Package ‘ABSSeq’
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Type Package

Title ABSSeq: a new RNA-Seq analysis method based on modelling absolute expression differences

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Author Wentao Yang

Maintainer Wentao Yang <wyang@zoologie.uni-kiel.de>

Description Inferring differential expression genes by absolute counts difference between two groups, utilizing Negative binomial distribution and moderating fold-change according to heterogeneity of dispersion across expression level.

License GPL (>= 3)

biocViews DifferentialExpression

Imports locfit, limma

Depends R (>= 2.10), methods

Suggests

NeedsCompilation no

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ABSDataSet

Description

ABSDataSet object and constructors

Usage

ABSDataSet(counts, groups, normMethod = c("user", "total", "quartile", "geometric"), sizeFactor = 0, minDispersion = NULL, minRates = 0.1, maxRates = 0.3, LevelstoNormFC = 100)

Arguments

counts a matrix or table with at least two columns and one row,
groups a factor with two groups, whose length should be equal with sample size
normMethod method for estimating the size factors, should be one of ‘user’, ‘total’, ‘quartile’ and ‘geometric’. See normalFactors for description.
sizeFactor size factors for ‘user’ method, self-defined size factors by user.
minDispersion a positive double for user-defined penalty of dispersion estimation
minRates low bounder rate of baseline estimation for counts difference, default is 0.1
maxRates up bounder rate of baseline estimation for counts difference, default is 0.3. Setting minRates equal with maxRates will result in a testing on user-define rate,
LevelstoNormFC maximal level of average standard deviation in fold-change normalization according to expression level, default is 100.

Details

The function constructs an ABSDataSet object with counts table and groups. It also checks the structure of counts and groups. The ABSDataSet is a class, used to store the input values, intermediate calculations and results of an analysis of differential expression. It also contains information for the running time of an analysis.

Value

An ABSDataSet object.

Examples

counts <- matrix(1:4,ncol=2)
groups <- factor(c("a","b"))
obj <- ABSDataSet(counts, groups)
**Description**

This function performs a default analysis by calling, in order, the functions: `normalFactors`, `callParameter`, `callDEs`.

**Usage**

```r
ABSSeq(object, adjmethod = "BH", replaceOutliers = TRUE, quiet = FALSE, ...)
```

**Arguments**

- `object`: an `ABSDataSet` object, contains the reads count matrix, groups and normalization method.
- `adjmethod`: default is 'BH', method for p-value adjusted, see `p.adjust.methods` for details
- `replaceOutliers`: default is TRUE, switch for outlier replacement.
- `quiet`: default is FALSE, whether to print messages at each step
- `...`: parameters passed to `ReplaceOutliersByMAD` from `callParameter`

**Details**

The differential expression analysis models the total counts difference by a Negative binomail distribution

\[ NB(\mu, r) \]

**Value**

an `ABSDataSet` object with additional elements, which can be retrieved by `results`: Amean and Bmean, mean of log2 normalized reads count for group A and B, foldChange, shrinked (expression level and gene-specific) log2 of fold-change, B - A, rawFC, raw log2 of fold-change, B-A (without shrinkage), lowFC, expression level corrected log2 fold-change, pvalue, pvalue from NB distribution model, adj.pvalue, adjusted p-value used p.adjust method.

**Author(s)**

Wentao Yang

**References**

Wentao Yang, Philip Rosenstiel & Hinrich Schulenburg: ABSSeq: a new RNA-Seq analysis method based on modelling absolute expression differences
Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
obj <- ABSSeq(obj)
res <- results(obj,c("Amean","Bmean","foldChange","pvalue","adj.pvalue"))
head(res)

---

callDEs Test the differential expression by counts difference

Description
Using NB distribution to calculate p-value for each gene as well as adjust p-value

Usage

callDEs(object, adjmethod = "BH")

Arguments

object an ABSDataSet object.
adjmethod the method for adjusting p-value, default is 'BH'. For details, see p.adjust.methods.

Details
This function firstly calls p-value used pnbinom to call pvalue based on sum of counts difference between two groups, then adjusts the pvalues via p.adjust method. In addition, it also shrink the log2 fold-change towards a common dispersion after pvalue calling.

Value
an ABSDataSet object with additional elements: shrinked log2 fold-change, pvalue and adjusted p-value, denoted by foldChange value and adj-pvalue, respectively. Use the results method to get access it.

Note
this function should run after callParameter

Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
obj <- normalFactors(obj)
obj <- callParameter(obj)
obj <- callDEs(obj)
head(results(obj))
callParameter

Calculate parameters for differential expression test base on absolute counts differences

Description

Calculate parameters for each gene (the moderating basemean and dispersions)

Usage

callParameter(object, replaceOutliers = TRUE, ...)

Arguments

object
  a ABSDataSet object.
replaceOutliers
  switch for outlier replacement, default is TRUE.
...
parameters past to ReplaceOutliersByMAD

Details

shifted and calculate a set of parameters from normalized counts table before callDEs

Value

A ABSDataSet object with absolute differences, basemean, mean of each group, variance, log2 of foldchange, named as 'absD', 'baseMean', 'Amean', 'Bmean', 'Variance' and 'foldChange', respectively. Use the results to get access it and plotDifftoBase to plot it.

Note

This function should run after normalFactors or providing size factors.

Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
obj <- normalFactors(obj)
obj <- callParameter(obj)
head(results(obj,c("foldChange","absD","baseMean")))
plotDifftoBase(obj)
callParameterwithoutReplicates

*Calculate parameters for differential expression test base on absolute counts differences without replicates*

**Description**

Calculate parameters for each gene (the moderating basemean and dispersions), without replicates

**Usage**

```
callParameterwithoutReplicates(object)
```

**Arguments**

- `object` a *ABSDataSet* object.

**Details**

Building a pseudo group to estimate parameter by mean difference. Shifted and calculate a set of parameters from normalized counts table before *callDEs*.

**Value**

A *ABSDataSet* object with absolute differences, basemean, mean of each group, variance, log2 of foldchange, named as 'absD', 'baseMean', 'Amean', 'Bmean', 'Variance' and 'foldChange', respectively. Use the `results` to get access it.

**Note**

This function should run after *normalFactors* or providing size factors. This function firstly constructs an expression level depended fold-change cutoffs and then separate the data into two groups. The group with fold-change less than cutoffs is used to training the dispersion. However, the cutoff might be too small when applied on data set without or with less DEs. To avoid it, we set a prior value (0.5) to it.

**Examples**

```
data(simuN5)
oj <- ABSDataSet(counts=(simuN5$counts)[,c(1,2)], groups=factor(c(1,2)))
oj <- normalFactors(oj)
oj <- callParameterwithoutReplicates(oj)
oj <- callDEs(oj)
head(results(oj))
```
### Accessors for the 'counts' slot of a ABSDataSet object.

**Description**

Accessors for the 'counts' slot of a ABSDataSet object, return a matrix.

**Usage**

```r
## S4 method for signature 'ABSDataSet'
counts(object, norm=FALSE)
```

```r
## S4 replacement method for signature 'ABSDataSet, matrix'
counts(object)<-value
```

**Arguments**

- `object` a ABSDataSet object.
- `norm` logical indicating whether or not to normalize the counts before returning.
- `value` an numeric matrix.

**Details**

The counts slot holds the count data as a matrix of non-negative integer count values, rows and columns for genes and samples, respectively.

**See Also**

`sFactors, normalFactors`

**Examples**

```r
data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
head(counts(obj))
counts(obj)<- matrix(1:50, nrow=5, ncol=10)
head(counts(obj))
```

---

### estimateSizeFactorsForMatrix

*Low-level function to estimate size factors with robust regression.*

**Description**

This function is borrowed from DESeq.

**Usage**

```r
estimateSizeFactorsForMatrix(counts, locfunc = median)
```
Arguments

- **counts**: a matrix or data frame of counts, i.e., non-negative integer values
- **locfunc**: a function to compute a location for a sample. By default, the median is used.

Details

Given a matrix or data frame of count data, this function estimates the size factors as follows: Each column is divided by the geometric means of the rows. The median (or, if requested, another location estimator) of these ratios (skipping the genes with a geometric mean of zero) is used as the size factor for this column. Typically, you will not call this function directly.

Value

a vector with the estimates size factors, one element per column

Author(s)

Simon Anders

References


Examples

data(simuN5)
dat <- simuN5
estimateSizeFactorsForMatrix(dat$counts)

---

**excounts**

Accessors for the 'excounts' slot of an ABSDataSet object.

Description

Accessors for the 'excounts' slot of an ABSDataSet object, return a matrix

Usage

```r
## S4 replacement method for signature 'ABSDataSet,matrix'
excounts(object)<-value
```

Arguments

- **object**: a ABSDataSet object.
- **value**: an numeric matrix

Details

The excounts slot holds the normalized (trimmed or not) count data as a matrix of non-negative integer count values, rows and columns for genes and samples, respectively.
groups

See Also

ABSDataSet, ReplaceOutliersByMAD

Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
obj <- normalFactors(obj)
obj <- ReplaceOutliersByMAD(obj)
head(excounts(obj))

groups

Accessors for the 'groups' slot of a ABSDataSet object.

Description

Accessor functions for the 'groups' information in a ABSDataSet object.

Usage

## S4 method for signature 'ABSDataSet'
groups(object)

## S4 replacement method for signature 'ABSDataSet,factor'
groups(object)<-value

Arguments

object an ABSDataSet object.

value a factor object, includes two groups, equal with the number of samples

Details

The 'groups' is a factor object, contains the experiment design for differential expression analysis. Its length should be equal with the sample size.

Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
groups(obj)
groups(obj) <- factor(rep(c("A","B"),c(5,5)))
groups(obj)
**LevelstoNormFC**

*Accessors for the 'LevelstoNormFC' slot of a ABSDataSet object.*

**Description**

Accessor functions for the 'LevelstoNormFC' slot of a ABSDataSet object.

**Usage**

```r
## S4 method for signature 'ABSDataSet'
LevelstoNormFC(object)

## S4 replacement method for signature 'ABSDataSet,numeric'
LevelstoNormFC(object) <- value
```

**Arguments**

- `object`: an ABSDataSet object.
- `value`: a positive numeric object.

**Details**

The 'LevelstoNormFC' is maximal level of average standard deviation in fold-change normalization according to expression level.

**See Also**

ABSDataSet, callParameter

**Examples**

```r
data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
LevelstoNormFC(obj)
LevelstoNormFC(obj) <- 200
LevelstoNormFC(obj)
```

**maxRates**

*Accessors for the 'maxRates' slot of a ABSDataSet object.*

**Description**

Accessor functions for the 'maxRates' slot of a ABSDataSet object.

**Usage**

```r
## S4 method for signature 'ABSDataSet'
maxRates(object)

## S4 replacement method for signature 'ABSDataSet,numeric'
maxRates(object) <- value
```

**Examples**

```r
data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
maxRates(obj)
maxRates(obj) <- 200
maxRates(obj)
```
minimalDispersion

Arguments

object an ABSDataSet object.
value a positive numeric object

Details

The 'maxRates' is the upper bound of rate for baseline of counts difference estimation.

See Also

callParameter, ABSDataSet

Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
maxRates(obj)
maxRates(obj) <- 0.4
maxRates(obj)

minimalDispersion  Accessors for the 'minDispersion' slot of a ABSDataSet object.

Description

Accessor functions for the 'minDispersion' slot of a ABSDataSet object.

Usage

## S4 method for signature 'ABSDataSet'
minimalDispersion(object)

## S4 replacement method for signature 'ABSDataSet,numeric'
minimalDispersion(object)<-value

Arguments

object an ABSDataSet object.
value a positive numeric object

Details

The 'minimalDispersion' is the penalty of dispersion estimation. User can set the penalty of dispersion by this function

See Also

callParameter, ABSDataSet
minRates

Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
minimalDispersion(obj)
minimalDispersion(obj) <- 0.2
minimalDispersion(obj)

minRates

Accessors for the 'minRates' slot of a ABSDataSet object.

Description

Accessor functions for the 'minRates' slot of a ABSDataSet object.

Usage

## S4 method for signature 'ABSDataSet'
minRates(object)

## S4 replacement method for signature 'ABSDataSet,numeric'
minRates(object)<-value

Arguments

object an ABSDataSet object.
value a positive numeric object

Details

The 'minRates' is the lower bound of rate for baseline of counts difference estimation.

See Also

callParameter.ABSDataSet

Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
minRates(obj)
minRates(obj) <- 0.3
minRates(obj)
normalFactors

Estimating size factors from the reads count table

Description
Function for estimating size factors

Usage

normalFactors(object)

Arguments

object a ABSSeq object with element of 'counts' and 'normMethod', see the constructor functions ABSDataSet.

Details
Given a matrix of count data, this function estimates the size factors by selected method. It also provides four different methods for normalizing according to user-defined size factors, total reads, up quantile (75)

Value
a ABSDataSet object with the estimates size factors, one element per column. Use the sFactors to show it.

Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
obj <- normalFactors(obj)
sFactors(obj)

normMethod
Accessors for the 'normMethod' slot of a ABSDataSet object.

Description
Accessor functions for the 'normMethod' information in a ABSDataSet object.

Usage

## S4 method for signature 'ABSDataSet'
normMethod(object)

## S4 replacement method for signature 'ABSDataSet,character'
normMethod(object)<-value
plotDifftoBase

Arguments

- **object**: an ABSDataSet object.
- **value**: a character object, should be one of 'user', 'total', 'quartile' and 'geometric'. See `normalFactors`

Details

The 'normMethod' is the method for calculating the size factors. Currently, Four methods: 'user', 'total', 'quartile' and 'DESeq' are available.

Examples

```r
data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
normMethod(obj)
normMethod(obj) <- "geometric"
normMethod(obj)
```

plotDifftoBase: *Plot absolute log2 fold-change against base mean of expression*

Description

Plot absolute differences against expression levels

Usage

```r
plotDifftoBase(object, foldname = "foldChange", adj.pcut = 0.05,
cole = c("black", "red"), pch = 16, xlab = "log2 of Expression level",
ylab = "log2 fold-change", ...)
```

Arguments

- **object**: a ABSDataSet
- **foldname**: indicates kind of fold-change in plotting, default is 'foldChange', see results
- **adj.pcut**: cutoff for differential expressed genes, marked by different color, default is 0.05
- **cols**: the colors to mark the non-DE and DE genes, default is black and red, respectively
- **pch**: pch, default is 16
- **xlab**: xlab, default is 'log2 of Expression level'
- **ylab**: ylab, default is 'log2 fold-change'
- **...**: further arguments to `plot`

Details

Plot absolute differences against expression levels and mark the gene with a color at a given cutoff of fold-change
ReplaceOutliersByMAD

Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
obj <- ABSSeq(obj)
plotDifftoBase(obj)

ReplaceOutliersByMAD Replacing outliers by moderated MAD

Description

Function for replacing the outliers by MAD

Usage

ReplaceOutliersByMAD(object, replaceOutlier = TRUE, cutoff = 2,
baseMean = 100, limitMad = 0.707, spriors = 2, Caseon = TRUE)

Arguments

object a ABSSeq object with element of 'counts' and 'normMethod', see the constructor functions ABSDataSet.
replaceOutlier switch for replacing, default is TRUE.
cutoff cutoff of moderating MAD for outliers, default is 2
baseMean parameter for limiting the trimming at low expression level by baseMean/(sample size), default is 100.
limitMad the minimal prior for moderating MAD, default is set to 0.707, which is usually the highest standard deviation at expression level of 1
spriors prior weight size for prior MAD, default is 2
Caseon switch for dealing with outlier trimming at sample size of 2

Details

Given a matrix of count data, this function replacing the outliers by MAD. Noticely, this function also provides part of parameters for DEs calling. It is called by callParameter

Value

a ABSDataSet object with normalized counts after trimming (replaceOutlier=TRUE) or not (replaceOutlier=FALSE). Use the excounts to show it. Use results with name 'trimmed' to view the trimming status.

Examples

data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
obj <- normalFactors(obj)
obj <- ReplaceOutliersByMAD(obj)
head(excounts(obj))
head(results(obj,c("trimmed")))
results

Accessor functions for the result from a ABSDataSet

Description

Accessor functions for the result from a ABSDataSet by given names

Usage

```r
## S4 method for signature 'ABSDataSet'
results(object, cnames = c("Amean", "Bmean", "baseMean", "absD", "Variance", "rawFC", "lowFC", "foldChange", "pvalue", "adj.pvalue", "trimmed"))
```

Arguments

- **object**: a ABSDataSet
- **cnames**: a vector of names for output, which are among: 'Amean', 'Bmean', log2 of mean counts for group A and B, 'baseMean', estimated mean for absolute counts difference (absD), used for mu in `pnbinom` 'absD', absolute counts difference in total 'Variance', pooled Variance for two groups 'rawFC', lowFC', 'foldChange', log2 fold-change of original (Bmean-Amean), corrected by expression level and corrected by both expression level and gene-specific dispersion 'pvalue', 'adj.pvalue', pvalue and adjusted pvalue 'trimmed', number of trimmed outliers

Details

This function returns the result of ABSSeq as a table or a vector depended on the given names, see `ABSSeq`

Value

a table according to canmes.

See Also

`ABSSeq`

Examples

```r
data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
obj <- normalFactors(obj)
obj <- callParameter(obj)
obj <- callDEs(obj)
head(results(obj))
```
sFactors

Accessors for the 'sizeFactor' slot of a ABSDataSet object.

Description

Accessor functions for the 'sizeFactor' slot of a ABSDataSet object.

Usage

```r
## S4 method for signature 'ABSDataSet'
sFactors(object)

## S4 replacement method for signature 'ABSDataSet,numeric'
sFactors(object)<-value
```

Arguments

- `object` an ABSDataSet object.
- `value` a numeric object, one for each sample

Details

The sizeFactors vector assigns to each sample a value, used to normalize the counts in each sample according to selected normMethod.

See Also

normalFactors

Examples

```r
data(simuN5)
obj <- ABSDataSet(counts=simuN5$counts, groups=factor(simuN5$groups))
obj <- normalFactors(obj)
sFactors(obj)
sFactors(obj) <- runif(10,1,2)
sFactors(obj)
```

simuN5

Simulated study with random outliers

Description

Simulated study with random outliers, include five samples for two groups. It contains counts table, groups and defined differential expression genes.

Usage

```r
data(simuN5)
```
Format

The format is: List of 3
$ counts: integer, reads count matrix
$ groups: two groups
$ DEs : differential expression genes

Details

Multiple each gene with a value from 5-10 by chance at pvalue of 0.05.

Source

http://bcf.isb-sib.ch/data/compcodeR/

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