Package 'RUVSeq'

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Title Remove Unwanted Variation from RNA-Seq Data

Description This package implements the remove unwanted variation (RUV) methods of Risso et al. (2014) for the normalization of RNA-Seq read counts between samples.

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`RUVSeq-package` *Remove Unwanted Variation from RNA-Seq Data*

Description

This package implements the remove unwanted variation (RUV) methods of Risso et al. (2014) for the normalization of RNA-Seq read counts between samples.

Details

Package:	RUVSeq
Type:	Package
Version:	0.99.1
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License:	Artistic-2.0

The `RUVg` function implements the RUVg normalization procedure of Risso et al. (2014), by using control genes to remove unwanted variation from the RNA-Seq read counts.

See also `RUVr` and `RUVs` for the "residual" and "sample" methods, based, respectively, on residuals (e.g., deviance residuals from a first-pass GLM regression of the unnormalized counts on the covariates of interest) and replicate/negative control samples for which the covariates of interest are constant.

Author(s)

Davide Risso and Sandrine Dudoit

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References

D. Risso, J. Ngai, T. P. Speed, and S. Dudoit. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nature Biotechnology*, 2014. (In press).

D. Risso, J. Ngai, T. P. Speed, and S. Dudoit. The role of spike-in standards in the normalization of RNA-Seq. In D. Nettleton and S. Datta, editors, *Statistical Analysis of Next Generation Sequence Data*. Springer, 2014. (In press).

See Also

RUVg, RUVr, RUVs

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makeGroups *Make a matrix suitable for use with RUVSeq methods such as RUVs.*

Description

Each row in the returned matrix corresponds to a set of replicate samples. The number of columns is the size of the largest set of replicates; rows for smaller sets are padded with -1 values.

Usage

```
makeGroups(xs)
```

Arguments

xs	A vector indicating membership in a group.

Author(s)

Kamil Slowikowski

See Also

RUVs

Examples

```
makeGroups(c("A","B","B","C","C","D","D","D","A"))
```

---

residuals.DGEGLM *Deviance and Pearson Residuals for the Negative Binomial Model of* **edgeR**

Description

This function implements the residuals method for the **edgeR** function glmFit.

Usage

```
## S3 method for class 'DGEGLM'
residuals(object, type = c("deviance", "pearson"), ...)
```

Arguments

object	An object of class DGEGLM as created by the glmFit function of **edgeR**.
type	Compute deviance or Pearson residuals.
...	Additional arguments to be passed to the generic function.

Value

A genes-by-samples numeric matrix with the negative binomial residuals for each gene and sample.

Author(s)

Davide Risso

References

McCullagh P, Nelder J (1989). *Generalized Linear Models*. Chapman and Hall, New York.

Venables, W. N. and Ripley, B. D. (1999). *Modern Applied Statistics with S-PLUS*. Third Edition. Springer.

Examples

```
library(edgeR)
library(zebrafishRNASeq)
data(zfGenes)

## run on a subset genes for time reasons
## (real analyses should be performed on all genes)
genes <- rownames(zfGenes)[grep("^ENS", rownames(zfGenes))]
spikes <- rownames(zfGenes)[grep("^ERCC", rownames(zfGenes))]
set.seed(123)
idx <- c(sample(genes, 1000), spikes)
seq <- newSeqExpressionSet(as.matrix(zfGenes[idx,]))

x <- as.factor(rep(c("Ctl", "Trt"), each=3))
design <- model.matrix(~x)
y <- DGEList(counts=counts(seq), group=x)
y <- calcNormFactors(y, method="upperquartile")
y <- estimateGLMCommonDisp(y, design)
y <- estimateGLMTagwiseDisp(y, design)

fit <- glmFit(y, design)
res <- residuals(fit, type="deviance")
head(res)
```

RUVg-methods *Remove Unwanted Variation Using Control Genes*

Description

This function implements the RUVg method of Risso et al. (2014).

Usage

```
RUVg(x, cIdx, k, drop=0, center=TRUE, round=TRUE, epsilon=1, tolerance=1e-8, isLog=FALSE)
```

Arguments

x	Either a genes-by-samples numeric matrix or a SeqExpressionSet object containing the read counts.
cIdx	A character, logical, or numeric vector indicating the subset of genes to be used as negative controls in the estimation of the factors of unwanted variation.
k	The number of factors of unwanted variation to be estimated from the data.
drop	The number of singular values to drop in the estimation of the factors of unwanted variation. This number is usually zero, but might be set to one if the first singular value captures the effect of interest. It must be less than k.
center	If TRUE, the counts are centered, for each gene, to have mean zero across samples. This is important to ensure that the first singular value does not capture the average gene expression.
round	If TRUE, the normalized measures are rounded to form pseudo-counts.
epsilon	A small constant (usually no larger than one) to be added to the counts prior to the log transformation to avoid problems with log(0).
tolerance	Tolerance in the selection of the number of positive singular values, i.e., a singular value must be larger than tolerance to be considered positive.
isLog	Set to TRUE if the input matrix is already log-transformed.

Details

The RUVg procedure performs factor analysis of the read counts based on a suitably-chosen subset of negative control genes known a priori not be differentially expressed (DE) between the samples under consideration.

Several types of controls can be used, including housekeeping genes, spike-in sequences (e.g., ERCC), or "in-silico" empirical controls (e.g., least significantly DE genes based on a DE analysis performed prior to RUV normalization).

Note that one can relax the negative control gene assumption by requiring instead the identification of a set of positive or negative controls, with a priori known expression fold-changes between samples. RUVg can then simply be applied to control-centered log counts, as detailed in the vignette.

Methods

`signature(x = "matrix", cIdx = "ANY", k = "numeric")` It returns a list with

- A samples-by-factors matrix with the estimated factors of unwanted variation (`W`).
- The genes-by-samples matrix of normalized expression measures (possibly rounded) obtained by removing the factors of unwanted variation from the original read counts (`normalizedCounts`).

`signature(x = "SeqExpressionSet", cIdx = "character", k="numeric")` It returns a SeqExpressionSet with

- The normalized counts in the `normalizedCounts` slot.
- The estimated factors of unwanted variation as additional columns of the `phenoData` slot.

Author(s)

Davide Risso

References

D. Risso, J. Ngai, T. P. Speed, and S. Dudoit. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nature Biotechnology*, 2014. (In press).

D. Risso, J. Ngai, T. P. Speed, and S. Dudoit. The role of spike-in standards in the normalization of RNA-Seq. In D. Nettleton and S. Datta, editors, *Statistical Analysis of Next Generation Sequence Data*. Springer, 2014. (In press).

See Also

RUVr, RUVs.

Examples

```
library(zebrafishRNASeq)
data(zfGenes)

## run on a subset of genes for time reasons
## (real analyses should be performed on all genes)
genes <- rownames(zfGenes)[grep("^ENS", rownames(zfGenes))]
spikes <- rownames(zfGenes)[grep("^ERCC", rownames(zfGenes))]
set.seed(123)
idx <- c(sample(genes, 1000), spikes)
seq <- newSeqExpressionSet(as.matrix(zfGenes[idx,]))

# RUVg normalization
seqRUVg <- RUVg(seq, spikes, k=1)

pData(seqRUVg)
head(normCounts(seqRUVg))

plotRLE(seq, outline=FALSE, ylim=c(-3, 3))
plotRLE(seqRUVg, outline=FALSE, ylim=c(-3, 3))

barplot(as.matrix(pData(seqRUVg)), beside=TRUE)
```

RUVr-methods *Remove Unwanted Variation Using Residuals*

Description

This function implements the RUVr method of Risso et al. (2014).

Usage

```
RUVr(x, cIdx, k, residuals, center=TRUE, round=TRUE, epsilon=1, tolerance=1e-8, isLog=FALSE)
```

Arguments

x	Either a genes-by-samples numeric matrix or a SeqExpressionSet object containing the read counts.
cIdx	A character, logical, or numeric vector indicating the subset of genes to be used as negative controls in the estimation of the factors of unwanted variation.
k	The number of factors of unwanted variation to be estimated from the data.
residuals	A genes-by-samples matrix of residuals obtained from a first-pass regression of the counts on the covariates of interest, usually the negative binomial deviance residuals obtained from **edgeR** with the `residuals` method.
center	If `TRUE`, the residuals are centered, for each gene, to have mean zero across samples.
round	If `TRUE`, the normalized measures are rounded to form pseudo-counts.
epsilon	A small constant (usually no larger than one) to be added to the counts prior to the log transformation to avoid problems with log(0).
tolerance	Tolerance in the selection of the number of positive singular values, i.e., a singular value must be larger than `tolerance` to be considered positive.
isLog	Set to `TRUE` if the input matrix is already log-transformed.

Details

The RUVr procedure performs factor analysis on residuals, such as deviance residuals from a first-pass GLM regression of the counts on the covariates of interest using **edgeR**. The counts may be either unnormalized or normalized with a method such as upper-quartile (UQ) normalization.

Methods

`signature(x = "matrix", cIdx = "ANY", k = "numeric", residuals = "matrix")` It returns a list with

- A samples-by-factors matrix with the estimated factors of unwanted variation (`W`).
- The genes-by-samples matrix of normalized expression measures (possibly rounded) obtained by removing the factors of unwanted variation from the original read counts (`normalizedCounts`).

`signature(x = "SeqExpressionSet", cIdx = "character", k="numeric", residuals = "matrix")`
It returns a SeqExpressionSet with

- The normalized counts in the `normalizedCounts` slot.
- The estimated factors of unwanted variation as additional columns of the `phenoData` slot.

Author(s)

Davide Risso

References

D. Risso, J. Ngai, T. P. Speed, and S. Dudoit. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nature Biotechnology*, 2014. (In press).

D. Risso, J. Ngai, T. P. Speed, and S. Dudoit. The role of spike-in standards in the normalization of RNA-Seq. In D. Nettleton and S. Datta, editors, *Statistical Analysis of Next Generation Sequence Data*. Springer, 2014. (In press).

See Also

RUVg, RUVs, residuals.

Examples

```
library(edgeR)
library(zebrafishRNASeq)
data(zfGenes)

## run on a subset of genes for time reasons
## (real analyses should be performed on all genes)
genes <- rownames(zfGenes)[grep("^ENS", rownames(zfGenes))]
spikes <- rownames(zfGenes)[grep("^ERCC", rownames(zfGenes))]
set.seed(123)
idx <- c(sample(genes, 1000), spikes)
seq <- newSeqExpressionSet(as.matrix(zfGenes[idx,]))

# Residuals from negative binomial GLM regression of UQ-normalized
# counts on covariates of interest, with edgeR
x <- as.factor(rep(c("Ctl", "Trt"), each=3))
design <- model.matrix(~x)
y <- DGEList(counts=counts(seq), group=x)
y <- calcNormFactors(y, method="upperquartile")
y <- estimateGLMCommonDisp(y, design)
y <- estimateGLMTagwiseDisp(y, design)

fit <- glmFit(y, design)
res <- residuals(fit, type="deviance")

# RUVr normalization (after UQ)
seqUQ <- betweenLaneNormalization(seq, which="upper")
controls <- rownames(seq)
seqRUVr <- RUVr(seqUQ, controls, k=1, res)
```

```
pData(seqRUVr)
head(normCounts(seqRUVr))
```

RUVs-methods *Remove Unwanted Variation Using Replicate/Negative Control Samples*

Description

This function implements the RUVs method of Risso et al. (2014).

Usage

```
RUVs(x, cIdx, k, scIdx, round=TRUE, epsilon=1, tolerance=1e-8, isLog=FALSE)
```

Arguments

x	Either a genes-by-samples numeric matrix or a SeqExpressionSet object containing the read counts.
cIdx	A character, logical, or numeric vector indicating the subset of genes to be used as negative controls in the estimation of the factors of unwanted variation.
k	The number of factors of unwanted variation to be estimated from the data.
scIdx	A numeric matrix specifying the replicate samples for which to compute the count differences used to estimate the factors of unwanted variation (see details).
round	If TRUE, the normalized measures are rounded to form pseudo-counts.
epsilon	A small constant (usually no larger than one) to be added to the counts prior to the log transformation to avoid problems with log(0).
tolerance	Tolerance in the selection of the number of positive singular values, i.e., a singular value must be larger than tolerance to be considered positive.
isLog	Set to TRUE if the input matrix is already log-transformed.

Details

The RUVs procedure performs factor analysis on a matrix of count differences for replicate/negative control samples, for which the biological covariates of interest are constant.

Each row of scIdx should correspond to a set of replicate samples. The number of columns is the size of the largest set of replicates; rows for smaller sets are padded with -1 values.

For example, if the sets of replicate samples are (1,11,21),(2,3),(4,5),(6,7,8), then scIdx should be

1 11 21
2 3 -1
4 5 -1
6 7 8

Methods

`signature(x = "matrix", cIdx = "ANY", k = "numeric", scIdx = "matrix")` It returns a list with

- A samples-by-factors matrix with the estimated factors of unwanted variation (`W`).
- The genes-by-samples matrix of normalized expression measures (possibly rounded) obtained by removing the factors of unwanted variation from the original read counts (`normalizedCounts`).

`signature(x = "SeqExpressionSet", cIdx = "character", k="numeric", scIdx = "matrix")`
It returns a SeqExpressionSet with

- The normalized counts in the `normalizedCounts` slot.
- The estimated factors of unwanted variation as additional columns of the `phenoData` slot.

Author(s)

Davide Risso (building on a previous version by Laurent Jacob).

References

D. Risso, J. Ngai, T. P. Speed, and S. Dudoit. Normalization of RNA-seq data using factor analysis of control genes or samples. *Nature Biotechnology*, 2014. (In press).

D. Risso, J. Ngai, T. P. Speed, and S. Dudoit. The role of spike-in standards in the normalization of RNA-Seq. In D. Nettleton and S. Datta, editors, *Statistical Analysis of Next Generation Sequence Data*. Springer, 2014. (In press).

See Also

RUVg, RUVr.

Examples

```
library(zebrafishRNASeq)
data(zfGenes)

## run on a subset of genesfor time reasons
## (real analyses should be performed on all genes)
genes <- rownames(zfGenes)[grep("^ENS", rownames(zfGenes))]
spikes <- rownames(zfGenes)[grep("^ERCC", rownames(zfGenes))]
set.seed(123)
idx <- c(sample(genes, 1000), spikes)
seq <- newSeqExpressionSet(as.matrix(zfGenes[idx,]))

# RUVs normalization
controls <- rownames(seq)
differences <- matrix(data=c(1:3, 4:6), byrow=TRUE, nrow=2)
seqRUVs <- RUVs(seq, controls, k=1, differences)

pData(seqRUVs)
head(normCounts(seqRUVs))
```

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