, relQuantPCR)
geneStabM(relData[,c(3,4)])
exprData <- normPCR(SLqPCRdata, c(3,4))
```

relQuantPCR	<i>Compute relative expression values for realtime quantitative RT-PCR data</i>
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Description

Compute relative expression values for realtime quantitative RT-PCR data based on Ct or take-off values, respectively. The computations use the PCR efficiency.

Usage

```
relQuantPCR(x, E = 2, na.rm = FALSE)
```

Arguments

x	numeric vector containing raw data
E	PCR efficiency
na.rm	a logical value indicating whether NA values should be stripped before the computation proceeds.

Value

vector of relative expression values w.r.t. specified PCR efficiency.

Author(s)

Dr. Matthias Kohl (SIRS-Lab GmbH) <kohl@sirs-lab.com>

References

Jo Vandesompele, Katleen De Preter, Filip Pattyn et al. (2002). Accurate normalization of realtime 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/>

selectHKgenes	<i>Selection of reference/housekeeping genes</i>
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Description

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

Usage

```
selectHKgenes(relData, method = "Vandesompele", minNrHK = 2, geneSymbol,  
              trace = TRUE, na.rm = FALSE)
```

Arguments

<code>relData</code>	matrix or data.frame containing relative expression values
<code>method</code>	method to compute most stable genes
<code>minNrHK</code>	minimum number of HK genes that should be considered
<code>geneSymbol</code>	gene symbols
<code>trace</code>	logical, print additional information
<code>na.rm</code>	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 "Vandesompele" was proposed by Vandesompele et al. (2002).

Currently, only the method by Vandesompele et al. (2002) is 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 = "Vandesompele"` a list with the following components is returned

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

Author(s)

Dr. Matthias Kohl (SIRS-Lab GmbH) <kohl@sirs-lab.com>

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(vandesompele)
res.BM <- selectHKgenes(vandesompele[1:9,], method = "Vandesompele", geneSymbol = names(v
```

vandesompele

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(vandesompele)

Format

A data frame with 85 observations on the following 10 variables which stand for expression data of ten commonly used 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(vandesompele)
str(vandesompele)
rownames(vandesompele)
```


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