Package ‘ccfindR’

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Description A collection of tools for cancer genomic data clustering analyses, including those for single cell RNA-seq. Cell clustering and feature gene selection analysis employ Bayesian (and maximum likelihood) non-negative matrix factorization (NMF) algorithm. Input data set consists of RNA count matrix, gene, and cell bar code annotations. Analysis outputs are factor matrices for multiple ranks and marginal likelihood values for each rank. The package includes utilities for downstream analyses, including meta-gene identification, visualization, and construction of rank-based trees for clusters.
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assignCelltype

Cell type assignment via GSEA

Description

Computes GSEA enrichment score of marker sets in meta gene list

Usage

assignCelltype(obj, rank, gset, gene_names = NULL, p = 0, remove.na = FALSE, p.value = FALSE, nperm = 1000, progress.bar = TRUE, grp.prefix = c("IG"))

Arguments

obj Object of class scNMFSet.
rank Rank to examine
gset List of gene sets to be used as markers
gene_names Names of genes to be used for meta-gene identification
p Enrichment score exponent.
Remove gene sets with no overlap
Estimate p values using permutation
No. of permutation replicates
Display progress bar for p value computation
Gene name prefix to search for with wildcard matches in query

Details
If obj is of class scNMFSet, it computes meta gene list using meta_gene.cv. Otherwise, obj is expected to be a data frame of the same structure as the output of meta_gene.cv; the number of rows same as the total number of metagenes per cluster, three columns per each cluster (gene name, meta-gene score, and coefficient of variation). The argument gset is a list of gene sets to be checked for enrichment in each cluster meta gene list. The enrichment score is computed using the GSEA algorithm (Subramanian et al. 2005).

Value
Matrix of enrichment score statistics with cell types in rows and clusters in columns

References

Examples
dir <- system.file('extdata', package='ccfindR')
pbmc <- read_10x(dir)
pbmc <- vb_factorize(pbmc, ranks=5)
meta <- meta_gene.cv(pbmc, rank=5, gene_names=rowData(pbmc)[,2])
markers <- list('B cell'=c('CD74', 'IG', 'HLA'),
                    'CD8+ T'=c('CD8A', 'CD8B', 'GZMK', 'CCR7', 'LTB'),
                    'CD4+ T'=c('CD3D', 'CD3E', 'IL7R', 'LEF1'),
                    'NK'=c('GNLY', 'NKG7', 'GZMA', 'GZMH'),
                    'Macrophage'=c('S100A8', 'S100A9', 'CD14', 'LYZ', 'CFD'))
gsea <- assignCelltype(meta, rank=5, gset=markers, grp.prefix=c('IG', 'HLA'))
gsea

basis

Basis matrices in an Object

Description
Retrieve or set the basis matrices W from factorization in an object
Usage

basis(object)

Arguments

object Object of class scNMFSet

Details

After factorization, basis matrices corresponding to each rank value are stored as elements of a list, which is in slot basis of object of class scNMFSet. basis(object) will return the list of matrices. basis(object) <- value can be used to modify it.

Value

Either NULL or a list of same length as ranks(object), whose elements are basis matrices derived from factorization under each rank value.

Examples

s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s,ranks=seq(2,4))
basis(s)[[1]]
Generics for basis matrix assignment

Description
Access and modify basis matrices

Usage
basis(object) <- value

Arguments
object Object of class scNMFSet
value Basis matrix to be substituted

Value
Input object with updated basis matrices

Examples
set.seed(1)
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=3)
basis(s)[[1]] <- apply(basis(s)[[1]],seq(1,2),round,digits=3)
basis(s)

Modify basis matrices

Description
Access and modify basis matrices

Usage
## S4 replacement method for signature 'scNMFSet'
basis(object) <- value

Arguments
object Object of class scNMFSet
value Basis matrix to be substituted
**build_tree**

**Value**

Input object with updated basis matrices

**Examples**

```r
set.seed(1)
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=3)
basis(s)[[1]] <- apply(basis(s)[[1]],c(1,2),round,digits=3)
basis(s)
```

---

**build_tree**

*Build tree connecting clusters at different ranks*

**Description**

Build tree connecting clusters at different ranks

**Usage**

```r
build_tree(object, rmax)
```

**Arguments**

- `object`: Object of class `scNMFSet`
- `rmax`: Maximum rank at which tree branching stops

**Value**

List containing the tree structure

**Examples**

```r
set.seed(1)
x <- simulate_whx(nrow=50,ncol=100,rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s,ranks=seq(2,8),nrun=5)
tree <- build_tree(s,rmax=5)
tree
```
ccfindR  
\textit{ccfindR: Cancer Clone Find\textit{e}R}

\section*{Description}

This package contains tools and utilities for cell-type discovery using single-cell transcriptomic data while evaluating significance of the depth of clustering (Woo et al. 2019).

\section*{References}


\section*{cell_map}

\textit{Plot heatmap of clustering coefficient matrix}

\section*{Description}

Retrieve a coefficient matrix $H$ derived from factorization by rank value and generate heatmap of its elements.

\section*{Usage}

\begin{verbatim}
cell_map(object, rank, main = "Cells", ...)
\end{verbatim}

\section*{Arguments}

- \textbf{object} Object of class \texttt{scNMFSet}.
- \textbf{rank} Rank value for which the cell map is to be displayed. The object must contain the corresponding slot: one element of \texttt{coeff(object)[[k]]} for which \texttt{ranks(object)[[k]]==rank}.
- \textbf{main} Title of plot.
- \textbf{...} Other arguments to be passed to \texttt{heatmap}, \texttt{image}, and \texttt{plot}.

\section*{Value}

\texttt{NULL}
Examples

```r
set.seed(1)
x <- simulate_data(nfeatures=10, nsamples=c(20,20,60))
rownames(x) <- seq_len(10)
colnames(x) <- seq_len(100)
s <- scNMFSet(count=x, rowData=seq_len(10), colData=seq_len(100))
s <- vb_factorize(s, ranks=seq(2,5))
plot(s)
cell_map(s, rank=3)
```

---

cluster_id

Assign cells into clusters

Description

Use factorization results in an object to assign cells into clusters.

Usage

```r
cluster_id(object, rank = 2)
```

Arguments

- `object`: Object of class `scNMFSet`
- `rank`: Rank value whose factor matrices are to be used for assignment.

Value

Vector of length equal to the number of cells containing cluster ID numbers of each cell.

Examples

```r
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(count=x$x)
s <- vb_factorize(s, ranks=seq(2,8), nrun=5)
cid <- cluster_id(s, ranks=seq(2,5))
table(cid)
```
Description

Retrieve or set the coefficient matrices from factorization in an object

Usage

coeff(object)

Arguments

object Object of class scNMFSet.

Details

After factorization, coefficient matrices H corresponding to each rank value are stored as elements of a list, which is in slot coeff of object of class scNMFSet. coeff(object) will return the list of matrices. coeff(object) <- value can be used to modify it.

Value

Either NULL or a list of same length as ranks(object), whose elements are coefficient matrices derived from factorization under each rank value.

Examples

s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))s <- vb_factorize(s,ranks=seq(2,4))coeff(s)[[1]]

Description

Coefficient matrix accessor

Usage

## S4 method for signature 'scNMFSet'
coeff(object)

Arguments

object Object containing coefficient matrix
coeff <-

Value

List of coefficient matrices

Description

Access and modify coefficient matrices

Usage

coeff(object) <- value

Arguments

object Object of class scNMFSet
value Coefficient matrix to be substituted

Value

Input object with updated coefficient matrices

Examples

s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=3)
coeff(s)[[1]] <- apply(coeff(s)[[1]],c(1,2),round,digits=2)
coeff(s)

coeff<-,scNMFSet-method

Modify coefficient matrices

Description

Can be used to access and modify coefficient matrices

Usage

## S4 replacement method for signature 'scNMFSet'
coeff(object) <- value
Arguments

object Object of class scNMFSet
value Coefficient matrix to be substituted

Value

Input object with updated coefficient matrices

Examples

```r
s <- scNMFSet(count=matrix(rpois(n=12, lambda=3), 4, 3))
s <- vb_factorize(s, ranks=3)
coeff(s)[[1]] <- apply(coeff(s)[[1]], c(1, 2), round, digits=2)
coeff(s)
```

colData, scNMFSet-method

Sample annotation accessor

Description

Sample annotation accessor

Usage

```r
## S4 method for signature 'scNMFSet'
colData(x)
```

Arguments

x Object containing sample annotation

Value

Column annotation DataFrame

Examples

```r
library(S4Vectors)
x <- matrix(rpois(n=12, lambda=3), 4, 3)
rownames(x) <- seq_len(4)
colnames(x) <- c('a', 'b', 'c')
s <- scNMFSet(count=x, rowData=seq_len(4), colData=c('a', 'b', 'c'))
cols <- DataFrame(tissue=c('tissue1', 'tissue1', 'tissue2'))
rownames(cols) <- c('a', 'b', 'c')
colData(s) <- cols
s
```
colData<-,scNMFSet,ANY-method  

Cell annotation assignment

Description

Cell annotation assignment

Usage

## S4 replacement method for signature 'scNMFSet,ANY'

colData(x) <- value

Arguments

x
Object containing cell annotation

value
DataFrame to be substituted

Value

Updated column annotation

Examples

library(S4Vectors)
x <- matrix(rpois(n=12,lambda=3),4,3)
rownames(x) <- seq_len(4)
colnames(x) <- c('a','b','c')
s <- scNMFSet(count=x,rowData=seq_len(4),colData=c('a','b','c'))
cols <- DataFrame(tissue=c('tissue1','tissue1','tissue2'))
rownames(cols) <- c('a','b','c')
colData(s) <- cols
s

counts,scNMFSet-method  

Accessor for count matrix

Description

Accessor for count matrix

Usage

## S4 method for signature 'scNMFSet'
counts(object)
Arguments

object Object containing count matrix

Value

Count matrix

Examples

s <- scNMFSet(count = matrix(rpois(n=12,lambda=3),3,4))
counts(s)

mat <- matrix(rpois(n=12,lambda=3),3,4)
s <- scNMFSet(count = mat)
counts(s) <- mat^2
counts(s)
**dbasis**  
*Basis SD matrix accessor*

### Description
Basis SD matrix accessor

### Usage
```
dbasis(object)
```

### Arguments
- **object**  
  Object containing dbasis matrix

### Value
List of dbasis matrices

---

**dbasis,scNMFSset-method**  
*Basis SD matrix accessor*

### Description
Basis SD matrix accessor

### Usage
```
## S4 method for signature 'scNMFSset'
dbasis(object)
```

### Arguments
- **object**  
  Object containing basis standard deviation (SD) matrix

### Value
List of dbasis matrices
**dbasis<-,scNMFSets-method**

---

**dbasis<-& Basis SD matrix assignment**

**Description**

Basis SD matrix assignment

**Usage**

```r
dbasis(object) <- value
```

**Arguments**

- `object` Object containing dbasis matrix
- `value` List for assignment

**Value**

Updated object

---

**dbasis<-,scNMFSets-method**

*Modify dbasis matrices*

**Description**

Access and modify dbasis matrices

**Usage**

```r
## S4 replacement method for signature 'scNMFSets'
dbasis(object) <- value
```

**Arguments**

- `object` Object of class scNMFSet
- `value` Basis SD matrix to be substituted

**Value**

Modified object
dcoeff

**Coeff SD matrix accessor**

**Description**

Coeff SD matrix accessor

**Usage**

dcoeff(object)

**Arguments**

- **object**
  Object containing dcoeff matrix

**Value**

List of dcoeff matrices

---

dcoeff,scNMFSset-method

**Coefficient SD matrix accessor**

**Description**

Coefficient SD matrix accessor

**Usage**

```r
## S4 method for signature 'scNMFSset'
dcoeff(object)
```

**Arguments**

- **object**
  Object containing coefficient standard deviation (SD) matrix

**Value**

List of dcoeff matrices
dcoeff<-, scNMFSet-method

**dcoeff<-
Coeff SD matrix assignment**

**Description**
Coeff SD matrix assignment

**Usage**
dcoeff(object) <- value

**Arguments**
- object: Object containing dcoeff matrix
- value: List for assignment

**Value**
Updated object

---

dcoeff<-, scNMFSet-method

**Modify dcoeff matrices**

**Description**
Access and modify dcoeff matrices

**Usage**
```
## S4 replacement method for signature 'scNMFSet'
dcoeff(object) <- value
```

**Arguments**
- object: Object of class scNMFSet
- value: Coeff SD matrix to be substituted

**Value**
Updated object
factorize

Maximum likelihood factorization

Description
Performs single or multiple rank NMF factorization of count matrix using maximum likelihood

Usage
factorize(object, ranks = 2, nrun = 20, randomize = FALSE,
nsmpl = 1, verbose = 2, progress.bar = TRUE, Itmax = 10000,
ncnn.step = 40, criterion = "likelihood", linkage = "average",
Tol = 1e-05, store.connectivity = FALSE)

Arguments
object scNMFSet object containing count matrix.
ranks Rank for factorization; can be a vector of multiple values.
nrun No. of runs with different initial guess.
randomize Boolean; if TRUE, input matrix is randomized.
nsmpl No. of randomized samples to average over.
verbose The verbosity level: 3, each iteration output printed; 2, each run output printed;
1, each randomized sample output printed; 0, silent.
progress.bar Display progress bar when nrun > 1 and verbose = 1.
Itmax Maximum no. of iteration.
ncnn.step Minimum no. of steps with no change in connectivity matrix to achieve convergence.
criterion If 'likelihood', iteration stops when fractional changes in likelihood is below
tolerance Tol. If criterion = 'connectivity', iteration stops when connectivity matrix does not change for at least ncnn.step steps.
linkage Method to be sent to hclust in calculating cophenetic correlation.
Tol Tolerance for checking convergence with criterion = 'likelihood'.
store.connectivity Returns a list also containing connectivity data.

Details
The main input is the scNMFSet object with count matrix. This function performs non-negative
factorization and fills in the empty slots \texttt{basis}, \texttt{coeff}, and \texttt{ranks}.

When run with multiple values of \texttt{ranks}, factorization is repeated for each rank and the slot \texttt{measure}
contains quality measures of the ranks. The quality measure \texttt{likelihood} is negative the KL distance
of the fit to the target. With \texttt{nrun > 1}, the likelihood is the maximum among all runs.
The quality measure dispersion is the scalar measure of how far the connectivity matrix is from 0, 1. With increasing nrun, dispersion decreases from 1. nrun should be chosen such that dispersion does not change appreciably. With randomization, count matrix of object is shuffled. nsmpl can be used to average over multiple permutations. This averaging applies to each quality measure under a given rank.

Value

Object of class scNMFSet with factorization slots filled.

Examples

```r
set.seed(1)
x <- simulate_data(nfeatures=10,nsamples=c(20,20,60,40,30))
s <- scNMFSet(count=x)
s <- factorize(s,ranks=seq(2,8),nrun=5)
plot(s)
```

---

**feature_map**

*Plot heatmap of basis matrix*

Description

Generate heatmap of features derived from factorization of count data.

Usage

```r
feature_map(object, basis.matrix = NULL, rank, markers = NULL,
subtract.mean = TRUE, log = TRUE, max.per.cluster = 10,
feature.names = NULL, perm = NULL, main = "Feature map",
cscale = NULL, cex.cluster = 1, cex.feature = 0.5, mar = NULL,
...)
```

Arguments

- **object**
  - Object of class scNMFSet.

- **basis.matrix**
  - Basis matrix can be supplied instead of object.

- **rank**
  - Rank value for which the gene map is to be displayed. The object must contain the corresponding slot (one element of basis(object)[[k]] for which ranks(object)[[k]]==rank.

- **markers**
  - Vector of gene names containing markers to be included in addition to the metagene list. All entries of rowData(object) matching them will be added to the metagene list.

- **subtract.mean**
  - Process each rows of basis matrix W by standardization using the mean of elements within the row.

- **log**
  - If TRUE, subtract.mean uses geometric mean and division. Otherwise, use arithmetic mean and subtraction.
filter_cells

max.per.cluster  Maximum number of metagenes per cluster.
feature.names    Names to be used in the plot for features.
perm             Permutation of cluster IDs.
main             Main title.
cscale           Colors for heatmap.
cex.cluster      Cluster ID label size.
cex.feature      Feature ID label size.
mar              Margins for graphics::par.
...              Other arguments to be passed to image, and plot.

Details

This function uses image() and is more flexible than gene_map.
If object contains multiple ranks, only the requested rank’s basis matrix W will be displayed. As in gene_map, the features displayed in rows are selected by "max" scheme.

Value

NULL

Examples

set.seed(1)
x <- simulate_data(nfeatures=10, nsamples=c(20,20,60))
rownames(x) <- seq_len(10)

set.seed(1)
x <- simulate_data(nfeatures=10, nsamples=c(20,20,60))
rownames(x) <- seq_len(10)
colnames(x) <- seq_len(100)
s <- scNMFSet(count=x, rowData=seq_len(10), colData=seq_len(100))
s <- vb_factorize(s, ranks=seq(2,5))
plot(s)
feature_map(s, rank=3)

filter_cells  Filter cells with quality control criteria

Description

Remove low quality cell entries from object

Usage

filter_cells(object, umi.min = 0, umi.max = Inf, plot = TRUE,
             remove.zeros = TRUE)
filter_genes

Arguments

- **object**: scNMFSet object
- **umi.min**: Minimum UMI count for cell filtering
- **umi.max**: Maximum UMI count for cell filtering
- **plot**: If TRUE, the UMI count distribution of all cells will be displayed. Cells selected are colored red.
- **remove.zeros**: Remove rows/columns containing zeros only

Details

Takes as input scNMFSet object and plots histogram of UMI counts for each cell. Optionally, cells are filtered using minimum and maximum UMI counts. The resulting object is returned after removing empty rows and columns, if any.

Value

scNMFSet object with cells filtered.

Examples

```r
set.seed(1)
s <- scNMFSet(matrix(stats::rpois(n=1200,lambda=3),40,30))
s <- filter_cells(s,umi.min=10^2.0,umi.max=10^2.1)
```

filter_genes  
Filter genes with quality control criteria

Description

Select genes with high relative variance in count data for further analysis

Usage

```r
filter_genes(object, markers = NULL, vmr.min = 0, 
min.cells.expressed = 0, max.cells.expressed = Inf, 
rescue.genes = FALSE, progress.bar = TRUE, save.memory = FALSE, 
plot = TRUE, log = "xy", cex = 0.5)
```

Arguments

- **object**: scNMFSet object.
- **markers**: A vector containing marker genes to be selected. All rows in rowData that contain columns matching this set will be selected.
- **vmr.min**: Minimum variance-to-mean ratio for gene filtering.
- **min.cells.expressed**: Minimum no. of cells expressed for gene filtering.
max.cells.expressed

Maximum no. of cells expressed for gene filtering.

rescue.genes

Selected additional genes whose (non-zero) count distributions have at least one mode.

progress.bar

Display progress of mode-gene scan or VMR calculation with save.memory = TRUE.

save.memory

For a very large number of cells, calculate VMR row by row while avoiding calls to as.matrix(). Progress bar will be displayed unless progress.bar=FALSE.

plot

Plot the distribution of no. of cells expressed vs. VMR.

log

Axis in log-scale, c('x','y','xy').

cex

Symbol size for each gene in the plot.

Details

Takes as input scNMFSet object and scatterplot no. of cells expressed versus VMR (variance-to-mean ratio) for each gene. Optionally, genes are filtered using minimum VMR together with a range of no. of cells expressed.

Value

Object of class scNMFSet.

Examples

```r
set.seed(1)

s <- scNMFSet(matrix(stats::rpois(n=1200,lambda=3),40,30))

s <- filter_genes(s,vmr.min=1.0,min.cells.expressed=28,
                 rescue.genes=FALSE)
```

---

**gene_map**

*Plot heatmap of metagene matrix*

**Description**

Generate heatmap of metagenes derived from factorization of count data.

**Usage**

```r
gene_map(object, rank, markers = NULL, subtract.mean = TRUE,
         log = TRUE, max.per.cluster = 10, Colv = NA, gene.names = NULL,
         main = "Genes", col = NULL, ...)
```
Arguments

- **object**: Object of class scNMFSet.
- **rank**: Rank value for which the gene map is to be displayed. The object must contain the corresponding slot (one element of basis(object)[[k]] for which ranks(object)[[k]]==rank.
- **markers**: Vector of gene names containing markers to be included in addition to the metagenes. All entries of rowData(object) matching them will be added to the metagene list.
- **subtract.mean**: Process each rows of basis matrix \( W \) by standardization using the mean of elements within the row.
- **log**: If TRUE, subtract.mean uses geometric mean and division. Otherwise, use arithmetic mean and subtraction.
- **max.per.cluster**: Maximum number of metagenes per cluster.
- **Colv**: NA suppresses reordering and dendrogram of clusters along the column. See heatmap.
- **gene.names**: Names to be used in the plot for genes.
- **main**: Title of plot.
- **col**: Colors for the cluster panels on the left and top.
- **...**: Other arguments to be passed to heatmap, image, and plot.

Details

Wrapper for heatmap to display metagenes and associated basis matrix element magnitudes. Factorization results inside an object specified by its rank value will be retrieved, and metagene sets identified from clusters.

If object contains multiple ranks, only the requested rank’s basis matrix \( W \) will be displayed. The genes displayed in rows are selected by "max" scheme [Carmona-Saez, BMC Bioinformatics (2006), https://doi.org/10.1186/1471-2105-7-54]: for each cluster \( (k \text{ in } 1: ncol) \), rows of \( W \) are sorted by decreasing order of \( W[,k] \). Marker genes for \( k \) are those among the top \( nmarker \) for which \( W[,k] \) is maximum within each row.

Value

NULL

Examples

```r
set.seed(1)
x <- simulate_data(nfeatures=10,nsamples=c(20,20,60))
rownames(x) <- seq_len(10)
colnames(x) <- seq_len(100)
s <- scNMFSet(count=x,rowData=seq_len(10), colData=seq_len(100))
s <- vb_factorize(s,ranks=seq(2,5))
plot(s)
gene_map(s, rank=3)
```
**measure**

*Factorization measures in an Object*

**Description**
Retrieve or set factorization measures in an object

**Usage**

measure(object)

**Arguments**

object Object of class scNMFSet.

**Details**
Factorization under multiple rank values lead to measures stored in a data frame inside a slot measure. In maximum likelihood using factorize, this set of quality measures include dispersion and cophenetic coefficients for each rank. In Bayesian factorization using vb_factorize, log evidence for each rank is stored. measure(object) will return the data frame. measure(object) <- value can be used to modify it.

**Value**
Either NULL or a data frame containing measures.

**Examples**

```r
s <- scNMFSet(count=matrix(rpois(n=12, lambda=3), 4, 3))
s <- vb_factorize(s, ranks=seq(2, 4))
measure(s)
```

---

**measure,scNMFSet-method**

*Rank measure accessor*

**Description**
Rank measure accessor

**Usage**

```r
## S4 method for signature 'scNMFSet'
measure(object)
```
**Arguments**
- `object` Object containing measure

**Value**
- Data frame of measure

---

**Description**
Can be used to access and modify factorization measure

**Usage**
```r
measure(object) <- value
```

**Arguments**
- `object` Object of class `scNMFSet`
- `value` Measure to be substituted

**Value**
- Input object with updated measure

**Examples**
```r
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=3)
measure(s)[,-1] <- apply(measure(s)[,-1], c(1,2), round,digits=3)
measure(s)
```

---

**Description**
Modify factorization measure

**Usage**
```r
## S4 replacement method for signature 'scNMFSet'
measure(object) <- value
```
Arguments

- **object**: Object of class `scNMFSet`
- **value**: Measure to be substituted

Value

Input object with updated measure

Examples

```r
s <- scNMFSet(count=matrix(rpois(n=12, lambda=3), 4, 3))
s <- vb_factorize(s, ranks=3)
measure(s)[,-1] <- apply(measure(s)[,-1], c(1,2), round, digits=3)
measure(s)
```

---

**meta_gene.cv**

Meta gene table with CV

Description

Generates meta gene table with coefficient of variation

Usage

```r
meta_gene.cv(object = NULL, rank, basis.matrix = NULL, dbasis = NULL, max.per.cluster = 100, gene_names = NULL, subtract.mean = TRUE, log = TRUE, cv.max = Inf)
```

Arguments

- **object**: Main object containing factorization outcome
- **rank**: Rank for which meta gene is to be found
- **basis.matrix**: Basis matrix to work with. Only necessary when `object` is `NULL`
- **dbasis**: Variance of basis matrix. Only necessary when `object` is `NULL`
- **max.per.cluster**: Maximum meta genes per cluster.
- **gene_names**: Name of genes. If `NULL`, will be taken from row names.
- **subtract.mean**: Standardize magnitudes of basis elements by subtracting mean
- **log**: Use geometric mean.
- **cv.max**: Upper bound for CV in selecting meta genes.

Value

Data frame with meta genes and their CV in each column.
Examples

```r
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s, ranks=seq(2,8), nrun=5)
plot(s)
meta_gene.cv(s, rank=5)
```

---

meta_genes  
*Find metagenes from basis matrix*

Description

Retrieve a basis matrix from an object and find metagenes.

Usage

```r
meta_genes(object, rank, basis.matrix = NULL, max.per.cluster = 10,
gene_names = NULL, subtract.mean = TRUE, log = TRUE)
```

Arguments

- **object**: Object of class `scNMFSet`.
- **rank**: Rank value for which metagenes are to be found.
- **basis.matrix**: Instead of an object containing basis matrices, the matrix itself can be provided.
- **max.per.cluster**: Maximum number of metagenes per cluster.
- **gene_names**: Names of genes to replace row names of basis matrix.
- **subtract.mean**: Standardize the matrix elements with means within each row.
- **log**: Use geometric mean and division instead of arithmetic mean and subtraction with `subtract.mean`.

Value

List of vectors each containing metagene names of clusters.

Examples

```r
set.seed(1)
x <- simulate_data(nfeatures=10,nsamples=c(20,20,60))
rownames(x) <- seq_len(10)
colnames(x) <- seq_len(100)
s <- scNMFSet(count=x,rowData=seq_len(10),colData=seq_len(100))
s <- vb_factorize(s, ranks=seq(2,5))
meta_genes(s, rank=4)
```
newick

Generate Newick format tree string from tree list object

Description
Generate Newick format tree string from tree list object

Usage
newick(tree, parent = "1.1", string = "")

Arguments
- **tree**: Tree list object from `build_tree`
- **parent**: Parent ID
- **string**: Newick string of parent tree

Value
String of newick tree

Examples
```r
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s, ranks=seq(2, 8), nrun=5)
tree <- build_tree(s, rmax=5)
new <- newick(tree=tree)
new
```

normalize_count

Normalize count data

Description
Rescale count matrix entries such that all cells have the same library size.

Usage
normalize_count(object)

Arguments
- **object**: scNMFSet object.
Details

For analysis purposes, it is sometimes useful to rescale integer count data into floats such that all cells have the same median counts. This function will calculate the median of all UMI counts of cells (total number of RNAs derived from each cell). All count data are then rescaled such that cells have uniform UMI count equal to the median.

Value

scNMFSet object with normalized count data.

Examples

```r
library(Matrix)
set.seed(1)
s <- scNMFSet(count=matrix(rpois(n=1200,lambda=3),40,30))
colMeans(counts(s))
s <- normalize_count(s)
colMeans(counts(s))
```

<table>
<thead>
<tr>
<th>optimal_rank</th>
<th>Determine optimal rank</th>
</tr>
</thead>
</table>

Description

Takes as main argument scNMFSet object containing factorized output and estimate the optimal rank.

Usage

`optimal_rank(object, df = 10, BF.threshold = 3, type = NULL, m = NULL)`

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>object</td>
<td>scNMFSet object containing factorization output, or data frame containing the rank-evidence profile.</td>
</tr>
<tr>
<td>df</td>
<td>Degrees of freedom for split fit. Upper bound is the total number of data points (number of rank values scanned).</td>
</tr>
<tr>
<td>BF.threshold</td>
<td>Bayes factor threshold for statistical threshold.</td>
</tr>
<tr>
<td>type</td>
<td>c(1, 2). Type 1 is where there is a clear maximum. Type 2 is where marginal likelihood reaches a maximal level and stays constant. If omitted, the type will be inferred from data.</td>
</tr>
<tr>
<td>m</td>
<td>Number of features (e.g., genes) in the count matrix. Only necessary when object is of type data.frame.</td>
</tr>
</tbody>
</table>
Details

The input object is used along with Bayes factor threshold to determine the heterogeneity type (1 or 2) and the optimal rank. If evidence(rank 1)/evidence(rank2) > BF.threshold, rank 1 is favorable than rank 2.

Value

List containing type and ropt (optimal rank).

Examples

```r
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s, ranks=seq(2,8), nrun=5)
plot(s)
optimal_rank(s)
```

Description

Gene variance to mean ratio and the number of expressing cells are plotted.

Usage

```r
plot_genes(object, vmr = NULL, ncexpr = NULL, selected_genes = NULL,
variable_genes = NULL, mode_genes = NULL, marker_genes = NULL,
save.memory = FALSE, progress.bar = TRUE, log = "xy", cex = 0.5)
```

Arguments

- **object**: Object containing count data
- **vmr**: Variance to mean ratio (VMR)
- **ncexpr**: Number of cells expressing each gene
- **selected_genes**: Logical vector specifying genes selected
- **variable_genes**: Logical vector specifying genes with high VMR
- **mode_genes**: Logical vector specifying genes with nonzero modes
- **marker_genes**: Logical vector specifying marker genes
- **save.memory**: If TRUE, calculate VMR using slower method to save memory. Not used when gene lists are supplied.
- **progress.bar**: Display progress bar for VMR calculation. Not used when gene lists are supplied.
- **log**: Axis in log-scale, c(‘x’, ‘y’, ‘xy’).
- **cex**: Symbol size for genes (supplied to plot()).
Details

This function can be called separately or is also called within `filter_genes` by default. In the latter case, parameters other than object will have been already filled. If called separately with NULL gene lists, VMR is recalculated but gene selection is not done.

Value

NULL

Examples

```r
set.seed(1)
s <- scNMFSet(matrix(stats::rpois(n=1200,lambda=3),40,30))
plot_genes(s)

plot_tree(tree, direction = "rightwards", cex = 0.7, ...)
```

Description

Visualize the output of `build_tree` as a dendrogram.

Usage

`plot_tree(tree, direction = "rightwards", cex = 0.7, ...)`

Arguments

- `tree` List containing tree structure. Output from `build_tree`
- `direction` c('rightwards', 'downwards'); the direction of dendrogram
- `cex` Font size of edge/tip labels
- `...` Other parameters to `plot.phylo`

Details

Uses `plot.phylo` to visualize cluster tree.

Value

NULL

Examples

```r
set.seed(1)
x <- simulate_whx(nrow=50,ncol=100,rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s,ranks=seq(2,8),nrun=5)
```
ranks

Description
Retrieve or set the rank values in an object

Usage
ranks(object)

Arguments
object Object of class scNMFSet.

Details
Ranks for which factorization has been performed are stored in slot ranks of scNMFSet object. ranks(object) will return the rank vector. ranks(object) <- value can be used to modify it.

Value
Either NULL or vector.

Examples
s <- scNMFSet(matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s,ranks=seq(2,4))
ranks(s)

ranks,scNMFSet-method Rank accessor

Description
Rank accessor

Usage
## S4 method for signature 'scNMFSet'
ranks(object)

Arguments
object Object containing rank values

Value
Vector of rank values
Generics for ranks assignment

**Description**
Replace ranks slot of `scNMFSset` object

**Usage**
```
ranks(object) <- value
```

**Arguments**
- `object`: Object of class `scNMFSset`
- `value`: Rank values (vector) to be substituted

**Value**
Input object with updated ranks

**Examples**
```
s <- scNMFSset(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=seq(2,3))
ranks(s) <- c('two','three')
ranks(s)
```

Modify ranks

**Description**
Replace ranks slot of `scNMFSset` object

**Usage**
```
## S4 replacement method for signature 'scNMFSset'
ranks(object) <- value
```

**Arguments**
- `object`: Object of class `scNMFSset`
- `value`: Rank values (vector) to be substituted
Value

Input object with updated ranks

Examples

```r
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
s <- vb_factorize(s, ranks=seq(2,3))
ranks(s) <- c('two','three')
ranks(s)
```

Description

Read count, gene, and barcode annotation data in 10x format and create an object of class `scNMFSet`.

Usage

```r
read_10x(dir, count = "matrix.mtx", genes = "genes.tsv",
barcodes = "barcodes.tsv", remove.zeros = TRUE)
```

Arguments

- `dir` Name of directory containing data files.
- `count` Name of count matrix file.
- `genes` Name of gene annotation file.
- `barcodes` Name of cell annotation file.
- `remove.zeros` If `TRUE`, empty rows/columns are removed.

Details

Files for `count`, `genes`, and `barcodes` are assumed to be present in `dir`. Count data are in sparse "Matrix Market" format (https://math.nist.gov/MatrixMarket/formats.html).

Value

Object of class `scNMFSet`

Examples

```r
library(S4Vectors)
s <- scNMFSet(count=matrix(rpois(n=12,lambda=3),4,3))
rowData(s) <- DataFrame(seq_len(4))
colData(s) <- DataFrame(seq_len(3))
write_10x(s,dir='.')
s <- read_10x(dir='.
```

```r
```
**remove_zeros**

*Remove rows or columns that are empty from an object*

**Description**

Remove rows or columns that are empty from an object

**Usage**

```r
remove_zeros(object)
```

**Arguments**

- `object` Object containing data

**Value**

Object with empty rows/columns removed

**Examples**

```r
set.seed(1)
x <- matrix(rpois(n=100,lambda=0.1),10,10)
s <- scNMFSet(count=x,remove.zeros=FALSE)
s2 <- remove_zeros(s)
s2
```

---

**rename_tips**

*Rename tips of trees with cell types*

**Description**

Rename tips of trees with cell types

**Usage**

```r
rename_tips(tree, rank, tip.labels)
```

**Arguments**

- `tree` List containing tree
- `rank` Rank value of which tip names are to be replaced
- `tip.labels` Vector of new names for tips

**Value**

List containing tree with updated tip labels
Examples

```r
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s, ranks=seq(2, 8), nrun=5)
tree <- build_tree(s, rmax=5)
tree <- rename_tips(tree, rank=5, tip.labels=letters[seq_len(5)])
tree
```

rowData,scNMFSet-method

**Feature annotation accessor**

Description

Feature annotation accessor

Usage

```r
## S4 method for signature 'scNMFSet'
rowData(x)
```

Arguments

- `x` Object containing data

Value

DataFrame of feature annotation

Examples

```r
x <- matrix(rpois(n=12, lambda=3), 4, 3)
rownames(x) <- seq_len(4)
colnames(x) <- seq_len(3)
s <- scNMFSet(count=x, rowData=seq_len(4), colData=seq_len(3))
rowData(s)
```
rowData<-,scNMFSet-method

Gene annotation assignment

Description
Gene annotation assignment

Usage
## S4 replacement method for signature 'scNMFSet'
rowData(x) <- value

Arguments
x Object containing data
value DataFrame of row annotation to be substituted

Value
Row annotation DataFrame

scNMFSet Create scNMFSet object

Description
Object derived from SingleCellExperiment

Usage
scNMFSet(count = NULL, ..., remove.zeros = TRUE)

Arguments
count Count matrix
... Other parameters of SingleCellExperiment
remove.zeros Remove empty rows and columns

Value
Object of class scNMFSet.

Examples
count <- matrix(rpois(n=12,lambda=2),4,3)
s <- scNMFSet(count=count)
s
Description

S4 class derived from SingleCellExperiment that can store single-cell count matrix, gene and cell annotation data frames, and factorization factors as well as quality measures for rank determination.

Usage

```r
## S4 method for signature 'scNMFSet,ANY'
plot(x)
```

Arguments

- `x` Object containing measure

Value

Object of class scNMFSet

NULL

Methods (by generic)

- `plot`: Plot measures of an object. For quality measures derived from maximum likelihood inference, dispersion and cophenetic will be plotted separately.
  For measure derived from Bayesian inference, log evidence as a function of rank values will be plotted.

Slots

- `assays` Named list for count matrix counts.
- `rowData` DataFrame for gene (feature) names and annotations in columns.
- `colData` DataFrame for cell IDs and other annotations in columns (e.g., barcodes, cell types).
- `ranks` Vector for rank values for which factorization has been performed.
- `basis` List (of length equal to that of ranks) of basis matrices $W$ from factorization; dimension `nrow x rank`, where `nrow` is no. of rows in count.
- `coeff` List (of length equal to that of ranks) of coefficient matrices $H$ from factorization; dimension `rank x ncol`, where `ncol` is no. of columns in count.
- `measure` Data frame of factorization quality measures for each rank (likelihood and dispersion).

Other slots inherited from SingleCellExperiment class are not explicitly used.
Examples

library(S4Vectors)
# toy matrix
ngenes <- 8
ncells <- 5
mat <- matrix(rpois(n=ngenes*ncells,lambda=3),ngenes,ncells)

abc <- letters[seq_len(ngenes)]
ABC <- LETTERS[seq_len(ncells)]
gen <- DataFrame(gene_id=abc)
cell <- DataFrame(cell_id=ABC)
rownames(mat) <- rownames(gen) <- abc
colnames(mat) <- rownames(cell) <- ABC

# create scNMFSet object
s <- scNMFSet(count=mat,rowData=gen,colData=cell)
# alternative ways
s2 <- scNMFSet(count=mat)
s2 <- scNMFSet(assays=list(counts=mat))

# show dimensions
dim(s)

# show slots
rowData(s)

# modify slots
colData(s) <- DataFrame(cell_id=seq_len(ncells),
    cell_type=c(rep('tissue1',2),
        rep('tissue2',ncells-2)))
colData(s)

show,scNMFSet-method  Display object

Description

Display the class and dimension of an object

Object name itself on command line or (show(object)) will display class and dimensionality

Usage

## S4 method for signature 'scNMFSet'
show(object)

Arguments

object  Object of class scNMFSet
simulate_data

Value

NULL

Examples

s <- scNMFSets(matrix(rpois(n=12,lambda=3),4,3))
show(s)

simulate_data

Generate simulated data for factorization

Description

Use one of two schemes to generate simulated data suitable for testing factorization.

Usage

simulate_data(nfeatures, nsamples, generate.factors = FALSE,
               nfactor = 10, alpha0 = 0.5, shuffle = TRUE)

Arguments

nfeatures Number of features m (e.g., genes).
nsamples Vector of sample sizes in each cluster. Rank r is equal to the length of this
            vector. Sum of elements is the total sample size n.
generate.factors
               Generate factor matrices W and H, each with dimension n x r and r x n. If FALSE,
               factor matrices are not used and count data are generated directly from r multi-
               nomials for m genes.
nfactor Total RNA count of multinomials for each cluster with generate.factors =
            FALSE. Small nfactor will yield sparse count matrix.
alpha0 Variance parameter of Dirichlet distribution from which multinomial probabili-
            ties are sampled with generate.factors = FALSE.
shuffle Randomly permute rows and columns of count matrix.

Details

In one scheme (generate.factors = TRUE), simulated factor matrices W and H are used to build
count data X = WH. In the second scheme, factor matrices are not used and X is sampled directly from
r (rank requested) sets of multinomial distributions.

Value

If generate.factors = TRUE, list of components w (basis matrix, nfeatures x rank), h (coefficient
matrix, rank x ncells, where ncells is equal to n, the sum of nsamples), and x, a matrix of
Poisson deviates with mean W x H. If generate.factors = FALSE, only the count matrix x is in the
list.
simulate_whx

Simulate factor matrices and data using priors

Description

Under Bayesian formulation, use prior distributions of factor matrices and generate simulated data

Usage

simulate_whx(nrow, ncol, rank, aw = 0.1, bw = 1, ah = 0.1, bh = 1)

Arguments

nrow Number of features (genes).
ncol Number of cells (samples).
rank Rank (ncol of W, nrow of H).
aw Shape parameter of basis prior.
bw Mean of basis prior. Scale parameter is equal to aw/bw.
ah Shape parameter of coefficient prior.
bh Mean of coefficient prior. Scale parameter is equal to ah/bh.

Details

Basis W and coefficient matrices H are sampled from gamma distributions (priors) with shape (aw, ah) and mean (bw, bh) parameters. Count data X are sampled from Poisson distribution with mean values given by WH.

Value

List with elements w, h, and x, each containing basis, coefficient, and count matrices.

Examples

set.seed(1)
x <- simulate_data(nfeatures=10,nsamples=c(20,20,60,40,30))
s <- scNMFSets(x)
s

set.seed(1)
x <- simulate_whx(nrow=50,ncol=100,rank=5)
s <- scNMFSets(count=x$x)
s <- vb_factorize(s,ranks=seq(2,8),nrun=5)
plot(s)
vb_factorize

Bayesian NMF inference of count matrix

Description
Perform variational Bayes NMF and store factor matrices in object

Usage
vb_factorize(object, ranks = 2, nrun = 1, verbose = 2,
   progress.bar = TRUE, initializer = "random", Itmax = 10000,
   hyper.update = rep(TRUE, 4), gamma.a = 1, gamma.b = 1,
   Tol = 1e-05, hyper.update.n0 = 10, hyper.update.dn = 1,
   connectivity = TRUE, fudge = NULL, ncores = 1, useC = TRUE,
   unif.stop = TRUE)

Arguments
- object: scNMFSet object containing count matrix.
- ranks: Rank for factorization; can be a vector of multiple values.
- nrun: No. of runs with different initial guesses.
- verbose: The verbosity level: 3, each iteration output printed; 2, each run output printed; 1, each randomized sample output printed; 0, silent.
- progress.bar: Display progress bar with verbose = 1 for multiple runs.
- initializer: If 'random', randomized initial conditions; 'svd2' for singular value decomposed initial condition.
- Itmax: Maximum no. of iteration.
- hyper.update: Vector of four logicals, each indicating whether hyperparameters c(aw, bw, ah, bh) should be optimized.
- gamma.a: Gamma distribution shape parameter.
- gamma.b: Gamma distribution mean. These two parameters are used for fixed hyperparameters with hyper.update elements FALSE.
- Tol: Tolerance for terminating iteration.
- hyper.update.n0: Initial number of steps in which hyperparameters are fixed.
- hyper.update.dn: Step intervals for hyperparameter updates.
- connectivity: If TRUE, connectivity and dispersion will be calculated after each run. Can be turned off to save memory.
- fudge: Small positive number used as lower bound for factor matrix elements to avoid singularity. If fudge = NULL (default), it will be replaced by .Machine$double.eps. Can be set to 0 to skip regularization.
- ncores: Number of processors (cores) to run. If ncores > 1, parallelization is attempted.
- useC: Use C++ version of updates for speed.
- unif.stop: Terminate if any of columns in basis matrix is uniform.
Details

The main input is the scNMFSet object with count matrix. This function performs non-negative factorization using Bayesian algorithm and gamma priors. Slots basis, coeff, and ranks are filled.

When run with multiple values of ranks, factorization is repeated for each rank and the slot measure contains log evidence and optimal hyperparameters for each rank. With \( nrun > 1 \), the solution with the maximum log evidence is stored for a given rank.

Value

Object of class scNMFSet with factorization slots filled.

Examples

```r
set.seed(1)
x <- simulate_whx(nrow=50,ncol=100,rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s,ranks=seq(2,8),nrun=5)
plot(s)
```

visualize_clusters

**Visualize clusters**

Description

Use tSNE to generate two-dimensional map of coefficient matrix.

Usage

```r
visualize_clusters(object, rank, verbose = FALSE, cex = 1, cex.names = 0.7, ...)
```

Arguments

- **object**: scNMF object.
- **rank**: Rank value to extract from object.
- **verbose**: Print tSNE messages.
- **cex**: Symbol size in tSNE plot.
- **cex.names**: Font size of labels in count barplot.
- **...**: Other parameters to send to Rtsne.

Details

It retrieves a coefficient matrix \( H \) from an object and use its elements to assign each cell into clusters. t-Distributed Stochastic Neighbor Embedding (t-SNE; [https://lvdmaaten.github.io/tsne/](https://lvdmaaten.github.io/tsne/)) is used to visualize the clustering in 2D. Also plotted is the distribution of cell counts for all clusters.
write_10x

Value

NULL

Examples

set.seed(1)
x <- simulate_data(nfeatures=10,nsamples=c(20,20,60,40,30))
rownames(x) <- seq_len(10)
colnames(x) <- seq_len(170)
s <- scNMFSet(count=x,rowData=seq_len(10),colData=seq_len(170))
s <- vb_factorize(s,ranks=seq(2,5))
visualize_clusters(s,rank=5)

write_10x

Write 10x data files

Description

Use an object and write count and annotation files in 10x format.

Usage

write_10x(object, dir, count = "matrix.mtx", genes = "genes.tsv",
           barcodes = "barcodes.tsv", quote = FALSE)

Arguments

  object Object of class scNMFSet containing count data
  dir Directory where files are to be written.
  count File name for count matrix.
  genes File name for gene annotation.
  barcodes File name for cell annotation.
  quote Suppress quotation marks in output files.

Value

NULL

Examples

set.seed(1)
x <- matrix(rpois(n=12,lambda=3),4,3)
rownames(x) <- seq_len(4)
colnames(x) <- seq_len(3)
s <- scNMFSet(count=x,rowData=seq_len(4),colData=seq_len(3))
write_10x(s,dir='.')
write_meta  
Write meta genes to a file

Description
Write a csv file of meta gene lists from input list

Usage
write_meta(meta, file)

Arguments
meta  List of meta genes output from meta_genes
file  Output file name

Value
NULL

Examples
set.seed(1)
x <- simulate_whx(nrow=50, ncol=100, rank=5)
s <- scNMFSet(x$x)
s <- vb_factorize(s, ranks=seq(2,8), nrun=5)
plot(s)
m <- meta_genes(s, rank=5)
write_meta(m, file='meta.csv')

Subsetting scNMFSet object

Description
Subsetting scNMFSet object

Usage
## S4 method for signature 'scNMFSet,ANY,ANY,ANY'
x[i, j]
Arguments

\( x \)  
Object to be subsetted

\( i \)  
row index

\( j \)  
column index

Value

Subsetted object
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