

# Package ‘dandelionR’

April 9, 2025

**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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'projectProbability.R' 'maxMinSampling.R' 'minMaxScale.R'  
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---

<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**

<code>wp_data</code>	Multi scale data of the waypoints
<code>knn.</code>	Number of nearest neighbors for graph construction
<code>pseudotime</code>	pseudotime ordering of cells
<code>waypoints</code>	integer vector, index of selected waypoint used to
<code>vb</code>	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**

<code>extract_cols</code>	names of columns in the colData where the main VDJ information stores, passed from vdjPseudobulk
<code>mode_option</code>	Specifies the mode for extracting V(D)J genes
<code>milo</code>	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>	<i>.getPbsPerCol</i>
----------------------------	----------------------

---

**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')
```

## Author(s)

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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"> <li>• If is not specified, the number of eigen vectors will be determined using the eigen gap.</li> </ul>
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 'UMAP_knngraph'. <ul style="list-style-type: none"> <li>The slot name in <code>reduceDim</code> where the result store</li> </ul>
<code>n_neighbors</code>	integer, with default 50L. <ul style="list-style-type: none"> <li>the size of local neighborhood (in terms of number of neighboring sample points) used for manifold approximation.</li> <li>Here, the goal is to create large enough neighborhoods to capture the local manifold structure to allow for hypersampling.</li> </ul>
<code>metric</code>	character, with default 'euclidean' <ul style="list-style-type: none"> <li>the choice of metric used to measure distance to find nearest neighbors. Default is 'euclidean'.</li> </ul>
<code>min_dist</code>	numeric, with default 0.3 <ul style="list-style-type: none"> <li>the minimum distance between points in the low dimensional space</li> </ul>
<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"> <li>• If the first element is TRUE, function will filter the unmapped data in V gene of the VJ chain</li> <li>• If the second element is TRUE, function will filter the unmapped data in J gene of the VJ chain</li> </ul>
check_vdj_mapping	Logic vector. Specifies columns to check for VDJ mapping. Default is c(TRUE, FALSE, 'TRUE'). <ul style="list-style-type: none"> <li>• If the first element is TRUE, function will filter the unmapped data in V gene of the VDJ chain</li> <li>• If the second element is TRUE, function will filter the unmapped data in D gene of the VDJ chain</li> <li>• If the third element is TRUE, function will filter the unmapped data in J gene of the VDJ chain</li> </ul>
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"> <li>• If <code>milo</code> is a Milo object, this parameter is not required.</li> <li>• If <code>milo</code> is a SingleCellExperiment object, either <code>pbs</code> or <code>col_to_bulk</code> must be provided.</li> </ul>
<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"> <li>• If <code>milo</code> is a Milo object, this parameter is not required.</li> <li>• If <code>milo</code> is a SingleCellExperiment object, either <code>pbs</code> or <code>col_to_bulk</code> must be provided.</li> </ul>
<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"> <li>• Note: This parameter is considered only when <code>extract_cols = NULL</code>.</li> <li>• If <code>NULL</code>, uses column names such as <code>v_call_VDJ</code> instead of <code>v_call_abT_VDJ</code>.</li> </ul>
<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")
```

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