# **Package**

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Title ScRNA-seq workflow CONCLUS - from CONsensus CLUSters to a meaningful CONCLUSion
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<b>Description</b> CONCLUS is a tool for robust clustering, positive marker features selection and collecting info about marker genes using web scraping from MGI, UniProt and NCBI websites. The tool was developed to distinguish not only cell types from different tissues but also characterize heterogeneity within a tissue or characterize stages of a differentiation process.
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addClusteringManually
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 $add {\tt Clustering Manually} \\ add {\tt Clustering Manually}$ 

### Description

The function replaces the content of the column "clusters" in the colData(sceObject) with the clustering provided in the user table. The function will return the sceObject with cells which intersect with the cells from the input table.

### Usage

```
addClusteringManually(fileName, sceObject, dataDirectory, experimentName,
    columnName = "clusters")
```

### **Arguments**

fileName a file with the clustering solution (for example, from previous CONCLUS runs).

sceObject a SingleCellExperiment object with your experiment.

dataDirectory output directory (supposed to be the same for one experiment during the workflow).

experimentName name of the experiment which appears in filenames (supposed to be the same for one experiment during the workflow).

columnName name of the column with the clusters.

#### Value

A SingleCellExperiment object with the created/renewed column "clusters" in the colData(sceObject).

```
calculateClustersSimilarity
```

Having cells similarity, calculate clusters similarity.

#### **Description**

Having cells similarity, calculate clusters similarity.

### Usage

```
calculateClustersSimilarity(cellsSimilarityMatrix, sceObject,
   clusteringMethod)
```

#### **Arguments**

```
cellsSimilarityMatrix
```

a similarity matrix, one of the results of conclus::clusterCellsInternal() function.

sceObject

a SingleCellExperiment object with your experiment.

clusteringMethod

a clustering methods passed to hclust() function.

#### Value

A list contating the cluster similarity matrix and cluster names (order).

choosePalette

Choose palette for a plot.

#### **Description**

It is an internal function usually applied for choosing the palette for clusters. Depending if the number of clusters is more than 12 or not, one of two built-in palettes will be applied. If you give your vector of colors, the function will not change them. If the number of clusters is more than 26, it will copy colors to get the needed length of the palette.

### Usage

```
choosePalette(colorPalette, clustersNumber)
```

### Arguments

```
colorPalette Either "default" or a vector of colors, for example c("yellow", "#CC79A7"). clustersNumber
```

number of clusters in the output palette.

#### Value

Color palette with the number of colors equal to the clusterNumber parameter.

```
clusterCellsInternal
```

Cluster cells and get similarity matrix of cells.

### **Description**

The function returns consensus clusters by using hierarchical clustering on the similarity matrix of cells. It provides two options: to specify an exact number of clusters (with clusterNumber parameter) or to select the depth of splitting (deepSplit parameter).

### Usage

```
clusterCellsInternal(dbscanMatrix, sceObject, clusterNumber = 0,
  deepSplit, cores = 14, clusteringMethod = "ward.D2")
```

#### **Arguments**

dbscanMatrix an output matrix of conclus::runDBSCAN() function.

sceObject a SingleCellExperiment object with your experiment.

clusterNumber

a parameter, specifying the exact number of cluster.

deepSplit a parameter, specifying how deep we will split the clustering tree. It takes inte-

gers from 1 to 4.

cores maximum number of jobs that CONCLUS can run in parallel.

clusteringMethod

a clustering methods passed to hclust() function.

#### Value

A SingleCellExperiment object with modified/created "clusters" column in the colData, and cells similarity matrix.

```
exportClusteringResults
```

exportClusteringResults

#### **Description**

The function saves clustering results into a table. Row names are cell names in the same order as in the sceObject.

```
exportClusteringResults(sceObject, dataDirectory, experimentName, fileName)
```

exportMatrix 5

#### **Arguments**

sceObject a SingleCellExperiment object with your experiment. dataDirectory

output directory (supposed to be the same for one experiment during the work-flow).

experimentName

name of the experiment which appears at the beginning of the file name (supposed to be the same for one experiment during the workflow).

fileName the rest of output file name.

exportMatrix

Export matrix to a file.

### Description

The function allows you to export a matrix to a .csv file with a hard-coded filename (according to experimentName) in the "dataDirectory/output\_tables" directory for further analysis.

### Usage

```
exportMatrix(matrix, dataDirectory, experimentName, name)
```

#### **Arguments**

```
matrix your matrix (e.g., expression matrix)
dataDirectory
```

CONCLUS output directory for a given experiment (supposed to be the same for one experiment during the workflow).

experimentName

name of the experiment which will appear at the beginning of the filenames (supposed to be the same for one experiment during the workflow).

name

name of the file. Will be placed after the experimentName header.

```
generateTSNECoordinates
```

*Generate and save t-SNE coordinates with selected parameters.* 

### **Description**

The function generates several t-SNE coordinates based on given perplexity and ranges of PCs. Final number of t-SNE plots is length(PCs)\*length(perplexities) It writes coordinates in "dataDirectory/tsnes" subfolder.

```
generateTSNECoordinates(sceObject, dataDirectory, experimentName, randomSeed = 42, cores = 14, PCs = c(4, 6, 8, 10, 20, 40, 50), perplexities = c(30, 40))
```

6 getGenesInfo

#### **Arguments**

sceObject a SingleCellExperiment object with your experiment.

dataDirectory

output directory for CONCLUS (supposed to be the same for one experiment

during the workflow).

experimentName

name of the experiment which will appear in filenames (supposed to be the same

for one experiment during the workflow).

randomSeed random seed for reproducibility.

cores maximum number of jobs that CONCLUS can run in parallel.

PCs a vector of first principal components. For example, to take ranges 1:5 and 1:10

write c(5, 10).

perplexities a vector of perplexity (t-SNE parameter).

#### Value

An object with t-SNE results (coordinates for each plot).

getGenesInfo

Collect genes information to one table.

#### **Description**

The function takes a data frame containing gene symbols and (or) ENSEMBL IDs and returns a data frame with such information as gene name, feature type, chromosome, gene IDs in different annotations, knockout information from MGI, a summary from NCBI and UniProt, and whether or not a gene belongs to GO terms containing proteins on the cell surface or involved in secretion.

### Usage

```
getGenesInfo(genes, databaseDir = system.file("extdata", package =
   "conclus"), groupBy = "clusters", orderGenes = "initial",
   getUniprot = TRUE, silent = FALSE, coresGenes = 20)
```

#### **Arguments**

genes

	and (or) ENSEMBL IDs. Other columns are optional. For example, the second
	column could be "clusters" with the name of the cluster for which the gene is a
	marker.
databaseDir	a path to the database provided with CONCLUS called "Mmus_gene_database_secretedMol.tsv".

a data frame with the first column called "geneName" containing gene symbols

groupBy a column in the input table used for grouping the genes in the output tables. This

option is useful if a table contains genes from different clusters.

orderGenes if "initial" then the order of genes will not be changed.

getUniprot boolean, whether to get information from UniProt or not. Default is TRUE.

Sometimes, the connection to the website is not reliable. If you tried a couple of

times and it failed, select FALSE.

silent whether to show messages from intermediate steps or not.

coresGenes maximum number of jobs that the function can run in parallel.

getMarkerGenes 7

#### Value

Returns a data frame.

getMarkerGenes

Get top N marker genes from each cluster.

### **Description**

This function reads results of conclus::rankGenes() from "dataDirectory/marker\_genes" and selects top N markers for each cluster.

### Usage

```
getMarkerGenes(dataDirectory, sceObject, genesNumber = 14,
    experimentName, removeDuplicates = TRUE)
```

#### **Arguments**

dataDirectory

output directory for a run of CONCLUS (supposed to be the same for one ex-

periment during the workflow).

sceObject a SingleCellExperiment object with your experiment.

genesNumber top N number of genes to get from one cluster.

experimentName

name of the experiment which appears in filenames (supposed to be the same

for one experiment during the workflow).

removeDuplicates

boolean, if duplicated genes must be deleted or not.

### Value

A data frame where the first columns are marker genes ("geneName") and the second column is the groups ("clusters").

initialisePath

Create all needed directories for CONCLUS output.

#### **Description**

Create all needed directories for CONCLUS output.

### Usage

```
initialisePath(dataDirectory)
```

### **Arguments**

```
dataDirectory
```

output directory for a given CONCLUS run (supposed to be the same for one experiment during the workflow).

8 normaliseCountMatrix

```
normaliseCountMatrix

normaliseCountMatrix
```

#### **Description**

Create a SingleCellExperiment object and perform normalization. The same as conclus::normalizeCountMatrix.

#### Usage

```
normaliseCountMatrix(countMatrix, species, method = "default",
    sizes = c(20, 40, 60, 80, 100), rowData = NULL, colData = NULL,
    alreadyCellFiltered = FALSE, runQuickCluster = TRUE,
    databaseDir = system.file("extdata", package = "conclus"))
```

### Arguments

countMatrix a matrix with non-normalised gene expression.

species either 'mmu' or 'human'.

method a method of clustering: available option is "default" using scran and scater.

sizes a vector of size factors from scran::computeSumFactors() function.

rowData a data frame with information about genes
colData a data frame with information about cells

alreadyCellFiltered

if TRUE, cells quality check and filtering will not be applied. However, the func-

tion may delete some cells if they have negative size factors after scran::computeSumFactors.

runQuickCluster

if scran::quickCluster() function must be applied. Usually, it allows to improve normalization for medium-size count matrices. However, it is not recommended for datasets with less than 200 cells and may take too long for datasets with more

than 10000 cells.

databaseDir

a path to annotation database provided with CONCLUS called "Mmus\_gene\_database\_secretedMol.to (only for MusMusculus 'mmu'). The function will work also without the database

but slower because it will retrieve genes info from biomaRt.

### Value

A SingleCellExperiment object with normalized gene expression, colData, and rowData.

plotCellHeatmap 9

|--|--|

#### **Description**

This function plots heatmap with marker genes on rows and clustered cells on columns.

### Usage

```
plotCellHeatmap(markersClusters, sceObject, dataDirectory, experimentName,
  fileName, meanCentered = TRUE, colorPalette = "default",
  statePalette = "default", clusteringMethod = "ward.D2",
  orderClusters = FALSE, orderGenes = FALSE, returnPlot = FALSE,
  saveHeatmapTable = FALSE, width = 10, height = 8.5, ...)
```

### **Arguments**

markersClusters

a data frame where the first column is "geneName" containing genes names from sceObject, and the second column is corresponding "clusters". All names from that column must come from the column "clusters" in the colData(sceObject). The data frame can be obtained from conclus::getMarkerGenes() function or created manually.

 $\verb|sceObject| a SingleCellExperiment| object with your experiment. \\ \verb|dataDirectory|$ |

output directory of a given CONCLUS run (supposed to be the same for one experiment during the workflow).

experimentName

name of the experiment which appears in filenames (supposed to be the same for one experiment during the workflow).

fileName name of the ouput file

meanCentered boolean, should mean centering be applied to the expression data or not.

colorPalette "default" or a vector of colors for the column "clusters" in the colData, for example c("yellow", "#CC79A7").

statePalette "default" or a vector of colors for the column "state" in the colData, for example c("yellow", "#CC79A7").

clusteringMethod

a clustering methods passed to hclust() function.

orderClusters

boolean, should the heatmap be structured by clusters.

orderGenes boolean, should the heatmap be structured by genes.

returnPlot boolean, whether to return a ggplot object with the plot or not.

saveHeatmapTable

boolean, whether to save the expression matrix used for heatmap into a .csv file or not. The file will be saved into 'dataDirectory/output\_tables' with the same name as the .pdf plot.

width plot width. height plot height.

... other parameters from pdf() and pheatmap() functions.

10 plotCellSimilarity

#### Value

A ggplot object of the plot if needed. The function saves pdf in "dataDirectiry/pictures" folder.

```
plotCellSimilarity Save a cells similarity matrix.
```

#### **Description**

This function plots similarity matrix as a heatmap, so one can see similarity between parts of different clusters.

### Usage

```
plotCellSimilarity(sceObject, cellsSimilarityMatrix, dataDirectory,
   experimentName, colorPalette = "default", statePalette = "default",
   clusteringMethod = "ward.D2", orderClusters = FALSE,
   plotPDF = TRUE, returnPlot = FALSE, width = 7, height = 6, ...)
```

### **Arguments**

. . .

a clustering methods passed to hclust() function.

orderClusters

boolean, order clusters or not.

plotPDF if TRUE export to pdf, if FALSE export to png. FALSE is recommended for

datasets with more than 2500 cells due to large pdf file size.

returnPlot boolean, return plot or not. Default if FALSE.

width plot width. height plot height.

... other parameters of pdf(), pheatmap() and png() functions.

#### Value

A ggplot object or nothing (depends on the returnPlot parameter). It saves the pdf in "dataDirectory/pictures" folder.

plotClusteredTSNE 11

```
plotClusteredTSNE Plot t-SNE. Additionally, it can highlight clusters or states.
```

#### **Description**

Plot t-SNE. Additionally, it can highlight clusters or states.

#### Usage

```
plotClusteredTSNE(sceObject, dataDirectory, experimentName,
  tSNEresExp = "", colorPalette = "default", PCs = c(4, 6, 8, 10, 20,
  40, 50), perplexities = c(30, 40), columnName = "clusters",
  returnPlot = FALSE, width = 6, height = 5, ...)
```

### Arguments

```
sceObject
                  a SingleCellExperiment object with your experiment.
dataDirectory
                  output directory for CONCLUS (supposed to be the same for one experiment
                  during the workflow).
experimentName
                  name of the experiment which will appear in filenames (supposed to be the same
                  for one experiment during the workflow).
                  if t-SNE coordinates were generated in a different CONCLUS run, you can use
tSNEresExp
                  them without renaming the files. Please copy tsnes folder from the source run to
                  the current one and write that experimentName in the tSNEresExp argument.
colorPalette "default" or a vector of colors for the column "clusters" in the colData, for ex-
                  ample c("yellow", "#CC79A7").
PCs
                  vector of PCs (will be specified in filenames).
perplexities vector of perplexities (will be specified in filenames).
                  name of the column to plot on t-SNE dimensions.
columnName
returnPlot
                  boolean, return plot or not.
width
                  plot width.
height
                  plot height.
                  other arguments of the pdf() function.
```

#### Value

A ggplot object or nothing (depends on the returnPlot parameter).

plotClustersSimilarity

```
plotClustersSimilarity
```

Save a similarity cluster matrix.

#### **Description**

Save a similarity cluster matrix.

### Usage

```
plotClustersSimilarity(clustersSimilarityMatrix, sceObject, dataDirectory,
   experimentName, colorPalette, statePalette, clusteringMethod,
   returnPlot = FALSE, width = 7, height = 5.5, ...)
```

### **Arguments**

```
clustersSimilarityMatrix
```

a matrix, result of conclus::calculateClustersSimilarity() function.

sceObject a SingleCellExperiment object with your experiment.

dataDirectory

output directory for CONCLUS (supposed to be the same for one experiment during the workflow).

experimentName

name of the experiment which will appear in filenames (supposed to be the same for one experiment during the workflow).

colorPalette "default" or a vector of colors for the column "clusters" in the colData, for example c("yellow", "#CC79A7").

statePalette "default" or a vector of colors for the column "state" in the colData, for example c("yellow", "#CC79A7").

clusteringMethod

a clustering methods passed to hclust() function.

returnPlot boolean, return plot or not.

width plot width.
height plot height.

... other parameters of pdf() and pheatmap() functions.

### Value

A ggplot object or nothing (depends on returnPlot parameter). It saves the pdf in "dataDirectory/pictures" folder.

plotGeneExpression 13

```
plotGeneExpression plotGeneExpression
```

### **Description**

The function saves a t-SNE plot colored by expression of a given gene. Warning: filename with t-SNE results is hardcoded, so please don't rename the output file.

#### Usage

```
plotGeneExpression(geneName, experimentName, dataDirectory,
  graphsDirectory = "pictures", sceObject, tSNEpicture = 1,
  commentName = "", palette = c("grey", "red", "#7a0f09", "black"),
  returnPlot = FALSE, savePlot = TRUE, alpha = 1, limits = NA,
  pointSize = 1, width = 6, height = 5, ...)
```

### Arguments

geneName name of the gene you want to plot.

experimentName

name of the experiment which appears in filenames (supposed to be the same

for one experiment during the workflow).

dataDirectory

output directory for CONCLUS (supposed to be the same for one experiment during the workflow).

graphsDirectory

name of the subdirectory where to put graphs. Default is "dataDirectory/pictures".

sceObject a SingleCellExperiment object with your experiment.

tSNEpicture number of the picture you want to use for plotting. Please check "dataDirec-

tory/tsnes" or "dataDirectory/pictures/tSNE\_pictures/clusters" to get the num-

ber, it is usually from 1 to 14.

comment Name comment you want to specify in the filename.

palette color palette for the legend.

returnPlot boolean, should the function return a ggplot object or not. savePlot boolean, should the function export the plot to pdf or not.

alpha opacity of the points of the plot.

limits range of the gene expression shown in the legend. This option allows generating

t-SNE plots with equal color scale to compare the expression of different genes.

By default, limits are the range of expression of a selected gene.

pointSize size of the point.
width plot width.
height plot height.

... other parameters of the pdf() function.

### Value

A ggplot object of the plot if needed.

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rankGenes

Rank marker genes by statistical significance.

### **Description**

This function searches marker genes for each cluster. It saves tables in the "dataDirectory/marker\_genes" directory, one table per cluster.

### Usage

```
rankGenes (sceObject, clustersSimilarityMatrix, dataDirectory,
  experimentName, column = "clusters")
```

#### Arguments

```
sceObject
                 a SingleCellExperiment object with your experiment.
clustersSimilarityMatrix
                 matrix, result of conclus::calculateClustersSimilarity() function.
dataDirectory
                 output directory for CONCLUS (supposed to be the same for one experiment
                 during the workflow).
experimentName
                 name of the experiment which will appear in filenames (supposed to be the same
                 for one experiment during the workflow).
                 name of the column with a clustering result.
```

runClustering

column

DBSCAN clustering on t-SNE results.

#### **Description**

This function provides consensus DBSCAN clustering based on the results of t-SNE. You can tune algorithm parameters in options to get the number of clusters you want.

```
runClustering(tSNEResults, sceObject, dataDirectory, experimentName,
  epsilon = c(1.3, 1.4, 1.5), minPoints = c(3, 4), k = 0,
  deepSplit = 4, clusteringMethod = "ward.D2", cores = 14,
  deleteOutliers = TRUE, PCs = c(4, 6, 8, 10, 20, 40, 50),
 perplexities = c(30, 40), randomSeed = 42)
```

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#### **Arguments**

```
the result of conclus::generateTSNECoordinates() function.
tSNEResults
                  a SingleCellExperiment object with your experiment.
sceObject
dataDirectory
                  output directory of a given CONCLUS run (supposed to be the same for one
                  experiment during the workflow).
experimentName
                  name of the experiment which appears in filenames (supposed to be the same
                  for one experiment during the workflow).
                  a parameter of fpc::dbscan() function.
epsilon
minPoints
                  a parameter of fpc::dbscan() function.
                  preferred number of clusters. Alternative to deepSplit.
                  intuitive level of clustering depth. Options are 1, 2, 3, 4.
deepSplit
clusteringMethod
                  a clustering methods passed to hclust() function.
                  maximum number of jobs that CONCLUS can run in parallel.
deleteOutliers
                  Whether cells which were often defined as outliers by dbscan must be deleted.
                  It will require recalculating of the similarity matrix of cells. Default is FALSE.
                  Usually those cells appear in an "outlier" cluster and can be easier distinguished
                  and deleted later if necessary.
                  a vector of first principal components. For example, to take ranges 1:5 and 1:10
PCs
                  write c(5, 10).
perplexities a vector of perplexity for t-SNE.
```

#### Value

randomSeed

A list containing filtered from outliers SingleCellExperiment object and cells similarity matrix.

random seed for reproducibility.

runCONCLUS Run CONCLUS in one click

#### **Description**

This function performs core CONCLUS workflow. It generates PCA and t-SNE coordinates, runs DBSCAN, calculates similarity matrices of cells and clusters, assigns cells to clusters, searches for positive markers for each cluster. The function saves plots and tables into dataDirectory.

```
runCONCLUS(sceObject, dataDirectory, experimentName,
  colorPalette = "default", statePalette = "default",
  clusteringMethod = "ward.D2", epsilon = c(1.3, 1.4, 1.5),
  minPoints = c(3, 4), k = 0, PCs = c(4, 6, 8, 10, 20, 40, 50),
  perplexities = c(30, 40), randomSeed = 42, deepSplit = 4,
  preClustered = F, orderClusters = FALSE, cores = 14,
  plotPDFcellSim = TRUE, deleteOutliers = TRUE,
  tSNEalreadyGenerated = FALSE, tSNEresExp = "")
```

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#### **Arguments**

sceObject a SingleCellExperiment object with your data.

dataDirectory

CONCLUS will create this directory if it doesn't exist and store there all output files.

experimentName

most of output file names of CONCLUS are hardcoded. experimentName will stay at the beginning of each output file name to distinguish different runs easily.

colorPalette a vector of colors for clusters.

statePalette a vector of colors for states.

clusteringMethod

a clustering methods passed to hclust() function.

a parameter of fpc::dbscan() function. epsilon a parameter of fpc::dbscan() function. minPoints

k preferred number of clusters. Alternative to deepSplit. A parameter of cutree()

function.

PCs a vector of first principal components. For example, to take ranges 1:5 and 1:10

write c(5, 10).

perplexities a vector of perplexity for t-SNE. random seed for reproducibility. randomSeed

deepSplit intuitive level of clustering depth. Options are 1, 2, 3, 4.

preClustered if TRUE, it will not change the column clusters after the run. However, it will

anyway run DBSCAN to calculate similarity matrices.

orderClusters

can be either FALSE (default) of "name". If "name", clusters in the similarity

matrix of cells will be ordered by name.

maximum number of jobs that CONCLUS can run in parallel. cores

plotPDFcellSim

if FALSE, the similarity matrix of cells will be saved in png format. FALSE is recommended for count matrices with more than 2500 cells due to large pdf file

deleteOutliers

whether cells which were often defined as outliers by dbscan must be deleted. It will require recalculating of the similarity matrix of cells. Default is FALSE. Usually those cells form a separate "outlier" cluster and can be easier distinguished and deleted later if necessary.

tSNEalreadyGenerated

if you already ran CONCLUS ones and have t-SNE coordinated saved You can set TRUE to run the function faster since it will skip the generation of t-SNE coordinates and use the stored ones. Option TRUE requires t-SNE coordinates to be located in your 'dataDirectory/tsnes' directory.

experimentName of t-SNE coordinates which you want to use. This argument tSNEresExp

allows copying and pasting t-SNE coordinates between different CONCLUS runs without renaming the files.

#### Value

A SingleCellExperiment object.

runDBSCAN 17

runDBSCAN

Run clustering iterations with selected parameters using DBSCAN.

#### **Description**

This function returns a matrix of clustering iterations of DBSCAN.

#### Usage

```
runDBSCAN(tSNEResults, sceObject, dataDirectory, experimentName, cores = 14, epsilon = c(1.3, 1.4, 1.5), minPoints = c(3, 4))
```

#### Arguments

### Value

A matrix of DBSCAN results.

saveGenesInfo

Save gene information into a table or tables for multiple inputs.

### **Description**

This function runs conclus::getGenesInfo() function for all tables into the inputDir and saves the result into the outputDir.

```
saveGenesInfo(dataDirectory = "", inputDir = "", outputDir = "",
pattern = "", databaseDir = system.file("extdata", package =
"conclus"), sep = ";", header = TRUE, startFromFile = 1,
groupBy = "clusters", orderGenes = "initial", getUniprot = TRUE,
silent = FALSE, coresGenes = 20)
```

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#### **Arguments**

dataDirectory

a directory with CONCLUS output. You can specify either dataDirectory, then inputDir and outputDir will be hardcoded, or inputDir and outputDir only. The first is recommended during running CONCLUS workflow when the second option is comfortable when you created input tables with genes manually.

input Dir input directory containing text files. These files can be obtained by applying

conclus::saveMarkersLists() function or created manually. Each file must be a data frame with the first column called "geneName" containing gene symbols

and (or) ENSEMBL IDs.

outputDir output directory.

pattern a pattern of file names to take.

databaseDir a path to the database "Mmus\_gene\_database\_secretedMol.tsv". It is provided

with the conclus package.

sep a parameter of read.delim() function.

header whether or not your input files have a header.

startFromFile

number of the input file to start with. The function approaches files one by one. It uses web scraping method to collect publicly available info from MGI, NCBI and UniProt websites. Sometimes, if the Internet connection is not reliable, the function can drop. In this case, it is comfortable to start from the failed file and

not to redo the previous ones.

groupBy a column in the input table used for grouping the genes in the output tables.

orderGenes if "initial" then the order of genes will not be changed.

getUniprot boolean, whether to get information from UniProt or not. Default is TRUE.

Sometimes, the connection to the website is not reliable. If you tried a couple of

times and it failed, select FALSE.

silent whether to show messages from intermediate steps or not.

coresGenes maximum number of jobs that the function can run in parallel.

#### Value

It saves text files either in the 'dataDirectory/marker\_genes/saveGenesInfo' or outputDir depending on whether you specify dataDirectory or (inpitDir and outputDir) explicitly.

saveMarkersLists Save top N marker genes for each cluster into a format suitable for conclus::saveGenesInfo() function.

### **Description**

The function takes the output files of conclus::rankGenes(), extracts top N markers and saves them into the first "geneName" column of the output table. The second column "clusters" contains the name of the corresponding cluster.

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#### Usage

```
saveMarkersLists(experimentName, dataDirectory,
  inputDir = file.path(dataDirectory, "marker_genes"),
  outputDir = file.path(dataDirectory,
  paste0("marker_genes/markers_lists")), pattern = "genes.tsv",
  Ntop = 100)
```

#### **Arguments**

experimentName

name of the experiment which appears at the beginning of the file name (supposed to be the same for one experiment during the workflow).

dataDirectory

experiment directory (supposed to be the same for one experiment during the

workflow).

input Dir input directory, usually "marker\_genes" created automatically after conclus::runCONCLUS().

outputDir output directory.

pattern a pattern of the input file names to take.

Ntop number of top markers to take from each cluster.

#### Value

It saves files into the outputDir. The number of files is equal to the number of clusters.

testClustering  $\it To~check~one~iteration~of~clustering~before~running~full~workflow~CON-CLUS.$ 

### **Description**

This function generates a single clustering iteration of CONCLUS to check whether chosen parameters for dbscan are suitable for your data.

#### Usage

```
testClustering(sceObject, dataDirectory, experimentName,
  dbscanEpsilon = 1.4, minPts = 5, perplexities = c(30),
  PCs = c(4), randomSeed = 42, width = 7, height = 7, ...)
```

#### **Arguments**

```
sceObject a SingleCellExperiment object with your experiment.
```

dataDirectory

output directory (supposed to be the same for one experiment during the work-flow)

experimentName

name of the experiment which will appear in filenames (supposed to be the same for one experiment during the workflow).

dbscanEpsilon

a parameter of fpc::dbscan() function.

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minPts a parameter of fpc::dbscan() function.
perplexities vector of perplexities (t-SNE parameter).

PCs a vector of PCs for plotting.

randomSeed random seed for reproducibility.

width plot width. height plot height.

... other pdf() arguments.

### Value

t-SNE results, a distance graph plot, a t-SNE plot colored by test clustering solution.

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