

Package ‘dandelionR’

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Title Single-cell Immune Repertoire Trajectory Analysis in R

Version 0.99.11

Description dandelionR is an R package for performing single-cell immune repertoire trajectory analysis, based on the original python implementation. It provides the necessary functions to interface with scRepertoire and a custom implementation of an absorbing Markov chain for pseudo-time inference, inspired by the Palantir Python package.

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'differentiationProbabilities.R' 'filterCells.R' 'getPbs.R'
'projectProbability.R' 'maxMinSampling.R' 'minMaxScale.R'
'markovProbability.R' 'miloUmap.R' 'projectPseudotimeToCell.R'
'setupVdjPseudobulk.R' 'splitCTgene.R' 'vdjPseudobulk.R'

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Contents

.addColData	3
.allowedChain	4
.calDif	4
.classCheck	5
.collapse_nested_list	5
.constructMarkovChain	6
.determExtractColN	6
.determineMultiscaleSpace	7
.determTerminal	7
.extractVdj	8
.featureSpaceConstruct	8
.filterCells	9
.filterProductivity	9
.filterUnmapped	10
.findNewWaypoints	11
.generateExtractColumn	12
.generateExtractName	12
.getPbs	13
.getPbsCol	13
.getPbsPerCol	14
.KNNind	14
.maxMinSampling	15
.minMaxScale	15
.normalizeFeatureSpace	16
.normalizePerVDJ	16
.packFeatureSpace	17
.removeEdge	18
.subsetSce	18
.terminalStateFromMarkovChain	19
.typeCheck	20
.waypiontsPerCol	20
chainAssign	21
dandelionR	21
demo_airr	22
demo_sce	23
differentiationProbabilities	24

<code>.addColData</code>	3
<code>formatVdj</code>	24
<code>markovProbability</code>	25
<code>miloUmap</code>	27
<code>projectProbability</code>	28
<code>projectPseudotimeToCell</code>	29
<code>sce_vdj</code>	31
<code>setupVdjPseudobulk</code>	32
<code>splitCTgene</code>	35
<code>vdjPseudobulk</code>	35
Index	38

<code>.addColData</code>	<i>add the calculated probability to the colData</i>
--------------------------	--

Description

add the calculated probability to the colData

Usage

```
.addColData(probabilities_proj, terminal_state, milo, verbose)
```

Arguments

<code>probabilities_proj</code>	the probabilities need to be stored
<code>terminal_state</code>	Integer. The index of the terminal state in the Markov chain, passed from <code>markovProbability</code>
<code>milo</code>	the milo object provided by user
<code>verbose</code>	logical, print warnings.

Value

a Milo object with probabilities and pseudotime in its colData slot

`.allowedChain` *filtering cell without allowed chain status*

Description

filtering cell without allowed chain status

Usage

```
.allowedChain(sce, allowed_chain_status, verbose)
```

Arguments

<code>sce</code>	SingleCellExperiment object input
<code>allowed_chain_status</code>	the chain needs to be retain, passed from <code>setupVdjPseudobulk</code>
<code>verbose</code>	logical, print messages. Default is TRUE.

Value

SingleCellExperiment object with allowed chain status

`.calDif` *function help to reconstruct diffusion distance using Reduce*

Description

function help to reconstruct diffusion distance using Reduce

Usage

```
.calDif(dfm, i, j, eigenvector, lambda_t, K)
```

Arguments

<code>dfm</code>	an <code>nrow(eigenvectors) x nrow(eigenvectors)</code> matrix need to be filled with diffusion distance with iteration
<code>i</code>	integer the row selected in this iteration
<code>j</code>	integer the col selected in this iteration
<code>eigenvector</code>	numeric vector, the eigenvector from diffusion map
<code>lambda_t</code>	eigenvalues to the power of <code>t</code> (diffusion time)
<code>K</code>	The number of the eigenvectors to be used in calculation

Value

updated diffusion distance matrix after one iteration

`.classCheck` *.classCheck*

Description

check whether the input is with the correct class

Usage

`.classCheck(input, must)`

Arguments

<code>input</code>	the input need to be check
<code>must</code>	the type we need

Value

whether or not the input is the correct class

`.collapse_nested_list` *Collapse a nested list*

Description

Collapse a nested list

Usage

`.collapse_nested_list(input_list)`

Arguments

<code>input_list</code>	input nested list.
-------------------------	--------------------

Value

collapsed list

```
.constructMarkovChain .constructMarkovChain
```

Description

Markov chain construction

Usage

```
.constructMarkovChain(wp_data, knn., pseudotime, waypoints, vb)
```

Arguments

wp_data	Multi scale data of the waypoints
knn.	Number of nearest neighbors for graph construction
pseudotime	pseudotime ordering of cells
waypoints	integer vector, index of selected waypoint used to
vb	whether to print messages

Value

transition matrix of the markov chain

```
.determExtractColN determine the columns in the colData where the main VDJ information is stored
```

Description

determine the columns in the colData where the main VDJ information is stored

Usage

```
.determExtractColN(extract_cols, mode_option, milo)
```

Arguments

extract_cols	names of columns in the colData where the main VDJ information stores, passed from vdjPseudobulk
mode_option	Specifies the mode for extracting V(D)J genes
milo	Milo or SingleCellExperiment object provided by user

Value

a character vector stores the names of columns in the colData where the main VDJ information stores

`.determineMultiscaleSpace`
.determineMultiscaleSpace

Description

`.determineMultiscaleSpace`

Usage

`.determineMultiscaleSpace(diffusionmap, n_eigs = NULL)`

Arguments

`diffusionmap` DiffusionMap object
`n_eigs` integer, default is NULL. Number of eigen vectors to use.

- If is not specified, the number of eigen vectors will be determined using the eigen gap.

Value

dataframe

`.determTerminal` *.determTerminal*

Description

function in Reduce to provide waypoints

Usage

`.determTerminal(terminal_states, i, dm_boudaries, wp_data)`

Arguments

`terminal_states` integer vector to store the generated waypoint index
`i` iteration index
`dm_boudaries` index of the maxium or minium value of transition matrix per row
`wp_data` Multi scale data of the waypoints

Value

integer vector store the index of waypoints serve as terminal state

<code>.extractVdj</code>	<i>Specify the columns which store VDJ information, and extract the main chain from it</i>
--------------------------	--

Description

Specify the columns which store VDJ information, and extract the main chain from it

Usage

```
.extractVdj(sce, extract_cols, mode_option, verbose)
```

Arguments

<code>sce</code>	SingleCellExperiment object input
<code>extract_cols</code>	The <code>setupVdjPseudobulk</code> transferred parameter given by user to specify the VDJ information columns
<code>mode_option</code>	see document of <code>setupVdjPseudobulk</code> for detailed explanation
<code>verbose</code>	logical, print messages. Default is TRUE.

Value

SingleCellExperiment objects with column stores the information of the main VDJ information in `colData` slot

<code>.featureSpaceConstruct</code>	<i>Construct VDJ feature space</i>
-------------------------------------	------------------------------------

Description

Construct VDJ feature space

Usage

```
.featureSpaceConstruct(milo, extract_cols, pbs)
```

Arguments

<code>milo</code>	Milo or SingleCellExperiment object provided by user
<code>extract_cols</code>	columns of names where to extract the VDJ information
<code>pbs</code>	cell x pseudobulk adjacent matrix

Value

constructed feature space

.filterCells *.filterCells*

Description

Helper function that identifies filter_pattern hits in determined column of sce, and then either removes the offending cells or masks the matched values with a uniform value of '(column's name)_missing'

Usage

```
.filterCells(  
  sce,  
  col_n,  
  filter_pattern = ",|None|No_contig",  
  remove_missing = TRUE  
)
```

Arguments

sce SingleCellExperiment object, adata in python data after combineTCR, contain both vdj and seq

col_n mode for extraction the V(D)J genes.

filter_pattern character string, optional ',|None|No_contig' by default

remove_missing bool, True by default

- If TRUE, will remove cells with contigs matching the filter from the object.
- If FALSE, will mask them with a uniform value dependent on the column name.

Value

filtered SingleCellExperiment object according to the parameter.

.filterProductivity *filer out cell with unproductive chain*

Description

filer out cell with unproductive chain

Usage

```
.filterProductivity(
  sce,
  mode_option,
  productive_cols,
  productive_vj,
  productive_vdj,
  verbose
)
```

Arguments

sce	SingleCellExperiment input
mode_option	check setupVdjPseudobulk for detailed explanation
productive_vj	If TRUE, retains cells where the main VJ chain is productive.
productive_vdj	If TRUE, retains cells where the main VDJ chain is productive.
verbose	logical, print messages. Default is TRUE.

Value

SingleCellExperiment object after filtering on productive chain

<i>.filterUnmapped</i>	<i>Filter out cell with unclear mapping in VDJ information</i>
------------------------	--

Description

Filter out cell with unclear mapping in VDJ information

Usage

```
.filterUnmapped(
  sce,
  mode_option,
  check_vj_mapping,
  check_vdj_mapping,
  main_cols,
  check_extract_cols_mapping,
  remove_missing,
  verbose
)
```

Arguments

- sce SingleCellExperiment object input
- mode_option see document of setupVdjPseudobulk for explanation
- check_vj_mapping logical vector to set whether to check V and J gene in VJ chain, passed from setupVdjPseudobulk
- check_vdj_mapping logical vector to set whether to check V, D and J gene in VDJ chain, passed from setupVdjPseudobulk
- main_cols column names in colData in which The information of main chain stores
- check_extract_cols_mapping character vector,the names of columns that needs to be checked, passed from setupVdjPseudobulk
- remove_missing option for removing the unclear mappin or just mask it, passed from setupVdjPseudobulk
- verbose logical, print messages.

Value

filtered SingleCellExperiment object

.findNewWaypoints *function used in Reduce to find new waypoint in an iteration*

Description

function used in Reduce to find new waypoint in an iteration

Usage

.findNewWaypoints(iterdists, k, vecs, ind, datas)

Arguments

- iterdists a list containing both waypoints deteted in the former iterations and the distance matrix used to find waypoints
- k the iteration number
- vecs a numeric vector used to calculate distance of waypoints to each points
- ind colnames

Value

a list containing updated distance matrix and new waypoints

```
.generateExtractColumn
```

Check whether the columns with specified names exist, if not, create them with CTgene columns

Description

Check whether the columns with specified names exist, if not, create them with CTgene columns

Usage

```
.generateExtractColumn(sce, extract_cols, verbose)
```

Arguments

sce	SingleCellExperiment object input
extract_cols	column names we aim to extract information from
verbose	logical, print messages. Default is TRUE.

Value

SingleCellExperiment with columns containing VDJ information in the names we've specified.

```
.generateExtractName Generate the name of columns with given parameter
```

Description

Generate the name of columns with given parameter

Usage

```
.generateExtractName(sce, mode_option, verbose)
```

Arguments

sce	SingleCellExperiment object input
mode_option	see document of setupVdjPseudobulk for explanation
verbose	logical, print messages. Default is TRUE.

Value

a vector of colnames we need to perform main chain extraction

.getPbs	<i>.getPbs</i>
---------	----------------

Description

Helper function to ensure we have cells by pseudobulks matrix which we can use for pseudobulking.

Usage

```
.getPbs(pbs, col_to_bulk, milo, verbose = TRUE)
```

Arguments

pbs	pbs parameter provided by vdjPseudobulk(), cells by pseudobulks matrix or NULL
col_to_bulk	col_to_bulk parameter provided by vdjPseudobulk(), column's name of colData from milo
milo	SingleCellExperiment object
verbose	logical, whether to print messages

Value

a cell x pseudobulk matrix

.getPbsCol	<i>.getPbsCol</i>
------------	-------------------

Description

Helper function to create the new pseudobulk object's coldata.

Usage

```
.getPbsCol(pbs, col_to_take, milo)
```

Arguments

pbs	dgeMatrix, cell x pseudobulk binary matrix
col_to_take	character vector, names of colData of milo that need to be processed
milo	Milo or SingleCellExperiment object

Value

pbs_col, a DataFrame which will be passed to the new SingleCellExperiment object as colData of vdj x pseudobulk assays

<code>.getPbsPerCol</code>	<code>.getPbsPerCol</code>
----------------------------	----------------------------

Description

function used in Reduce to get the PbsCol

Usage

```
.getPbsPerCol(pbs.col, anno_col, milo, pbs)
```

Arguments

<code>pbs.col</code>	DataFrame object used to store the result of each iteration
<code>anno_col</code>	colname where to generate the metadata from
<code>milo</code>	milo or SingleCellExperiment objects provided by user
<code>pbs</code>	dgeMatrix, cell x pseudobulk binary matrix

Value

DataFrame object, serve as part of metadata of the new milo object

<code>.KNNind</code>	<i>Calculate the weight adjacent matrix of knn graph and its index</i>
----------------------	--

Description

Calculate the weight adjacent matrix of knn graph and its index

Usage

```
.KNNind(wp_data, knn.)
```

Arguments

<code>wp_data</code>	Multi scale data of the waypoints
<code>knn.</code>	Number of nearest neighbors for graph construction

Value

a list containing the weight adjacent matrix and index

`.maxMinSampling` *.maxMinSampling*

Description

function for max min sampling of waypoints

Usage

```
.maxMinSampling(datas, num_waypoints, verbose = TRUE)
```

Arguments

`datas` data matrix along which to sample the waypoints, usually diffusion components
`num_waypoints` number of waypoints to sample
`verbose` logical, print progress

Value

Series representing the sampled waypoints

`.minMaxScale` *minMaxScale*

Description

scale the value to range 0 to 1

Usage

```
.minMaxScale(data)
```

Arguments

`data` dataframe need to be scale

Value

scaled value

```
.normalizeFeatureSpace
```

Normalize Feature Space

Description

Make sure the sum of each V, D, and J gene within a pseudobulk equals to 1

Usage

```
.normalizeFeatureSpace(  
  pseudo_vdj_feature,  
  extract_cols,  
  min_count,  
  renormalize,  
  milo  
)
```

Arguments

pseudo_vdj_feature	constructed feature space
extract_cols	names of columns to extract the VDJ information
min_count	the minim count of a V/D/J gene
renormalize	Whether to renormalize the matrix
milo	Milo or SingleCellExperiment object provided by user

Value

normalized VDJ feature space

```
.normalizePerVDJ
```

function to normalize a specific kind of VDJ gene in feature space

Description

function to normalize a specific kind of VDJ gene in feature space

Usage

```
.normalizePerVDJ(  
  pseudo_vdj_feature,  
  col_n,  
  renormalize,  
  define.mask,  
  milo,  
  min_count  
)
```

Arguments

- pseudo_vdj_feature constructed feature space
- col_n name of column to extract the VDJ information
- renormalize Whether to renormalize the matrix
- define.mask logical vector determine whether the V/D/J gene should be masked when normalizing
- milo Milo or SingleCellExperiment object provided by user
- min_count the minim count of a V/D/J gene

Value

feature space normalized on specified V/D/J gene

.packFeatureSpace *Pack the normalized feature space into new Milo object*

Description

The metadata will derived from the original milo

Usage

```
.packFeatureSpace(pbs, col_to_take, milo, pseudo_vdj_feature)
```

Arguments

- pbs cell x pseudobulk adjacent matrix
- col_to_take Optional character or vector of characters. Specifies names of colData of milo that need to identify the most common value for each pseudobulk
- milo Milo or SingleCellExperiment object provided by user
- pseudo_vdj_feature VDJ feature space

Value

Milo object with VDJ feature space stored in its assay

<code>.removeEdge</code>	<i>function used in Reduce to remove KNN's backward edges except for edges that are within the computed standard deviation</i>
--------------------------	--

Description

function used in Reduce to remove KNN's backward edges except for edges that are within the computed standard deviation

Usage

```
.removeEdge(Knn, i, rem_edges)
```

Arguments

<code>Knn</code>	weight KNN adjacent matrix
<code>i</code>	the iteration number
<code>rem_edges</code>	the edges that need to be removes

Value

an updated matrix after one round of iteration

<code>.subsetSce</code>	<i>Subset sce with given parameter</i>
-------------------------	--

Description

Subset sce with given parameter

Usage

```
.subsetSce(sce, subsetby, groups, verbose)
```

Arguments

<code>sce</code>	SingleCellExperiment object input
<code>subsetby</code>	subsetby Character. Name of a colData column for subsetting. given by setupVdjPsudobulk.
<code>groups</code>	Character vector. Specifies the subset condition for filtering. given by setupVdjPsudobulk.
<code>verbose</code>	logical, print messages. Default is TRUE.

Value

subsetting SingleCellExperiment object

.terminalStateFromMarkovChain

Determine terminal states using Markov chain if end states are not provided.

Description

Determine terminal states using Markov chain if end states are not provided.

Usage

```
.terminalStateFromMarkovChain(  
  Transmat,  
  wp_data,  
  pseudotime,  
  waypoints,  
  verbose  
)
```

Arguments

Transmat	Transition matrix
wp_data	Multi scale data of the waypoints
pseudotime	numeric vector, pseudotime of each pseudobulk
waypoints	integer vector, waypoint selected to construct markov chain.
verbose	Boolean, whether to print messages/warnings.

Value

terminal_state

`.typeCheck`*.typeCheck*

Description

check whether the input has the correct type

Usage

```
.typeCheck(input, must)
```

Arguments

input the input need to be check

must the type we need

Value

whether or not the input is the correct type

`.waypiontsPerCol`*find the waypoints according to certain columns of data*

Description

find the waypoints according to certain columns of data

Usage

```
.waypiontsPerCol(waypoints, ind, datas, no.iterations)
```

Arguments

waypoints integer vector used to store waypoints

ind columns' colnames

datas scaled diffusionmap

Value

a numeric vector containing waypoints' index

chainAssign	<i>Assign the V(D)J gene to the right chain.</i>
-------------	--

Description

Assign the V(D)J gene to the right chain.

Usage

```
chainAssign(vec, num)
```

Arguments

vec	vector of V(D)J genes to assign to the right chain.
num	number of genes to return. should be 2(vj) or 3(vdj)

Value

list contain vector of VJ + VDJ of the cell input

dandelionR	<i>dandelionR: Single-cell immune repertoire trajectory analysis</i>
------------	--

Description

dandelionR is an R package for performing single-cell immune repertoire trajectory analysis, based on the original python implementation. It provides the necessary functions to interface with scRepertoire and a custom implementation of an absorbing Markov chain for pseudotime inference, inspired by the Palantir Python package.

Main functions

- [setupVdjPseudobulk](#): Preprocess V(D)J Data for Pseudobulk Analysis.
- [vdjPseudobulk](#): Generate Pseudobulk V(D)J Feature Space.
- [markovProbability](#): Markov Chain Construction and Probability Calculation.
- [projectPseudotimeToCell](#): Project Pseudotime and Branch Probabilities to Single Cells.

Vignettes

See the package vignettes for detailed workflows: `vignette('dandelionR')`

Installation

To install from Bioconductor, use:

```
if (!requireNamespace('BiocManager', quietly = TRUE))
  install.packages('BiocManager')
BiocManager::install('dandelionR')
```

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See Also

Useful links:

- <https://www.github.com/tuonglab/dandelionR/>
- Report bugs at <https://www.github.com/tuonglab/dandelionR/issues>

demo_airr

Example AIRR Dataset for V(D)J Analysis

Description

The `demo_airr` object is a list of AIRR data frames from a down-sampled demo dataset derived from Suo et al., 2024, *Nature Biotechnology*.

This dataset is used in vignettes to demonstrate workflows for V(D)J analysis.

For details, see the original publication at <https://www.nature.com/articles/s41587-023-01734-7>.

The original files are available at <https://github.com/zktuong/dandelion-demo-files>.

Usage

```
data(demo_airr)
```

Format

A `SingleCellExperiment` object with the following slots:

`list` List of `DataFrames` containing the standardised AIRR data for each sample.

For information of AIRR rearrangements, see the AIRR Community standards at <https://docs.airr-community.org/>.

Source

Suo et al., 2024, *Nature Biotechnology*.
<https://www.nature.com/articles/s41587-023-01734-7>.

Examples

```
data(demo_airr)
```

demo_sce

Example SCE Dataset that does not contain V(D)J information

Description

The `demo_sce` object is a down-sampled demo dataset derived from Suo et al., 2024, *Nature Biotechnology*.

This dataset is used in vignettes to demonstrate workflows for V(D)J analysis.

For details, see the original publication at <https://www.nature.com/articles/s41587-023-01734-7>.

The original Lymphoid cells data in h5ad format is available at <https://developmental.cellatlas.io/fetal-immune>.

Usage

```
data(demo_sce)
```

Format

A `SingleCellExperiment` object with the following slots:

`colData` A minimal `DataFrame` containing metadata about each sample, corresponding to `obs` in `AnnData` (Python). The following columns are relevant for vignette usage:

`anno_lvl1_2_final_clean` Cell type annotations.

`int_colData` A `DataFrame` containing additional assay metadata important for further analysis.

Includes:

- `X_scvi`: A dimensionality reduction matrix from the scVI model.
- `UMAP`: A UMAP reduction matrix.

Source

Suo et al., 2024, *Nature Biotechnology*.
<https://www.nature.com/articles/s41587-023-01734-7>.

Examples

```
data(demo_sce)
```

differentiationProbabilities

Compute Branch Probabilities Using Markov Chain

Description

This function calculates branch probabilities for differentiation trajectories based on a Markov chain constructed from waypoint data and pseudotime ordering.

Usage

```
differentiationProbabilities(
  wp_data,
  terminal_states = NULL,
  knn = 30L,
  pseudotime,
  waypoints,
  verbose = TRUE
)
```

Arguments

wp_data	A multi-scale data matrix or data frame representing the waypoints.
terminal_states	Integer vector. Indices of the terminal states. Default is NULL.
knn	Integer. Number of nearest neighbors for graph construction. Default is 30L.
pseudotime	Numeric vector. Pseudotime ordering of cells.
waypoints	Integer vector. Indices of selected waypoints used to construct the Markov chain.
verbose	Boolean, whether to print messages/warnings.

Value

A numeric matrix or data frame containing branch probabilities for each waypoint.

formatVdj

Change the format of splitCTgene output.

Description

Change the format of splitCTgene output.

Usage

```
formatVdj(gene_list)
```


Arguments

gene_list list containing the output from splitCTgene.

Value

list contain vector of VJ + VDJ information of the cell input

markovProbability *Markov Chain Construction and Probability Calculation*

Description

This function preprocesses data, constructs a Markov chain, and calculates transition probabilities based on pseudotime information.

Usage

```
markovProbability(
  milo,
  diffusionmap,
  terminal_state = NULL,
  root_cell,
  knn = 30L,
  diffusiontime = NULL,
  pseudotime_key = "pseudotime",
  scale_components = TRUE,
  num_waypoints = 500,
  n_eigs = NULL,
  verbose = TRUE
)
```

Arguments

milo A Milo or SingleCellExperiment object. This object should have pseudotime stored in colData, which will be used to calculate probabilities. If pseudotime is available in milo, it takes precedence over the value provided through the diffusiontime parameter.

diffusionmap A DiffusionMap object corresponding to the milo object. Used for Markov chain construction.

terminal_state Integer. The index of the terminal state in the Markov chain.

root_cell Integer. The index of the root state in the Markov chain.

knn Integer. The number of nearest neighbors for graph construction. Default is 30L.

diffusiontime Numeric vector. If pseudotime is not stored in milo, this parameter can be used to provide pseudotime values to the function.

pseudotime_key	Character. The name of the column in colData that contains the inferred pseudotime.
scale_components	Logical. If TRUE, the components will be scaled before constructing the Markov chain. Default is FALSE.
num_waypoints	Integer. The number of waypoints to sample when constructing the Markov chain. Default is 500L.
n_eigs	integer, default is NULL. Number of eigen vectors to use. <ul style="list-style-type: none"> • If is not specified, the number of eigen vectors will be determined using the eigen gap.
verbose	Logical. If TRUE, print progress. Default is TRUE.

Value

milo or SingleCellExperiment object with pseudotime, probabilities in its colData

Examples

```

data(sce_vdj)
# downsample to first 2000 cells
sce_vdj <- sce_vdj[, 1:2000]
sce_vdj <- setupVdjPseudobulk(sce_vdj,
  already.productive = FALSE,
  allowed_chain_status = c("Single pair", "Extra pair")
)
# Build Milo Object
set.seed(100)
milo_object <- miloR::Milo(sce_vdj)
milo_object <- miloR::buildGraph(milo_object,
  k = 50, d = 20,
  reduced.dim = "X_scvi"
)
milo_object <- miloR::makeNhoods(milo_object,
  reduced_dims = "X_scvi",
  d = 20
)

# Construct Pseudobulked VDJ Feature Space
pb.milo <- vdjPseudobulk(milo_object, col_to_take = "anno_lvl_2_final_clean")
pb.milo <- scater::runPCA(pb.milo, assay.type = "Feature_space")

# Define root and branch tips
pca <- t(as.matrix(SingleCellExperiment::reducedDim(pb.milo, type = "PCA")))
branch.tips <- c(which.min(pca[, 2]), which.max(pca[, 2]))
names(branch.tips) <- c("CD8+T", "CD4+T")
root <- which.min(pca[, 1])

# Construct Diffusion Map
dm <- destiny::DiffusionMap(t(pca), n_pcs = 10, n_eigs = 5)
dif.pse <- destiny::DPT(dm, tips = c(root, branch.tips), w_width = 0.1)

```

```

# Markov Chain Construction
pb.milo <- markovProbability(
  milo = pb.milo,
  diffusionmap = dm,
  diffusiontime = dif.pse[[paste0("DPT", root)]],
  terminal_state = branch.tips,
  root_cell = root,
  pseudotime_key = "pseudotime"
)

```

miloUmap

Perform UMAP on the Adjacency Matrix of a Milo Object

Description

This function uses `uwot::umap` to perform UMAP dimensionality reduction on the adjacency matrix of the KNN graph in a Milo object.

Usage

```

miloUmap(
  milo,
  slot_name = "UMAP_knngraph",
  n_neighbors = 50L,
  metric = "euclidean",
  min_dist = 0.3,
  ...
)

```

Arguments

<code>milo</code>	the milo object with knn graph that needed to conduct umap on.
<code>slot_name</code>	character, with default <code>'UMAP_knngraph'</code> . <ul style="list-style-type: none"> The slot name in <code>reduceDim</code> where the result store
<code>n_neighbors</code>	integer, with default 50L. <ul style="list-style-type: none"> the size of local neighborhood (in terms of number of neighboring sample points) used for manifold approximation. Here, the goal is to create large enough neighborhoods to capture the local manifold structure to allow for hypersampling.
<code>metric</code>	character, with default <code>'euclidean'</code> <ul style="list-style-type: none"> the choice of metric used to measure distance to find nearest neighbors. Default is <code>'euclidean'</code>.
<code>min_dist</code>	numeric, with default 0.3 <ul style="list-style-type: none"> the minimum distance between points in the low dimensional space
<code>...</code>	other parameters passed to <code>uwot::umap</code>

Value

milo object with umap reduction

Examples

```
data(sce_vdj)
# downsample to just 1000 cells
sce_vdj <- sce_vdj[, 1:1000]
sce_vdj <- setupVdjPseudobulk(sce_vdj,
  already.productive = FALSE,
  allowed_chain_status = c("Single pair", "Extra pair")
)
# Build Milo Object
milo_object <- miloR::Milo(sce_vdj)
milo_object <- miloR::buildGraph(milo_object,
  k = 50, d = 20,
  reduced.dim = "X_scvi"
)
milo_object <- miloR::makeNhoods(milo_object,
  reduced_dims = "X_scvi", d = 20
)

# Construct UMAP on Milo Neighbor Graph
milo_object <- miloUmap(milo_object)
```

projectProbability *Project Probabilities from Markov Chain to Pseudobulks*

Description

This function projects probabilities calculated from a Markov chain onto each pseudobulk based on a diffusion distance matrix.

Usage

```
projectProbability(
  diffusionmap,
  waypoints,
  probabilities,
  t = 1,
  verbose = TRUE
)
```

Arguments

`diffusionmap` diffusion map, used to reconstruct diffusion distance matrix
`waypoints` Integer vector. Indices of the waypoints used in the Markov chain.

probabilities	Numeric vector. Probabilities associated with the waypoints, calculated from the Markov chain.
t	Numeric. The diffusion time to be used in the projection.
verbose	Boolean, whether to print messages/warnings.

Value

each pseudobulk's probabilities

projectPseudotimeToCell

Project Pseudotime and Branch Probabilities to Single Cells

Description

This function projects pseudotime and branch probabilities from pseudobulk data to single-cell resolution (milo). The results are stored in the colData of the milo object.

Usage

```
projectPseudotimeToCell(
  milo,
  pb_milo,
  term_states = NULL,
  pseudotime_key = "pseudotime",
  suffix = "",
  verbose = TRUE
)
```

Arguments

milo	A SingleCellExperiment or Milo object. Represents single-cell data where pseudotime and branch probabilities will be projected.
pb_milo	A pseudobulk Milo object. Contains aggregated branch probabilities and pseudotime information to be transferred to single cells.
term_states	Named vector of terminal states, with branch probabilities to be transferred. The names should correspond to branches of interest.
pseudotime_key	Character. The column name in colData of pb_milo that contains the pseudotime information which was used in the markovProbability function. Default is "pseudotime".
suffix	Character. A suffix to be added to the new column names in colData. Default is an empty string ('').
verbose	Boolean, whether to print messages/warnings.

Value

subset of milo or SingleCellExperiment object where cell that do not belong to any neighbourhood are removed and projected pseudotime information stored colData

Examples

```

data(sce_vdj)
# downsample to first 2000 cells
sce_vdj <- sce_vdj[, 1:2000]
sce_vdj <- setupVdjPseudobulk(sce_vdj,
  already.productive = FALSE,
  allowed_chain_status = c("Single pair", "Extra pair")
)
# Build Milo Object
set.seed(100)
milo_object <- miloR::Milo(sce_vdj)
milo_object <- miloR::buildGraph(milo_object,
  k = 50, d = 20,
  reduced.dim = "X_scvi"
)
milo_object <- miloR::makeNhoods(milo_object,
  reduced_dims = "X_scvi",
  d = 20
)

# Construct Pseudobulked VDJ Feature Space
pb.milo <- vdjPseudobulk(milo_object, col_to_take = "anno_lvl_2_final_clean")
pb.milo <- scater::runPCA(pb.milo, assay.type = "Feature_space")

# Define root and branch tips
pca <- t(as.matrix(SingleCellExperiment::reducedDim(pb.milo, type = "PCA")))
branch.tips <- c(which.min(pca[, 2]), which.max(pca[, 2]))
names(branch.tips) <- c("CD8+T", "CD4+T")
root <- which.min(pca[, 1])

# Construct Diffusion Map
dm <- destiny::DiffusionMap(t(pca), n_pcs = 10, n_eigs = 5)
dif.pse <- destiny::DPT(dm, tips = c(root, branch.tips), w_width = 0.1)

# Markov Chain Construction
pb.milo <- markovProbability(
  milo = pb.milo,
  diffusionmap = dm,
  diffusiontime = dif.pse[[paste0("DPT", root)]],
  terminal_state = branch.tips,
  root_cell = root,
  pseudotime_key = "pseudotime"
)
# Project Pseudobulk Data
projected_milo <- projectPseudotimeToCell(
  milo_object,
  pb.milo,

```

```

    branch.tips,
    pseudotime_key = "pseudotime"
)

```

sce_vdj

*Example Dataset for V(D)J Analysis***Description**

The `sce_vdj` object is a down-sampled demo dataset derived from Suo et al., 2024, *Nature Biotechnology*.

This dataset is used in vignettes to demonstrate workflows for V(D)J analysis.

For details, see the original publication at <https://www.nature.com/articles/s41587-023-01734-7>.

Usage

```
data(sce_vdj)
```

Format

A `SingleCellExperiment` object with the following slots:

`colData` A `DataFrame` containing metadata about each sample, corresponding to `obs` in `AnnData` (Python). The following columns are relevant for vignette usage:

`productive_(mode)_VDJ`, `productive_(mode)_VJ` Factors indicating whether the heavy or light chain is productive. `mode` refers to the extraction mode for V(D)J genes and can be one of:

- 'abT': TCR alpha-beta
- 'gdT': TCR gamma-delta
- 'B': BCR

`Gene segment fields` Gene segment annotations with column names in the format `(v/d/j)_call_(mode)_(VDJ/VJ)`.

Examples include:

- `v_call_abT_VDJ`: V gene for TCR alpha-beta VDJ recombination
- `d_call_abT_VJ`: D gene for TCR alpha-beta VJ recombination

`chain_status` A factor describing the receptor chain's status.

`anno_lvl_2_final_clean` Cell type annotations.

`int_colData` A `DataFrame` containing additional assay metadata important for further analysis.

Includes:

- `X_scvi`: A dimensionality reduction matrix from the scVI model.
- `UMAP`: A UMAP reduction matrix.

Source

Suo et al., 2024, *Nature Biotechnology*.

<https://www.nature.com/articles/s41587-023-01734-7>.

Examples

```
data(sce_vdj)
```

```
setupVdjPseudobulk    Preprocess V(D)J Data for Pseudobulk Analysis
```

Description

This function preprocesses single-cell V(D)J sequencing data for pseudobulk analysis. It filters data based on productivity and chain status, subsets data, extracts main V(D)J genes, and removes unmapped entries.

Usage

```
setupVdjPseudobulk(
  sce,
  mode_option = c("abT", "gdT", "B"),
  already.productive = TRUE,
  productive_cols = NULL,
  productive_vj = TRUE,
  productive_vdj = TRUE,
  allowed_chain_status = NULL,
  subsetby = NULL,
  groups = NULL,
  extract_cols = NULL,
  filter_unmapped = TRUE,
  check_vj_mapping = c(TRUE, TRUE),
  check_vdj_mapping = c(TRUE, FALSE, TRUE),
  check_extract_cols_mapping = NULL,
  remove_missing = TRUE,
  verbose = TRUE
)
```

Arguments

sce	A SingleCellExperiment object. V(D)J data should be contained in colData for filtering.
mode_option	Optional character. Specifies the mode for extracting V(D)J genes. If NULL, extract_cols must be specified. Default is NULL.
already.productive	Logical. Whether the data has already been filtered for productivity. If TRUE, skips productivity filtering. Default is FALSE.
productive_cols	Character vector. Names of colData columns used for productivity filtering. Default is NULL.

productive_vj	Logical. If TRUE, retains cells where the main VJ chain is productive. Default is TRUE.
productive_vdj	Logical. If TRUE, retains cells where the main VDJ chain is productive. Default is TRUE.
allowed_chain_status	Character vector. Specifies chain statuses to retain. Valid options include `c('single pair', 'Extra pair', 'Extra pair-exception', 'Orphan VDJ', 'Orphan VDJ-exception')`. Default is NULL.
subsetby	Character. Name of a colData column for subsetting. Default is NULL.
groups	Character vector. Specifies the subset condition for filtering. Default is NULL.
extract_cols	Character vector. Names of colData columns where V(D)J information is stored, used instead of the standard columns. Default is NULL.
filter_unmapped	Logic. Whether to filter unmapped data. Default is TRUE.
check_vj_mapping	Logic vector. Whether to check for VJ mapping. Default is c(TRUE, TRUE). <ul style="list-style-type: none"> • If the first element is TRUE, function will filter the unmapped data in V gene of the VJ chain • If the second element is TRUE, function will filter the unmapped data in J gene of the VJ chain
check_vdj_mapping	Logic vector. Specifies columns to check for VDJ mapping. Default is c(TRUE, FALSE, 'TRUE'). <ul style="list-style-type: none"> • If the first element is TRUE, function will filter the unmapped data in V gene of the VDJ chain • If the second element is TRUE, function will filter the unmapped data in D gene of the VDJ chain • If the third element is TRUE, function will filter the unmapped data in J gene of the VDJ chain
check_extract_cols_mapping	Character vector. Specifies columns related to extract_cols for mapping checks. Default is NULL.
remove_missing	Logical. If TRUE, removes cells with contigs matching the filter. If FALSE, masks them with uniform values. Default is TRUE.
verbose	Logical. Whether to print messages. Default is TRUE.

Details

The function performs the following preprocessing steps:

- **Productivity Filtering:**

- Skipped if already.productive = TRUE.
- Filters cells based on productivity using productive_cols or standard colData columns named productive_{mode_option}_{type} (where type is 'VDJ' or 'VJ').
- *mode_option*

- * function will check colData(s) named `productive_{mode_option}_{type}`, where type should be 'VDJ' or 'VJ' or both, depending on values of `productive_vj` and `productive_vdj`.
- * If set as NULL, the function needs the option 'extract_cols' to be specified
- *productive_cols*
 - * must be specified when productivity filtering is need to conduct and mode_option is NULL.
 - * where VDJ/VJ information is stored so that this will be used instead of the standard columns.
- *productive_vj, productive_vdj*
 - * If TRUE, cell will only be kept if the main V(D)J chain is productive
- **Chain Status Filtering:**
 - Retains cells with chain statuses specified by `allowed_chain_status`.
- **Subsetting:**
 - Conducted only if both `subsetby` and `groups` are provided.
 - Retains cells matching the `groups` condition in the `subsetby` column.
- **Main V(D)J Extraction:**
 - Uses `extract_cols` to specify custom columns for extracting V(D)J information.
- **Unmapped Data Filtering:**
 - decided to removes or masks cells based on `filter_unmapped`.
 - Checks specific columns for unclear mappings using `check_vj_mapping`, `check_vdj_mapping`, or `check_extract_cols_mapping`.
 - *filter_unmapped*
 - * pattern to be filtered from object.
 - * If is set to be NULL, the filtering process will not start
 - *check_vj_mapping, check_vdj_mapping*
 - * only colData specified by these arguments (`check_vj_mapping` and `check_vdj_mapping`) will be checked for unclear mappings
 - *check_extract_cols_mapping, related to extract_cols*
 - * Only colData specified by the argument will be checked for unclear mapping, the colData should first specified by `extract_cols`
 - `remove_missing`
 - * If TRUE, will remove cells with contigs matching the filter from the object.
 - * If FALSE, will mask them with a uniform value dependent on the column name.

Value

filtered SingleCellExperiment object

Examples

```
# load data
data(sce_vdj)
# check the dimension
```

```

dim(sce_vdj)
# filtered the data
sce_vdj <- setupVdjPseudobulk(
  sce = sce_vdj,
  mode_option = "abT", # set the mode to alpha-beta TCR
  allowed_chain_status = c("Single pair", "Extra pair"),
  already.productive = FALSE
) # need to filter the unproductive cells
# check the remaining dim
dim(sce_vdj)

```

splitCTgene	<i>Split the V(D)J genes from CTgene column and store them separately.</i>
-------------	--

Description

Split the V(D)J genes from CTgene column and store them separately.

Usage

```
splitCTgene(sce)
```

Arguments

sce SingleCellExperiment object after conducting scRepertoire::combineTCR()

Value

list contain vector of VJ & VDJ genes from each cell

vdpseudobulk	<i>Generate Pseudobulk V(D)J Feature Space</i>
--------------	--

Description

This function creates a pseudobulk V(D)J feature space from single-cell data, aggregating V(D)J information into pseudobulk groups. It supports input as either a Milo object or a SingleCellExperiment object.

Usage

```

vdjPseudobulk(
  milo,
  pbs = NULL,
  col_to_bulk = NULL,
  extract_cols = c("v_call_abT_VDJ_main", "j_call_abT_VDJ_main", "v_call_abT_VJ_main",
    "j_call_abT_VJ_main"),
  mode_option = c("abT", "gdT", "B"),
  col_to_take = NULL,
  normalise = TRUE,
  renormalize = FALSE,
  min_count = 1L,
  verbose = TRUE
)

```

Arguments

<code>milo</code>	A Milo or SingleCellExperiment object containing V(D)J data.
<code>pbs</code>	Optional. A binary matrix with cells as rows and pseudobulk groups as columns. <ul style="list-style-type: none"> If <code>milo</code> is a Milo object, this parameter is not required. If <code>milo</code> is a SingleCellExperiment object, either <code>pbs</code> or <code>col_to_bulk</code> must be provided.
<code>col_to_bulk</code>	Optional character or character vector. Specifies <code>colData</code> column(s) to generate pbs. If multiple columns are provided, they will be combined. Default is <code>NULL</code> . <ul style="list-style-type: none"> If <code>milo</code> is a Milo object, this parameter is not required. If <code>milo</code> is a SingleCellExperiment object, either <code>pbs</code> or <code>col_to_bulk</code> must be provided.
<code>extract_cols</code>	Character vector. Specifies column names where V(D)J information is stored. Default is <code>c('v_call_abT_VDJ_main', 'j_call_abT_VDJ_main', 'v_call_abT_VJ_main', 'j_ca</code>
<code>mode_option</code>	Character. Specifies the mode for extracting V(D)J genes. Must be one of <code>c('B', 'abT', 'gdT')</code> . Default is <code>'abT'</code> . <ul style="list-style-type: none"> Note: This parameter is considered only when <code>extract_cols = NULL</code>. If <code>NULL</code>, uses column names such as <code>v_call_VDJ</code> instead of <code>v_call_abT_VDJ</code>.
<code>col_to_take</code>	Optional character or vector of characters. Specifies names of <code>colData</code> of <code>milo</code> that need to identify the most common value for each pseudobulk. Default is <code>NULL</code> .
<code>normalise</code>	Logical. If <code>TRUE</code> , scales the counts of each V(D)J gene group to 1 for each pseudobulk. Default is <code>TRUE</code> .
<code>renormalize</code>	Logical. If <code>TRUE</code> , rescales the counts of each V(D)J gene group to 1 for each pseudobulk after removing 'missing' calls. Useful when <code>setupVdjPseudobulk()</code> was run with <code>remove_missing = FALSE</code> . Default is <code>FALSE</code> .
<code>min_count</code>	Integer. Sets pseudobulk counts in V(D)J gene groups with fewer than this many non-missing calls to 0. Default is 1.
<code>verbose</code>	Logical. If <code>TRUE</code> , prints messages and warnings. Default is <code>TRUE</code> .

Details

This function aggregates V(D)J data into pseudobulk groups based on the following logic:

- **Input Requirements:**
 - If `milo` is a `Milo` object, neither `pbs` nor `col_to_bulk` is required.
 - If `milo` is a `SingleCellExperiment` object, the user must provide either `pbs` or `col_to_bulk`.
- **Normalization:**
 - When `normalise = TRUE`, scales V(D)J counts to 1 for each pseudobulk group.
 - When `renormalize = TRUE`, rescales the counts after removing 'missing' calls.
- **Mode Selection:**
 - If `extract_cols = NULL`, the function relies on `mode_option` to determine which V(D)J columns to extract.
- **Filtering:**
 - Uses `min_count` to filter pseudobulks with insufficient counts for V(D)J groups.

Value

`SingleCellExperiment` object

Examples

```
data(sce_vdj)
sce_vdj <- setupVdjPseudobulk(sce_vdj,
  already.productive = FALSE,
  allowed_chain_status = c("Single pair", "Extra pair")
)
# Build Milo Object
milo_object <- miloR::Milo(sce_vdj)
milo_object <- miloR::buildGraph(milo_object,
  k = 50, d = 20,
  reduced.dim = "X_scvi"
)
milo_object <- miloR::makeNhoods(milo_object,
  reduced_dims = "X_scvi",
  d = 20
)

# Construct pseudobulked VDJ feature space
pb.milo <- vdjPseudobulk(milo_object, col_to_take = "anno_lvl_2_final_clean")
```

Index

* datasets

- demo_airr, 22
- demo_sce, 23
- sce_vdj, 31

* internal

- .KNNind, 14
- .addColData, 3
- .allowedChain, 4
- .calDif, 4
- .classCheck, 5
- .collapse_nested_list, 5
- .constructMarkovChain, 6
- .determExtractColN, 6
- .determTerminal, 7
- .determineMultiscaleSpace, 7
- .extractVdj, 8
- .featureSpaceConstruct, 8
- .filterCells, 9
- .filterProductivity, 9
- .filterUnmapped, 10
- .findNewWaypoints, 11
- .generateExtractColumn, 12
- .generateExtractName, 12
- .getPbs, 13
- .getPbsCol, 13
- .getPbsPerCol, 14
- .maxMinSampling, 15
- .minMaxScale, 15
- .normalizeFeatureSpace, 16
- .normalizePerVDJ, 16
- .packFeatureSpace, 17
- .removeEdge, 18
- .subsetSce, 18
- .terminalStateFromMarkovChain, 19
- .typeCheck, 20
- .waypiontsPerCol, 20
- chainAssign, 21
- dandelionR, 21
- formatVdj, 24

- splitCTgene, 35

- .KNNind, 14
- .addColData, 3
- .allowedChain, 4
- .calDif, 4
- .classCheck, 5
- .collapse_nested_list, 5
- .constructMarkovChain, 6
- .determExtractColN, 6
- .determTerminal, 7
- .determineMultiscaleSpace, 7
- .extractVdj, 8
- .featureSpaceConstruct, 8
- .filterCells, 9
- .filterProductivity, 9
- .filterUnmapped, 10
- .findNewWaypoints, 11
- .generateExtractColumn, 12
- .generateExtractName, 12
- .getPbs, 13
- .getPbsCol, 13
- .getPbsPerCol, 14
- .maxMinSampling, 15
- .minMaxScale, 15
- .normalizeFeatureSpace, 16
- .normalizePerVDJ, 16
- .packFeatureSpace, 17
- .removeEdge, 18
- .subsetSce, 18
- .terminalStateFromMarkovChain, 19
- .typeCheck, 20
- .waypiontsPerCol, 20
- chainAssign, 21
- dandelionR, 21
- dandelionR-package (dandelionR), 21
- demo_airr, 22
- demo_sce, 23
- differentiationProbabilities, 24

`formatVdj`, [24](#)

`markovProbability`, [21](#), [25](#)

`miUmap`, [27](#)

`projectProbability`, [28](#)

`projectPseudotimeToCell`, [21](#), [29](#)

`sce_vdj`, [31](#)

`setupVdjPseudobulk`, [21](#), [32](#)

`splitCTgene`, [35](#)

`vdjPseudobulk`, [21](#), [35](#)