

# Package ‘cellmarkeraccordion’

January 10, 2024

**Title** Annotation of normal and aberrant hematopoietic cell types in single-cell datasets

**Version** 0.9.0

**Description** cellmarkeraccordion is an R package designed to obtain a robust identification of normal and aberrant hematopoietic cell types in single-cell datasets and 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.

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Seurat (>= 4.0.0),  
ggplot2 (>= 3.0.0),  
stringr (>= 1.5.0),  
ontoProc (>= 1.20.0),  
ontologyIndex (>= 2.10),  
igraph (>= 1.4.2),  
ontologyPlot (>= 1.6),  
ggraph (>= 2.1.0),  
cowplot (>= 1.1.0),  
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accordion	<i>Automatically annotating and interpreting single-cell populations with the built-in Cell Marker Accordion database</i>
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## 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 informations of the annotation results (added to the Seurat object or as a list).

## Usage

```
accordion(
  data,
  cluster_info = "seurat_clusters",
  assay = "RNA",
  cell_types = NULL,
  species = "Human",
  evidence_consistency_score_threshold = NULL,
  specificity_score_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,
  cell_type_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 4.9) 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 <code>NormalizeData()</code> function from the Seurat package.
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cluster_info	in case object 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 object 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".
cell_types	Character string or character string vector specifying the cell types to annotate. 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".
evidence_consistency_score_threshold	Integer value (currently in 1,7) specifying the minimum evidence consistency (EC) score for each marker. Only markers $\geq$ this threshold are kept. If NULL, no filter is applied. Default is NULL.
specificity_score_threshold	numeric value in (0,1] specifying the minimum specificity score for each marker. Only markers $\leq$ this threshold 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 $\geq$ 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 specificity 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 specificity 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 is 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 object 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 object 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.

cell\_type\_group\_info

in case object is a Seurat object, cell\_type\_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 object is a count matrix, cell\_type\_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\_threshold 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 <code>annotation_resolution</code> parameter. Default is 5.
<code>n_top_markers</code>	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 <code>annotation_resolution</code> and <code>group_markers_by</code> parameters. Default is 5.
<code>top_marker_score_quantile_threshold</code>	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 <code>n_top_markers</code> for a cluster <code>cl</code> or a cell type <code>ct</code> , the <code>top_marker_score_quantile_threshold</code> is computed across cells belonging to that cluster or labeled as <code>ct</code> , and the <code>n_top_markers</code> with the maximum score are reported. Default is 0.75.
<code>plot</code>	Logical value indicating whether to store plots displaying detailed annotation information. This parameter can be set to TRUE only when <code>include_detailed_annotation_info</code> is set to TRUE. If TRUE, lollipop plots displaying the top <code>n_top_markers</code> group by <code>group_markers_by</code> and top <code>n_top_celltypes</code> for each <code>annotation_resolution</code> together with the cell types hierarchies based on the cell ontology structure are stored in the <code>annotation_name</code> 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_info` 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

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accordion_annotation	<i>Automatically annotating single-cell populations with the built-in Cell Marker Accordion database</i>
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## 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 (added to the Seurat object or as a list).

## Usage

```
accordion_annotation(
  data,
  cluster_info = "seurat_clusters",
  assay = "RNA",
  cell_types = NULL,
  species = "Human",
  evidence_consistency_score_threshold = NULL,
  specificity_score_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,
  cell_type_group_info = NULL,
  group_markers_by = "celltype_cluster",
  n_top_celltypes = 5,
  n_top_markers = 5,
  top_marker_score_quantile_threshold = 0.75
)
```

## Arguments

data	Either a Seurat object (version 4.9) 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 <code>NormalizeData()</code> function from the Seurat package.
cluster_info	in case object 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 object 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”.
cell_types	Character string or character string vector specifying the cell types to annotate. 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”.
evidence_consistency_score_threshold	Integer value (currently in 1,7) specifying the minimum evidence consistency (EC) score for each marker. Only markers $\geq$ this threshold are kept. If NULL, no filter is applied. Default is NULL.
specificity_score_threshold	numeric value in (0,1] specifying the minimum specificity score for each marker. Only markers $\leq$ this threshold 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 $\geq$ 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 specificity 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 specificity 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 object 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 object 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.

**cell\_type\_group\_info**

in case object is a Seurat object, `cell_type_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 object is a count matrix, `cell_type_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_threshold` are retrieved. Either "celltype\_cluster", "celltype\_cell", "cluster", "cell" or "score\_cell". Default is "celltype\_cluster".

**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.

**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_threshold`



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.

## 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_info` was set to TRUE, a list containing cell types and markers information 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

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<code>accordion_cell_cycle</code>	<i>Automatically identify and interpreting cell cycle state of single-cell populations</i>
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## 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_cell_cycle(
  data,
  cluster_info = "seurat_clusters",
  assay = "RNA",
  species = "Human",
  annotation_resolution = "cell",
  annotation_name = "accordion_cell_cycle",
  cluster_score_quantile_threshold = 0.75,
  allow_unknown = FALSE,
  include_detailed_annotation_info = FALSE,
```

```

condition_group_info = NULL,
cell_type_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 4.9) 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 <code>NormalizeData()</code> function from the Seurat package.
cluster_info	in case object is a Seurat object, <code>cluster_info</code> should be need to be a character string specifying the name of the column in the metadata that contains cluster ids; if object is a count matrix, <code>cluster_info</code> 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 <code>annotation_resolution</code> 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 <code>cluster_info</code> where the annotation will be stored. Per cluster and per cell annotation results will be stored in the <code>annotation_name_per_cluster</code> and <code>annotation_name_per_cell</code> columns respectively. If <code>include_detailed_annotation_info</code> parameter is set to TRUE, the detailed information the stored in a list named <code>annotation_name</code> . Default is "accordion_cell_cycle".
cluster_score_quantile_threshold	numeric value in <a href="#">0,1</a> 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 <code>cluster_score_quantile_threshold</code> 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.
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 <code>annotation_name</code> is created. If <code>resolution_annotation</code> 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. @param `condition_group_info` in case object 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 object 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. @param `cell_type_group_info` in case object is a Seurat object, `cell_type_group_info` should be need to be a character string specifying the name of the column in the metadata that contains cell types for each cell; if object is a count matrix, `cell_type_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.

<code>group_markers_by</code>	Character string or character string vector specifying the classification of marker genes. It possible to retrieve <code>n_top_markers</code> marker genes for each cell type identified with cluster ("celltype_cluster") or cell ("celltype_cell") resolution; <code>n_top_markers</code> 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".
<code>n_top_celltypes</code>	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 <code>annotation_resolution</code> parameter Default is 5.
<code>n_top_markers</code>	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 <code>annotation_resolution</code> and <code>group_markers_by</code> parameters. Default is 5.
<code>top_marker_score_quantile_threshold</code>	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 <code>n_top_markers</code> for a cluster <code>cl</code> or a cell type <code>ct</code> , the <code>top_marker_score_quantile_threshold</code> is computed across cells belonging to that cluster or labeled as <code>ct</code> , and the <code>n_top_markers</code> with the maximum score are reported. Default is 0.75.
<code>plot</code>	Logical value indicating whether to store plots displaying detailed annotation information. This parameter can be set to TRUE only when <code>include_detailed_annotation_info</code> is set to TRUE. If TRUE, lollipop plots displaying the top <code>n_top_markers</code> group by <code>group_markers_by</code> and <code>n_top_celltypes</code> for each <code>annotation_resolution</code> together with the cell types hierarchies based on the cell ontology structure are stored in the <code>annotation_name</code> 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

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accordion_custom	<i>Automatically annotating and interpreting single-cell populations with custom marker genes sets</i>
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### 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 informations 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,
  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,
```

```

cell_type_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 4.9) 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 object 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 object 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 $\geq$ 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 specificity score. 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 object 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 object 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.
- cell\_type\_group\_info**  
in case object is a Seurat object, `cell_type_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 object is a count matrix, `cell_type_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

	<p><code>n_top_markers</code> marker genes only for cells with a score greater than <code>top_cell_score_quantile_threshold</code> are retrieved. Either "celltype_cluster", "celltype_cell", "cluster", "cell" or "score_cell". Default is "celltype_cluster".</p>
<code>top_cell_score_quantile_threshold</code>	<p>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 <code>top_cell_score_quantile_threshold</code> is computed across cells belonging to the same cell type/signature, and only cells with a score greater than the <code>top_cell_score_quantile_threshold</code> are kept. This parameter is necessary only when <code>group_markers_by</code> is set to "score_cell". Default is 0.90.</p>
<code>n_top_celltypes</code>	<p>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 <code>annotation_resolution</code> parameter. Default is 5.</p>
<code>n_top_markers</code>	<p>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 <code>annotation_resolution</code> and <code>group_markers_by</code> parameters. Default is 5.</p>
<code>top_marker_score_quantile_threshold</code>	<p>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 <code>n_top_markers</code> for a cluster <code>cl</code> or a cell type <code>ct</code>, the <code>top_marker_score_quantile_threshold</code> is computed across cells belonging to that cluster or labeled as <code>ct</code>, and the <code>n_top_markers</code> with the maximum score are reported. Default is 0.75.</p>
<code>plot</code>	<p>Logical value indicating whether to store plots displaying detailed annotation information. This parameter can be set to TRUE only when <code>include_detailed_annotation_info</code> is set to TRUE. If TRUE, lollipop plots displaying the top <code>n_top_markers</code> group by <code>group_markers_by</code> and <code>op</code> <code>n_top_celltypes</code> for each <code>annotation_resolution</code> together with the cell types hierarchies based on the cell ontology structure are stored in the <code>annotation_name</code> list. Default is TRUE.</p>

## 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_info` 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

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accordion\_custom\_annotation

*Automatically annotating 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 (added to the Seurat object or as a list).

## Usage

```
accordion_custom_annotation(
  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,
  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,
  cell_type_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
)
```

## Arguments

data	Either a Seurat object (version 4.9) 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 <code>NormalizeData()</code> 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 <code>category_column</code> , which specifies cell types or categories, and the <code>marker_column</code> , 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 object 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 object is a count matrix,
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 $\geq$ 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 specificity score. 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 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 object 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 object 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.

`cell_type_group_info`

in case object is a Seurat object, `cell_type_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 object is a count matrix, `cell_type_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_threshold` 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/signature a score specific for each cell is computed. The `top_cell_score_quantile_threshold` 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.

## 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_info` 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

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<code>accordion_disease</code>	<i>Automatically annotating and interpreting aberrant single-cell populations with the built-in Cell Marker Accordion disease database</i>
--------------------------------	--

---

## 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 informations of the annotation results (added to the Seurat object or as a list).

## Usage

```
accordion_disease(
  data,
  disease = NULL,
  cluster_info = "seurat_clusters",
  assay = "RNA",
  cell_types = NULL,
  species = "Human",
  evidence_consistency_score_threshold = NULL,
  specificity_score_threshold = NULL,
  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,
cell_type_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 4.9) 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 <code>NormalizeData()</code> function from the Seurat package.
disease	Character string or character string vector specifying diseases to consider. If NULL, the full disease Accordion database is considered. Default is NULL.
cluster_info	in case object 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 object 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".
cell_types	Character string or character string vector specifying the cell types to annotate. 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".
evidence_consistency_score_threshold	Integer value (currently in 1,7) specifying the minimum evidence consistency (EC) score for each marker. Only markers $\geq$ this threshold are kept. If NULL, no filter is applied. Default is NULL.
specificity_score_threshold	numeric value in (0,1] specifying the minimum specificity score for each marker. Only markers $\leq$ this threshold 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 $\geq$ 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 specificity 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 specificity 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 specificity 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 is 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 object 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 object 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.

#### cell\_type\_group\_info

in case object is a Seurat object, cell\_type\_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 object is a count matrix, cell\_type\_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\_threshold 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 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 "accordion" 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\_annotation

*Automatically annotating 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 (added to the Seurat object or as a list).

## Usage

```
accordion_disease_annotation(
  data,
  disease = NULL,
  cluster_info = "seurat_clusters",
  assay = "RNA",
  cell_types = NULL,
  species = "Human",
  evidence_consistency_score_threshold = NULL,
  specificity_score_threshold = NULL,
  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,
cell_type_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
)

```

## Arguments

data	Either a Seurat object (version 4.9) 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 <code>NormalizeData()</code> function from the Seurat package.
disease	Character string or character string vector specifying diseases to consider. If <code>NULL</code> , the full disease Accordion database is considered. Default is <code>NULL</code> .
cluster_info	in case object is a Seurat object, <code>cluster_info</code> should be need to be a character string specifying the name of the column in the metadata that contains cluster ids; if object is a count matrix, <code>cluster_info</code> 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 <code>annotation_resolution</code> 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".
cell_types	Character string or character string vector specifying the cell types to annotate. If this parameter is not specified, all cell types present in the Accordion database are used for the annotation. Default is <code>NULL</code> .
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".
evidence_consistency_score_threshold	Integer value (currently in 1,7) specifying the minimum evidence consistency (EC) score for each marker. Only markers $\geq$ this threshold are kept. If <code>NULL</code> , no filter is applied. Default is <code>NULL</code> .
specificity_score_threshold	numeric value in (0,1] specifying the minimum specificity score for each marker. Only markers $\leq$ this threshold are kept. If <code>NULL</code> , no filter is applied. Default is <code>NULL</code> .
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 $\geq$ 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 specificity score. If <code>NULL</code> , no filter is applied. Default is <code>NULL</code> .
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 specificity 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 specificity 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 object 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 object 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.

cell\_type\_group\_info

in case object is a Seurat object, cell\_type\_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 object is a count matrix, cell\_type\_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\_threshold 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.

## 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\_info 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

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accordion_plot	<i>Interpreting annotation results</i>
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## 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"
)
```

## Arguments

data	A Seurat object or a list containing “detailed_annotation_info”, from either <code>accordion()</code> , <code>accordion_disease()</code> or <code>accordion_custom()</code> functions, run with <code>include_detailed_annotation_info</code> 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”. Default is “accordion”.
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 <code>group_markers_by</code> to “score_cell”, the <code>n_top_markers</code> marker genes only for cells with a score greater than <code>top_cell_score_quantile_threshold</code> are retrieved. Either “celltype_cluster”, “celltype_cell”, “cluster”, “cell” or “score_cell”. Default is “celltype_cluster”.

## 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 informations 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.

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