# Package 'ClustAssess'

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Type Package

**Title** Tools for Assessing Clustering

Version 1.1.0

Description A set of tools for evaluating clustering robustness using proportion of ambiguously clustered pairs (Senbabaoglu et al. (2014) <doi:10.1038/srep06207>), as well as similarity across methods and method stability using element-centric clustering comparison (Gates et al. (2019) <doi:10.1038/s41598-019-44892-y>). Additionally, this package enables stability-based parameter assessment for graph-based clustering pipelines typical in single-cell data analysis.

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**Encoding UTF-8** 

Additional\_repositories https://blaserlab.r-universe.dev

biocViews Software, SingleCell, RNASeq, ATACSeq, Normalization,
 Preprocessing, DimensionReduction, Visualization,
 QualityControl, Clustering, Classification, Annotation,
 GeneExpression, DifferentialExpression

**Depends** R (>= 4.0.0), methods, stats

Imports dplyr, DT, fastcluster, foreach, glue, Gmedian, ggnewscale, ggplot2, ggrastr, ggrepel, ggtext, gprofiler2, igraph, jsonlite, leiden, Matrix (>= 1.5.0), matrixStats, progress, stringr, paletteer, plotly, qualpalr, RANN, reshape2, rlang, Seurat, shiny, shinyjs, shinyLP, shinyWidgets, utils, uwot, vioplot

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LinkingTo Rcpp, RcppEigen

**Suggests** BiocManager, colourpicker, ComplexHeatmap, data.table, DelayedMatrixStats, devtools, doParallel, leidenbase, monocle3, patchwork, ragg, reticulate, rhdf5, RhpcBLASctl, rmarkdown, scales, SeuratObject, SharedObject, styler, testthat (>= 3.0.0)

URL https://github.com/Core-Bioinformatics/ClustAssess,
 https://core-bioinformatics.github.io/ClustAssess/

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$\pmb{BugReports} \   \texttt{https://github.com/Core-Bioinformatics/ClustAssess/issues}$
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add	metadata Add metadata to ClustAssess ShinyApp	

# Description

Adds new metadata into the ClustAssess ShinyApp without having to update the object and re-create the app.

# Usage

```
add_metadata(app_folder, metadata, qualpalr_colorspace = "pretty")
```

# Arguments

app\_folder The folder containing the ClustAssess ShinyApp

metadata The new metadata to be added. This parameter should be a dataframe that fol-

lows the same row ordering as the already existing metadata from the Clus-

tAssess app.

qualpalr\_colorspace

The colorspace to be used for the metadata

#### Value

NULL - the metadata object is updated in the app folder

```
assess_clustering_stability
```

Assessment of Stability for Graph Clustering

# **Description**

Evaluates the stability of different graph clustering methods in the clustering pipeline. The method will iterate through different values of the resolution parameter and compare, using the EC Consistency score, the partitions obtained at different seeds.

# Usage

```
assess_clustering_stability(
  graph_adjacency_matrix,
  resolution,
  n_repetitions = 100,
  seed_sequence = NULL,
  ecs_thresh = 1,
  clustering_algorithm = 1:3,
  clustering_arguments = list(),
  verbose = TRUE
)
```

### **Arguments**

graph\_adjacency\_matrix

A square adjacency matrix based on which an igraph object will be built. The matrix should have rownames and colnames that correspond to the names of the

cells.

resolution A sequence of resolution values. The resolution parameter controls the coarse-

ness of the clustering. The higher the resolution, the more clusters will be obtained. The resolution parameter is used in the community detection algorithms.

n\_repetitions The number of repetitions of applying the pipeline with different seeds; ignored

if seed\_sequence is provided by the user. Defaults to 100.

seed\_sequence A custom seed sequence; if the value is NULL, the sequence will be built starting from 1 with a step of 100.

An index or a list of indexes indicating which community detection algorithm will be used: Louvain (1), Louvain refined (2), SLM (3) or Leiden (4). More details can be found in the Seurat's FindClusters function. Defaults to 1:3.

clustering\_arguments

A list of additional arguments that will be passed to the clustering method. More details can be found in the Seurat's FindClusters function.

verbose

Boolean value used for displaying the progress bar.

#### Value

A list having two fields:

- all a list that contains, for each clustering method and each resolution value, the EC consistency between the partitions obtained by changing the seed
- filtered similar to all, but for each configuration, we determine the number of clusters that appears the most and use only the partitions with this size

#### **Examples**

```
set.seed(2024)
# create an artificial PCA embedding
pca_embedding <- matrix(runif(100 * 30), nrow = 100)</pre>
rownames(pca_embedding) <- paste0("cell_", seq_len(nrow(pca_embedding)))</pre>
colnames(pca_embedding) <- paste0("PC_", 1:30)</pre>
adj_matrix <- getNNmatrix(</pre>
    RANN::nn2(pca\_embedding, k = 10)nn.idx,
    10.
    0,
    -1
)$nn
rownames(adj_matrix) <- paste0("cell_", seq_len(nrow(adj_matrix)))</pre>
colnames(adj_matrix) <- paste0("cell_", seq_len(ncol(adj_matrix)))</pre>
# alternatively, the adj_matrix can be calculated
# using the `Seurat::FindNeighbors` function.
clust_diff_obj <- assess_clustering_stability(</pre>
    graph_adjacency_matrix = adj_matrix,
    resolution = c(0.5, 1),
    n_repetitions = 10,
    clustering_algorithm = 1:2,
    verbose = TRUE
)
plot_clustering_overall_stability(clust_diff_obj)
```

```
assess_feature_stability
```

Assess the stability for configurations of feature types and sizes

### **Description**

Evaluate the stability of clusterings obtained based on incremental subsets of a given feature set.

# Usage

```
assess_feature_stability(
  data_matrix,
  feature_set,
  steps,
  feature_type,
  resolution,
 n_repetitions = 100,
  seed_sequence = NULL,
  graph_reduction_type = "PCA",
  ecs_{thresh} = 1,
 matrix_processing = function(dt_mtx, actual_npcs = 30, ...) {
     actual_npcs <-
    min(actual_npcs, ncol(dt_mtx)%/%2)
    RhpcBLASctl::blas_set_num_threads(foreach::getDoParWorkers())
     embedding <-</pre>
    stats::prcomp(x = dt_mtx, rank. = actual_npcs)$x
    RhpcBLASctl::blas_set_num_threads(1)
     rownames(embedding) <- rownames(dt_mtx)</pre>
     colnames(embedding) <- paste0("PC_", seq_len(ncol(embedding)))</pre>
    return(embedding)
},
  umap_arguments = list(),
 prune_value = -1,
  clustering_algorithm = 1,
  clustering_arguments = list(),
  verbose = FALSE
)
```

#### **Arguments**

data\_matrix A data matrix having the features on the rows and the observations on the columns.

feature\_set A set of feature names that can be found on the rownames of the data matrix.

steps Vector containing the sizes of the subsets; negative values will be interpreted as

using all features.

feature\_type A name associated to the feature\_set.

resolution A vector containing the resolution values used for clustering.

n\_repetitions The number of repetitions of applying the pipeline with different seeds; ignored

if seed\_sequence is provided by the user. Defaults to 100.

seed\_sequence A custom seed sequence; if the value is NULL, the sequence will be built starting

from 1 with a step of 100. Defaults to NULL.

graph\_reduction\_type

The graph reduction type, denoting if the graph should be built on either the

PCA or the UMAP embedding. Defaults to PCA.

ecs\_thresh The ECS threshold used for merging similar clusterings. We recommend using

the 1 value. Defaults to 1.

matrix\_processing

A function that will be used to process the data matrix by using a dimensionality reduction technique. The function should have one parameter, the data matrix, and should return an embedding describing the reduced space. By default, the

function will use the precise PCA method with prcomp.

umap\_arguments A list containing the arguments that will be passed to the UMAP function. Refer

to the uwot::umap function for more details.

prune\_value Argument indicating whether to prune the SNN graph. If the value is 0, the

graph won't be pruned. If the value is between 0 and 1, the edges with weight under the pruning value will be removed. If the value is -1, the highest pruning

value will be calculated automatically and used.

clustering\_algorithm

An index indicating which community detection algorithm will be used: Louvain (1), Louvain refined (2), SLM (3) or Leiden (4). More details can be found

in the Seurat's FindClusters function.

clustering\_arguments

A list containing the arguments that will be passed to the community detection algorithm, such as the number of iterations and the number of starts. Refer to

the Seurat's FindClusters function for more details.

verbose A boolean indicating if the intermediate progress will be printed or not.

# Value

A list having one field associated with a step value. Each step contains a list with three fields:

- ecc the EC-Consistency of the partitions obtained on all repetitions
- embedding one UMAP embedding generated on the feature subset
- most\_frequent\_partition the most common partition obtained across repetitions

### Note

The algorithm assumes that the feature\_set is already sorted when performing the subsetting based on the steps values. For example, if the user wants to analyze highly variable feature set, they should provide them sorted by their variability.

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### **Examples**

```
set.seed(2024)
# create an artificial expression matrix
expr_matrix <- matrix(</pre>
   c(runif(100 * 10), runif(100 * 10, min = 3, max = 4)),
    nrow = 200, byrow = TRUE
)
rownames(expr_matrix) <- as.character(1:200)</pre>
colnames(expr_matrix) <- paste("feature", 1:10)</pre>
feature_stability_result <- assess_feature_stability(</pre>
    data_matrix = t(expr_matrix),
    feature_set = colnames(expr_matrix),
    steps = 5,
    feature_type = "feature_name",
    resolution = c(0.1, 0.5, 1),
   n_repetitions = 10,
   umap_arguments = list(
        # the following parameters are used by the umap function
        # and are not mandatory
        n_neighbors = 3,
        approx_pow = TRUE,
        n_{epochs} = 0,
        init = "random",
        min_dist = 0.3
   ),
    clustering\_algorithm = 1
)
plot_feature_overall_stability_boxplot(feature_stability_result)
```

# **Description**

Evaluates clustering stability when changing the values of different parameters involved in the graph building step, namely the base embedding, the graph type and the number of neighbours.

# Usage

```
assess_nn_stability(
  embedding,
  n_neigh_sequence,
  n_repetitions = 100,
  seed_sequence = NULL,
  graph_reduction_type = "PCA",
  ecs_thresh = 1,
  graph_type = 2,
  prune_value = -1,
```

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```
clustering_algorithm = 1,
  clustering_arguments = list(),
  umap_arguments = list()
)
```

#### **Arguments**

embedding A matrix associated with a PCA embedding. Embeddings from other dimen-

sionality reduction techniques (such as LSI) can be used.

n\_neigh\_sequence

A sequence of the number of nearest neighbours.

n\_repetitions The number of repetitions of applying the pipeline with different seeds; ignored

if seed\_sequence is provided by the user.

seed\_sequence A custom seed sequence; if the value is NULL, the sequence will be built starting

from 1 with a step of 100.

graph\_reduction\_type

The graph reduction type, denoting if the graph should be built on either the

PCA or the UMAP embedding.

ecs\_thresh The ECS threshold used for merging similar clusterings.

graph\_type Argument indicating whether the graph should be unweighted (0), weighted (1)

or both (2).

prune\_value Argument indicating whether to prune the SNN graph. If the value is 0, the

graph won't be pruned. If the value is between 0 and 1, the edges with weight under the pruning value will be removed. If the value is -1, the highest pruning

value will be calculated automatically and used.

clustering\_algorithm

An index indicating which community detection algorithm will be used: Louvain (1), Louvain refined (2), SLM (3) or Leiden (4). More details can be found

in the Seurat's FindClusters function.

clustering\_arguments

A list of arguments that will be passed to the clustering algorithm. See the FindClusters function in Seurat for more details.

 $\verb|umap_arguments|| Additional|| arguments|| passed to the the \verb|uwot::umap|| method.$ 

#### Value

A list having three fields:

- n\_neigh\_k\_corresp list containing the number of the clusters obtained by running the pipeline multiple times with different seed, number of neighbours and graph type (weighted vs unweigted)
- n\_neigh\_ec\_consistency list containing the EC consistency of the partitions obtained at multiple runs when changing the number of neighbours or the graph type
- n\_different\_partitions the number of different partitions obtained by each number of neighbours

# **Examples**

```
set.seed(2024)
# create an artificial PCA embedding
pca_emb <- matrix(runif(100 * 30), nrow = 100, byrow = TRUE)
rownames(pca_emb) <- as.character(1:100)
colnames(pca_emb) <- paste0("PC_", 1:30)

nn_stability_obj <- assess_nn_stability(
    embedding = pca_emb,
    n_neigh_sequence = c(10, 15, 20),
    n_repetitions = 10,
    graph_reduction_type = "PCA",
    clustering_algorithm = 1
)
plot_n_neigh_ecs(nn_stability_obj)</pre>
```

automatic\_stability\_assessment

Assessment of Stability for Graph Clustering

# Description

Evaluates the stability of different graph clustering methods in the clustering pipeline. The method will iterate through different values of the resolution parameter and compare, using the EC Consistency score, the partitions obtained at different seeds.

# Usage

```
automatic_stability_assessment(
  expression_matrix,
  n_repetitions,
  n_neigh_sequence,
  resolution_sequence,
  features_sets,
  steps,
  seed_sequence = NULL,
  graph_reduction_embedding = "PCA",
  include_umap_nn_assessment = FALSE,
  n_{top_{configs} = 3,
  ranking_criterion = "iqr",
  overall_summary = "median",
  ecs_{threshold} = 1,
  matrix_processing = function(dt_mtx, actual_npcs = 30, ...) {
     actual_npcs <-
    min(actual_npcs, ncol(dt_mtx)%/%2)
    RhpcBLASctl::blas_set_num_threads(foreach::getDoParWorkers())
     embedding <-
```

```
stats::prcomp(x = dt_mtx, rank. = actual_npcs)$x
    RhpcBLASctl::blas_set_num_threads(1)
     rownames(embedding) <- rownames(dt_mtx)</pre>
     colnames(embedding) <- paste0("PC_", seq_len(ncol(embedding)))</pre>
    return(embedding)
},
  umap_arguments = list(),
  prune_value = -1,
  algorithm_dim_reduction = 1,
  algorithm_graph_construct = 1,
  algorithms_clustering_assessment = 1:3,
  clustering_arguments = list(),
  verbose = TRUE,
  temp_file = NULL,
  save\_temp = TRUE
)
```

### **Arguments**

expression\_matrix

An expression matrix having the features on the rows and the cells on the columns.

n\_repetitions

The number of repetitions of applying the pipeline with different seeds; ignored if seed\_sequence is provided by the user. Defaults to 100.

n\_neigh\_sequence

A sequence of the number of nearest neighbours.

resolution\_sequence

A sequence of resolution values. The resolution parameter controls the coarseness of the clustering. The higher the resolution, the more clusters will be obtained. The resolution parameter is used in the community detection algorithms.

features\_sets

A list of the feature sets. A feature set is a list of genes from the expression matrix that will be used in the dimensionality reduction.

steps

A list with the same names as feature\_sets. Each name has assigned a ector containing the sizes of the subsets; negative values will be interpreted as using all features.

seed\_sequence

A custom seed sequence; if the value is NULL, the sequence will be built starting from 1 with a step of 100.

graph\_reduction\_embedding

The type of dimensionality reduction used for the graph construction. The options are "PCA" and "UMAP". Defaults to PCA.

include\_umap\_nn\_assessment

A boolean value indicating if the UMAP embeddings will be used for the nearest neighbours assessment. Defaults to FALSE.

n\_top\_configs

The number of top configurations that will be used for the downstream analysis in the dimensionality reduction step. Defaults to 3.

ranking\_criterion

The criterion used for ranking the configurations from the dimensionality reduction step. The options are "iqr", "median", "max", "top\_qt", "top\_qt\_max", "iqr median", "iqr median coeff" and "mean". Defaults to iqr.

overall\_summary

A function used to summarize the stability of the configurations from the dimensionality reduction step across the different resolution values. The options are "median", "max", "top\_qt", "top\_qt\_max", "iqr", "iqr\_median", "iqr\_median\_coeff" and "mean". Defaults to median.

ecs\_threshold The ECS threshold used for merging similar clusterings.

matrix\_processing

prune\_value

A function that will be used to process the data matrix by using a dimensionality reduction technique. The function should have one parameter, the data matrix, and should return an embedding describing the reduced space. By default, the function will use the precise PCA method with prcomp.

umap\_arguments A list containing the arguments that will be passed to the UMAP function. Refer to the uwot::umap function for more details.

Argument indicating whether to prune the SNN graph. If the value is 0, the graph won't be pruned. If the value is between 0 and 1, the edges with weight under the pruning value will be removed. If the value is -1, the highest pruning value will be calculated automatically and used.

algorithm\_dim\_reduction

An index indicating the community detection algorithm that will be used in the Dimensionality reduction step.

algorithm\_graph\_construct

An index indicating the community detection algorithm that will be used in the Graph construction step.

algorithms\_clustering\_assessment

An index indicating which community detection algorithm will be used for the clustering step: Louvain (1), Louvain refined (2), SLM (3) or Leiden (4). More details can be found in the Seurat's FindClusters function.

clustering\_arguments

A list containing the arguments that will be passed to the community detection algorithm, such as the number of iterations and the number of starts. Refer to the Seurat's FindClusters function for more details.

verbose Boolean value used for displaying the progress of the assessment.

temp\_file The path to the file where the object will be saved.

save\_temp A boolean value indicating if the object will be saved to a file.

# Value

A list having two fields:

- all a list that contains, for each clustering method and each resolution value, the EC consistency between the partitions obtained by changing the seed
- filtered similar to all, but for each configuration, we determine the number of clusters that appears the most and use only the partitions with this size

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### **Examples**

```
## Not run:
set.seed(2024)
# create an already-transposed artificial expression matrix
expr_matrix <- matrix(</pre>
    c(runif(20 * 10), runif(30 * 10, min = 3, max = 4)),
    nrow = 10, byrow = FALSE
)
colnames(expr_matrix) <- as.character(seq_len(ncol(expr_matrix)))</pre>
rownames(expr_matrix) <- paste("feature", seq_len(nrow(expr_matrix)))</pre>
autom_object <- automatic_stability_assessment(</pre>
    expression_matrix = expr_matrix,
   n_repetitions = 3,
   n_{\text{neigh\_sequence}} = c(5),
    resolution_sequence = c(0.1, 0.5),
    features_sets = list(
        "set1" = rownames(expr_matrix)
   ),
    steps = list(
        "set1" = c(5, 7)
    ),
    umap_arguments = list(
        # the following parameters have been modified
        # from the default values to ensure that
        # the function will run under 5 seconds
        n_neighbors = 3,
        approx_pow = TRUE,
        n_{epochs} = 0,
        init = "random",
        min_dist = 0.3
   ),
    n_{top\_configs} = 1,
    algorithms_clustering_assessment = 1,
    save\_temp = FALSE,
    verbose = FALSE
)
# the object can be further used to plot the assessment results
plot_feature_overall_stability_boxplot(autom_object$feature_stability)
plot_n_neigh_ecs(autom_object$set1$"5"$nn_stability)
plot_k_n_partitions(autom_object$set1$"5"$clustering_stability)
## End(Not run)
```

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### **Description**

Performs the Wilcoxon rank sum test to identify differentially expressed genes between two groups of cells

### Usage

```
calculate_markers(
  expression_matrix,
  cells1,
  cells2,
  logfc_threshold = 0,
 min_pct_threshold = 0.1,
  avg_expr_threshold_group1 = 0,
 min_diff_pct_threshold = -Inf,
  rank_matrix = NULL,
  feature names = NULL.
  used_slot = "data",
  norm_method = "SCT",
  pseudocount_use = 1,
  base = 2,
  adjust_pvals = TRUE,
  check_cells_set_diff = TRUE
)
```

# Arguments

expression\_matrix

A matrix of gene expression values having genes in rows and cells in columns.

cells1

A vector of cell indices for the first group of cells. A vector of cell indices for the second group of cells.

cells2
logfc\_threshold

The minimum absolute log fold change to consider a gene as differentially expressed. Defaults to 0, meaning all genes are taken into considereation.

min\_pct\_threshold

The minimum fraction of cells expressing a gene form each cell population to consider the gene as differentially expressed. Increasing the value will speed up the function. Defaults to 0.1.

avg\_expr\_threshold\_group1

The minimum average expression that a gene should have in the first group of cells to be considered as differentially expressed. Defaults to  $\emptyset$ .

min\_diff\_pct\_threshold

The minimum difference in the fraction of cells expressing a gene between the two cell populations to consider the gene as differentially expressed. Defaults to -Inf.

rank\_matrix

A matrix where the cells are ranked based on their expression levels with respect to each gene. Defaults to NULL, in which case the function will calculate the rank matrix. We recommend calculating the rank matrix beforehand and passing it to the function to speed up the computation.

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feature\_names

A vector of gene names. Defaults to NULL, in which case the function will use the row names of the expression matrix as gene names.

used\_slot

Parameter that provides additional information about the expression matrix, whether it was scaled or not. The value of this parameter impacts the calculation of the fold change. If data, the function will calculates the fold change as the fraction between the log value of the average of the expression raised to exponential for the two cell groups. If scale.data, the function will calculate the fold change as the fraction between the average of the expression values for the two cell groups. Other options will default to calculating the fold change as the fraction between the log value of the average of the expression values for the two cell groups. Defaults to data.

norm\_method

The normalization method used to normalize the expression matrix. The value of this parameter impacts the calculation of the average expression of the genes when used\_slot = "data". If LogNormalize, the log fold change will be calculated as described for the used\_slot parameter. Otherwise, the log fold change will be calculated as the fraction between the log value of the average of the expression values for the two cell groups. Defaults to SCT.

pseudocount\_use

The pseudocount to add to the expression values when calculating the average expression of the genes, to avoid the 0 value for the denominator. Defaults to 1.

base

The base of the logharithm. Defaults to 2.

adjust\_pvals

A logical value indicating whether to adjust the p-values for multiple testing using the Bonferonni method. Defaults to TRUE.

check\_cells\_set\_diff

A logical value indicating whether to check if thw two cell groups are disjoint or not. Defaults to TRUE.

#### Value

A data frame containing the following columns:

- gene: The gene name.
- avg\_log2FC: The average log fold change between the two cell groups.
- p\_val: The p-value of the Wilcoxon rank sum test.
- p\_val\_adj: The adjusted p-value of the Wilcoxon rank sum test.
- pct.1: The fraction of cells expressing the gene in the first cell group.
- pct.2: The fraction of cells expressing the gene in the second cell group.
- avg\_expr\_group1: The average expression of the gene in the first cell group.

# **Examples**

```
set.seed(2024)
# create an artificial expression matrix
expr_matrix <- matrix(
    c(runif(100 * 50), runif(100 * 50, min = 3, max = 4)),
    ncol = 200, byrow = FALSE</pre>
```

```
colnames(expr_matrix) <- as.character(1:200)
rownames(expr_matrix) <- paste("feature", 1:50)

calculate_markers(
    expression_matrix = expr_matrix,
    cells1 = 101:200,
    cells2 = 1:100
)
# TODO should be rewritten such that you don't create new matrix objects inside
# just</pre>
```

calculate\_markers\_shiny

Calculate markers - Shiny

### **Description**

Performs the Wilcoxon rank sum test to identify differentially expressed genes between two groups of cells in the shiny context. The method can be also used outside the shiny context, as long as the expression matrix is stored in a h5 file.

# Usage

```
calculate_markers_shiny(
  cells1,
  cells2,
  logfc_threshold = 0,
 min_pct_threshold = 0.1,
  average_expression_threshold = 0,
  average_expression_group1_threshold = 0,
  min_diff_pct_threshold = -Inf,
  used_slot = "data",
  norm_method = "SCT",
  expression_h5_path = "expression.h5",
  pseudocount_use = 1,
  base = 2,
  verbose = TRUE,
  check_difference = TRUE
)
```

### **Arguments**

cells1 A vector of cell indices for the first group of cells.

cells2 A vector of cell indices for the second group of cells.

logfc\_threshold

The minimum absolute log fold change to consider a gene as differentially expressed. Defaults to 0, meaning all genes are taken into considereation.

calculate\_markers\_shiny

min\_pct\_threshold

The minimum fraction of cells expressing a gene form each cell population to consider the gene as differentially expressed. Increasing the value will speed up the function. Defaults to  $\emptyset$ .1.

average\_expression\_threshold

The minimum average expression that a gene should have in order to be considered as differentially expressed.

 $average\_expression\_group1\_threshold$ 

The minimum average expression that a gene should have in the first group of cells to be considered as differentially expressed. Defaults to 0.

min\_diff\_pct\_threshold

The minimum difference in the fraction of cells expressing a gene between the two cell populations to consider the gene as differentially expressed. Defaults to -Inf

used\_slot

Parameter that provides additional information about the expression matrix, whether it was scaled or not. The value of this parameter impacts the calculation of the fold change. If data, the function will calculates the fold change as the fraction between the log value of the average of the expression raised to exponential for the two cell groups. If scale.data, the function will calculate the fold change as the fraction between the average of the expression values for the two cell groups. Other options will default to calculating the fold change as the fraction between the log value of the average of the expression values for the two cell groups. Defaults to data.

norm\_method

The normalization method used to normalize the expression matrix. The value of this parameter impacts the calculation of the average expression of the genes when used\_slot = "data". If LogNormalize, the log fold change will be calculated as described for the used\_slot parameter. Otherwise, the log fold change will be calculated as the fraction between the log value of the average of the expression values for the two cell groups. Defaults to SCT.

expression\_h5\_path

The path to the h5 file containing the expression matrix. The h5 file should contain the following fields: expression\_matrix, rank\_matrix, average\_expression, genes. The file path defaults to expression.h5.

pseudocount\_use

The pseudocount to add to the expression values when calculating the average expression of the genes, to avoid the 0 value for the denominator. Defaults to 1.

base

The base of the logharithm. Defaults to 2.

verbose 'check\_difference

Whether to print messages about the progress of the function. Defaults to TRUE.

Whether to perform set difference between the two cells. Defaults to TRUE.

#### Value

A data frame containing the following columns:

- gene: The gene name.
- avg\_log2FC: The average log fold change between the two cell groups.

- p\_val: The p-value of the Wilcoxon rank sum test.
- p\_val\_adj: The adjusted p-value of the Wilcoxon rank sum test.
- pct.1: The fraction of cells expressing the gene in the first cell group.
- pct. 2: The fraction of cells expressing the gene in the second cell group.
- avg\_expr\_group1: The average expression of the gene in the first cell group.
- avg\_expr: The average expression of the gene.

```
choose_stable_clusters
```

Choose stable clusters based on ECC and frequency

# Description

Filter the list of clusters obtained by the automatic ClustAssess pipeline using the ECC and frequency thresholds. The ECC threshold is meant to filter out the partitions that are highly sensitive to the change of the random seed, while the purpose of the frequency threshold is to assure a statistical significance of the inferred stability.

### Usage

```
choose_stable_clusters(
  clusters_list,
  ecc_threshold = 0.9,
  freq_threshold = 30,
  summary_function = mean
)
```

#### **Arguments**

```
{\tt clusters\_list} \quad {\tt List~of~clusters~obtained~from~the~get\_clusters\_from\_clustassess\_object~function.}
```

ecc\_threshold Minimum ECC value to consider a cluster as stable. Default is 0.9.

freq\_threshold Minimum total frequency of the partitions to consider. Default is 30. summary\_function

Function to summarize the ECC values. Default is mean. To match the results from the ClustAssess Shiny App, use median.

### Value

A list of stable clusters that satisfy the ECC and frequency.

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consensus\_cluster

Consensus Clustering and Proportion of Ambiguously Clustered Pairs

# **Description**

Calculate consensus clustering and proportion of ambiguously clustered pairs (PAC) with hierarchical clustering.

# Usage

```
consensus_cluster(
    x,
    k_min = 3,
    k_max = 100,
    n_reps = 100,
    p_sample = 0.8,
    p_feature = 1,
    p_minkowski = 2,
    dist_method = "euclidean",
    linkage = "complete",
    lower_lim = 0.1,
    upper_lim = 0.9,
    verbose = TRUE
)
```

# Arguments

X	A samples x features normalized data matrix.
k_min	The minimum number of clusters calculated.
k_max	The maximum number of clusters calculated.
n_reps	The total number of subsamplings and reclusterings of the data; this value needs to be high enough to ensure PAC converges; convergence can be assessed with pac_convergence.
p_sample	The proportion of samples included in each subsample.
p_feature	The proportion of features included in each subsample.
p_minkowski	The power of the Minkowski distance.
dist_method	The distance measure for the distance matrix used in hclust; must be one of "euclidean", "maximum", "manhattan", "canberra", "binary" or "minkowski".
linkage	The linkage method used in hclust; must be one of "ward.D", "ward.D2", "single", "complete", "average", "mcquitty", "median" or "centroid"
lower_lim	The lower limit for determining whether a pair is clustered ambiguously; the lower this value, the higher the PAC.
upper_lim	The upper limit for determining whether a pair is clustered ambiguously; the higher this value, the higher the PAC.
verbose	Logical value used for choosing to display a progress bar or not.

#### Value

A data.frame with PAC values across iterations, as well as parameter values used when calling the method.

#### References

Monti, S., Tamayo, P., Mesirov, J., & Golub, T. (2003). Consensus clustering: a resampling-based method for class discovery and visualization of gene expression microarray data. Machine learning, 52(1), 91-118. https://doi.org/10.1023/A:1023949509487

Senbabaoglu, Y., Michailidis, G., & Li, J. Z. (2014). Critical limitations of consensus clustering in class discovery. Scientific reports, 4(1), 1-13. https://doi.org/10.1038/srep06207

### **Examples**

```
pac.res <- consensus_cluster(iris[, 1:4], k_max = 20)
pac_convergence(pac.res, k_plot = c(3, 5, 7, 9))

create_monocle_default

Create monocle object</pre>
```

### **Description**

Use a normalized expression matrix and, potentially, an already generated PCA / UMAP embedding, to create a Monocle object.

### Usage

```
create_monocle_default(
  normalized_expression_matrix,
  count_matrix = NULL,
  pca_embedding = NULL,
  umap_embedding = NULL,
  metadata_df = NULL
)
```

# **Arguments**

```
The normalized expression matrix having genes on rows and cells on columns.

The count matrix having genes on rows and cells on columns. If NULL, the normalized_expression_matrix will be used.

pca_embedding The PCA embedding of the expression matrix. If NULL, the pca will be created using the monocle3 package (default parameters).

umap_embedding The UMAP embedding of the expression matrix. If NULL, the umap will be created using the monocle3 package (default parameters).

metadata_df The metadata dataframe having the cell names as rownames. If NULL, a dataframe with a single column named identical_ident will be created.
```

### Value

A Monocle object of the expression matrix, having the stable number of clusters identified by ClustAssess.

# **Examples**

```
## Not run:
set.seed(2024)
# create an already-transposed artificial expression matrix
expr_matrix <- matrix(</pre>
    c(runif(20 * 10), runif(30 * 10, min = 3, max = 4)),
   nrow = 10, byrow = FALSE
colnames(expr_matrix) <- as.character(seq_len(ncol(expr_matrix)))</pre>
rownames(expr_matrix) <- paste("feature", seq_len(nrow(expr_matrix)))</pre>
# uncomment to create the monocle object
mon_obj <- create_monocle_default(</pre>
    normalized_expression_matrix = expr_matrix,
   pca_emb = NULL,
   umap_emb = NULL,
   metadata_df = NULL
)
## End(Not run)
```

create\_monocle\_from\_clustassess

Create monocle object from a ClustAssess object

# **Description**

Use the object generated using the ClustAssess automatic\_stability\_assessment function to create a Monocle object which has the stable number of clusters.

# Usage

```
create_monocle_from_clustassess(
  normalized_expression_matrix,
  count_matrix = NULL,
  clustassess_object,
  metadata_df,
  stable_feature_type,
  stable_feature_set_size,
  stable_clustering_method,
  stable_n_clusters = NULL,
  use_all_genes = FALSE
)
```

#### **Arguments**

```
normalized_expression_matrix
                  The normalized expression matrix having genes on rows and cells on columns.
count_matrix
                  The count matrix having genes on rows and cells on columns. If NULL, the
                  normalized_expression_matrix will be used.
clustassess_object
                  The output of the automatic_stability_assessment.
metadata_df
                  The metadata dataframe having the cell names as rownames. If NULL, a dataframe
                  with a single column named identical_ident will be created.
stable_feature_type
                  The feature type which leads to stable clusters.
stable_feature_set_size
                  The feature size which leads to stable clusters.
stable_clustering_method
                 The clustering method which leads to stable clusters.
stable_n_clusters
                  The number of clusters that are stable. If NULL, all the clusters will be provided.
                  Defaults to NULL.
                 A boolean value indicating if the expression matrix should be truncated to the
use_all_genes
                  genes used in the stability assessment. Defaults to FALSE.
```

#### Value

A Monocle object of the expression matrix, having the stable number of clusters identified by ClustAssess.

# **Examples**

```
## Not run:
set.seed(2024)
# create an already-transposed artificial expression matrix
expr_matrix <- matrix(</pre>
    c(runif(20 * 10), runif(30 * 10, min = 3, max = 4)),
    nrow = 10, byrow = FALSE
)
colnames(expr_matrix) <- as.character(seq_len(ncol(expr_matrix)))</pre>
rownames(expr_matrix) <- paste("feature", seq_len(nrow(expr_matrix)))</pre>
autom_object <- automatic_stability_assessment(</pre>
    expression_matrix = expr_matrix,
   n_{repetitions} = 3,
   n_{\text{neigh\_sequence}} = c(5),
    resolution_sequence = c(0.1, 0.5),
    features_sets = list(
        "set1" = rownames(expr_matrix)
    ),
    steps = list(
        "set1" = c(5, 7)
```

```
),
   umap_arguments = list(
        # the following parameters have been modified
        # from the default values to ensure that the function
        # will run under 5 seconds
        n_neighbors = 3,
        approx_pow = TRUE,
        n_{epochs} = 0,
        init = "random",
       min_dist = 0.3
   ),
   n_top_configs = 1,
   algorithms_clustering_assessment = 1,
    save_temp = FALSE,
    verbose = FALSE
)
# uncomment to create the monocle object
# mon_obj <- create_monocle_from_clustassess(</pre>
      normalized_expression_matrix = expr_matrix,
      clustassess_object = autom_object,
     metadata = NULL,
      stable_feature_type = "set1",
      stable_feature_set_size = "5",
      stable_clustering_method = "Louvain"
#)
## End(Not run)
```

create\_monocle\_from\_clustassess\_app

Create monocle object from a ClustAssess shiny app

# **Description**

Use the files generated in the ClustAssess app to create a Monocle object which has the stable number of clusters.

# Usage

```
create_monocle_from_clustassess_app(
   app_folder,
   stable_feature_type,
   stable_feature_set_size,
   stable_clustering_method,
   stable_n_clusters = NULL,
   use_all_genes = FALSE
)
```

# **Arguments**

### Value

A Monocle object of the expression matrix, having the stable number of clusters identified by ClustAssess.

```
create_seurat_object_default

Create Seurat object
```

# Description

Use a normalized expression matrix and, potentially, an already generated PCA / UMAP embedding, to create a Seurat object.

### Usage

```
create_seurat_object_default(
  normalized_expression_matrix,
  count_matrix = NULL,
  pca_embedding = NULL,
  umap_embedding = NULL,
  metadata_df = NULL
)
```

# **Arguments**

 ${\tt normalized\_expression\_matrix}$ 

The normalized expression matrix having genes on rows and cells on columns.

normalized\_expression\_matrix will be used.

pca\_embedding 
The PCA embedding of the expression matrix. If NULL, the pca will be created

using the Seurat package (default parameters).

umap\_embedding The UMAP embedding of the expression matrix. If NULL, the umap will be created using the Seurat package (default parameters).

metadata\_df The metadata dataframe having the cell names as rownames. If NULL, a dataframe with a single column named identical\_ident will be created.

#### Value

A Seurat object of the expression matrix, having the stable number of clusters identified by ClustAssess

# **Description**

Use the files generated in the ClustAssess app to create a Seurat object which has the stable number of clusters.

#### Usage

```
create_seurat_object_from_clustassess_app(
   app_folder,
   stable_feature_type,
   stable_feature_set_size,
   stable_clustering_method,
   stable_n_clusters = NULL,
   use_all_genes = FALSE
)
```

# **Arguments**

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#### Value

A Seurat object of the expression matrix, having the stable number of clusters identified by ClustAssess.

element\_agreement

Element-Wise Average Agreement Between a Set of Clusterings

# **Description**

Inspect how consistently of a set of clusterings agree with a reference clustering by calculating their element-wise average agreement.

# Usage

```
element_agreement(
  reference_clustering,
  clustering_list,
  alpha = 0.9,
  r = 1,
  rescale_path_type = "max",
  ppr_implementation = "prpack",
  dist_rescaled = FALSE,
  row_normalize = TRUE
)
```

# **Arguments**

reference\_clustering

The reference clustering, that each clustering in clustering\_list is compared to. It can be either:

- A numeric/character/factor vector of cluster labels for each element.
- A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
- · An helust object.

clustering\_list

The list of clustering results, each of which is either:

- A numeric/character/factor vector of cluster labels for each element.
- A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
- An helust object.

alpha

A numeric giving the personalized PageRank damping factor; 1 - alpha is the restart probability for the PPR random walk.

r

A numeric hierarchical scaling parameter.

rescale\_path\_type

A string; rescale the hierarchical height by:

• "max": the maximum path from the root.

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- "min": the minimum path form the root.
- "linkage": use the linkage distances in the clustering.

ppr\_implementation

Choose a implementation for personalized page-rank calculation:

- "prpack": use PPR algorithms in igraph.
- "power\_iteration": use power\_iteration method.

dist\_rescaled

A logical: if TRUE, the linkage distances are linearly rescaled to be in-between 0 and 1.

row\_normalize

Whether to normalize all rows in clustering\_result so they sum to one before calculating ECS. It is recommended to set this to TRUE, which will lead to slightly different ECS values compared to clusim.

### Value

A vector containing the element-wise average agreement.

### References

Gates, A. J., Wood, I. B., Hetrick, W. P., & Ahn, Y. Y. (2019). Element-centric clustering comparison unifies overlaps and hierarchy. Scientific reports, 9(1), 1-13. https://doi.org/10.1038/s41598-019-44892-y

# **Examples**

```
# perform k-means clustering across 20 random seeds
reference.clustering <- iris$Species
clustering.list <- lapply(1:20, function(x) kmeans(iris[, 1:4], centers = 3)$cluster)
element_agreement(reference.clustering, clustering.list)</pre>
```

element\_consistency

Element-Wise Consistency Between a Set of Clusterings

# Description

Inspect the consistency of a set of clusterings by calculating their element-wise clustering consistency (also known as element-wise frustration).

# Usage

```
element_consistency(
  clustering_list,
  alpha = 0.9,
  r = 1,
  rescale_path_type = "max",
  ppr_implementation = "prpack",
  dist_rescaled = FALSE,
  row_normalize = TRUE
)
```

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# **Arguments**

clustering\_list

The list of clustering results, each of which is either:

- A numeric/character/factor vector of cluster labels for each element.
- A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
- An helust object.

alpha

A numeric giving the personalized PageRank damping factor; 1 - alpha is the restart probability for the PPR random walk.

r

A numeric hierarchical scaling parameter.

rescale\_path\_type

A string; rescale the hierarchical height by:

- "max": the maximum path from the root.
- "min": the minimum path form the root.
- "linkage": use the linkage distances in the clustering.

ppr\_implementation

Choose a implementation for personalized page-rank calculation:

- "prpack": use PPR algorithms in igraph.
- "power\_iteration": use power\_iteration method.

dist\_rescaled

A logical: if TRUE, the linkage distances are linearly rescaled to be in-between 0 and 1.

row\_normalize

Whether to normalize all rows in clustering\_result so they sum to one before calculating ECS. It is recommended to set this to TRUE, which will lead to slightly different ECS values compared to clusim.

### Value

A vector containing the element-wise consistency. If calculate\_sim\_matrix is set to TRUE, the element similarity matrix will be returned as well.

#### References

Gates, A. J., Wood, I. B., Hetrick, W. P., & Ahn, Y. Y. (2019). Element-centric clustering comparison unifies overlaps and hierarchy. Scientific reports, 9(1), 1-13. https://doi.org/10.1038/s41598-019-44892-y

### **Examples**

```
# cluster across 20 random seeds
clustering.list <- lapply(1:20, function(x) kmeans(mtcars, centers = 3)$cluster)
element_consistency(clustering.list)</pre>
```

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element\_sim

The Element-Centric Clustering Similarity

### **Description**

Calculates the average element-centric similarity between two clustering results

### Usage

```
element_sim(
   clustering1,
   clustering2,
   alpha = 0.9,
   r_cl1 = 1,
   rescale_path_type_cl1 = "max",
   ppr_implementation_cl1 = "prpack",
   dist_rescaled_cl1 = FALSE,
   row_normalize_cl1 = TRUE,
   r_cl2 = 1,
   rescale_path_type_cl2 = "max",
   ppr_implementation_cl2 = "prpack",
   dist_rescaled_cl2 = FALSE,
   row_normalize_cl2 = TRUE
)
```

# **Arguments**

clustering1

The first clustering result, which can be one of:

- A numeric/character/factor vector of cluster labels for each element.
- A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
- · An helust object.

clustering2

The second clustering result, which can be one of:

- A numeric/character/factor vector of cluster labels for each element.
- A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
- An helust object.

alpha

A numeric giving the personalized PageRank damping factor; 1 - alpha is the restart probability for the PPR random walk.

r\_cl1 A numeric hierarchical scaling parameter for the first clustering. rescale\_path\_type\_cl1

A string; rescale the hierarchical height of the first clustering by:

- "max": the maximum path from the root.
- "min": the minimum path form the root.
- "linkage": use the linkage distances in the clustering.

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#### ppr\_implementation\_cl1

Choose a implementation for personalized page-rank calculation for the first clustering:

- "prpack": use PPR algorithms in igraph.
- "power\_iteration": use power\_iteration method.

#### dist\_rescaled\_cl1

A logical: if TRUE, the linkage distances of the first clustering are linearly rescaled to be in-between 0 and 1.

#### row\_normalize\_cl1

Whether to normalize all rows in the first clustering so they sum to one before calculating ECS. It is recommended to set this to TRUE, which will lead to slightly different ECS values compared to clusim.

r\_cl2 A numeric hierarchical scaling parameter for the second clustering.

rescale\_path\_type\_cl2

A string; rescale the hierarchical height of the second clustering by:

- "max": the maximum path from the root.
- "min": the minimum path form the root.
- "linkage": use the linkage distances in the clustering.

# ppr\_implementation\_cl2

Choose a implementation for personalized page-rank calculation for the second clustering:

- "prpack": use PPR algorithms in igraph.
- "power\_iteration": use power\_iteration method.

#### dist\_rescaled\_cl2

A logical: if TRUE, the linkage distances of the second clustering are linearly rescaled to be in-between 0 and 1.

row\_normalize\_cl2

Whether to normalize all rows in the second clustering so they sum to one before calculating ECS. It is recommended to set this to TRUE, which will lead to slightly different ECS values compared to clusim.

#### Value

The average element-wise similarity between the two Clusterings.

### **Examples**

```
km.res <- kmeans(mtcars, centers = 3)$cluster
hc.res <- hclust(dist(mtcars))
element_sim(km.res, hc.res)</pre>
```

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element\_sim\_elscore

The Element-Centric Clustering Similarity for each Element

### **Description**

Calculates the element-wise element-centric similarity between two clustering results.

### Usage

```
element_sim_elscore(
  clustering1,
  clustering2,
  alpha = 0.9,
  r_cl1 = 1,
  rescale_path_type_cl1 = "max",
  ppr_implementation_cl1 = "prpack",
  dist_rescaled_cl1 = FALSE,
  row_normalize_cl1 = TRUE,
  r_c12 = 1,
  rescale_path_type_cl2 = "max",
  ppr_implementation_cl2 = "prpack",
  dist_rescaled_cl2 = FALSE,
  row_normalize_cl2 = TRUE
)
```

### **Arguments**

clustering1

The first clustering result, which can be one of:

- A numeric/character/factor vector of cluster labels for each element.
- A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
- · An helust object.

clustering2

The second clustering result, which can be one of:

- A numeric/character/factor vector of cluster labels for each element.
- A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
- An helust object.

alpha

A numeric giving the personalized PageRank damping factor; 1 - alpha is the restart probability for the PPR random walk.

r\_cl1 rescale\_path\_type\_cl1

A numeric hierarchical scaling parameter for the first clustering. A string; rescale the hierarchical height of the first clustering by:

• "max": the maximum path from the root.

- "min": the minimum path form the root.
- "linkage": use the linkage distances in the clustering.

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ppr\_implementation\_cl1

Choose a implementation for personalized page-rank calculation for the first clustering:

- "prpack": use PPR algorithms in igraph.
- "power\_iteration": use power\_iteration method.

dist\_rescaled\_cl1

A logical: if TRUE, the linkage distances of the first clustering are linearly rescaled to be in-between 0 and 1.

row\_normalize\_cl1

Whether to normalize all rows in the first clustering so they sum to one before calculating ECS. It is recommended to set this to TRUE, which will lead to slightly different ECS values compared to clusim.

r\_cl2 A numeric hierarchical scaling parameter for the second clustering.

rescale\_path\_type\_cl2

A string; rescale the hierarchical height of the second clustering by:

- "max": the maximum path from the root.
- "min": the minimum path form the root.
- "linkage": use the linkage distances in the clustering.

ppr\_implementation\_cl2

Choose a implementation for personalized page-rank calculation for the second clustering:

- "prpack": use PPR algorithms in igraph.
- "power\_iteration": use power\_iteration method.

dist rescaled cl2

A logical: if TRUE, the linkage distances of the second clustering are linearly rescaled to be in-between 0 and 1.

row\_normalize\_cl2

Whether to normalize all rows in the second clustering so they sum to one before calculating ECS. It is recommended to set this to TRUE, which will lead to slightly different ECS values compared to clusim.

#### Value

Vector of element-centric similarity between the two clusterings for each element.

### References

Gates, A. J., Wood, I. B., Hetrick, W. P., & Ahn, Y. Y. (2019). Element-centric clustering comparison unifies overlaps and hierarchy. Scientific reports, 9(1), 1-13. https://doi.org/10.1038/s41598-019-44892-y

### **Examples**

```
km.res <- kmeans(iris[, 1:4], centers = 8)$cluster
hc.res <- hclust(dist(iris[, 1:4]))
element_sim_elscore(km.res, hc.res)</pre>
```

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element\_sim\_matrix

Pairwise Comparison of Clusterings

### **Description**

Compare a set of clusterings by calculating their pairwise average element-centric clustering similarities.

# Usage

```
element_sim_matrix(
  clustering_list,
  output_type = "matrix",
  alpha = 0.9,
  r = 1,
  rescale_path_type = "max",
  ppr_implementation = "prpack",
  dist_rescaled = FALSE,
  row_normalize = TRUE
)
```

### **Arguments**

clustering\_list

The list of clustering results, each of which is either:

- A numeric/character/factor vector of cluster labels for each element.
- A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
- An helust object.

output\_type

A string specifying whether the output should be a matrix or a data.frame.

alpha

A numeric giving the personalized PageRank damping factor; 1 - alpha is the restart probability for the PPR random walk.

A numeric hierarchical scaling parameter.

rescale\_path\_type

A string; rescale the hierarchical height by:

- "max": the maximum path from the root.
- "min": the minimum path form the root.
- "linkage": use the linkage distances in the clustering.

ppr\_implementation

Choose a implementation for personalized page-rank calculation:

- "prpack": use PPR algorithms in igraph.
- "power\_iteration": use power\_iteration method.

dist\_rescaled

A logical: if TRUE, the linkage distances are linearly rescaled to be in-between 0 and 1.

row\_normalize

Whether to normalize all rows in clustering\_result so they sum to one before calculating ECS. It is recommended to set this to TRUE, which will lead to slightly different ECS values compared to clusim.

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# Value

A matrix or data.frame containing the pairwise ECS values.

### References

Gates, A. J., Wood, I. B., Hetrick, W. P., & Ahn, Y. Y. (2019). Element-centric clustering comparison unifies overlaps and hierarchy. Scientific reports, 9(1), 1-13. https://doi.org/10.1038/s41598-019-44892-y

# **Examples**

```
# cluster across 20 random seeds
clustering.list <- lapply(1:20, function(x) kmeans(mtcars, centers = 3)$cluster)
element_sim_matrix(clustering.list, output_type = "matrix")</pre>
```

getNNmatrix

Computes the NN adjacency matrix given the neighbours

# **Description**

Computes the NN adjacency matrix given the neighbours

# Usage

```
getNNmatrix(nnRanked, k = -1L, start = 0L, prune = 0)
```

# Arguments

nnRanked	A matrix with the lists of the nearest neighbours for each point
k	The number of neighbours to consider. Defaults to -1, which means all neighbours.
start	The index of the first neighbour to consider. Defaults to 0.
prune	The threshold to prune the SNN matrix. If -1, the function will only return the NN matrix. Defaults to 0

# Value

A list with the NN and SNN adjacency matrices.

```
{\tt get\_clusters\_from\_clustassess\_object}
```

Extract config-specific clusters from a ClustAssess object

# Description

Given the output of the automatic\_stability\_assessment function, extract the clusters that are specific to a particular configuration of feature type, feature size, clustering method and, optionally, the number of clusters.

# Usage

```
get_clusters_from_clustassess_object(
  clustassess_object,
  feature_type = NULL,
  feature_size = NULL,
  clustering_method = NULL,
  nclusters = NULL
)
```

### **Arguments**

clustassess\_object

Output of the  $automatic\_stability\_assessment$ .

available feature.

feature\_size Size of the feature set used for clustering. If NULL, it will select the first available

feature size.

clustering\_method

Clustering method used. If NULL, it will select the first available clustering

method.

nclusters Number of clusters to extract. If NULL, all clusters are returned.

#### Value

A list of clusters that are specific to the given configuration. Each number of cluster will contain the list of partitions with that specific k and the ECC value indicating the overall stability of k.

```
get_colour_vector_from_palette
```

Get the vector of colours from a palette

# **Description**

Using the paletteer package, this function retrieves a vector of colours from a specified palette. The function will look for both discrete and continuous palettes. If the palette is not found, a default option will be used.

### Usage

```
get_colour_vector_from_palette(
  palette_name,
  is_inverse = FALSE,
  placeholder = "viridis::viridis"
)
```

## **Arguments**

palette\_name The name of the palette to retrieve. The naming should follow the guidelines

described in the paletteer package.

is\_inverse Logical. If TRUE, the colours will be reversed.

placeholder The default palette to use if the specified palette is not found. The default is

"viridis::viridis".

#### Value

A vector of colours from the specified palette. If the palette is not found, a default palette will be used. If paletter is not installed, an error will be thrown.

```
get_highest_prune_param
```

Calculate the highest pruning parameter for the SNN graph given NN matrix

# **Description**

Given a NN adjacency matrix, the function calculates the highest pruning parameter for the SNN graph that preserves the connectivity of the graph.

### Usage

```
get_highest_prune_param(nn_matrix, n_neigh)
```

### **Arguments**

nn\_matrix The adjacency matrix of the nearest neighbour graph.

n\_neigh The number of nearest neighbours.

#### Value

A list with the following fields:

- prune\_value: The value of the highest pruning parameter.
- adj\_matrix: The adjacency matrix of the SNN graph after pruning.

### Note

Given the way the SNN graph is built, the possible values for the pruning parameter are limited and can be determined by the formula i / (2 \* n\_neigh - i), where i is a number of nearest neighbours between 0 and n\_neigh.

### **Examples**

```
set.seed(2024)
# create an artificial pca embedding
pca_embedding <- matrix(
          c(runif(100 * 10), runif(100 * 10, min = 3, max = 4)),
          nrow = 200, byrow = TRUE
)
rownames(pca_embedding) <- as.character(1:200)
colnames(pca_embedding) <- paste("PC", 1:10)

# calculate the nn adjacency matrix
nn_matrix <- getNNmatrix(
          RANN::nn2(pca_embedding, k = 5)$nn.idx,
          5,
          0,
          -1
)$nn

get_highest_prune_param(nn_matrix, 5)$prune_value</pre>
```

```
get_highest_prune_param_embedding
```

Calculate the highest pruning parameter for the SNN graph given Embedding

### **Description**

Given an embedding, the function calculates the highest pruning parameter for the SNN graph that preserves the connectivity of the graph.

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### Usage

```
get_highest_prune_param_embedding(embedding, n_neigh)
```

### **Arguments**

embedding A matrix associated with a PCA embedding. Embeddings from other dimen-

sionality reduction techniques (such as LSI) can be used.

n\_neigh The number of nearest neighbours.

#### Value

The value of the highest pruning parameter.

### Note

Given the way the SNN graph is built, the possible values for the pruning parameter are limited and can be determined by the formula  $i / (2 * n_neigh - i)$ , where i is a number of nearest neighbours between 0 and  $n_neigh$ .

# **Examples**

get\_nn\_conn\_comps

Relationship Between Nearest Neighbours and Connected Components

### **Description**

One of the steps in the clustering pipeline is building a k-nearest neighbour graph on a reduced-space embedding. This method assesses the relationship between different number of nearest neighbours and the connectivity of the graph. In the context of graph clustering, the number of connected components can be used as a lower bound for the number of clusters. The calculations are performed multiple times by changing the seed at each repetition.

get\_nn\_conn\_comps 39

### Usage

```
get_nn_conn_comps(
  embedding,
  n_neigh_sequence,
  n_repetitions = 100,
  seed_sequence = NULL,
  include_umap = FALSE,
  umap_arguments = list()
)
```

### **Arguments**

umap\_arguments Additional arguments passed to the the uwot::umap method.

#### Value

A list having one field associated with a number of nearest neighbours. Each value contains an array of the number of connected components obtained on the specified number of repetitions.

```
set.seed(2024)
# create an artificial PCA embedding
pca_emb <- matrix(runif(100 * 30), nrow = 100, byrow = TRUE)</pre>
rownames(pca_emb) <- as.character(1:100)</pre>
colnames(pca_emb) <- paste0("PCA_", 1:30)</pre>
nn_conn_comps_obj <- get_nn_conn_comps(</pre>
    embedding = pca_emb,
    n_{\text{neigh\_sequence}} = c(2, 5),
    n_repetitions = 3,
    # arguments that are passed to the uwot function
    umap_arguments = list(
        min_dist = 0.3,
        metric = "cosine"
    )
)
plot_connected_comps_evolution(nn_conn_comps_obj)
```

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marker\_overlap

Cell-Wise Marker Gene Overlap

# Description

Calculates the per-cell overlap of previously calculated marker genes.

# Usage

```
marker_overlap(
  markers1,
  markers2,
  clustering1,
  clustering2,
  n = 25,
  overlap_type = "jsi",
  rank_by = "-p_val",
  use_sign = TRUE
)
```

# Arguments

markers1	The first data frame of marker genes, must contain columns called 'gene' and 'cluster'.
markers2	The second data frame of marker genes, must contain columns called 'gene' and 'cluster'.
clustering1	The first vector of cluster assignments.
clustering2	The second vector of cluster assignments.
n	The number of top n markers (ranked by $rank\_by$ ) to use when calculating the overlap.
overlap_type	The type of overlap to calculated: must be one of 'jsi' for Jaccard similarity index and 'intersect' for intersect size.
rank_by	A character string giving the name of the column to rank marker genes by. Note the sign here: to rank by lowest p-value, preface the column name with a minus sign; to rank by highest value, where higher value indicates more discriminative genes (for example power in the ROC test), no sign is needed.
use_sign	A logical: should the sign of markers match for overlap calculations? So a gene must be a positive or a negative marker in both clusters being compared. If TRUE, markers1 and markers2 must have a 'avg_logFC' or 'avg_log2FC' column, from which the sign of the DE will be extracted.

# Value

A vector of the marker gene overlap per cell.

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### **Examples**

```
suppressWarnings({
    set.seed(1234)
    library(Seurat)
    data("pbmc_small")
    # cluster with Louvain algorithm
    pbmc_small <- FindClusters(pbmc_small, resolution = 0.8, verbose = FALSE)</pre>
    # cluster with k-means
    pbmc.pca <- Embeddings(pbmc_small, "pca")</pre>
   pbmc_small@meta.data$kmeans_clusters <- kmeans(pbmc.pca, centers = 3)$cluster</pre>
    # compare the markers
    Idents(pbmc_small) <- pbmc_small@meta.data$seurat_clusters</pre>
    louvain.markers <- FindAllMarkers(pbmc_small,</pre>
        logfc.threshold = 1,
        test.use = "t",
        verbose = FALSE
   )
    Idents(pbmc_small) <- pbmc_small@meta.data$kmeans_clusters</pre>
    kmeans.markers <- FindAllMarkers(pbmc_small,</pre>
        logfc.threshold = 1,
        test.use = "t",
        verbose = FALSE
    )
   pbmc_small@meta.data$jsi <- marker_overlap(</pre>
        louvain.markers, kmeans.markers,
        pbmc_small@meta.data$seurat_clusters, pbmc_small@meta.data$kmeans_clusters
    )
    # which cells have the same markers, regardless of clustering?
    FeaturePlot(pbmc_small, "jsi")
})
```

merge\_partitions

Merge Partitions

# **Description**

Merge flat disjoint clusterings whose pairwise ECS score is above a given threshold. The merging is done using a complete linkage approach.

### Usage

```
merge_partitions(
  partition_list,
```

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```
ecs_thresh = 1,
  order_logic = c("freq", "avg_agreement", "none"),
  return_ecs_matrix = FALSE,
  check_ties = TRUE
)
```

# **Arguments**

partition\_list A list of flat disjoint membership vectors.

ecs\_thresh

A numeric: the ecs threshold.

order\_logic

Variable indicating the method of ordering the partitions. It can take these three values:

- "freq": order the partitions based on their frequencies. The partition with the highest frequency will be the first on the list (default).
- "avg\_agreement": order the partitions based on their average agreement index. The average agreement index of a partition is calculated as the mean of the ECS scores between that partition and the other partitions from the list. The partition with the highest agreement will be the first on the list.
- "none": do not perform any ordering (not recommended). If selected, the average agreement scores will not be calculated.

return\_ecs\_matrix

A logical: if TRUE, the function will add the ECS matrix to the return list. Defaults to FALSE.

check\_ties

A logical value that indicates whether to check for ties in the highest frequency partitions or not. If TRUE, the function will put at the first position the partition that has the highest similarity with the other partitions. Defaults to FALSE.

### Value

a list of the merged partitions, together with their associated ECC score. If return\_ecs\_matrix is set to TRUE, the function will also return the ECS matrix.

# **Examples**

```
initial_list <- list(c(1, 1, 2), c(2, 2, 2), c("B", "B", "A")) merge_partitions(initial_list, 1)
```

merge\_resolutions

Merge Partitions from different Resolutions

### Description

Merge partitions obtained with different resolution values. The partitions will be grouped based on the number of clusters. The identical partitions will be merged into a single partition by updating the frequency using the merge\_partitions method.

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### Usage

```
merge_resolutions(res_obj)
```

# **Arguments**

res\_obj

A list associated to a configuration field from the object returned by the assess\_clustering\_importance method.

#### Value

A list having one field assigned to each number of clusters. A number of cluster will contain a list of all merged partitions. To avoid duplicates, merged\_partitions with threshold 1 is applied.

pac\_convergence

PAC Convergence Plot

# Description

Plot PAC across iterations for a set of k to assess convergence.

# Usage

```
pac_convergence(pac_res, k_plot)
```

### **Arguments**

pac\_res [

The data.frame output by consensus\_cluster.

k\_plot

A vector with values of k to plot.

### Value

A ggplot2 object with the convergence plot. Convergence has been reached when the lines flatten out across k-plot values. out across

```
pac.res <- consensus_cluster(iris[, 1:4], k_max = 20)
pac_convergence(pac.res, k_plot = c(3, 5, 7, 9))</pre>
```

pac\_landscape

PAC Landscape Plot

# Description

Plot final PAC values across range of k to find optimal number of clusters.

# Usage

```
pac_landscape(pac_res, n_shade = max(pac_res$iteration)/5)
```

# **Arguments**

pac\_res

The data.frame output by consensus\_cluster.

n\_shade

The PAC values across the last n\_shade iterations will be shaded to illustrate the

how stable the PAC score is.

#### Value

A ggplot2 object with the final PAC vs k plot. A local minimum in the landscape indicates an especially stable value of k.

# **Examples**

```
pac.res <- consensus_cluster(iris[, 1:4], k_max = 20)
pac_landscape(pac.res)</pre>
```

```
plot_clustering_difference_facet
```

Clustering Method Stability Facet Plot

# Description

Display the distribution of the EC consistency for each clustering method and each resolution value on a given embedding The all field of the object returned by the get\_clustering\_difference\_object method is used.

# Usage

```
plot_clustering_difference_facet(
  clust_object,
  embedding,
  low_limit = 0,
  high_limit = 1,
  grid = TRUE
)
```

### **Arguments**

clust_object	An object returned by the assess_clustering_stability method.
embedding	An embedding (only the first two dimensions will be used for visualization).
low_limit	The lowest value of ECC that will be displayed on the embedding.
high_limit	The highest value of ECC that will be displayed on the embedding.
grid	Boolean value indicating whether the facet should be a grid (where each row is associated with a resolution value and each column with a clustering method) or a wrap.

#### Value

A ggplot2 object. #TODO should export

### **Examples**

```
# FIXME fix the examples
# set.seed(2021)
# # create an artificial PCA embedding
# pca_embedding <- matrix(runif(100 * 30), nrow = 100)</pre>
# rownames(pca_embedding) <- as.character(1:100)</pre>
# colnames(pca_embedding) <- paste0("PCA_", 1:30)</pre>
# adj_matrix <- Seurat::FindNeighbors(pca_embedding,</pre>
      k.param = 10,
      nn.method = "rann",
      verbose = FALSE,
#
      compute.SNN = FALSE
# )$nn
# clust_diff_obj <- assess_clustering_stability(</pre>
      graph_adjacency_matrix = adj_matrix,
      resolution = c(0.5, 1),
      n_repetitions = 10,
      algorithm = 1:2,
#
      verbose = FALSE
# )
# plot_clustering_difference_facet(clust_diff_obj, pca_embedding)
```

```
{\it plot\_clustering\_overall\_stability} \\ {\it Clustering\ Method\ Overall\ Stability\ Boxplot}
```

# **Description**

Display EC consistency across clustering methods by summarising the distribution of the EC consistency for each number of clusters.

### Usage

```
plot_clustering_overall_stability(
  clust_object,
  value_type = c("k", "resolution"),
  summary_function = stats::median
)
```

### **Arguments**

clust\_object An object returned by the assess\_clustering\_stability method.

value\_type A string that specifies the type of value that was used for grouping the partitions

and calculating the ECC score. It can be either k or resolution. Defaults to k.

summary\_function

The function that will be used to summarize the distribution of the ECC values obtained for each number of clusters. Defaults to median.

#### Value

A ggplot2 object with the EC consistency distributions grouped by the clustering methods. Higher consistency indicates a more stable clustering.

```
set.seed(2024)
# create an artificial PCA embedding
pca_embedding <- matrix(runif(100 * 30), nrow = 100)</pre>
rownames(pca_embedding) <- paste0("cell_", seq_len(nrow(pca_embedding)))</pre>
colnames(pca_embedding) <- paste0("PC_", 1:30)</pre>
adj_matrix <- getNNmatrix(</pre>
    RANN::nn2(pca\_embedding, k = 10)$nn.idx,
    10,
    0,
    -1
)$nn
rownames(adj_matrix) <- paste0("cell_", seq_len(nrow(adj_matrix)))</pre>
colnames(adj_matrix) <- paste0("cell_", seq_len(ncol(adj_matrix)))</pre>
# alternatively, the adj_matrix can be calculated
# using the `Seurat::FindNeighbors` function.
clust_diff_obj <- assess_clustering_stability(</pre>
    graph_adjacency_matrix = adj_matrix,
    resolution = c(0.5, 1),
   n_{repetitions} = 10,
   clustering_algorithm = 1:2,
    verbose = FALSE
plot_clustering_overall_stability(clust_diff_obj)
```

```
plot_clustering_per_value_stability

Clustering Method per value Stability Boxplot
```

### **Description**

Display EC consistency across clustering methods, calculated for each value of the resolution parameter or the number of clusters.

# Usage

```
plot_clustering_per_value_stability(
  clust_object,
  value_type = c("k", "resolution")
)
```

### **Arguments**

clust\_object An object returned by the assess\_clustering\_stability method.

value\_type A string that specifies the type of value that was used for grouping the partitions

and calculating the ECC score. It can be either k or resolution. Defaults to  $k.\,$ 

#### Value

A ggplot2 object with the EC consistency distributions grouped by the clustering methods. Higher consistency indicates a more stable clustering. The X axis is decided by the value\_type parameter.

```
set.seed(2024)
# create an artificial PCA embedding
pca_embedding <- matrix(runif(100 * 30), nrow = 100)</pre>
rownames(pca_embedding) <- paste0("cell_", seq_len(nrow(pca_embedding)))</pre>
colnames(pca_embedding) <- paste0("PC_", 1:30)</pre>
adj_matrix <- getNNmatrix(</pre>
    RANN::nn2(pca\_embedding, k = 10)$nn.idx,
    10,
    0,
    -1
)$nn
rownames(adj_matrix) <- paste0("cell_", seq_len(nrow(adj_matrix)))</pre>
colnames(adj_matrix) <- paste0("cell_", seq_len(ncol(adj_matrix)))</pre>
# alternatively, the adj_matrix can be calculated
# using the `Seurat::FindNeighbors` function.
clust_diff_obj <- assess_clustering_stability(</pre>
```

```
graph_adjacency_matrix = adj_matrix,
  resolution = c(0.5, 1),
  n_repetitions = 10,
  clustering_algorithm = 1:2,
  verbose = FALSE
)
plot_clustering_per_value_stability(clust_diff_obj)
```

plot\_clust\_hierarchical

Hierarchical relationship between partitions with different number of clusters

### **Description**

After assessing the stability of the clustering step, the user can visualise the relationship between the partitions as the number of clusters changes. The aim is to understand the hierarchical relationship between super and sub celltypes. The function will create a plot that will represent the clusters of each partition as nodes. The colours of the nodes will indicate the stability of the cluster. The size is proportional to the number of cells in the cluster. The edges will represent the relationship between the clusters of two partitions. The colour of the edges will indicate the stability of the relationship between the clusters. The thickness of the edges will indicate the number of cells that are shared between the two clusters.

### Usage

```
plot_clust_hierarchical(
   clustering_assessment,
   clustering_method = NULL,
   k = NULL,
   edge_threshold = 0.3,
   range_point_size = c(1, 6),
   range_edge_width = c(0.01, 3),
   edge_palette_name = "RColorBrewer::Greys",
   edge_palette_inverse = FALSE,
   node_palette_name = "viridis::rocket",
   node_palette_inverse = TRUE
)
```

### **Arguments**

clustering\_assessment

An object returned by the assess\_clustering\_stability method.

clustering\_method

A string that specifies the clustering method. Should be one of the following: 'Louvain', 'Louvain.refined', 'SLM', 'Leiden'. If NULL, the first clustering method will be used. Defaults to NULL.

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A vector of integers that specifies the number of clusters. If NULL, all available values will be used. Defaults to NULL.

edge\_threshold A numeric value that specifies the quantile threshold for the edges. The edges with the intersection size below the quantile threshold will be removed. Defaults to 0.3.

range\_point\_size

A numeric vector of length 2 that specifies the minimum and the maximum size of the nodes. Defaults to c(1, 6).

range\_edge\_width

A numeric vector of length 2 that specifies the minimum and the maximum width of the edges. Defaults to c(0.01, 3).

edge\_palette\_name

A string that specifies the name of the palette that will be used for the edges. Defaults to "RColorBrewer::Greys".

edge\_palette\_inverse

A boolean value that specifies whether the palette should be inverted. Defaults

node\_palette\_name

A string that specifies the name of the palette that will be used for the nodes. Defaults to "viridis::rocket".

node\_palette\_inverse

A boolean value that specifies whether the palette should be inverted. Defaults to TRUE.

### Value

A ggplot object following the details from description.

### Note

The names of the colour palettes should follow the format defined in the paletteer package.

```
set.seed(2024)
# create an artificial PCA embedding
pca_embedding <- matrix(runif(100 * 30), nrow = 100)</pre>
rownames(pca_embedding) <- paste0("cell_", seq_len(nrow(pca_embedding)))</pre>
colnames(pca_embedding) <- paste0("PC_", 1:30)</pre>
adj_matrix <- getNNmatrix(</pre>
    RANN::nn2(pca\_embedding, k = 10)nn.idx,
    10,
    0,
)$nn
rownames(adj_matrix) <- paste0("cell_", seq_len(nrow(adj_matrix)))</pre>
colnames(adj_matrix) <- paste0("cell_", seq_len(ncol(adj_matrix)))</pre>
```

```
# alternatively, the adj_matrix can be calculated
# using the `Seurat::FindNeighbors` function.

clust_diff_obj <- assess_clustering_stability(
    graph_adjacency_matrix = adj_matrix,
    resolution = c(0.5, 1),
    n_repetitions = 10,
    clustering_algorithm = 1:2,
    verbose = TRUE
)
plot_clust_hierarchical(clust_diff_obj)</pre>
```

```
plot_connected_comps_evolution
```

Relationship Between Number of Nearest Neighbours and Graph Connectivity

# **Description**

Display the distribution of the number connected components obtained for each number of neighbours across random seeds.

### Usage

```
plot_connected_comps_evolution(nn_conn_comps_object)
```

# Arguments

```
nn_conn_comps_object
```

An object or a concatenation of objects returned by the get\_nn\_conn\_comps method.

#### Value

A ggplot2 object with boxplots for the connected component distributions.

# Note

The number of connected components is displayed on a logarithmic scale.

```
set.seed(2024)
# create an artificial PCA embedding
pca_emb <- matrix(runif(100 * 30), nrow = 100, byrow = TRUE)
rownames(pca_emb) <- as.character(1:100)
colnames(pca_emb) <- paste0("PCA_", 1:30)
nn_conn_comps_obj <- get_nn_conn_comps(</pre>
```

```
embedding = pca_emb,
    n_neigh_sequence = c(2, 5),
    n_repetitions = 3,
    # arguments that are passed to the uwot function
    umap_arguments = list(
        min_dist = 0.3,
        metric = "cosine"
    )
)
plot_connected_comps_evolution(nn_conn_comps_obj)
```

### **Description**

Display EC consistency for each feature set and for each step. Above each boxplot there is a number representing the step (or the size of the subset). The ECC values are extracted for each resolution value and summarized using the summary\_function parameter.

### Usage

```
plot_feature_overall_stability_boxplot(
  feature_object_list,
  summary_function = stats::median,
  text_size = 4,
  boxplot_width = 0.4,
  dodge_width = 0.7,
  return_df = FALSE
)
```

### **Arguments**

 $\label{list-constraint} An \ object \ or \ a \ concatenation \ of \ objects \ returned \ by \ the \ assess\_feature\_stability \\ method \\ summary\_function$ 

The function that will be used to summarize the ECC values. Defaults to median.

text\_size The size of the labels above boxplots

boxplot\_width Used for adjusting the width of the boxplots; the value will be passed to the

width argument of the ggplot2::geom\_boxplot method.

dodge\_width Used for adjusting the horizontal position of the boxplot; the value will be

passed to the width argument of the ggplot2::position\_dodge method.

return\_df If TRUE, the function will return the ECS values as a dataframe. Default is

FALSE.

#### Value

A ggplot2 object.

### **Examples**

```
set.seed(2024)
# create an artificial expression matrix
expr_matrix <- matrix(</pre>
    c(runif(100 * 10), runif(100 * 10, min = 3, max = 4)),
    nrow = 200, byrow = TRUE
)
rownames(expr_matrix) <- as.character(1:200)</pre>
colnames(expr_matrix) <- paste("feature", 1:10)</pre>
feature_stability_result <- assess_feature_stability(</pre>
    data_matrix = t(expr_matrix),
    feature_set = colnames(expr_matrix),
    steps = 5,
    feature_type = "feature_name",
    resolution = c(0.1, 0.5, 1),
    n_repetitions = 10,
    umap_arguments = list(
        # the following parameters are used by the umap function
        # and are not mandatory
        n_neighbors = 3,
        approx_pow = TRUE,
        n_{epochs} = 0,
        init = "random",
        min_dist = 0.3
    ),
    clustering_algorithm = 1
plot_feature_overall_stability_boxplot(feature_stability_result)
```

### Description

Perform an incremental ECS between two consecutive feature steps. The ECS values are extracted for every resolution value and summarized using a function (e.g. median, mean, etc.).

### Usage

```
plot_feature_overall_stability_incremental(
  feature_object_list,
  summary_function = stats::median,
  dodge_width = 0.7,
```

```
text_size = 4,
boxplot_width = 0.4,
return_df = FALSE
)
```

### **Arguments**

feature\_object\_list

An object or a concatenation of objects returned by the assess\_feature\_stability method.

summary\_function

The function used to summarize the ECS values. Default is median.

dodge\_width Used for adjusting the horizontal position of the boxplot; the value will be

passed to the width argument of the ggplot2::position\_dodge method.

text\_size The size of the labels above boxplots.

boxplot\_width Used for adjusting the width of the boxplots; the value will be passed to the

width argument of the ggplot2::geom\_boxplot method.

return\_df If TRUE, the function will return the ECS values as a dataframe. Default is

FALSE.

#### Value

A ggplot2 object with ECS distribution will be displayed as a boxplot. Above each boxplot there will be a pair of numbers representing the two steps that are compared.

```
set.seed(2024)
# create an artificial expression matrix
expr_matrix <- matrix(</pre>
    c(runif(50 * 10), runif(50 * 10, min = 3, max = 4)),
    nrow = 100, byrow = TRUE
rownames(expr_matrix) <- as.character(1:100)</pre>
colnames(expr_matrix) <- paste("feature", 1:10)</pre>
feature_stability_result <- assess_feature_stability(</pre>
    data_matrix = t(expr_matrix),
    feature_set = colnames(expr_matrix),
    steps = c(5, 10),
    feature_type = "feature_name",
    resolution = c(0.1, 0.5),
    n_repetitions = 3,
    umap_arguments = list(
        # the following parameters are used by the umap function
        # and are not mandatory
        n_neighbors = 3,
        approx_pow = TRUE,
        n_{epochs} = 0,
        init = "random",
```

```
min_dist = 0.3
),
  clustering_algorithm = 1
)
plot_feature_overall_stability_incremental(feature_stability_result)
```

```
plot_feature_per_resolution_stability_boxplot

Per resolution Feature Stability Boxplot
```

### **Description**

Display EC consistency for each feature set and for each step. Above each boxplot there is a number representing the step (or the size of the subset). The ECC values are extracted depdening on the resolution value provided by the user.

# Usage

```
plot_feature_per_resolution_stability_boxplot(
  feature_object_list,
  resolution,
  violin_plot = FALSE,
  text_size = 4,
  boxplot_width = 0.4,
  dodge_width = 0.7,
  return_df = FALSE
)
```

### **Arguments**

feature\_object\_list

An object or a concatenation of objects returned by the assess\_feature\_stability method

resolution The resolution value for which the ECC will be extracted.

violin\_plot If TRUE, the function will return a violin plot instead of a boxplot. Default is FALSE.

text\_size The size of the labels above boxplots

boxplot\_width Used for adjusting the width of the boxplots; the value will be passed to the width argument of the ggplot2::geom\_boxplot method.

Used for adjusting the horizontal position of the boxplot; the value will be passed to the width argument of the ggplot2::position\_dodge method.

FALSE.

### Value

A ggplot2 object.

dodge\_width

### **Examples**

```
set.seed(2024)
# create an artificial expression matrix
expr_matrix <- matrix(</pre>
   c(runif(100 * 10), runif(100 * 10, min = 3, max = 4)),
    nrow = 200, byrow = TRUE
)
rownames(expr_matrix) <- as.character(1:200)</pre>
colnames(expr_matrix) <- paste("feature", 1:10)</pre>
feature_stability_result <- assess_feature_stability(</pre>
    data_matrix = t(expr_matrix),
    feature_set = colnames(expr_matrix),
    steps = 5,
    feature_type = "feature_name",
    resolution = c(0.1, 0.5, 1),
   n_repetitions = 10,
   umap_arguments = list(
        # the following parameters are used by the umap function
        # and are not mandatory
        n_neighbors = 3,
        approx_pow = TRUE,
        n_{epochs} = 0,
        init = "random",
        min_dist = 0.3
   ),
    clustering_algorithm = 1
)
plot_feature_per_resolution_stability_boxplot(feature_stability_result, 0.5)
```

```
plot_feature_per_resolution_stability_incremental

Per resolution - Feature Stability Incremental Boxplot
```

# Description

Perform an incremental ECS between two consecutive feature steps. The ECS values are extracted only for a specified resolution value.

# Usage

```
plot_feature_per_resolution_stability_incremental(
  feature_object_list,
  resolution,
  dodge_width = 0.7,
  text_size = 4,
  boxplot_width = 0.4,
  return_df = FALSE
)
```

### **Arguments**

feature\_object\_list An object or a concatenation of objects returned by the assess\_feature\_stability method. resolution The resolution value for which the ECS will be extracted. dodge\_width Used for adjusting the horizontal position of the boxplot; the value will be passed to the width argument of the ggplot2::position\_dodge method. text\_size The size of the labels above boxplots. boxplot\_width Used for adjusting the width of the boxplots; the value will be passed to the width argument of the ggplot2::geom\_boxplot method. return\_df If TRUE, the function will return the ECS values as a dataframe. Default is FALSE.

#### Value

A ggplot2 object with ECS distribution will be displayed as a boxplot. Above each boxplot there will be a pair of numbers representing the two steps that are compared.

```
set.seed(2024)
# create an artificial expression matrix
expr_matrix <- matrix(</pre>
    c(runif(50 * 10), runif(50 * 10, min = 3, max = 4)),
    nrow = 100, byrow = TRUE
)
rownames(expr_matrix) <- as.character(1:100)</pre>
colnames(expr_matrix) <- paste("feature", 1:10)</pre>
feature_stability_result <- assess_feature_stability(</pre>
    data_matrix = t(expr_matrix),
    feature_set = colnames(expr_matrix),
    steps = c(5, 10),
    feature_type = "feature_name",
    resolution = c(0.1, 0.5),
    n_{repetitions} = 3,
    umap_arguments = list(
        # the following parameters are used by the umap function
        # and are not mandatory
        n_neighbors = 3,
        approx_pow = TRUE,
        n_{epochs} = 0,
        init = "random",
        min_dist = 0.3
    clustering_algorithm = 1
plot_feature_per_resolution_stability_incremental(feature_stability_result, 0.1)
```

# **Description**

Display a facet of plots where each subpanel is associated with a feature set and illustrates the distribution of the EC consistency score over the UMAP embedding.

# Usage

```
plot_feature_stability_ecs_facet(
  feature_object_list,
  resolution,
  n_facet_cols = 3,
  point_size = 0.3
)
```

# **Arguments**

feature\_object\_list

An object or a concatenation of objects returned by the assess\_feature\_stability

method

resolution The resolution value for which the ECS will be extracted.

 $n\_facet\_cols \qquad The \ number \ of \ facet \lq s \ columns.$ 

point\_size The size of the points displayed on the plot.

### Value

A ggplot2 object

```
n_repetitions = 10,
    clustering_algorithm = 1
)
plot_feature_stability_ecs_facet(
    feature_stability_result,
    0.5,
    point_size = 2
)
```

# **Description**

Display a facet of plots where each subpanel is associated with a feature set and illustrates the distribution of the most frequent partition over the UMAP embedding.

# Usage

```
plot_feature_stability_mb_facet(
  feature_object_list,
  resolution,
  text_size = 5,
  n_facet_cols = 3,
  point_size = 0.3
)
```

# Arguments

feature\_object\_list

An object or a concatenation of objects returned by the assess\_feature\_stability

method

resolution The resolution value for which the ECS will be extracted.

text\_size The size of the cluster label n\_facet\_cols The number of facet's columns.

point\_size The size of the points displayed on the plot.

### Value

A ggplot2 object.

plot\_k\_n\_partitions 59

### **Examples**

```
set.seed(2024)
# create an artificial expression matrix
expr_matrix <- matrix(</pre>
    c(runif(100 * 10), runif(50 * 10, min = 3, max = 4)),
    nrow = 150, byrow = TRUE
)
rownames(expr_matrix) <- as.character(1:150)</pre>
colnames(expr_matrix) <- paste("feature", 1:10)</pre>
feature_stability_result <- assess_feature_stability(</pre>
    data_matrix = t(expr_matrix),
    feature_set = colnames(expr_matrix),
    steps = 5,
    feature_type = "feature_name",
    resolution = c(0.1, 0.5, 1),
    n_repetitions = 10,
    clustering_algorithm = 1
plot_feature_stability_mb_facet(
    feature_stability_result,
    0.5,
    point_size = 2
)
```

plot\_k\_n\_partitions

Relationship Between the Number of Clusters and the Number of Unique Partitions

# Description

For each configuration provided in clust\_object, display how many different partitions with the same number of clusters can be obtained by changing the seed.

# Usage

```
plot_k_n_partitions(
  clust_object,
  colour_information = c("ecc", "freq_part"),
  dodge_width = 0.3,
  pt_size_range = c(1.5, 4),
  summary_function = stats::median,
  y_step = 5
)
```

# **Arguments**

clust\_object An object returned by the assess\_clustering\_stability method.

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colour\_information

String that specifies the information type that will be illustrated using gradient colour: either freq\_part for the frequency of the most common partition or ecc for the Element-Centric Consistency of the partitions obtained when the the number of clusters is fixed. Defaults to ecc.

dodge\_width

Used for adjusting the distance between the boxplots representing a clustering method. Defaults to 0.3.

pt\_size\_range

Indicates the minimum and the maximum size a point on the plot can have. Defaults to c(1.5, 4).

mmany function

summary\_function

The function that will be used to summarize the distribution of the ECC values obtained for each number of clusters. Defaults to median.

y\_step

The step used for the y-axis. Defaults to 5.

#### Value

A ggplot2 object. The color gradient suggests the frequency of the most common partition relative to the total number of appearances of that specific number of clusters or the Element-Centric Consistency of the partitions. The size illustrates the frequency of the partitions with k clusters relative to the total number of partitions. The shape of the points indicates the clustering method.

```
set.seed(2024)
# create an artificial PCA embedding
pca_embedding <- matrix(runif(100 * 30), nrow = 100)</pre>
rownames(pca_embedding) <- paste0("cell_", seq_len(nrow(pca_embedding)))</pre>
colnames(pca_embedding) <- paste0("PC_", 1:30)</pre>
adj_matrix <- getNNmatrix(</pre>
    RANN::nn2(pca\_embedding, k = 10)$nn.idx,
    10,
    0,
    -1
)$nn
rownames(adj_matrix) <- paste0("cell_", seq_len(nrow(adj_matrix)))</pre>
colnames(adj_matrix) <- paste0("cell_", seq_len(ncol(adj_matrix)))</pre>
# alternatively, the adj_matrix can be calculated
# using the `Seurat::FindNeighbors` function.
clust_diff_obj <- assess_clustering_stability(</pre>
    graph_adjacency_matrix = adj_matrix,
    resolution = c(0.5, 1),
   n_repetitions = 10,
    clustering_algorithm = 1:2,
    verbose = FALSE
plot_k_n_partitions(clust_diff_obj)
```

```
plot_k_resolution_corresp
```

Correspondence Between Resolution and the Number of Clusters

### **Description**

For each configuration provided in the clust\_object, display what number of clusters appear for different values of the resolution parameters.

### Usage

```
plot_k_resolution_corresp(
  clust_object,
  colour_information = c("ecc", "freq_k"),
  dodge_width = 0.3,
  pt_size_range = c(1.5, 4),
  summary_function = stats::median
)
```

### **Arguments**

 $\verb|clust_object| An object returned by the assess\_clustering\_stability method.$ 

colour\_information

String that specifies the information type that will be illustrated using gradient colour: either freq\_part for the frequency of the most common partition or ecc for the Element-Centric Consistency of the partitions obtained when the the number of clusters is fixed. Defaults to ecc.

dodge\_width

Used for adjusting the distance between the boxplots representing a clustering method. Defaults to 0.3.

pt\_size\_range

Indicates the minimum and the maximum size a point on the plot can have. Defaults to c(1.5, 4).

summary\_function

The function that will be used to summarize the distribution of the ECC values obtained for each number of clusters. Defaults to median.

### Value

A ggplot2 object. Different shapes of points indicate different parameter configuration, while the color illustrates the frequency of the most common partition or the Element-Centric Consistency of the partitions. The frequency is calculated as the fraction between the number of total appearances of partitions with a specific number of clusters and resolution value and the number of runs. The size illustrates the frequency of the most common partition with k clusters relative to the partitions obtained with the same resolution value and have k clusters.

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### **Examples**

```
set.seed(2024)
# create an artificial PCA embedding
pca_embedding <- matrix(runif(100 * 30), nrow = 100)</pre>
rownames(pca_embedding) <- paste0("cell_", seq_len(nrow(pca_embedding)))</pre>
colnames(pca_embedding) <- paste0("PC_", 1:30)</pre>
adj_matrix <- getNNmatrix(</pre>
    RANN::nn2(pca\_embedding, k = 10)nn.idx,
    0.
    -1
)$nn
rownames(adj_matrix) <- paste0("cell_", seq_len(nrow(adj_matrix)))</pre>
colnames(adj_matrix) <- paste0("cell_", seq_len(ncol(adj_matrix)))</pre>
# alternatively, the adj_matrix can be calculated
# using the `Seurat::FindNeighbors` function.
clust_diff_obj <- assess_clustering_stability(</pre>
    graph_adjacency_matrix = adj_matrix,
    resolution = c(0.5, 1),
    n_repetitions = 10,
    clustering_algorithm = 1:2,
    verbose = FALSE
)
plot_k_resolution_corresp(clust_diff_obj)
```

plot\_n\_neigh\_ecs

Graph construction parameters - ECC facet

### **Description**

Display, for all configurations consisting in different number of neighbours, graph types and base embeddings, the EC Consistency of the partitions obtained over multiple runs on an UMAP embedding.

# Usage

```
plot_n_neigh_ecs(nn_ecs_object, boxplot_width = 0.5)
```

### **Arguments**

nn\_ecs\_object An object or a concatenation of objects returned by the get\_nn\_importance method.

boxplot\_width

Used for adjusting the width of the boxplots; the value will be passed to the width argument of the ggplot2::geom\_boxplot method.

# Value

A ggplot2 object.

### **Examples**

```
set.seed(2024)
# create an artificial PCA embedding
pca_emb <- matrix(runif(100 * 30), nrow = 100, byrow = TRUE)
rownames(pca_emb) <- as.character(1:100)
colnames(pca_emb) <- paste0("PC_", 1:30)

nn_stability_obj <- assess_nn_stability(
    embedding = pca_emb,
    n_neigh_sequence = c(10, 15, 20),
    n_repetitions = 10,
    graph_reduction_type = "PCA",
    clustering_algorithm = 1
)
plot_n_neigh_ecs(nn_stability_obj)</pre>
```

plot\_n\_neigh\_k\_correspondence

Relationship Between Number of Nearest Neighbours and Number of Clusters

### **Description**

Display the distribution of the number of clusters obtained for each number of neighbours across random seeds.

### Usage

```
plot_n_neigh_k_correspondence(nn_object_n_clusters)
```

# Arguments

```
nn_object_n_clusters
```

An object or a concatenation of objects returned by the get\_nn\_importance method.

### Value

A ggplot2 object with the distributions displayed as boxplots.

### Note

The number of clusters is displayed on a logarithmic scale.

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### **Examples**

```
set.seed(2024)
# create an artificial PCA embedding
pca_emb <- matrix(runif(100 * 30), nrow = 100, byrow = TRUE)
rownames(pca_emb) <- as.character(1:100)
colnames(pca_emb) <- paste0("PC_", 1:30)

nn_stability_obj <- assess_nn_stability(
    embedding = pca_emb,
    n_neigh_sequence = c(10, 15, 20),
    n_repetitions = 10,
    graph_reduction_type = "PCA",
    clustering_algorithm = 1
)
plot_n_neigh_k_correspondence(nn_stability_obj)</pre>
```

server\_comparisons

Server - Comparison module

### **Description**

Creates the backend interface for the comparison module inside the ClustAssess Shiny application.

### Usage

```
server_comparisons(id, chosen_config, chosen_method)
```

#### **Arguments**

id The id of the module, used to acess the UI elements.

chosen\_config A reactive object that contains the chosen configuration from the Dimensionality

Reduction tab.

chosen\_method A reactive object that contains the chosen method from the Clustering tab.

### Note

server\_dimensionality\_reduction

Server - Dimensionality reduction module

### **Description**

Creates the backend interface for the dimensionality reduction module inside the ClustAssess Shiny application.

### Usage

```
server_dimensionality_reduction(id, parent_session)
```

### **Arguments**

id The id of the module, used to acess the UI elements.

parent\_session The session of the parent module, used to control the tabs of the application.

#### Note

This function should not be called directly, but in the context of the app that is created using the write\_shiny\_app function.

```
server_graph_clustering
```

Server - Graph clustering module

### Description

Creates the backend interface for the graph clustering module inside the ClustAssess Shiny application.

### Usage

```
server_graph_clustering(id, feature_choice, parent_session)
```

### **Arguments**

id The id of the module, used to acess the UI elements.

feature\_choice A reactive object that contains the chosen configuration from the Dimensionality

Reduction tab.

parent\_session The session of the parent module, used to control the tabs of the application.

### Note

server\_landing\_page

```
server_graph_construction
```

Server - Graph construction module

# Description

Creates the backend interface for the graph construction module inside the ClustAssess Shiny application.

# Usage

```
server_graph_construction(id, chosen_config)
```

# **Arguments**

id The id of the module, used to acess the UI elements.

chosen\_config A reactive object that contains the chosen configuration from the Dimensionality

Reduction tab.

### Note

This function should not be called directly, but in the context of the app that is created using the write\_shiny\_app function.

# Description

Creates the backend interface for the landing page module inside the ClustAssess Shiny application.

### Usage

```
server_landing_page(
   id,
   height_ratio,
   dimension,
   parent_session,
   organism = "hsapiens"
)
```

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### **Arguments**

id The id of the module, used to acess the UI elements.

height\_ratio A reactive object that contains the height ratio of the plots in the application

(the height of the plot is calculated using the height ratio and the height of the

webpage).

dimension A reactive object that contains the dimensions of the webpage.

parent\_session The session of the parent module, used to control the tabs of the application.

organism The organism of the dataset, which will be used in the enrichment analysis.

#### Note

This function should not be called directly, but in the context of the app that is created using the write\_shiny\_app function.

server\_sandbox Server - Sandbox module

### **Description**

Creates the backend interface for the sandbox module inside the ClustAssess Shiny application.

# Usage

server\_sandbox(id)

### **Arguments**

id The id of the module, used to acess the UI elements.

### Note

ui\_comparisons

UI - Comparison module

# **Description**

Creates the UI interface for the comparison module inside the ClustAssess Shiny application.

# Usage

```
ui_comparisons(id)
```

### **Arguments**

id

The id of the module, used to identify the UI elements.

#### Note

This function should not be called directly, but in the context of the app that is created using the write\_shiny\_app function.

```
ui_dimensionality_reduction
```

UI - Dimensionality reduction module

# **Description**

Creates the UI interface for the dimensionality reduction module inside the ClustAssess Shiny application.

# Usage

```
ui_dimensionality_reduction(id)
```

### **Arguments**

id

The id of the module, used to identify the UI elements.

### Note

ui\_graph\_clustering 69

# **Description**

Creates the UI interface for the graph clustering module inside the ClustAssess Shiny application.

# Usage

```
ui_graph_clustering(id)
```

# Arguments

id

The id of the module, used to identify the UI elements.

# Note

This function should not be called directly, but in the context of the app that is created using the write\_shiny\_app function.

 $\verb"ui_graph_construction" \textit{UI-Graph construction module}$ 

# **Description**

Creates the UI interface for the graph construction module inside the ClustAssess Shiny application.

### Usage

```
ui_graph_construction(id)
```

### **Arguments**

id

The id of the module, used to identify the UI elements.

### Note

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ui\_landing\_page

UI - Landing page module

# Description

Creates the UI interface for the landing page module inside the ClustAssess Shiny application.

# Usage

```
ui_landing_page(id)
```

# Arguments

id

The id of the module, used to identify the UI elements.

# Note

This function should not be called directly, but in the context of the app that is created using the write\_shiny\_app function.

ui\_sandbox

UI - Sandbox module

# **Description**

Creates the UI interface for the sandbox module inside the ClustAssess Shiny application.

### Usage

```
ui_sandbox(id)
```

### **Arguments**

id

The id of the module, used to identify the UI elements.

# Note

weighted\_element\_consistency

Weighted Element-Centric Consistency

### **Description**

Calculate the weighted element-centric consistency of a set of clusterings. The weights are used to give more importance to some clusterings over others.

# Usage

```
weighted_element_consistency(
  clustering_list,
  weights = NULL,
  calculate_sim_matrix = FALSE
)
```

### **Arguments**

clustering\_list

The list of clustering results, each of which is either:

- A numeric/character/factor vector of cluster labels for each element.
- A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
- An helust object.

weights

A numeric vector of weights for each clustering in clustering\_list. If NULL, then all weights will be equal to 1. Defaults to NULL.

calculate\_sim\_matrix

A logical value that indicates whether to calculate the similarity matrix or not along with the consistency score. Defaults to FALSE.

#### Value

A vector containing the weighted element-wise consistency. If calculate\_sim\_matrix is set to TRUE, the element similarity matrix will be returned as well.

# Note

```
The weighted ECC will be calculated as \frac{\sum_{i} \sum_{j} w_{i} w_{j} ECS(i,j)}{\sum_{i} w_{i}}
```

```
# cluster across 20 random seeds
clustering_list <- lapply(1:20, function(x) kmeans(mtcars, centers = 3)$cluster)
weights <- sample(1:10, 20, replace = TRUE)
weighted_element_consistency(clustering_list, weights = weights)</pre>
```

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write\_objects

Write the objects for the ClustAssess ShinyApp

### **Description**

Given the output of the ClustAssess pipeline, the expression matrix and the metadata, this function creates the files needed for the ClustAssess ShinyApp. The files are written in the project\_folder and are the following:

- metadata.rds: the metadata file
- stability.h5: contains the stability results
- expression.h5: contains the expression matrix and the rank matrix

# Usage

```
write_objects(
  clustassess_object,
  expression_matrix,
  metadata,
  project_folder = ".",
  compression_level = 6,
  chunk_size = 100,
  gene_variance_threshold = 0,
  summary_function = stats::median,
  qualpalr_colorspace = "pretty"
)
```

### **Arguments**

clustassess\_object

The output of the ClustAssess automatic pipeline

expression\_matrix

The expression matrix

metadata The metadata

project\_folder The folder where the files will be written

compression\_level

The compression level for the h5 files (See 'rhdf5::h5createFile" for more de-

tai

The chunk size for the rank matrix (See rhdf5::h5createDataset for more details)

gene\_variance\_threshold

The threshold for the gene variance; genes with variance below this threshold will be removed

summary\_function

chunk\_size

The function used for summarizing the stability values; the default is median qualpalr\_colorspace

The colorspace used for generating the colors; the default is pretty

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### Value

NULL (the files are written in the project\_folder)

write\_shiny\_app

Create the ClustAssess ShinyApp

# **Description**

Creates the ClustAssess ShinyApp based on the output of the automatic ClustAssess pipeline. In addition to that, the expression matrix and the metadata dataframe are provided as input to the ShinyApp. If the clustassess object is not provided, the function will create the light version of the ClustAssess ShinyApp, that will not contain the assessment results. For this case, the metadata parameter should contain two aditional columns named 'UMAP\_1" and 'UMAP\_2' that will correspond to the 2D embedding of the cells.

### Usage

```
write_shiny_app(
  object,
 metadata = NULL,
  assay_name = NULL,
  clustassess_object,
  project_folder,
  compression\_level = 6,
  summary_function = stats::median,
  shiny_app_title = "",
  organism_enrichment = "hsapiens",
  height_ratio = 0.6,
  qualpalr_colorspace = "pretty",
  prompt_feature_choice = TRUE
)
## S3 method for class 'Seurat'
write_shiny_app(
  object,
 metadata = NULL,
  assay_name,
  clustassess_object = NULL,
  project_folder,
  compression_level = 6,
  summary_function = stats::median,
  shiny_app_title = "",
  organism_enrichment = "hsapiens",
  height_ratio = 0.6,
  qualpalr_colorspace = "pretty",
  prompt_feature_choice = TRUE
```

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```
)
## Default S3 method:
write_shiny_app(
  object,
 metadata = NULL,
  assay_name = NULL,
  clustassess_object = NULL,
  project_folder,
  compression_level = 6,
  summary_function = stats::median,
  shiny_app_title = "",
  organism_enrichment = "hsapiens",
  height_ratio = 0.6,
  qualpalr_colorspace = "pretty",
  prompt_feature_choice = TRUE
)
```

### **Arguments**

object A Seurat object or an expression matrix

metadata The metadata dataframe. This parameter will be ignored if the object is a Seurat

object.

assay\_name The name of the assay to be used to extract the expression matrix from the Seurat

object. This parameter will be ignored if the object is not a Seurat object.

clustassess\_object

The output of the ClustAssess automatic pipeline. If the ClustAssess object is not provided (NULL), the function will create the light version of the ShinyApp, that will not contain the assessment results.

that will not contain the assessment results.

project\_folder The folder where the files will be written
compression\_level

The compression level for the h5 files (See 'rhdf5::h5createFile" for more details)

summary\_function

The function used for summarizing the stability values; the default is median

shiny\_app\_title

The title of the shiny app

organism\_enrichment

The organism used for the enrichment analysis; the default is hsapiens

height\_ratio The ratio of the height of the plot to the height of the browser; the default is 0.6 qualpalr\_colorspace

The colorspace used for generating the colors; the default is pretty

prompt\_feature\_choice

Should the user be prompted to choose if he wants to continue with the selection of features even if it is lower than median sequence depth; the default is TRUE

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