Package 'ccfindR'

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Title Cancer Clone Finder

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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.

License GPL (>= 2)

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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.na	Remove gene sets with no overlap
p.value	Estimatte p values using permutation
nperm	No. of permutation replicates
progress.bar	Display progress bar for p value computation
grp.prefix	Gene name prefix to search for with wildcard matches in query

Details

If obj is of clas 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

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP (2005). "Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles." *Proc. Natl. Acad. Sci., USA*, **102**(43), 15545–15550. doi:10.1073/pnas.0506580102.

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]]
```

---

basis,scNMFSet-method *Basis matrix accessor*

Description

Basis matrix accessor

Usage

```
## S4 method for signature 'scNMFSet'
basis(object)
```

Arguments

object	Object containing basis matrix

Value

List of basis matrices

basis<- *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)
```

basis<-,scNMFSet-method
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

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]],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

```
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

```
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 *ccfindR: Cancer Clone FindeR*

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).

References

Woo J, Winterhoff BJ, Starr TK, Aliferis C, Wang J (2019). "De novo prediction of cell-type complexity in single-cell RNA-seq and tumor microenvironments." *Life Sci. Alliance*, **2**, e201900443. http://dx.doi.org/10.26508/lsa.201900443.

cell_map *Plot heatmap of clustering coefficient matrix*

Description

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

Usage

```
cell_map(object, rank, main = "Cells", ...)
```

Arguments

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

Value

NULL

Examples

```
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

```
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

```
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, rank=5)
table(cid)
```

coeff *Coefficient matrices in an Object*

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]]
```

coeff,scNMFSet-method *Coefficient matrix accessor*

Description

Coefficient matrix accessor

Usage

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

Arguments

object	Object containing coefficient matrix

Value

List of coefficient matrices

`coeff<-` *Generics for coefficient matrix assignment*

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

```
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

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

Arguments

x	Object containing sample annotation

Value

Column annotation DataFrame

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
```

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)
```

---

counts<-,scNMFSet-method

Assignment of count matrix

Description

Count matrix can be modified

Usage

```
## S4 replacement method for signature 'scNMFSet'
counts(object) <- value
```

Arguments

object	Object containing count
value	Matrix-like object for replacement

Value

Object with updated count

Examples

```
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,scNMFSet-method *Basis SD matrix accessor*

Description

Basis SD matrix accessor

Usage

```
## S4 method for signature 'scNMFSet'
dbasis(object)
```

Arguments

object	Object containing basis standard deviation (SD) matrix

Value

List of dbasis matrices

dbasis<- *Basis SD matrix assignment*

Description

Basis SD matrix assignment

Usage

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

Arguments

object	Object containing dbasis matrix
value	List for assignment

Value

Updated object

dbasis<-,scNMFSet-method *Modify dbasis matrices*

Description

Access and modify dbasis matrices

Usage

```
## S4 replacement method for signature 'scNMFSet'
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,scNMFSet-method *Coeffcient SD matrix accessor*

Description

Coeffcient SD matrix accessor

Usage

```
## S4 method for signature 'scNMFSet'
dcoeff(object)
```

Arguments

object	Object containing coeffient standard deviation (SD) matrix

Value

List of dcoeff matrices

`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 `basis`, `coeff`, and `ranks`.

When run with multiple values of `ranks`, factorization is repeated for each rank and the slot `measure` contains quality measures of the ranks. The quality measure `likelihood` is negative the KL distance of the fit to the target. With `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

```
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

```
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 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.
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)
```

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

```
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

```
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

```
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

```
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 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

```
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 coeeficients 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

```
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

```
## S4 method for signature 'scNMFSet'
measure(object)
```

Arguments

object	Object containing measure

Value

Data frame of measure

---

measure<- *Generics for factorization measure assignment*

Description

Can be used to access and modify factorization measure

Usage

```
measure(object) <- value
```

Arguments

object	Object of class scNMFSet
value	Measure to be substituted

Value

Input object with updated measure

Examples

```
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)
```

---

measure<-,scNMFSet-method *Modify factorization measure*

Description

Can be used to access and modify factorization measure

Usage

```
## 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

```
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

```
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

```
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

```
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

```
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

```
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)
nw <- newick(tree=tree)
nw
```

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

```
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))
```

`optimal_rank` *Determine optimal rank*

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

`object`	`scNMFSet` object containing factorization output, or data frame containing the rank-evidence profile.
`df`	Degrees of freedom for split fit. Upper bound is the total number of data points (number of rank values scanned).
`BF.threshold`	Bayes factor threshold for statistical threshold.
`type`	`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.
`m`	Number of features (e.g., genes) in the count matrix. Only necessary when `object` is of type `data.frame`.

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.treshold`, rank 1 is favorable than rank 2.

Value

List containing `type` and `ropt` (optimal rank).

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)
optimal_rank(s)
```

---

plot_genes *Plot gene variance distributions*

Description

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

Usage

```
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 specifing genes selected
`variable_genes`	Logical vector specifing 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

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

---

plot_tree *Plot cluster tree*

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

```
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)
plot_tree(tree)
```

ranks *Rank values in an Object*

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

ranks<- *Generics for ranks assignment*

Description

Replace ranks slot of scNMFSet object

Usage

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

Arguments

object	Object of class scNMFSet
value	Rank values (vector) to be substituted

Value

Input object with updated ranks

Examples

```
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)
```

ranks<-,scNMFSet-method *Modify ranks*

Description

Replace ranks slot of scNMFSet object

Usage

```
## S4 replacement method for signature 'scNMFSet'
ranks(object) <- value
```

Arguments

object	Object of class scNMFSet
value	Rank values (vector) to be substituted

Value

Input object with updated ranks

Examples

```
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)
```

`read_10x` *Read 10x data and generate scNMF object*

Description

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

Usage

```
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

```
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='.')
s
```

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

Description

Remove rows or columns that are empty from an object

Usage

```
remove_zeros(object)
```

Arguments

object	Object containing data

Value

Object with empty rows/columns removed

Examples

```
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

```
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

```
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

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

Arguments

x	Object containing data

Value

DataFrame of feature annotation

Examples

```
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
```

scNMFSet-class *Class* scNMFSet *for storing input data and results*

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

```
## 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)]
genes <- DataFrame(gene_id=abc)
cells <- DataFrame(cell_id=ABC)
rownames(mat) <- rownames(genes) <- abc
colnames(mat) <- rownames(cells) <- ABC

# create scNMFSet object
s <- scNMFSet(count=mat,rowData=genes,colData=cells)
# 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

Value

NULL

Examples

```
s <- scNMFSet(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 multinomials 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 probabilities 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.

Examples

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

---

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_whx(nrow=50,ncol=100,rank=5)
s <- scNMFSet(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 indcating 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

```
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

```
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/) is used to visualize the clustering in 2D. Also plotted is the distribution of cell counts for all clusters.

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')
```

[,scNMFSet,ANY,ANY,ANY-method *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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