

Package ‘ASURAT’

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Type Package

Title Functional annotation-driven unsupervised clustering for single-cell data

Version 1.11.0

Description ASURAT is a software for single-cell data analysis. Using ASURAT, one can simultaneously perform unsupervised clustering and biological interpretation in terms of cell type, disease, biological process, and signaling pathway activity. Inputting a single-cell RNA-seq data and knowledge-based databases, such as Cell Ontology, Gene Ontology, KEGG, etc., ASURAT transforms gene expression tables into original multivariate tables, termed sign-by-sample matrices (SSMs).

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| | |
|--------------|---|
| add_metadata | <i>Add metadata of variables and samples.</i> |
|--------------|---|

Description

This function adds metadata of variables and samples.

Usage

```
add_metadata(sce = NULL, mitochondria_symbol = NULL)
```

Arguments

`sce` A SingleCellExperiment object.

`mitochondria_symbol` A string representing for mitochondrial genes. This function computes percents of reads that map to the mitochondrial genes. Examples are `^MT-`, `^mt-`, etc.

Value

A SingleCellExperiment object.

Examples

```
data(pbmc_eg)
pbmc <- add_metadata(sce = pbmc_eg, mitochondria_symbol = "^MT-")
```

| | |
|--------|---|
| ASURAT | <i>Functional annotation-driven unsupervised clustering of SingleCell data.</i> |
|--------|---|

Description

ASURAT is a software for single-cell data analysis. Using ASURAT, one can simultaneously perform unsupervised clustering and biological interpretation in terms of cell type, disease, biological process, and signaling pathway activity. Inputting a single-cell RNA-seq data and knowledge-based databases, such as Cell Ontology, Gene Ontology, KEGG, etc., ASURAT transforms gene expression tables into original multivariate tables, termed sign-by-sample matrices (SSMs).

| | |
|--------------------------|--|
| <code>bubble_sort</code> | <i>Perform bubble sorting, counting the number of steps.</i> |
|--------------------------|--|

Description

Perform bubble sorting, counting the number of steps.

Usage

```
bubble_sort(listdata)
```

Arguments

`listdata` A list of vector and integer. For example, in R code, `listdata = list(vec = c(1, 0, 1, ...), cnt = 0)`. The integer (`cnt = 0`) is the initial number of steps for bubble sorting.

Value

A List.

Examples

```
bubble_sort(list(vec = c(1, 1, 0), cnt = 0))
```

| | |
|------------------|--|
| cluster_genesets | <i>Cluster each functional gene set into three groups.</i> |
|------------------|--|

Description

This function clusters each functional gene set into strongly, variably, and weakly correlated gene sets.

Usage

```
cluster_genesets(sce = NULL, cormat = NULL, th_posi = NULL, th_neg = NULL)
```

Arguments

| | |
|---------|--|
| sce | A SingleCellExperiment object. |
| cormat | A correlation matrix of gene expressions. |
| th_posi | A threshold of positive correlation coefficient. |
| th_neg | A threshold of negative correlation coefficient. |

Value

A SingleCellExperiment object.

Examples

```
data(pbmc_eg)
data(human_GO_eg)
mat <- t(as.matrix(SummarizedExperiment::assay(pbmc_eg, "centered")))
pbmc_cormat <- cor(mat, method = "spearman")
pbmcs <- list(GO = pbmc_eg)
S4Vectors::metadata(pbmcs$GO) <- list(sign = human_GO_eg[["BP"]])
pbmcs$GO <- remove_signs(sce = pbmcs$GO, min_genes = 2, max_genes = 1000)
pbmcs$GO <- cluster_genesets(sce = pbmcs$GO, cormat = pbmc_cormat,
                             th_posi = 0.24, th_neg = -0.20)
# The results are stored in `metadata(pbmcs$GO)$sign`.
```

compute_sepI_all *Compute separation indices for each cluster against the others.*

Description

This function computes separation indices for each cluster versus the others.

Usage

```
compute_sepI_all(sce = NULL, labels = NULL, nrand_samples = NULL)
```

Arguments

sce A SingleCellExperiment object.
 labels A vector of labels of all the samples (cells).
 nrand_samples An integer for the number of samples used for random sampling, which samples at least one sample per cluster.

Value

A SingleCellExperiment object.

Examples

```
data(pbmcs_eg)
labels <- SummarizedExperiment::colData(pbmcs_eg$GO)$seurat_clusters
pbmcs_eg$GO <- compute_sepI_all(sce = pbmcs_eg$GO, labels = labels,
                               nrand_samples = 10)
# The results are stored in `metadata(pbmcs_eg$GO)$marker_signs`.
```

compute_sepI_clusters *Compute separation indices of sign scores for given two clusters.*

Description

This function computes separation indices of sign scores for given two clusters.

Usage

```
compute_sepI_clusters(
  sce = NULL,
  labels = NULL,
  nrand_samples = NULL,
  ident_1 = NULL,
  ident_2 = NULL
)
```

Arguments

| | |
|---------------|---|
| sce | A SingleCellExperiment object. |
| labels | A vector of labels of all the samples. |
| nrand_samples | An integer for the number of samples used for random sampling, which samples at least one sample per cluster. |
| ident_1 | Label names identifying cluster numbers, e.g., <code>ident_1 = 1</code> , <code>ident_1 = c(1, 3)</code> . |
| ident_2 | Label names identifying cluster numbers, e.g., <code>ident_2 = 2</code> , <code>ident_2 = c(2, 4)</code> . |

Value

A SingleCellExperiment object.

Examples

```
data(pbmcs_eg)
labels <- SummarizedExperiment::colData(pbmcs_eg$G0)$seurat_clusters
pbmcs_eg$G0 <- compute_sepI_clusters(sce = pbmcs_eg$G0, labels = labels,
                                   nrand_samples = 10, ident_1 = 1,
                                   ident_2 = c(0, 2))
# The results are stored in `metadata(pbmcs_eg$G0)$marker_signs`.
```

create_signs

Define signs for strongly and variably correlated gene sets.

Description

This function define signs for strongly and variably correlated gene sets.

Usage

```
create_signs(sce = NULL, min_cnt_strg = 2, min_cnt_vari = 2)
```

Arguments

| | |
|--------------|--|
| sce | A SingleCellExperiment object. |
| min_cnt_strg | An integer for the cutoff value for strongly correlated gene sets. |
| min_cnt_vari | An integer for the cutoff value for variably correlated gene sets. |

Value

A SingleCellExperiment object.

Examples

```

data(pbmc_eg)
data(human_GO_eg)
mat <- t(as.matrix(SummarizedExperiment::assay(pbmc_eg, "centered")))
pbmc_cormat <- cor(mat, method = "spearman")
pbmcs <- list(GO = pbmc_eg)
S4Vectors::metadata(pbmcs$GO) <- list(sign = human_GO_eg[["BP"]])
pbmcs$GO <- remove_signs(sce = pbmcs$GO, min_ngenes = 2, max_ngenes = 1000)
pbmcs$GO <- cluster_genesets(sce = pbmcs$GO, cormat = pbmc_cormat,
                             th_posi = 0.24, th_negs = -0.20)
pbmcs$GO <- create_signs(sce = pbmcs$GO, min_cnt_strg = 2, min_cnt_vari = 2)
# The results are stored in `metadata(pbmcs$GO)$sign_all`.

```

| | |
|-----------------|--|
| human_COMSig_eg | <i>A list of small Cell Ontology and MSigDB databases for human.</i> |
|-----------------|--|

Description

A list of small Cell Ontology and MSigDB databases for human.

Usage

```
human_COMSig_eg
```

Format

A list of dataframe.

| | |
|-------------|--|
| human_GO_eg | <i>A list of small Gene Ontology database for human.</i> |
|-------------|--|

Description

A list of small Gene Ontology database for human.

Usage

```
human_GO_eg
```

Format

A list of dataframe.

| | |
|---------------|---|
| human_KEGG_eg | <i>A list of small KEGG database for human.</i> |
|---------------|---|

Description

A list of small KEGG database for human.

Usage

```
human_KEGG_eg
```

Format

A list of dataframe.

| | |
|----------------|--|
| makeSignMatrix | <i>Create a new SingleCellExperiment object for sign-by-sample matrices.</i> |
|----------------|--|

Description

This function creates a new `SingleCellExperiment` object for sign-by-sample matrices (SSM) by concatenating SSMs for strongly and variably correlated gene sets.

Usage

```
makeSignMatrix(sce = NULL, weight_strg = 0.5, weight_vari = 0.5)
```

Arguments

| | |
|-------------|---|
| sce | A <code>SingleCellExperiment</code> object. |
| weight_strg | A weight parameter for strongly correlated gene sets. |
| weight_vari | A weight parameter for variably correlated gene sets. |

Value

A `SingleCellExperiment` object.

Examples

```

data(pbmcs_eg)
data(human_GO_eg)
mat <- t(as.matrix(SummarizedExperiment::assay(pbmcs_eg, "centered")))
pbmc_cormat <- cor(mat, method = "spearman")
pbmcs <- list(GO = pbmc_eg)
S4Vectors::metadata(pbmcs$GO) <- list(sign = human_GO_eg[["BP"]])
pbmcs$GO <- remove_signs(sce = pbmcs$GO, min_ngenes = 2, max_ngenes = 1000)
pbmcs$GO <- cluster_genesets(sce = pbmcs$GO, cormat = pbmc_cormat,
                             th_posi = 0.24, th_negs = -0.20)
pbmcs$GO <- create_signs(sce = pbmcs$GO, min_cnt_strg = 2, min_cnt_vari = 2)
pbmcs$GO <- makeSignMatrix(sce = pbmcs$GO, weight_strg = 0.5,
                           weight_vari = 0.5)
# The results can be check by, e.g., assay(pbmcs$GO, "counts").

```

| | |
|----------|---|
| pbmcs_eg | <i>A list of SingleCellExperiment objects made from sign-sample matrices.</i> |
|----------|---|

Description

A list of SingleCellExperiment objects, consisting of small sign-by-sample matrices, pbmcs_eg\$CM (using Cell Ontology and MSigDB databases), pbmcs_eg\$GO (using Gene Ontology database), and pbmcs_eg\$KG (KEGG). Here, pbmcs_eg\$CM, pbmcs_eg\$GO, and pbmcs_eg\$KG include 87, 72, and 64 signs, respectively, and 50 cells.

Usage

```
pbmcs_eg
```

Format

A list of SingleCellExperiment objects.

| | |
|---------|---|
| pbmc_eg | <i>A SingleCellExperiment object made from a gene expression table.</i> |
|---------|---|

Description

A SingleCellExperiment object, including 50 genes and 50 cells. The original data "4k PBMCs from a Healthy Donor" was downloaded from 10x Genomics database.

Usage

```
pbmc_eg
```

Format

SingleCellExperiment object.

Source

<https://support.10xgenomics.com/single-cell-gene-expression>

| | |
|------------------|---|
| plot_dataframe3D | <i>Visualize a three-dimensional data with labels and colors.</i> |
|------------------|---|

Description

This function visualizes a three-dimensional data with labels and colors.

Usage

```
plot_dataframe3D(  
  dataframe3D = NULL,  
  labels = NULL,  
  colors = NULL,  
  theta = 30,  
  phi = 30,  
  title = "",  
  xlabel = "",  
  ylabel = "",  
  zlabel = ""  
)
```

Arguments

| | |
|-------------|---|
| dataframe3D | A dataframe with three columns. |
| labels | NULL or a vector of labels of all the samples, corresponding to colors. |
| colors | NULL or a vector of colors of all the samples, corresponding to labels. |
| theta | Angle of the plot. |
| phi | Angle of the plot. |
| title | Title. |
| xlabel | x-axis label. |
| ylabel | y-axis label. |
| zlabel | z-axis label. |

Value

A scatter3D object in plot3D package.

Examples

```

data(pbmcs_eg)
mat <- SingleCellExperiment::reducedDim(pbmcs_eg$CM, "UMAP")[, 1:3]
dataframe3D <- as.data.frame(mat)
labels <- SummarizedExperiment::colData(pbmcs_eg$CM)$seurat_clusters
plot_dataframe3D(dataframe3D = dataframe3D, labels = labels, colors = NULL,
                 theta = 45, phi = 20, title = "PBMC (CO & MSigDB)",
                 xlabel = "UMAP_1", ylabel = "UMAP_2", zlabel = "UMAP_3")

```

plot_multiheatmaps *Visualize multivariate data by heatmaps.*

Description

This function visualizes multivariate data by heatmaps.

Usage

```

plot_multiheatmaps(
  ssm_list = NULL,
  gem_list = NULL,
  ssmlabel_list = NULL,
  gemlabel_list = NULL,
  nrand_samples = NULL,
  show_row_names = FALSE,
  title = NULL
)

```

Arguments

| | |
|----------------|--|
| ssm_list | A list of sign-by-sample matrices. |
| gem_list | A list of gene-by-sample matrices. |
| ssmlabel_list | NULL or a list of dataframes of sample (cell) labels and colors. The length of the list must be as same as that of ssm_list, and the order of labels in each list must be as same as those in ssm_list. |
| gemlabel_list | NULL or a list of dataframes of sample (cell) annotations and colors. The length of the list must be as same as that of gem_list, and the order of labels in each list must be as same as those in gem_list. |
| nrand_samples | Number of samples (cells) used for random sampling. |
| show_row_names | TRUE or FALSE: if TRUE, row names are shown. |
| title | Title. |

Value

A ComplexHeatmap object.

Examples

```

data(pbmcs_eg)
mat_CM <- SummarizedExperiment::assay(pbmcs_eg$CM, "counts")
mat_GO <- SummarizedExperiment::assay(pbmcs_eg$GO, "counts")
mat_KG <- SummarizedExperiment::assay(pbmcs_eg$KG, "counts")
ssm_list <- list(SSM_COMSig = mat_CM, SSM_GO = mat_GO, SSM_KEGG = mat_KG)
se <- SingleCellExperiment::altExp(pbmcs_eg$CM, "logcounts")
mat <- SummarizedExperiment::assay(se, "counts")
se <- SingleCellExperiment::altExp(pbmcs_eg$CM, "logcounts")
gem_list <- list(GeneExpr = SummarizedExperiment::assay(se, "counts"))
labels <- list() ; ssmlabel_list <- list()
for(i in seq_along(pbmcs_eg)){
  fa <- SummarizedExperiment::colData(pbmcs_eg[[i]])$seurat_clusters
  labels[[i]] <- data.frame(label = fa)
  colors <- rainbow(length(unique(labels[[i]]$label)))[labels[[i]]$label]
  labels[[i]]$color <- colors
  ssmlabel_list[[i]] <- labels[[i]]
}
names(ssmlabel_list) <- c("Label_COMSig", "Label_GO", "Label_KEGG")
phases <- SummarizedExperiment::colData(pbmcs_eg$CM)$Phase
label_CC <- data.frame(label = phases, color = NA)
gemlabel_list <- list(CellCycle = label_CC)
plot_multiheatmaps(ssm_list = ssm_list, gem_list = gem_list,
                   ssmlabel_list = ssmlabel_list,
                   gemlabel_list = gemlabel_list, nrand_samples = 50,
                   show_row_names = FALSE, title = "PBMC")

```

remove_samples

Remove samples based on expression profiles across variables.

Description

This function removes sample data by setting minimum and maximum threshold values for the metadata.

Usage

```

remove_samples(
  sce = NULL,
  min_nReads = NULL,
  max_nReads = NULL,
  min_nGenes = NULL,
  max_nGenes = NULL,
  min_percMT = NULL,
  max_percMT = NULL
)

```

Arguments

| | |
|------------|--|
| sce | A SingleCellExperiment object. |
| min_nReads | A minimum threshold value of the number of reads. |
| max_nReads | A maximum threshold value of the number of reads. |
| min_nGenes | A minimum threshold value of the number of non-zero expressed genes. |
| max_nGenes | A maximum threshold value of the number of non-zero expressed genes. |
| min_percMT | A minimum threshold value of the percent of reads that map to mitochondrial genes. |
| max_percMT | A maximum threshold value of the percent of reads that map to mitochondrial genes. |

Value

A SingleCellExperiment object.

Examples

```
data(pbmc_eg)
pbmc <- add_metadata(sce = pbmc_eg, mitochondria_symbol = "^MT-")
pbmc <- remove_samples(sce = pbmc, min_nReads = 0, max_nReads = 1e+10,
                      min_nGenes = 0, max_nGenes = 1e+10,
                      min_percMT = NULL, max_percMT = NULL)
```

remove_signs

Remove signs including too few or too many genes.

Description

This function removes signs including too few or too many genes.

Usage

```
remove_signs(sce = NULL, min_ngenes = 2, max_ngenes = 1000)
```

Arguments

| | |
|------------|--|
| sce | A SingleCellExperiment object. |
| min_ngenes | Minimum number of genes, which must be greater than one. |
| max_ngenes | Maximum number of genes, which must be greater than one. |

Value

A SingleCellExperiment object.

Examples

```

data(pbmc_eg)
data(human_GO_eg)
pbmcs <- list(GO = pbmc_eg)
S4Vectors::metadata(pbmcs$GO) <- list(sign = human_GO_eg[["BP"]])
pbmcs$GO <- remove_signs(sce = pbmcs$GO, min_ngenes = 2, max_ngenes = 1000)
# The results are stored in `metadata(pbmcs$GO)$sign`.

```

remove_signs_manually *Remove signs by specifying keywords.*

Description

This function removes signs by specifying keywords.

Usage

```
remove_signs_manually(sce = NULL, keywords = NULL)
```

Arguments

| | |
|----------|--------------------------------------|
| sce | A SingleCellExperiment object. |
| keywords | keywords separated by pipes ' '. |

Value

A SingleCellExperiment object.

Examples

```

data(pbmc_eg)
data(human_GO_eg)
mat <- t(as.matrix(SummarizedExperiment::assay(pbmc_eg, "centered")))
pbmc_cormat <- cor(mat, method = "spearman")
pbmcs <- list(GO = pbmc_eg)
S4Vectors::metadata(pbmcs$GO) <- list(sign = human_GO_eg[["BP"]])
pbmcs$GO <- remove_signs(sce = pbmcs$GO, min_ngenes = 2, max_ngenes = 1000)
pbmcs$GO <- cluster_genesets(sce = pbmcs$GO, cormat = pbmc_cormat,
                             th_posi = 0.24, th_neg = -0.20)
pbmcs$GO <- create_signs(sce = pbmcs$GO, min_cnt_strg = 2, min_cnt_vari = 2)
keywords <- "Covid19|foofoo|hogegege"
pbmcs$GO <- remove_signs_manually(sce = pbmcs$GO, keywords = keywords)
# The results are stored in `metadata(pbmcs$GO)$sign_SCG`,
# `metadata(pbmcs$GO)$sign_VCG`, and `metadata(pbmcs$GO)$sign_all`.

```

 remove_signs_redundant

Remove redundant signs using semantic similarity matrices.

Description

This function removes redundant signs using semantic similarity matrices.

Usage

```
remove_signs_redundant(
  sce = NULL,
  similarity_matrix = NULL,
  threshold = NULL,
  keep_rareID = NULL
)
```

Arguments

| | |
|-------------------|---|
| sce | A SingleCellExperiment object. |
| similarity_matrix | A semantic similarity matrix. |
| threshold | A threshold value of semantic similarity, used for regarding biological terms as similar ones |
| keep_rareID | If TRUE, biological terms with the larger ICs are kept. |

Value

A SingleCellExperiment object.

Examples

```
data(pbmc_eg)
data(human_GO_eg)
mat <- t(as.matrix(SummarizedExperiment::assay(pbmc_eg, "centered")))
pbmc_cormat <- cor(mat, method = "spearman")
pbmcs <- list(GO = pbmc_eg)
S4Vectors::metadata(pbmcs$GO) <- list(sign = human_GO_eg[["BP"]])
pbmcs$GO <- remove_signs(sce = pbmcs$GO, min_ngenes = 2, max_ngenes = 1000)
pbmcs$GO <- cluster_genesets(sce = pbmcs$GO, cormat = pbmc_cormat,
  th_posi = 0.24, th_neg = -0.20)
pbmcs$GO <- create_signs(sce = pbmcs$GO, min_cnt_strg = 2, min_cnt_vari = 2)
pbmcs$GO <- remove_signs_redundant(
  sce = pbmcs$GO, similarity_matrix = human_GO_eg$similarity_matrix$BP,
  threshold = 0.80, keep_rareID = TRUE)
# The results are stored in `metadata(pbmcs$GO)$sign_SCG`,
# `metadata(pbmcs$GO)$sign_VCG`, `metadata(pbmcs$GO)$sign_all`,
# and if there exist, `metadata(pbmcs$GO)$sign_SCG_redundant` and
```

```
# `metadata(pbmcs$GO)$sign_VCG_redundant`.
```

remove_variables *Remove variables based on expression profiles across samples.*

Description

This function removes low expressed variable data.

Usage

```
remove_variables(sce = NULL, min_nsamples = 0)
```

Arguments

sce A SingleCellExperiment object.

min_nsamples An integer. This function removes variables for which the numbers of non-zero expressing samples are less than this value.

Value

A SingleCellExperiment object.

Examples

```
data(pbmc_eg)
pbmc <- add_metadata(sce = pbmc_eg, mitochondria_symbol = "^MT-")
pbmc <- remove_variables(sce = pbmc, min_nsamples = 10)
```

remove_variables_second *Remove variables based on the mean expression levels across samples.*

Description

This function removes variable data such that the mean expression levels across samples are less than 'min_meannReads'.

Usage

```
remove_variables_second(sce = NULL, min_meannReads = 0)
```


Arguments

sce A SingleCellExperiment object.

min_meannReads An integer. This function removes variables for which the mean read counts are less than this value.

Value

A SingleCellExperiment object.

Examples

```
data(pbmc_eg)
pbmc <- remove_variables_second(sce = pbmc_eg, min_meannReads = 0.01)
```

select_signs_manually *Select signs by specifying keywords.*

Description

This function selects signs by specifying keywords.

Usage

```
select_signs_manually(sce = NULL, keywords = NULL)
```

Arguments

sce An ASURAT object.

keywords Keywords separated by a pipe.

Value

An ASURAT object.

Examples

```
data(pbmc_eg)
data(human_GO_eg)
mat <- t(as.matrix(SummarizedExperiment::assay(pbmc_eg, "centered")))
pbmc_cormat <- cor(mat, method = "spearman")
pbmcs <- list(GO = pbmc_eg)
S4Vectors::metadata(pbmcs$GO) <- list(sign = human_GO_eg[["BP"]])
pbmcs$GO <- remove_signs(sce = pbmcs$GO, min_ngenes = 2, max_ngenes = 1000)
pbmcs$GO <- cluster_genesets(sce = pbmcs$GO, cormat = pbmc_cormat,
                           th_posi = 0.24, th_neg = -0.20)
pbmcs$GO <- create_signs(sce = pbmcs$GO, min_cnt_strg = 2, min_cnt_vari = 2)
keywords <- "cell|process"
```

```
pbmcs$GO <- select_signs_manually(sce = pbmcs$GO, keywords = keywords)
# The results are stored in `metadata(pbmcs$GO)$sign_SCG`,
# `metadata(pbmcs$GO)$sign_VCG`, and `metadata(pbmcs$GO)$sign_all`.
```

swap_pass

Perform one-shot adjacent swapping for each element.

Description

Perform one-shot adjacent swapping for each element.

Usage

```
swap_pass(listdata)
```

Arguments

listdata A list of vector and integer.

Value

A List.

Examples

```
swap_pass(list(vec = c(1, 1, 0), cnt = 0))
```

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