SLqPCR

October 5, 2010

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
Version:	1.0.0
Date:	2007-01-02
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 quantitativ RT-PCR data. For more details we refer to Vandesompele et al. (2002).

Usage

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

Arguments

relData	matrix or data.frame containing real-time quantitative RT-PCR data
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).

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

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

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

relData	matrix or data.frame containing relative quantities (genes in columns)
HKs	integer, column numbers of housekeeping genes
method	method for the computation
na.rm	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` *Compute relative expression values for realtime quantitative RT-PCR data*

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 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/

`selectHKgenes` *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

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

Arguments

relData	matrix or data.frame containing relative expression values
method	method to compute most stable genes
minNrHK	minimum number of HK genes that should be considered
geneSymbol	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 "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 returnd

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

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)
```

Index