# Package 'escape'

April 12, 2022

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

**Version** 1.4.0

**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, msigdbr, 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_14

**git_last_commit** b3d13db

**git_last_commit_date** 2021-10-26

**Date/Publication** 2022-04-12

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

**Maintainer** Nick Borcherding <ncborch@gmail.com>

# R topics documented:



---

`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 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 `getGeneSets` 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 enrichmenet scores (NES)

## Author(s)

Nick Borcherding, 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 correcent gene nomenclature |
| `library` | Individual collection(s) of gene sets, e.g. c("H", "C5"). See https://www.gsea-msigdb.org/gsea/msigdb/collections.jsp for all MSigDB collections. |
| `gene.sets` | Select gene sets or pathways, using specific names, example: pathways = c("HALLMARK_TNFA_SIGNA Will only be honored if library is set, too. |

## Value

A `GeneSetCollection` object containing the requested `GeneSet` objects.

## Author(s)

Nick Borcherding, Jared Andrews

## Examples

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

## getSignificance *Perform significance testing between groups and enrichement scores.*

### 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 enrichIt. |
| 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 *Visualize the components of the PCA analysis of the enrichment results*

### Description

Graph the major gene set contributors to the pcaEnrichment.

### Usage

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

### Arguments

| | |
|---|---|
| enriched | The output of enrichIt. |
| 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 sumamrizing 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 performPCA |
| 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 enrichIt. |
| groups | The column headers to use in future graphing functions. |

## Value

Data frame of principal compoenents

## 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"))
```

`ridgeEnrichment` *Generate a ridge plot to examine enrichment distributions*

## 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 `enrichIt` |
| `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 seocndary bracket is autommatically 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` *Generate a split violin plot examine enrichment distributions*

---

## 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 `enrichIt` |
| `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