

# Package ‘ccfindR’

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(>= 0.7)

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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	<i>Cell type assignment via GSEA</i>
----------------	--------------------------------------

---

### 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	Estimate 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 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**

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 <code>coeff(object)[[k]]</code> for which <code>ranks(object)[[k]]==rank</code> .
main	Title of plot.
...	Other arguments to be passed to <a href="#">heatmap</a> , <a href="#">image</a> , and <a href="#">plot</a> .

**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	<i>Assign cells into clusters</i>
------------	-----------------------------------

---

**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	<i>Coefficient matrices in an Object</i>
-------	--

---

**Description**

Retrieve or set the coefficient matrices from factorization in an object

**Usage**

```
coeff(object)
```



**Arguments**

object            Object of class scNMFSets.

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

---

coeff,scNMFSets-method    *Coefficient matrix accessor*

---

**Description**

Coefficient matrix accessor

**Usage**

```
## S4 method for signature 'scNMFSets'
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	<i>Coeff SD matrix accessor</i>
--------	---------------------------------

---

**Description**

Coeff SD matrix accessor

**Usage**

```
dcoeff(object)
```

**Arguments**

object	Object containing dcoeff matrix
--------	---------------------------------

**Value**

List of dcoeff matrices

---

dcoeff, scNMFSet-method

*Coefficient SD matrix accessor*

---

### Description

Coefficient SD matrix accessor

### Usage

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

### Arguments

object            Object containing coefficient 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<- ,scNMFSets-method  
*Modify dcoeff matrices*

---

**Description**

Access and modify dcoeff matrices

**Usage**

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

**Arguments**

object	Object of class scNMFSets
value	Coeff SD matrix to be substituted

**Value**

Updated object

---

factorize	<i>Maximum likelihood factorization</i>
-----------	---

---

**Description**

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

**Usage**

```
factorize(object, ranks = 2, nrun = 20, randomize = FALSE,
  nsmp1 = 1, verbose = 2, progress.bar = TRUE, Itmax = 10000,
  ncnns.step = 40, criterion = "likelihood", linkage = "average",
  Tol = 1e-05, store.connectivity = FALSE)
```

**Arguments**

object	scNMFSets 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.
nsmp1	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 <code>basis(object)[[k]]</code> for which <code>ranks(object)[[k]]==rank</code> ).
markers	Vector of gene names containing markers to be included in addition to the metagenes. All entries of <code>rowData(object)</code> matching them will be added to the metagene list.
subtract.mean	Process each rows of basis matrix <i>W</i> by standardization using the mean of elements within the row.
log	If TRUE, <code>subtract.mean</code> 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: <code>:par</code> .
...	Other arguments to be passed to <code>image</code> , and <code>plot</code> .

**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	scNMFSets 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 scNMFSets 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

scNMFSets object with cells filtered.

### Examples

```
set.seed(1)
s <- scNMFSets(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 <code>scNMFSet</code> .
rank	Rank value for which the gene map is to be displayed. The object must contain the corresponding slot (one element of <code>basis(object)[[k]]</code> for which <code>ranks(object)[[k]]==rank</code> ).
markers	Vector of gene names containing markers to be included in addition to the metagenes. All entries of <code>rowData(object)</code> matching them will be added to the metagene list.
subtract.mean	Process each rows of basis matrix <i>W</i> by standardization using the mean of elements within the row.
log	If TRUE, <code>subtract.mean</code> 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 <a href="#">heatmap</a> .
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 <a href="#">heatmap</a> , <a href="#">image</a> , and <a href="#">plot</a> .

**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	<i>Factorization measures in an Object</i>
---------	--

---

**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**

```
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	<i>Find metagenes from basis matrix</i>
------------	---

---

### 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	<i>Generate Newick format tree string from tree list object</i>
--------	---

---

### Description

Generate Newick format tree string from tree list object

### Usage

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

**Arguments**

tree	Tree list object from <a href="#">build_tree</a>
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	<i>Normalize count data</i>
-----------------	-----------------------------

---

**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	<i>Determine optimal rank</i>
--------------	-------------------------------

---

### Description

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

### Usage

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

### Arguments

<code>object</code>	<code>scNMFSets</code> object containing factorization output, or data frame containing the rank-evidence profile.
<code>df</code>	Degrees of freedom for split fit. Upper bound is the total number of data points (number of rank values scanned).
<code>BF.threshold</code>	Bayes factor threshold for statistical threshold.
<code>type</code>	<code>c(1, 2)</code> . 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.
<code>m</code>	Number of features (e.g., genes) in the count matrix. Only necessary when object is of type <code>data.frame</code> .

### Details

The input object is used along with Bayes factor threshold to determine the heterogeneity type (1 or 2) and the optimal rank. If  $\text{evidence}(\text{rank } 1)/\text{evidence}(\text{rank } 2) > \text{BF.threshold}$ , 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 <- scNMFSets(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, ncepr = 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)
ncepr	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

```
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 <code>build_tree</code>
direction	c('rightwards', 'downwards'); the direction of dendrogram
cex	Font size of edge/tip labels
...	Other parameters to <code>plot.phylo</code>

### 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 <code>scNMFSet</code> .
--------	---



**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	<i>Read 10x data and generate scNMF object</i>
----------	--

---

**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	<i>Remove rows or columns that are empty from an object</i>
--------------	---

---

**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	<i>Rename tips of trees with cell types</i>
-------------	---

---

**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,scNMFSets-method

*Feature annotation accessor*

---

### Description

Feature annotation accessor

### Usage

```
## S4 method for signature 'scNMFSets'  
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 <- scNMFSets(count=x,rowData=seq_len(4),colData=seq_len(3))  
rowData(s)
```

---

rowData<-,scNMFSets-method

*Gene annotation assignment*

---

### Description

Gene annotation assignment

### Usage

```
## S4 replacement method for signature 'scNMFSets'  
rowData(x) <- value
```

### Arguments

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

### Value

Row annotation DataFrame

---

scNMFSet	<i>Create scNMFSet object</i>
----------	-------------------------------

---

**Description**

Object derived from [SingleCellExperiment](#)

**Usage**

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

**Arguments**

count	Count matrix
...	Other parameters of <a href="#">SingleCellExperiment</a>
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	<i>Class scNMFSet for storing input data and results</i>
----------------	--

---

**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



---

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  $n \times r$  and  $r \times n$ . 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 <code>generate.factors = FALSE</code> .
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,  $n_{\text{features}} \times \text{rank}$ ),  $h$  (coefficient matrix,  $\text{rank} \times n_{\text{cells}}$ , where  $n_{\text{cells}}$  is equal to  $n$ , the sum of  $n_{\text{samples}}$ ), and  $x$ , a matrix of Poisson deviates with mean  $W \times H$ . 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 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 <code>.Machine\$double.eps</code> . Can be set to 0 to skip regularization.
ncores	Number of processors (cores) to run. If <code>ncores &gt; 1</code> , 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 <code>Rtsne</code> .

**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	<i>Write meta genes to a file</i>
------------	-----------------------------------

---

**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 <- scNMFSets(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')
```

---

[,scNMFSets,ANY,ANY,ANY-method	<i>Subsetting scNMFSets object</i>
--------------------------------	------------------------------------

---

**Description**

Subsetting scNMFSets object

**Usage**

```
## S4 method for signature 'scNMFSets,ANY,ANY,ANY'
x[i, j]
```

**Arguments**

x	Object to be subsetted
i	row index
j	column index

**Value**

Subsetting object

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