

Package ‘singleCellHaystack’

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Type Package

Title Finding Needles (=differentially Expressed Genes) in Haystacks
(=single Cell Data)

Version 0.3.4

Description Identification of differentially expressed genes (DEGs) is a key step in single-cell transcriptomics data analysis. 'singleCellHaystack' predicts DEGs without relying on clustering of cells into arbitrary clusters. Single-cell RNA-seq (scRNA-seq) data is often processed to fewer dimensions using Principal Component Analysis (PCA) and represented in 2-dimensional plots (e.g. t-SNE or UMAP plots). 'singleCellHaystack' uses Kullback-Leibler divergence to find genes that are expressed in subsets of cells that are non-randomly positioned in a these multi-dimensional spaces or 2D representations. For the theoretical background of 'singleCellHaystack' we refer to Vandenberg and Diez (Nature Communications, 2020) <doi:10.1038/s41467-020-17900-3>.

Imports methods, Matrix, splines, ggplot2, reshape2

Suggests knitr, rmarkdown, SummarizedExperiment, SingleCellExperiment,
SeuratObject, Rtsne, cowplot, testthat, wrswor

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| | |
|----------------|-------------------------------------|
| dat.expression | <i>Single cell RNA-seq dataset.</i> |
|----------------|-------------------------------------|

Description

Single cell RNA-seq dataset.

| | |
|----------|---------------------------------------|
| dat.tsne | <i>Single cell tSNE coordingates.</i> |
|----------|---------------------------------------|

Description

Single cell tSNE coordingates.

default_bandwidth.nrd *Default function given by function bandwidth.nrd in MASS. No changes were made to this function.*

Description

Default function given by function bandwidth.nrd in MASS. No changes were made to this function.

Usage

```
default_bandwidth.nrd(x)
```

Arguments

x A numeric vector

Value

A suitable bandwidth.

extract_row_dgRMatrix *Returns a row of a sparse matrix of class dgRMatrix. Function made by Ben Bolker and Ott Toomet (see <https://stackoverflow.com/questions/47997184/>)*

Description

Returns a row of a sparse matrix of class dgRMatrix. Function made by Ben Bolker and Ott Toomet (see <https://stackoverflow.com/questions/47997184/>)

Usage

```
extract_row_dgRMatrix(m, i = 1)
```

Arguments

m a sparse matrix of class dgRMatrix
i the index of the row to return

Value

A row (numerical vector) of the sparse matrix

`extract_row_lgRMatrix` Returns a row of a sparse matrix of class `lgRMatrix`. Function made by Ben Bolker and Ott Toomet (see <https://stackoverflow.com/questions/47997184/>)

Description

Returns a row of a sparse matrix of class `lgRMatrix`. Function made by Ben Bolker and Ott Toomet (see <https://stackoverflow.com/questions/47997184/>)

Usage

```
extract_row_lgRMatrix(m, i = 1)
```

Arguments

| | |
|----------------|---|
| <code>m</code> | a sparse matrix of class <code>lgRMatrix</code> |
| <code>i</code> | the index of the row to return |

Value

A row (logical vector) of the sparse matrix

`get_density` Function to get the density of points with value `TRUE` in the (x,y) plot

Description

Function to get the density of points with value `TRUE` in the (x,y) plot

Usage

```
get_density(  
  x,  
  y,  
  detection,  
  rows.subset = 1:nrow(detection),  
  high.resolution = FALSE  
)
```

Arguments

| | |
|-----------------|---|
| x | x-axis coordinates of cells in a 2D representation (e.g. resulting from PCA or t-SNE) |
| y | y-axis coordinates of cells in a 2D representation |
| detection | A logical matrix or dgRMatrix showing which gens (rows) are detected in which cells (columns) |
| rows.subset | Indices of the rows of 'detection' for which to get the densities. Default: all. |
| high.resolution | Logical: should high resolution be used? Default is FALSE. |

Value

A 3-dimensional array (dim 1: genes/rows of expression, dim 2 and 3: x and y grid points) with density data

| | |
|-------------------|---|
| get_dist_two_sets | <i>Calculate the pairwise Euclidean distances between the rows of 2 matrices.</i> |
|-------------------|---|

Description

Calculate the pairwise Euclidean distances between the rows of 2 matrices.

Usage

```
get_dist_two_sets(set1, set2)
```

Arguments

| | |
|------|---------------------|
| set1 | A numerical matrix. |
| set2 | A numerical matrix. |

Value

A matrix of pairwise distances between the rows of 2 matrices.

| | |
|----------|--|
| get_D_KL | <i>Calculates the Kullback-Leibler divergence between distributions.</i> |
|----------|--|

Description

Calculates the Kullback-Leibler divergence between distributions.

Usage

```
get_D_KL(classes, parameters, reference.prob, pseudo)
```

Arguments

| | |
|----------------|--|
| classes | A logical vector. Values are T is the gene is expressed in a cell, F is not. |
| parameters | Parameters of the analysis, as set by function 'get_parameters_haystack' |
| reference.prob | A reference distribution to calculate the divergence against. |
| pseudo | A pseudocount, used to avoid log(0) problems. |

Value

A numerical value, the Kullback-Leibler divergence

| | |
|----------------|---|
| get_D_KL_highD | <i>Calculates the Kullback-Leibler divergence between distributions for the high-dimensional version of haystack().</i> |
|----------------|---|

Description

Calculates the Kullback-Leibler divergence between distributions for the high-dimensional version of haystack().

Usage

```
get_D_KL_highD(classes, density.contributions, reference.prob, pseudo = 0)
```

Arguments

| | |
|-----------------------|---|
| classes | A logical vector. Values are T is the gene is expressed in a cell, F is not. |
| density.contributions | A matrix of density contributions of each cell (rows) to each center point (columns). |
| reference.prob | A reference distribution to calculate the divergence against. |
| pseudo | A pseudocount, used to avoid log(0) problems. |

Value

A numerical value, the Kullback-Leibler divergence

`get_euclidean_distance`*Calculate the Euclidean distance between x and y.*

Description

Calculate the Euclidean distance between x and y.

Usage

```
get_euclidean_distance(x, y)
```

Arguments

| | |
|---|---------------------|
| x | A numerical vector. |
| y | A numerical vector. |

Value

A numerical value, the Euclidean distance.

`get_grid_points`*A function to decide grid points in a higher-dimensional space*

Description

A function to decide grid points in a higher-dimensional space

Usage

```
get_grid_points(input, method = "centroid", grid.points = 100)
```

Arguments

| | |
|-------------|---|
| input | A numerical matrix with higher-dimensional coordinates (columns) of points (rows) |
| method | The method to decide grid points. Should be "centroid" (default) or "seeding". |
| grid.points | The number of grid points to return. Default is 100. |

Value

Coordinates of grid points in the higher-dimensional space.

| | |
|----------------|--|
| get_log_p_D_KL | <i>Estimates the significance of the observed Kullback-Leibler divergence by comparig to randomizations.</i> |
|----------------|--|

Description

Estimates the significance of the observed Kullback-Leibler divergence by comparig to randomizations.

Usage

```
get_log_p_D_KL(T.counts, D_KL.observed, D_KL.randomized, output.dir = NULL)
```

Arguments

| | |
|-----------------|---|
| T.counts | The number of cells in which a gene is detected. |
| D_KL.observed | A vector of observed Kullback-Leibler divergences. |
| D_KL.randomized | A matrix of Kullback-Leibler divergences of randomized datasets. |
| output.dir | Optional parameter. Default is NULL. If not NULL, some files will be written to this directory. |

Value

A vector of log₁₀ p values, not corrected for multiple testing using the Bonferroni correction.

| | |
|-------------------------|--|
| get_parameters_haystack | <i>Function that decides most of the parameters that will be during the "Haystack" analysis.</i> |
|-------------------------|--|

Description

Function that decides most of the parameters that will be during the "Haystack" analysis.

Usage

```
get_parameters_haystack(x, y, high.resolution = FALSE)
```

Arguments

| | |
|-----------------|---|
| x | x-axis coordinates of cells in a 2D representation (e.g. resulting from PCA or t-SNE) |
| y | y-axis coordinates of cells in a 2D representation |
| high.resolution | Logical: should high resolution be used? Default is FALSE. |

Value

A list containing various parameters to use in the analysis.

| | |
|---------------|-----------------------------------|
| get_reference | <i>Get reference distribution</i> |
|---------------|-----------------------------------|

Description

Get reference distribution

Usage

```
get_reference(param, use.advanced.sampling = NULL)
```

Arguments

| | |
|-----------------------|---|
| param | Parameters of the analysis, as set by function 'get_parameters_haystack' |
| use.advanced.sampling | If NULL naive sampling is used. If a vector is given (of length = no. of cells) sampling is done according to the values in the vector. |

Value

A list with two components, Q for the reference distribution and pseudo.

| | |
|----------|-----------------------------------|
| haystack | <i>The main Haystack function</i> |
|----------|-----------------------------------|

Description

The main Haystack function

Usage

```
haystack(x, ...)

## S3 method for class 'matrix'
haystack(
  x,
  dim1 = 1,
  dim2 = 2,
  detection,
  method = "highD",
  use.advanced.sampling = NULL,
  dir.randomization = NULL,
```

```
    scale = TRUE,
    grid.points = 100,
    grid.method = "centroid",
    ...
)

## S3 method for class 'data.frame'
haystack(
  x,
  dim1 = 1,
  dim2 = 2,
  detection,
  method = "highD",
  use.advanced.sampling = NULL,
  dir.randomization = NULL,
  scale = TRUE,
  grid.points = 100,
  grid.method = "centroid",
  ...
)

## S3 method for class 'Seurat'
haystack(
  x,
  assay = "RNA",
  slot = "data",
  coord = "pca",
  dims = NULL,
  cutoff = 1,
  method = NULL,
  use.advanced.sampling = NULL,
  ...
)

## S3 method for class 'SingleCellExperiment'
haystack(
  x,
  assay = "counts",
  coord = "TSNE",
  dims = NULL,
  cutoff = 1,
  method = NULL,
  use.advanced.sampling = NULL,
  ...
)
```

Arguments

x a matrix or other object from which coordinates of cells can be extracted.

| | |
|-----------------------|---|
| ... | further parameters passed down to methods. |
| dim1 | column index or name of matrix for x-axis coordinates. |
| dim2 | column index or name of matrix for y-axis coordinates. |
| detection | A logical matrix showing which genes (rows) are detected in which cells (columns) |
| method | choose between highD (default) and 2D haystack. |
| use.advanced.sampling | If NULL naive sampling is used. If a vector is given (of length = no. of cells) sampling is done according to the values in the vector. |
| dir.randomization | If NULL, no output is made about the random sampling step. If not NULL, files related to the randomizations are printed to this directory. |
| scale | Logical (default=TRUE) indicating whether input coordinates in x should be scaled to mean 0 and standard deviation 1. |
| grid.points | An integer specifying the number of centers (gridpoints) to be used for estimating the density distributions of cells. Default is set to 100. |
| grid.method | The method to decide grid points for estimating the density in the high-dimensional space. Should be "centroid" (default) or "seeding". |
| assay | name of assay data for Seurat method. |
| slot | name of slot for assay data for Seurat method. |
| coord | name of coordinates slot for specific methods. |
| dims | dimensions from coord to use. By default, all. |
| cutoff | cutoff for detection. |

Value

An object of class "haystack"

haystack_2D

The main Haystack function, for 2-dimensional spaces.

Description

The main Haystack function, for 2-dimensional spaces.

Usage

```
haystack_2D(
  x,
  y,
  detection,
  use.advanced.sampling = NULL,
  dir.randomization = NULL
)
```

Arguments

| | |
|-----------------------|--|
| x | x-axis coordinates of cells in a 2D representation (e.g. resulting from PCA or t-SNE) |
| y | y-axis coordinates of cells in a 2D representation |
| detection | A logical matrix showing which genes (rows) are detected in which cells (columns) |
| use.advanced.sampling | If NULL naive sampling is used. If a vector is given (of length = no. of cells) sampling is done according to the values in the vector. |
| dir.randomization | If NULL, no output is made about the random sampling step. If not NULL, files related to the randomizations are printed to this directory. |

Value

An object of class "haystack"

Examples

```
# using the toy example of the singleCellHaystack package
# define a logical matrix with detection of each gene (rows) in each cell (columns)
dat.detection <- dat.expression > 1

# running haystack in default mode
res <- haystack(dat.tsne, detection=dat.detection, method = "2D")
# list top 10 biased genes
show_result_haystack(res, n =10)
```

| | |
|----------------|---|
| haystack_highD | <i>The main Haystack function, for higher-dimensional spaces.</i> |
|----------------|---|

Description

The main Haystack function, for higher-dimensional spaces.

Usage

```
haystack_highD(
  x,
  detection,
  grid.points = 100,
  use.advanced.sampling = NULL,
  dir.randomization = NULL,
  scale = TRUE,
  grid.method = "centroid"
)
```

Arguments

| | |
|-----------------------|---|
| x | Coordinates of cells in a 2D or higher-dimensional space. Rows represent cells, columns the dimensions of the space. |
| detection | A logical matrix showing which genes (rows) are detected in which cells (columns) |
| grid.points | An integer specifying the number of centers (gridpoints) to be used for estimating the density distributions of cells. Default is set to 100. |
| use.advanced.sampling | If NULL naive sampling is used. If a vector is given (of length = no. of cells) sampling is done according to the values in the vector. |
| dir.randomization | If NULL, no output is made about the random sampling step. If not NULL, files related to the randomizations are printed to this directory. |
| scale | Logical (default=TRUE) indicating whether input coordinates in x should be scaled to mean 0 and standard deviation 1. |
| grid.method | The method to decide grid points for estimating the density in the high-dimensional space. Should be "centroid" (default) or "seeding". |

Value

An object of class "haystack", including the results of the analysis, and the coordinates of the grid points used to estimate densities.

Examples

```
# I need to add some examples.
# A toy example will be added too.
```

| | |
|-----------------|--|
| hclust_haystack | <i>Function for hierarchical clustering of genes according to their expression distribution in 2D or multi-dimensional space</i> |
|-----------------|--|

Description

Function for hierarchical clustering of genes according to their expression distribution in 2D or multi-dimensional space

Usage

```
hclust_haystack(x, ...)

## S3 method for class 'matrix'
hclust_haystack(x, dim1 = 1, dim2 = 2, ...)

## S3 method for class 'data.frame'
hclust_haystack(x, dim1 = 1, dim2 = 2, ...)
```

Arguments

| | |
|------|--|
| x | a matrix or other object from which coordinates of cells can be extracted. |
| ... | further parameters passed down to methods. |
| dim1 | column index or name of matrix for x-axis coordinates. |
| dim2 | column index or name of matrix for y-axis coordinates. |

hclust_haystack_highD *Function for hierarchical clustering of genes according to their distribution in a higher-dimensional space.*

Description

Function for hierarchical clustering of genes according to their distribution in a higher-dimensional space.

Usage

```
hclust_haystack_highD(
  x,
  detection,
  genes,
  method = "ward.D",
  grid.coordinates = NULL,
  scale = TRUE
)
```

Arguments

| | |
|------------------|--|
| x | Coordinates of cells in a 2D or higher-dimensional space. Rows represent cells, columns the dimensions of the space. |
| detection | A logical matrix showing which genes (rows) are detected in which cells (columns) |
| genes | A set of genes (of the 'detection' data) which will be clustered. |
| method | The method to use for hierarchical clustering. See '?hclust' for more information. Default: "ward.D". |
| grid.coordinates | Coordinates of grid points in the same space as 'x', to be used to estimate densities for clustering. |
| scale | whether to scale data. |

Value

An object of class hclust, describing a hierarchical clustering tree.

Examples

```
# to be added
```

`hclust_haystack_raw` *Function for hierarchical clustering of genes according to their distribution on a 2D plot.*

Description

Function for hierarchical clustering of genes according to their distribution on a 2D plot.

Usage

```
hclust_haystack_raw(x, y, detection, genes, method = "ward.D")
```

Arguments

| | |
|------------------------|---|
| <code>x</code> | x-axis coordinates of cells in a 2D representation (e.g. resulting from PCA or t-SNE) |
| <code>y</code> | y-axis coordinates of cells in a 2D representation |
| <code>detection</code> | A logical matrix showing which genes (rows) are detected in which cells (columns) |
| <code>genes</code> | A set of genes (of the 'detection' data) which will be clustered. |
| <code>method</code> | The method to use for hierarchical clustering. See '?hclust' for more information. Default: "ward.D". |

Value

An object of class `hclust`, describing a hierarchical clustering tree.

Examples

```
# using the toy example of the singleCellHaystack package
# define a logical matrix with detection of each gene (rows) in each cell (columns)
dat.detection <- dat.expression > 1

# running haystack in default mode
res <- haystack(dat.tsne, detection=dat.detection, method = "2D")

# get biased genes, store in variable gene.subset
sorted.table <- show_result_haystack(res, p.value.threshold = 1e-5)
gene.subset <- row.names(sorted.table)

# hierarchical clustering, and cutting into 5 clusters
hc <- hclust_haystack(dat.tsne, detection=dat.detection, genes=gene.subset)
hc.clusters <- cutree(hc,k = 5)
```

| | |
|--------------|--|
| kde2d_faster | <i>Based on the MASS kde2d() function, but heavily simplified; it's just tcrossprod() now.</i> |
|--------------|--|

Description

Based on the MASS kde2d() function, but heavily simplified; it's just tcrossprod() now.

Usage

```
kde2d_faster(dens.x, dens.y)
```

Arguments

| | |
|--------|---|
| dens.x | Contribution of all cells to densities of the x-axis grid points. |
| dens.y | Contribution of all cells to densities of the y-axis grid points. |

| | |
|-----------------|---|
| kmeans_haystack | <i>Function for k-means clustering of genes according to their expression distribution in 2D or multi-dimensional space</i> |
|-----------------|---|

Description

Function for k-means clustering of genes according to their expression distribution in 2D or multi-dimensional space

Usage

```
kmeans_haystack(x, ...)

## S3 method for class 'matrix'
kmeans_haystack(x, dim1 = 1, dim2 = 2, ...)

## S3 method for class 'data.frame'
kmeans_haystack(x, dim1 = 1, dim2 = 2, ...)
```

Arguments

| | |
|------|--|
| x | a matrix or other object from which coordinates of cells can be extracted. |
| ... | further parameters passed down to methods. |
| dim1 | column index or name of matrix for x-axis coordinates. |
| dim2 | column index or name of matrix for y-axis coordinates. |

kmeans_haystack_highD *Function for k-means clustering of genes according to their distribution in a higher-dimensional space.*

Description

Function for k-means clustering of genes according to their distribution in a higher-dimensional space.

Usage

```
kmeans_haystack_highD(  
  x,  
  detection,  
  genes,  
  grid.coordinates = NULL,  
  k,  
  scale = TRUE,  
  ...  
)
```

Arguments

| | |
|------------------|--|
| x | Coordinates of cells in a 2D or higher-dimensional space. Rows represent cells, columns the dimensions of the space. |
| detection | A logical matrix showing which genes (rows) are detected in which cells (columns) |
| genes | A set of genes (of the 'detection' data) which will be clustered. |
| grid.coordinates | Coordinates of grid points in the same space as 'x', to be used to estimate densities for clustering. |
| k | The number of clusters to return. |
| scale | whether to scale data. |
| ... | Additional parameters which will be passed on to the kmeans function. |

Value

An object of class kmeans, describing a clustering into 'k' clusters

Examples

```
# to be added
```

kmeans_haystack_raw *Function for k-means clustering of genes according to their distribution on a 2D plot.*

Description

Function for k-means clustering of genes according to their distribution on a 2D plot.

Usage

```
kmeans_haystack_raw(x, y, detection, genes, k, ...)
```

Arguments

| | |
|-----------|---|
| x | x-axis coordinates of cells in a 2D representation (e.g. resulting from PCA or t-SNE) |
| y | y-axis coordinates of cells in a 2D representation |
| detection | A logical matrix showing which genes (rows) are detected in which cells (columns) |
| genes | A set of genes (of the 'detection' data) which will be clustered. |
| k | The number of clusters to return. |
| ... | Additional parameters which will be passed on to the kmeans function. |

Value

An object of class kmeans, describing a clustering into 'k' clusters

Examples

```
# using the toy example of the singleCellHaystack package
# define a logical matrix with detection of each gene (rows) in each cell (columns)
dat.detection <- dat.expression > 1

# running haystack in default mode
res <- haystack(dat.tsne, detection=dat.detection, method = "2D")

# get biased genes, store in variable gene.subset
sorted.table <- show_result_haystack(res, p.value.threshold = 1e-5)
gene.subset <- row.names(sorted.table)

# k-means clustering into 5 clusters
km <- kmeans_haystack(dat.tsne, detection=dat.detection, genes=gene.subset, k=5)
km.clusters <- km$cluster
```

plot_gene_haystack *Visualizing the detection/expression of a gene in a 2D plot*

Description

Visualizing the detection/expression of a gene in a 2D plot

Usage

```
plot_gene_haystack(x, ...)  
  
## S3 method for class 'matrix'  
plot_gene_haystack(x, dim1 = 1, dim2 = 2, ...)  
  
## S3 method for class 'data.frame'  
plot_gene_haystack(x, dim1 = 1, dim2 = 2, ...)  
  
## S3 method for class 'SingleCellExperiment'  
plot_gene_haystack(  
  x,  
  dim1 = 1,  
  dim2 = 2,  
  assay = "counts",  
  coord = "TSNE",  
  ...  
)  
  
## S3 method for class 'Seurat'  
plot_gene_haystack(  
  x,  
  dim1 = 1,  
  dim2 = 2,  
  assay = "RNA",  
  slot = "data",  
  coord = "tsne",  
  ...  
)
```

Arguments

| | |
|-------|--|
| x | a matrix or other object from which coordinates of cells can be extracted. |
| ... | further parameters passed to plot_gene_haystack_raw(). |
| dim1 | column index or name of matrix for x-axis coordinates. |
| dim2 | column index or name of matrix for y-axis coordinates. |
| assay | name of assay data for Seurat method. |

coord name of coordinates slot for specific methods.
slot name of slot for assay data for Seurat method.

plot_gene_haystack_raw

Visualizing the detection/expression of a gene in a 2D plot

Description

Visualizing the detection/expression of a gene in a 2D plot

Usage

```
plot_gene_haystack_raw(  
  x,  
  y,  
  gene,  
  expression,  
  detection = NULL,  
  high.resolution = FALSE,  
  point.size = 1,  
  order.by.signal = FALSE  
)
```

Arguments

| | |
|-----------------|--|
| x | x-axis coordinates of cells in a 2D representation (e.g. resulting from PCA or t-SNE) |
| y | y-axis coordinates of cells in a 2D representation |
| gene | name of a gene that is present in the input expression data, or a numerical index |
| expression | a logical/numerical matrix showing detection/expression of genes (rows) in cells (columns) |
| detection | an optional logical matrix showing detection of genes (rows) in cells (columns). If left as NULL, the density distribution of the gene is not plotted. |
| high.resolution | logical (default: FALSE). If set to TRUE, the density plot will be of a higher resolution |
| point.size | numerical value to set size of points in plot. Default is 1. |
| order.by.signal | If TRUE, cells with higher signal will be put on the foreground in the plot. Default is FALSE. |

Value

A plot

Examples

```
# using the toy example of the singleCellHaystack package
# define a logical matrix with detection of each gene (rows) in each cell (columns)
dat.detection <- dat.expression > 1
# various ways of plotting gene expression patterns
plot_gene_haystack(dat.tsne, expression=dat.expression, gene="gene_242",
  detection = dat.detection, high.resolution = TRUE)
plot_gene_haystack(dat.tsne, expression=dat.expression, gene="gene_242",
  detection = dat.detection, high.resolution = TRUE, point.size = .1)
```

```
plot_gene_set_haystack
```

Visualizing the detection/expression of a set of genes in a 2D plot

Description

Visualizing the detection/expression of a set of genes in a 2D plot

Usage

```
plot_gene_set_haystack(x, ...)

## S3 method for class 'matrix'
plot_gene_set_haystack(x, dim1 = 1, dim2 = 2, ...)

## S3 method for class 'data.frame'
plot_gene_set_haystack(x, dim1 = 1, dim2 = 2, ...)

## S3 method for class 'SingleCellExperiment'
plot_gene_set_haystack(
  x,
  dim1 = 1,
  dim2 = 2,
  assay = "counts",
  coord = "TSNE",
  ...
)

## S3 method for class 'Seurat'
plot_gene_set_haystack(
  x,
  dim1 = 1,
  dim2 = 2,
  assay = "RNA",
  slot = "data",
  coord = "tsne",
  ...
)
```

Arguments

| | |
|-------|--|
| x | a matrix or other object from which coordinates of cells can be extracted. |
| ... | further parameters passed to plot_gene_haystack_raw(). |
| dim1 | column index or name of matrix for x-axis coordinates. |
| dim2 | column index or name of matrix for y-axis coordinates. |
| assay | name of assay data for Seurat method. |
| coord | name of coordinates slot for specific methods. |
| slot | name of slot for assay data for Seurat method. |

plot_gene_set_haystack_raw

Visualizing the detection/expression of a set of genes in a 2D plot

Description

Visualizing the detection/expression of a set of genes in a 2D plot

Usage

```
plot_gene_set_haystack_raw(
  x,
  y,
  genes = NA,
  detection,
  high.resolution = TRUE,
  point.size = 1,
  order.by.signal = FALSE
)
```

Arguments

| | |
|-----------------|--|
| x | x-axis coordinates of cells in a 2D representation (e.g. resulting from PCA or t-SNE) |
| y | y-axis coordinates of cells in a 2D representation |
| genes | Gene names that are present in the input expression data, or a numerical indices. If NA, all genes will be used. |
| detection | a logical matrix showing detection of genes (rows) in cells (columns) |
| high.resolution | logical (default: TRUE). If set to FALSE, the density plot will be of a lower resolution |
| point.size | numerical value to set size of points in plot. Default is 1. |
| order.by.signal | If TRUE, cells with higher signal will be put on the foreground in the plot. Default is FALSE. |

Value

A plot

Examples

```
# using the toy example of the singleCellHaystack package
# define a logical matrix with detection of each gene (rows) in each cell (columns)
dat.detection <- dat.expression > 1

# define a set of genes that we want to visualize
# this might be a set of differentially expressed genes
# predicted by haystack and clustered together by hclust_haystack
gene_set <- c("gene_9", "gene_59", "gene_112", "gene_137", "gene_155",
             "gene_216", "gene_234", "gene_275", "gene_291", "gene_317",
             "gene_339", "gene_340", "gene_351", "gene_400", "gene_424", "gene_479")

# visualize the expression pattern of the set of genes
plot_gene_set_haystack(dat.tsne, detection=dat.detection, genes=gene_set)
```

read_haystack

Function to read haystack results from file.

Description

Function to read haystack results from file.

Usage

```
read_haystack(file)
```

Arguments

file A file containing 'haystack' results to read

Value

An object of class "haystack"

Examples

```
# using the toy example of the singleCellHaystack package
# define a logical matrix with detection of each gene (rows) in each cell (columns)
dat.detection <- dat.expression > 1

# running haystack in default mode
res <- haystack(dat.tsne, detection=dat.detection, method = "2D")

outfile <- file.path(tempdir(), "output.csv")
```

```
# write result to file outfile.csv
write_haystack(res, file = outfile)

# read in result from file
res.copy <- read_haystack(file = outfile)
```

`show_result_haystack` *Shows the results of the 'haystack' analysis in various ways, sorted by significance. Priority of params is genes > p.value.threshold > n.*

Description

Shows the results of the 'haystack' analysis in various ways, sorted by significance. Priority of params is genes > p.value.threshold > n.

Usage

```
show_result_haystack(res.haystack, n = NA, p.value.threshold = NA, gene = NA)
```

Arguments

`res.haystack` A 'haystack' result variable

`n` If defined, the top "n" significant genes will be returned. Default: NA, which shows all results.

`p.value.threshold` If defined, genes passing this p-value threshold will be returned.

`gene` If defined, the results of this (these) gene(s) will be returned.

Value

A table with a sorted subset of the 'haystack' result according to input parameters.

Examples

```
# using the toy example of the singleCellHaystack package
# define a logical matrix with detection of each gene (rows) in each cell (columns)
dat.detection <- dat.expression > 1

# running haystack in default mode
res <- haystack(dat.tsne, detection=dat.detection, method = "2D")

# below are variations for showing the results in a table
# 1. list top 10 biased genes
show_result_haystack(res.haystack = res, n =10)
# 2. list genes with p value below a certain threshold
show_result_haystack(res.haystack = res, p.value.threshold=1e-10)
# 3. list a set of specified genes
set <- c("gene_497", "gene_386", "gene_275")
show_result_haystack(res.haystack = res, gene = set)
```

write_haystack *Function to write haystack result data to file.*

Description

Function to write haystack result data to file.

Usage

```
write_haystack(res.haystack, file)
```

Arguments

res.haystack A 'haystack' result variable
file A file to write to

Examples

```
# using the toy example of the singleCellHaystack package
# define a logical matrix with detection of each gene (rows) in each cell (columns)
dat.detection <- dat.expression > 1

# running haystack in default mode
res <- haystack(dat.tsne, detection=dat.detection, method = "2D")

outfile <- file.path(tempdir(), "output.csv")

# write result to file outfile.csv
write_haystack(res, file = outfile)

# read in result from file
res.copy <- read_haystack(file = outfile)
```

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