# Package 'SuperCell'

September 5, 2024

```
Cells
Version 1.0
Description Aggregates large single-cell data into metacell dataset by merging together gene expres-
      sion of very similar cells. 'SuperCell' uses 'velocyto.R' <doi:10.1038/s41586-018-0414-
      6> <a href="https://github.com/velocyto-team/velocyto.R">https://github.com/velocyto-team/velocyto.R</a> for RNA velocity. We also recom-
      mend installing 'scater' Bioconductor package <doi:10.18129/B9.bioc.scater> <https:
      //bioconductor.org/packages/release/bioc/html/scater.html>.
License GPL-3
BugReports https://github.com/GfellerLab/SuperCell/issues
Encoding UTF-8
LazyData true
LazyDataCompression xz
biocViews Software
Additional_repositories https://mteleman.github.io/drat
Imports igraph, RANN, WeightedCluster, corpcor, weights, Hmisc,
      Matrix, matrixStats, plyr, irlba, grDevices, patchwork,
      ggplot2, umap, entropy, Rtsne, dbscan, scales, plotfunctions,
      proxy, methods, rlang,
RoxygenNote 7.3.2
Suggests SingleCellExperiment, SummarizedExperiment, cowplot, scater,
      Seurat, knitr, rmarkdown, remotes, bluster, velocyto.R,
      testthat (>= 3.0.0)
Depends R (>= 4.0.0)
VignetteBuilder knitr
Config/testthat/edition 3
NeedsCompilation no
Author Mariia Bilous [aut],
      Leonard Herault [cre]
```

Title Simplification of scRNA-Seq Data by Merging Together Similar

Type Package

2 Contents

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anndata\_2\_supercell

Convert Anndata metacell object (Metacell-2 or SEACells) to Supercell like object

#### **Description**

Convert Anndata metacell object (Metacell-2 or SEACells) to Super-cell like object

#### Usage

```
anndata_2_supercell(adata, simplification.algo = "unknown")
```

### **Arguments**

adata

anndata object of metacells (for example, the output of collect\_metacells() for Metacells or the output of SEACells.core.summarize\_by\_SEACell)

Please, \*\*make sure\*\*, adata has 'uns['sc.obs']' field containing observation information of single-cell data, in particular, a column 'membership' (single-cell assignemnt to metacells)

simplification.algo

metacell construction algorithm (i.e., Metacell2 or SEACells)

#### Value

a list of super-cell like object (similar to the output of SCimplify)

build\_knn\_graph

Build kNN graph

### **Description**

Build kNN graph either from distance (from == "dist") or from coordinates (from == "coordinates")

```
build_knn_graph(
   X,
   k = 5,
   from = c("dist", "coordinates"),
   use.nn2 = TRUE,
   return_neighbors_order = FALSE,
   dist_method = "euclidean",
   cor_method = "pearson",
   p = 2,
   directed = FALSE,
```

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```
DoSNN = FALSE,
which.snn = c("bluster", "dbscan"),
pruning = NULL,
kmin = 0,
...
)
```

#### **Arguments**

X either distance or matrix of coordinates (rows are samples and cols are coordi-

nates)

k kNN parameter

from which data type to build kNN network: "dist" if X is a distance (dissim-

ilarity) or "coordinates" if X is a matrix with coordinates as cols and cells as

rows

use.nn2 whether use nn2 method to build kNN network faster (available only for "coor-

dinates" option)

return\_neighbors\_order

whether return order of neighbors (not available for nn2 option)

dist\_method method to compute dist (if X is a matrix of coordinates) available: c("cor",

"euclidean", "maximum", "manhattan", "canberra", "binary", "minkowski")

cor\_method if distance is computed as correlation (dist\_method == "cor), which type of cor-

relation to use (available: "pearson", "kendall", "spearman")

p param in "dist" function

directed whether to build a directed graph

DoSNN whether to apply shared nearest neighbors (default is FALSE)

which.snn whether to use neighborsToSNNGraph or sNN for sNN graph construction

pruning quantile to perform edge pruning (default is NULL - no pruning applied) based

on PCA distance distribution

kmin keep at least kmin edges in single-cell graph when pruning applied (idnored if

is.null(pruning))

... other parameters of neighborsToSNNGraph or sNN

#### Value

a list with components

- graph.knn igraph object
- order Nxk matrix with indices of k nearest neighbors ordered by relevance (from 1st to k-th)

build\_knn\_graph\_nn2 5

build\_knn\_graph\_nn2

Build kNN graph using RANN::nn2 (used in "build\_knn\_graph")

### Description

Build kNN graph using RANN::nn2 (used in "build\_knn\_graph")

### Usage

```
build_knn_graph_nn2(
    X,
    k = min(5, ncol(X)),
    mode = "all",
    DoSNN = FALSE,
    which.snn = c("bluster", "dbscan"),
    pruning = NULL,
    kmin = 0,
    ...
)
```

### Arguments

X	matrix of coordinates (rows are samples and cols are coordinates)
k	kNN parameter
mode	mode of graph_from_adj_list ('all' – undirected graph, 'out' – directed graph)
DoSNN	whether to apply shared nearest neighbors (default is FALSE)
which.snn	whether to use neighborsToSNNGraph or sNN for sNN graph construction
pruning	quantile to perform edge pruning (default is $\ensuremath{NULL}$ - no pruning applied) based on PCA distance distribution
kmin	keep at least kmin edges in single-cell graph when pruning applied (idnored if $is.null(pruning)$ )
	other parameters of neighborsToSNNGraph or sNN

#### Value

a list with components

• graph.knn - igraph object

knn\_graph\_from\_dist

cell\_lines

Cancer cell lines dataset

### Description

ScRNA-seq data of 5 cancer cell lines from [Tian et al., 2019](https://doi.org/10.1038/s41592-019-0425-8).

### Usage

cell\_lines

#### **Format**

A list with gene expression (i.e., log-normalized counts) (GE), and metadata data (meta):

**GE** gene expression (log-normalized counts) matrix **meta** cells metadata (cell line annotation)

#### **Details**

Data available at authors' [GitHub](https://github.com/LuyiTian/sc\_mixology/blob/master/data/) under file name \*sincell\_with\_class\_5cl.Rdata\*.

### Source

doi:10.1038/s4159201904258

knn\_graph\_from\_dist

Build kNN graph from distance (used in "build\_knn\_graph")

### Description

Build kNN graph from distance (used in "build\_knn\_graph")

### Usage

```
knn_graph_from_dist(D, k = 5, return_neighbors_order = TRUE, mode = "all")
```

#### Arguments

D dist matrix or dist object (preferentially)

k kNN parameter

return\_neighbors\_order

whether return order of neighbors (not available for nn2 option)

mode mode of graph\_from\_adj\_list ('all' – undirected graph, 'out' – directed graph)

#### Value

- a list with components
  - graph.knn igraph object
  - order Nxk matrix with indices of k nearest neighbors ordered by relevance (from 1st to k-th)

```
metacell2_anndata_2_supercell
```

Convert Metacells (Metacell-2) to Super-cell like object

### **Description**

Convert Metacells (Metacell-2) to Super-cell like object

### Usage

```
metacell2_anndata_2_supercell(adata, obs.sc)
```

#### **Arguments**

adata anndata object of metacells (the output of collect\_metacells())

obs.sc a dataframe of the single-cell anndata object used to compute metacells (anndata after applying divide\_and\_conquer\_pipeline() function)

### Value

a list of super-cell like object (similar to the output of SCimplify)

SCimplify

Detection of metacells with the SuperCell approach

#### **Description**

This function detects metacells (former super-cells) from single-cell gene expression matrix

```
SCimplify(
   X,
   genes.use = NULL,
   genes.exclude = NULL,
   cell.annotation = NULL,
   cell.split.condition = NULL,
   n.var.genes = min(1000, nrow(X)),
   gamma = 10,
```

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```
k.knn = 5,
do.scale = TRUE,
n.pc = 10,
fast.pca = TRUE,
do.approx = FALSE,
approx.N = 20000,
block.size = 10000,
seed = 12345,
igraph.clustering = c("walktrap", "louvain"),
return.singlecell.NW = TRUE,
return.hierarchical.structure = TRUE,
...
)
```

#### **Arguments**

X log-normalized gene expression matrix with rows to be genes and cols to be

cells

genes.use a vector of genes used to compute PCA

genes.exclude a vector of genes to be excluded when computing PCA

cell.annotation

a vector of cell type annotation, if provided, metacells that contain single cells of different cell type annotation will be split in multiple pure metacell (may result in slightly larger numbe of metacells than expected with a given gamma)

cell.split.condition

a vector of cell conditions that must not be mixed in one metacell. If provided, metacells will be split in condition-pure metacell (may result in significantly(!)

larger number of metacells than expected)

n.var.genes if "genes.use" is not provided, "n.var.genes" genes with the largest variation

are used

gamma graining level of data (proportion of number of single cells in the initial dataset

to the number of metacells in the final dataset)

k.knn parameter to compute single-cell kNN network

do.scale whether to scale gene expression matrix when computing PCA

n.pc number of principal components to use for construction of single-cell kNN net-

work

fast.pca use irlba as a faster version of prcomp (one used in Seurat package)
do.approx compute approximate kNN in case of a large dataset (>50'000)
approx.N number of cells to subsample for an approximate approach

block.size number of cells to map to the nearest metacell at the time (for approx coarse-

graining)

seed seed to use to subsample cells for an approximate approach

igraph.clustering

clustering method to identify metacells (available methods "walktrap" (default)

and "louvain" (not recommended, gamma is ignored)).

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```
return.singlecell.NW

whether return single-cell network (which consists of approx.N if "do.approx" or all cells otherwise)

return.hierarchical.structure

whether return hierarchical structure of metacell
```

other parameters of build\_knn\_graph function

#### Value

a list with components

- graph.supercells igraph object of a simplified network (number of nodes corresponds to number of metacells)
- membership assignment of each single cell to a particular metacell
- graph.singlecells igraph object (kNN network) of single-cell data
- supercell\_size size of metacells (former super-cells)
- gamma requested graining level
- N.SC number of obtained metacells
- genes.use used genes
- do.approx whether approximate coarse-graining was perfirmed
- n.pc number of principal components used for metacells construction
- k.knn number of neighbors to build single-cell graph
- sc.cell.annotation. single-cell cell type annotation (if provided)
- sc.cell.split.condition. single-cell split condition (if provided)
- SC.cell.annotation. super-cell cell type annotation (if was provided for single cells)
- SC.cell.split.condition. super-cell split condition (if was provided for single cells)

### **Examples**

```
SCimplify_for_velocity
```

Construct super-cells from spliced and un-spliced matrices

#### **Description**

Construct super-cells from spliced and un-spliced matrices

### Usage

```
SCimplify_for_velocity(emat, nmat, gamma = NULL, membership = NULL, ...)
```

### **Arguments**

emat spliced (exonic) count matrix

nmat unspliced (nascent) count matrix

gamma graining level of data (proportion of number of single cells in the initial dataset to the number of super-cells in the final dataset)

membership metacell membership vector (if provided, will be used for emat, nmat metacell matrices averaging)

... other parameters from SCimplify

#### Value

list containing vector of membership, spliced count and un-spliced count matrices

```
SCimplify_from_embedding
```

Detection of metacells with the SuperCell approach from low dim representation

#### **Description**

This function detects metacells (former super-cells) from single-cell gene expression matrix

```
SCimplify_from_embedding(
   X,
   cell.annotation = NULL,
   cell.split.condition = NULL,
   gamma = 10,
   k.knn = 5,
   n.pc = 10,
```

```
do.approx = FALSE,
approx.N = 20000,
block.size = 10000,
seed = 12345,
igraph.clustering = c("walktrap", "louvain"),
return.singlecell.NW = TRUE,
return.hierarchical.structure = TRUE,
...
)
```

#### **Arguments**

X low dimensional embedding matrix with rows to be cells and cols to be low-dim components

cell.annotation

a vector of cell type annotation, if provided, metacells that contain single cells of different cell type annotation will be split in multiple pure metacell (may result in slightly larger numbe of metacells than expected with a given gamma)

cell.split.condition

a vector of cell conditions that must not be mixed in one metacell. If provided, metacells will be split in condition-pure metacell (may result in significantly(!)

larger number of metacells than expected)

gamma graining level of data (proportion of number of single cells in the initial dataset

to the number of metacells in the final dataset)

k.knn parameter to compute single-cell kNN network

n.pc number of principal components to use for construction of single-cell kNN net-

work

do.approx compute approximate kNN in case of a large dataset (>50'000)

approx.N number of cells to subsample for an approximate approach

block.size number of cells to map to the nearest metacell at the time (for approx coarse-

graining)

seed seed to use to subsample cells for an approximate approach

igraph.clustering

clustering method to identify metacells (available methods "walktrap" (default)

and "louvain" (not recommended, gamma is ignored)).

return.singlecell.NW

whether return single-cell network (which consists of approx.N if "do.approx" or all cells otherwise)

return.hierarchical.structure

whether return hierarchical structure of metacell

... other parameters of build\_knn\_graph function

#### Value

a list with components

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• graph.supercells - igraph object of a simplified network (number of nodes corresponds to number of metacells)

- membership assignment of each single cell to a particular metacell
- graph.singlecells igraph object (kNN network) of single-cell data
- supercell\_size size of metacells (former super-cells)
- · gamma requested graining level
- N.SC number of obtained metacells
- genes.use used genes (NA due to low-dim representation)
- do.approx whether approximate coarse-graining was perfirmed
- n.pc number of principal components used for metacells construction
- k.knn number of neighbors to build single-cell graph
- sc.cell.annotation. single-cell cell type annotation (if provided)
- sc.cell.split.condition. single-cell split condition (if provided)
- SC.cell.annotation. super-cell cell type annotation (if was provided for single cells)
- SC.cell.split.condition. super-cell split condition (if was provided for single cells)

sc\_mixing\_score

Compute mixing of single-cells within supercell

#### **Description**

Compute mixing of single-cells within supercell

#### Usage

```
sc_mixing_score(SC, clusters)
```

### **Arguments**

sc super-cell object (output of SCimplify function)

clusters vector of clustering assignment (reference assignment)

### Value

a vector of single-cell mixing within super-cell it belongs to, which is defined as: 1 - proportion of cells of the same annotation (e.g., cell type) within the same super-cell With 0 meaning that super-cell consists of single cells from one cluster (reference assignment) and higher values correspond to higher cell type mixing within super-cell

supercell\_2\_sce 13

CII	perc	11م	2	SCA
Su	perc	$e_{TT}$		SCC

Super-cells to SingleCellExperiment object

### Description

This function transforms super-cell gene expression and super-cell partition into SingleCellExperiment object

### Usage

```
supercell_2_sce(
   SC.GE,
   SC,
   fields = c(),
   var.genes = NULL,
   do.preproc = TRUE,
   is.log.normalized = TRUE,
   do.center = TRUE,
   do.scale = TRUE,
   ncomponents = 50
)
```

### **Arguments**

	SC.GE	gene expression matrix with genes as rows and cells as columns
	SC	super-cell (output of SCimplify function)
	fields	which fields of SC to use as cell metadata
	var.genes	set of genes used as a set of variable features of SingleCellExperiment (by default is the set of genes used to generate super-cells)
	do.preproc	whether to do prepocessing, including data normalization, scaling, HVG, PCA, nearest neighbors, TRUE by default, change to FALSE to speed up conversion
is.log.normalized		
		whether SC.GE is log-normalized counts. If yes, then SingleCellExperiment field assay name = 'logcounts' else assay name = 'counts' $$
	do.center	whether to center gene expression matrix to compute PCA
	do.scale	whether to scale gene expression matrix to compute PCA
	ncomponents	number of principal components to compute

#### Value

SingleCellExperiment object

supercell\_2\_Seurat

### **Examples**

supercell\_2\_Seurat Super-cells to Seurat object

#### **Description**

This function transforms super-cell gene expression and super-cell partition into Seurat object

### Usage

```
supercell_2_Seurat(
   SC.GE,
   SC,
   fields = c(),
   var.genes = NULL,
   do.preproc = TRUE,
   is.log.normalized = TRUE,
   do.center = TRUE,
   do.scale = TRUE,
   N.comp = NULL,
   output.assay.version = "v4"
)
```

### Arguments

SC.GE	gene expression matrix with genes as rows and cells as columns	
SC	super-cell (output of SCimplify function)	
fields	which fields of SC to use as cell metadata	
var.genes	set of genes used as a set of variable features of Seurat (by default is the set of genes used to generate super-cells), ignored if !do.preproc	
do.preproc	whether to do prepocessing, including data normalization, scaling, HVG, PCA, nearest neighbors, TRUE by default, change to FALSE to speed up conversion	
is.log.normalized		
	whether SC.GE is log-normalized counts. If yes, then Seurat field data is replaced with counts after normalization (see 'Details' section), ignored if !do.preproc	
do.center	whether to center gene expression matrix to compute PCA, ignored if !do.preproc	
do.scale	whether to scale gene expression matrix to compute PCA, ignored if !do.preproc	

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```
N. comp number of principal components to use for construction of single-cell kNN network, ignored if !do.preproc output.assay.version version of the seurat assay in output, "v4" by default, "v5" requires Seurat v5 installed
```

#### **Details**

Since the input of CreateSeuratObject should be unnormalized count matrix (UMIs or TPMs, see CreateSeuratObject). Thus, we manually set field `assays\$RNA@data` to SC.GE if is.log.normalized == TRUE. Avoid running NormalizeData for the obtained Seurat object, otherwise this will overwrite field `assays\$RNA@data`. If you have run NormalizeData, then make sure to replace `assays\$RNA@data` with correct matrix by running `your\_seurat@assays\$RNA@data <- your\_seurat@assays\$RNA@counts`.

Since super-cells have different size (consist of different number of single cells), we use sample-weighted algorithms for all possible steps of the downstream analysis, including scaling and dimensionality reduction. Thus, generated Seurat object comes with the results of sample-wighted scaling (available as `your\_seurat@assays\$RNA@scale.data` or `your\_seurat@assays\$RNA@misc[["scale.data.weighted" to reproduce if the first one has been overwritten) and PCA (available as `your\_seurat@reductions\$pca` or `your\_seurat@reductions\$pca\_weighted` to reproduce if the first one has been overwritten).

#### Value

Seurat object

#### **Examples**

supercell\_assign

Assign super-cells to the most aboundant cluster

#### **Description**

Assign super-cells to the most aboundant cluster

```
supercell_assign(
  clusters,
  supercell_membership,
  method = c("jaccard", "relative", "absolute")
)
```

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### **Arguments**

```
clusters a vector of clustering assignment supercell_membership
```

a vector of assignment of single-cell data to super-cells (membership field of SCimplify function output)

method

method to define the most abuldant cell cluster within super-cells. Available: "jaccard" (default), "relative", "absolute".

- jaccard assignes super-cell to cluster with the maximum jaccard coefficient (recommended)
- relative assignes super-cell to cluster with the maximum relative abundance (normalized by cluster size), may result in assignment of super-cells to poorly represented (small) cluser due to normalizetaion
- absolute assignes super-cell to cluster with the maximum absolute abundance within super-cell, may result in disappearence of poorly represented (small) clusters

#### Value

a vector of super-cell assignment to clusters

supercell\_cluster

Cluster super-cell data

### Description

Cluster super-cell data

#### Usage

```
supercell_cluster(
   D,
   k = 5,
   supercell_size = NULL,
   algorithm = c("hclust", "PAM"),
   method = NULL,
   return.hcl = TRUE
)
```

### **Arguments**

D a dissimilarity matrix or a dist object

k number of clusters

supercell\_size a vector with supercell size (ordered the same way as in D)

algorithm which algorithm to use to compute clustering: "hclust" (default) or "PAM" (see

wcKMedoids)

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```
    which method of algorithm to use:
    for "hclust": "ward.D", "ward.D2" (default), "single", "complete", "average", "mcquitty", "median" or "centroid", (see hclust)
    for "PAM": "KMedoids", "PAM" or "PAMonce" (default), (see wcKMedoids)
    return.hcl

whether to return a result of "hclust" (only for "hclust" algorithm)
```

#### Value

a list with components

- clustering vector of clustering assignment of super-cells
- algo the algorithm used
- method method used with an algorithm
- hlc hclust result (only for "hclust" algorithm when return. hcl is TRUE)

supercell\_DimPlot

Plot metacell 2D plot (PCA, UMAP, tSNE etc)

### Description

Plots 2d representation of metacells

#### Usage

```
supercell_DimPlot(
   SC,
   groups = NULL,
   dim.name = "PCA",
   dim.1 = 1,
   dim.2 = 2,
   color.use = NULL,
   asp = 1,
   alpha = 0.7,
   title = NULL,
   do.sqtr.rescale = FALSE
)
```

### Arguments

SC	SuperCell computed metacell object (the output of SCimplify)	
groups	an assignment of metacells to any group (for ploting in different colors)	
dim.name	name of the dimensionality reduction to plot (must be a field in SC)	
dim.1	dimension to plot on X-axis	
dim.2	dimension to plot on Y-axis	

```
color.use colros to use for groups, if NULL, an automatic palette of colors will be applied asp aspect ratio
alpha a rotation of the layout (either provided or computed)
title a title of a plot
do.sqtr.rescale
whether to sqrt-scale node size (to balance plot if some metacells are large and covers smaller metacells)
```

### Value

ggplot

```
supercell_estimate_velocity

Run RNAvelocity for super-cells (slightly modified from https:
//github.com/velocyto-team/velocyto.R) Not yet adjusted for
super-cell size (not sample-weighted)
```

#### **Description**

Run RNAvelocity for super-cells (slightly modified from <a href="https://github.com/velocyto-team/velocyto-team/velocyto-R">https://github.com/velocyto-team/velocyto-team/velocyto-team/velocyto-R</a>) Not yet adjusted for super-cell size (not sample-weighted)

#### Usage

```
supercell_estimate_velocity(
  emat,
  nmat,
  smat = NULL,
  membership = NULL,
  supercell_size = NULL,
  do.run.avegaring = (ncol(emat) == length(membership)),
  kCells = 10,
  ...
)
```

#### Arguments

#### Value

results of https://github.com/velocyto-team/velocyto.R plus metacell size vector

```
supercell_FindAllMarkers
```

Differential expression analysis of supep-cell data. Most of the parameters are the same as in Seurat FindAllMarkers (for simplicity)

### **Description**

Differential expression analysis of supep-cell data. Most of the parameters are the same as in Seurat FindAllMarkers (for simplicity)

#### Usage

```
supercell_FindAllMarkers(
   ge,
   clusters,
   supercell_size = NULL,
   genes.use = NULL,
   logfc.threshold = 0.25,
   min.expr = 0,
   min.pct = 0.1,
   seed = 12345,
   only.pos = FALSE,
   return.extra.info = FALSE,
   do.bootstrapping = FALSE
)
```

### **Arguments**

```
gene expression matrix for super-cells (rows - genes, cols - super-cells)

clusters a vector with clustering information (ordered the same way as in ge)

supercell_size a vector with supercell size (ordered the same way as in ge)

genes.use set of genes to test. Defeult – all genes in ge

logfc.threshold
```

log fold change threshold for genes to be considered in the further analysis

min.expr minimal expression (default 0)

min.pct remove genes with lower percentage of detection from the set of genes which will be tested

seed random seed to use

only.pos whether to compute only positive (upregulated) markers

return.extra.info

whether to return extra information about test and its statistics. Default is FALSE.

do.bootstrapping

whether to perform bootstrapping when computing standard error and p-value in wtd.t.test

#### Value

list of results of supercell\_FindMarkers

supercell\_FindMarkers Differential expression analysis of supep-cell data. Most of the parameters are the same as in Seurat FindMarkers (for simplicity)

### Description

Differential expression analysis of supep-cell data. Most of the parameters are the same as in Seurat FindMarkers (for simplicity)

```
supercell_FindMarkers(
   ge,
   supercell_size = NULL,
   clusters,
   ident.1,
   ident.2 = NULL,
   genes.use = NULL,
   logfc.threshold = 0.25,
   min.expr = 0,
   min.pct = 0.1,
   seed = 12345,
   only.pos = FALSE,
   return.extra.info = FALSE,
   do.bootstrapping = FALSE
)
```

supercell\_GE 21

### Arguments

ge	gene expression matrix for super-cells (rows - genes, cols - super-cells)	
supercell_size	a vector with supercell size (ordered the same way as in ge)	
clusters	a vector with clustering information (ordered the same way as in ge)	
ident.1	name(s) of cluster for which markers are computed	
ident.2	$name(s) \ of \ clusters \ for \ comparison. \ If \ \ \ NULL \ (defauld), \ then \ all \ the \ other \ clusters \\ used$	
genes.use	set of genes to test. Defeult – all genes in ge	
logfc.threshold		
	log fold change threshold for genes to be considered in the further analysis	
min.expr	minimal expression (default 0)	
min.pct	remove genes with lower percentage of detection from the set of genes which will be tested	
seed	random seed to use	
only.pos	whether to compute only positive (upregulated) markers	
return.extra.info		
	whether to return extra information about test and its statistics. Default is FALSE.	
do.bootstrapping		
	whether to perform bootstrapping when computing standard error and p-value in wtd.t.test	

### Value

a matrix with a test name (t-test), statisctics, adjusted p-values, logFC, percenrage of detection in eacg ident and mean expresiion

supercell\_GE Simplification of scRNA-seq dataset

### Description

This function converts (i.e., averages or sums up) gene-expression matrix of single-cell data into a gene expression matrix of metacells

```
supercell_GE(
   ge,
   groups,
   mode = c("average", "sum"),
   weights = NULL,
   do.median.norm = FALSE
)
```

### **Arguments**

ge	gene expression matrix (or any coordinate matrix) with genes as rows and cells as cols
groups	vector of membership (assignment of single-cell to metacells)
mode	string indicating whether to average or sum up 'ge' within metacells
weights	vector of a cell weight (NULL by default), used for computing average gene expression withing cluster of metaells
do.median.norm	whether to normalize by median value (FALSE by default)

### Value

a matrix of simplified (averaged withing groups) data with ncol equal to number of groups and nrows as in the initial dataset

```
supercell_GeneGenePlot
```

Gene-gene correlation plot

### Description

Plots gene-gene expression and computes their correaltion

```
supercell_GeneGenePlot(
 ge,
  gene_x,
 gene_y,
 supercell_size = NULL,
 clusters = NULL,
  color.use = NULL,
  idents = NULL,
 pt.size = 1,
  alpha = 0.9,
 x.max = NULL,
 y.max = NULL,
  same.x.lims = FALSE,
  same.y.lims = FALSE,
 ncol = NULL,
 combine = TRUE,
  sort.by.corr = TRUE
)
```

### **Arguments**

a gene expression matrix of super-cells (ncol same as number of super-cells) ge gene or vector of genes (if vector, has to be the same lenght as gene\_y) gene\_x gene or vector of genes (if vector, has to be the same lenght as gene\_x) gene\_y supercell\_size a vector with supercell size (ordered the same way as in ge) clusters a vector with clustering information (ordered the same way as in ge) color.use colors for idents idents idents (clusters) to plot (default all) point size (if supercells have identical sizes) pt.size transparency alpha max of x axis x.max y.max max of y axis same.x.lims same x axis for all plots same y axis for all plots same.y.lims ncol number of colums in combined plot combine plots into a single patchworked ggplot object. If FALSE, return a list combine of ggplot whether to sort plots by absolute value of correlation (fist plot genes with largest sort.by.corr (anti-)correlation)

#### Value

a list with components

- p is a combined ggplot or list of ggplots if combine = TRUE
- w.cor weighted correlation between genes

a list, where

supercell\_GeneGenePlot\_single

Plot Gene-gene correlation plot for 1 feature

#### **Description**

Used for supercell\_GeneGenePlot

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#### Usage

```
supercell_GeneGenePlot_single(
   ge_x,
   ge_y,
   gene_x_name,
   gene_y_name,
   supercell_size = NULL,
   clusters = NULL,
   color.use = NULL,
   x.max = NULL,
   y.max = NULL,
   pt.size = 1,
   alpha = 0.9
)
```

### **Arguments**

```
first gene expression vector (same length as number of super-cells)
ge_x
                  second gene expression vector (same length as number of super-cells)
ge_y
gene_x_name
                  name of gene x
gene_y_name
                  name of gene y
supercell_size a vector with supercell size (ordered the same way as in ge)
clusters
                  a vector with clustering information (ordered the same way as in ge)
color.use
                  colors for idents
x.max
                  max of x axis
y.max
                  max of y axis
pt.size
                  point size (0 by default)
alpha
                  transparency of dots
```

```
supercell_GE_idx Simplification of scRNA-seq dataset (old version, not used since 12.02.2021)
```

### Description

This function converts gene-expression matrix of single-cell data into a gene expression matrix of super-cells

```
supercell_GE_idx(ge, groups, weights = NULL, do.median.norm = FALSE)
```

supercell\_merge 25

#### **Arguments**

ge gene expression matrix (or any coordinate matrix) with genes as rows and cells

as cols

groups vector of membership (assignment of single-cell to super-cells)

weights vector of a cell weight (NULL by default), used for computing average gene

expression withing cluster of super-cells

do.median.norm whether to normalize by median value (FALSE by default)

#### Value

a matrix of simplified (averaged withing groups) data with ncol equal to number of groups and nrows as in the initial dataset

supercell\_merge

Merging independent SuperCell objects

### **Description**

This function merges independent SuperCell objects

### Usage

```
supercell_merge(SCs, fields = c())
```

### **Arguments**

SCs list of SuperCell objects (results of SCimplify )

fields which additional fields (e.g., metadata) of the the SuperCell objects to keep

when merging

#### Value

a list with components

- membership assignment of each single cell to a particular metacell
- cell.ids the original ids of single-cells
- supercell\_size size of metacells (former super-cells)
- gamma graining level of the merged object (estimated as an average size of metacells as the independent SuperCell objects might have different graining levels)
- N.SC number of obtained metacells

26 supercell\_mergeGE

#### **Examples**

```
data(cell_lines) # list with GE - gene expression matrix (logcounts), meta - cell meta data
GE <- cell lines$GE
cell.meta <- cell_lines$meta</pre>
cell.idx.HCC827 <- which(cell.meta == "HCC827")</pre>
cell.idx.H838 <- which(cell.meta == "H838")</pre>
SC.HCC827 <- SCimplify(GE[,cell.idx.HCC827], # log-normalized gene expression matrix
                gamma = 20, # graining level
                n.var.genes = 1000,
                k.knn = 5, # k for kNN algorithm
                n.pc = 10) # number of principal components to use
SC.HCC827$cell.line <- supercell_assign(</pre>
    cell.meta[cell.idx.HCC827],
    supercell_membership = SC.HCC827$membership)
SC.H838 <- SCimplify(GE[,cell.idx.H838], # log-normalized gene expression matrix
                gamma = 30, # graining level
               n.var.genes = 1000, # number of top var genes to use for the dim reduction
                k.knn = 5, # k for kNN algorithm
                n.pc = 15) # number of proncipal components to use
SC.H838$cell.line <- supercell_assign(</pre>
    cell.meta[cell.idx.H838],
    supercell_membership = SC.H838$membership)
SC.merged <- supercell_merge(list(SC.HCC827, SC.H838), fields = c("cell.line"))</pre>
# compute metacell gene expression for SC.HCC827
SC.GE.HCC827 <- supercell_GE(GE[, cell.idx.HCC827], groups = SC.HCC827$membership)
# compute metacell gene expression for SC.H838
SC.GE.H838 <- supercell_GE(GE[, cell.idx.H838], groups = SC.H838$membership)</pre>
# merge GE matricies
SC.GE.merged <- supercell_mergeGE(list(SC.GE.HCC827, SC.GE.H838))</pre>
```

supercell\_mergeGE

Merging metacell gene expression matrices from several independent SuperCell objects

#### **Description**

This function merges independent SuperCell objects

```
supercell_mergeGE(SC.GEs)
```

supercell\_plot 27

### **Arguments**

SC.GEs

list of metacell gene expression matricies (result of supercell\_GE ), make sure the order of the gene expression metricies is the same as in the call of supercell\_merge

#### Value

a merged matrix of gene expression

### **Examples**

```
# see examples in \link{supercell_merge}
```

supercell\_plot

Plot metacell NW

### **Description**

Plot metacell NW

```
supercell_plot(
  SC.nw,
 group = NULL,
 color.use = NULL,
 lay.method = c("nicely", "fr", "components", "drl", "graphopt"),
 lay = NULL,
 alpha = 0,
  seed = 12345,
 main = NA,
 do.frames = TRUE,
  do.extra.log.rescale = FALSE,
  do.directed = FALSE,
  log.base = 2,
 do.extra.sqtr.rescale = FALSE,
  frame.color = "black",
 weights = NULL,
 min.cell.size = 0,
  return.meta = FALSE
)
```

28 supercell\_plot

### **Arguments**

SC.nw a super-cell (metacell) network (a field supercell\_network of the output of SCimplify) an assignment of metacells to any group (for ploting in different colors) group color.use colros to use for groups, if NULL, an automatic palette of colors will be applied lay.method method to compute layout of the network (for the moment there several available: "nicely" for layout\_nicely and "fr" for layout\_with\_fr, "components" for layout\_components, "drl" for layout\_with\_drl, "graphopt" for layout\_with\_graphopt). If your dataset has clear clusters, use "components" a particular layout of a graph to plot (in is not NULL, lay.method is ignored and lay new layout is not computed) alpha a rotation of the layout (either provided or computed) seed a random seed used to compute graph layout main a title of a plot do.frames whether to keep vertex.frames in the plot do.extra.log.rescale whether to log-scale node size (to balance plot if some metacells are large and covers smaller metacells) do.directed whether to plot edge direction log.base base with thich to log-scale node size do.extra.sqtr.rescale whether to sqrt-scale node size (to balance plot if some metacells are large and covers smaller metacells) frame.color color of node frames, black by default weights edge weights used for some layout algorithms min.cell.size do not plot cells with smaller size

#### Value

plot of a super-cell network

return.meta

#### **Examples**

whether to return all the meta data

supercell\_plot\_GE 29

supercell\_plot\_GE

Plot super-cell NW colored by an expression of a gene (gradient color)

### **Description**

Plot super-cell NW colored by an expression of a gene (gradient color)

### Usage

```
supercell_plot_GE(
   SC.nw,
   ge,
   color.use = c("gray", "blue"),
   n.color.gradient = 10,
   main = NA,
   legend.side = 4,
   gene.name = NULL,
   ...
)
```

#### **Arguments**

```
sc.nw a super-cell network (a field supercell_network of the output of SCimplify)

ge a gene expression vector (same length as number of super-cells)

color.use colors of gradient

n.color.gradient

number of bins of the gradient, default is 10

main plot title

legend.side a side parameter of gradientLegend function (default is 4)

gene.name name of gene of for which gene expression is plotted

... rest of the parameters of supercell_plot function
```

### Value

plot of a super-cell network with color representing an expression level

```
supercell\_plot\_tSNE \qquad \textit{Plot super-cell tSNE (Use supercell\_DimPlot instead) Plots super-cell} \\ \textit{tSNE (result of supercell\_tSNE)}
```

#### **Description**

Plot super-cell tSNE (Use supercell\_DimPlot instead) Plots super-cell tSNE (result of supercell\_tSNE)

### Usage

```
supercell_plot_tSNE(
   SC,
   groups,
   tSNE_name = "SC_tSNE",
   color.use = NULL,
   asp = 1,
   alpha = 0.7,
   title = NULL
)
```

#### **Arguments**

```
SC super-cell structure (output of SCimplify) with a field tSNE_name containing tSNE result
groups coloring metacells by groups
tSNE_name the mane of the field containing tSNE result
color.use colors of groups
asp plot aspect ratio
alpha transparency of
title title of the plot
```

### Value

ggplot

#### **Description**

Plot super-cell UMAP (Use supercell\_DimPlot instead) Plots super-cell UMAP (result of supercell\_UMAP)

supercell\_prcomp 31

### Usage

```
supercell_plot_UMAP(
   SC,
   groups,
   UMAP_name = "SC_UMAP",
   color.use = NULL,
   asp = 1,
   alpha = 0.7,
   title = NULL
)
```

#### **Arguments**

SC super-cell structure (output of SCimplify) with a field UMAP\_name containing UMAP result
groups coloring metacells by groups
UMAP\_name the mane of the field containing UMAP result
color.use colors of groups
asp plot aspect ratio
alpha transparency of
title title of the plot

#### Value

ggplot

supercell\_prcomp

compute PCA for super-cell data (sample-weighted data)

### Description

compute PCA for super-cell data (sample-weighted data)

```
supercell_prcomp(
   X,
   genes.use = NULL,
   genes.exclude = NULL,
   supercell_size = NULL,
   k = 20,
   do.scale = TRUE,
   do.center = TRUE,
   fast.pca = TRUE,
   seed = 12345
)
```

32 supercell\_purity

#### **Arguments**

X super-cell transposed gene expression matrix (! where rows represent super-cells

and cols represent genes)

genes.use genes to use for dimensionality reduction

genes.exclude genes to exclude from dimensionaloty reduction

supercell\_size a vector with supercell sizes (ordered the same way as in X)

k number of components to compute

do.scale scale data before PCA do.center center data before PCA

fast.pca whether to run fast PCA (works for datasets with |super-cells| > 50)

seed a seed to use for set. seed

#### Value

the same object as prcomp result

supercell\_purity

Compute purity of super-cells

#### **Description**

Compute purity of super-cells

### Usage

```
supercell_purity(
  clusters,
  supercell_membership,
  method = c("max_proportion", "entropy")[1]
)
```

### **Arguments**

clusters vector of clustering assignment (reference assignment)

supercell\_membership

vector of assignment of single-cell data to super-cells (membership field of

**SCimplify** function output)

method method to compute super-cell purity. "max\_proportion" if the purity is de-

fined as a proportion of the most abundant cluster (cell type) within super-cell or "entropy" if the purity is defined as the Shanon entropy of the cell types

super-cell consists of.

supercell\_rescale 33

#### Value

a vector of super-cell purity, which is defined as: - proportion of the most abundant cluster within super-cell for method = "max\_proportion" or - Shanon entropy for method = "entropy". With 1 meaning that super-cell consists of single cells from one cluster (reference assignment)

supercell\_rescale

Rescale supercell object

### **Description**

This function recomputes super-cell structure at a different graining level (gamma) or for a specific number of super-cells (N.SC)

### Usage

```
supercell_rescale(SC.object, gamma = NULL, N.SC = NULL)
```

### **Arguments**

SC. object super-cell object (an output from SCimplify function) new grainig level (provide either gamma or N.SC)

N. SC new number of super-cells (provide either gamma or N. SC)

#### Value

the same object as SCimplify at a new graining level

#### **Description**

Compute Silhouette index accounting for samlpe size (super cells size) ###

#### Usage

```
supercell_silhouette(x, dist, supercell_size = NULL)
```

#### Arguments

```
x - clusteringdist - distance among super-cellssupercell_size - super-cell size
```

#### Value

silhouette result

34 supercell\_UMAP

supercell\_tSNE

Compute tSNE of super-cells

### Description

Computes tSNE of super-cells

### Usage

```
supercell_tSNE(
   SC,
   PCA_name = "SC_PCA",
   n.comp = NULL,
   perplexity = 30,
   seed = 12345,
   ...
)
```

### Arguments

SC super-cell structure (output of SCimplify) with a field PCA\_name containig PCA

result

PCA\_name name of SC field containing result of supercell\_prcomp

n. comp number of vector of principal components to use for computing tSNE

perplexity perplexity parameter (parameter of Rtsne)

seed random seed

... other parameters of Rtsne

### Value

Rtsne result

supercell\_UMAP

Compute UMAP of super-cells

### Description

Computes UMAP of super-cells

```
supercell_UMAP(SC, PCA_name = "SC_PCA", n.comp = NULL, n_neighbors = 15, ...)
```

supercell\_VlnPlot 35

### **Arguments**

SC super-cell structure (output of SCimplify) with a field PCA\_name containing PCA result

PCA\_name name of SC field containing result of supercell\_prcomp

n. comp number of vector of principal components to use for computing UMAP

n\_neighbors number of neighbors (parameter of umap)

... other parameters of umap

### Value

umap result

supercell\_VlnPlot

Violin plots

#### **Description**

Violin plots (similar to VlnPlot with some changes for super-cells)

### Usage

```
supercell_VlnPlot(
  ge,
  supercell_size = NULL,
  clusters,
  features = NULL,
  idents = NULL,
  color.use = NULL,
 pt.size = 0,
 pch = "o",
 y.max = NULL,
  y.min = NULL,
  same.y.lims = FALSE,
  adjust = 1,
  ncol = NULL,
  combine = TRUE,
 angle.text.y = 90,
  angle.text.x = 45
)
```

### **Arguments**

```
ge a gene expression matrix (ncol same as number of super-cells)
supercell_size a vector with supercell size (ordered the same way as in ge)
clusters a vector with clustering information (ordered the same way as in ge)
```

features name of genes of for which gene expression is plotted idents idents (clusters) to plot (default all) color.use colors for idents pt.size point size (0 by default) pch shape of jitter dots max of y axis y.max min of y axis y.min same.y.lims same y axis for all plots adjust param of geom\_violin ncol number of columns in combined plot combine combine plots into a single patchworked ggplot object. If FALSE, return a list of ggplot angle.text.y rotation of y text

#### Value

angle.text.x

combined ggplot or list of ggplots if combine = TRUE

rotation of x text

```
supercell_VlnPlot_single

Plot Violin plot for 1 feature
```

### **Description**

Used for supercell\_VlnPlot

```
supercell_VlnPlot_single(
   ge1,
   supercell_size = NULL,
   clusters,
   feature = NULL,
   color.use = NULL,
   pt.size = 0,
   pch = "o",
   y.max = NULL,
   y.min = NULL,
   adjust = 1,
   angle.text.y = 90,
   angle.text.x = 45
)
```

### Arguments

ge1 a gene expression vector (same length as number of super-cells) supercell\_size a vector with supercell size (ordered the same way as in ge)

clusters a vector with clustering information (ordered the same way as in ge)

feature gene to plot color.use colors for idents

pt.size point size (0 by default)
pch shape of jitter dots
y.max max of y axis
y.min min of y axis

adjust param of geom\_violin angle.text.y rotation of y text angle.text.x rotation of x text

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