

Package ‘singleCellHaystack’

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Type Package

Title A Universal Differential Expression Prediction Tool for
Single-Cell and Spatial Genomics Data

Version 1.0.3

Description One key exploratory analysis step in single-cell genomics data analysis is the prediction of features with different activity levels. For example, we want to predict differentially expressed genes (DEGs) in single-cell RNA-seq data, spatial DEGs in spatial transcriptomics data, or differentially accessible regions (DARs) in single-cell ATAC-seq data. 'singleCellHaystack' predicts differentially active features in single cell omics datasets without relying on the clustering of cells into arbitrary clusters. 'singleCellHaystack' uses Kullback-Leibler divergence to find features (e.g., genes, genomic regions, etc) that are active in subsets of cells that are non-randomly positioned inside an input space (such as 1D trajectories, 2D tissue sections, multi-dimensional embeddings, etc). For the theoretical background of 'singleCellHaystack' we refer to our original paper Vandenbon and Diez (Nature Communications, 2020) <[doi:10.1038/s41467-020-17900-3](https://doi.org/10.1038/s41467-020-17900-3)> and our update Vandenbon and Diez (Scientific Reports, 2023) <[doi:10.1038/s41598-023-38965-2](https://doi.org/10.1038/s41598-023-38965-2)>.

Imports methods, Matrix, splines, ggplot2, reshape2

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SingleCellExperiment, SeuratObject, cowplot, wrswoR,
sparseMatrixStats, ComplexHeatmap, patchwork

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URL <https://alexisvdb.github.io/singleCellHaystack/>,
<https://github.com/alexisvdb/singleCellHaystack>

BugReports <https://github.com/alexisvdb/singleCellHaystack/issues>

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`dat.expression`

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

Single cell RNA-seq dataset.

<code>dat.tsne</code>	<i>Single cell tSNE coordingates.</i>
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Description

Single cell tSNE coordingates.

<code>default_bandwidth.nrd</code>	<i>Default function given by function <code>bandwidth.nrd</code> in MASS. No changes were made to this function.</i>
------------------------------------	--

Description

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

Usage

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

Arguments

<code>x</code>	A numeric vector
----------------	------------------

Value

A suitable bandwith.

`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

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

Value

A row (logical vector) of the sparse matrix

get_density	<i>Function to get the density of points with value TRUE in the (x,y) plot</i>
-------------	--

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 genes (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 if the gene is expressed in a cell, F if 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_continuous_highD	<i>Calculates the Kullback-Leibler divergence between distributions for the high-dimensional continuous version of haystack.</i>
---------------------------	--

Description

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

Usage

```
get_D_KL_continuous_highD(
  weights,
  density.contributions,
  reference.prob,
  pseudo = 0
)
```

Arguments

`weights` A numerical vector with expression values of a gene.

`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

<code>get_D_KL_highD</code>	<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 comparing to randomizations.</i>
----------------	---

Description

Estimates the significance of the observed Kullback-Leibler divergence by comparing 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_log_p_D_KL_continuous	<i>Estimates the significance of the observed Kullback-Leibler divergence by comparing to randomizations for the continuous version of haystack.</i>
---------------------------	--

Description

Estimates the significance of the observed Kullback-Leibler divergence by comparing to randomizations for the continuous version of haystack.

Usage

```
get_log_p_D_KL_continuous(
  D_KL.observed,
  D_KL.randomized,
  all.coeffVar,
  train.coeffVar,
  output.dir = NULL,
  spline.method = "ns"
)
```

Arguments

D_KL.observed	A vector of observed Kullback-Leibler divergences.
D_KL.randomized	A matrix of Kullback-Leibler divergences of randomized datasets.
all.coeffVar	Coefficients of variation of all genes. Used for fitting the Kullback-Leibler divergences.
train.coeffVar	Coefficients of variation of genes that will be used for fitting the Kullback-Leibler divergences.
output.dir	Optional parameter. Default is NULL. If not NULL, some files will be written to this directory.
spline.method	Method to use for fitting splines "ns" (default): natural splines, "bs": B-splines.

Value

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

get_parameters_haystack

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

Description

Function that decides most of the parameters that will be used 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,
  expression,
  weights.advanced.Q = NULL,
  dir.randomization = NULL,
  scale = TRUE,
  grid.points = 100,
  grid.method = "centroid",
  ...
)
```

```

## S3 method for class 'data.frame'
haystack(
  x,
  expression,
  weights.advanced.Q = NULL,
  dir.randomization = NULL,
  scale = TRUE,
  grid.points = 100,
  grid.method = "centroid",
  ...
)

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

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

```

Arguments

<code>x</code>	a matrix or other object from which coordinates of cells can be extracted.
<code>...</code>	further parameters passed down to methods.
<code>expression</code>	a matrix with expression data of genes (rows) in cells (columns)
<code>weights.advanced.Q</code>	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.
<code>dir.randomization</code>	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".
coord	name of coordinates slot for specific methods.
assay	name of assay data for Seurat method.
slot	name of slot for assay data for Seurat method.
dims	dimensions from coord to use. By default, all.
cutoff	cutoff for detection.
method	choose between highD (default) and 2D haystack.

Value

An object of class "haystack"

haystack_2D	<i>The main Haystack function, for 2-dimensional spaces.</i>
-------------	--

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"

haystack_continuous_highD

The main Haystack function, for higher-dimensional spaces and continuous expression levels.

Description

The main Haystack function, for higher-dimensional spaces and continuous expression levels.

Usage

```
haystack_continuous_highD(
  x,
  expression,
  grid.points = 100,
  weights.advanced.Q = NULL,
  dir.randomization = NULL,
  scale = TRUE,
  grid.method = "centroid",
  randomization.count = 100,
  n.genes.to.randomize = 100,
  selection.method.genes.to.randomize = "heavytails",
  grid.coord = NULL,
  spline.method = "ns"
)
```

Arguments

x	Coordinates of cells in a 2D or higher-dimensional space. Rows represent cells, columns the dimensions of the space.
expression	a matrix with expression data of genes (rows) in cells (columns)
grid.points	An integer specifying the number of centers (grid points) to be used for estimating the density distributions of cells. Default is set to 100.
weights.advanced.Q	(Default: NULL) Optional weights of cells for calculating a weighted distribution of expression.
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".
`randomization.count` Number of randomizations to use. Default: 100
`n.genes.to.randomize` Number of genes to use in randomizations. Default: 100
`selection.method.genes.to.randomize` Method used to select genes for randomization.
`grid.coord` matrix of grid coordinates.
`spline.method` Method to use for fitting splines "ns" (default): natural splines, "bs": B-splines.

Value

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

Examples

```
# using the toy example of the singleCellHaystack package

# running haystack
res <- haystack(dat.tsne, dat.expression)
# 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

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

<code>hclust_haystack</code>	<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,
  expression,
  grid.coordinates,
  hclust.method = "ward.D",
  cor.method = "spearman",
  ...
```

```

)

## S3 method for class 'matrix'
hclust_haystack(
  x,
  expression,
  grid.coordinates,
  hclust.method = "ward.D",
  cor.method = "spearman",
  ...
)

## S3 method for class 'data.frame'
hclust_haystack(
  x,
  expression,
  grid.coordinates,
  hclust.method = "ward.D",
  cor.method = "spearman",
  ...
)

```

Arguments

<code>x</code>	a matrix or other object from which coordinates of cells can be extracted.
<code>expression</code>	expression matrix.
<code>grid.coordinates</code>	coordinates of the grid points.
<code>hclust.method</code>	method used with <code>hclust</code> .
<code>cor.method</code>	method used with <code>cor</code> .
<code>...</code>	further parameters passed down to methods.

`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

<code>x</code>	Coordinates of cells in a 2D or higher-dimensional space. Rows represent cells, columns the dimensions of the space.
<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".
<code>grid.coordinates</code>	Coordinates of grid points in the same space as 'x', to be used to estimate densities for clustering.
<code>scale</code>	whether to scale data.

Value

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

Examples

```
# to be added
```

<code>hclust_haystack_raw</code>	<i>Function for hierarchical clustering of genes according to their distribution on a 2D plot.</i>
----------------------------------	--

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.

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

<code>dens.x</code>	Contribution of all cells to densities of the x-axis grid points.
<code>dens.y</code>	Contribution of all cells to densities of the y-axis grid points.

<code>kmeans_haystack</code>	<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, expression, grid.coordinates, k, ...)
```

```
## S3 method for class 'matrix'
```

```
kmeans_haystack(x, expression, grid.coordinates, k, ...)
```

```
## S3 method for class 'data.frame'
```

```
kmeans_haystack(x, expression, grid.coordinates, k, ...)
```

Arguments

<code>x</code>	a matrix or other object from which coordinates of cells can be extracted.
<code>expression</code>	expression matrix.
<code>grid.coordinates</code>	coordinates of the grid points.
<code>k</code>	number of clusters.
<code>...</code>	further parameters passed down to methods.

`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

<code>x</code>	Coordinates of cells in a 2D or higher-dimensional space. Rows represent cells, columns the dimensions of the space.
<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>grid.coordinates</code>	Coordinates of grid points in the same space as 'x', to be used to estimate densities for clustering.
<code>k</code>	The number of clusters to return.
<code>scale</code>	whether to scale data.
<code>...</code>	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	<i>Function for k-means clustering of genes according to their distribution on a 2D plot.</i>
---------------------	---

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

plot_compare_ranks	<i>plot_compare_ranks</i>
--------------------	---------------------------

Description

plot_compare_ranks

Usage

```
plot_compare_ranks(res1, res2, sort_by = "log.p.vals")
```

Arguments

res1	haystack result.
res2	haystack result.
sort_by	column to sort results (default: log.p.vals).

plot_gene_haystack	<i>Visualizing the detection/expression of a gene in a 2D plot</i>
--------------------	--

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

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

plot_rand_fit	<i>plot_rand_fit</i>
---------------	----------------------

Description

plot_rand_fit

Usage

```
plot_rand_fit(x, type = c("mean", "sd"))
```

```
## S3 method for class 'haystack'  
plot_rand_fit(x, type = c("mean", "sd"))
```

Arguments

x	haystack object.
type	whether to plot mean or sd.

plot_rand_KLD	<i>plot_rand_KLD</i>
---------------	----------------------

Description

Plots the distribution of randomized KLD for each of the genes, together with the mean and standard deviation, the 0.95 quantile and the 0.95 quantile from a normal distribution with mean and standard deviations from the distribution of KLDs. The logCV is indicated in the subtitle of each plot.

Usage

```
plot_rand_KLD(x, n = 12, log = TRUE, tail = FALSE)
```

Arguments

x	haystack result.
n	number of genes from randomization set to plot.
log	whether to use log of KLD.
tail	whether the genes are chosen from the tail of randomized genes.

read_haystack	<i>Function to read haystack results from file.</i>
---------------	---

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"

show_result_haystack	<i>show_result_haystack</i>
----------------------	-----------------------------

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 = NULL,
  p.value.threshold = NULL,
  gene = NULL
)

## S3 method for class 'haystack'
show_result_haystack(
  res.haystack,
  n = NULL,
  p.value.threshold = NULL,
  gene = NULL
)
```

Arguments

res.haystack	A 'haystack' result object.
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.

Details

The output is a data.frame with the following columns: * D_KL the calculated KL divergence. * log.p.vals log10 p.values calculated from randomization. * log.p.adj log10 p.values adjusted by Bonferroni correction.

Value

A data.frame with 'haystack' results sorted by log.p.vals.

Examples

```
# using the toy example of the singleCellHaystack package

# running haystack
res <- haystack(dat.tsne, dat.expression)

# 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	<i>Function to write haystack result data to file.</i>
----------------	--

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

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