

# Package ‘fgsea’

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**Title** Fast Gene Set Enrichment Analysis

**Version** 1.35.8

**Description** The package implements an algorithm for fast gene set enrichment analysis. Using the fast algorithm allows to make more permutations and get more fine grained p-values, which allows to use accurate standart approaches to multiple hypothesis correction.

**biocViews** GeneExpression, DifferentialExpression, GeneSetEnrichment, Pathways

**Depends** R (>= 4.1)

**Imports** Rcpp, data.table, BiocParallel, stats, ggplot2 (>= 2.2.0), cowplot, grid, fastmatch, Matrix, scales, utils

**Suggests** testthat, knitr, rmarkdown, reactome.db, AnnotationDbi, parallel, org.Mm.eg.db, limma, GEOquery, msigdb, aggregation, Seurat

**License** MIT + file LICENCE

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**LinkingTo** Rcpp, BH

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**BugReports** <https://github.com/ctlab/fgsea/issues>

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## Contents

|                                            |           |
|--------------------------------------------|-----------|
| calcGseaStat . . . . .                     | 2         |
| calcGseaStatBatchCpp . . . . .             | 3         |
| collapsePathways . . . . .                 | 4         |
| collapsePathwaysGeseca . . . . .           | 5         |
| collapsePathwaysORA . . . . .              | 6         |
| exampleExpressionMatrix . . . . .          | 7         |
| examplePathways . . . . .                  | 7         |
| exampleRanks . . . . .                     | 7         |
| fgsea . . . . .                            | 8         |
| fgseaLabel . . . . .                       | 9         |
| fgseaMultilevel . . . . .                  | 10        |
| fgseaSimple . . . . .                      | 12        |
| fgseaSimpleImpl . . . . .                  | 13        |
| fora . . . . .                             | 14        |
| geseca . . . . .                           | 15        |
| gesecaSimple . . . . .                     | 17        |
| gmtPathways . . . . .                      | 18        |
| mapIdsList . . . . .                       | 18        |
| multilevelError . . . . .                  | 19        |
| multilevelImpl . . . . .                   | 20        |
| plotCoregulationProfile . . . . .          | 20        |
| plotCoregulationProfileImage . . . . .     | 21        |
| plotCoregulationProfileReduction . . . . . | 22        |
| plotCoregulationProfileSpatial . . . . .   | 23        |
| plotEnrichment . . . . .                   | 24        |
| plotEnrichmentData . . . . .               | 25        |
| plotGesecaTable . . . . .                  | 26        |
| plotGseaTable . . . . .                    | 27        |
| reactomePathways . . . . .                 | 28        |
| writeGmtPathways . . . . .                 | 29        |
| <b>Index</b>                               | <b>30</b> |

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|              |                                                              |
|--------------|--------------------------------------------------------------|
| calcGseaStat | <i>Calculates GSEA statistics for a given query gene set</i> |
|--------------|--------------------------------------------------------------|

---

### Description

Takes  $O(k \log k)$  time, where  $k$  is a size of ‘selectedSize’.

### Usage

```
calcGseaStat(
  stats,
  selectedStats,
  gseaParam = 1,
  returnAllExtremes = FALSE,
  returnLeadingEdge = FALSE,
  scoreType = c("std", "pos", "neg")
)
```

**Arguments**

|                   |                                                                                                    |
|-------------------|----------------------------------------------------------------------------------------------------|
| stats             | Named numeric vector with gene-level statistics sorted in decreasing order (order is not checked). |
| selectedStats     | Indexes of selected genes in the 'stats' array.                                                    |
| gseaParam         | GSEA weight parameter (0 is unweighted, suggested value is 1).                                     |
| returnAllExtremes | If TRUE return not only the most extreme point, but all of them. Can be used for enrichment plot   |
| returnLeadingEdge | If TRUE return also leading edge genes.                                                            |
| scoreType         | This parameter defines the GSEA score type. Possible options are ("std", "pos", "neg")             |

**Value**

Value of GSEA statistic if both returnAllExtremes and returnLeadingEdge are FALSE. Otherwise returns list with the following elements:

- res – value of GSEA statistic
- tops – vector of top peak values of cumulative enrichment statistic for each gene;
- bottoms – vector of bottom peak values of cumulative enrichment statistic for each gene;
- leadingGene – vector with indexes of leading edge genes that drive the enrichment, see [http://software.broadinstitute.org/gsea/doc/GSEAUserGuideTEXT.htm#\\_Running\\_a\\_Leading](http://software.broadinstitute.org/gsea/doc/GSEAUserGuideTEXT.htm#_Running_a_Leading).

**Examples**

```
data(exampleRanks)
data(examplePathways)
ranks <- sort(exampleRanks, decreasing=TRUE)
es <- calcGseaStat(ranks, na.omit(match(examplePathways[[1]], names(ranks))))
```

---

calcGseaStatBatchCpp    *Calculates GSEA statistic value for all gene sets in 'selectedStats' list.*

---

**Description**

Takes  $O(n + mK \log K)$  time, where  $n$  is the number of genes,  $m$  is the number of gene sets, and  $k$  is the mean gene set size.

**Usage**

```
calcGseaStatBatchCpp(stats, selectedGenes, geneRanks)
```

**Arguments**

|               |                                                                    |
|---------------|--------------------------------------------------------------------|
| stats         | Numeric vector of gene-level statistics sorted in decreasing order |
| selectedGenes | List of integer vector with integer gene IDs (from 1 to $n$ )      |
| geneRanks     | Integer vector of gene ranks                                       |

**Value**

Numeric vector of GSEA statistics of the same length as 'selectedGenes' list

---

|                  |                                                                |
|------------------|----------------------------------------------------------------|
| collapsePathways | <i>Collapse list of enriched pathways to independent ones.</i> |
|------------------|----------------------------------------------------------------|

---

## Description

Collapse list of enriched pathways to independent ones.

## Usage

```
collapsePathways(
  fgseaRes,
  pathways,
  stats,
  pval.threshold = 0.05,
  nperm = 10/pval.threshold,
  gseaParam = 1
)
```

## Arguments

|                |                                                                                                                                               |
|----------------|-----------------------------------------------------------------------------------------------------------------------------------------------|
| fgseaRes       | Table with results of running fgsea(), should be filtered by p-value, for example by selecting ones with padj < 0.01.                         |
| pathways       | List of pathways, should contain all the pathways present in 'fgseaRes'.                                                                      |
| stats          | Gene-level statistic values used for ranking, the same as in 'fgsea()'.                                                                       |
| pval.threshold | Two pathways are considered dependent when p-value of enrichment of one pathways on background of another is greater then 'pval.threshold'.   |
| nperm          | Number of permutations to test for independence, should be several times greater than '1/pval.threshold'. Default value: '10/pval.threshold'. |
| gseaParam      | GSEA parameter, same as for 'fgsea()'                                                                                                         |

## Value

Named list with two elements: 'mainPathways' containing IDs of pathways not reducable to each other, and 'parentPathways' with vector describing for all the pathways to which ones they can be reduced. For pathways from 'mainPathways' vector 'parentPathways' contains 'NA' values.

## Examples

```
data(examplePathways)
data(exampleRanks)
fgseaRes <- fgsea(examplePathways, exampleRanks, nperm=10000, maxSize=500)
collapsedPathways <- collapsePathways(fgseaRes[order(pval)][padj < 0.01],
                                     examplePathways, exampleRanks)
mainPathways <- fgseaRes[pathway %in% collapsedPathways$mainPathways][
  order(-NES), pathway]
```

---

collapsePathwaysGeseca

*Collapse list of enriched pathways to independent ones (GESECA version, highly experimental).*

---

## Description

Collapse list of enriched pathways to independent ones (GESECA version, highly experimental).

## Usage

```
collapsePathwaysGeseca(
  gesecaRes,
  pathways,
  E,
  center = TRUE,
  scale = FALSE,
  eps = min(c(1e-50, gesecaRes$pval)),
  checkDepth = 10,
  nproc = 0,
  BPPARAM = NULL
)
```

## Arguments

|            |                                                                                                                                                                                                     |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| gesecaRes  | Table with results of running geseca(), should be filtered by p-value, for example by selecting ones with padj < 0.01.                                                                              |
| pathways   | List of pathways, should contain all the pathways present in 'gesecaRes'.                                                                                                                           |
| E          | expression matrix, the same as in 'geseca()'.                                                                                                                                                       |
| center     | a logical value indicating whether the gene expression should be centered to have zero mean before the analysis takes place. The default is TRUE. The value is passed to <a href="#">scale</a> .    |
| scale      | a logical value indicating whether the gene expression should be scaled to have unit variance before the analysis takes place. The default is FALSE. The value is passed to <a href="#">scale</a> . |
| eps        | eps parameter for internal gesecaMultilevel runs. Default: min(c(1e-50, gesecaRes\$pval))                                                                                                           |
| checkDepth | how much pathways to check against                                                                                                                                                                  |
| nproc      | If not equal to zero sets BPPARAM to use nproc workers (default = 0).                                                                                                                               |
| BPPARAM    | Parallelization parameter used in bplapply.                                                                                                                                                         |

---

|                     |                                                                                                     |
|---------------------|-----------------------------------------------------------------------------------------------------|
| collapsePathwaysORA | <i>Collapse list of enriched pathways to independent ones. Version for ORA hypergeometric test.</i> |
|---------------------|-----------------------------------------------------------------------------------------------------|

---

## Description

Collapse list of enriched pathways to independent ones. Version for ORA hypergeometric test.

## Usage

```
collapsePathwaysORA(forRes, pathways, genes, universe, pval.threshold = 0.05)
```

## Arguments

|                |                                                                                                                                             |
|----------------|---------------------------------------------------------------------------------------------------------------------------------------------|
| forRes         | Table with results of running fgsea(), should be filtered by p-value, for example by selecting ones with $p_{adj} < 0.01$ .                 |
| pathways       | List of pathways, should contain all the pathways present in 'fgseaRes'.                                                                    |
| genes          | Set of query genes, same as in 'fora()'.                                                                                                    |
| universe       | A universe from which 'genes' were selected, same as in 'fora()'.                                                                           |
| pval.threshold | Two pathways are considered dependent when p-value of enrichment of one pathways on background of another is greater than 'pval.threshold'. |

## Value

Named list with two elements: 'mainPathways' containing IDs of pathways not reducible to each other, and 'parentPathways' with vector describing for all the pathways to which ones they can be reduced. For pathways from 'mainPathways' vector 'parentPathways' contains 'NA' values.

## Examples

```
data(examplePathways)
data(exampleRanks)
forRes <- fora(examplePathways, genes=tail(names(exampleRanks), 200), universe=names(exampleRanks))
collapsedPathways <- collapsePathwaysORA(forRes[order(pval)][pval < 0.01],
                                         examplePathways,
                                         genes=tail(names(exampleRanks), 200),
                                         universe=names(exampleRanks))

mainPathways <- forRes[pathway %in% collapsedPathways$mainPathways][
  order(pval), pathway]
```

---

`exampleExpressionMatrix`*Example of expression values obtained for GSE14308.*

---

**Description**

Expression data was obtained by preprocessing the GSE14308 dataset. For the matrix of gene expression value, the following steps were performed:

- expression values were log2-scaled
- quantile-type normalization was performed between arrays
- rows were collapsed by 'ENTREZID'
- rows were sorted in descending order by mean expression value per gene
- finally, top-10\_000 genes were taken

The exact script is available as `system.file("gen_gse14308_expression_matrix.R", package="fgsea")`

---

`examplePathways`*Example list of mouse Reactome pathways.*

---

**Description**

The list was obtained by selecting all the pathways from 'reactome.db' package that contain mouse genes. The exact script is available as `system.file("gen_reactome_pathways.R", package="fgsea")`

---

`exampleRanks`*Example vector of gene-level statistics obtained for Th1 polarization.*

---

**Description**

The data were obtained by doing differential expression between Naive and Th1-activated states for GEO dataset GSE14308. The exact script is available as `system.file("gen_gene_ranks.R", package="fgsea")`

---

|       |                                                                           |
|-------|---------------------------------------------------------------------------|
| fgsea | <i>Wrapper to run methods for preranked gene set enrichment analysis.</i> |
|-------|---------------------------------------------------------------------------|

---

## Description

This function provide an interface to two existing functions: [fgseaSimple](#), [fgseaMultilevel](#). By default, the [fgseaMultilevel](#) function is used for analysis. For compatibility with the previous implementation you can pass the ‘nperm’ argument to the function.

## Usage

```
fgsea(
  pathways,
  stats,
  minSize = 1,
  maxSize = length(stats) - 1,
  gseaParam = 1,
  ...
)
```

## Arguments

|           |                                                                                                |
|-----------|------------------------------------------------------------------------------------------------|
| pathways  | List of gene sets to check.                                                                    |
| stats     | Named vector of gene-level stats. Names should be the same as in ‘pathways’                    |
| minSize   | Minimal size of a gene set to test. All pathways below the threshold are excluded.             |
| maxSize   | Maximal size of a gene set to test. All pathways above the threshold are excluded.             |
| gseaParam | GSEA parameter value, all gene-level statis are raised to the power of ‘gseaParam’             |
| ...       | optional arguments for functions <a href="#">fgseaSimple</a> , <a href="#">fgseaMultilevel</a> |

## Value

A table with GSEA results. Each row corresponds to a tested pathway.

## Examples

```
data(examplePathways)
data(exampleRanks)
fgseaRes <- fgsea(examplePathways, exampleRanks, maxSize=500)
# Testing only one pathway is implemented in a more efficient manner
fgseaRes1 <- fgsea(examplePathways[1], exampleRanks)
```

---

|            |                                                           |
|------------|-----------------------------------------------------------|
| fgseaLabel | <i>Runs label-permuring gene set enrichment analysis.</i> |
|------------|-----------------------------------------------------------|

---

## Description

Runs label-permuring gene set enrichment analysis.

## Usage

```
fgseaLabel(
  pathways,
  mat,
  labels,
  nperm,
  minSize = 1,
  maxSize = nrow(mat) - 1,
  nproc = 0,
  gseaParam = 1,
  BPPARAM = NULL
)
```

## Arguments

|           |                                                                                                                                                                           |
|-----------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathways  | List of gene sets to check.                                                                                                                                               |
| mat       | Gene expression matrix. Row name should be the same as in 'pathways'                                                                                                      |
| labels    | Numeric vector of labels for the correlation score of the same length as the number of columns in 'mat'                                                                   |
| nperm     | Number of permutations to do. Minimal possible nominal p-value is about 1/nperm                                                                                           |
| minSize   | Minimal size of a gene set to test. All pathways below the threshold are excluded.                                                                                        |
| maxSize   | Maximal size of a gene set to test. All pathways above the threshold are excluded.                                                                                        |
| nproc     | If not equal to zero sets BPPARAM to use nproc workers (default = 0).                                                                                                     |
| gseaParam | GSEA parameter value, all gene-level statis are raised to the power of 'gseaParam' before calculation of GSEA enrichment scores.                                          |
| BPPARAM   | Parallelization parameter used in bplapply. Can be used to specify cluster to run. If not initialized explicitly or by setting 'nproc' default value 'bpparam()' is used. |

## Value

A table with GSEA results. Each row corresponds to a tested pathway. The columns are the following:

- pathway – name of the pathway as in 'names(pathway)';
- pval – an enrichment p-value;
- padj – a BH-adjusted p-value;

- ES – enrichment score, same as in Broad GSEA implementation;
- NES – enrichment score normalized to mean enrichment of random samples of the same size;
- nMoreExtreme – a number of times a random gene set had a more extreme enrichment score value;
- size – size of the pathway after removing genes not present in 'names(stats)'.
- leadingEdge – vector with indexes of leading edge genes that drive the enrichment, see [http://software.broadinstitute.org/gsea/doc/GSEAUserGuideTEXT.htm#\\_Running\\_a\\_Leading](http://software.broadinstitute.org/gsea/doc/GSEAUserGuideTEXT.htm#_Running_a_Leading).

## Examples

```
library(limma)
library(GEOquery)
es <- getGEO("GSE19429", AnnotGPL = TRUE)[[1]]
exprs(es) <- normalizeBetweenArrays(log2(exprs(es)+1), method="quantile")
es <- es[!grepl("///", fData(es)$`Gene ID`), ]
es <- es[fData(es)$`Gene ID` != "", ]
es <- es[order(apply(exprs(es), 1, mean), decreasing=TRUE), ]
es <- es[!duplicated(fData(es)$`Gene ID`), ]
rownames(es) <- fData(es)$`Gene ID`

pathways <- reactomePathways(rownames(es))
mat <- exprs(es)
labels <- as.numeric(as.factor(gsub(".*", "", es$title)))
fgseaRes <- fgseaLabel(pathways, mat, labels, nperm = 1000, minSize = 15, maxSize = 500)
```

---

fgseaMultilevel

*Runs preranked gene set enrichment analysis.*


---

## Description

This feature is based on the adaptive multilevel splitting Monte Carlo approach. This allows us to exceed the results of simple sampling and calculate arbitrarily small P-values.

## Usage

```
fgseaMultilevel(
  pathways,
  stats,
  sampleSize = 101,
  minSize = 1,
  maxSize = length(stats) - 1,
  eps = 1e-50,
  scoreType = c("std", "pos", "neg"),
  nproc = 0,
  gseaParam = 1,
  BPPARAM = NULL,
  nPermSimple = 1000,
  absEps = NULL
)
```

**Arguments**

|             |                                                                                                                                                                                                                                                                                                                                          |
|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathways    | List of gene sets to check.                                                                                                                                                                                                                                                                                                              |
| stats       | Named vector of gene-level stats. Names should be the same as in 'pathways'                                                                                                                                                                                                                                                              |
| sampleSize  | The size of a random set of genes which in turn has size = pathwaySize                                                                                                                                                                                                                                                                   |
| minSize     | Minimal size of a gene set to test. All pathways below the threshold are excluded.                                                                                                                                                                                                                                                       |
| maxSize     | Maximal size of a gene set to test. All pathways above the threshold are excluded.                                                                                                                                                                                                                                                       |
| eps         | This parameter sets the boundary for calculating the p value.                                                                                                                                                                                                                                                                            |
| scoreType   | This parameter defines the GSEA score type. Possible options are ("std", "pos", "neg"). By default ("std") the enrichment score is computed as in the original GSEA. The "pos" and "neg" score types are intended to be used for one-tailed tests (i.e. when one is interested only in positive ("pos") or negative ("neg") enrichment). |
| nproc       | If not equal to zero sets BPPARAM to use nproc workers (default = 0).                                                                                                                                                                                                                                                                    |
| gseaParam   | GSEA parameter value, all gene-level statis are raised to the power of 'gsea-Param' before calculation of GSEA enrichment scores.                                                                                                                                                                                                        |
| BPPARAM     | Parallelization parameter used in bplapply. Can be used to specify cluster to run. If not initialized explicitly or by setting 'nproc' default value 'bpparam()' is used.                                                                                                                                                                |
| nPermSimple | Number of permutations in the simple fgsea implementation for preliminary estimation of P-values.                                                                                                                                                                                                                                        |
| absEps      | deprecated, use 'eps' parameter instead                                                                                                                                                                                                                                                                                                  |

**Value**

A table with GSEA results. Each row corresponds to a tested pathway. The columns are the following

- pathway – name of the pathway as in 'names(pathway)';
- pval – an enrichment p-value;
- padj – a BH-adjusted p-value;
- log2err – the expected error for the standard deviation of the P-value logarithm.
- ES – enrichment score, same as in Broad GSEA implementation;
- NES – enrichment score normalized to mean enrichment of random samples of the same size;
- size – size of the pathway after removing genes not present in 'names(stats)';
- leadingEdge – vector with indexes of leading edge genes that drive the enrichment, see [http://software.broadinstitute.org/gsea/doc/GSEAUserGuideTEXT.htm#\\_Running\\_a\\_Leading](http://software.broadinstitute.org/gsea/doc/GSEAUserGuideTEXT.htm#_Running_a_Leading).

**Examples**

```
data(examplePathways)
data(exampleRanks)
fgseaMultilevelRes <- fgseaMultilevel(examplePathways, exampleRanks, maxSize=500)
```

fgseaSimple

*Runs preranked gene set enrichment analysis.***Description**

The function takes about  $O(nk^{3/2})$  time, where  $n$  is number of permutations and  $k$  is a maximal size of the pathways. That means that setting 'maxSize' parameter with a value of ~500 is strongly recommended.

**Usage**

```
fgseaSimple(
  pathways,
  stats,
  nperm,
  minSize = 1,
  maxSize = length(stats) - 1,
  scoreType = c("std", "pos", "neg"),
  nproc = 0,
  gseaParam = 1,
  BPPARAM = NULL
)
```

**Arguments**

|           |                                                                                                                                                                                                                                                                                                                                          |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathways  | List of gene sets to check.                                                                                                                                                                                                                                                                                                              |
| stats     | Named vector of gene-level stats. Names should be the same as in 'pathways'                                                                                                                                                                                                                                                              |
| nperm     | Number of permutations to do. Minimal possible nominal p-value is about $1/nperm$                                                                                                                                                                                                                                                        |
| minSize   | Minimal size of a gene set to test. All pathways below the threshold are excluded.                                                                                                                                                                                                                                                       |
| maxSize   | Maximal size of a gene set to test. All pathways above the threshold are excluded.                                                                                                                                                                                                                                                       |
| scoreType | This parameter defines the GSEA score type. Possible options are ("std", "pos", "neg"). By default ("std") the enrichment score is computed as in the original GSEA. The "pos" and "neg" score types are intended to be used for one-tailed tests (i.e. when one is interested only in positive ("pos") or negative ("neg") enrichment). |
| nproc     | If not equal to zero sets BPPARAM to use nproc workers (default = 0).                                                                                                                                                                                                                                                                    |
| gseaParam | GSEA parameter value, all gene-level statis are raised to the power of 'gseaParam' before calculation of GSEA enrichment scores.                                                                                                                                                                                                         |
| BPPARAM   | Parallelization parameter used in bplapply. Can be used to specify cluster to run. If not initialized explicitly or by setting 'nproc' default value 'bpparam()' is used.                                                                                                                                                                |

**Value**

A table with GSEA results. Each row corresponds to a tested pathway. The columns are the following:

- pathway – name of the pathway as in ‘names(pathway)’;
- pval – an enrichment p-value;
- padj – a BH-adjusted p-value;
- ES – enrichment score, same as in Broad GSEA implementation;
- NES – enrichment score normalized to mean enrichment of random samples of the same size;
- nMoreExtreme – a number of times a random gene set had a more extreme enrichment score value;
- size – size of the pathway after removing genes not present in ‘names(stats)’.
- leadingEdge – vector with indexes of leading edge genes that drive the enrichment, see [http://software.broadinstitute.org/gsea/doc/GSEAUserGuideTEXT.htm#\\_Running\\_a\\_Leading](http://software.broadinstitute.org/gsea/doc/GSEAUserGuideTEXT.htm#_Running_a_Leading).

**Examples**

```
data(examplePathways)
data(exampleRanks)
fgseaRes <- fgseaSimple(examplePathways, exampleRanks, nperm=10000, maxSize=500)
# Testing only one pathway is implemented in a more efficient manner
fgseaRes1 <- fgseaSimple(examplePathways[1], exampleRanks, nperm=10000)
```

---

|                 |                                                                                 |
|-----------------|---------------------------------------------------------------------------------|
| fgseaSimpleImpl | <i>Runs preranked gene set enrichment analysis for preprocessed input data.</i> |
|-----------------|---------------------------------------------------------------------------------|

---

**Description**

Runs preranked gene set enrichment analysis for preprocessed input data.

**Usage**

```
fgseaSimpleImpl(
  pathwayScores,
  pathwaysSizes,
  pathwaysFiltered,
  leadingEdges,
  permPerProc,
  seeds,
  toKeepLength,
  stats,
  BPPARAM,
  scoreType
)
```

## Arguments

|                  |                                                                                                                                                                                                                      |
|------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathwayScores    | Vector with enrichment scores for the 'pathways'.                                                                                                                                                                    |
| pathwaysSizes    | Vector of pathways sizes.                                                                                                                                                                                            |
| pathwaysFiltered | Filtered pathways.                                                                                                                                                                                                   |
| leadingEdges     | Leading edge genes.                                                                                                                                                                                                  |
| permPerProc      | Parallelization parameter for permutations.                                                                                                                                                                          |
| seeds            | Seed vector                                                                                                                                                                                                          |
| toKeepLength     | Number of 'pathways' that meet the condition for 'minSize' and 'maxSize'.                                                                                                                                            |
| stats            | Named vector of gene-level stats. Names should be the same as in 'pathways'                                                                                                                                          |
| BPPARAM          | Parallelization parameter used in bplapply.                                                                                                                                                                          |
| scoreType        | This parameter defines the GSEA score type. Possible options are ("std", "pos", "neg") Can be used to specify cluster to run. If not initialized explicitly or by setting 'nproc' default value 'bpparam()' is used. |

## Value

A table with GSEA results. Each row corresponds to a tested pathway. The columns are the following:

- pathway – name of the pathway as in 'names(pathway)';
- pval – an enrichment p-value;
- padj – a BH-adjusted p-value;
- ES – enrichment score, same as in Broad GSEA implementation;
- NES – enrichment score normalized to mean enrichment of random samples of the same size;
- nMoreExtreme – a number of times a random gene set had a more extreme enrichment score value;
- size – size of the pathway after removing genes not present in 'names(stats)';
- leadingEdge – vector with indexes of leading edge genes that drive the enrichment, see [http://software.broadinstitute.org/gsea/doc/GSEAUserGuideTEXT.htm#\\_Running\\_a\\_Leading](http://software.broadinstitute.org/gsea/doc/GSEAUserGuideTEXT.htm#_Running_a_Leading).

---

fora

---

Simple overrepresentation analysis based on hypergeometric test

---

## Description

Simple overrepresentation analysis based on hypergeometric test

## Usage

```
fora(pathways, genes, universe, minSize = 1, maxSize = length(universe) - 1)
```

**Arguments**

|          |                                                                                    |
|----------|------------------------------------------------------------------------------------|
| pathways | List of gene sets to check.                                                        |
| genes    | Set of query genes                                                                 |
| universe | A universe from which 'genes' were selected                                        |
| minSize  | Minimal size of a gene set to test. All pathways below the threshold are excluded. |
| maxSize  | Maximal size of a gene set to test. All pathways above the threshold are excluded. |

**Value**

A table with ORA results. Each row corresponds to a tested pathway. The columns are the following:

- pathway – name of the pathway as in 'names(pathway)';
- pval – an enrichment p-value from hypergeometric test;
- padj – a BH-adjusted p-value;
- foldEnrichment – degree of enrichment relative to background;
- overlap – size of the overlap;
- size – size of the gene set;
- leadingEdge – vector with overlapping genes.

**Examples**

```
data(examplePathways)
data(exampleRanks)
foraRes <- fora(examplePathways, genes=tail(names(exampleRanks), 200), universe=names(exampleRanks))
```

---

|        |                                                                                            |
|--------|--------------------------------------------------------------------------------------------|
| geseca | <i>Runs multilevel Monte-Carlo variant for performing gene sets co-regulation analysis</i> |
|--------|--------------------------------------------------------------------------------------------|

---

**Description**

This function is based on the adaptive multilevel splitting Monte Carlo approach and allows to estimate arbitrarily small P-values for the task of analyzing variance along a set of genes.

**Usage**

```
geseca(
  pathways,
  E,
  minSize = 1,
  maxSize = nrow(E) - 1,
  center = TRUE,
  scale = FALSE,
  sampleSize = 101,
  eps = 1e-50,
```

```

nproc = 0,
BPPARAM = NULL,
nPermSimple = 1000
)

```

### Arguments

|             |                                                                                                                                                                                                     |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathways    | List of gene sets to check.                                                                                                                                                                         |
| E           | expression matrix, rows corresponds to genes, columns corresponds to samples.                                                                                                                       |
| minSize     | Minimal size of a gene set to test. All pathways below the threshold are excluded.                                                                                                                  |
| maxSize     | Maximal size of a gene set to test. All pathways above the threshold are excluded.                                                                                                                  |
| center      | a logical value indicating whether the gene expression should be centered to have zero mean before the analysis takes place. The default is TRUE. The value is passed to <a href="#">scale</a> .    |
| scale       | a logical value indicating whether the gene expression should be scaled to have unit variance before the analysis takes place. The default is FALSE. The value is passed to <a href="#">scale</a> . |
| sampleSize  | sample size for conditional sampling.                                                                                                                                                               |
| eps         | This parameter sets the boundary for calculating P-values.                                                                                                                                          |
| nproc       | If not equal to zero sets BPPARAM to use nproc workers (default = 0).                                                                                                                               |
| BPPARAM     | Parallelization parameter used in bplapply.                                                                                                                                                         |
| nPermSimple | Number of permutations in the simple geseca implementation for preliminary estimation of P-values.                                                                                                  |

### Value

A table with GESECA results. Each row corresponds to a tested pathway. The columns are the following

- pathway – name of the pathway as in ‘names(pathways)’;
- pctVar – percent of explained variance along gene set;
- pval – P-value that corresponds to the gene set score;
- padj – a BH-adjusted p-value;
- size – size of the pathway after removing genes not present in ‘rownames(E)’.

### Examples

```

data("exampleExpressionMatrix")
data("examplePathways")
gr <- geseca(examplePathways, exampleExpressionMatrix, minSize=15, maxSize=500)

```

gesecaSimple

*Runs simple variant for performing gene sets co-regulation analysis***Description**

This function is based on the rude Monte Carlo sampling approach and P-value calculation accuracy is limited to '1 / nperm' value.

**Usage**

```
gesecaSimple(
  pathways,
  E,
  minSize = 1,
  maxSize = nrow(E) - 1,
  center = TRUE,
  scale = FALSE,
  nperm = 1000,
  nproc = 0,
  BPPARAM = NULL
)
```

**Arguments**

|          |                                                                                                                                                                                                     |
|----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathways | List of gene sets to check.                                                                                                                                                                         |
| E        | expression matrix, rows corresponds to genes, columns corresponds to samples.                                                                                                                       |
| minSize  | Minimal size of a gene set to test. All pathways below the threshold are excluded.                                                                                                                  |
| maxSize  | Maximal size of a gene set to test. All pathways above the threshold are excluded.                                                                                                                  |
| center   | a logical value indicating whether the gene expression should be centered to have zero mean before the analysis takes place. The default is TRUE. The value is passed to <a href="#">scale</a> .    |
| scale    | a logical value indicating whether the gene expression should be scaled to have unit variance before the analysis takes place. The default is FALSE. The value is passed to <a href="#">scale</a> . |
| nperm    | Number of permutations to do. Minimal possible nominal p-value is about 1/nperm                                                                                                                     |
| nproc    | If not equal to zero sets BPPARAM to use nproc workers (default = 0).                                                                                                                               |
| BPPARAM  | Parallelization parameter used in bplapply.                                                                                                                                                         |

**Value**

A table with GESECA results. Each row corresponds to a tested pathway. The columns are the following

- pathway – name of the pathway as in 'names(pathways)';
- pctVar – percent of explained variance along gene set;

- pval – P-value that corresponds to the gene set score;
- padj – a BH-adjusted p-value;
- size – size of the pathway after removing genes not present in ‘rownames(E)’.

### Examples

```
data("exampleExpressionMatrix")
data("examplePathways")
gesecaRes <- gesecaSimple(examplePathways, exampleExpressionMatrix, minSize=15, maxSize=500)
```

---

|             |                                                    |
|-------------|----------------------------------------------------|
| gmtPathways | <i>Returns a list of pathways from a GMT file.</i> |
|-------------|----------------------------------------------------|

---

### Description

Returns a list of pathways from a GMT file.

### Usage

```
gmtPathways(gmt.file)
```

### Arguments

|          |                     |
|----------|---------------------|
| gmt.file | Path to a GMT file. |
|----------|---------------------|

### Value

A list of vectors with gene sets.

### Examples

```
pathways <- gmtPathways(system.file(
  "extdata", "mouse.reactome.gmt", package="fgsea"))
```

---

|            |                                                                                                                                                                                            |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| mapIdsList | <i>Effeciently converts collection of pathways using AnnotationDbi::mapIds function. Parameters are the sames as for mapIds except for keys, which is assumed to be a list of vectors.</i> |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

---

### Description

Effeciently converts collection of pathways using AnnotationDbi::mapIds function. Parameters are the sames as for mapIds except for keys, which is assumed to be a list of vectors.

### Usage

```
mapIdsList(x, keys, column, keytype, ...)
```

**Arguments**

|         |                                                                                                                                         |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------|
| x       | the AnnotationDb object. But in practice this will mean an object derived from an AnnotationDb object such as a OrgDb or ChipDb object. |
| keys    | a list of vectors with gene ids                                                                                                         |
| column  | the column to search on                                                                                                                 |
| keytype | the keytype that matches the keys used                                                                                                  |
| ...     | other parameters passed to AnnotationDbi::mapIds                                                                                        |

**See Also**

AnnotationDbi::mapIds

**Examples**

```
library(org.Mm.eg.db)
data(exampleRanks)
fgseaRes <- fgsea(examplePathways, exampleRanks, maxSize=500, eps=1e-4)
fgseaRes[, leadingEdge := mapIdsList(org.Mm.eg.db, keys=leadingEdge, column="SYMBOL", keytype="ENTREZID")]
```

---

|                 |                                                                                           |
|-----------------|-------------------------------------------------------------------------------------------|
| multilevelError | <i>Calculates the expected error for the standard deviation of the P-value logarithm.</i> |
|-----------------|-------------------------------------------------------------------------------------------|

---

**Description**

Calculates the expected error for the standard deviation of the P-value logarithm.

**Usage**

```
multilevelError(pval, sampleSize)
```

**Arguments**

|            |                                              |
|------------|----------------------------------------------|
| pval       | P-value                                      |
| sampleSize | equivavlent to sampleSize in fgseaMultilevel |

**Value**

The value of the expected error

**Examples**

```
expectedError <- multilevelError(pval=1e-10, sampleSize=1001)
```

---

|                |                                                   |
|----------------|---------------------------------------------------|
| multilevelImpl | <i>Calculates P-values for preprocessed data.</i> |
|----------------|---------------------------------------------------|

---

### Description

Calculates P-values for preprocessed data.

### Usage

```
multilevelImpl(
  multilevelPathwaysList,
  stats,
  sampleSize,
  seed,
  eps,
  sign = FALSE,
  BPPARAM = NULL
)
```

### Arguments

|                        |                                                                                                                                                                           |
|------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| multilevelPathwaysList | List of pathways for which P-values will be calculated.                                                                                                                   |
| stats                  | Named vector of gene-level stats. Names should be the same as in 'pathways'                                                                                               |
| sampleSize             | The size of a random set of genes which in turn has size = pathwaySize                                                                                                    |
| seed                   | 'seed' parameter from 'fgseaMultilevel'                                                                                                                                   |
| eps                    | This parameter sets the boundary for calculating the p value.                                                                                                             |
| sign                   | This option will be used in future implementations.                                                                                                                       |
| BPPARAM                | Parallelization parameter used in bplapply. Can be used to specify cluster to run. If not initialized explicitly or by setting 'nproc' default value 'bpparam()' is used. |

### Value

List of P-values.

---

|                         |                                               |
|-------------------------|-----------------------------------------------|
| plotCoregulationProfile | <i>Plots expression profile of a gene set</i> |
|-------------------------|-----------------------------------------------|

---

### Description

Plots expression profile of a gene set

**Usage**

```
plotCoregulationProfile(
  pathway,
  E,
  center = TRUE,
  scale = FALSE,
  titles = colnames(E),
  conditions = NULL
)
```

**Arguments**

|            |                                                                                                                                                                                                     |
|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathway    | Gene set to plot.                                                                                                                                                                                   |
| E          | matrix with gene expression values                                                                                                                                                                  |
| center     | a logical value indicating whether the gene expression should be centered to have zero mean before the analysis takes place. The default is TRUE. The value is passed to <a href="#">scale</a> .    |
| scale      | a logical value indicating whether the gene expression should be scaled to have unit variance before the analysis takes place. The default is FALSE. The value is passed to <a href="#">scale</a> . |
| titles     | sample titles to use for labels                                                                                                                                                                     |
| conditions | sample grouping to use for coloring                                                                                                                                                                 |

**Value**

ggplot object with the coregulation profile plot

---

plotCoregulationProfileImage

*Spatial visualization of GESECA scores for individual cells*

---

**Description**

This function computes GESECA scores for one or more gene sets and overlays those scaled scores onto the spatial image.

**Usage**

```
plotCoregulationProfileImage(
  pathway,
  object,
  title = NULL,
  assay = DefaultAssay(object),
  colors = rdbuColors,
  guide = "colourbar",
  minLimit = -3,
  maxLimit = 3,
  ...
)
```

**Arguments**

|          |                                                                                                                                                                                                           |
|----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathway  | Gene set (vector of gene names) or a named list of gene sets to plot. If a list is provided, each element is treated as a separate pathway and yields its own plot.                                       |
| object   | Seurat object                                                                                                                                                                                             |
| title    | Optional title for the plot. If 'pathway' is a list, 'title' should be a character vector of the same length; otherwise, the list element names are used.                                                 |
| assay    | assay to use for obtaining scaled data, preferably with the same universe of genes in the scaled data                                                                                                     |
| colors   | vector of colors to use in the color scheme (default is similar to "RdBu" Brewer's color palette)                                                                                                         |
| guide    | option for 'ggplot2::scale_color_gradientn' to control for presence of the color legend the same universe of genes in the scaled data                                                                     |
| minLimit | Numeric value specifying the minimum limit for the color scale. This defines the lower bound of the z-score used in coloring the feature plot. Values below this limit are squished to the minimum color. |
| maxLimit | Numeric value specifying the maximum limit for the color scale. This defines the upper bound of the z-score used in coloring the feature plot. Values above this limit are squished to the maximum color. |
| ...      | Additional arguments passed to <a href="#">ImageFeaturePlot</a>                                                                                                                                           |

**Value**

ggplot object (or a list of objects) with the spatial image plot of scaled geseca scores

When the input is a list of pathways, pathway names are used for titles. A list of ggplot objects a returned in that case.

---

plotCoregulationProfileReduction

*Plot a spatial expression profile of a gene set*

---

**Description**

Plot a spatial expression profile of a gene set

**Usage**

```
plotCoregulationProfileReduction(
  pathway,
  object,
  title = NULL,
  assay = DefaultAssay(object),
  reduction = NULL,
  colors = rdbuColors,
  guide = "colourbar",
  minLimit = -3,
  maxLimit = 3,
  ...
)
```

**Arguments**

|           |                                                                                                                                                                                                           |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathway   | Gene set to plot or a list of gene sets (see details below)                                                                                                                                               |
| object    | Seurat object                                                                                                                                                                                             |
| title     | plot title                                                                                                                                                                                                |
| assay     | assay to use for obtaining scaled data, preferably with                                                                                                                                                   |
| reduction | reduction to use for plotting (one of the 'Seurat::Reductions(object)')                                                                                                                                   |
| colors    | vector of colors to use in the color scheme (default is similar to "RdBu" Brewer's color palette)                                                                                                         |
| guide     | option for 'ggplot2::scale_color_gradientn' to control for presence of the color legend the same universe of genes in the scaled data                                                                     |
| minLimit  | Numeric value specifying the minimum limit for the color scale. This defines the lower bound of the z-score used in coloring the feature plot. Values below this limit are squished to the minimum color. |
| maxLimit  | Numeric value specifying the maximum limit for the color scale. This defines the upper bound of the z-score used in coloring the feature plot. Values above this limit are squished to the maximum color. |
| ...       | additional arguments for Seurat::FeaturePlot                                                                                                                                                              |

**Value**

ggplot object (or a list of objects) with the coregulation profile plot

When the input is a list of pathways, pathway names are used for titles. A list of ggplot objects is returned in that case.

---

plotCoregulationProfileSpatial

*Plot a spatial expression profile of a gene set*

---

**Description**

Plot a spatial expression profile of a gene set

**Usage**

```
plotCoregulationProfileSpatial(
  pathway,
  object,
  title = NULL,
  assay = DefaultAssay(object),
  colors = rdbuColors,
  guide = "colourbar",
  image.alpha = 0,
  minLimit = -3,
  maxLimit = 3,
  ...
)
```

**Arguments**

|             |                                                                                                                                                                                                           |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathway     | Gene set to plot or a list of gene sets (see details below)                                                                                                                                               |
| object      | Seurat object                                                                                                                                                                                             |
| title       | plot title                                                                                                                                                                                                |
| assay       | assay to use for obtaining scaled data, preferably with the same universe of genes in the scaled data                                                                                                     |
| colors      | vector of colors to use in the color scheme (default is similar to "RdBu" Brewer's color palette)                                                                                                         |
| guide       | option for 'ggplot2::scale_color_gradientn' to control for presence of the color legend the same universe of genes in the scaled data                                                                     |
| image.alpha | adjust the opacity of the background images                                                                                                                                                               |
| minLimit    | Numeric value specifying the minimum limit for the color scale. This defines the lower bound of the z-score used in coloring the feature plot. Values below this limit are squished to the minimum color. |
| maxLimit    | Numeric value specifying the maximum limit for the color scale. This defines the upper bound of the z-score used in coloring the feature plot. Values above this limit are squished to the maximum color. |
| ...         | optional arguments for <a href="#">SpatialFeaturePlot</a>                                                                                                                                                 |

**Value**

ggplot object (or a list of objects) with the coregulation profile plot

When the input is a list of pathways, pathway names are used for titles. A list of ggplot objects are returned in that case.

---

|                |                                                                                            |
|----------------|--------------------------------------------------------------------------------------------|
| plotEnrichment | <i>Plots GSEA enrichment plot. For more flexibility use 'plotEnrichmentData' function.</i> |
|----------------|--------------------------------------------------------------------------------------------|

---

**Description**

Plots GSEA enrichment plot. For more flexibility use 'plotEnrichmentData' function.

**Usage**

```
plotEnrichment(pathway, stats, gseaParam = 1, ticksSize = 0.2)
```

**Arguments**

|           |                                                               |
|-----------|---------------------------------------------------------------|
| pathway   | Gene set to plot.                                             |
| stats     | Gene-level statistics.                                        |
| gseaParam | GSEA parameter.                                               |
| ticksSize | width of vertical line corresponding to a gene (default: 0.2) |

**Value**

ggplot object with the enrichment plot.



```

      fill="grey") +
    geom_segment(data=ticks,
      mapping=aes(x=rank, y=-spreadES/16,
        xend=rank, yend=spreadES/16),
      size=0.2) +
    geom_hline(yintercept=posES, colour="red", linetype="dashed") +
    geom_hline(yintercept=negES, colour="red", linetype="dashed") +
    geom_hline(yintercept=0, colour="black") +
    theme(
      panel.background = element_blank(),
      panel.grid.major=element_line(color="grey92")
    ) +
    labs(x="rank", y="enrichment score"))

```

---

|                 |                                          |
|-----------------|------------------------------------------|
| plotGesecaTable | <i>Plots table of gene set profiles.</i> |
|-----------------|------------------------------------------|

---

## Description

Plots table of gene set profiles.

## Usage

```

plotGesecaTable(
  gesecaRes,
  pathways,
  E,
  center = TRUE,
  scale = FALSE,
  colwidths = c(5, 3, 0.8, 1.2, 1.2),
  titles = colnames(E),
  colors = rdbuColors,
  pathwayLabelStyle = NULL,
  headerLabelStyle = NULL,
  valueStyle = NULL,
  axisLabelStyle = NULL,
  axisLabelHeightScale = NULL,
  minLimit = -3,
  maxLimit = 3
)

```

## Arguments

|           |                                                                                                                                                                                                     |
|-----------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| gesecaRes | Table with geseca results.                                                                                                                                                                          |
| pathways  | Pathways to plot table, as in ‘geseca’ function.                                                                                                                                                    |
| E         | gene expression matrix, as in ‘geseca’ function.                                                                                                                                                    |
| center    | a logical value indicating whether the gene expression should be centered to have zero mean before the analysis takes place. The default is TRUE. The value is passed to <a href="#">scale</a> .    |
| scale     | a logical value indicating whether the gene expression should be scaled to have unit variance before the analysis takes place. The default is FALSE. The value is passed to <a href="#">scale</a> . |

|                      |                                                                                                                                                                                                                                        |
|----------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| colwidths            | Vector of five elements corresponding to column width for grid.arrange. Can be both units and simple numeric vector, in latter case it defines proportions, not actual sizes. If column width is set to zero, the column is not drawn. |
| titles               | sample titles to use an axis labels. Default to 'colnames(E)'                                                                                                                                                                          |
| colors               | vector of colors to use in the color scheme (default is similar to "RdBu" Brewer's color palette)                                                                                                                                      |
| pathwayLabelStyle    | list with style parameter adjustments for pathway labels. For example, 'list(size=10, color="red")' set the font size to 10 and color to red. See 'cowplot::draw_text' for possible options.                                           |
| headerLabelStyle     | similar to 'pathwayLabelStyle' but for the table header.                                                                                                                                                                               |
| valueStyle           | similar to 'pathwayLabelStyle' but for pctVar and p-value columns.                                                                                                                                                                     |
| axisLabelStyle       | list with style parameter adjustments for sample labels. See 'ggplot2::element_text' for possible options.                                                                                                                             |
| axisLabelHeightScale | height of the row with axis labels compared to other rows. When set to 'NULL' the value is determined automatically.                                                                                                                   |
| minLimit             | Numeric value specifying the minimum limit for the color scale. This defines the lower bound of the z-score used in coloring the feature plot. Values below this limit are squished to the minimum color.                              |
| maxLimit             | Numeric value specifying the maximum limit for the color scale. This defines the upper bound of the z-score used in coloring the feature plot. Values above this limit are squished to the maximum color.                              |

### Value

ggplot object with gene set profile plots

---

|               |                                                                     |
|---------------|---------------------------------------------------------------------|
| plotGseaTable | <i>Plots table of enrichment graphs using ggplot and gridExtra.</i> |
|---------------|---------------------------------------------------------------------|

---

### Description

Plots table of enrichment graphs using ggplot and gridExtra.

### Usage

```
plotGseaTable(
  pathways,
  stats,
  fgseaRes,
  gseaParam = 1,
  colwidths = c(5, 3, 0.8, 1.2, 1.2),
  pathwayLabelStyle = NULL,
  headerLabelStyle = NULL,
  valueStyle = NULL,
  axisLabelStyle = NULL,
  render = NULL
)
```

**Arguments**

|                   |                                                                                                                                                                                                                                        |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| pathways          | Pathways to plot table, as in 'fgsea' function.                                                                                                                                                                                        |
| stats             | Gene-level stats, as in 'fgsea' function.                                                                                                                                                                                              |
| fgseaRes          | Table with fgsea results.                                                                                                                                                                                                              |
| gseaParam         | GSEA-like parameter. Adjusts displayed statistic values, values closer to 0 flatten plots. Default = 1, value of 0.5 is a good choice too.                                                                                             |
| colwidths         | Vector of five elements corresponding to column width for grid.arrange. Can be both units and simple numeric vector, in latter case it defines proportions, not actual sizes. If column width is set to zero, the column is not drawn. |
| pathwayLabelStyle | list with style parameter adjustments for pathway labels. For example, 'list(size=10, color="red")' set the font size to 10 and color to red. See 'cowplot::draw_text' for possible options.                                           |
| headerLabelStyle  | similar to 'pathwayLabelStyle' but for the table header.                                                                                                                                                                               |
| valueStyle        | similar to 'pathwayLabelStyle' but for NES and p-value columns.                                                                                                                                                                        |
| axisLabelStyle    | list with style parameter adjustments for stats axis labels. See 'ggplot2::element_text' for possible options.                                                                                                                         |
| render            | (deprecated)                                                                                                                                                                                                                           |

**Value**

ggplot object with enrichment barcode plots

**Examples**

```
data(examplePathways)
data(exampleRanks)
fgseaRes <- fgsea(examplePathways, exampleRanks, minSize=15, maxSize=500)
topPathways <- fgseaRes[head(order(pval), n=15)][order(NES), pathway]
plotGseaTable(examplePathways[topPathways], exampleRanks,
              fgseaRes, gseaParam=0.5)
```

---

reactomePathways

*Returns a list of Reactome pathways for given Entrez gene IDs*

---

**Description**

Returns a list of Reactome pathways for given Entrez gene IDs

**Usage**

```
reactomePathways(genes)
```

**Arguments**

|       |                            |
|-------|----------------------------|
| genes | Entrez IDs of query genes. |
|-------|----------------------------|

**Value**

A list of vectors with gene sets.

**Examples**

```
data(exampleRanks)
pathways <- reactomePathways(names(exampleRanks))
```

---

|                  |                                                                     |
|------------------|---------------------------------------------------------------------|
| writeGmtPathways | <i>Write collection of pathways (list of vectors) to a gmt file</i> |
|------------------|---------------------------------------------------------------------|

---

**Description**

Write collection of pathways (list of vectors) to a gmt file

**Usage**

```
writeGmtPathways(pathways, gmt.file)
```

**Arguments**

|          |                                       |
|----------|---------------------------------------|
| pathways | a named list of vectors with gene ids |
| gmt.file | name of the output file               |

**Examples**

```
data(examplePathways)
writeGmtPathways(examplePathways, tempfile("examplePathways", fileext=".gmt"))
```

# Index

[calcGseaStat](#), [2](#)  
[calcGseaStatBatchCpp](#), [3](#)  
[collapsePathways](#), [4](#)  
[collapsePathwaysGeseca](#), [5](#)  
[collapsePathwaysORA](#), [6](#)  
  
[exampleExpressionMatrix](#), [7](#)  
[examplePathways](#), [7](#)  
[exampleRanks](#), [7](#)  
  
[fgsea](#), [8](#)  
[fgseaLabel](#), [9](#)  
[fgseaMultilevel](#), [8](#), [10](#)  
[fgseaSimple](#), [8](#), [12](#)  
[fgseaSimpleImpl](#), [13](#)  
[fora](#), [14](#)  
  
[geseca](#), [15](#)  
[gesecaSimple](#), [17](#)  
[gmtPathways](#), [18](#)  
  
[ImageFeaturePlot](#), [22](#)  
  
[mapIdsList](#), [18](#)  
[multilevelError](#), [19](#)  
[multilevelImpl](#), [20](#)  
  
[plotCoregulationProfile](#), [20](#)  
[plotCoregulationProfileImage](#), [21](#)  
[plotCoregulationProfileReduction](#), [22](#)  
[plotCoregulationProfileSpatial](#), [23](#)  
[plotEnrichment](#), [24](#)  
[plotEnrichmentData](#), [25](#)  
[plotGesecaTable](#), [26](#)  
[plotGseaTable](#), [27](#)  
  
[reactomePathways](#), [28](#)  
  
[scale](#), [5](#), [16](#), [17](#), [21](#), [26](#)  
[SpatialFeaturePlot](#), [24](#)  
  
[writeGmtPathways](#), [29](#)