

# Package ‘escape’

September 16, 2021

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

**Version** 1.2.0

**Date** 2020-11-05

**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** Apache License 2.0

**Encoding** UTF-8

**LazyData** true

**RoxygenNote** 7.1.1

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

**Depends** R (>= 4.0)

**Imports** grDevices, dplyr, ggplot2, GSEABase, GSVA,  
SingleCellExperiment, limma, ggridges, msigdb, stats,  
BiocParallel, Matrix

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

**VignetteBuilder** knitr

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

**git\_branch** RELEASE\_3\_13

**git\_last\_commit** eac1d27

**git\_last\_commit\_date** 2021-05-19

**Date/Publication** 2021-09-16

**Author** Nick Borcharding [aut, cre],  
Jared Andrews [aut]

**Maintainer** Nick Borcharding <[ncborch@gmail.com](mailto:ncborch@gmail.com)>

## R topics documented:

enrichIt . . . . .	2
getGeneSets . . . . .	3
getSignificance . . . . .	4
masterPCAPlot . . . . .	4
pcaEnrichment . . . . .	5
performPCA . . . . .	6
ridgeEnrichment . . . . .	7
splitEnrichment . . . . .	8

<b>Index</b>	<b>10</b>
--------------	-----------

---

enrichIt	<i>Calculate gene set enrichment scores for single-cell data</i>
----------	--

---

### 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 gsva R package and the poisson distribution for RNA.

### Usage

```
enrichIt(obj, gene.sets = NULL, groups = 1000, cores = 2)
```

### Arguments

obj	The count matrix, Seurat, or SingleCellExperiment object.
gene.sets	Gene sets from <a href="#">getGeneSets</a> to use for the enrichment analysis. Alternatively a simple base R list where the names of 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.
groups	The number of cells to separate the enrichment calculation.
cores	The number of cores to use for parallelization.

### Value

Data frame of normalized enrichment scores (NES)

### Author(s)

Nick Borcharding, Jared Andrews

### See Also

[getGeneSets](#) to collect gene sets.

## Examples

```
# download HALLMARK gene set collection
GS <- getGeneSets(library = "H")
GS <- GS[c(1:2)] #Reduce list size for example
seurat_ex <- suppressWarnings(SeuratObject::pbmc_small)
ES <- enrichIt(obj = seurat_ex, gene.sets = GS)

# alternatively, construct your own list of gene sets
myGS <- list(Housekeeping = c("ACTA1", "ACTN1", "GAPDH"),
  Cancer = c("TP53", "BRCA2", "ERBB2", "MYC"))
```

---

getGeneSets

*Get a collection of gene sets to perform enrichment on*

---

## 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, gene.sets = NULL)
```

## Arguments

species	The scientific name of the species of interest in order to get correct gene nomenclature
library	Individual collection(s) of gene sets, e.g. c("H", "C5"). See <a href="https://www.gsea-msigdb.org/gsea/msigdb/collections.jsp">https://www.gsea-msigdb.org/gsea/msigdb/collections.jsp</a> for all MSigDB collections.
gene.sets	Select gene sets or pathways, using specific names, example: pathways = c("HALLMARK_TNFA_SIGNAL"). 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 3 tests: 1) linear model based on the limma package, 2) Welch's T test, and 3) one-way ANOVA. The output includes adjusted p-values based on the Benjamini Hochberg method.

### Usage

```
getSignificance(enriched, group = NULL, fit = "linear.model")
```

### Arguments

enriched	The output of <a href="#">enrichIt</a> .
group	The parameter to group for the comparison, should a column of the enriched input
fit	The test used for significance, either linear.model, ANOVA, or T.test

### 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 = "linear.model")
```

---

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, PCx, PCy, top.contribution = 10)
```

**Arguments**

enriched	The output of <a href="#">enrichIt</a> .
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", top.contribution = 10)
```

---

pcaEnrichment

*Density plot of the principal components*

---

**Description**

Density plot of the principal components

**Usage**

```
pcaEnrichment(
  PCAout,
  PCx,
  PCy,
  colors = c("#0348A6", "#7AC5FF", "#C6FDEC", "#FFB433", "#FF4B20"),
  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

*Calculate Principal Components for the Enrichment Scores*

---

**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, groups)
```

**Arguments**

enriched	The output of <a href="#">enrichIt</a> .
groups	The column headers to use in future graphing functions.

**Value**

Data frame of principal components

**Author(s)**

Nick Borchering

**Examples**

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

---

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("#0348A6", "#7AC5FF", "#C6FDEC", "#FFB433", "#FF4B20")  
)
```

## 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)
```

---

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("#0348A6", "#7AC5FF", "#C6FDEC", "#FFB433", "#FF4B20")
)
```

**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 seocndary bracket is autommatically 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")
```

# Index

`enrichIt`, [2](#), [4-8](#)

`getGeneSets`, [2](#), [3](#)

`getSignificance`, [4](#)

`masterPCAPlot`, [4](#)

`pcaEnrichment`, [4](#), [5](#)

`performPCA`, [5](#), [6](#), [6](#)

`ridgeEnrichment`, [7](#)

`splitEnrichment`, [8](#)