Package ‘SeqGSEA’

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Type Package

Title Gene Set Enrichment Analysis (GSEA) of RNA-Seq Data: integrating differential expression and splicing

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Description The package generally provides methods for gene set enrichment analysis of high-throughput RNA-Seq data by integrating differential expression and splicing. It uses negative binomial distribution to model read count data, which accounts for sequencing biases and biological variation. Based on permutation tests, statistical significance can also be achieved regarding each gene’s differential expression and splicing, respectively.

License GPL (>= 3)

Depends Biobase, doParallel, DESeq2

Imports methods, biomaRt

Suggests GenomicRanges

biocViews Sequencing, RNASeq, GeneSetEnrichment, GeneExpression, DifferentialExpression, DifferentialSplicing, ImmunoOncology

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R topics documented:

SeqGSEA-package .................................................. 3
calES ............................................................... 6
calES.perm .......................................................... 6
convertEnsembl2Symbol ............................................ 7
convertSymbol2Ensembl ........................................... 8
counts-methods ..................................................... 9
DENBStat4GSEA .................................................... 9
DENBStatPermut4GSEA ............................................ 10
DENBTest .......................................................... 11
DEpermutePval ...................................................... 12
DEscore ............................................................. 13
DSpermute4GSEA ................................................... 14
DSpermutePval ....................................................... 15
DSresultExonTable ................................................ 16
DSresultGeneTable ................................................ 17
estiExonNBstat ...................................................... 18
estiGeneNBstat ...................................................... 19
exonID ............................................................... 20
exonTestability ..................................................... 20
geneID ............................................................... 21
geneList ............................................................. 22
genePermuteScore ................................................ 23
geneScore ........................................................... 24
geneSetDescs ......................................................... 25
geneSetName ......................................................... 26
geneSetSize ........................................................ 27
geneTestability ..................................................... 28
genePermuteMat ..................................................... 29
getGeneCount ........................................................ 30
GSEAresultTable ................................................... 31
GSEnrichAnalyze .................................................... 32
GS_example .......................................................... 33
label ................................................................. 33
loadExonCountData ................................................ 34
loadGenesets ........................................................ 35
newGeneSets ........................................................ 36
newReadCountSet ................................................... 37
normES ............................................................... 38
normFactor .......................................................... 39
plotES ............................................................... 40
plotGeneScore ....................................................... 40
plotSig .............................................................. 41
plotSigGeneSet ..................................................... 42
rankCombine ......................................................... 43
RCS_example ........................................................ 44
ReadCountSet-class ................................................ 45
**Description**

SeqGSEA is an R package for gene set enrichment analysis of RNA-Seq data with the ability to integrate differential expression and differential splice in functional analysis.

**Details**

- **Package**: SeqGSEA
- **Type**: Package
- **License**: GPL (>= 3)

A User’s Guide is available as well as the usual help page documentation for each of the individual functions.

The most useful functions are listed below:

* ReadCountSet class
  - ReadCountSet-class
  - ReadCountSet
  - exonID
  - geneID
  - counts-methods
  - label
  - subsetByGenes
* SeqGeneSet class
  - SeqGeneSet-class
  - geneSetDescs
  - geneSetNames
  - geneSetSize
  - size

* Load data
  - newReadCountSet
  - loadExonCountData
  - newGeneSets
  - loadGenesets

* DE analysis
  - getGeneCount
  - runDESeq
  - DENBStat4GSEA
  - DENBStatPermut4GSEA
  - DENBTest
  - DEpermutePval

* DS analysis
  - DSpermute4GSEA
  - DSpermutePval
  - exonTestability
  - geneTestability
  - estiExonNBstat
  - estiGeneNBstat

* GSEA main
  - GSEnrichAnalyze
  - calES
  - calES.perm
  - genePermuteScore
  - geneScore
  - rankCombine
  - normES
  - normFactor
  - scoreNormalization
• signifES

* Result tables
  • GSEAresultTable
  • DSresultExonTable
  • DSresultGeneTable
  • topDEGenes
  • topDSExons
  • topDSGenes
  • topGeneSets

* Result displays
  • plotES
  • plotGeneScore
  • plotSig
  • plotSigGeneSet
  • writeSigGeneSet

* Miscellaneous
  • genpermuteMat
  • convertEnsembl2Symbol
  • convertSymbol2Ensembl

Author(s)

Xi Wang and Murray J. Cairns

Maintainer: Xi Wang <xi.wang@newcastle.edu.au>

References

calES

*Calculate running enrichment scores of gene sets*

**Description**

This is an internal function to calculate running enrichment scores of each gene set in the SeqGeneSet object specified.

**Usage**

```r
calES(gene.set, gene.score, weighted.type = 1)
```

**Arguments**

- `weighted.type`: gene score weight type.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

GSEnrichAnalyze, calES.perm.

**Examples**

```r
data(DEscore, package="SeqGSEA")
data(DSscore, package="SeqGSEA")
gene.score <- geneScore(DEscore, DSscore, method="linear", DEweight = 0.3)
data(GS_example, package="SeqGSEA")
rES <- calES(GS_example, gene.score)
rES[1,]
```

calES.perm

*Calculate enrichment scores for gene sets in the permutation data sets*

**Description**

This is an internal function to calculate enrichment scores for gene sets in the permutation data sets.

**Usage**

```r
calES.perm(gene.set, gene.score.perm, weighted.type = 1)
```
**convertEnsembl2Symbol**

Convert ensembl gene IDs to gene symbols

**Description**

Convert ensembl gene IDs to gene symbols

**Usage**

`convertEnsembl2Symbol(ensembl.genes)`

**Arguments**

- `ensembl.genes`  
  ensembl gene ID(s).

**Value**

A 2-column matrix showing the correspondence of ensembl gene IDs and gene symbols.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

`convertSymbol2Ensembl`
convertSymbol2Ensembl

Convert gene symbols to ensembl gene IDs

Description

Convert gene symbols to ensembl gene IDs

Usage

convertSymbol2Ensembl(symbols)

Arguments

symbols gene symbol(s).

Value

A 2-column matrix showing the correspondence of gene symbols and ensembl gene IDs.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

convertEnsembl2Symbol

Examples

## Not run:
convertSymbol2Ensembl("DISC1") #ENSG00000162946

## End(Not run)
counts-methods

Accessors for the 'counts' slot of a ReadCountSet object.

Description

Accessors for the 'counts' slot of a ReadCountSet object.

Usage

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

Arguments

- `object`: a ReadCountSet object
- `value`: a matrix of read counts

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

Examples

```r
data(RCS_example, package="SeqGSEA")
readCounts <- counts(RCS_example)
head(readCounts)
```

DENBStat4GSEA

Calculate NB-statistics quantifying differential expression for each gene.

Description

Calculate NB-statistics quantifying differential expression between two groups of samples compared. The results will be used for GSEA run. Comparing with `DENBTest`, this function will not calculate NB test p-values.

This function only works with two-group comparison.

Usage

`DENBStat4GSEA(dds)`
**DENBStatPermut4GSEA**

**Arguments**

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>dds</td>
<td>A DESeqDataSet object with size factors and dispersion parameters estimated. Recommended to take the output of <code>runDESeq</code>.</td>
</tr>
</tbody>
</table>

**Value**

A data frame containing each gene’s expression means and variances in each group, and each gene’s DE NB-statistics.

**Note**

The results with the output of `DENBStatPermut4GSEA` can also be used to run `DEpermutePval`.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**References**


**See Also**

`DENBTest`, `runDESeq`, `DENBStatPermut4GSEA`

**Examples**

```r
data(RCS_example, package="SeqGSEA")
geneCounts <- getGeneCount(RCS_example)
label <- label(RCS_example)
DEG <- runDESeq(geneCounts, label)
DEGres <- DENBStat4GSEA(DEG)
head(DEGres)
```

---

**DENBStatPermut4GSEA**

*Calculate NB-statistics quantifying DE for each gene in the permutation data sets*

**Description**

Calculate NB-statistics quantifying differential expression for each gene in the permutation data sets. The results will be used for GSEA run.

**Usage**

`DENBStatPermut4GSEA(dds, permuteMat)`
**DENBTest**

**Arguments**

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>dds</td>
<td>a DESeqDataSet object, can be the output of runDESeq.</td>
</tr>
<tr>
<td>permuteMat</td>
<td>a permutation matrix generated by genpermuteMat.</td>
</tr>
</tbody>
</table>

**Value**

A matrix of NB-statistics. Each row corresponds to each gene, and each column to each permutation.

**Note**

The results with the output of DENBStat4GSEA can also be used to run DEpermutePval.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**References**


**See Also**

DENBStat4GSEA, runDESeq, DEpermutePval, genpermuteMat

**Examples**

```r
data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10)
genCounts <- getGeneCount(RCS_example)
label <- label(RCS_example)
dds <- runDESeq(geneCounts, label)
DEpermNBstat <- DENBStatPermut4GSEA(dds, permuteMat)
DEpermNBstat[1:10,1:10]
```

---

**DENBTest**  
*Perform negative binomial exact test for differential expression*

**Description**

Perform negative binomial exact test for differential expression - a modified version of nbinomTest in DESeq package.

**Usage**

DENBTest(dds)
DEpermutePval

Arguments

dds
A DESeqDataSet object with size factors and dispersion parameters estimated. Recommended to take the output of runDESeq.

Value

A data frame of the test results. Information contains mean expression values, NB-statistics, (log) fold-changes, p-values, and adjusted p-values.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

References


See Also

runDESeq, DENBStat4GSEA

Examples

data(RCS_example, package="SegGSEA")
geneCounts <- getGeneCount(RCS_example)
label <- label(RCS_example)
DEG <- runDESeq(geneCounts, label)
DEGres <- DENBTest(DEG)
head(DEGres)

DEpermutePval

Permutation for p-values in differential expression analysis

Description

Calculate permutation p-values in differential expression analysis for each genes.

Usage

DEpermutePval(DEGres, permuteNBstat)

Arguments

DEGres
the output of DENBStat4GSEA.

permuteNBstat
the output of DENBStatPermut4GSEA.
**Value**

A data frame containing the expression means and variances for each gene in each group compared, and NB-stats, permutation p-values and adjusted p-values for each gene.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

runDESeq, DENBStat4GSEA, DENBStatPermut4GSEA, DENBTest

**Examples**

```r
data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10)
geneCounts <- getGeneCount(RCS_example)
label <- label(RCS_example)
DEG <- runDESeq(geneCounts, label)
DEGres <- DENBStat4GSEA(DEG)
DEpermNBstat <- DENBStatPermut4GSEA(DEG, permuteMat)
DEGres <- DEpermutePval(DEGres, DEpermNBstat)
head(DEGres)
```

**Description**

DEscore and DSscore are pre-calculated DE and DS scores, respectively; DEscore.perm and DSscore.perm are pre-calculated DE and DS scores on the permutation data sets, respectively; They are used in examples of the SeqGSEA package. Note that these scores are of no meaning but to demonstrate the usage of functions.

**Usage**

```r
data("DEscore")
data("DEscore.perm")
data("DSscore")
data("DSscore.perm")
```

**References**

DSpermute4GSEA

Description

This function is to calculate NB-statistics quantifying differential splicing for each gene on each permutation data set. The results will be used for GSEA run as DS background.

Usage

DSpermute4GSEA(RCS, permuteMat)

Arguments

RCS a ReadCountSet object after running exonTestability.
permuteMat a permutation matrix generated by genpermuteMat.

Details

Parallel running configuration: TODO

Value

A ReadCountSet object with slot permute_NBstat_gene updated.

Note

Please run exonTestability before run this function.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

References


See Also

exonTestability, genpermuteMat, DENBStatPermut4GSEA, DSpermutePval
**Examples**

```r
data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10)
RCS_example <- exonTestability(RCS_example)
RCS_example <- DSpermute4GSEA(RCS_example, permuteMat)
head(RCS_example@permute_NBstat_gene)
```

---

**DSpermutePval**  
Permutation for p-values in differential splicing analysis

**Description**

Calculate permutation p-values in differential splicing analysis.

**Usage**

`DSpermutePval(RCS, permuteMat)`

**Arguments**

- `RCS`: a ReadCountSet object after running `estiExonNBstat` and `estiGeneNBstat`.
- `permuteMat`: a permutation matrix generated by `genpermuteMat`.

**Details**

Permutation p-values are computed based on NB-statistics for comparison of the studied groups and NB-statistics from the permutation data sets.

**Value**

A ReadCountSet object with slots `permute_NBstat_exon`, `permute_NBstat_gene`, `featureData`, and `featureData_gene` updated.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**References**


**See Also**

`estiExonNBstat`, `estiGeneNBstat`, `genpermuteMat`, `DSpermute4GSEA`
Examples

```r
data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10)
RCS_example <- exonTestability(RCS_example)
RCS_example <- estiExonNBstat(RCS_example)
RCS_example <- estiGeneNBstat(RCS_example)
RCS_example <- DSpermutePval(RCS_example, permuteMat)
head(DSresultExonTable(RCS_example))
head(DSresultGeneTable(RCS_example))
```

---

**DSresultExonTable**

*Form a table for DS analysis results at the Exon level*

**Description**

Form a table for differential splicing analysis results at the Exon level.

**Usage**

```r
DSresultExonTable(RCS)
```

**Arguments**

- **RCS**
  
  A ReadCountSet object with **DSpermutePval** done.

**Details**

A data frame containing each exon’s NB-statistics, p-values and adjusted p-values for differential splicing analysis.

**Value**

A matrix containing exon DS analysis results, including testability, NBstats, p-values and adjusted p-values.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

- **DSresultGeneTable**
- **DSpermutePval**
**Examples**

```r
data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10)
RCS_example <- exonTestability(RCS_example)
RCS_example <- estiExonNBstat(RCS_example)
RCS_example <- estiGeneNBstat(RCS_example)
RCS_example <- DSpermutePval(RCS_example, permuteMat)
head(DSresultExonTable(RCS_example))
```

---

**DSresultGeneTable**  
*Form a table for DS analysis results at the gene level*

**Description**

Form a table for differential splicing analysis results at the gene level.

**Usage**

```r
DSresultGeneTable(RCS)
```

**Arguments**

- **RCS**  
  A ReadCountSet object with **DSpermutePval** done.

**Value**

A data frame containing each gene’s NB-statistics, p-values and adjusted p-values for differential splicing analysis.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

*DSresultExonTable, DSpermutePval*

**Examples**

```r
data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10)
RCS_example <- exonTestability(RCS_example)
RCS_example <- estiExonNBstat(RCS_example)
RCS_example <- estiGeneNBstat(RCS_example)
RCS_example <- DSpermutePval(RCS_example, permuteMat)
head(DSresultGeneTable(RCS_example))
```
Description

Calculate NB-statistics quantifying differential splicing for individual exons between two groups of samples compared.

Usage

estiExonNBstat(RCS)

Arguments

RCS a ReadCountSet object after running exonTestability.

Value

A ReadCountSet object with the slot featureData updated.

Note

Please run exonTestability before you run this function.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

References


See Also

exonTestability, estiGeneNBstat

Examples

data(RCS_example, package="SeqGSEA")
RCS_example <- exonTestability(RCS_example, cutoff=5)
RCS_example <- estiExonNBstat(RCS_example)
head(fData(RCS_example))
Description

Calculate NB-statistics quantifying differential splicing for each gene between two groups of samples compared. The results will be used for GSEA run (as DS-scores).

Usage

estiGeneNBstat(RCS)

Arguments

RCS a ReadCountSet object after running estiExonNBstat.

Value

A ReadCountSet object with slot featureData_gene updated.

Note

Please run estiExonNBstat before run this function.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

References


See Also

estiExonNBstat

Examples

data(RCS_example, package="SeqGSEA")
RCS_example <- exonTestability(RCS_example, cutoff=5)
RCS_example <- estiExonNBstat(RCS_example)
RCS_example <- estiGeneNBstat(RCS_example)
head(RCS_example@featureData_gene)
**exonID**  
*Accessor to the exonID slot of ReadCountSet objects*

**Description**  
Accessor to the exonID slot of ReadCountSet objects

**Usage**

```r
exonID(RCS)
exonID(RCS) <- value
```

**Arguments**

- **RCS**: a ReadCountSet object
- **value**: a vector of exon IDs

**Value**

A character vector of exon IDs; or a ReadCountSet object.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

`newReadCountSet`, `geneID`

**Examples**

```r
data(RCS_example, package="SeqGSEA")
exonID(RCS_example)
```

---

**exonTestability**  
*Check exon testability*

**Description**  
Check exon testability, filtering out exons with very few (default: 5) read counts

**Usage**

```r
exonTestability(RCS, cutoff = 5)
```
geneID

Arguments
RCS a ReadCountSet object.
cutoff exons with read counts less than this cutoff are to be marked as untestable.

Value
a ReadCountSet object with slot fData updated.

Author(s)
Xi Wang, xi.wang@newcastle.edu.au

See Also
geneTestability

Examples
data(RCS_example, package="SeqGSEA")
RCS_example <- exonTestability(RCS_example, cutoff=5)
head(fData(RCS_example))

---

**geneID**

*Accessor to the geneID slot of ReadCountSet objects*

Description
Accessor to the geneID slot of ReadCountSet objects

Usage
geneID(RCS)
geneID(RCS) <- value

Arguments
RCS a ReadCountSet object
value a vector of gene IDs

Value
A character vector of gene IDs, which can be duplicated; or a ReadCountSet object.

Author(s)
Xi Wang, xi.wang@newcastle.edu.au
geneList

See Also
   newReadCountSet, exonID

Examples
   data(RCS_example, package="SeqGSEA")
   geneID(RCS_example)

---

geneList  Get the gene list in a SeqGeneSet object

Description
   Get the gene list in a SeqGeneSet object

Usage
   geneList(GS)

Arguments
   GS  A SeqGeneSet object.

Details
   TBA

Value
   A vector of gene IDs.

Author(s)
   Xi Wang, xi.wang@newcastle.edu.au

See Also
   loadGenesets, SeqGeneSet-class

Examples
   ##
   gs <- newGeneSets(GS=list(1:10, 6:15, 11:20),
                     geneList=paste("Gene", 1:22, sep=""),
                     GSNames=c("gs1","gs2","gs3"),
                     GSDescs=c("test1","test2","test3"),
                     name="gs examples")
   geneList(gs)
   ## End
genePermuteScore  

Calculate gene scores on permutation data sets

Description

Calculate gene scores on permutation data sets

Usage

genePermuteScore(DEscoreMat, DSscoreMat = NULL, method = c("linear", "quadratic", "rank"), DEweight = 0.5)

Arguments

- DEscoreMat  normalized DE scores on permutation data sets.
- DSscoreMat  normalized DS scores on permutation data sets.
- method      one of the integration methods: linear, quadratic, or rank; default: linear.
- DEweight    any number between 0 and 1 (included), the weight of differential expression scores (the weight for differential splice is (1-DEweight)).

Details

The integration methods including "linear", "quadratic", and "rank" are detailed in Wang and Cairns (2013). Here the rank method refers only to the method using data-set-specific ranks.

For DE-only analysis, just specify DEweight to be 1, and the DSscoreMat value can be NULL.

Value

A gene score matrix.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

References


See Also

geneScore
geneScore

Examples

```r
data(DEscore.perm, package="SeqGSEA")
data(DSscore.perm, package="SeqGSEA")
# linear combination with weight for DE 0.3
gene.score.perm <- genePermuteScore(DEscore.perm, DSscore.perm, method="linear", DEweight=0.3)
# DE only analysis
gene.score.perm <- genePermuteScore(DEscore.perm, DEweight=1)
```

geneScore

Calculate gene scores by integrating DE and DS scores

Description

Calculate gene scores by integrating DE and DS scores

Usage

```r
geneScore(DEscore, DSscore = NULL, method = c("linear", "quadratic", "rank"), DEweight = 0.5)
```

Arguments

- **DEscore**: normalized DE scores.
- **DSscore**: normalized DS scores.
- **method**: one of the integration methods: linear, quadratic, or rank; default: linear.
- **DEweight**: any number between 0 and 1 (included), the weight of differential expression scores (the weight for differential splice is (1-DEweight)).

Details

The integration methods including "linear", "quadratic", and "rank" are detailed in Wang and Cairns (2013). Here the rank method refers only to the method using data-set-specific ranks.

For DE-only analysis, just specify DEweight to be 1, and the DSscore value can be NULL.

Value

A vector of gene scores.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

References

geneSetDescs

See Also
genePermuteScore

Examples
data(DEscore, package="SeqGSEA")
data(DSscore, package="SeqGSEA")
# linear combination with weight for DE 0.3
gene.score <- geneScore(DEscore, DSscore, method="linear", DEweight = 0.3)
# DE only analysis
gene.score <- geneScore(DEscore, DEweight = 1)

geneSetDescs

Get the descriptions of gene sets in a SeqGeneSet object

Description

Get the descriptions of gene sets in a SeqGeneSet object

Usage
geneSetDescs(GS)

Arguments

GS a SeqGeneSet object.

Details

Gene sets with size less than GSSizeMin or more than GSSizeMax are not included.

Value

A vector of descriptions of each gene set in the SeqGeneSet object.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also
geneSetNames, geneSetSize, SeqGeneSet-class, loadGenesets

Examples
data(GS_example, package="SeqGSEA")
geneSetDescs(GS_example)
geneSetNames

Get the names of gene set in a SeqGeneSet object

Description

Get the names of gene set in a SeqGeneSet object

Usage

geneSetNames(GS)

Arguments

GS       a SeqGeneSet object.

Details

Gene sets with size less than GSSizeMin or more than GSSizeMax are not included.

Value

A vector of gene set names in this SeqGeneSet object.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

geneSetDescs, geneSetSize, SeqGeneSet-class, loadGenesets

Examples

data(GS_example, package="SeqGSEA")
geneSetNames(GS_example)
**geneSetSize**

*Get the numbers of genes in each gene set in a SeqGeneSet object*

**Description**

Get the numbers of genes in each gene set in a SeqGeneSet object.

**Usage**

```
geneSetSize(GS)
```

**Arguments**

- `GS` a SeqGeneSet object.

**Details**

Gene sets with size less than `GSSizeMin` or more than `GSSizeMax` are not included.

**Value**

A vector of integers indicating the number of genes in each gene set in this SeqGeneSet object.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

`geneSetNames`, `geneSetDescs`, `SeqGeneSet-class`, `loadGenesets`

**Examples**

```r
data(GS_example, package="SeqGSEA")
geneSetSize(GS_example)
```
geneTestability  

Description

This function is to determine each gene’s testability. A gene is testable if at least one of its exons are testable.

Usage

geneTestability(RCS)

Arguments

RCS  
a ReadCountSet object after exon testability checked, usually the output of exonTestability.

Details

This result can applied to filter out genes not expressed.

Value

A logical vector indicating which genes are testable, i.e., having at least one exon testable.

Note

Please run exonTestability before run this function.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

exonTestability, subsetByGenes

Examples

data(RCS_example, package="SeqGSEA")
RCS_example <- exonTestability(RCS_example, cutoff=5)
geneTestable <- geneTestability(RCS_example)
head(geneTestable)
genpermuteMat

Generate permutation matrix

Description
Generate permutation matrix from ReadCountSet objects or from label vectors.

Usage

genpermuteMat(obj, times = 1000, seed = NULL)

Arguments

obj  
a ReadCountSet object or a label vector. This function needs the original sample label information to generate permutation matrix.
times  
an integer indication the times of permutation.
seed  
an integer or NULL, to produce the random seed (an integer vector) for generating random permutation matrix: the same seed generates the same permutation matrix, which is introduced for reproducibility.

Value
A sample label shuffled matrix, rows corresponding to samples and columns for each permutation.

Author(s)
Xi Wang, xi.wang@newcastle.edu.au

See Also
DSpermute4GSEA, DENBStatPermut4GSEA

Examples

data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10, seed=0)
RCS_example <- exonTestability(RCS_example)
RCS_example <- DSpermute4GSEA(RCS_example, permuteMat)
getGeneCount

Calculate read counts of genes from a ReadCountSet object

Description

Calculate read counts of genes from a ReadCountSet object

Usage

getGeneCount(RCS)

Arguments

RCS a ReadCountSet object

Details

This function can be used to get gene read counts from exon read counts.

Value

a matrix of gene read counts for each gene (row) and each sample (col).

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

loadExonCountData, runDESeq

Examples

data(RCS_example, package="SeqGSEA")
geneCounts <- getGeneCount(RCS_example)
GSEAresultTable

Description

Form a table for GSEA results.

Usage

GSEAresultTable(gene.set, GSDesc = FALSE)

Arguments

gene.set  a SeqGeneSet object after running GSEnrichAnalyze.
GSDesc    logical indicating whether to output gene set descriptions. default: FALSE

Value

A data frame containing columns of GSName, GSSize, ES, ES.pos, pval, FDR, and FWER.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

GSEnrichAnalyze, topGeneSets

Examples

data(DEscore, package="SeqGSEA")
data(DSscore, package="SeqGSEA")
gene.score <- geneScore(DEscore, DSscore, method="linear", DEweight = 0.3)
data(DEscore.perm, package="SeqGSEA")
data(DSscore.perm, package="SeqGSEA")
gene.score.perm <- genePermuteScore(DEscore.perm, DSscore.perm, method="linear", DEweight=0.3)
data(GS_example, package="SeqGSEA")
GS_example <- GSEnrichAnalyze(GS_example, gene.score, gene.score.perm)
head(GSEAresultTable(GS_example))
GSEnrichAnalyze  

Main function of gene set enrichment analysis

Description
The main function of gene set enrichment analysis

Usage
GSEnrichAnalyze(gene.set, gene.score, gene.score.perm, weighted.type = 1)

Arguments
- **gene.set**: a SeqGeneSet object.
- **gene.score**: a vector of integrated gene scores in the same order as genes listed in the geneList slot of gene.set.
- **gene.score.perm**: a matrix of integrated gene scores on permutation data sets; row: genes; col: permutation.
- **weighted.type**: weight type for gene scores; default: 1.

Value
A SeqGeneSet object with many slots updated, such as GSEA.ES and GSEA.pval.

Author(s)
Xi Wang, xi.wang@newcastle.edu.au

References

See Also
normES, signifES

Examples
```
data(DEscore, package="SeqGSEA")
data(DSscore, package="SeqGSEA")
gene.score <- geneScore(DEscore, DSscore, method="linear", DEweight = 0.3)
data(DEscore.perm, package="SeqGSEA")
data(DSscore.perm, package="SeqGSEA")
gene.score.perm <- genePermuteScore(DEscore.perm, DSscore.perm, method="linear", DEweight=0.3)
data(GS_example, package="SeqGSEA")
GS_example <- GSEnrichAnalyze(GS_example, gene.score, gene.score.perm)
topGeneSets(GS_example, 5)
```
Description

An exemplified SeqGeneSet object to demonstrate functions in the SeqGSEA package. This object was generated with collection #6 (C6) gene sets of the Molecular Signatures Database (MSigDB) v3.1.

Usage

data("GS_example")

References


label

Get the labels of samples in a ReadCountSet object

Description

Get the labels of samples in a ReadCountSet object

Usage

label(RCS)

Arguments

RCS a ReadCountSet object

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

eNewReadCountSet

Examples

data(RCS_example, package="SeqGSEA")
label(RCS_example)
loadExonCountData  

Description

This function is used to load (sub-)exon count data. Exon count data can be got by the Python script `count_in_exons.py`.

Usage

loadExonCountData(case.files, control.files)

Arguments

case.files  
a character vector containing the exon count file names for case samples

control.files  
a character vector containing the exon count file names for control samples

Details

You may need the Python script `count_in_exons.py` (released with this package) to generate your exon count files from read mapping results (say BAM files). The detailed usage can be obtained by simply typing `python \path\to\count_in_exons.py`. Users can also use other scripts or software for exon read counting.

The format of the exon count file is:

```
GeneName1:001[tab]Count11
GeneName1:002[tab]Count12
...
GeneName1:00N[tab]Count1N
GeneName2:001[tab]Count21
...
```

Value

This function returns a ReadCountSet object.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

newReadCountSet, ReadCountSet-class
loadGenesets

Examples

library(SeqGSEA)
dat.dir = system.file("extdata", package="SeqGSEA", mustWork=TRUE)
case.pattern <- "^SC"
ctrl.pattern <- "^SN"
case.files <- dir(dat.dir, pattern=case.pattern, full.names = TRUE)
control.files <- dir(dat.dir, pattern=ctrl.pattern, full.names = TRUE)

## Not run:
RCS <- loadExonCountData(case.files, control.files)
RCS

## End(Not run)

loadGenesets

Load gene sets from files

Description

This function is to load annotation of gene sets from files. The files are in the format of Molecular Signatures Database (MSigDB), and those files can be downloaded at http://www.broadinstitute.org/gsea/msigdb/index.jsp.

Usage

loadGenesets(geneset.file, geneIDs, geneID.type = c("gene.symbol", "ensembl"),
genetsize.min = 5, genesetsize.max = 1000, singleCell = FALSE)

Arguments

geneset.file the file containing the gene set annotation.
geneIDs gene IDs that have expression values in the studied data set.
geneID.type indicating the type of gene IDs, gene symbol or ensembl gene IDs.
genetsize.min the minimum number of genes in a gene set that will be treated in the analysis.
genetsize.max the maximum number of genes in a gene set that will be treated in the analysis.
singleCell logical, whether to creat a SeqGeneSet object for scGSEA.

Details

TBA

Value

A SeqGeneSet object.
newGeneSets

Initialize a new SeqGeneSet object

Description

This is an internal function to generate a new SeqGeneSet object.

Usage

```r
newGeneSets(GS, GSNames, GSDescs, geneList, scGSEA = FALSE,
            name = NA_character_, sourceFile = NA_character_,
            GSSizeMin = 5, GSSizeMax = 1000)
```

Arguments

- **GS**
  - a list, each element is an integer vector, indicating the indexes of genes in each gene set. See Details below.
- **GSNames**
  - a character string vector, each is the name of each gene set.
- **GSDescs**
  - a character string vector, each is the description of each gene set.
- **geneList**
  - a character string vector of gene IDs. See Details below.
- **scGSEA**
  - logical, if this object used for scGSEA.
- **name**
  - the name of this category of gene sets.
- **sourceFile**
  - the source file name of this category of gene sets.
- **GSSizeMin**
  - the minimum number of genes in a gene set to be analyzed. Default: 5
- **GSSizeMax**
  - the maximum number of genes in a gene set to be analyzed. Default: 1000
newReadCountSet

Generate a new ReadCountSet object

Description

This is a internal function to generate a new ReadCountSet object.

Usage

newReadCountSet(readCounts, exonIDs, geneIDs)

Arguments

readCounts  a data frame, read counts for each exon of each samples. Must have colnames, which indicate the label of samples.

exonIDs     a character vector indicating exon IDs.

geneIDs     a character vector indicating gene IDs.

Value

A object of the ReadCountSet class.
normES

Normalize enrichment scores

Description

This is an internal function to normalize enrichment scores. For advanced users only.

Usage

normES(gene.set)

Arguments

gene.set a SeqGeneSet object after running calES and calES.perm.

Value

A SeqGeneSet object with ES scores normalized.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

GSEnrichAnalyze, signifES
**normFactor**

Get normalization factors for normalization DE or DS scores

**Description**

Get normalization factors from permutation scores for normalization DE or DS scores

**Usage**

```r	normFactor(permStat)
```

**Arguments**

- `permStat` a matrix of NB-statistics from permutation data sets, with row corresponding to genes and columns to permutations.

**Value**

A vector of normalization factors, each for one gene.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**References**


**See Also**

- `scoreNormalization`

**Examples**

```r
data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10)
RCS_example <- exonTestability(RCS_example)
RCS_example <- estiExonNBstat(RCS_example)
RCS_example <- estiGeneNBstat(RCS_example)
RCS_example <- DSpermute4GSEA(RCS_example, permuteMat)
## (not run)
DSscore.normFac <- normFactor(RCS_example@permute_NBstat_gene)
DSscore <- scoreNormalization(RCS_example@featureData_gene$NBstat, DSScore.normFac)
DSscore.perm <- scoreNormalization(RCS_example@permute_NBstat_gene, DSScore.normFac)
## End (not run)
```
plotES  
Plot the distribution of enrichment scores

Description
This function is to plot the distribution of enrichment scores, with comparison with permutation enrichment scores.

Usage
plotES(gene.set, pdf = NULL)

Arguments
- gene.set: a SeqGeneSet object after running `GSEnrichAnalyze`.
- pdf: whether to save the plot to PDF file; if yes, provide the name of the PDF file.

Author(s)
Xi Wang, xi.wang@newcastle.edu.au

See Also
`GSEnrichAnalyze`, `plotSigGeneSet`

Examples
```r
data(DEscore, package="SeqGSEA")
data(DSscore, package="SeqGSEA")
gene.score <- geneScore(DEscore, DSscore, method="linear", DEweight = 0.3)
data(DEscore.perm, package="SeqGSEA")
data(DSscore.perm, package="SeqGSEA")
gene.score.perm <- genePermuteScore(DEscore.perm, DSscore.perm, method="linear", DEweight=0.3)
data(GS_example, package="SeqGSEA")
GS_example <- GSEnrichAnalyze(GS_example, gene.score, gene.score.perm)
plotES(GS_example)
```

plotGeneScore  
Plot gene (DE/DS) scores

Description
This function is to plot gene scores, as well as DE scores and DS scores.
Usage

plotGeneScore(score, perm.score = NULL, pdf = NULL,
              main = c("Overall", "Expression", "Splicing"))

Arguments

score | the gene/DE/DS score vector.
perm.score | a matrix of the corresponding gene/DE/DS scores on the permutation data sets.
pdf | if a PDF file name provided, plot will be save to that file.
main | the key words representing the type of scores that will be shown in the plot main title.

Details

The plot shows the ranked scores from the largest to the smallest. Lines also show the maximum and average scores, values shown on the top left.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

Examples

data(DEscore, package="SeqGSEA")
plotGeneScore(DEscore, main="Expression")
data(DSscore, package="SeqGSEA")
gene.score <- geneScore(DEscore, DSscore, method="linear", DEweight = 0.3)
plotGeneScore(gene.score)

plotSig

Plot showing SeqGeneSet’s p-values/FDRs vs. NESs

Description

The function is to generate a plot of p-values (FDRs) versus normalized enrichment scores (NES). It also shows the distribution of p-values (FDRs) in this gene set category.

Usage

plotSig(gene.set, pdf = NULL)

Arguments

gene.set | a SeqGeneSet object after running GSEnrichAnalyze.
pdf | whether to save the plot to PDF file; if yes, provide the name of the PDF file.
Author(s)
Xi Wang, xi.wang@newcastle.edu.au

See Also
GSEnrichAnalyze, plotSigGeneSet

Examples

data(DEscore, package="SeqGSEA")
data(DSscore, package="SeqGSEA")
gene.score <- geneScore(DEscore, DSscore, method="linear", DEweight = 0.3)
data(DEscore.perm, package="SeqGSEA")
data(DSscore.perm, package="SeqGSEA")
gene.score.perm <- genePermuteScore(DEscore.perm, DSscore.perm, method="linear", DEweight=0.3)
data(GS_example, package="SeqGSEA")
GS_example <- GSEnrichAnalyze(GS_example, gene.score, gene.score.perm)
plotSig(GS_example)

plotSigGeneSet  Plot gene set details

Description
This function is to generate a two-panel plot showing detailed information of the gene set specified.
One panel is showing the running enrichment scores and the position where the ES appear. The other panel shows the significance level of the ES, comparing with permutation ESs.

Usage
plotSigGeneSet(gene.set, i, gene.score, pdf = NULL)

Arguments
gene.set a SeqGeneSet object after running GSEnrichAnalyze.
i the i-th gene set in the SeqGeneSet object. topGeneSets is useful to find the most significantly overrepresented gene set.
gene.score the gene score vector containing gene scores for each gene.
pdf whether to save the plot to PDF file; if yes, provide the name of the PDF file.

Details
See writeSigGeneSet, which writes the detailed gene set information to a file or to the screen.

Author(s)
Xi Wang, xi.wang@newcastle.edu.au
rankCombine

Description

Integration of differential expression and differential splice scores with a rank-based strategy, which simultaneously integrates observed scores and permutation scores using the same ranks.

Usage

rankCombine(DEscore, DSscore, DEscoreMat, DSscoreMat, DEweight = 0.5)

Arguments

- **DEscore**: differential expression scores, normalized.
- **DSscore**: differential splice scores, normalized.
- **DEscoreMat**: differential expression scores in permuted data sets, normalized.
- **DSscoreMat**: differential splice scores in permuted data sets, normalized.
- **DEweight**: any number between 0 and 1 (included), the weight of differential expression scores (so the weight for differential splice is (1-DEweight)).

Details

This integration method is also known as integration with global ranks. See Wang and Cairns (2013) for details.

Value

A list with two elements geneScore and genePermuteScore.

Examples

data(DEscore, package="SeqGSEA")
data(DSscore, package="SeqGSEA")
gene.score <- geneScore(DEscore, DSscore, method="linear", DEweight = 0.3)
data(DEscore.perm, package="SeqGSEA")
data(DSscore.perm, package="SeqGSEA")
gene.score.perm <- genePermuteScore(DEscore.perm, DSscore.perm, method="linear", DEweight=0.3)
data(GS_example, package="SeqGSEA")
GS_example <- GSEnrichAnalyze(GS_example, gene.score, gene.score.perm)
topGeneSets(GS_example, n=5)
plotSigGeneSet(GS_example, 9, gene.score) # 9th gene set is the most significant one.
Author(s)

Xi Wang, xi.wang@newcastle.edu.au

References


See Also
geneScore, genePermuteScore

Examples

```r
data(DEscore, package="SeqGSEA")
data(DSscore, package="SeqGSEA")
data(DEscore.perm, package="SeqGSEA")
data(DSscore.perm, package="SeqGSEA")
combine <- rankCombine(DEscore, DSscore, DEscore.perm, DSscore.perm, DEweight=0.3)
gene.score <- combine$geneScore
gene.score.perm <- combine$genePermuteScore
```

---

RCS_example  
ReadCountSet object example

Description

An exemplified ReadCountSet object to demonstrate functions in the SeqGSEA package. This object is comprised of 20 samples across 5,000 exons, a part of the prostate cancer RNA-Seq data set from Kannan et al (2011). Please note that the count data in this example object is incomplete.

Usage

```r
data("RCS_example")
```

References

ReadCountSet-class

Class "ReadCountSet"

Description
ReadCountSet class

Objects from the Class
Objects can be created by calls of the form newReadCountSet.

Slots
featureData_gene: Object of class "data.frame". Data for each genes.
permute_NBstat_exon: Object of class "matrix". NB statistics of exons on the permutation data sets.
permute_NBstat_gene: Object of class "matrix". NB statistics of genes on the permutation data sets.
assayData: Object of class "AssayData". The read count data.
phenoData: Object of class "AnnotatedDataFrame". Data for each samples.
featureData: Object of class "AnnotatedDataFrame". Data for each exons.
experimentData: Object of class "MIAXE". Experiment data.
annotation: Object of class "character". Not used.
protocolData: Object of class "AnnotatedDataFrame". Protocol information.
__classVersion__: Object of class "Versions". Version information.

Methods

counts Get counts from a ReadCountSet object. See counts.
counts<- Set counts to a ReadCountSet object. See counts.

Extends
Class "eSet", directly.

Author(s)
Xi Wang, xi.wang@newcastle.edu.au

References
runDESeq

See Also

newReadCountSet, loadExonCountData, exonID, geneID, counts-methods, label, subsetByGenes

Examples

showClass("ReadCountSet")

data(RCS_example, package="SegGSEA")
geneCounts <- getGeneCount(RCS_example)
label <- label(RCS_example)
.dds <- runDESeq(geneCounts, label)

Description

This function provides a wrapper to run DESeq for differential expression analysis. It includes two steps, DESeq::estimateSizeFactors and DESeq::estimateDispersions.

Usage

runDESeq(geneCounts, label)

Arguments

geneCounts a matrix containing read counts for each gene, can be the output of getGeneCount.
label the sample classification labels.

Value

A DESeqDataSet object with size factors and dispersion parameters been estimated.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

References


See Also

geneCounts, DENBTest, DENBStat4GSEA

Examples

data(RCS_example, package="SegGSEA")
geneCounts <- getGeneCount(RCS_example)
label <- label(RCS_example)
.dds <- runDESeq(geneCounts, label)
runSeqGSEA

An all-in function that allows end users to apply SeqGSEA to their data with one step.

Description

This function provides typical SeqGSEA analysis pipelines for end users to apply the SeqGSEA method in the easiest fashion. It assumes the pipelines start with exon reads counts, even for the DE-only analysis. Users should specify their file locations and a few parameters before running this pipeline.

It allows DE-only analysis, which will skip the DS analysis portion, and it also allows users to try different weights in integrating DE and DS scores, which will save time in computing the DE and DS scores.

The function returns a list of SeqGSEA analysis results in the format of `GSEAresultTable`, and generates a few plots and writes a few files, whose name prefix can be specified. The output files will either be in PDF format or TXT format, and generated by `plotGeneScore`, `writeScores`, `plotES`, `plotSig`, `plotSigGeneSet`, and `writeSigGeneSet`.

Usage

```
runSeqGSEA(data.dir, case.pattern, ctrl.pattern, geneset.file, output.prefix, topGS=10,
        geneID.type=c("gene.symbol", "ensembl"), nCores=1, perm.times=1000, seed=NULL,
        minExonReadCount=5, integrationMethod=c("linear", "quadratic", "rank"),
        DEweight=c(0.5), DEonly=FALSE, minGSsize=5, maxGSsize=1000, GSEA.WeightedType=1)
```

Arguments

data.dir a character vector, the path to your count data directory.

case.pattern a character vector, the unique pattern in the file names of case samples. E.g, if file names starting with "SC", the pattern writes "^SC".

ctrl.pattern a character vector, the unique pattern in the file names of control samples.


output.prefix a character vector, the path with prefix for output files.

topGS an integer, this number of top ranked gene sets will be output with details; if geneset.file contains less than this number of gene sets, all gene sets’ result details will be output. Default: 10.

geneID.type the gene ID type in geneset.file. Currently only support "gene.symbol" and "ensembl". Default: gene.symbol.

nCores an integer. The number of cores for running SeqGSEA. Default: 1

perm.times an integer. The number of times for permutation, which will be used for normalizing DE and DS scores and for GSEA significance analysis. Recommended values are greater than 1000. Default: 1000.
seed

an integer or NULL, used for setting the seeds to generate random numbers. The same seed will guarantee the same analysis results given by SeqGSEA. Default: NULL.

minExonReadCount

an integer. An exon with total read count across all samples less than this number will be marked as untestable and be excluded in SeqGSEA analysis. Default: 5.

integrationMethod

one of the three integration methods for DE and DS score integration: linear, quadratic, or rank. Default: linear.

DEweight

a real number between 0 and 1 OR a vector of those. Each number is the DE weight in DE and DS integration. If using a vector of real numbers, SeqGSEA will run with each of them individually. Default: 0.5.

DEonly

logical, whether to run SeqGSEA only considering DE. Default: FALSE

minGSsize

an integer. The minimum gene set size: gene sets with genes less than this number will be skipped. Default: 5.

maxGSsize

an integer. The maximum gene set size: gene sets with genes greater than this number will be skipped. Default: 1000.

GSEA.WeightedType

the weight type of the main GSEA algorithm, can be 0 (unweighted = Kolmogorov-Smirnov), 1 (weighted), and 2 (over-weighted). Default: 1. It is recommended not to change it.

Value

A list of SeqGSEA analysis results in the format of `GSEAresultTable`, which allows users for meta-analysis.

Author(s)

Xi Wang, xi.wang@mdc-berlin.de

References


See Also

`GSEAresultTable`, `geneScore`, `GSEnrichAnalyze`

Examples

```r
### Initialization ###
# input file location and pattern
data.dir <- system.file("extdata", package="SeqGSEA", mustWork=TRUE)
case.pattern <- "^SC" # file name starting with "SC"
ctrl.pattern <- "^SN" # file name starting with "SN"
# gene set file and type
```
geneset.file <- system.file("extdata", "gs_symb.txt", package="SeqGSEA", mustWork=TRUE)
geneID.type <- "ensembl"
# output file prefix
output.prefix <- "SeqGSEAexample"
# analysis parameters
nCores <- 1
perm.times <- 10
DEonly <- FALSE
DEweight <- c(0.2, 0.5, 0.8) # a vector for different weights
integrationMethod <- "linear"

### one step SeqGSEA running ###
# Caution: if running the following command line, it will generate many files in your working directory
## Not run:
runSeqGSEA(data.dir=data.dir, case.pattern=case.pattern, ctrl.pattern=ctrl.pattern,
    geneset.file=geneset.file, geneID.type=geneID.type, output.prefix=output.prefix,
    nCores=nCores, perm.times=perm.times, integrationMethod=integrationMethod,
    DEonly=DEonly, DEweight=DEweight)
## End(Not run)

## scoreNormalization

### Normalization of DE/DS scores

**Description**
Normalization of DE/DS scores or permutation DE/DS scores.

**Usage**
scoreNormalization(scores, norm.factor)

**Arguments**

- `scores`: a vector (a nX1 matrix) of a matrix of scores, rows corresponding to genes and columns corresponding to a study or permutation.
- `norm.factor`: normalization factor, output of the function `normFactor`.

**Value**
A normalized vector or matrix depending on the input: with the same dimensions as the input.

**Author(s)**
Xi Wang, xi.wang@newcastle.edu.au
References


See Also

normFactor

Examples

data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10)
RCS_example <- exonTestability(RCS_example)
RCS_example <- estiExonNBstat(RCS_example)
RCS_example <- estiGeneNBstat(RCS_example)
RCS_example <- DSpermute4GSEA(RCS_example, permuteMat)
## (not run)
DSscore.normFac <- normFactor(RCS_example@permute_NBstat_gene)
DSscore <- scoreNormalization(RCS_example@featureData_gene$NBstat, DSscore.normFac)
DSscore.perm <- scoreNormalization(RCS_example@permute_NBstat_gene, DSscore.normFac)
## End (not run)

SeqGeneSet-class

Class "SeqGeneSet"

Description

SeqGeneSet class

Objects from the Class

Objects can be created by calls of the function newGeneSets.

Slots

name: Object of class "character" the name of this gene set category
sourceFile: Object of class "character" the source file of gene set category
geneList: Object of class "character" the gene ID list indicating genes involved in this GSEA
GS: Object of class "list" a list of gene indexes corresponding to geneList, each element in the list indicating which genes are in each gene set of this SeqGeneSet object
GSNames: Object of class "character". Gene set names.
GSDescs: Object of class "character". Gene set descriptions.
GSSize: Object of class "numeric". Gene set sizes.
GSSizeMin: Object of class "numeric". The minimum gene set size to be analyzed.
GSSizeMax: Object of class "numeric". The maximum gene set size to be analyzed.


Methods

[ Get a sub-list of gene sets, and return a SeqGeneSet object.

  show Show basic information of the SeqGeneSet object.

Author(s)

  Xi Wang, xi.wang@newcastle.edu.au

References


See Also

  newGeneSets, size, geneSetNames, geneSetDescs, geneSetSize

Examples

  showClass("SeqGeneSet")
**signifES**  
*Calculate significance of ESs*

**Description**  
The is an internal function to calculate significance of ESs of each gene set. For advanced users only.

**Usage**  
`signifES(gene.set)`

**Arguments**  
gene.set a GeneSet object after running `normES`.

**Value**  
A SeqGeneSet object with gene set enrichment significance metrics calculated.

**Author(s)**  
Xi Wang, xi.wang@newcastle.edu.au

**See Also**  
`GSEnrichAnalyze`, `normES`

---

**size**  
*Number of gene sets in a SeqGeneSet object*

**Description**  
This function to get the number of gene sets in a SeqGeneSet object.

**Usage**  
`sise(GS)`

**Arguments**  
GS an object of class SeqGeneSet.

**Details**  
Gene sets with size less than GSSizeMin or more than GSSizeMax are not included.
**subsetByGenes**

**Value**

The number of gene sets in this SeqGeneSet object.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

`SeqGeneSet-class`, `loadGenesets`

**Examples**

```r
data(GS_example, package="SeqGSEA")
size(GS_example)
```

---

**subsetByGenes**  
*Get a new ReadCountSet with specified gene IDs.*

**Description**

Get a new ReadCountSet with specified gene IDs.

**Usage**

`subsetByGenes(RCS, genes)`

**Arguments**

- `RCS`: a ReadCountSet object.
- `genes`: a list of gene IDs.

**Value**

This function returns a new ReadCountSet object, with changes in slots `assayData`, `featureData`, `featureData_gene`, and `permute_NBstat_exon` and `permute_NBstat_gene` if they have been calculated.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

`newReadCountSet`, `ReadCountSet`
Examples

```r
data(RCS_example, package="SeqGSEA")
RCS_example
genes <- c("ENSG00000000938", "ENSG00000000005")
RCS_sub <- subsetByGenes(RCS_example, genes)
RCS_sub
```

---

**topDEGenes**

*Extract top differentially expressed genes.*

**Description**

This function is to extract top n differentially expressed genes, ranked by either DESeq p-values, DESeq adjusted p-values, permutation p-values, permutation adjusted p-values, or NB-statistics.

**Usage**

```r
topDEGenes(DEGres, n = 20,
    sortBy = c("padj", "pval", "perm.pval", "perm.padj", "NBstat", "foldChange")
```

**Arguments**

- `DEGres`: DE analysis results.
- `n`: the number of top DE genes.
- `sortBy`: indicating which method to rank genes.

**Details**

If the `sortBy` method is not among the column names, the function will result in an error.

**Value**

A table for top n DE genes with significance metrics.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

topDSGenes, topDSExons
**Examples**

```r
data(RCS_example, package="SeqGSEA")
geneCounts <- getGeneCount(RCS_example)
label <- label(RCS_example)
DEG <- runDESeq(geneCounts, label)
permuteMat <- genpermuteMat(RCS_example, times=10)
DEGres <- DENBTest(DEG)
DEpermNBstat <- DENBStatPermut4GSEA(DEG, permuteMat)
DEGres <- DEpermutePval(DEGres, DEpermNBstat)
```

```r
topDEGenes(DEGres, n = 10, sortBy = "NBstat")
```

---

**Description**

This function is to extract top n differentially spliced exons, ranked by p-values or NB-stats.

**Usage**

```r
topDSExons(RCS, n = 20, sortBy = c("pvalue", "NBstat"))
```

**Arguments**

- **RCS**
  - a ReadCountSet object after running `DSpermutePval`.
- **n**
  - the number of top genes.
- **sortBy**
  - indicating whether p-value or NBstat to be used for ranking genes.

**Value**

A table for top n exons. Columns include: geneID, exonID, testable, NBstat, pvalue, padjust, and meanCounts.

**Author(s)**

Xi Wang, xi.wang@newcastle.edu.au

**See Also**

`topDSGenes`, `DSpermutePval`

**Examples**

```r
data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10)
RCS_example <- exonTestability(RCS_example)
RCS_example <- estiExonNBstat(RCS_example)
RCS_example <- estiGeneNBstat(RCS_example)
RCS_example <- DSpermutePval(RCS_example, permuteMat)
topDSExons(RCS_example, 10, "NB")
```
Extract top differentially spliced genes

Description

This function to extract top n differentially spliced genes, ranked by p-values or NBstats.

Usage

topDSGenes(RCS, n = 20, sortBy = c("pvalue", "NBstat"))

Arguments

RCS  
a ReadCountSet object after running DSpermutePval.

n  
the number of top genes.

sortBy  
indicating whether p-value or NBstat to be used for ranking genes.

Value

A table for top n genes. Columns include: geneID, NBstat, pvalue, and padjust.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

topDSExons, DSpermutePval

Examples

data(RCS_example, package="SeqGSEA")
permuteMat <- genpermuteMat(RCS_example, times=10)
RCS_example <- exonTestability(RCS_example)
RCS_example <- estiExonNBstat(RCS_example)
RCS_example <- estiGeneNBstat(RCS_example)
RCS_example <- DSpermutePval(RCS_example, permuteMat)
topDSGenes(RCS_example, 10, "NB")
topGeneSets

Extract top significant gene sets

Description
This function is to extract n top significant gene sets overrepresented in the samples studied, ranked by FDR, p-values, or FWER.

Usage
topGeneSets(gene.set, n = 20, sortBy = c("FDR", "pvalue", "FWER"), GSDesc = FALSE)

Arguments
gene.set an object of class SeqGeneSet after GSEA runs.
n the number of top gene sets.
sortBy indicating which method to rank gene sets.
GSDesc logical indicating whether or not to output gene set descriptions.

Value
A data frame for top n gene sets detected with respect to the ranking method specified. Information includes: GSName, GSSize, ES, ES.pos, pval, FDR, and FWER.

Author(s)
Xi Wang, xi.wang@newcastle.edu.au

See Also
GSEnrichAnalyze, GSEAreultTable

Examples

data(DEscore, package="SeqGSEA")
data(DDscore, package="SeqGSEA")
gene.score <- geneScore(DEscore, DDScore, method="linear", DEweight = 0.3)
data(DEscore.perm, package="SeqGSEA")
data(DDscore.perm, package="SeqGSEA")
gene.score.perm <- genePermuteScore(DEscore.perm, DDScore.perm, method="linear", DEweight=0.3)
data(GS_example, package="SeqGSEA")
GS_example <- GSEnrichAnalyze(GS_example, gene.score, gene.score.perm)
topGeneSets(GS_example, n=5)
writeScores  Write DE/DS scores and gene scores

Description

This function is to write DE and DS scores, and optionally gene scores.

Usage

writeScores(DEscore, DSscore, geneScore=NULL, geneScoreAttr=NULL, file="")

Arguments

DEscore normalized DE scores.
DSscore normalized DS scores.
geneScore gene scores integrated from DE and DS scores.
geneScoreAttr the parameters for integrating DE and DS scores.
file output file name, if not specified print to screen.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

DEscore, geneScore

Examples

data(DEscore, package="SeqGSEA")
data(DSscore, package="SeqGSEA")
gene.score <- geneScore(DEscore, DSscore, method="linear", DEweight = 0.3)
writeScores(DEscore, DSscore) # without gene scores
writeScores(DEscore, DSscore, geneScore = gene.score,
            geneScoreAttr = "linear,0.3") # gene scores with attr.
writeSigGeneSet

Write gene set supporting information

Description

This function is to write the specified gene set (whose index is i) with significance information, including p-value and FDR, and gene scores for each gene in this set.

Usage

writeSigGeneSet(gene.set, i, gene.score, file = "")

Arguments

gene.set an object of class SeqGeneSet with GSEnrichAnalyze done.
i the i-th gene set in the SeqGeneSet object. topGeneSets is useful to find the most significantly overrepresented gene set.
gene.score the vector of gene scores for running GSEA.
file output file name, if not specified print to screen.

Details

See plotSigGeneSet, which shows graphic information of the gene set specified.

Author(s)

Xi Wang, xi.wang@newcastle.edu.au

See Also

GSEnrichAnalyze, topGeneSets, plotSigGeneSet

Examples

data(DEscore, package="SeqGSEA")
data(DSscore, package="SeqGSEA")
gene.score <- geneScore(DEscore, DSscore, method="linear", DEweight = 0.3)
data(DEscore.perm, package="SeqGSEA")
data(DSscore.perm, package="SeqGSEA")
gene.score.perm <- genePermuteScore(DEscore.perm, DSscore.perm, method="linear", DEweight=0.3)
data(GS_example, package="SeqGSEA")
GS_example <- GSEnrichAnalyze(GS_example, gene.score, gene.score.perm)
topGeneSets(GS_example, n=5)
writeSigGeneSet(GS_example, 9, gene.score) # 9th gene set is the most significant one.
Index

* classes
  ReadCountSet-class, 45
  SeqGeneSet-class, 50

* datasets
  DEscore, 13
  GS_example, 33
  RCS_example, 44
  [,SeqGeneSet,numeric,ANY,ANY-method (SeqGeneSet-class), 50
  [,SeqGeneSet,numeric-method (SeqGeneSet-class), 50
  calES, 4, 6, 7, 38
  calES.perm, 4, 6, 6, 38
  convertEnsembl2Symbol, 5, 7, 8
  convertSymbol2Ensembl, 5, 7, 8
  counts, 45
  counts (counts-methods), 9
  counts,ReadCountSet-method (counts-methods), 9
  counts-methods, 9
  counts<-,ReadCountSet,matrix-method (counts-methods), 9
  DENBStat4GSEA, 4, 9, 11–13, 46
  DENBStatPermut4GSEA, 4, 10, 10, 12–14, 29
  DENBTest, 4, 9, 10, 11, 13, 46
  DEpermutePval, 4, 10, 11, 12
  DEscore, 13, 58
  DSpermute4GSEA, 4, 14, 15, 29
  DSpermutePval, 4, 14, 15, 16, 17, 55, 56
  DResultExonTable, 5, 16, 17
  DScore (DEscore), 13
  eSet, 45
  estiExonNBstat, 4, 15, 18, 19
  estiGeneNBstat, 4, 15, 18, 19
  exonID, 3, 20, 22, 46
  exonID<-(exonID), 20
  exonTestability, 4, 14, 18, 20, 28
  geneID, 3, 20, 21, 46
  geneID<-(geneID), 21
  geneList, 22
  genePermuteScore, 4, 23, 25, 44
  geneScore, 4, 23, 24, 44, 48, 58
  geneSetDescs, 4, 25, 26, 27, 51
  geneSetNames, 4, 25, 26, 27, 51
  geneSetSize, 4, 25, 26, 27, 51
  geneTestability, 4, 21, 28
  genpermuteMat, 5, 11, 14, 15, 29
  getGeneCount, 4, 30, 46
  GS_example, 33
  GSEAresultTable, 5, 31, 47, 48, 57
  GSEnrichAnalyze, 4, 6, 7, 31, 32, 38, 40–43, 48, 52, 57, 59
  label, 3, 33, 46
  loadExonCountData, 4, 30, 34, 38, 46
  loadGenesets, 4, 22, 25–27, 35, 37, 53
  newGeneSets, 4, 36, 36, 50, 51
  newReadCountSet, 4, 20, 22, 33, 34, 37, 45, 46, 53
  normES, 4, 32, 38, 52
  normFactor, 4, 39, 49, 50
  plotES, 5, 40, 43, 47
  plotGeneScore, 5, 40, 47
  plotSig, 5, 41, 43, 47
  plotSigGeneSet, 5, 40, 42, 42, 47, 59
  rankCombine, 4, 43
  RCS_example, 44
  ReadCountSet, 3, 53
  ReadCountSet (ReadCountSet-class), 45
  ReadCountSet-class, 45
  runDESeq, 4, 10–13, 30, 46
  runSeqGSEA, 47

60
scoreNormalization, 4, 39, 49
SeqGeneSet (SeqGeneSet-class), 50
SeqGeneSet-class, 50
SeqGSEA (SeqGSEA-package), 3
SeqGSEA-package, 3
show, SeqGeneSet-method
(SeqGeneSet-class), 50
signifES, 5, 32, 38, 52
size, 4, 51, 52
subsetByGenes, 3, 28, 46, 53
topDEGenes, 5, 54
topDSExons, 5, 54, 55, 56
topDSGenes, 5, 54, 55, 56
topGeneSets, 5, 31, 42, 43, 57, 59
writeScores, 47, 58
writeSigGeneSet, 5, 42, 43, 47, 59