# Package ‘ClustAssess’

January 26, 2022

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<td>Tools for Assessing Clustering</td>
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<tr>
<td>Version</td>
<td>0.3.0</td>
</tr>
<tr>
<td>Maintainer</td>
<td>Arash Shahsavari <a href="mailto:as3006@cam.ac.uk">as3006@cam.ac.uk</a></td>
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<tr>
<td>Description</td>
<td>A set of tools for evaluating clustering robustness using proportion of ambiguously clustered pairs (Senbabaoglu et al. (2014) <a href="">doi:10.1038/srep06207</a>), as well as similarity across methods and method stability using element-centric clustering comparison (Gates et al. (2019) <a href="">doi:10.1038/s41598-019-44892-y</a>). Additionally, this package enables stability-based parameter assessment for graph-based clustering pipelines typical in single-cell data analysis.</td>
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<td>Imports</td>
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<td><a href="https://github.com/Core-Bioinformatics/ClustAssess">https://github.com/Core-Bioinformatics/ClustAssess</a></td>
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<tr>
<td>NeedsCompilation</td>
<td>yes</td>
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<tr>
<td>Author</td>
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</tr>
<tr>
<td>Repository</td>
<td>CRAN</td>
</tr>
<tr>
<td>Date/Publication</td>
<td>2022-01-26 16:52:46 UTC</td>
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Description

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

Usage

```r
consensus_cluster(
  x,
  k_min = 3,
  k_max = 100,
  n_reps = 100,
  p_sample = 0.8,
  p_feature = 1,
)```

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```r
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 reclustering 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**


**Examples**

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

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

**Usage**

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

**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 hclust 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 hclust 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.
element_consistency

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

Usage
```r
element_consistency(
  clustering_list,
  alpha = 0.9,
  r = 1,
  rescale_path_type = "max",
  ppr_implementation = "prpack",
)```

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.

ncores
the number of parallel R instances that will run the code. If the value is set to 1, the code will be run sequentially.

Value
A vector containing the element-wise average agreement.

References

Examples
```r
reference.clustering = iris$Species
clustering.list = list()
for (i in 1:20){
  clustering.list[[i]] = kmeans(iris[,1:4], 3)$cluster
}

element_agreement(reference.clustering, clustering.list)
```
dist_rescaled = FALSE,
row_normalize = TRUE,
ncores = 1
}

**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 hclust 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 from 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.

- **ncores**
  The number of parallel R instances that will run the code. If the value is set to 1, the code will be run sequentially.

**Value**

A vector containing the element-wise consistency.

**References**

Examples

```r
clustering.list = list()
for (i in 1:20){
    clustering.list[[i]] = kmeans(mtcars, 3)$cluster
}
element_consistency(clustering.list)
```

The Element-Centric Clustering Similarity

Description

Calculates the average element-centric similarity between two clustering results

Usage

```r
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 hclust 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 hclust 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.

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, 3)$cluster
   hc.res = hclust(dist(mtcars))
   element_sim(km.res, hc.res)
element_sim_elscore  \hspace{1cm} \textit{The Element-Centric Clustering Similarity for each Element}

\section*{Description}
Calculates the element-wise element-centric similarity between two clustering results.

\section*{Usage}

\begin{verbatim}
  
  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_cl2 = 1, 
    rescale_path_type_cl2 = "max", 
    ppr_implementation_cl2 = "prpack", 
    dist_rescaled_cl2 = FALSE, 
    row_normalize_cl2 = TRUE 
  )
\end{verbatim}

\section*{Arguments}

\begin{itemize}
  \item \textbf{clustering1}  \hspace{1cm} The first clustering result, which can be one of:
    \begin{itemize}
      \item A numeric/character/factor vector of cluster labels for each element.
      \item A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
      \item An hclust object.
    \end{itemize}
  \item \textbf{clustering2}  \hspace{1cm} The second clustering result, which can be one of:
    \begin{itemize}
      \item A numeric/character/factor vector of cluster labels for each element.
      \item A samples x clusters matrix/Matrix::Matrix of nonzero membership values.
      \item An hclust object.
    \end{itemize}
  \item \textbf{alpha}  \hspace{1cm} A numeric giving the personalized PageRank damping factor; 1 - alpha is the restart probability for the PPR random walk.
  \item \textbf{r_cl1}  \hspace{1cm} A numeric hierarchical scaling parameter for the first clustering.
  \item \textbf{rescale_path_type_cl1}  \hspace{1cm} A string; rescale the hierarchical height of the first clustering by:
    \begin{itemize}
      \item "max" : the maximum path from the root.
      \item "min" : the minimum path form the root.
      \item "linkage" : use the linkage distances in the clustering.
    \end{itemize}
  \item \textbf{ppr_implementation_cl1}  \hspace{1cm} A string; the method for computing PPR.
  \item \textbf{dist_rescaled_cl1}  \hspace{1cm} A boolean; rescale the distance matrix.
  \item \textbf{row_normalize_cl1}  \hspace{1cm} A boolean; normalize the rows of the matrix.
  \item \textbf{r_cl2}  \hspace{1cm} A numeric hierarchical scaling parameter for the second clustering.
  \item \textbf{rescale_path_type_cl2}  \hspace{1cm} A string; rescale the hierarchical height of the second clustering by:
    \begin{itemize}
      \item "max" : the maximum path from the root.
      \item "min" : the minimum path form the root.
      \item "linkage" : use the linkage distances in the clustering.
    \end{itemize}
  \item \textbf{ppr_implementation_cl2}  \hspace{1cm} A string; the method for computing PPR.
  \item \textbf{dist_rescaled_cl2}  \hspace{1cm} A boolean; rescale the distance matrix.
  \item \textbf{row_normalize_cl2}  \hspace{1cm} A boolean; normalize the rows of the matrix.
\end{itemize}
Choose a implementation for personalized page-rank calculation for the first clustering:

- "prpack": use PPR algorithms in igraph.
- "power_iteration": use power_iteration method.

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

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.

A numeric hierarchical scaling parameter for the second clustering.

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.

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

- "prpack": use PPR algorithms in igraph.
- "power_iteration": use power_iteration method.

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

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.

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


```r
km.res = kmeans(iris[,1:4], centers=8)$cluster
hc.res = hclust(dist(iris[,1:4]))
element_sim_elscore(km.res, hc.res)
```
element_sim_matrix

Pairwise Comparison of Clusterings

Description

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

Usage

```r
element_sim_matrix(
  clustering_list,
  output_type = "matrix",  # A string specifying whether the output should be a matrix or a data.frame.
  alpha = 0.9,            # A numeric giving the personalized PageRank damping factor; 1 - alpha is the restart probability for the PPR random walk.
  r = 1,                 # A numeric hierarchical scaling parameter.
  rescale_path_type = "max",  # A string: rescale the hierarchical height by:
  ppr_implementation = "prpack",  # Choose a implementation for personalized page-rank calculation:
  dist_rescaled = FALSE,  # A logical: if TRUE, the linkage distances are linearly rescaled to be in-between 0 and 1.
  row_normalize = TRUE,
  ncores = 1
)
```

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 hclust object.

- **output_type**
  A string giving the output type: "matrix" or "data.frame".

- **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 from 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.
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.

the number of parallel R instances that will run the code. If the value is set to 1, the code will be run sequentially.

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


clustering.list = list()
for (i in 1:20) {
  clustering.list[[i]] = kmeans(mtcars, 3)$cluster
}
element_sim_matrix(clustering.list, output_type="matrix")

get_clustering_difference

Description
Evaluatores 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
get_clustering_difference(
  graph_adjacency_matrix,
  resolution,
  n_repetitions = 100,
  seed_sequence = NULL,
  ecs_thresh = 1,
  ncores = 1,
  algorithm = 1:4,
  verbose = TRUE
)
**get_clustering_difference**

**Arguments**

- `graph_adjacency_matrix`: A square adjacency matrix based on which an igraph object will be built.
- `resolution`: A sequence of resolution values.
- `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.
- `ecs_thresh`: The ECS threshold used for merging similar clusterings.
- `ncores`: The number of parallel R instances that will run the code. If the value is set to 1, the code will be run sequentially.
- `algorithm`: 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.
- `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**

```r
set.seed(2021)
# create an artificial expression matrix
eexpr_matrix = matrix(runif(100*10), nrow = 100)
rownames(expr_matrix) = as.character(1:100)

adj_matrix = Seurat::FindNeighbors(expr_matrix,
 k.param = 10,
 nn.method = "rann",
 verbose = FALSE,
 compute.SNN = FALSE)$nn

clust_diff_obj = get_clustering_difference(graph_adjacency_matrix = adj_matrix,
 resolution = c(0.5, 1),
 n_repetitions = 10,
 algorithm = 1:2,
 verbose = FALSE)

plot_clustering_difference_boxplot(clust_diff_obj)
```
get_feature_stability  Evaluate Feature Set Stability

Description

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

Usage

```r
get_feature_stability(
  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.
  n_repetitions = 100,  # The number of repetitions of applying the pipeline with different seeds; ignored if seed_sequence is provided by the user.
  seed_sequence = NULL,  # 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 = "PCA",  # The graph reduction type, denoting if the graph should be built on either the PCA or the UMAP embedding.
  npcs = 30,  # The number of principal components.
  ecs_thresh = 1,  # The ECS threshold used for merging similar clusterings.
  ncores = 1,  # The number of parallel R instances that will run the code. If the value is set to 1, the code will be run sequentially.
  ...)
```

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.
- **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.
- **npcs**: The number of principal components.
- **ecs_thresh**: The ECS threshold used for merging similar clusterings.
- **ncores**: The number of parallel R instances that will run the code. If the value is set to 1, the code will be run sequentially.
get_nn_conn_comps

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.

... additional arguments passed to the umap method.

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. For example, if the user wants to analyze highly variable feature set, they should provide them sorted by their variability.

Examples

set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(c(runif(100*10), runif(100*10, min = 3, max = 4)), nrow = 200, byrow = TRUE)
rownames(expr_matrix) = as.character(1:200)
colnames(expr_matrix) = paste("feature", 1:10)

feature_stability_result = get_feature_stability(data_matrix = t(expr_matrix),
feature_set = colnames(expr_matrix),
feature_type = "feature_name",
steps = 5,
npcs = 2,
n_repetitions = 10,
algorithm = 1,
# 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)
plot_feature_stability_boxplot(feature_stability_result)

---

get_nn_conn_comps  Relationship Between Nearest Neighbors and Connected Components
Description

One of the steps in the clustering pipeline is building a k-nearest neighbor graph on a reduced-space embedding. This method assesses the relationship between different number of nearest neighbors 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.

Usage

get.nn.conn.comps(
  object,
  n_neigh_sequence,
  config_name = "",
  n_repetitions = 100,
  seed_sequence = NULL,
  graph_reduction_type = "UMAP",
  transpose = (graph_reduction_type == "PCA"),
  ncores = 1,
  ...
)

Arguments

object A data matrix. If the graph reduction type is PCA, the object should be an expression matrix, with features on rows and observations on columns; in the case of UMAP, the user could also provide a matrix associated to a PCA embedding. See also the transpose argument.

n_neigh_sequence A sequence of the number of nearest neighbors.

config_name User specified string that uniquely describes the embedding characteristics.

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.

core_reduction_type The graph reduction type, denoting if the graph should be built on either the PCA or the UMAP embedding.

transpose Logical: whether the input object will be transposed or not. Set to FALSE if the input is an observations X features matrix, and set to TRUE if the input is a features X observations matrix.

ncores The number of parallel R instances that will run the code. If the value is set to 1, the code will be run sequentially.

... Additional arguments passed to the ‘irlba::irlba’ or the ‘uwot::umap’ method, depending on the value of graph_reduction_type.
Value

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

Examples

```r
set.seed(2021)
# create an artificial expression matrix
eexpr_matrix = matrix(c(runif(50*10), runif(50*10, min = 1, max = 2)), nrow = 100, byrow = TRUE)
rownames(expr_matrix) = as.character(1:100)

# the graph reduction type is PCA, so we can provide the expression matrix as argument
nn_conn_comps_obj = get_nn_conn_comps(object = expr_matrix,
                               n_neigh_sequence = c(2,3,5),
                               config_name = "example_config",
                               n_repetitions = 10,
                               graph_reduction_type = "PCA",
                               transpose = FALSE,
                               # the following parameter is used by the irlba function and is not mandatory
                               nv = 3)
plot_connected_comps_evolution(nn_conn_comps_obj)
```

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

```r
get_nn_importance(
  object,
  n_neigh_sequence,
  n_repetitions = 100,
  seed_sequence = NULL,
  graph_reduction_type = "PCA",
  ecs_thresh = 1,
  ncores = 1,
  transpose = (graph_reduction_type == "PCA"),
  graph_type = 2,
  algorithm = 4,
  ...
)
```
Arguments

object

The data matrix. If the graph reduction type is PCA, the object should be an expression matrix, with features on rows and observations on columns; in the case of UMAP, the user could also provide a matrix associated to a PCA embedding. See also the transpose argument.

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.

ces_thresh

The ECS threshold used for merging similar clusterings.

ncores

The number of parallel R instances that will run the code. If the value is set to 1, the code will be run sequentially.

transpose

Logical: whether the input object will be transposed or not. Set to FALSE if the input is an observations X features matrix, and set to TRUE if the input is a features X observations matrix.

graph_type

Argument indicating whether the graph should be unweighted (0), weighted (1) or both (2).

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.

... Additional arguments passed to the irlba::irlba or the uwot::umap method, depending on the value of graph_reduction_type.

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 neighbors and graph type (weighted vs unweighted)
- n_neigh_ec_consistency - list containing the EC consistency of the partitions obtained at multiple runs when changing the number of neighbors or the graph type
- n_different_partitions - the number of different partitions obtained by each number of neighbors

Examples

set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(c(runif(100*10), runif(100*10, min=5, max=6)), nrow = 200)
get_resolution_importance

Evaluate Stability Across Resolution, Number of Neighbors, and Graph Type

Description

Perform a grid search over the resolution, number of neighbors and graph type.

Usage

get_resolution_importance(
  embedding,
  resolution,
  n_neigh,
  n_repetitions = 100,
  seed_sequence = NULL,
  clustering_method = 4,
  graph_type = 0,
  object_name = NULL,
  ecs_thresh = 1,
  ncores = 1
)

Arguments

embedding  The base embedding for the graph construction.
resolution  A sequence of resolution values.
n_neigh    A value or a sequence of number of neighbors used for graph construction.
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.
clusterings_method
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.

graph_type
Argument indicating whether the graph should be unweighted (0), weighted (1) or both (2).

object_name
User specified string that uniquely describes the embedding characteristics.

ces_thresh
The ECS threshold used for merging similar clusterings.

ncores
The number of parallel R instances that will run the code. If the value is set to 1, the code will be run sequentially.

Value
A list having two fields:

- split_by_resolution: A five-level list. The hierarchy is as follows:
  - the configuration name: concatenation between the object name provided by the user, the number of neighbors, the graph type and the clustering method
  - the resolution value $\gamma$
  - the number of clusters $k$ that can be obtained using the specified resolution
  - the partitions obtained with resolution $\gamma$ and have $k$ clusters
  - the structure of a partitions, which consists in having a mb field with the flat membership vector, freq denoting its frequency and seed, that is the seed used to obtain this partition in this configuration.

- split_by_k: has a similar structure, but the resolution level is removed. The partitions obtained in a configuration with the same number of clusters will be merged into the same list.

Examples

```r
set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(runif(500*10), nrow = 500)

# get the PCA embedding of the data
pca_embedding = irlba::irlba(expr_matrix, nv = 2)
pca_embedding = pca_embedding$u %*% diag(pca_embedding$d)
rownames(pca_embedding) = as.character(1:500)

# run the function on the pca embedding
resolution_result = get_resolution_importance(embedding = pca_embedding, resolution = c(0.8, 1), n_neigh = c(5, 7), n_repetitions = 5, clustering_method = 1, graph_type = 2, object_name = "name_example")

plot_k_resolution_corresp(resolution_result)
```

marker_overlap

**Cell-Wise Marker Gene Overlap**

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

**Usage**
```r
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' column, from which the sign of the DE will be extracted.

**Value**
A vector of the marker gene overlap per cell.
Examples

```r
suppressWarnings(
  set.seed(1234)
  library(Seurat)

  # cluster with Louvain algorithm
  pbmc_small = FindClusters(pbmc_small, resolution=0.8, verbose=FALSE)
  pbmc_pca = Embeddings(pbmc_small, 'pca')
  pbmc_small@meta.data$kmeans_clusters = kmeans(pbmc_pca, centers=3)$cluster

  # compare the markers
  Idents(pbmc_small) = pbmc_small@meta.data$seurat_clusters
  louvain.markers = FindAllMarkers(pbmc_small,
    logfc.threshold=1,
    test.use='t',
    verbose=FALSE)
  kmeans.markers = FindAllMarkers(pbmc_small,
    logfc.threshold=1,
    test.use='t',
    verbose=FALSE)
  pbmc_small@meta.data$jsi = marker_overlap(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

```r
merge_partitions(partition_list, ecs_thresh = 1, ncores = 1, order = TRUE)
```

Arguments

- `partition_list` A list of flat disjoint membership vectors.
- `ecs_thresh` A numeric: the ecs threshold.
**ncores**  
The number of parallel R instances that will run the code. If the value is set to 1, the code will be run sequentially.

**order**  
A logical: if TRUE, order the partitions based on their frequencies.

**Value**

a list of the merged partitions

**Examples**

```r
initial_list = list(c(1,1,2), c(2,2,2), c('B','B','A'))
merge_partitions(initial_list, 0.99)
```

---

**pac_convergence**  
 **PAC Convergence Plot**

**Description**

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

**Usage**

```r
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.

**Examples**

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

**PAC Landscape Plot**

**Description**

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

**Usage**

```r
call_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**

```r
pac.res = consensus_cluster(iris[,1:4], k_max=20)
pac_landscape(pac.res)
```

plot_clustering_difference_boxplot

**Clustering Method Stability Boxplot**

**Description**

Display EC consistency across clustering method and resolution values. The ‘filtered’ field of the object returned by the ‘get_clustering_difference_object’ method is used. Above each boxplot, the number of clusters is displayed.

**Usage**

```r
plot_clustering_difference_boxplot(clustering_difference_object, text_size = 3)
```

**Arguments**

- `clustering_difference_object`: An object returned by the ‘get_clustering_difference_object’ method.
- `text_size`: The size of the labels above boxplots.
Value

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

Examples

```r
set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(runif(500*10), nrow = 500)
rownames(expr_matrix) = as.character(1:500)

adj_matrix = Seurat::FindNeighbors(expr_matrix,
  k.param = 10,
  nn.method = "rann",
  verbose = FALSE,
  compute.SNN = FALSE)$nn

clust_diff_obj = get_clustering_difference(graph_adjacency_matrix = adj_matrix,
  resolution = c(0.5, 1),
  n_repetitions = 10,
  algorithm = 1:2,
  verbose = FALSE)

plot_clustering_difference_boxplot(clust_diff_obj)
```

---

**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

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

Arguments

- **clustering_difference_object**
  - An object returned by the 'get_clustering_difference_object' method.
- **embedding**
  - An embedding (only the first two dimensions will be used for visualisation).
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.

Examples

```r
set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(c(runif(250*10), runif(250*10, min = 5, max = 7)), nrow = 500)
rownames(expr_matrix) = as.character(1:500)

pca_embedding = irlba::irlba(expr_matrix, nv = 2)
pca_embedding = pca_embedding$u %*% diag(pca_embedding$d)
rownames(pca_embedding) = as.character(1:500)

adj_matrix = Seurat::FindNeighbors(pca_embedding,
    k.param = 10,
    nn.method = "rann",
    verbose = FALSE,
    compute.SNN = FALSE)$nn

clust_diff_obj = get_clustering_difference(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)
```

plot_connected_comps_evolution  

*Relationship Between Number of Nearest Neighbors and Graph Connectivity*

Description

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

Usage

```r
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.

Examples

set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(c(runif(50*10), runif(50*10, min = 1, max = 2)), nrow = 100, byrow = TRUE)
rownames(expr_matrix) = as.character(1:100)

# the graph reduction type is PCA, so we can provide the expression matrix as argument
nn_conn_comps_obj = get_nn_conn_comps(object = expr_matrix,
                              n_neigh_sequence = c(2,3,5),
                              config_name = "example_config",
                              n_repetitions = 10,
                              graph_reduction_type = "PCA",
                              transpose = FALSE,
                              # the following parameter is used by the irlba function and is not mandatory
                              nv = 3)
plot_connected_comps_evolution(nn_conn_comps_obj)

plot_feature_stability_boxplot

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)

Usage

plot_feature_stability_boxplot(feature_object_list, text_size = 4)

Arguments

feature_object_list
An object or a concatenation of objects returned by the ‘get_feature_stability’ method

text_size
The size of the labels above boxplots.
plot_feature_stability_ecs_facet

Value

A ggplot2 object.

Examples

```r
set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(c(runif(100*10), runif(100*10, min = 3, max = 4)), nrow = 200, byrow = TRUE)
rownames(expr_matrix) = as.character(1:200)
colnames(expr_matrix) = paste("feature", 1:10)

feature_stability_result = get_feature_stability(data_matrix = t(expr_matrix),
feature_set = colnames(expr_matrix),
feature_type = "feature_name",
steps = 5,
npcs = 2,
n_repetitions = 10,
algorithm = 1,
# 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)
plot_feature_stability_boxplot(feature_stability_result)
```

plot_feature_stability_ecs_facet

*Feature Stability - EC Consistency Facet Plot*

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

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

Arguments

- **feature_object_list**: An object or a concatenation of objects returned by the `get_feature_stability` method.
- **n_facet_cols**: The number of facet's columns.
- **point_size**: The size of the points displayed on the plot.
**plot_feature_stability_ecs_incremental**

**Value**

A ggplot2 object

**Examples**

```r
set.seed(2021)
# create an artificial expression matrix
data_matrix = matrix(c(runif(100*10), runif(50*10, min = 5, max = 7)), nrow = 150, byrow = TRUE)
rownames(data_matrix) = as.character(1:150)
colnames(data_matrix) = paste("feature", 1:10)

# calculate feature stability
feature_stability_result = get_feature_stability(data_matrix = t(data_matrix),
                                             feature_set = colnames(data_matrix),
                                             feature_type = "feature_name",
                                             steps = c(5,10),
                                            npcs = 2,
n_repetitions = 3,
algorithm = 1,
# 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)
plot_feature_stability_ecs_facet(feature_stability_result)
plot_feature_stability_ecs_incremental

Feature Stability Incremental Boxplot

**Description**

Perform an incremental ECS between two consecutive feature steps.

**Usage**

```r
plot_feature_stability_ecs_incremental(
  feature_object_list,
  dodge_width = 0.7,
  text_size = 4
)
```

**Arguments**

- `feature_object_list`: An object or a concatenation of objects returned by the `get_feature_stability` method.
- `dodge_width`: Used for adjusting the horizontal position of the boxplot; the value will be passed in the `width` argument of the `ggplot2::position_dodge` method.
- `text_size`: The size of the labels above boxplots.
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.

Examples

```r
set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(c(runif(25*10), runif(75*10, min = 5, max = 7)), nrow = 100, byrow = TRUE)
rownames(expr_matrix) = as.character(1:100)
colnames(expr_matrix) = paste("feature", 1:10)

feature_stability_result = get_feature_stability(data_matrix = t(expr_matrix),
feature_set = colnames(expr_matrix),
feature_type = "feature_name",
steps = c(5,10),
npcs = 2,
n_repetitions = 3,
algorithm = 1,
# 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)
plot_feature_stability_ecs_incremental(feature_stability_result)
```

---

```
plot_feature_stability_mb_facet

Feature Stability - Cluster Membership Facet Plot
```

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

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

Arguments

- **feature_object_list**: An object or a concatenation of objects returned by the 'get_feature_stability' method.
- **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.

Examples

```r
set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(c(runif(100*10), runif(50*10, min = 5, max = 7)), nrow = 150, byrow = TRUE)
rownames(expr_matrix) = as.character(1:150)
colnames(expr_matrix) = paste("feature", 1:10)

feature_stability_result = get_feature_stability(data_matrix = t(expr_matrix),
feature_set = colnames(expr_matrix),
feature_type = "feature_name",
steps = c(5,10),
npcs = 2,
n_repetitions = 3,
algorithm = 1,
# 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)
plot_feature_stability_mb_facet(feature_stability_result)
```

Description

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

Usage

```r
plot_k_n_partitions(partition_obj_list, object_names = NULL)
```
plot_k_resolution_corresp

Arguments

partition_obj_list
An object or a concatenation of objects returned by the ‘merge_resolutions’ method.

object_names
Custom names that the user could assign to each configuration; if not specified, the plot will use the generated configuration names.

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.

Examples

```
set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(runif(500*10), nrow = 500)

# get the PCA embedding of the data
pca_embedding = irlba::irlba(expr_matrix, nv = 2)
pca_embedding = pca_embedding$u %*% diag(pca_embedding$d)
rownames(pca_embedding) = as.character(1:500)

# run the function on the pca embedding
resolution_result = get_resolution_importance(embedding = pca_embedding,
                                          resolution = c(0.8, 1),
                                          n_neigh = c(5, 7),
                                          n_repetitions = 5,
                                          clustering_method = 1,
                                          graph_type = 2,
                                          object_name = "name_example")

plot_k_n_partitions(resolution_result)
```

Description

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

Usage

```
plot_k_resolution_corresp(
  res_object_list,
  res_object_names = NULL,
  given_height = 0.7
)
```
**Arguments**

- **res_object_list**: An object returned by the `get_resolution_importance` method.

- **res_object_names**: Custom names that the user could assign to each configuration; if not specified, the plot will use the generated configuration names.

- **given_height**: Used for adjusting the vertical position of the boxplot; the value will be passed in the `width` argument of the `ggplot::position_dodge` method.

**Value**

A ggplot2 object. Different shapes of points indicate different parameter configuration, while the color illustrates the frequency of the most common partition. The frequency is calculated as the fraction between the number of total appearances and the number of runs.

**Examples**

```r
set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(runif(500*10), nrow = 500)

# get the PCA embedding of the data
pca_embedding = irlba::irlba(expr_matrix, nv = 2)
pca_embedding = pca_embedding%*%diag(pca_embedding$D)
rownames(pca_embedding) = as.character(1:500)

# run the function on the pca embedding
resolution_result = get_resolution_importance(embedding = pca_embedding,
                                          resolution = c(0.8, 1),
                                          n_neigh = c(5, 7),
                                          n_repetitions = 5,
                                          clustering_method = 1,
                                          graph_type = 2,
                                          object_name = "name_example")

plot_k_resolution_corresp(resolution_result)
```

---

**Description**

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

**Usage**

```r
plot_n_neigh_ecs(nn_ecs_object)
```
Arguments

nn ecs object  An object or a concatenation of objects returned by the ‘get_nn_importance’ method.

Value

A ggplot2 object.

Examples

set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(c(runif(100*10), runif(100*10, min=5, max=6)), nrow = 200)
rownames(expr_matrix) = as.character(1:200)

nn_importance_obj = get_nn_importance(object = expr_matrix,
n_neigh_sequence = c(10,15,20),
n_repetitions = 10,
graph_reduction_type = "PCA",
algorithm = 1,
transpose = FALSE, # the matrix is already observations x features, so we won't transpose it
# the following parameter is used by the irlba function and is not mandatory
nv = 2)
plot_n_neigh_ecs(nn_importance_obj)

plot_n_neigh_k_correspondence

Relationship Between Number of Nearest Neighbors and Number of Clusters

Description

Display the distribution of the number of clusters obtained for each number of neighbors 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.

**Examples**

```r
set.seed(2021)
# create an artificial expression matrix
expr_matrix = matrix(runif(100*10), nrow = 100)
rownames(expr_matrix) = as.character(1:100)

nn_importance_obj = get_nn_importance(object = expr_matrix,
               n_neigh_sequence = c(2,5),
               n_repetitions = 5,
               graph_reduction_type = "PCA",
               algorithm = 1,
               transpose = FALSE, # the matrix is already observations x features, so we won't transpose it
               # the following parameter is used by the irlba function and is not mandatory
               nv = 2)

plot_n_neigh_k_correspondence(nn_importance_obj)
```
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