Package 'cellmarkeraccordion'

March 24, 2025

Title Single-cell and spatial omics annotation in health and disease

Version 0.9.7

Description cellmarkeraccordion is an R package designed to obtain a robust identification of healthy and disease critical populations in singlecell and spatial omics datasets in multiple tissues.

Additionally, the cellmarkeraccordion allows an easy interpretation of the results. The cellmarkeraccordion package allows to automatically annotate normal and disease critical cell populations based on the built-in Accordion gene marker database. It requires in input only the counts matrix (raw or normalized) or a Seurat object. In addition, by exploiting the built-in cell cycle labeling you can easily assign the cell cycle phase to each cell. The users can also customize the annotation by simply providing their own genes list as input, which can be associated with cell types or even to specific pathways or signatures of interest. Importantly, the Accordion implements novel options to explore annotation results by inspecting for each group of cells the top marker genes which mostly impacted the annotation, together with the top cell types and their relationship based on the cell ontology tree.

```
License MIT + file LICENSE
Encoding UTF-8
Roxygen list(markdown = TRUE)
RoxygenNote 7.3.1
Depends R (>= 4.0.0)
Imports scales (>= 1.2.1),
      plyr (>= 1.8.8),
      data.table (>= 1.12.0),
      Seurat (>= 4.0.0),
      ggplot2 (>= 3.0.0),
      stringr (>= 1.5.0),
      ontologyIndex (\geq 2.10),
      igraph (>= 1.4.2),
      ontologyPlot (>= 1.6),
      ggraph (>= 2.1.0),
      cowplot(>= 1.1.0),
      ggnewscale (>= 0.4.0),
      purrr (>= 1.0.0),
      Rgraphviz (>= 2.42.0),
      knitr (>= 1.45),
      methods (>= 4.2.3),
      stats (>= 4.2.3)
```

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Suggests testthat (>= 3.0.0)

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the built-in Cell Marker Accordion database

Description

This function performs cell types annotation exploiting the built-in Accordion gene marker database. It takes in input either a Seurat object or a raw or normalized count matrix and return in output the cell types assignment and the detailed information of the annotation results (added to the Seurat object or as a list).

Usage

```
accordion(
  data,
  cluster_info = "seurat_clusters",
  assay = "RNA",
  database = NULL,
  CL_celltypes = NULL,
  species = "Human",
  tissue = NULL,
  include_descendants = FALSE,
  ECs_threshold = NULL,
  SPs_threshold = NULL,
  log2FC_threshold = NULL,
```

```
min_n_marker = 5,
max n marker = NULL.
combined_score_quantile_threshold = NULL,
annotation_resolution = "cluster",
cluster_score_quantile_threshold = 0.75,
allow_unknown = TRUE,
annotation_name = "accordion",
include_detailed_annotation_info = TRUE,
condition_group_info = NULL,
celltype_group_info = NULL,
group_markers_by = "celltype_cluster",
top_cell_score_quantile_threshold = 0.9,
n_{top_celltypes} = 5,
n_{top_markers} = 5,
top_marker_score_quantile_threshold = 0.75,
plot = TRUE,
color_by = "cell_type"
```

Arguments

data

Either a Seurat object (version 4 or 5) or a raw or normalized count matrix with genes on rows and cells on columns. If raw counts are provided, data are lognormalized exploiting the NormalizeData() function from the Seurat package.

cluster_info

in case data is a Seurat object, cluster_info should be need to be a character string specifying the name of the column in the metadata that contains cluster ids; if data is a count matrix, cluster_info should be need to be a data frame or data table containing cluster identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "cluster", which specifies the clustering id's for each cell. This parameter is necessary only when the input is a count matrix and only if the annotation_resolution parameter is set to "cluster". Default is "seurat_clusters".

assay

Character string specifying the Assay of the Seurat object. This parameter is necessary only in case data is a Seurat object. Default is "RNA".

database

Data table returns from the "marker_database_integration()" function. By default is set to NULL and the Accordion database is used for the annotation.

CL_celltypes

Character string or character string vector specifying the cell types to annotate. Run the function "list_celltypes()" to obtain the available cell types. If this parameter is not specified, all cell types present in the Accordion database are used for the annotation. Default is NULL.

species

Character string or character string vector specifying the species. Currently, either "Human" and/or "Mouse" are supported. If multiple species are selected, marker genes are merged together. Default is "Human".

tissue

Character string or character string vector specifying the tissue. Run the function "list_tissues()" to obtain the available tissues. If multiple tissues are selected cell types and markers from the selected tissues are aggregated. If NULL, all tissues are considered. Default is NULL.

include_descendants

Logical value indicating whether include all the tissues that are descendants of the selected tissue(s) according to the uberon ontology. If TRUE,cell types and

markers from the selected tissues and their descendants are aggregated. Default is FALSE

ECs_threshold

Integer value (currently in (1,17)) specifying the minimum evidence consistency score (ECs) for each marker. Only markers >= this threshold are kept. If NULL, no filter is applied. Default is NULL.

SPs_threshold

numeric value in (0,1) specifying the minimum specificity score (SPs) for each marker. Only markers <= this threshold are kept. If NULL, no filter is applied. Default is NULL.

log2FC_threshold

numeric value specifying the minimum log2FC threshold for each marker reporting this information. Only markers <= this threshold or without any log2FC are kept. If NULL, no filter is applied. Default is NULL.

min_n_marker

Integer value specifying the minimum number of markers to keep for each cell type. Only cell types with a number of markers >= this threshold are kept. Default is 5.

max_n_marker

Integer value specifying the maximum number of markers to keep for each cell type. For the selection, markers are ranked according to their combined score, obtained by multiplying evidence consistency score and SPs score. If NULL, no filter is applied. Default is NULL.

combined_score_quantile_threshold

numeric value in (0,1) specifying the combined score quantile threshold. For the selection, markers are ranked according to their combined score, obtained by multiplying evidence consistency score and SPs score. Only markers > the quantile_threshold are kept. If NULL, no filter is applied. Default is NULL.

annotation_resolution

Character string or character string vector specifying the resolution of the annotation. Either "cluster" and/or "cell" are supported. Default is "cluster".

cluster_score_quantile_threshold

numeric value in (0,1) specifying the cluster score quantile threshold. For each cell a score specific for each cell type is computed. To annotate a cluster cl, for each cell type the cluster_score_quantile_threshold is computed across cells belonging to that cluster and the cell type with the maximum score is then assigned to the cluster cl. Default is 0.75.

allow_unknown

Logical value indicating whether to allow cells or clusters to be labeled as "unknown". If it is set to TRUE, cells or clusters with negative scores are assigned to the "unknown" category. Default is TRUE.

annotation_name

Character string specifying the name of the column in either the metadata of the input Seurat object or in the input cluster_info where the annotation will be stored. Per cluster and per cell annotation results will be stored in the annotation_name_per_cluster and annotation_name_per_cell columns respectively. If include_detailed_annotation_info parameter is set to TRUE, the detailed information the stored in a list named annotation_name. Default is "accordion".

include_detailed_annotation_info

Logical value indicating whether to store information on the top cell types and markers in the output. If TRUE, a nested list named annotation_name is created. If resolution_annotation is set to "cluster" and/or "cell, sublists named "cluster_resolution" and/or "cell_resolution" are then added. Inside the sublist "detailed_annotation_info" the n_top_markers markers, group by group_markers_by

and the n_top_celltypes cell types are then included. If a Seurat object is provided as input the list is stored in the misc slot of the object (object@misc@annotation_name). If the input is a count matrix, the list is returned in the final output. Default is TRUE.

condition_group_info

in case data is a Seurat object, condition_group_info should be need to be a character string specifying the name of the column in the metadata that contains condition ids for each cell; if data is a count matrix, condition_group_info should be need to be a data frame or data table containing condition identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "condition", which specifies the condition id's for each cell. Default is NULL.

celltype_group_info

in case data is a Seurat object, celltype_group_info should be need to be a character string specifying the name of the column in the metadata that contains cell types ids for each cell; if data is a count matrix, celltype_group_info should be need to be a data frame or data table containing cell types identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "CL_celltype", which specifies the cell types for each cell. Default is NULL.

group_markers_by

Character string or character string vector specifying the classification of marker genes. It possible to retrieve n_top_markers marker genes for each cell type identified with cluster ("celltype_cluster") or cell ("celltype_cell") resolution; n_top_markers marker genes per cluster ("cluster") or per cell ("cell") can be also obtained. Additionally, by setting group_markers_by to "score_cell", the n_top_markers marker genes only for cells with a score greater than top_cell_score_quantile_the are retrieved. Either "celltype_cluster", "celltype_cell", "cluster", "cell" or "score_cell". Default is "celltype_cluster".

top_cell_score_quantile_threshold

numeric value in (0,1) specifying the cell score quantile threshold. For each cell type a score specific for each cell is computed. The top_cell_score_quantile_threshold is computed across cells belonging to the same cell type, and only cells with a score greater than the top_cell_score_quantile_threshold are kept. This parameter is necessary only when group_markers_by is set to "score_cell". Default is 0.90.

n_top_celltypes

Integer value specifying the number of the top cell types to be included in the output for each cluster and cell depending on the selected annotation_resolution parameter Default is 5.

n_top_markers

Integer value specifying the number of the top markers to be included in the output for each cell type, cluster or cell depending on the selected annotation_resolution and group_markers_by parameters. Default is 5.

top_marker_score_quantile_threshold

numeric value in (0,1) specifying the marker score quantile threshold. For each marker a score specific for each cell is computed. To identify the n_top_markers for a cluster cl or a cell type ct, the top_marker_score_quantile_threshold is computed across cells belonging to that cluster or labeled as ct, and the n_top_markers with the maximum score are reported. Default is 0.75.

plot

Logical value indicating whether to store plots displaying detailed annotation information. This parameter can be set to TRUE only when include_detailed_annotation_info

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is set to TRUE. If TRUE, lollipop plots displaying the top n_top_markers group by group_markers_by and top n_top_celltypes for each annotation_resolution together with the cell types hierarchies based on the cell ontology structure are stored in the annotation_name list. Default is TRUE.

color_by

Character string specifying if the plot reporting the top cell types for each cluster/cell is colored based on the assigned cell type ("CL_celltype") or on cluster id ("cluster"). Default is "CL_celltype.

Details

If a Seurat object was provided in input, the function returns the Seurat object with markers-based scaled data in the scale.data slot and cell types annotation results in the metadata. If include_detailed_annotation_in and plot were set to TRUE, a list containing cell types and markers information, together with ggplot objects, is stored in the "misc@annotation_name" slot. If a count matrix was provided in input, the function returns a list containing the following elements:

"scaled_matrix": normalized and scaled expression matrix;

If annotation_resolution is set to "cell":

"cell_annotation": data table containing cell types annotation results for each cell;

If annotation_resolution is set to "cluster":

"cluster annotation": data table containing cell types annotation results for each cell;

If include_detailed_annotation_info is set to TRUE:

"annotation_name": list containing detailed information of cell types annotation.

Value

A Seurat object or a list

accordion_cellcycle

Automatically identify and interpreting cell cycle state of single-cell populations

Description

This function identifies cell cycle states exploiting the collection of marker genes associated to each phase, including G0. It takes in input either a Seurat object or a raw or normalized count matrix and return in output the cell cycle assignment and the detailed informations of the annotation results (added to the Seurat object or as a list).

Usage

```
accordion_cellcycle(
  data,
  cluster_info = "seurat_clusters",
  assay = "RNA",
  species = "Human",
  annotation_resolution = "cell",
```

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```
annotation_name = "accordion_cell_cycle",
  cluster_score_quantile_threshold = 0.75,
  allow_unknown = FALSE,
  include_detailed_annotation_info = FALSE,
  condition_group_info = NULL,
  celltype_group_info = NULL,
  group_markers_by = "celltype_cell",
  n_top_celltypes = 5,
  n_top_markers = 5,
  top_marker_score_quantile_threshold = 0.75,
  plot = FALSE
)
```

Arguments

data

Either a Seurat object (version 4 or 5) or a raw or normalized count matrix with genes on rows and cells on columns. If raw counts are provided, data are lognormalized exploiting the NormalizeData() function from the Seurat package.

cluster_info

in case data is a Seurat object, cluster_info should be need to be a character string specifying the name of the column in the metadata that contains cluster ids; if data is a count matrix, cluster_info should be need to be a data frame or data table containing cluster identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "cluster", which specifies the clustering id's for each cell. This parameter is necessary only when the input is a count matrix and only if the annotation_resolution parameter is set to "cluster". Default is "seurat_clusters".

assay

Character string specifying the Assay of the Seurat object. This parameter is necessary only in case data is a Seurat object. Default is "RNA".

species

Character string or character string vector specifying the species. Currently, either "Human" and/or "Mouse" are supported. If multiple species are selected, marker genes are merged together. Default is "Human".

annotation_resolution

Character string or character string vector specifying the resolution of the annotation. Either "cluster" and/or "cell" are supported. Default is "cell".

annotation_name

Character string specifying the name of the column in either the metadata of the input Seurat object or in the input cluster_info where the annotation will be stored. Per cluster and per cell annotation results will be stored in the annotation_name_per_cluster and annotation_name_per_cell columns respectively. If include_detailed_annotation_info parameter is set to TRUE, the detailed information the stored in a list named annotation_name. Default is "accordion_cell_cycle".

cluster_score_quantile_threshold

numeric value in (0,1) specifying the cluster score quantile threshold. For each cell a score specific for each cell type is computed. To annotate a cluster cl, for each cell type the cluster_score_quantile_threshold is computed across cells belonging to that cluster and the cell type with the maximum score is then assigned to the cluster cl. Default is 0.75.

allow_unknown

Logical value indicating whether to allow cells or clusters to be labeled as "unknown". If it is set to TRUE, cells or clusters with negative scores are assigned to the "unknown" category. Default is TRUE.

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include_detailed_annotation_info

Logical value indicating whether to store information on the top cell types and markers in the output. If TRUE, a nested list named annotation_name is created. If resolution_annotation is set to "cluster" and/or "cell, sublists named "cluster_resolution" and/or "cell_resolution" are then added. Inside the sublist "detailed_annotation_info" the n_top_markers markers, group by group_markers_by and the n_top_celltypes cell types are then included. If a Seurat object is provided as input the list is stored in the misc slot of the object (object@misc@annotation_name). If the input is a count matrix, the list is returned in the final output. Default is FALSE.

condition_group_info

in case data is a Seurat object, condition_group_info should be need to be a character string specifying the name of the column in the metadata that contains condition ids for each cell; if data is a count matrix, condition_group_info should be need to be a data frame or data table containing condition identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "condition", which specifies the condition id's for each cell. Default is NULL.

celltype_group_info

in case data is a Seurat object, celltype_group_info should be need to be a character string specifying the name of the column in the metadata that contains cell types ids for each cell; if data is a count matrix, celltype_group_info should be need to be a data frame or data table containing cell types identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "cell_type", which specifies the cell types for each cell. Default is NULL.

group_markers_by

Character string or character string vector specifying the classification of marker genes. It possible to retrieve n_top_markers marker genes for each cell type identified with cluster ("celltype_cluster") or cell ("celltype_cell") resolution; n_top_markers marker genes per cluster ("cluster") or per cell ("cell") can be also obtained. Either "celltype_cluster", "celltype_cell", "cluster" and/or "cell". Default is "celltype_cell".

n_top_celltypes

Integer value specifying the number of the top cell types to be included in the output for each cluster and cell depending on the selected annotation_resolution parameter Default is 5.

n_top_markers

Integer value specifying the number of the top markers to be included in the output for each cell type, cluster or cell depending on the selected annotation_resolution and group_markers_by parameters. Default is 5.

top_marker_score_quantile_threshold

numeric value in (0,1] specifying the marker score quantile threshold. For each marker a score specific for each cell is computed. To identify the n_top_markers for a cluster cl or a cell type ct, the top_marker_score_quantile_threshold is computed across cells belonging to that cluster or labeled as ct, and the n_top_markers with the maximum score are reported. Default is 0.75.

plot

Logical value indicating whether to store plots displaying detailed annotation information. This parameter can be set to TRUE only when include_detailed_annotation_info is set to TRUE. If TRUE, lollipop plots displaying the top n_top_markers group by group_markers_by and n_top_celltypes for each annotation_resolution together with the cell types hierarchies based on the cell ontology structure are stored in the annotation_name list. Default is FALSE.

Details

If a Seurat object was provided in input, the function returns the Seurat object with markers-based scaled data in the scale.data slot and cell types annotation results in the metadata. If include_detailed_annotation_in and plot were set to TRUE, a list named annotation_name containing cell types and markers information, together with ggplot objects, is stored in the "misc" slot. If a count matrix was provided in input, the function returns a list containing the following elements:

"scaled_matrix": normalized and scaled expression matrix;

If annotation_resolution is set to "cell":

"cell_annotation": data table containing cell types annotation results for each cell;

If annotation_resolution is set to "cluster":

"cluster_annotation": data table containing cell types annotation results for each cell;

If include_detailed_annotation_info is set to TRUE:

"annotation_name": list containing detailed information of cell types annotation.

Value

A Seurat object or a list

accordion_custom

Automatically annotating and interpreting single-cell populations with custom marker genes sets

Description

This function performs cell types or signatures/pathways annotation based on custom marker genes set. It takes in input either a Seurat object or a raw or normalized count matrix and a table of marker genes associated to cell types or even to pathways and return in output the cell types/pathways assignment and the detailed information of the annotation results (added to the Seurat object or as a list).

Usage

```
accordion_custom(
  data,
  marker_table,
  category_column = "cell_type",
  marker_column = "marker",
  marker_type_column = "marker_type",
  weight_column = "weight",
  cluster_info = "seurat_clusters",
  assay = "RNA",
  min_n_marker = 5,
  max_n_marker = NULL,
  combined_score_quantile_threshold = NULL,
  annotation_resolution = "cluster",
  cluster_score_quantile_threshold = 0.75,
```

```
allow_unknown = TRUE,
annotation_name = "accordion_custom",
include_detailed_annotation_info = TRUE,
condition_group_info = NULL,
celltype_group_info = NULL,
group_markers_by = "celltype_cluster",
top_cell_score_quantile_threshold = 0.9,
n_top_celltypes = 5,
n_top_markers = 5,
top_marker_score_quantile_threshold = 0.75,
plot = TRUE
```

Arguments

data

Either a Seurat object (version 4 or 5) or a raw or normalized count matrix with genes on rows and cells on columns. If raw counts are provided, data are log-normalized exploiting the NormalizeData() function from the Seurat package.

marker_table

Data table or data frame containing cell type markers. The table needs to have at least two columns, the category_column, which specifies cell types or categories, and the marker_column, which specifies the corresponding markers on each row. Columns indicating the marker type (either positive or negative), and the marker weight can be optionally included.

category_column

String characters specifying the name of the marker_table column containing cell types or categories. Default is "cell_type".

marker_column

String characters specifying the name of the marker_table column containing markers. Default is "marker".

marker_type_column

Optional string characters specifying the name of the marker_table column containing string characters indicating the type of markers, either "positive" or "negative". If no marker_type_column is found in the marker_table all markers are considered "positive". Default is "marker_type".

weight_column

Optional string characters specifying the name of the marker_table column containing numeric value indicating the weight for each marker. If no weight_column is found in the marker_table all markers are equally weighted as 1. Default is "weight".

cluster_info

in case data is a Seurat object, cluster_info should be need to be a character string specifying the name of the column in the metadata that contains cluster ids; if data is a count matrix, cluster_info should be need to be a data frame or data table containing cluster identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "cluster", which specifies the clustering id's for each cell. This parameter is necessary only when the input is a count matrix and only if the annotation_resolution parameter is set to "cluster". Default is "seurat_clusters".

assay

Character string specifying the Assay of the Seurat object. This parameter is necessary only in case data is a Seurat object. Default is "RNA".

min_n_marker

Integer value specifying the minimum number of markers to keep for each cell type. Only cell types with a number of markers >= this threshold are kept. Default is 5.

max_n_marker

Integer value specifying the maximum number of markers to keep for each cell type. For the selection, markers are ranked according to their combined score, obtained by multiplying evidence consistency score and SPs score. If NULL, no filter is applied. Default is NULL.

combined_score_quantile_threshold

numeric value in (0,1] specifying the combined score quantile threshold. For the selection, markers are ranked according to their combined score, obtained by multiplying weight and SPs score. Only markers > the quantile_threshold are kept. If NULL, no filter is applied. Default is NULL.

annotation_resolution

Character string or character string vector specifying the resolution of the annotation. Either "cluster" and/or "cell" are supported. Default is "cluster".

cluster_score_quantile_threshold

numeric value in (0,1) specifying the cluster score quantile threshold. For each cell a score specific for each cell type is computed. To annotate a cluster cl, for each cell type the cluster_score_quantile_threshold is computed across cells belonging to that cluster and the cell type with the maximum score is then assigned to the cluster cl. Default is 0.75.

allow_unknown

Logical value indicating whether to allow cells or clusters to be labeled as "unknown". If it is set to TRUE, cells or clusters with negative scores are assigned to the "unknown" category. Default is TRUE.

annotation_name

Character string specifying the name of the column in either the metadata of the input Seurat object or in the input cluster_info where the annotation will be stored. Per cluster and per cell annotation results will be stored in the annotation_name_per_cluster and annotation_name_per_cell columns respectively. If include_detailed_annotation_info parameter is set to TRUE, the detailed information the stored in a list named annotation_name. Default is "accordion custom".

include_detailed_annotation_info

Logical value indicating whether to store information on the top cell types and markers in the output. If TRUE, a list named annotation_name is created. If resolution_annotation is set to "cluster" and/or "cell, sublists named "cluster_resolution" and/or "cell_resolution" are then added. Inside the sublist "detailed_annotation_info" the n_top_markers markers, group by group_markers_by and the n_top_celltypes cell types are then included. If a Seurat object is provided as input the list is stored in the misc slot of the object (object@misc@annotation_name). If the input is a count matrix, the list is returned in the final output. Default is TRUE.

condition_group_info

in case data is a Seurat object, condition_group_info should be need to be a character string specifying the name of the column in the metadata that contains condition ids for each cell; if data is a count matrix, condition_group_info should be need to be a data frame or data table containing condition identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "condition", which specifies the condition id's for each cell. Default is NULL.

celltype_group_info

in case data is a Seurat object, celltype_group_info should be need to be a character string specifying the name of the column in the metadata that contains cell types ids for each cell; if data is a count matrix, celltype_group_info

should be need to be a data frame or data table containing cell types identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "cell_type", which specifies the cell types for each cell. Default is NULL.

group_markers_by

Character string or character string vector specifying the classification of marker genes. It possible to retrieve n_top_markers marker genes for each cell type identified with cluster ("celltype_cluster") or cell ("celltype_cell") resolution; n_top_markers marker genes per cluster ("cluster") or per cell ("cell") can be also obtained. Additionally, by setting group_markers_by to "score_cell", the n_top_markers marker genes only for cells with a score greater than top_cell_score_quantile_there retrieved. Either "celltype_cluster", "celltype_cell", "cluster", "cell" or "score_cell". Default is "celltype_cluster".

top_cell_score_quantile_threshold

numeric value in (0,1] specifying the cell score quantile threshold. For each cell type/signature a score specific for each cell is computed. The top_cell_score_quantile_threshol is computed across cells belonging to the same cell type/signature, and only cells with a score greater than the top_cell_score_quantile_threshold are kept. This parameter is necessary only when group_markers_by is set to "score_cell". Default is 0.90.

n_top_celltypes

Integer value specifying the number of the top cell types to be included in the output for each cluster and cell depending on the selected annotation_resolution parameter Default is 5.

n_top_markers

Integer value specifying the number of the top markers to be included in the output for each cell type, cluster or cell depending on the selected annotation_resolution and group_markers_by parameters. Default is 5.

top_marker_score_quantile_threshold

numeric value in (0,1] specifying the marker score quantile threshold. For each marker a score specific for each cell is computed. To identify the n_top_markers for a cluster cl or a cell type ct, the top_marker_score_quantile_threshold is computed across cells belonging to that cluster or labeled as ct, and the n_top_markers with the maximum score are reported. Default is 0.75.

plot

Logical value indicating whether to store plots displaying detailed annotation information. This parameter can be set to TRUE only when include_detailed_annotation_info is set to TRUE. If TRUE, lollipop plots displaying the top n_top_markers group by group_markers_by and op n_top_celltypes for each annotation_resolution together with the cell types hierarchies based on the cell ontology structure are stored in the annotation_name list. Default is TRUE.

Details

If a Seurat object was provided in input, the function returns the Seurat object with markers-based scaled data in the scale.data slot and cell types annotation results in the metadata. If include_detailed_annotation_in and plot were set to TRUE, a list containing cell types and markers information, together with ggplot objects, is stored in the "misc@annotation_name" slot. If a count matrix was provided in input, the function returns a list containing the following elements:

"scaled_matrix": normalized and scaled expression matrix;

If annotation_resolution is set to "cell":

"cell_annotation": data table containing cell types annotation results for each cell;

```
If annotation_resolution is set to "cluster":
```

"cluster_annotation": data table containing cell types annotation results for each cell;

If include_detailed_annotation_info is set to TRUE:

"annotation_name": list containing detailed information of cell types annotation.

Value

A Seurat object or a list

accordion_disease

Automatically annotating and interpreting aberrant single-cell populations with the built-in Cell Marker Accordion disease database

Description

This function identified aberrant populations exploiting the built-in Accordion gene marker disease database. It takes in input either a Seurat object or a raw or normalized count matrix and return in output the cell types assignment and the detailed information of the annotation results (added to the Seurat object or as a list).

Usage

```
accordion_disease(
 data.
 disease = NULL,
  tissue = NULL,
  cluster_info = "seurat_clusters",
  assay = "RNA",
  database = NULL,
 NCIT_celltypes = NULL,
  species = "Human",
  include_descendants = FALSE,
 ECs_threshold = NULL,
  SPs_threshold = NULL,
  log2FC_threshold = NULL,
 malignant_quantile_threshold = 0.95,
 min_n_marker = 5,
 max_n_marker = NULL,
 combined_score_quantile_threshold = NULL,
 disease_vs_healthy = TRUE,
  annotation_resolution = "cluster",
  cluster_score_quantile_threshold = 0.75,
  allow_unknown = TRUE,
  annotation_name = "accordion_disease",
  include_detailed_annotation_info = TRUE,
  condition_group_info = NULL,
  celltype_group_info = NULL,
  group_markers_by = "celltype_cluster",
  top_cell_score_quantile_threshold = 0.9,
```

```
n_top_celltypes = 5,
n_top_markers = 5,
top_marker_score_quantile_threshold = 0.75,
plot = TRUE,
color_by = "cell_type"
)
```

Arguments

data Either a Seurat obj

Either a Seurat object (version 4 or 5) or a raw or normalized count matrix with genes on rows and cells on columns. If raw counts are provided, data are lognormalized exploiting the NormalizeData() function from the Seurat package.

disease Character string or character string vector specifying diseases to consider. Run

the function "list_diseases()" to obtain the available diseases. If NULL, all dis-

eases are considered. Default is NULL.

tissue Character string or character string vector specifying the tissue. Run the function

"list_tissues()" to obtain the available tissues. If multiple tissues are selected cell types and markers from the selected tissues are aggregated. If NULL, all tissues

are considered. Default is NULL.

cluster_info in case data is a Seurat object, cluster_info should be need to be a character

string specifying the name of the column in the metadata that contains cluster ids; if data is a count matrix, cluster_info should be need to be a data frame or data table containing cluster identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "cluster", which specifies the clustering id's for each cell. This parameter is necessary only when the input is a count matrix and only if the annotation_resolution parameter is set to "cluster". Default is

"seurat clusters".

assay Character string specifying the Assay of the Seurat object. This parameter is

necessary only in case data is a Seurat object. Default is "RNA".

database Data table returns from the "marker_database_integration()" function. By de-

fault is set to NULL and the Accordion database is used for the annotation.

NCIT_celltypes Character string or character string vector specifying the cell types to annotate.

Run the function "list_aberrant_celltypes()" to obtain the available aberrant cell types. If this parameter is not specified, all aberrant cell types are used for the

annotation. Default is NULL.

species Character string or character string vector specifying the species. Currently,

either "Human" and/or "Mouse" are supported. If multiple species are selected,

marker genes are merged together. Default is "Human".

include_descendants

Logical value indicating whether include all the tissues that are descendants of the selected tissue(s) according to the uberon ontology. If TRUE,cell types and markers from the selected tissues and their descendants are aggregated. Default

is FALSE

 ${\tt ECs_threshold} \quad \text{Integer value specifying the minimum evidence consistency score (ECs) for each} \\$

marker. Only markers >= this threshold are kept. If NULL, no filter is applied.

Default is NULL.

SPs_threshold numeric value in (0,1) specifying the minimum specificity score (SPs) for each

marker. Only markers <= this threshold are kept. If NULL, no filter is applied.

Default is NULL.

log2FC_threshold

numeric value specifying the minimum log2FC threshold for each marker reporting this information. Only markers <= this threshold or without any log2FC are kept. If NULL, no filter is applied. Default is NULL.

$malignant_quantile_threshold$

numeric value in (0,1) specifying the malignant quantile threshold. If an aberant cell type is provide in input, a malignant score specific for each cell is computed. The malignant_quantile_threshold is computed across cells, and only cells with a score greater than the top_cell_score_quantile_threshold are labeled as malignant. If annotation_resolution is set to "cell", a cell

min_n_marker

Integer value specifying the minimum number of markers to keep for each cell type. Only cell types with a number of markers >= this threshold are kept. Default is 5.

max_n_marker

Integer value specifying the maximum number of markers to keep for each cell type. For the selection, markers are ranked according to their combined score, obtained by multiplying evidence consistency score and SPs score. If NULL, no filter is applied. Default is NULL.

combined_score_quantile_threshold

numeric value in (0,1) specifying the combined score quantile threshold. For the selection, marers are ranked according to their combined score, obtained by multiplying evidence consistency score and SPs score. Only markers > the quantile_threshold are kept. If NULL, no filter is applied. Default is NULL.

disease_vs_healthy

Logical value indicating whether to compare the markers associated with disease cell types with respect to the markers associated with the corresponding healthy cell types. If TRUE the SPs score is calculated considering if a gene is also a marker for the corresponding healthy cell type. For each cell, a specific score for disease cell types and another score for the healthy types (named as the cell types label) are returned. Default is TRUE.

annotation_resolution

Character string or character string vector specifying the resolution of the annotation. Either "cluster" and/or "cell" are supported. Default is "cluster".

cluster_score_quantile_threshold

numeric value in (0,1) specifying the cluster score quantile threshold. For each cell a score specific for each cell type is computed. To annotate a cluster cl, for each cell type the cluster_score_quantile_threshold is computed across cells belonging to that cluster and the cell type with the maximum score is then assigned to the cluster cl. Default is 0.75.

allow_unknown

Logical value indicating whether to allow cells or clusters to be labeled as "unknown". If it is set to TRUE, cells or clusters with negative scores are assigned to the "unknown" category. Default is TRUE.

annotation_name

Character string specifying the name of the column in either the metadata of the input Seurat object or in the input cluster_info where the annotation will be stored. Per cluster and per cell annotation results will be stored in the annotation_name_per_cluster and annotation_name_per_cell columns respectively. If include_detailed_annotation_info parameter is set to TRUE, the detailed information the stored in a list named annotation_name. Default is "accordion_disease".

include_detailed_annotation_info

Logical value indicating whether to store information on the top cell types and markers in the output. If TRUE, a nested list named annotation_name is created. If resolution_annotation is set to "cluster" and/or "cell, sublists named "cluster_resolution" and/or "cell_resolution" are then added. Inside the sublist "detailed_annotation_info" the n_top_markers markers, group by group_markers_by and the n_top_celltypes cell types are then included. If a Seurat object is provided as input the list is stored in the misc slot of the object (object@misc@annotation_name). If the input is a count matrix, the list is returned in the final output. Default is TRUE.

condition_group_info

in case data is a Seurat object, condition_group_info should be need to be a character string specifying the name of the column in the metadata that contains condition ids for each cell; if data is a count matrix, condition_group_info should be need to be a data frame or data table containing condition identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "condition", which specifies the condition id's for each cell. Default is NULL.

celltype_group_info

in case data is a Seurat object, celltype_group_info should be need to be a character string specifying the name of the column in the metadata that contains cell types ids for each cell; if data is a count matrix, celltype_group_info should be need to be a data frame or data table containing cell types identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "NCIT_celltype", which specifies the cell types for each cell. Default is NULL.

group_markers_by

Character string or character string vector specifying the classification of marker genes. It possible to retrieve n_top_markers marker genes for each cell type identified with cluster ("celltype_cluster") or cell ("celltype_cell") resolution; n_top_markers marker genes per cluster ("cluster") or per cell ("cell") can be also obtained. Additionally, by setting group_markers_by to "score_cell", the n_top_markers marker genes only for cells with a score greater than top_cell_score_quantile_the are retrieved. Either "celltype_cluster", "celltype_cell", "cluster", "cell" or "score_cell". Default is "celltype_cluster".

top_cell_score_quantile_threshold

numeric value in (0,1) specifying the cell score quantile threshold. For each cell type a score specific for each cell is computed. The top_cell_score_quantile_threshold is computed across cells belonging to the same cell type, and only cells with a score greater than the top_cell_score_quantile_threshold are kept. This parameter is necessary only when group_markers_by is set to "score_cell". Default is 0.90.

n_top_celltypes

Integer value specifying the number of the top cell types to be included in the output for each cluster and cell depending on the selected annotation_resolution parameter Default is 5.

n_top_markers

Integer value specifying the number of the top markers to be included in the output for each cell type, cluster or cell depending on the selected annotation_resolution and group_markers_by parameters. Default is 5.

top_marker_score_quantile_threshold

numeric value in (0,1) specifying the marker score quantile threshold. For each marker a score specific for each cell is computed. To identify the n_top_markers

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for a cluster cl or a cell type ct, the top_marker_score_quantile_threshold is computed across cells belonging to that cluster or labeled as ct, and the

n_top_markers with the maximum score are reported. Default is 0.75.

plot Logical value indicating whether to store plots displaying detailed annotation in-

formation. This parameter can be set to TRUE only when include_detailed_annotation_info

is set to TRUE. If TRUE, lollipop plots displaying the top <code>n_top_markers</code> group by <code>group_markers_by</code> and op <code>n_top_celltypes</code> for each annotation_resolution together with the cell types hierarchies based on the cell ontology structure are

stored in the "accordion" list. Default is TRUE.

color_by Character string specifying if the plot reporting the top cell types for each clus-

ter/cell is colored based on the assigned cell type ("CL_celltype") or on cluster

id ("cluster"). Default is "CL_celltype.

Details

If a Seurat object was provided in input, the function returns the Seurat object with markers-based scaled data in the scale.data slot and cell types annotation results in the metadata. If include_detailed_annotation_in and plot were set to TRUE, a list containing cell types and markers information, together with ggplot objects, is stored in the "misc@annotation_name" slot. If a count matrix was provided in input, the function returns a list containing the following elements:

"scaled_matrix": normalized and scaled expression matrix;

If annotation_resolution is set to "cell":

"cell_annotation": data table containing cell types annotation results for each cell;

If annotation_resolution is set to "cluster":

"cluster_annotation": data table containing cell types annotation results for each cell;

If $include_detailed_annotation_info$ is set to TRUE:

"annotation_name": list containing detailed information of cell types annotation.

Value

A Seurat object or a list

accordion_marker

accordion_marker: Cell Marker Accordion database

Description

accordion_marker: Cell Marker Accordion database

Usage

data(accordion_marker)

Format

A data table

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accordion_plot

Interpreting annotation results

Description

This function generates lollipop plots displaying the detailed annotation results obtained with the accordion_annotation, accordion_disease_annotation and accordion_custom_annotation functions.

Usage

```
accordion_plot(
  data,
  info_to_plot = "accordion",
  resolution = "cluster",
  group_markers_by = "celltype_cluster",
  color_by = "cell_type",
  condition_group_info = NULL,
  celltype_group_info = NULL
)
```

Arguments

data

A Seurat object or a list containing "detailed_annotation_info", from either accordion(), accordion_disease() or accordion_custom() functions, run with include_detailed_annotation_info parameter set to TRUE.

info_to_plot

Character string or character string vector specifying the list from which extract the detailed annotation information, either "accordion", "accordion_disease" or "accordion_custom", for which returns the plot, either "accordion", "accordion_disease" or "accordion_custom".

resolution

Character string or character string vector specifying the annotation resolution for which provided the plots. Either "cluster" and/or "cell" are supported. Default is "cluster".

group_markers_by

Character string or character string vector specifying the classification of marker genes. It is possible to retrieve top marker genes for each cell type identified with cluster ("celltype_cluster") or cell ("celltype_cell") resolution; top marker genes per cluster ("cluster") or per cell ("cell") can be also obtained. Additionally, by setting group_markers_by to "score_cell", the n_top_markers marker genes only for cells with a score greater than top_cell_score_quantile_threshold are retrieved. Either "celltype_cluster", "celltype_cell", "cluster", "cell" or "score_cell". Default is "celltype_cluster".

color_by

Character string specifying if the plot reporting the top cell types for each cluster/cell is colored based on the assigned cell type ("cell_type") or on cluster id ("cluster"). Default is "cell_type.

condition_group_info

in case data is a Seurat object, condition_group_info should be need to be a character string specifying the name of the column in the metadata that contains condition ids for each cell; if data is a count matrix, condition_group_info should be need to be a data frame or data table containing condition identity

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for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "condition", which specifies the condition id's for each cell. Default is NULL.

celltype_group_info

in case data is a Seurat object, celltype_group_info should be need to be a character string specifying the name of the column in the metadata that contains cell types ids for each cell; if data is a count matrix, celltype_group_info should be need to be a data frame or data table containing cell types identity for each cell. The data frame or data table should contain at least two columns, one named "cell", which specifies cell id's, and one named "CL_celltype" or "NCIT_celltype", which specifies the cell types for each cell. Default is NULL.

Details

top cell types (or pathways) and top markers It takes in input either a Seurat object or a raw or normalized count matrix and a table of marker genes associated to cell types or even to pathways and return in output the cell types/pathways assignment and the detailed information of the annotation results (added to the Seurat object or as a list).

If a Seurat object was provided in input, the function returns the Seurat object with a list of ggplot objects added to the "misc" slot in the info_to_plot list. If a list was provided in input, the function returns the same list with the addition of the ggplot objects.

Value

A Seurat object or a list.

cell_cycle_markers

cell_cycle_markers: Collection of markers assiciated to cell cycle

Description

cell_cycle_markers: Collection of markers assiciated to cell cycle

Usage

```
data(cell_cycle_markers)
```

Format

A data table

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cell_onto

cell_onto: Cell ontology

Description

cell_onto: Cell ontology

Usage

data(cell_onto)

Format

Cell ontology

disease_accordion_marker

disease_accordion_marker: Cell Marker Accordion disease database

Description

disease_accordion_marker: Cell Marker Accordion disease database

Usage

data(disease_accordion_marker)

Format

A data table

gene_description

gene_description: table containing gene description for human and mouse genes (human112_GRCh38.p14 and mouse112_GRCm39)

Description

 $gene_description: table \ containing \ gene \ description \ for \ human \ and \ mouse \ genes \ (human 112_GRCh 38.p14 \ and \ mouse \ 112_GRCm 39)$

Usage

data(gene_description)

Format

data table

list_aberrant_celltypes

List aberrant cell types available in the Cell Marker Accordion disease database

Description

List aberrant cell types available in the Cell Marker Accordion disease database

Usage

```
list_aberrant_celltypes(
  species = c("Human", "Mouse"),
  disease = NULL,
  tissue = NULL
)
```

Arguments

tissue

species	Character string or character string vector specifying the species for which to ex-
	tract the associate list of available cell types. Currently, either "Human" and/or
	"Mouse" are supported. Default is c("Mouse", "Human").
disease	Character string or character string vector specifying diseases to consider. If NULL, information from all diseases are considered. Default is NULL.

Character string or character string vector specifying the tissue for which to extract the associate list of available cell types. If NULL, information from all

tissues are retrieved.

Value

List of aberrant cell types available in the Cell Marker Accordion disease database

list_celltypes

List cell types available in the Cell Marker Accordion database

Description

List cell types available in the Cell Marker Accordion database

Usage

```
list_celltypes(species = c("Human", "Mouse"), tissue = NULL)
```

Arguments

species Character string or character string vector specifying the species for which to extract the associate list of available cell types. Currently, either "Human" and/or "Mouse" are supported. Default is c("Mouse", "Human").

tissue Character string or character string vector specifying the tissue for which to extract the associate list of available cell types. If NULL, information from all

tissues are retrieved.

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Value

List of cell types available in the Cell Marker Accordion database

list_diseases

List diseases available in the Cell Marker Accordion disease database

Description

List diseases available in the Cell Marker Accordion disease database

Usage

```
list_diseases(
  species = c("Human", "Mouse"),
  tissue = NULL,
  aberrant_celltype = NULL
)
```

Arguments

species

Character string or character string vector specifying the species for which to extract the associate list of available diseases Currently, either "Human" and/or "Mouse" are supported. Default is c("Mouse", "Human").

tissue

Character string or character string vector specifying the tissue for which to extract the associate list of available diseases. If NULL, information from all tissues are retrieved.

aberrant_celltype

Character string or character string vector specifying the aberrant celltype for which to extract the associate list of available tissues. If NULL, information from all aberrant cell types are retrieved. @return List of diseases available in the Cell Marker Accordion disease database

Description

List tissues available in the Cell Marker Accordion disease database

Usage

```
list_disease_tissues(
  species = c("Human", "Mouse"),
  disease = NULL,
  aberrant_celltype = NULL
)
```

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Arguments

species Character string or character string vector specifying the species for which to

extract the associate list of available tissues. Currently, either "Human" and/or

"Mouse" are supported. Default is c("Mouse", "Human").

disease Character string or character string vector specifying diseases to consider. If

NULL, information from all diseases are considered. Default is NULL.

aberrant_celltype

Character string or character string vector specifying the aberrant celltype for which to extract the associate list of available tissues. f NULL, information

from all cell types are retrieved.

Value

List of tissues available in the Cell Marker Accordion disease database

list_tissues

List tissues available in the Cell Marker Accordion database

Description

List tissues available in the Cell Marker Accordion database

Usage

```
list_tissues(species = c("Human", "Mouse"), celltype = NULL)
```

Arguments

species Character string or character string vector specifying the species for which to

extract the associate list of available tissues. Currently, either "Human" and/or

"Mouse" are supported. Default is c("Mouse", "Human").

celltype Character string or character string vector specifying the celltype for which to

extract the associate list of available tissues. If NULL, information from all cell

types are retrieved.

Value

List of tissues available in the Cell Marker Accordion database

```
marker_database_integration
```

Create an augmented database by integrated custom set of markers genes with the Accordion database

Description

This function performs markers integration provided by the users and the accordion database, either physiological ("healthy") or disease ("disease). It takes in input a table of marker genes associated to cell types and return the integrated database of custom and accordion markers. This new integrated database can be used as input for the "accordion" or "disease_accordion" function to provide the annotation.

Usage

```
marker_database_integration(
  marker_table,
  database = "healthy",
  species_column = "species",
  disease_column = "disease",
  tissue_column = "tissue",
  celltype_column = "cell_type",
  marker_column = "marker",
  marker_type_column = "marker_type",
  resource_column = "resource")
```

Arguments

marker table	Data table or data frame	e containing cell type	markers.	The table needs to have

at least two columns, the celltype_column, which specifies cell types, and the marker_column, which specifies the corresponding markers on each row. Columns indicating the marker type (either positive or negative), species, con-

dition, tissue and resource can be optionally included.

database String characters specifying the accordion database to use for integration. Either

"healthy" or "disease" are supported, where "healthy" refers to the physiological accordion database, while "disease" refers to the disease accordion database.

Default is "healthy"

species_column String characters specifying the name of the marker_table column containing

species. Default is "species".

disease_column String characters specifying the name of the marker_table column containing

diseases. Default is "disease".

tissue_column String characters specifying the name of the marker_table column containing

tissues. Default is "tissue".

celltype_column

String characters specifying the name of the marker_table column containing

cell types. Default is "cell_type".

marker_column String characters specifying the name of the marker_table column containing

markers. Default is "marker".

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marker_type_column

String characters specifying the name of the marker_table column containing marker types. Default is "marker_type".

resource_column

String characters specifying the name of the marker_table column containing resources. Default is "resource".

Value

A data table

uberon_onto

uberon_onto: Uberon anatomy ontology

Description

uberon_onto: Uberon anatomy ontology

Usage

data(uberon_onto)

Format

Uberon ontology

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