Package 'SPADEVizR'

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Type Package

Title Visualization and statistical analyses of cell clustering results generated by the SPADE clustering algorithm

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Description
Flow and mass cytometry are experimental techniques used for the characterization of cell phenotypes. Automatic gating algorithms can be used to automatically identified clusters of cells having similar phenotypes. The SPADE algorithm has been proposed as a new way to analysis and explore mass-cytometry data. This algorithm performs a density-based down-sampling combined with an agglomerative hierarchical clustering. XXX While SPADE offers new opportunities for identifying cell populations, complementary approaches are needed to improve the characterization of identified cell populations. SPADEVizR is an R package designed to better visualize and analyze SPADE clustering results. This package extends the original SPADE outputs with techniques such as parallel coordinates, heatmaps, multidimensional scaling, volcano plots or streamgraph representations. Moreover several statistical methods allow the identification of SPADE clusters with relevant biological behaviors.

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Imports data.table,
flowCore,
ggdendro,
ggnetwork,
ggplot2,
ggrepel,
gridExtra,
gtable,
gtools,
igraph,
methods,
MASS,
network,
plyr,
reshape2

LazyData true

Suggests knitr

VignetteBuilder knitr

RoxygenNote 5.0.1

R topics documented:

`abundantClustersViewer` *Visualization of abundant clusters*

Description

Generates a scatter plot representation showing for each cluster its mean abundance and associated p-value.

Usage

```
abundantClustersViewer(AC, show.cluster_sizes = TRUE,
  show.all_labels = FALSE)
```

Arguments

`AC` an object of class AC (object returned by the 'computeAC()' function)

`show.cluster_sizes` a logical specifying if dot sizes are proportional to cell counts

`show.all_labels` a logical specifying if all cluster labels must be shown (or just significant clusters)

Details

By default, only significant abundant clusters are labeled. Labels for all clusters can be displayed by setting the 'show.all_labels' parameter to TRUE.

Value

a 'ggplot' object

`AC-class` *Abundant Clusters (AC class) definition*

Description

The 'AC' object is a S4 object containing the information related to the abundant clusters in a given biological condition. Moreover this object contains all parameters used in the statistical analysis.

Details

A cluster is considered as a significant abundant cluster if its associated p-value and mean are below the specific thresholds 'th.pvalue' and 'th.mean'.

The 'print()' and 'show()' can be used to display a summury of this object. Moreover all information about this object could be saved as a tab separated file using the 'export()' method. This object is returned by the 'identifyAC()' function.

Slots

`sample.names` a character vector containing the samples used to compute the abundant clusters

`cluster.size` a numeric vector containing the number of cells (– sum of all samples –) for each cluster

`use.percentages` a logical specifying if computation was performed on percentage of cell abundance

`method` a character containing the name of the statistical test used to identify the abundant clusters

`method.adjust` a character containing the name of the multiple correction method used (if any)

`th.mean` a numeric value specifying the mean threshold

`th.pvalue` a numeric value specifying the p-value threshold

`result` a data.frame containing for each cluster (first column): the mean (second column) and the standard deviation (third column) of the biological condition, the associated p-value (fourth column) and a logical (fifth column) specifying if the cluster is significantly abundant.

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biplotViewer *biplotViewer*

Description

Generates a biplot representation with two markers

Usage

```
biplotViewer(SPADEResults, x.marker1, y.marker2, samples = NULL,
  clusters = NULL, sample.merge = FALSE, resample.ratio = NULL)
```

Arguments

`SPADEResults`	a SPADEResults object (Results object is not accepted)
`x.marker1`	a character indicating the marker name of the first dimension
`y.marker2`	a character indicating the marker name of the second dimension
`samples`	xxx
`clusters`	xxx
`sample.merge`	xxx
`resample.ratio`	a numric ratio (between 0 and 1) specifying the resample ratio to show less dots
`default.min`	a numeric value indicating the lower bound of the biplot representation

Details

In such representation, each dot corresponds to a cell profile and dot are ploted in a 2-dimentional space corresponding to the marker expressions. resample.ratio nb cell not the same than displayed dots

Value

if return.gg is TRUE, the function returns a list of ggplot objects

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`boxplotViewer` *Visualization of cluster enrichment profiles conditions*

Description

Generate a boxplot representation displaying the cell abundance for each cluster. Clusters are gathered by given biological conditions.

Usage

```
boxplotViewer(Results, conditions, clusters = NULL, use.percentages = TRUE,
  show.legend = FALSE, show.violin = TRUE)
```

Arguments

`Results`	a SPADEResults or Results object
`conditions`	conditions a named vector providing the correspondence between a sample name (in row names) and the condition of this sample : NA to exclude a sample from tests
`clusters`	a character vector containing the clusters names to be visualized (by default all clusters will be displayed)
`use.percentages`	a logical specifying if the visualization must be performed on percentage
`show.legend`	a logical specifying if the legend must be displayed
`show.violin`	a logical specifying if the count distribution must be displayed

Details

Cells clusters are colored based on theirs associated biological samples.

Value

a 'ggplot' object

CC-class *Correlated Clusters (CC class) definition*

Description

The 'CC' object is a S4 object containing coefficient of correlation associated between each cluster and a phenotypic variable. Moreover this object contains all parameters used in the statistical analysis.

Details

A cluster is considered as a significant correlated cluster if its associated p-value and correlation threshold are below the specific thresholds 'th.pvalue' and 'th.correlation'.

The 'print()' and 'show()' can be used to display a summury of this object. Moreover all information about this object could be saved as a tab separated file using the 'export()' method. This object is returned by the 'identifyCC()' function.

Slots

`sample.names` a character vector containing the samples used to compute correlated clusters

`variable` a numeric vector containing the expression values of the associated variable

`cluster.size` a numeric vector containing number of cells (– sum of all samples –) for each cluster

`use.percentages` a logical specifying if computation was performed on percentage of cell abundance

`method` a character containing the name of the statistical test used to identify the CC

`method.adjust` a character containing the name of the multiple correction method used (if any)

`th.correlation` a numeric value specifying the correlation threshold (R)

`th.pvalue` a numeric value specifying the p-value threshold

`result` a data.frame containing for each cluster (first column): the coefficiant of correlation R (second column) , the associated p-value (third column) and a logical (fourth column) specifying if the cluster is significantly correlated.

classifyEnrichmentProfiles *Classification of clusters based on their enrichment profiles*

Description

Classifies clusters based on their enrichment profiles.

Usage

```
classifyEnrichmentProfiles(Results, method = "hierarchical_h",
  method.parameter = NULL)
```

Arguments

`Results` a Results or SPADEResults object

`method` a character specifying the clustering method among one of those : 'hierarchical_h','hierarchical_k','k-means','eigencell','clique' (hierarchical_h by default)

`method.parameter`
a numeric specifying the numeric value required by the selected method

Details

The classification is done on cell abundances of each clusters and could be performed using 5 methods:

- "hierarchical_k" This method first compute the pearson correlation matrix and then use this matrix to performs a hierarchical classification. The hierarchical classification is cutted in order to return the desired number of classes. This number of classes must be provided as a numeric integer using the 'method.parameter' parameter. It is to note that negative correlations are considered as uncorrelated
- "hierarchical_k" This method works in the same way than 'hierarchical_k' but the height where the hierarchical tree is specified. This heigth is a correlation threshold (a numeric double between 0 and 1 included) provided using the 'method.parameter' parameter.
- "eigencell" This method compute the performs a eigen vector decomposition and then calculate the correlations between cluster expressions and these vectors. Clusters which correlate above a specific threshold with the same eigen vector are classified together. This correlation threshold (a numeric double between 0 and 1 included) provided using the 'method.parameter' parameter.
- "clique" This method first compute the Pearson correlation matrix and then use this matrix to generate an undirected graph. In this graph, an edge is drawn between two nodes if the correlation coefficient in the adjacency matrix is above a specific threshold. This correlation threshold (a numeric double between 0 and 1 included) provided using the 'method.parameter' parameter. After building the graph, the method looking for the largest cliques wich are considered as classes of nodes. Cliques correspond to subgraph in which every two distinct vertices are adjacent.

Value

a S4 object of class 'EnrichmentProfiles'

`classifyPhenoProfiles` *Classification of clusters based on their phenotype profiles*

Description

Classifies clusters based on their phenotype profiles.

Usage

```
classifyPhenoProfiles(Results, method = "hierarchical_h",
  method.parameter = NULL)
```

Arguments

`Results` a Results or SPADEResults object

`method` a character specifying the clustering method among one of those : "hierarchical_h", "hierarchical_k","k-means","eigencell","clique"

`method.parameter`
a numeric specifying the numeric value required by the selected method

Details

The classification is done on cell abundances of each clusters and could be performed using 5 methods:

- "hierarchical_k" This method first compute the pearson correlation matrix and then use this matrix to performs a hierarchical classification. The hierarchical classification is cutted in order to return the desired number of classes. This number of classes must be provided as a numeric integer using the 'method.parameter' parameter. It is to note that negative correlations are considered as uncorrelated
- "hierarchical_k" This method works in the same way than 'hierarchical_k' but the height where the hierarchical tree is specified. This heigth is a correlation threshold (a numeric double between 0 and 1 included) provided using the 'method.parameter' parameter.
- "eigencell" This method compute the performs a eigen vector decomposition and then calculate the correlations between cluster expressions and these vectors. Clusters which correlate above a specific threshold with the same eigen vector are classified together. This correlation threshold (a numeric double between 0 and 1 included) provided using the 'method.parameter' parameter.
- "clique" This method first compute the Pearson correlation matrix and then use this matrix to generate an undirected graph. In this graph, an edge is drawn between two nodes if the correlation coefficient in the adjacency matrix is above a specific threshold. This correlation threshold (a numeric double between 0 and 1 included) provided using the 'method.parameter' parameter. After building the graph, the method looking for the largest cliques wich are considered as classes of nodes. Cliques correspond to subgraph in which every two distinct vertices are adjacent.

Value

a S4 object of class 'PhenoProfiles'

`clusterViewer` *Visualization of cluster phenotypes*

Description

Generates a parallel coordinate plot representation showing for each cluster the marker median expressions.

Usage

```
clusterViewer(Results, clusters = NULL, samples = NULL, markers = NULL,
  show.mean = "both")
```

Arguments

`Results` a SPADEResults or Result object

`clusters` a character vector containing the clusters names to be visualized (by default all clusters will be displayed)

`samples` a named vector specifying the samples to used

`markers` a character vector specifying the markers to be displayed

`show.mean` a character specifying if marker means expression should be displayed, possible value are among : "none", "only" or "both"

Details

If 'Results' is an object of class 'SPADEResults' object then the quantile ranges will be displayed using a ribbon.

The 'samples' parameter required a named vector providing the correspondence between a sample name (in rowname) and the logical value TRUE to show this sample or FALSE otherwise.

The 'show.mean' parameter allows to visualize three kinds of information:

- "none" value will show marker median expressions for each selected samples;
- "only" value will show only the mean of median maker expressions for all selected samples (displayed as black dashed line);
- "both" value will show marker median expressions for each selected samples together with the mean of median maker expressions for all selected samples.

Value

a list of ggplot objects

`computeClique` *Internal - Clique percolation classification*

Description

This function is used internally to classify clusters enrichment profiles or phenotype profiles using a clique percolation algorithm.

Usage

```
computeClique(data, clique.correlation.th = 0.7)
```

Arguments

`data` a numeric matrix with all clusters in rownames

`clique.correlation.th`
a numeric value indicating the correlation coefficient threshold

Details

xxx

Value

a dataframe containing for each cluster, its name and class

computeEigenCellClusters *Internal - Eigen vector classification*

Description

This function is used internally to classify clusters enrichment profiles or phenotype profiles using eigen vector decomposition.

Usage

```
computeEigenCellClusters(data, eigencell.correlation.th = 0.8)
```

Arguments

`data`	a numeric matrix with all clusters in rownames
`eigencell.correlation.th`	a numeric value indicating the correlation coefficient threshold

Details

xxx

Value

a dataframe containing for each cluster, its name and class

computeHierarchicalClustering *Internal - Hierarchical classification*

Description

This function is used internally to classify clusters enrichment profiles or phenotype profiles using a hierarchical algorithm.

Usage

```
computeHierarchicalClustering(data, class.number = NULL,
  hierarchical.correlation.th = 0.8)
```

Arguments

`data`	a matrix with all clusters in rownames
`class.number`	a numeric specifying the number of classes
`hierarchical.correlation.th`	a numeric value specifying the cut height

Details

This function compute the Pearson correlation matrix associated to the provided matrix. It is to note that negative correlations are considered as uncorrelated. This correlation matrix is used to performs a hierarchical classification. If 'class.number' is NULL, classification will be determined base on the cut height correlation threshold.

Value

a dataframe containing for each cluster, its name and class

`computeKmeans` *Internal - Kmeans classification*

Description

This function is used internally to classify clusters enrichment profilies or phenotype profiles using a k-means algorithm.

Usage

```
computeKmeans(data, k = NULL)
```

Arguments

`data`	a numeric matrix with cluster names in rownames
`k`	a numeric specifying the desired number of classes

Details

xxx

Value

a dataframe containing for each cluster, its name and class

`computePhenoTable` *Internal - Generatate a matrix of marker expression scores describing phenotypes*

Description

This function generate a numeric matrix of discrete expression scores for each marker of each cluster.

Usage

```
computePhenoTable(SPADEResults, num = 5)
```

Arguments

`SPADEResults`	a SPADEResults object
`num`	a numeric value specifying the number of markers expression categories

Details

XXX

Value

a numeric matrix of expression scores

`computeQuantile` *Internal - Compute quantile with FCS flowset marker by marker*

Description

This function is used internally to compute the maker range quantiles.

Usage

```
computeQuantile(flowset, probs = c(0.05, 0.95))
```

Arguments

`flowset`	a flowCore flowset
`probs`	a numeric vector of 2 values specifying the quantiles to compute

Details

This function performs the exact calculation of quantiles with all cells but needs more memory than 'computeQuantile.approximation'.

Value

a numeric matrix containing the quantiles of each marker

computeQuantile.approximation

Internal - Compute quantile with FCS flowset sample by sample

Description

This function is used internally to provide the mean of quantiles from each sample to seed up computation.

Usage

```
computeQuantile.approximation(flowset, probs = c(0.05, 0.95))
```

Arguments

`flowset`	a flowCore flowset
`probs`	a numeric vector of 2 values specifying the quantiles to compute

Details

This function performs an approximate calculation of quantiles using less memory than computeQuantile.

Value

a numeric matrix containing the quantiles of each marker

correlatedClustersViewer

Visualization of correlated clusters

Description

Generate a scatter plot representation showing for each cluster

Usage

```
correlatedClustersViewer(CC, show.cluster.sizes = TRUE,
  show.all_labels = FALSE)
```

Arguments

`CC`	an object of class 'CC' (object returned by the 'computeCC()' function)
`show.cluster.sizes`	a logical specifying if dot sizes are proportional to cell counts
`show.all_labels`	a logical specifying if all cluster label must be show or just significant cluster

Details

By default, only significant correlated clusters are labeled. Labels for all clusters can be displayed by setting the 'all.label' parameter to TRUE.

Value

a 'ggplot' object

countViewer *Visualization of cluster sizes*

Description

Generate a two dimensional vizualisation showing the number of cells (sum of selected samples) of each cluster.

Usage

```
countViewer(Results, samples = NULL, clusters = NULL, min.cells = 0,
  sort = TRUE, show.samples = TRUE)
```

Arguments

`Results`	a SPADEResults or Results object
`samples`	a named vector providing the correspondence between samples name (in rownames) and the logical value TRUE to use these samples (all samples by default)
`clusters`	a character vector containing the clusters names to be visualized (by default all clusters will be displayed)
`min.cells`	a numeric specifying the minimum number of cell (sum of all selected samples) to display a cluster
`sort`	a logical specifying if clusters will be to be sorted (descending) based on the sum of all selected samples for each cluster
`show.samples`	a logical specifying if the number of cells for all selected samples will be displayed

Details

xxx

Value

a 'ggplot' object

`DEC-class` *Differentially Enriched Clusters (DEC class) definition*

Description

The 'DEC' object is a S4 object containing the information related to the differentially enriched clusters between two given biological conditions. Moreover this object contains all parameters used in the statistical analysis.

Details

A cluster is considered as a differentially enriched cluster if its associated p-value and fold-change are below the specific thresholds 'th.pvalue' and 'th.fc'.

The 'print()' and 'show()' can be used to display a summury of this object. Moreover all information about this object could be saved as a tab separated file using the 'export()' method. This object is returned by the 'identifyDEC()' function.

Slots

`sample.cond1` a character specifying the names of the samples of the first biological condition

`sample.cond2` a character specifying the names of the samples of the second biological condition

`cluster.size` a numeric vector containing number of cells (– sum of all samples –) for each cluster

`use.percentages` a logical specifying if computation was performed on percentage of cell abundance

`method` a character containing the name of the statistical test used to identify the DEC

`method.adjust` a character containing the name of the multiple correction method used (if any)

`method.paired` a logical indicating if the statistical test have been performed in a paired manner

`th.fc` a numeric value specifying the fold-change threshold

`th.pvalue` a numeric value specifying the p-value threshold

`result` a data.frame containing for each cluster (first column): the fold-change (second column) and the standard deviation (third column) for the first biological condition, the fold-change (fourth column) and the standard deviation (fifth column) for the second biological condition, the associated p-value (sixth column) and a logical (seventh column) specifying if the cluster is significantly differentially enriched.

`distogramViewer` *Visualization of marker co-expressions*

Description

Generate a distogram representation showing the marker co-expressions.

Usage

```
distogramViewer(Results, clusters = NULL, samples = NULL, markers = NULL)
```

Arguments

`Results`	a SPADEResults or Results object
`clusters`	a character vector containing the clusters names to be use (by default all clusters will be used)
`samples`	a character vector specifying the samples to use
`markers`	a character vector specifying the markers to be displayed

Details

A pearson correlation matrix is calculated between selected markers using selected clusters and samples. High positive correlated markers are shown by a green tile at their perpendicular intersection. In the same way, absence of correlation are shown by black tiles and negative correlation by red tiles.

Value

a list of 'ggplot' objects

`EnrichmentProfiles-class` *Classification of clusters based on their enrichment profiles (EnrichmentProfiles class) definition*

Description

The 'EnrichmentProfiles' is a S4 object containing the information related to the cluster classification based on theirs enrichment profiles.

This object contains all information about the classification method and parameters used.

Details

Five methods are available to classify cellular clusters: 'hierarchical_k', 'hierarchical_h', 'kmeans', 'eigencell' and 'clique'. Each method can parameterized using the 'method.parameter' parameter.

The 'print()' and 'show()' can be used to display a summary of this object. Moreover all information about this object could be saved as a tab separated file using the 'export()' method. This object is returned by the 'classifyEnrichmentProfiles()' function.

Slots

`method` a character specifying the method used to classify cluster

`method.parameter` a numeric parameters associated with the chosen method

`cluster.size` a numeric vector with the number of cell in each cluster (sum of all samples)

`cluster.number` a numeric providing the number of cluster

`class.number` a numeric providing the number of classes

`classes` a two column dataframe with the cluster in first column and corresponding class in the second column

`exclude.markers` *Internal - Removing of cell markers to exclude from a matrix*

Description

This function is used internally to remove one or several cell markers.

Usage

```
exclude.markers(data, exclude, colnames.FCS = NULL)
```

Arguments

`data`	a numeric matrix or flowset
`exclude`	a character vector containing the cell markers to be excluded (case intensive)
`colnames.FCS`	a character vector containing column names if data is a FCS flowset

Details

If the data parameter is a dataframe the colnames.FCS parameter is ignored but if the data parameter is a flowset, the colnames.FCS parameter is required.

Value

a numeric matrix without the cell markers to exclude

`export` *Exportation of SPADEVizR objects*

Description

Exports a SPADEVizR object into a tab separated file.

Usage

```
export(object, filename = "export.txt")

## S4 method for signature 'Results'
export(object, filename = "export.txt")

## S4 method for signature 'AC'
export(object, filename = "export.txt")

## S4 method for signature 'DEC'
export(object, filename = "export.txt")

## S4 method for signature 'CC'
export(object, filename = "export.txt")
```

```
## S4 method for signature 'PhenoProfiles'
export(object, filename = "export.txt")

## S4 method for signature 'EnrichmentProfiles'
export(object, filename = "export.txt")
```

Arguments

`object`	a SPADEVizR object
`filename`	a character indicating the location of output file

Value

none

`filter.medians` *Internal - filter medians to exclude from a matrix*

Description

This function is used internally to remove raw or transform medians from SPADE matrix. CVS medians are always removed.

Usage

```
filter.medians(data, use.raw.medians = FALSE)
```

Arguments

`data`	a SPADE matrix
`use.raw.medians`	a logical specifying if "transformed" or "raw" medians will be use (FALSE by default)

Value

a numeric matrix without the cell markers to exclude `generateReport` *Generate a report including SPADEVizR plots.*

Description

Generate a customizable PDF report based on SPADEVizR vizualisation features. Available plots are :

"pheno" (included by default): Display an heatmap representation "kinetic": Display a kinetic representation for each cluster. This plot required to provide the 'assignment' parameter. "cluster" (included by default): Display a parallel coordinate representation showing for each cluster the marker median expression. "kinetic_cluster": Display a kinetic representation and a parallel coordinate juxtaposed (are arranged one on the side of the other) for each cluster "tree" (included by default): Display a tree representation showing combined SPADE trees. "disto" (included by default): Display a distogram representation showing the marker co-expressions. "stream" (included by default): Display a "MDS_clusters" (included by default): Display a Multidimensional Scaling (MDS) representation showing the "MDS_samples": Display a Multidimensional Scaling (MDS) representation showing the. This plot required to provide the 'assignment' parameter.

Usage

```
generateReport(results, PDFfile, plots.names = c("pheno", "cluster", "tree",
  "disto", "stream", "MDS_clusters"), clusters = NULL, markers = NULL,
  assignments = NULL, stat.objects = list(), profile.objects = list(),
  width = 29.7, height = 21)
```

Arguments

`PDFfile`	a character specifying the output path
`plots.names`	a character vector specifying the names (see details) and the order of the desired plots
`clusters`	a character vector of clusters to include in the report (all will be included by default)
`markers`	a character vector of markers to include in the report (all will be included by default)
`assignments`	a 2 column data.frame with the samples names in row names providing firstly the time-points (numeric) and secondly the individuals (character) of the experiment
`stat.objects`	a vector of stat.object to be displayed in the report (object of class 'DEC', 'AC' or 'CC')
`profile.objects`	a vector of profile.objects to be displayed in the report (object of class 'PhenoProfiles' or 'EnrichmentProfiles')
`width`	a numeric specifying the plot width
`height`	a numeric specifying the plot height
`Results`	a 'SPADEResults' or 'Result' object

`ggheatmap` *Internal - Create a list of elements allowing to build a heatmap*

Description

This function is used internally to build the element needed for an heatmap

Usage

```
ggheatmap(matrix, dendrogram.type = "rectangle", num = 5,
  clustering.markers = NULL)
```

Arguments

`matrix` a numeric matrix containing the markers expression categories

`dendrogram.type`
a character specifying the look of dendrograms ("rectangle" or "triangle", "rectangle" by default)

`num` a numeric value specifying the number of markers expression categories

`clustering.markers`
a character vector of clustering markers

Value

a list of 3 plots (top dendrogram, right dendrogram, heatmap)

`ggheatmap.plot` *Internal - Generate an heatmap by assembling elements*

Description

This function displays the heatmap elements build by 'ggheatmap()'

Usage

```
ggheatmap.plot(list, col.width = 0.15, row.width = 0.15)
```

Arguments

`list` the list of ggplot object provided by ggheatmap

`col.width` size of horizontal dendrogram

`row.width` size of vertical dendrogram

Value

a ggplot2 axis

`g_axis` *Internal - Extraction of ggplot axes*

Description

This function is used internally to extract axes from a 'ggplot' objet.

Usage

```
g_axis(gplot, x.axis = !y.axis, y.axis = !x.axis)
```

Arguments

`gplot`	a 'ggplot' plot
`x.axis`	a logical value specifying if the x-axis must be extract
`y.axis`	a logical value specifying if the y-axis must be extract

Details

It is to note that 'x' and 'y' are mutuality excluded (both cannot be both TRUE) with priority to 'x'.

Value

a 'ggplot' axis object

`g_dendro` *Internal - Build a dendrograms plot*

Description

This function is used internally to generate a 'ggplot' dendrogram.

Usage

```
g_dendro(dist, row = !col, col = !row)
```

Arguments

`dist`	a numeric matrix containing distances between objects
`row`	a logical value specifying if the horizontal dendrogram must be computed
`col`	a logical value specifying if the vertical dendrogram must be computed

Details

It is to note that 'row' and 'col' are mutuality excluded (both cannot be both TRUE) with priority to row.

Value

a 'ggplot' dendrogram object

g_legend *Internal - Extraction of ggplot legend*

Description

This function is used internally to extract the legend from a 'ggplot' object.

Usage

```
g_legend(gplot)
```

Arguments

gplot	a 'ggplot' plot

Value

a 'ggplot' legend object

identifyAC *Identification of the Abundant Clusters*

Description

This function is used to identify the abundant clusters. That is to say clusters that have cell abundance statistically greater than a specific threshold.

Usage

```
identifyAC(Results, condition, use.percentages = TRUE, method = "t.test",
  method.adjust = NULL, th.pvalue = 0.05, th.mean = 0)
```

Arguments

Results	a 'Results' or 'SPADEResults' object
condition	a named vector providing the correspondence between a sample name (in row names) and the logical value TRUE to test abundance for this sample or FALSE otherwise
use.percentages	a logical specifying if the computations should be performed on percentage
method	a character specifying the statistical method used to identify the abundant clusters. The parameter can take the values "t.test" or "wilcox.test"
method.adjust	a character specifying if the p-values should be corrected using multiple correction methods among : "holm", "hochberg", "hommel", "bonferroni", "BH", "BY" and "fdr" (from 'stats::p.adjust' method)
th.pvalue	a numeric specifying the p-value threshold
th.mean	a numeric specifying the abundance mean threshold

Value

a S4 object of class 'AC'

identifyCC *Identification of the correlation of SPADE cluster with a phenotype*

Description

This function is used to identify correlated clusters. That is to say clusters that correlate with a phenotypic variable.

Usage

```
identifyCC(Results, variable, use.percentages = TRUE, method = "pearson",
  method.adjust = NULL, th.pvalue = 0.05, th.correlation = 0.75)
```

Arguments

`Results`	a 'Results' or 'SPADEResults' object
`variable`	a numerical named vector providing the correspondence between a sample name (in rownames) and the specific phenotype or NA to ignore a sample
`use.percentages`	a logical specifying if the computations should be performed on percentage
`method`	a character indicating the correlation method to use : "pearson", "spearman"
`method.adjust`	a character specifying if the p-values should be corrected using multiple correction methods among : "holm", "hochberg", "hommel", "bonferroni", "BH", "BY" and "fdr" (from 'stats::p.adjust' method)
`th.pvalue`	a numeric specifying the p-value threshold
`th.correlation`	a numeric specifying the absolute value of the correlation coefficient threshold

Value

a S4 object of class 'CC'

identifyDEC *Identification of the Differentially Enriched Clusters*

Description

This function is used to identify differentially enriched clusters. That is to say clusters that are differentially abundant between two biologicals conditions.

Usage

```
identifyDEC(Results, conditions, use.percentages = TRUE, method = "t.test",
  method.adjust = NULL, method.paired = FALSE, th.pvalue = 0.05,
  th.fc = 1)
```

Arguments

`Results` a 'Results' or 'SPADEResults' object

`conditions` a named vector providing the correspondence between a sample name (in row names) and the condition of this sample : NA to exclude a sample from tests, 1 or 2 to attribute this sample, respectively to the first or second condition

`use.percentages` a logical specifying if the computations should be performed on percentage

`method` a character specifying the name of the statistical test to use "t.test" or "wilcox.test"

`method.adjust` a character specifying if the p-values should be corrected using multiple correction methods among : "holm", "hochberg", "hommel", "bonferroni", "BH", "BY" and "fdr" (from 'stats::p.adjust' method)

`method.paired` a logical indicating if the statistical test must be performed in a paired manner

`th.pvalue` a numeric specifying the p-value threshold

`th.fc` a numeric specifying the fold-change threshold

Value

a S4 object of class 'DEC'

importResults *Import clustering results generated by other algorithms*

Description

The 'importX()' function imports cell clustering results from two dataframes ('cells.count' and 'marker.expressions'). This function returns a 'Result' object.

Usage

```
importResults(cells.count, marker.expressions)
```

Arguments

`cells.count` a dataframe of cells abundances with clusters in row and samples in column

`marker.expressions` a dataframe containing median marker expression values for each cluster of each sample. In additions of markers, the 2 two first columns are are dedicated to "cluster" and "sample"

Details

The 'cells.count' dataframe must be formated as following:

cluster	sample1	sample1
cluster1	749	5421
cluster2	450	412

The 'marker.expressions' dataframe must be formated as following:

sample	cluster	marker1	marker2
sample1	cluster1	0.2	0.3
sample1	cluster2	0.1	0.3
sample2	cluster1	0.5	2.3
sample2	cluster2	1	1.3

Value

a S4 object of class 'Results'

`importSPADEResults` *Import clustering results generated by SPADE*

Description

The 'importSPADEResults()' function imports SPADE cell clustering results from a specified path. This function returns a 'SPADEResult' object.

This function import the expression matrix and count matrix as well as the SPADE tree. This function apply an hyperbolic sine transformation to imported FCS data and compute the maker range quantiles.

Usage

```
importSPADEResults(path, dict = data.frame(),
  exclude.markers = c("cell_length", "FileNum", "density", "time"),
  probs = c(0.05, 0.95), use.raw.medians = FALSE,
  quantile.approximation = FALSE)
```

Arguments

`path` a character specify the path of SPADE results folder

`dict` a two column dataframe providing the correspondence between the original marker names (first column) and the real marker names (second column)

`exclude.markers` a character vector of markers to exclude (case insensitive)

`probs` a vector of probabilities with 2 values in [0,1] to compute maker range quantiles. First is the lower bound and second is the upper bound.

`use.raw.medians` a logical specifying if "transformed" or "raw" medians will be use in the cluster expression matrix (FALSE by default)

`quantile.approximation` a logical specifying if maker range quantiles are computed using all cells (FALSE), or is the means of the quantile of each samples (TRUE)

Details

The computation of maker range quantiles can be approximated using 'quantile.approximation' parameter which is more efficient in term of loading time and memory usage.

Value

a S4 object of class 'SPADEResults'

---

kineticsViewer *Visualization of cluster enrichment profiles kinetics*

Description

Generates a kinetics plot representation showing for each cluster its enrichment profiles at each time-point of each individual.

Usage

```
kineticsViewer(Results, assignments, clusters = NULL,
  use.percentages = TRUE)
```

Arguments

`Results`	a SPADEResults or Results object
`assignments`	a 2 column data.frame with the samples names in row names providing firstly the time-points (numeric) and secondly the individuals (character) of the experiment
`clusters`	a character vector containing the clusters names to be visualized (by default all clusters will be displayed)
`use.percentages`	a logical specifying if the visualization should be performed on percentage

Details

xxx

Value

a 'ggplot' object

MDSViewer *Visualization of SPADE cluster similarities using MDS*

Description

Generate a Multidimensional Scaling (MDS) representation showing the similarities between SPADE results based on theirs phenotypes.

Usage

```
MDSViewer(Results, use.percentages = TRUE, assignments, clusters = NULL,
  space = "clusters", dist.method = "euclidean")
```

Arguments

`Results`	a SPADEResults or Results object
`use.percentages`	a logical specifying if the visualization should be performed on percentage
`assignments`	a 2 column data.frame with the samples names in row names providing firstly the time-points (numeric) and secondly the individuals (character) of the experiment
`clusters`	a character vector containing the clusters names to be visualized (by default all clusters will be displayed)
`space`	a character specifying the space ("clusters" or "samples", cluster by default)
`dist.method`	a character string containing the name of the distance measure to use

Details

talk about space xxx

Value

a list of ggplot objects

`names` *Definition of class names*

Description

Provides the name of each SPADEVizR object

Usage

```
## S4 method for signature 'Results'
names(x)

## S4 method for signature 'SPADEResults'
names(x)

## S4 method for signature 'AC'
names(x)

## S4 method for signature 'DEC'
names(x)

## S4 method for signature 'CC'
names(x)

## S4 method for signature 'PhenoProfiles'
names(x)

## S4 method for signature 'EnrichmentProfiles'
names(x)
```

Arguments

`x`	a SPADEVizR object

Value

a character providing the name of the object

`PhenoProfiles-class` *Classification of clusters based on their phenotype profiles (PhenoProfiles class) definition*

Description

The 'PhenoProfiles' is a S4 object containing the information related to the cluster classification based on theirs marker expressions.

This object contains all information about the classification method and parameters used.

Details

Five methods are available to classify cellular clusters: 'hierarchical_k', 'hierarchical_h', 'kmeans', 'eigencell' and 'clique'. Each method can parameterized using the 'method.parameter' parameter.

The 'print()' and 'show()' can be used to display a summary of this object. Moreover all information about this object could be saved as a tab separated file using the 'export()' method. This object is returned by the 'classifyPhenoProfiles()' function.

Slots

`cluster.number` a numeric providing the number of cluster

`method` a character specifying the method used to classify cluster

`method.parameter` a named list of parameters used by the classification method

`cluster.size` a numeric vector containing the number of cells associated with each cluster (– sum of all samples –)

`class.number` a numeric value specifying the number of clusters

`classes` a two column dataframe with the cluster in first colunm and corresponding classe in the second colunm

phenoViewer *Visualization of all clusters phenotypes as an heatmap*

Description

Generates an heatmap representation showing for all clusters the marker median expressions.

Usage

```
phenoViewer(SPADEResults, num = 5)
```

Arguments

`SPADEResults`	a SPADEResults object (Results object is not accepted)
`num`	a numeric value specifying the number of markers expression categories to use

Details

For each marker, median expressions are discretized in severals categories corresponding to the heat intensities. This number of categories is provided using 'num' parameter.

By default the 'pheno.table' parameter is null and will be automatically compute using the 'computePhenoTable' function.

Value

a list of ggplot objects

plot *Graphical representation for some SPADEVizR objects*

Description

This function generates a graphical representation for 'AC', 'DEC', 'CC', 'PhenoProfiles' and 'EnrichmentProfiles' objects.

Usage

```
plot(x, y = NULL, ...)

## S4 method for signature 'DEC,missing'
plot(x, y = NULL, ...)

## S4 method for signature 'AC,missing'
plot(x, y = NULL, ...)

## S4 method for signature 'CC,missing'
plot(x, y = NULL, ...)

## S4 method for signature 'PhenoProfiles,ANY'
```

```
plot(x, y = NULL, ...)

## S4 method for signature 'EnrichmentProfiles,ANY'
plot(x, y = NULL, ...)
```

Arguments

x	a 'AC', 'DEC', 'CC', 'PhenoProfiles' or 'EnrichmentProfiles' object

Value

a 'ggplot' object

---

print *Textual previews for all SPADEVizR objects*

Description

Prints a previews for a SPADEVizR object.

Usage

```
## S4 method for signature 'Results'
print(x)

## S4 method for signature 'SPADEResults'
print(x)

## S4 method for signature 'AC'
print(x)

## S4 method for signature 'DEC'
print(x)

## S4 method for signature 'CC'
print(x)

## S4 method for signature 'PhenoProfiles'
print(x)

## S4 method for signature 'EnrichmentProfiles'
print(x)
```

Arguments

x	a SPADEVizR object

Value

none

`profilesViewer` *profilesViewer*

Description

Generate a graph representation of PhenoProfiles classes

Usage

```
profilesViewer(profile.object, show.cluster.sizes = TRUE)
```

Arguments

`profile.object`	a PhenoProfiles object or an EnrichmentProfiles object
`show.cluster.sizes`	a logical specifying if dot sizes are proportional to cell counts

Details

xxx

Value

a 'ggplot' object

`rename.markers` *Internal - Renaming cell markers*

Description

This function is used internally to rename the cell markers based on a dictionary.

Usage

```
rename.markers(header, dictionary)
```

Arguments

`header`	a character vector containing the original maker names
`dictionary`	a character vector containing a correspondence between the original and the new marker names

Details

Dictionary is a data.frame used to rename the marker names. The first column must correspond to the original marker names, the second column must correspond to the new marker names.

Value

a character vector containing the renamed marker names

`Results-class` *Results class definition*

Description

The Results object is a S4 object containing cell clustering results obtained from various automatic gating algorithms.

This object mainly stores the count matrix (i.e. the number of cells associated with each cluster of each sample) and the cell cluster phenotypes (i.e. the marker median expressions for each cluster). It is to note that the Results object is a super class of the SPADEResult object.

Details

The 'cells.count' dataframe stores the number of cells associated with each cluster of each sample. This dataframe has in row the clusters and in column the samples.

The 'marker.expressions' dataframe stores the marker median expressions for each cluster. This dataframe has in the first the sample names, in the second column the cluster names, and the maker median expressions in the others columns.

The 'print()' and 'show()' can be used to display a summary of this object. Moreover all information about this object could be saved as a tab separated file using the 'export()' method. This object is returned by the 'importX()' function.

Slots

`cells.count` a dataframe containing the number of cells for each cluster of each sample

`marker.expressions` a numerical dataframe containing marker median expressions for each cluster of each sample

`sample.names` a character vector containing the sample names

`marker.names` a character vector containing the markers names

`cluster.number` a numeric specifying the number of cell clusters

`show` *Textual previews for SPADEVizR objects*

Description

Show a previews for a SPADEVizR object.

Usage

```
## S4 method for signature 'Results'
show(object)

## S4 method for signature 'SPADEResults'
show(object)

## S4 method for signature 'AC'
```

```
show(object)

## S4 method for signature 'DEC'
show(object)

## S4 method for signature 'CC'
show(object)

## S4 method for signature 'PhenoProfiles'
show(object)

## S4 method for signature 'EnrichmentProfiles'
show(object)
```

Arguments

`object`	a SPADEVizR object

Value

none

`SPADEResults-class` *SPADEResults class definition*

Description

The 'SPADEResults' object is a S4 object containing cell clustering results obtained from SPADE.

This object inherits from the 'Result' object and stores the count matrix (i.e. the number of cells associated with each cluster of each sample) and the cell cluster phenotypes (i.e. the marker median expressions for each cluster). In addition to the 'Result' object, the 'SPADEResults' object contains information about SPADE clustering results, such as the SPADE tree, the clustering makers and the FCS files.

Details

The 'print()' and 'show()' can be used to display a summury of this object. Moreover all information about this object could be saved as a tab separated file using the 'export()' method. This object is returned by the 'importSPADEResult()' function.

Slots

`cells.count` a dataframe containing the number of cells for each cluster of each sample

`marker.expressions` a numerical dataframe containing marker median expressions for each cluster of each sample

`sample.names` a character vector containing the sample names

`marker.names` a character vector containing the markers names

`cluster.number` a numeric specifying the number of cell clusters

`use.raw.medians` a logical specifying if the marker expressions correspond to the raw or transformed data `dictionary` a two column data.frame providing the correspondence between the original marker names (first column) and the real marker names (second column)

`marker.clustering` a logical vector specifying marker that have been used during the clustering precedure

`flowset` a flowSet object containing the imported SPADE FCS file

`fcs.files` a character vector containing the absolute path of the original FCS files

`quantiles` a numeric data.frame containing the quantiles for each each markers of cluster

`graph` a igraph object containing the SPADE tree

`graph.layout` a numeric matrix containing the layout of the SPADE tree

---

stat_steamgraph *Internal - Plot a steamgraph (from by ggTimeSeries : https://github.com/Ather-Energy/ggTimeSeries)*

Description

xxx

Usage

```
stat_steamgraph(mapping = NULL, data = NULL, show.legend = NA,
  inherit.aes = TRUE, na.rm = TRUE, ...)
```

Arguments

`mapping`	xxx
`data`	xxx
`show.legend`	xxx
`inherit.aes`	xxx
`na.rm`	xxx
`...`	xxx

Details

xxx

Value

xxx

`streamgraphViewer` *Visualization of cluster abundance dynamics*

Description

Generate a streamgraph representation showing the dynamic evolution of the number of cells in clusters across samples.

Usage

```
streamgraphViewer(Results, order = NULL, clusters = NULL)
```

Arguments

`Results`	a SPADEResults or Results object
`order`	a named vector providing the correspondence between a sample name (in rownames) and an integer ordering samples (NA to exclude a sample)
`clusters`	a character vector containing the clusters names to be vizualised (by default all clusters will be displayed)

Details

xxx

Value

a 'ggplot' object

`treeViewer` *Visualization of combined SPADE trees*

Description

Generates a tree representation showing combined SPADE trees.

Usage

```
treeViewer(SPADEResults, samples = NULL, stat.object = NULL)
```

Arguments

`SPADEResults`	a SPADEResults object (Results object is not accepted)
`samples`	a named vector providing the correspondence between samples name (in rowname) and the logical value TRUE to use these samples (all samples by default)
`stat.object`	an AC, DEC or CC object to highligth identified significant clusters in the SPADE tree

Details

The size of tree nodes are related to the number of cells in each cluster.

Value

a list of ggplot objects

volcanoViewer *Visualization of differentially enriched clusters*

Description

Generates a Volcano plot representation showing for each cluster

Usage

```
volcanoViewer(DEC = NULL, fc.log2 = TRUE, show.cluster.sizes = TRUE,
  show.all_labels = FALSE)
```

Arguments

`DEC`	an object of class 'DEC' (object returned by the 'computeDEC()' function)
`fc.log2`	a logical specifying if fold-change or log2(fold-change) is use
`show.cluster.sizes`	a logical specifying if dot sizes are proportional to cell counts
`show.all_labels`	a logical specifying if all cluster labels must be show or just significant cluster

Details

By default, only significant differentially enriched clusters are labeled. Labels for all clusters can be displayed by setting the 'all.label' parameter to TRUE.

Value

a 'ggplot' object

Index