

# Package ‘escape’

June 4, 2023

**Title** Easy single cell analysis platform for enrichment

**Version** 1.10.0

**Date** 2022-3-05

**Description** A bridging R package to facilitate gene set enrichment analysis (GSEA) in the context of single-cell RNA sequencing. Using raw count information, Seurat objects, or SingleCellExperiment format, users can perform and visualize GSEA across individual cells.

**License** GPL-2

**Encoding** UTF-8

**LazyData** false

**RoxygenNote** 7.1.2

**biocViews** Software, SingleCell, Classification, Annotation,  
GeneSetEnrichment, Sequencing, GeneSignaling, Pathways

**Depends** R (>= 4.1)

**Imports** grDevices, dplyr, ggplot2, GSEABase, GSVA,  
SingleCellExperiment, ggridges, msigdb, stats, BiocParallel,  
Matrix, UCell, broom, reshape2, patchwork, MatrixGenerics,  
utils, rlang, stringr, data.table, SummarizedExperiment,  
methods

**Suggests** Seurat, SeuratObject, knitr, rmarkdown, markdown, BiocStyle,  
testthat, dittoSeq (>= 1.1.2)

**VignetteBuilder** knitr

**git\_url** <https://git.bioconductor.org/packages/escape>

**git\_branch** RELEASE\_3\_17

**git\_last\_commit** 961805d

**git\_last\_commit\_date** 2023-04-25

**Date/Publication** 2023-06-04

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calculate_Uscore	<i>Calculate rankings and scores for query data and given signature set</i>
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**Description**

Calculate rankings and scores for query data and given signature set

**Usage**

```
calculate_Uscore(
  matrix,
  features,
  maxRank = 1500,
  chunk.size = 1000,
  ncores = 1,
  w_neg = 1,
  ties.method = "average",
  storeRanks = FALSE,
  force.gc = FALSE,
  name = ""
)
```

**Arguments**

matrix	Input data matrix
features	List of signatures
maxRank	Rank cutoff (1500)
chunk.size	Cells per sub-matrix (1000)
ncores	Number of cores to use for parallelization (1)
w_neg	Weight on negative signatures
ties.method	How to break ties, for data.table::frankv method ("average")
storeRanks	Store ranks? (FALSE)
force.gc	Force garbage collection? (FALSE)
name	Suffix for metadata columns ("_UCell")

**Value**

A list of signature scores

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check_genes	<i>Check if all genes in signatures are found in data matrix - otherwise add zero counts in data-matrix to complete it</i>
-------------	--

---

**Description**

Check if all genes in signatures are found in data matrix - otherwise add zero counts in data-matrix to complete it

**Usage**

```
check_genes(matrix, features)
```

**Arguments**

matrix	Input data matrix
features	List of genes that must be present (otherwise they are added)

**Value**

Same input matrix, extended to comprise any missing genes

check\_signature\_names *Check signature names and add standard names is missing*

---

**Description**

Check signature names and add standard names is missing

**Usage**

```
check_signature_names(features)
```

**Arguments**

features            List of signatures for scoring

**Value**

The input list of signatures, with standard names if provided un-named

---

data\_to\_ranks\_data\_table  
*Calculate per-cell feature rankings*

---

**Description**

Calculate per-cell feature rankings

**Usage**

```
data_to_ranks_data_table(data, ties.method = "average")
```

**Arguments**

data                Expression data matrix  
ties.method        How to break ties (passed on to data.table::frankv)

**Value**

A data.table of ranks

---

`enrichIt`*Calculate gene set enrichment scores for single-cell data*

---

## Description

This function allows users to input both the single-cell RNA-sequencing counts and any gene set pathways either from the stored data or from other sources. The enrichment calculation itself uses the two methods 1) gsva R package and the poisson distribution for RNA or the [UCell package](#).

## Usage

```
enrichIt(  
  obj,  
  gene.sets = NULL,  
  method = "ssGSEA",  
  groups = 1000,  
  cores = 2,  
  min.size = 5,  
  ssGSEA.norm = FALSE,  
  ...  
)
```

## Arguments

<code>obj</code>	The count matrix, Seurat, or SingleCellExperiment object.
<code>gene.sets</code>	Gene sets from <a href="#">getGeneSets</a> to use for the enrichment analysis. Alternatively a simple base R list where the list elements correspond to the name of the gene set and the elements themselves are simple vectors of gene names representing the gene set.
<code>method</code>	select the method to calculate enrichment, either "ssGSEA" or "UCell"
<code>groups</code>	The number of cells to separate the enrichment calculation.
<code>cores</code>	The number of cores to use for parallelization.
<code>min.size</code>	Minimum number of gene necessary to perform the enrichment calculation
<code>ssGSEA.norm</code>	normalized the enrichment score based on the range of the individual gene set. If TRUE, the returned enrichment score is based may change with cell composition.
<code>...</code>	pass arguments to ssGSEA or UCell call

## Value

Data frame of normalized enrichment scores (NES)

## Author(s)

Nick Borcharding, Jared Andrews

**See Also**

[getGeneSets](#) to collect gene sets.

**Examples**

```
GS <- list(Bcells = c("MS4A1", "CD79B", "CD79A", "IGH1", "IGH2"),
  Tcells = c("CD3E", "CD3D", "CD3G", "CD7", "CD8A"))
pbmc_small <- suppressWarnings(SeuratObject::pbmc_small)
ES <- enrichIt(obj = pbmc_small, gene.sets = GS, min.size = NULL)
```

---

enrichmentPlot	<i>Gene Rank Enrichment Plot Display the rank order density for individual gene sets by group identify. This function will use the group variable to take the mean rank order across all individual cells.</i>
----------------	--

---

**Description**

Gene Rank Enrichment Plot Display the rank order density for individual gene sets by group identify. This function will use the group variable to take the mean rank order across all individual cells.

**Usage**

```
enrichmentPlot(
  obj,
  gene.set,
  gene.sets,
  group,
  colors = c("#0D0887FF", "#7E03A8FF", "#CC4678FF", "#F89441FF", "#F0F921FF")
)
```

**Arguments**

obj	The Seurat or SingleCellExperiment object.
gene.set	The name of the specific gene set to visualize
gene.sets	Gene sets from <a href="#">getGeneSets</a> to use
group	The header in the meta data that will be used for the comparison
colors	The color palette for the enrichment plot

**Value**

ggplot2 object mean rank gene density

**Examples**

```
## Not run:
GS <- list(Housekeeping = c("ACTA1", "ACTN1", "GAPDH"),
Cancer = c("TP53", "BRCA2", "ERBB2", "MYC"))
pbmc_small <- suppressWarnings(SeuratObject::pbmc_small)

enrichmentPlot(pbmc_small gene.set = "Cancer",
               gene.sets = GS, group = "groups")

## End(Not run)
```

---

<code>escape.gene.sets</code>	<i>In-Built Gene Sets for Escape</i>
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**Description**

A list of gene sets derived from PMID: 29961579 relating to tumor immunity.

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<code>getGeneSets</code>	<i>Get a collection of gene sets to perform enrichment on</i>
--------------------------	---

---

**Description**

This function allows users to select libraries and specific gene.sets to form a GeneSetCollection that is a list of gene sets.

**Usage**

```
getGeneSets(
  species = "Homo sapiens",
  library = NULL,
  subcategory = NULL,
  gene.sets = NULL
)
```

**Arguments**

<code>species</code>	The scientific name of the species of interest in order to get correcent gene nomenclature
<code>library</code>	Individual collection(s) of gene sets, e.g. <code>c("H", "C5")</code> . See <a href="#">msigdb</a> for all MSigDB collections.
<code>subcategory</code>	MSigDB sub-collection abbreviation, such as CGP or BP.
<code>gene.sets</code>	Select gene sets or pathways, using specific names, example: <code>pathways = c("HALLMARK_TNFA_SIGNA")</code> . Will only be honored if library is set, too.

**Value**

A GeneSetCollection object containing the requested GeneSet objects.

**Author(s)**

Nick Borcharding, Jared Andrews

**Examples**

```
GS <- getGeneSets(library = "H")
```

---

getSignificance	<i>Perform significance testing between groups and enrichment scores.</i>
-----------------	---

---

**Description**

This functions takes the enrichment scores and performs statistical testing to evaluate the difference by group selected. The function can perform 5 tests: 1) Welch's T test (T.test), 2) Logistic Regression (LR), 3) Wilcoxon Rank Sum Test (Wilcoxon), 4) one-way ANOVA (ANOVA), and 5) Kruskal-Wallis (KW). The latter two output will include the individual comparisons between groups using TukeyHSD for ANOVA and pairwise Wilcoxon Rank Sum Test for KW. The output includes adjusted p-values based on the Benjamini Hochberg method.

**Usage**

```
getSignificance(enriched, group = NULL, gene.sets = NULL, fit = NULL)
```

**Arguments**

enriched	The output of <a href="#">enrichIt</a> .
group	The parameter to group for the comparison, should a column of the enriched input
gene.sets	Names of gene sets to compare
fit	The test used for significance, 2 group: Wilcoxon, LR, T.test. Multigroup: ANOVA or KW.

**Value**

Data frame of test statistics

**See Also**

[enrichIt](#) for generating enrichment scores.



## Examples

```
ES2 <- readRDS(url(
  "https://ncborcherding.github.io/vignettes/escape_enrichment_results.rds"))
output <- getSignificance(ES2, group = "Type", fit = "T.test")
```

---

masterPCAPlot	<i>Visualize the components of the PCA analysis of the enrichment results</i>
---------------	---

---

## Description

Graph the major gene set contributors to the [pcaEnrichment](#).

## Usage

```
masterPCAPlot(enriched, gene.sets, PCx, PCy, top.contribution = 10)
```

## Arguments

enriched	The output of <a href="#">enrichIt</a> .
gene.sets	Names of gene sets to include in the PCA
PCx	The principal component graphed on the x-axis.
PCy	The principal component graphed on the y-axis.
top.contribution	The number of gene sets to graph, organized by PCA contribution.

## Value

ggplot2 object summarizing the PCA for the enrichment scores

## See Also

[enrichIt](#) for generating enrichment scores.

## Examples

```
ES2 <- readRDS(url(
  "https://ncborcherding.github.io/vignettes/escape_enrichment_results.rds"))

masterPCAPlot(ES2, PCx = "PC1", PCy = "PC2", gene.sets = colnames(ES2),
  top.contribution = 10)
```

---

pcaEnrichment

*Density plot of the principal components*

---

### Description

Density plot of the principal components

### Usage

```
pcaEnrichment(  
  PCAout,  
  PCx,  
  PCy,  
  colors = c("#0D0887FF", "#7E03A8FF", "#CC4678FF", "#F89441FF", "#F0F921FF"),  
  contours = TRUE,  
  facet = NULL  
)
```

### Arguments

PCAout	The output of <a href="#">performPCA</a>
PCx	The principal component graphed on the x-axis
PCy	The principal component graphed on the y-axis
colors	The color palette for the density plot
contours	Binary classifier to add contours to the density plot
facet	A parameter to separate the graph

### Value

ggplot2 object of the results of PCA for the enrichment scores

### See Also

[performPCA](#) for generating PCA results.

### Examples

```
ES2 <- readRDS(url(  
  "https://ncborcherding.github.io/vignettes/escape_enrichment_results.rds"))  
PCA <- performPCA(enriched = ES2, groups = c("Type", "Cluster"))  
pcaEnrichment(PCA, PCx = "PC1", PCy = "PC2", contours = TRUE)
```

---

performPCA	<i>Calculate Principal Components for the Enrichment Scores</i>
------------	---

---

**Description**

Using all or selected enrichment scores of individual single-cells, this function will calculate principal components using scaled values and attach to the output columns to use to graph later.

**Usage**

```
performPCA(enriched, gene.sets = NULL, groups)
```

**Arguments**

enriched	The output of <a href="#">enrichIt</a> .
gene.sets	Names of gene sets to include in the PCA
groups	The column headers to use in future graphing functions.

**Value**

Data frame of principal components

**Author(s)**

Nick Borcherding

**Examples**

```
ES2 <- readRDS(url(
  "https://ncborcherding.github.io/vignettes/escape_enrichment_results.rds"))

PCA <- performPCA(enriched = ES2, groups = c("Type", "Cluster"),
  gene.sets = colnames(ES2))
```

---

rankings2Uscore	<i>Get signature scores from pre-computed rank matrix</i>
-----------------	---

---

**Description**

Get signature scores from pre-computed rank matrix

**Usage**

```
rankings2Uscore(
  ranks_matrix,
  features,
  chunk.size = 1000,
  w_neg = 1,
  ncores = 1,
  force.gc = FALSE,
  name = "_UCell"
)
```

**Arguments**

ranks_matrix	A rank matrix
features	List of signatures
chunk.size	How many cells per matrix chunk
w_neg	Weight on negative signatures
ncores	How many cores to use for parallelization
force.gc	Force garbage collection to recover RAM? (FALSE)
name	Name suffix for metadata columns ("_UCell")

**Value**

A list of signature scores

---

ridgeEnrichment	<i>Generate a ridge plot to examine enrichment distributions</i>
-----------------	--

---

**Description**

This function allows to the user to examine the distribution of enrichment across groups by generating a ridge plot.

**Usage**

```
ridgeEnrichment(
  enriched,
  group = "cluster",
  gene.set = NULL,
  scale.bracket = NULL,
  facet = NULL,
  add.rug = FALSE,
  colors = c("#0D0887FF", "#7E03A8FF", "#CC4678FF", "#F89441FF", "#F0F921FF")
)
```

**Arguments**

enriched	The output of <a href="#">enrichIt</a>
group	The parameter to group, displayed on the y-axis.
gene.set	The gene set to graph on the x-axis.
scale.bracket	This will filter the enrichment scores to remove extreme outliers. Values entered (1 or 2 numbers) will be the filtering parameter using z-scores of the selected gene.set. If only 1 value is given, a secondary bracket is automatically selected as the inverse of the number.
facet	A parameter to separate the graph.
add.rug	Binary classifier to add a rug plot to the x-axis.
colors	The color palette for the ridge plot.

**Value**

ggplot2 object with ridge-based distributions of selected gene.set

**See Also**

[enrichIt](#) for generating enrichment scores.

**Examples**

```
ES2 <- readRDS(url(
  "https://ncborcherding.github.io/vignettes/escape_enrichment_results.rds"))
ridgeEnrichment(ES2, gene.set = "HALLMARK_DNA_REPAIR", group = "cluster",
  facet = "Type", add.rug = TRUE)
```

---

ScoreSignatures\_UCell *Calculate module enrichment scores from single-cell data*

---

**Description**

Calculate module enrichment scores from single-cell data

**Usage**

```
ScoreSignatures_UCell(
  matrix = NULL,
  features,
  precalc.ranks = NULL,
  maxRank = 1500,
  w_neg = 1,
  name = "_UCell",
  assay = "counts",
  chunk.size = 1000,
```

```

ncores = 1,
ties.method = "average",
force.gc = FALSE,
seed = 123
)

```

### Arguments

matrix	Input matrix,
features	A list of signatures
precalc.ranks	If you have pre-calculated ranks
maxRank	Maximum number of genes to rank per cell; above this rank, a given gene is considered as not expressed.
w_neg	Weight on negative genes in signature. e.g. 'w_neg=1' weighs equally up- and down-regulated genes, '
name	Name suffix appended to signature names
assay	The sce object assay where the data is to be found
chunk.size	Number of cells to be processed simultaneously (lower size requires slightly more computation but reduces memory demands)
ncores	Number of processors to parallelize computation.
ties.method	How ranking ties should be resolved (passed on to [data.table::frank])
force.gc	Explicitly call garbage collector to reduce memory footprint
seed	Integer seed

### Value

Returns input SingleCellExperiment object with UCell scores added to altExp

---

splitEnrichment	<i>Generate a split violin plot examine enrichment distributions</i>
-----------------	--

---

### Description

This function allows to the user to examine the distribution of enrichment across groups by generating a split violin plot.

### Usage

```

splitEnrichment(
  enriched,
  x.axis = NULL,
  scale.bracket = NULL,
  split = NULL,
  gene.set = NULL,
  colors = c("#0D0887FF", "#7E03A8FF", "#CC4678FF", "#F89441FF", "#F0F921FF")
)

```

**Arguments**

enriched	The output of <a href="#">enrichIt</a>
x.axis	Optional parameter for seperation.
scale.bracket	This will filter the enrichment scores to remove extreme outliers. Values entered (1 or 2 numbers) will be the filtering parameter using z-scores of the selected gene.set. If only 1 value is given, a secondary bracket is automatically selected as the inverse of the number.
split	The parameter to split, must be binary.
gene.set	The gene set to graph on the y-axis.
colors	The color palette for the ridge plot.

**Value**

ggplot2 object violin-based distributions of selected gene.set

**See Also**

[enrichIt](#) for generating enrichment scores.

**Examples**

```
ES2 <- readRDS(url(
  "https://ncborcherding.github.io/vignettes/escape_enrichment_results.rds"))
splitEnrichment(ES2, x.axis = "cluster", split = "Type",
  gene.set = "HALLMARK_DNA_REPAIR")
```

---

StoreRankings\_UCell     *Calculate and store gene rankings for a single-cell dataset*

---

**Description**

Calculate and store gene rankings for a single-cell dataset

**Usage**

```
StoreRankings_UCell(
  matrix,
  maxRank = 1500,
  chunk.size = 1000,
  ncores = 1,
  assay = "counts",
  ties.method = "average",
  force.gc = FALSE,
  seed = 123
)
```

**Arguments**

matrix	Input matrix, either stored in a [SingleCellExperiment] object or as a raw matrix. dgCMatrx format supported.
maxRank	Maximum number of genes to rank per cell; above this rank, a given gene is considered as not expressed
chunk.size	Number of cells to be processed simultaneously (lower size requires slightly more computation but reduces memory demands)
ncores	Number of processors to parallelize computation
assay	Assay where the data is to be found (for input in 'sce' format)
ties.method	How ranking ties should be resolved (passed on to [data.table::frank])
force.gc	Explicitly call garbage collector to reduce memory footprint
seed	Integer seed

**Value**

Returns a sparse matrix of pre-calculated ranks that can be used multiple times to evaluate different signatures

Returns a sparse matrix of pre-calculated ranks that can be used multiple times to evaluate different signatures

---

u\_stat

*Calculate Mann Whitney U from a vector of ranks*


---

**Description**

Calculate Mann Whitney U from a vector of ranks

**Usage**

```
u_stat(rank_value, maxRank = 1000, sparse = FALSE)
```

**Arguments**

rank_value	A vector of ranks
maxRank	Max number of features to include in ranking
sparse	Whether the vector of ranks is in sparse format

**Value**

Normalized AUC (as U statistic) for the vector



---

u\_stat\_signature\_list *Calculate U scores for a list of signatures, given a rank matrix*

---

**Description**

Calculate U scores for a list of signatures, given a rank matrix

**Usage**

```
u_stat_signature_list(  
  sig_list,  
  ranks_matrix,  
  maxRank = 1000,  
  sparse = FALSE,  
  w_neg = 1  
)
```

**Arguments**

sig_list	A list of signatures
ranks_matrix	Matrix of pre-computed ranks
maxRank	Max number of features to include in ranking, for u_stat function
sparse	Whether the vector of ranks is in sparse format
w_neg	Weight on negative signatures

**Value**

A matrix of U scores

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