Package ‘escape’

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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 ssGSEA, GSVA, AUCell, and UCell-based enrichment calculations across individual cells.
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densityEnrichment

Visualize the mean density ranking of genes across gene set

Description
This function allows the user to examine the mean ranking within the groups across the gene set. The visualization uses the density function to display the relative position and distribution of rank.

Usage

densityEnrichment(
  input.data,
  gene.set.use = NULL,
  gene.sets = NULL,
  group.by = NULL,
  palette = "inferno"
)

Arguments

input.data The single-cell object to use.
gene.set.use Selected individual gene set.
gene.sets The gene set library to use to extract the individual gene set information from.
group.by Categorical parameter to plot along the x.axis. If input is a single-cell object the default will be cluster.
palette Colors to use in visualization - input any hcl.pals.

Value

ggplot2 object mean rank gene density across groups
### escape.gene.sets

**Built-In Gene Sets for escape**

**Description**

A list of gene sets derived from Azizi, et al 2018 (PMID: 29961579) relating to tumor immunity.

### escape.matrix

**Calculate gene set enrichment scores**

**Description**

This function allows users to input both the single-cell RNA-sequencing counts and output the enrichment scores as a matrix.

**Usage**

```r
escape.matrix(
  input.data,
  gene.sets = NULL,
  method = "ssGSEA",
  groups = 1000,
  min.size = 5,
  normalize = FALSE,
  make.positive = FALSE,
  BPPARAM = SerialParam(),
  ...
)
```
Arguments

input.data The count matrix, Seurat, or Single-Cell Experiment object.
gene.sets Gene sets can be a list, output from getGeneSets, or the built-in gene sets in the escape package escape.gene.sets.
method Select the method to calculate enrichment, AUCell, GSVA, ssGSEA or UCell.
groups The number of cells to separate the enrichment calculation.
min.size Minimum number of gene necessary to perform the enrichment calculation.
normalize Whether to divide the enrichment score by the number of genes TRUE or report unnormalized FALSE.
make.positive During normalization shift enrichment values to a positive range TRUE for downstream analysis or not TRUE (default). Will only be applied if normalize = TRUE.
BPPARAM A BiocParallel::bpparam() object that for parallelization.

Value

matrix of enrichment scores

Author(s)

Nick Borcherding, 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 <- SeuratObject::pbmc_small
ES <- escape.matrix(pbm_small,
                     gene.sets = GS,
                     min.size = NULL)

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.
geyserEnrichment

Usage

geneSets(
    species = "Homo sapiens",
    library = NULL,
    subcategory = 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 msigdb for all MSigDB collections.
subcategory MSigDB sub-collection abbreviation, such as CGP or BP.
gene.sets Select gene sets or pathways, using specific names, example: pathways = c("HALLMARK_TNFA_SIGNALING_VIA_NFKB"). Will only be honored if library is set, too.

Value

A list of gene sets from msigdb.

Author(s)

Nick Borcherding, Jared Andrews

Examples

GS <- getGeneSets(library = "H")

geyserEnrichment 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

geyserEnrichment(
    input.data,
    assay = NULL,
    group.by = NULL,
    gene.set = NULL,
geyserEnrichment

```r
color.by = "group",
order.by = NULL,
scale = FALSE,
facet.by = NULL,
palette = "inferno"
)
```

**Arguments**

- **input.data**: Enrichment output from `escape.matrix` or `runEscape`.
- **assay**: Name of the assay to plot if data is a single-cell object.
- **group.by**: Categorical parameter to plot along the x-axis. If input is a single-cell object the default will be cluster.
- **gene.set**: Gene set to plot (on y-axis).
- **color.by**: How the color palette applies to the graph - can be "group" for a categorical color palette based on the `group.by` parameter or use the `gene.set` name if wanting to apply a gradient palette.
- **order.by**: Method to organize the x-axis: "mean" will arrange the x-axis by the mean of the gene.set, while "group" will arrange the x-axis by in alphanumerical order. Using `NULL` will not reorder the x-axis.
- **scale**: Visualize raw values `FALSE` or Z-transform enrichment values `TRUE`.
- **facet.by**: Variable to facet the plot into n distinct graphs.
- **palette**: Colors to use in visualization - input any hcl.pals.

**Value**

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

**Examples**

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

geyserEnrichment(pbmc_small,
                 assay = "escape",
                 gene.set = "Tcells")
```
heatmapEnrichment

Generate a heatmap to visualize enrichment values

Description

This function allows the user to examine the heatmap with the mean enrichment values by group. The heatmap will have the gene sets as rows and columns will be the grouping variable.

Usage

heatmapEnrichment(
  input.data,
  assay = NULL,
  group.by = NULL,
  gene.set.use = "all",
  cluster.rows = FALSE,
  cluster.columns = FALSE,
  scale = FALSE,
  facet.by = NULL,
  palette = "inferno"
)

Arguments

input.data Enrichment output from escape.matrix or runEscape.
assay Name of the assay to plot if data is a single-cell object.
group.by Categorical parameter to plot along the x.axis. If input is a single-cell object the default will be cluster.
gene.set.use Selected gene sets to visualize. If "all", the heatmap will be generated across all gene sets.
cluster.rows Use Euclidean distance to order the row values.
cluster.columns Use Euclidean distance to order the column values.
scale Visualize raw values FALSE or Z-transform enrichment values TRUE.
facet.by Variable to facet the plot into n distinct graphs.
palette Colors to use in visualization - input any hcl.pals.

Value

ggplot2 object with heatmap of mean enrichment values
Examples

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

heatmapEnrichment(pbmc_small,
            assay = "escape")
```

**pcaEnrichment**

*Visualize the PCA of enrichment values*

**Description**

This function allows the user to examine the distribution of principal components run on the enrichment values.

**Usage**

```r
pcaEnrichment(
  input.data,
  dimRed = NULL,
  x.axis = "PC1",
  y.axis = "PC2",
  facet.by = NULL,
  style = "point",
  add.percent.contribution = TRUE,
  display.factors = FALSE,
  number.of.factors = 10,
  palette = "inferno"
)
```

**Arguments**

- `input.data` PCA from `performPCA`.
- `dimRed` Name of the dimensional reduction to plot if data is a single-cell object.
- `x.axis` Component to plot on the x.axis.
- `y.axis` Component set to plot on the y.axis.
- `facet.by` Variable to facet the plot into n distinct graphs.
- `style` Return a "hex" bin plot or a "point"-based plot.
- `add.percent.contribution` Add the relative percent of contribution of the selected components to the axis labels.
**performNormalization**

**display.factors**
Add an arrow overlay to show the direction and magnitude of individual gene sets on the PCA dimensions.

**number.of.factors**
The number of gene sets to display on the overlay.

**palette**
Colors to use in visualization - input any hcl.pals.

**Value**
ggplot2 object with PCA distribution

**Examples**

```r
GS <- list(Bcells = c("MS4A1", "CD79B", "CD79A", "IGH1", "IGH2"),
            Tcells = c("CD3E", "CD3D", "CD3G", "CD7", "CD8A"))
pbmc_small <- SeuratObject::pbmc_small
pbmc_small <- runEscape(pbmc_small,
                        gene.sets = GS,
                        min.size = NULL)
pbmc_small <- performPCA(pbmc_small,
                        assay = "escape")
pcaEnrichment(pbmc_small,
              x.axis = "PC1",
              y.axis = "PC2",
              dimRed = "escape.PCA")
```

---

**performNormalization**  
*Perform Normalization on Enrichment Data*

**Description**
This function allows users to normalize the enrichment calculations by accounting for single-cell dropout and producing positive values for downstream differential enrichment analyses. A positive range values is useful for several downstream analyses, like differential evaluation for log2-fold change, but will alter the original enrichment values.

**Usage**

```r
performNormalization(
  input.data,
  assay = NULL,
  gene.sets = NULL,
  make.positive = FALSE,
  scale.factor = NULL
)
```
Arguments

input.data  Enrichment output from escape.matrix or runEscape.
assay       Name of the assay to plot if data is a single-cell object.
gene.sets   The gene set library to use to extract the individual gene set information from.
make.positive Shift enrichment values to a positive range TRUE for downstream analysis or not TRUE (default).
scale.factor A vector to use for normalizing enrichment scores per cell.

Value

Single-cell object or matrix of normalized enrichment scores

Examples

GS <- list(Bcells = c("MS4A1", "CD79B", "CD79A", "IGH1", "IGH2"),
           Tcells = c("CD3E", "CD3D", "CD3G", "CD7", "CD8A"))
pbmc_small <- SeuratObject::pbmc_small
pbmc_small <- runEscape(pbmc_small,
                         gene.sets = GS,
                         min.size = NULL)
pbmc_small <- performNormalization(pbmc_small,
                                   assay = "escape",
                                   gene.sets = GS)

performPCA

Perform Principal Component Analysis on Enrichment Data

Description

This function allows users to calculate the principal components for the gene set enrichment values. For single-cell data, the PCA will be stored with the dimensional reductions. If a matrix is used as input, the output is a list for further plotting. Alternatively, users can use functions for PCA calculations based on their desired workflow in lieu of using performPCA, but will not be compatible with downstream pcaEnrichment visualization.

Usage

performPCA(
  input.data,
  assay = NULL,
  scale = TRUE,
  n.dim = 1:10,
  reduction.name = "escape.PCA",
  reduction.key = "PCA"
)
Arguments

input.data  
  Enrichment output from escape.matrix or runEscape.

assay  
  Name of the assay to plot if data is a single-cell object.

scale  
  Standardize the enrichment value (TRUE) or not (FALSE)

n.dim  
  The number of components to calculate.

reduction.name  
  Name of the reduced dimensions object to add if data is a single-cell object.

reduction.key  
  Name of the key to use with the components.

Value

single-cell object or list with PCA components to plot.

Examples

GS <- list(Bcells = c("MS4A1", "CD79B", "CD79A", "IGH1", "IGH2"),
            Tcells = c("CD3E", "CD3D", "CD3G", "CD7", "CD8A"))

pbmc_small <- SeuratObject::pbmc_small

pbmc_small <- runEscape(pbmc_small,
                        gene.sets = GS,
                        min.size = NULL)

pbmc_small <- performPCA(pbmc_small,
                         assay = "escape")

---

ridgeEnrichment  
  Visualize enrichment results with a ridge plot

Description

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

Usage

ridgeEnrichment(
  input.data,
  assay = NULL,
  group.by = NULL,
  gene.set = NULL,
  color.by = "group",
  order.by = NULL,
  scale = FALSE,
  facet.by = NULL,
  add.rug = FALSE,
  palette = "inferno"
)
**Arguments**

- **input.data**  
  Enrichment output from `escape.matrix` or `runEscape`.
- **assay**  
  Name of the assay to plot if data is a single-cell object.
- **group.by**  
  Categorical parameter to plot along the x-axis. If input is a single-cell object the default will be `cluster`.
- **gene.set**  
  Gene set to plot (on y-axis).
- **color.by**  
  How the color palette applies to the graph - can be "group" for a categorical color palette based on the `group.by` parameter or use the `gene.set` name if wanting to apply a gradient palette.
- **order.by**  
  Method to organize the x-axis: "mean" will arrange the x-axis by the mean of the gene.set, while "group" will arrange the x-axis by in alphanumerical order. Using `NULL` will not reorder the x-axis.
- **scale**  
  Visualize raw values `FALSE` or Z-transform enrichment values `TRUE`.
- **facet.by**  
  Variable to facet the plot into n distinct graphs.
- **add.rug**  
  Add visualization of the discrete cells along the ridge plot (`TRUE`).
- **palette**  
  Colors to use in visualization - input any `hcl.pals`.

**Value**

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

**Examples**

```r
GS <- list(Bcells = c("MS4A1", "CD79B", "CD79A", "IGH1", "IGH2"),
            Tcells = c("CD3E", "CD3D", "CD3G", "CD7", "CD8A"))

pbmc_small <- SeuratObject::pbmc_small
pbmc_small <- runEscape(pbmc_small,
                        gene.sets = GS,
                        min.size = NULL)

ridgeEnrichment(pbmc_small,
                 assay = "escape",
                 gene.set = "Tcells")

ridgeEnrichment(pbmc_small,
                 assay = "escape",
                 gene.set = "Tcells",
                 color.by = "Tcells")
```
runEscape: Enrichment calculation for single-cell workflows

Description

Run the escape-based gene-set enrichment calculation with Seurat or SingleCellExperiment pipelines

Usage

```r
runEscape(
  input.data,
  gene.sets = NULL,
  method = "ssGSEA",
  groups = 1000,
  min.size = 5,
  normalize = FALSE,
  make.positive = FALSE,
  new.assay.name = "escape",
  BPPARAM = SerialParam(),
  ...
)
```

Arguments

- `input.data`: The count matrix, Seurat, or Single-Cell Experiment object.
- `gene.sets`: Gene sets can be a list, output from `getGeneSets`, or the built-in gene sets in the escape package `escape.gene.sets`.
- `method`: Select the method to calculate enrichment, `AUCell`, `GSVA`, `ssGSEA` or `UCell`.
- `groups`: The number of cells to separate the enrichment calculation.
- `min.size`: Minimum number of gene necessary to perform the enrichment calculation.
- `normalize`: Whether to divide the enrichment score by the number of genes `TRUE` or report unnormalized `FALSE`.
- `make.positive`: During normalization shift enrichment values to a positive range `TRUE` for downstream analysis or not `TRUE` (default). Will only be applied if `normalize = TRUE`.
- `new.assay.name`: The new name of the assay to append to the single-cell object containing the enrichment scores.
- `BPPARAM`: A BiocParallel::bpparam() object that for parallelization.
- `...`: pass arguments to AUCell GSVA, ssGSEA or UCell call

Value

Seurat or Single-Cell Experiment object with escape enrichment scores in the assay slot.
Examples

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

---

scatterEnrichment

Generate a density-based scatter plot

Description

This function allows the user to examine the distribution of 2 gene sets along the x.axis and y.axis. The color gradient is generated using the a density estimate. See `ggpointdensity` for more information.

Usage

```r
scatterEnrichment(
  input.data,
  assay = NULL,
  x.axis = NULL,
  y.axis = NULL,
  scale = FALSE,
  facet.by = NULL,
  style = "point",
  palette = "inferno"
)
```

Arguments

- **input.data**: Enrichment output from `escape.matrix` or `runEscape`.
- **assay**: Name of the assay to plot if data is a single-cell object.
- **x.axis**: Gene set to plot on the x.axis.
- **y.axis**: Gene set to plot on the y.axis. **group.by** parameter or use the **gene.set** name if wanting to apply a gradient palette.
- **scale**: Visualize raw values **FALSE** or Z-transform enrichment values **TRUE**.
- **facet.by**: Variable to facet the plot into n distinct graphs.
- **style**: Return a "hex" bin plot or a "point"-based plot.
- **palette**: Colors to use in visualization - input any hcl.pals.

Value

`ggplot2` object with a scatter plot of selected gene.sets
Examples

```r
GS <- list(Bcells = c("MS4A1", "CD79B", "CD79A", "IGH1", "IGH2"),
            Tcells = c("CD3E", "CD3D", "CD3G", "CD7", "CD8A"))
pbmc_small <- SeuratObject::pbmc_small
pbmc_small <- runEscape(pbmc_small,
                       gene.sets = GS,
                       min.size = NULL)
scatterEnrichment(pbmc_small,
               assay = "escape",
               x.axis = "Tcells",
               y.axis = "Bcells")
```

splitEnrichment

Visualize enrichment results with a split violin plot

Description

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

Usage

```r
splitEnrichment(
  input.data,
  assay = NULL,
  split.by = NULL,
  group.by = NULL,
  gene.set = NULL,
  order.by = NULL,
  facet.by = NULL,
  scale = TRUE,
  palette = "inferno"
)
```

Arguments

- **input.data**: Enrichment output from *escape.matrix* or *runEscape*.
- **assay**: Name of the assay to plot if data is a single-cell object.
- **split.by**: Variable to form the split violin, must have 2 levels.
- **group.by**: Categorical parameter to plot along the x.axis. If input is a single-cell object the default will be cluster.
- **gene.set**: Gene set to plot (on y-axis).
- **order.by**: Method to organize the x-axis - "mean" will arrange the x-axis by the mean of the gene.set, while "group" will arrange the x-axis by in alphanumerical order. Using NULL will not reorder the x-axis.
splitEnrichment

- **facet.by** Variable to facet the plot into n distinct graphs.
- **scale** Visualize raw values **FALSE** or Z-transform enrichment values **TRUE**.
- **palette** Colors to use in visualization - input any hcl.pals.

**Value**

`ggplot2` object violin-based distributions of selected gene.set

**Examples**

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

splitEnrichment(pbmc_small,
                assay = "escape",
                split.by = "groups",
                gene.set = "Tcells")
```
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