Package ‘DR.SC’

Type Package

Title Joint Dimension Reduction and Spatial Clustering

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Description Joint dimension reduction and spatial clustering is conducted for
Single-cell RNA sequencing and spatial transcriptomics data, and more details can be referred to
Wei Liu, Xu Liao, Yi Yang, Huazhen Lin, Joe Yeong, Xi-
ang Zhou, Xingjie Shi and Jin Liu. (2022) <doi:10.1093/nar/gkac219>. It is not only computa-
tionally efficient and scalable to the sample size increment, but also is capable of choosing
the smoothness parameter and the number of clusters as well.

License GPL-3

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dlpfc151510               A human dorsolateral prefrontal cortex data

**Description**

A human dorsolateral prefrontal cortex dataset measured on the Visium platform, which includes 4634 spots and 500 genes, a subset of raw dataset at https://github.com/LieberInstitute/spatialLIBD.

**Note**

nothing

**Author(s)**

Wei Liu

**References**

None

**Examples**

data("dlpfc151510")
Joint dimension reduction and spatial clustering for scRNA-seq and spatial transcriptomics data

Usage

```r
## S3 method for class 'Var'
DR.SC(seu, K, q=15, platform= c('Visium', "ST", "Other_SRT", "scRNAseq"),...)
```

Arguments

- `seu` an object of class "Seurat". The details of this object are given under 'Details'.
- `q` a positive integer, specify the number of latent features to be extracted, default as 15.
- `K` a positive integer or integer vector, specify the number of clusters. When K is a vector, it is automatically selected by MBIC criteria. Users can also use BIC and AIC to select K or refine model with MBIC by argument `pen.const` in the function `selectModel`.
- `platform` a string, specify the platform of the provided data, default as "Visium". There are more platforms to be chosen, including ("Visium", "ST", "Other_SRT") and "scRNAseq", where the first group means there are spatial coordinates information in the metadata of seu, named "row" and "col" and a Hidden Markov random field is used to model the unobserved class label using spatial coordinates ("Other_SRT" represents the other SRT platforms except for 'Visium' and 'ST'), and the other group "scRNAseq" means there is no spatial information in object seu and a multinomial model is used to model the unobserved class labels. The platform helps to calculate the adjacency matrix.
- `...` Other arguments to pass into `DR.SC_fit` function.

Details

`seu` is an object named `Seurat`, which can easily be created by R package `Seurat`. DR-SC model can be applied to analyze both single cell RNA sequencing data and spatially resoved transcriptomics (SRT) data.

If the data are collected by the single cell RNA sequencing technologies which means there is no spatial information in object `seu` then a multinomial model is used to model the unobserved class labels.

If the data is collected by the spatial transcriptomics technologies, then there are spatial coordinates information in the metadata of `seu`, named "row" and "col". DR-SC model uses a Hidden Markov random field to model the spatial coordinates. DR.SC supports different platforms of SRT data, such as 'Visium', 'ST' and any other platforms 'Other_SRT'.

Description

Joint dimension reduction and spatial clustering for scRNA-seq and spatial transcriptomics data.
For lattice grids in ST platform (ST), the interior spot has four neighbors (left, right, up and down), the boundary spot has three neighbors, and the spot in the corner has two neighbors. For hexagon grids, such as spatial coordinate in 10X Visium platform (Visium), the interior spot has six neighbors. For the irregular coordinates in other platforms (Other_SRT), Euclidean distance is adopted to decide whether a spot is a neighbor of another spot. For example, if the Euclidean distance between spot A and B is less than a radius, then A is taken as the neighborhood of B. See function `getAdj` for more details.

**Value**

DR.SC returns a revised Seurat object. There are two revisions in the seu: 1. the metadata is added a new column named `spatial.drsc.cluster` that represents the clustering results from DR-SC model, and the `Ids(seu)` is assigned with `spatial.drsc.cluster`. 2. a DimReduc object named `dr-sc` is added in the slot `reductions`, which represents the features extracted by DR-SC model.

**Note**

nothing

**Author(s)**

Wei Liu

**References**


**See Also**

None

**Examples**

```r
## we generate the spatial transcriptomics data with lattice neighborhood, i.e. ST platform.
seu <- gendata_RNAExp(height=10, width=10,p=50, K=4,platform="ST")
library(Seurat)
seu <- NormalizeData(seu, verbose=FALSE)
# choose 100 highly variable features
# seu <- FindVariableFeatures(seu, nfeatures = 100)
# maxIter = 2 is only used for illustration, and user can use default.
# seu1 <- DR.SC(seu, K=4, platform = 'ST', maxIter=2,verbose=FALSE)

# choose spatially variable features (SVGs)
seu <- FindSVGs(seu, nfeatures = 40, verbose=FALSE)
# use SVGs to fit DR.SC model
# maxIter = 2 is only used for illustration, and user can use default.
seu1 <- DR.SC(seu, K=4,platform = 'ST', maxIter=2, verbose=TRUE)
```
Joint dimension reduction and spatial clustering for scRNA-seq and spatial transcriptomics data

Usage

```r
DR.SC_fit(X, K, Adj_sp=NULL, q=15,
  error.heter= TRUE, beta_grid=seq(0.5, 5, by=0.5),
  maxIter=25, epsLogLik=1e-5, verbose=FALSE, maxIter_ICM=6,
  wpca.int=FALSE, int.model="EEE", approxPCA=FALSE, coreNum = 5)
```

Arguments

- **X**: a sparse matrix with class `dgCMatrix` or `matrix`, specify the log-normalization gene expression matrix used for DR-SC model.
- **K**: a positive integer allowing scalar or vector, specify the number of clusters in model fitting.
- **Adj_sp**: an optional sparse matrix with class `dgCMatrix`, specify the adjoint matrix used for DR-SC model. We provide this interface for those users who would like to define the adjacency matrix by their own.
- **q**: a positive integer, specify the number of latent features to be extracted, default as 15. Usually, the choice of q is a trade-off between model complexity and fit to the data, and depends on the goals of the analysis and the structure of the data. A higher value will result in a more complex model with a higher number of parameters, which may lead to overfitting and poor generalization performance. On the other hand, a lower value will result in a simpler model with fewer parameters, but may also lead to underfitting and a poorer fit to the data.
- **error.heter**: an optional logical value, whether use the heterogenous error for DR-SC model, default as `TRUE`. If `error.heter=FALSE`, then the homogenous error is used for probabilistic PCA model in DR-SC.
- **beta_grid**: an optional vector of positive value, the candidate set of the smoothing parameter to be searched by the grid-search optimization approach.
- **maxIter**: an optional positive value, represents the maximum iterations of EM.
- **epsLogLik**: an optional positive value, tolerance value of relative variation rate of the observed pseudo log-loglikelihood value, default as `1e-5`.
- **verbose**: an optional logical value, whether output the information of the ICM-EM algorithm.
- **maxIter.ICM**: an optional positive value, represents the maximum iterations of ICM.
wpca.int: an optional logical value, means whether use the weighted PCA to obtain the initial values of loadings and other parameters, default as FALSE which means the ordinary PCA is used.

int.model: an optional string, specify which Gaussian mixture model is used in evaluating the initial values for DR-SC, default as "EEE"; and see Mclust for more models’ names.

approxPCA: an optional logical value, whether use approximated PCA to speed up the computation for initial values.

coreNum: an optional positive integer, means the number of thread used in parallel computing, default as 5. If the length of K is one, then coreNum will be set as 1 automatically.

Details

Nothing

Value

DR.SC_fit returns a list with class "drscObject" with the following three components:

Objdrsc: a list including the model fitting results, in which the number of elements is same as the length of K.

out_param: a numeric matrix used for model selection in MBIC.

K_set: a scalar or vector equal to input argument K.

In addition, each element of "Objdrsc" is a list with the following components:

cluster: inferred class labels

hZ: extracted latent features.

beta: estimated smoothing parameter

Mu: mean vectors of mixtures components.

Sigma: covariance matrix of mixtures components.

W: estimated loading matrix

Lam_vec: estimated variance of errors in probabilistic PCA model

loglik: pseudo observed log-likelihood.

Note

nothing

Author(s)

Wei Liu
## References


### Examples

```r
## we generate the spatial transcriptomics data with lattice neighborhood, i.e. ST platform.
seu <- gendata_RNAExp(height=10, width=10,p=50, K=4)
library(Seurat)
seu <- NormalizeData(seu, verbose=FALSE)
# choose 40 highly variable features using FindVariableFeatures in Seurat
# seu <- FindVariableFeatures(seu, nfeatures = 40)
# or choose 40 spatially variable features using FindSVGs in DR.SC
seu <- FindSVGs(seu, nfeatures = 40, verbose=FALSE)
# users define the adjacency matrix
Adj_sp <- getAdj(seu, platform = 'ST')
if(class(seu@assays$RNA)=="Assay5"){
  var.features <- seu@assays$RNA@meta.data$var.features
  var.features <- var.features[!is.na(var.features)]
dat <- GetAssayData(seu, assay = "RNA", slot='data')
  X <- Matrix::t(dat[var.features,])
} else{
  var.features <- seu@assays$RNA@var.features
  X <- Matrix::t(seu["RNA"]@data[var.features,])
}

# maxIter = 2 is only used for illustration, and user can use default.
drscList <- DR.SC_fit(X,Adj_sp=Adj_sp, K=4, maxIter=2, verbose=TRUE)
```

---

**drscPlot**

**tNSE or UMAP plot visualization**

### Description

Intuitive way of visualizing how cell types changes across the embeddings obtained by DR-SC.

### Usage

```
drscPlot(seu, dims=1:5, visu.method='tSNE',...)
```
Arguments

seu  an object of class "Seurat" obtained by DR.SC.
dims  a positive integer to specify the number of latent features for visualization.
visu.method  a string including 'tSNE' or "UMAP".
...  Other arguments passing to DimPlot function.

Details

Nothing

Value

return a ggplot2 object.

Note

nothing

Author(s)

Wei Liu

References

None

See Also

None

Examples

## we generate the spatial transcriptomics data with lattice neighborhood, i.e. ST platform.
seu <- gendata_RNAExp(height=10, width=10, p=50, K=4)
library(Seurat)
seu <- NormalizeData(seu)
# choose spatially variable features
seu <- FindSVGs(seu)
# use SVGs to fit DR.SC model
# maxIter = 2 is only used for illustration, and user can use default.
seu1 <- DR.SC(seu, K=4, platform = 'ST', maxIter = 2, verbose=FALSE)

drscPlot(seu1)
FindSVGs

**Find spatially variable genes**

**Description**
Identifies features that have spatially variation along spots using SPARK-X.

**Usage**

```r
FindSVGs(seu, nfeatures=2000, covariates=NULL,
        preHVGs=5000, num_core=1, verbose=TRUE)
```

**Arguments**
- **seu**: an object of class "Seurat".
- **nfeatures**: a positive integer, means how many spatially variable genes to be chosen. If there are less than 2000 features in seu, then all features are identified.
- **covariates**: a covariate matrix named control variable matrix whose number of rows is equal to the number of columns of seu.
- **preHVGs**: a positive integer, the number of highly variable genes selected for speeding up computation of SPARK-X in selecting spatially variable features.
- **num_core**: an optional positive integer, specify the cores used for identifying the SVGs in parallel.
- **verbose**: an optional logical value, whether output the related information.

**Details**
Nothing

**Value**
return a revised Seurat object by adding three columns named "is.SVGs", "order.SVGs" and "adjusted.pval.SVGs" in the meta.features of default Assay.

**Note**
nothing

**References**

**See Also**
- topSVGs
Examples

```r
gendata_RNAExp(height=20, width=20, p=200, K=4)
gendata_RNAExp(height=20, width=20, p=200, K=4)
seu<-FindSVGs(seu, nfeatures=100)
topSVGs(seu)
```

__gendata_RNAExp__

Generate simulated data

**Description**
Generate simulated spatial transcriptomics data or scRNAseq data.

**Usage**
```r
gendata_RNAExp(height=30, width=30, platform="ST", p =100, q=10, K=7,
G=4,sigma2=1, tau=8, seed=1, view=FALSE)
```

**Arguments**
- `height, width`: Height and width of lattice grids for generating spatial coordinates. `n=height * width` cells for scRNAseq data.
- `platform`: Set the platform for the simulated data, only support 'ST' and 'scRNAseq'.
- `p`: Number of genes to generate.
- `q`: Number of true latent features to generate gene expression.
- `K`: Number of clusters (cell types).
- `seed`: Random seed for generating data.
- `G`: The number of neighbors. The latter must be one of `G = 4` or `G = 8`, which respectively correspond to a first order and a second order dependency structure. By default, `G = 4`.
- `sigma2`: Variance of error term in probabilistic PCA model.
- `tau`: A positive factor of mixture mean values.
- `view`: Logical value indicating whether the draw should be printed. Do not display the optional borders.

**Details**
Nothing

**Value**
Return a "Seurat" object. If `platform="ST"`, then the metadata of this Seurat object will include two columns with names "row" and "col" which are the spatial coordinates; If `platform="scRNAseq"`, then the metadata of this Seurat object will not have them.
**getAdj**

**Note**

nothing

**Author(s)**

Wei Liu

**References**

None

**See Also**

None

**Examples**

```r
## we generate the spatial transcriptomics data with lattice neighborhood, i.e. ST platform.
seu <- gendata_RNAExp(height=20, width=20, p=200, K=4)
seu

## generate scRNAseq data
seu <- gendata_RNAExp(height=20, width=20, platform="scRNAseq", p=100, K=4)
seu
```

---

**getAdj**

*Calculate the adjacency matrix given the spatial coordinates*

**Description**

Calculate the adjacency matrix for the spatial transcriptomics data measured on 10X Visium, ST or other platforms as a Seurat object.

**Usage**

```r
## S3 method for class 'Seurat'
getAdj(obj, platform = c("Visium", "ST", "Other_SRT"), ...)
```

**Arguments**

- **obj**: an object with class "Seurat", there are spatial coordinates information in the metadata of obj, named "row" and "col", where first column is x-axis coordinate, the second column is y-axis coordinate. `getAdj_manual` and `getAdj_auto` supports multi-dimensional spatial coordinates with a matrix as input.
platform a string, specify the platform of the provided data, default as "Visium". There are more platforms to be chosen, including ("Visuim", "ST", "Other_SRT"), which means there are spatial coordinates information in the metadata of obj, named "row" and "col". The platform helps to calculate the adjacency matrix by defining the neighborhoods.

Other arguments to pass into getAdj_auto function.

Details
For lattice grids in ST platform (ST), the interior spot has four neighbors (left, right, up and down), the boundary spot has three neighbors, and the spot in the corner has two neighbors. For hexagon grids, such as spatial coordinate in 10X Visium platform (Visium), the interior spot has six neighbors. For the irregular coordinates in other platforms (Other_SRT), Euclidean distance is adopted to decide whether a spot is a neighbor of another spot. For example, if the Euclidean distance between spot A and B is less than a radius, then A is taken as the neighbourhood of B. See functions getAdj_auto and getAdj_manual for more details.

Value
Return a dgCMatrix object recording the information of neighborhoods about each spot.

Note
nothing

Author(s)
Wei Liu

References

See Also
getAdj_auto, getAdj_manual.

Examples
```r
## S3 method for class "Seurat"
seu <- gendata_RNAExp(height=20, width=20,p=200, K=4)
Adj_sp <- getAdj(seu, platform = 'ST')
```
getAdj_auto

*Calculate adjacency matrix by automatically choosing radius*

**Description**

An efficient function to find the radius by bi-section method, then find neighbors based on the matrix of position, which ensures that each spot has approximately lower.med~upper.med neighbors in the sense of median.

**Usage**

getAdj_auto(pos, lower.med=4, upper.med=6, radius.upper= NULL)

**Arguments**

- `pos`: A n-by-2 matrix of position.
- `lower.med`: An integer, the lower bound of median number of neighbors among all spots.
- `upper.med`: An integer, the upper bound of median number of neighbors among all spots.
- `radius.upper`: A real, the upper bound of radius, default as NULL. If `radius.upper= NULL`, the upper bound is automatically determined by algorithm.

**Value**

A sparse adjacency matrix containing the neighbourhood.

**See Also**

getAdj, getAdj_manual.

getAdj_manual

*Calculate adjacency matrix by user-specified radius*

**Description**

An efficient function to find the neighbors based on the matrix of position and a pre-defined radius.

**Usage**

getAdj_manual(pos, radius)

**Arguments**

- `pos`: A n-by-d matrix of position, where n is the number of spots, and d is the dimension of coordinates.
- `radius`: Is a threshold of Euclidean distance to decide whether a spot is an neighborhood of another spot. For example, if the Euclidean distance between spot A and B is less than radius, then A is taken as the neighbourhood of B.
Value
A sparse matrix containing the neighbourhood

See Also
getAdj_auto, getAdj.

Description
an efficient function to find the neighborhood based on the matrix of position and a pre-defined
cutoff

Usage
getneighborhood_fast(x, radius)

Arguments
x is a n-by-2 matrix of position.
radius is a threshold of Euclidean distance to decide whether a spot is an neighborhood
of another spot. For example, if the Euclidean distance between spot A and B is
less than cutoff, then A is taken as the neighbourhood of B.

Value
A sparse matrix containing the neighbourhood

mbicPlot

Description
Intuitive way of visualizing how modified BIC values changes across different number of clusters

Usage
mbicPlot(seu, criteria="MBIC")

Arguments
seu an object of class Seurat revised by DR.SC with argument K=NULL.
criteria a string specifying the information criteria such as AIC, BIC and MBIC to be
plotted, default as MBIC.
Details

Nothing

Value

return a ggplot2 object.

Note

nothing

Author(s)

Wei Liu

References

None

See Also

None

Examples

```r
## we generate the spatial transcriptomics data with lattice neighborhood, i.e. ST platform.
seu <- gendata_RNAExp(height=20, width=20, p=100, K=4)
library(Seurat)
seu <- NormalizeData(seu)
# choose spatially variable features
seu <- FindSVGs(seu)
## Just for illustrating the usage of mbicPlot
seu[['RNA']]@misc[['icMat']] <- data.frame(K=2:5, MBIC=c(105, 101, 99, 108))
mbicPlot(seu)
```
readscRNAseq

**Arguments**

- **dirname**: A string, the directory of Visium datasets

**Details**

Nothing

**Value**

return a Seurat object.

**Note**

nothing

**Author(s)**

Wei Liu

**References**

None

**See Also**

None

**Examples**

```r
## Not run:
## set your file directory, then read it.
data_name <- "D/HCC"
HCC1 <- read10XVisium(data_name)
## End(Not run)
```

---

| readscRNAseq | Read the scRNAseq data measured on scRNA sequencing platform |

**Description**

Read the single cell RNA sequencing data measured on scRNA sequencing platform as a Seurat object.

**Usage**

```r
readscRNAseq(mtx, cells, features, ...)
```
**Arguments**

- `mtx` a string, name or remote URL of the mtx file
- `cells` a string, Name or remote URL of the cells/barcodes file
- `features` a string, Name or remote URL of the features/genes file
- ... the arguments passing to `ReadMtx`  

**Details**

Nothing

**Value**

return a Seurat object including expression matrix.

**Note**

nothing

**Author(s)**

Wei Liu

**References**

None

**See Also**

None

**Examples**

```r
## Not run:
### set the file directory, then read it.
seu <- readscRNAseq(mtx="GSM3755564_16_Liver_Treg_matrix.mtx.gz",  
                     features='GSM3755564_16_Liver_Treg_genes.tsv.gz',  
                     cells='GSM3755564_16_Liver_Treg_barcodes.tsv.gz')  
seu

## End(Not run)
```
RunWPCA

Run Weighted Principal Component Analysis

Description

Run a weighted PCA dimensionality reduction

Usage

RunWPCA(object, q=15)
### S3 method for class "Seurat"
## RunWPCA(object, q=15)
### S3 method for class "matrix"
## RunWPCA(object, q=15)
### S3 method for class "dgCMatrix"
## RunWPCA(object, q=15)

Arguments

- **object**: an object named "Seurat", "matrix" or "dgCMatrix". The object of class "Seurat" must include slot "scale.data".
- **q** : an optional positive integer, specify the number of features to be extracted.

Details

Nothing

Value

For Seurat object, return a Seurat object. For object "matrix" and "dgCMatrix", return a object "matrix" with rownames same as the colnames of X, and colnames "WPCA1" to "WPCAq".

Note

nothing

Author(s)

Wei Liu

References

selectModel

Description

Select the number of clusters by specified criteria.

Usage

```
selectModel(obj, criteria = 'MBIC', pen.const=1)
## S3 method for class 'drscObject'
selectModel(obj, criteria = 'MBIC', pen.const=1)
## S3 method for class 'Seurat'
selectModel(obj, criteria = 'MBIC', pen.const=1)
```
**selectModel**

**Arguments**

- `S`
  - `obj` an object with class `Seurat` by `DR.SC` or class `drscObject` by `DR.SC_fit`.
  - `criteria` a string, specify the criteria used for selecting the number of clusters, supporting "MBIC", "BIC" and "AIC".
  - `pen.const` an optional positive value, the adjusted constant used in the MBIC criteria. It usually takes value between 0.1 to 1.

**Value**

For S3 method of `Seurat`, it return a revised "Seurat" object with updated `Idents(seu)`, `spatial.drsc.cluster` in the metadata and `DimReduc object` named `dr-sc` in the slot `reductions`. For S3 method of `drscObject`, it returns a list with the following components:

- `bestK` the selected number of clusters.
- `cluster` inferred class labels.
- `hZ` extracted latent features.
- `icMat` a numeric matrix including the criteria value for each number of clusters K.

**Note**

nothing.

**Author(s)**

Wei Liu

**References**


**See Also**

`DR.SC`, `DR.SC_fit`.

**Examples**

```r
seu <- gendata_RNAExp(height=10, width=10,p=50, K=4)
library(Seurat)
seu <- NormalizeData(seu, verbose=FALSE)
# or choose 40 spatially variable features using FindSVGs in DR.SC
seu <- FindSVGs(seu, nfeatures = 40, verbose=FALSE)
# users define the adjacency matrix
Adj_sp <- getAdj(seu, platform = 'ST')
dat <- GetAssayData(seu, assay = "RNA", slot='data')
X <- Matrix::t(dat)
```
# maxIter = 2 is only used for illustration, and user can use default.
drscList <- DR.SC_fit(X, Adj_sp=Adj_sp, K=4, maxIter=2, verbose=TRUE)
drsc1 <- selectModel(drscList)
str(drsc1)

---

**spatialPlotClusters**  
*Spatial coordinates plot visualization*

**Description**
Intuitive way of visualizing how cell types changes across the spatial locations.

**Usage**

```r
spatialPlotClusters(seu)
```

**Arguments**

- `seu`  
an object of class "Seurat" obtained by `DR.SC`.

**Details**
Nothing

**Value**
return a ggplot2 object.

**Note**
nothing

**Author(s)**
Wei Liu

**References**
None

**See Also**
None
Examples

## we generate the spatial transcriptomics data with lattice neighborhood, i.e. ST platform.
seu <- gendata_RNAExp(height=10, width=10,p=50, K=4)
library(Seurat)
seu <- NormalizeData(seu)
# choose spatially variable features using Seurat
seu <- FindSVGs(seu)
# use SVGs to fit DR.SC model
# maxIter = 2 is only used for illustration, and user can use default.
seu1 <- DR.SC(seu, K=4,platform = 'ST', maxIter=2,verbose=FALSE)
spatialPlotClusters(seu1)

### sp_means_Rcpp

**Calculate column-wise or row-wise mean**

**Description**

Calculate column-wise or row-wise mean

**Usage**

```r
sp_means_Rcpp(sp_data, rowMeans = FALSE)
```

**Arguments**

- `sp_data`: A sparse matrix
- `rowMeans`: A boolean value, whether to calculate row-wise mean

**Value**

A n x 1 or p x 1 matrix

### sp_sums_Rcpp

**Calculate column-wise or row-wise sum**

**Description**

Calculate column-wise or row-wise sum

**Usage**

```r
sp_sums_Rcpp(sp_data, rowSums = FALSE)
```

**Arguments**

- `sp_data`: A sparse matrix
- `rowSums`: A boolean value, whether to calculate row-wise sum
topSVGs

Value

A n x 1 or p x 1 matrix

Description

Return top n spatially variable genes given a Seurat object performed by `FindSVGs`.

Usage

topSVGs(seu, ntop=5)

Arguments

- `seu`: an object of class "Seurat".
- `ntop`: an optional positive integer, means how many spatially variable genes to access.

Details

Nothing

Value

return a character vector including the names of SVGs.

Note

nothing

Author(s)

Wei Liu

References

None

See Also

topSVGs

Examples

```r
seu <- gendata_RNAExp(height=20, width=20,p=200, K=4)
seu <- FindSVGs(seu, nfeatures=100, verbose=FALSE)
(genes <- topSVGs(seu, ntop=10))
```
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