Package ‘ILoReg’

May 29, 2024

Type Package

Title ILoReg: a tool for high-resolution cell population identification from scRNA-Seq data

Version 1.14.0

Description ILoReg is a tool for identification of cell populations from scRNA-seq data. In particular, ILoReg is useful for finding cell populations with subtle transcriptomic differences. The method utilizes a self-supervised learning method, called Iterative Clustering Projection (ICP), to find cluster probabilities, which are used in noise reduction prior to PCA and the subsequent hierarchical clustering and t-SNE steps. Additionally, functions for differential expression analysis to find gene markers for the populations and gene expression visualization are provided.

License GPL-3


Depends R (>= 4.0.0)

Encoding UTF-8

LazyData TRUE

RoxygenNote 7.1.1

Suggests knitr, rmarkdown, BiocStyle

VignetteBuilder knitr

biocViews SingleCell, Software, Clustering, DimensionReduction, RNASeq, Visualization, Transcriptomics, DataRepresentation, DifferentialExpression, Transcription, GeneExpression

NeedsCompilation no

URL https://github.com/elolab/ILoReg

BugReports https://github.com/elolab/ILoReg/issues
**Contents**

- AnnotationScatterPlot ............................................. 3
- CalcSilhInfo ...................................................... 4
- ClusteringScatterPlot .............................................. 5
- DownOverSampling ................................................... 6
- FindAllGeneMarkers .................................................. 7
- FindGeneMarkers .................................................... 9
- GeneHeatmap .......................................................... 11
- GeneScatterPlot ..................................................... 12
- HierarchicalClustering ............................................. 13
- LogisticRegression ................................................... 14
- MergeClusters ........................................................ 15
- pbmc3k_500 ......................................................... 16
- PCAElbowPlot ....................................................... 17
- PrepareILoReg ....................................................... 17
- RenameAllClusters .................................................. 18
- RenameCluster ........................................................ 19
- RunICP ............................................................... 20
- RunParallelICP ...................................................... 21
- RunPCA ............................................................... 22
- RunTSNE .............................................................. 23
- RunUMAP ............................................................. 24
- SelectKClusters ..................................................... 25
- SelectTopGenes ..................................................... 25
- SilhouetteCurve .................................................... 26
- VlnPlot ............................................................. 27

**Index**

29
AnnotationScatterPlot

Visualisation of a custom annotation over nonlinear dimensionality reduction

Description

The AnnotationScatterPlot enables visualizing arbitrary class labels over the nonlinear dimensionality reduction, e.g. t-SNE or UMAP.

Usage

AnnotationScatterPlot.SingleCellExperiment(
  object,
  annotation,
  return.plot,
  dim.reduction.type,
  point.size,
  show.legend
)

## S4 method for signature 'SingleCellExperiment'
AnnotationScatterPlot(
  object,
  annotation = NULL,
  return.plot = FALSE,
  dim.reduction.type = "",
  point.size = 0.7,
  show.legend = FALSE
)

Arguments

- **object** of SingleCellExperiment class
- **annotation** a character vector, factor or numeric for the class labels.
- **return.plot** return.plot whether to return the ggplot2 object or just draw it. Default is FALSE.
- **dim.reduction.type** "tsne" or "umap". Default is tsne.
- **point.size** point size. Default is 0.7.
- **show.legend** a logical denoting whether to show the legend on the right side of the plot. Default is TRUE.

Value

ggplot2 object if return.plot=TRUE
Examples

```r
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- RunTSNE(sce)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce,K=5)
## Change the names to the first five alphabets and Visualize the annotation.
custom_annotation <- plyr::mapvalues(metadata(sce)$iloreg$clustering.manual,
c(1,2,3,4,5),
LETTERS[1:5])
AnnotationScatterPlot(sce,
anotation = custom.annotation,
return.plot = FALSE,
dim.reduction.type = "tsne",
show.legend = FALSE)
```

CalcSilhInfo

Estimating optimal K using silhouette

Description

The function estimates the optimal number of clusters K from the dendrogram of the hierarchical clustering using the silhouette method.

Usage

```r
CalcSilhInfo.SingleCellExperiment(object, K.start, K.end)
```

## S4 method for signature 'SingleCellExperiment'
CalcSilhInfo(object, K.start = 2, K.end = 50)

Arguments

- **object** of SingleCellExperiment class
- **K.start** a numeric for the smallest K value to be tested. Default is 2.
- **K.end** a numeric for the largest K value to be tested. Default is 50.

Value

object of SingleCellExperiment class
## Examples

```r
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
  ## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- CalcSilhInfo(sce)
```

## Description

ClusteringScatterPlot function enables visualizing the clustering over nonlinear dimensionality reduction (t-SNE or UMAP).

## Usage

```r
ClusteringScatterPlot.SingleCellExperiment(
  object,
  clustering.type,
  return.plot,
  dim.reduction.type,
  point.size,
  title,
  show.legend
)
```

```r
## S4 method for signature 'SingleCellExperiment'
ClusteringScatterPlot(
  object,
  clustering.type = "manual",
  return.plot = FALSE,
  dim.reduction.type = "",
  point.size = 0.7,
  title = "",
  show.legend = TRUE
)
```

## Arguments

- **object** of SingleCellExperiment class
clustering.type
"manual" or "optimal". "manual" refers to the clustering formed using the "SelectKClusters" function and "optimal" to the clustering formed using the "CalcSilhInfo" function. Default is "manual".

return.plot
a logical denoting whether to return the ggplot2 object. Default is FALSE.

dim.reduction.type
"tsne" or "umap". Default is "tsne".

point.size
point size. Default is Default is 0.7.

title
text to write above the plot

show.legend
whether to show the legend on the right side of the plot. Default is TRUE.

Value
ggplot2 object if return.plot=TRUE

Examples
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce,K=5)
sce <- RunTSNE(sce)
ClusteringScatterPlot(sce,"manual",dim.reduction.type="tsne")
sce <- RunUMAP(sce)
ClusteringScatterPlot(sce,"manual",dim.reduction.type="umap")

---

**DownOverSampling**

**Down- and oversample data**

**Description**

The function implements a script down- and oversamples data to include n cells.

**Usage**

DownOverSampling(x, n = 50)

**Arguments**

- **x**
  A character or numeric vector of data to down-and oversample.

- **n**
  How many cells to include per cluster.
Value

a list containing the output of the LiblineaR prediction

Description

FindAllGeneMarkers enables identifying gene markers for all clusters at once. This is done by differential expression analysis where cells from one cluster are compared against the cells from the rest of the clusters. Gene and cell filters can be applied to accelerate the analysis, but this might lead to missing weak signals.

Usage

```r
FindAllGeneMarkers.SingleCellExperiment(
  object,
  clustering.type,
  test,
  log2fc.threshold,
  min.pct,
  min.diff.pct,
  min.cells.group,
  max.cells.per.cluster,
  pseudocount.use,
  return.thresh,
  only.pos
)
```

```r
## S4 method for signature 'SingleCellExperiment'
FindAllGeneMarkers(
  object,
  clustering.type = "manual",
  test = "wilcox",
  log2fc.threshold = 0.25,
  min.pct = 0.1,
  min.diff.pct = NULL,
  min.cells.group = 3,
  max.cells.per.cluster = NULL,
  pseudocount.use = 1,
  return.thresh = 0.01,
  only.pos = FALSE
)
```
Arguments

object of SingleCellExperiment class

clustering.type "manual" or "optimal". "manual" refers to the clustering formed using the "SelectKClusters" function and "optimal" to the clustering formed using the "CalcSilhInfo" function. Default is "manual".

test Which test to use. Only "wilcoxon" (the Wilcoxon rank-sum test, AKA Mann-Whitney U test) is supported at the moment.

log2fc.threshold Filters out genes that have log2 fold-change of the averaged gene expression values (with the pseudo-count value added to the averaged values before division if pseudocount.use > 0) below this threshold. Default is 0.25.

min.pct Filters out genes that have dropout rate (fraction of cells expressing a gene) below this threshold in both comparison groups Default is 0.1.

min.diff.pct Filters out genes that do not have this minimum difference in the dropout rates (fraction of cells expressing a gene) between the two comparison groups. Default is NULL.

min.cells.group The minimum number of cells in the two comparison groups to perform the DE analysis. If the number of cells is below the threshold, the DE analysis of this cluster is skipped. Default is 3.

max.cells.per.cluster The maximum number of cells per cluster if downsampling is performed to speed up the DE analysis. Default is NULL, i.e. no downsampling.

pseudocount.use A positive integer, which is added to the average gene expression values before calculating the fold-change, assuring that no divisions by zero occur. Default is 1.

return.thresh If only.pos=TRUE, then return only genes that have the adjusted p-value (adjusted by the Bonferroni method) below or equal to this threshold. Default is 0.01.

only.pos Whether to return only genes that have an adjusted p-value (adjusted by the Bonferroni method) below or equal to the threshold. Default is FALSE.

Value

a data frame of the results if positive results were found, else NULL.

Examples

library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce, K=5)
gene_markers <- FindAllGeneMarkers(sce)

FindGeneMarkers

Identification of gene markers for a cluster or two arbitrary combinations of clusters

Description

FindGeneMarkers enables identifying gene markers for one cluster or two arbitrary combinations of clusters, e.g. 1_2 vs. 3_4_5. Gene and cell filters can be applied to accelerate the analysis, but this might lead to missing weak signals.

Usage

FindGeneMarkers.SingleCellExperiment(
  object,
  clusters.1,
  clusters.2,
  clustering.type,
  test,
  logfc.threshold,
  min.pct,
  min.diff.pct,
  min.cells.group,
  max.cells.per.cluster,
  pseudocount.use,
  return.thresh,
  only.pos
)

## S4 method for signature 'SingleCellExperiment'
FindGeneMarkers(
  object,
  clusters.1 = NULL,
  clusters.2 = NULL,
  clustering.type = "",
  test = "wilcox",
  logfc.threshold = 0.25,
  min.pct = 0.1,
  min.diff.pct = NULL,
  min.cells.group = 3,
  max.cells.per.cluster = NULL,
  pseudocount.use = 1,
  return.thresh = 0.01,
  only.pos = FALSE
)
Arguments

object of SingleCellExperiment class

clusters.1 a character or numeric vector denoting which clusters to use in the first group (named group.1 in the results)

clusters.2 a character or numeric vector denoting which clusters to use in the second group (named group.2 in the results)

clustering.type "manual" or "optimal". "manual" refers to the clustering formed using the "SelectKClusters" function and "optimal" to the clustering formed using the "CalcSilhInfo" function. Default is "manual".

test Which test to use. Only "wilcoxon" (the Wilcoxon rank-sum test, AKA Mann-Whitney U test) is supported at the moment.

logfc.threshold Filters out genes that have log2 fold-change of the averaged gene expression values (with the pseudo-count value added to the averaged values before division if pseudocount.use > 0) below this threshold. Default is 0.25.

min.pct Filters out genes that have dropout rate (fraction of cells expressing a gene) below this threshold in both comparison groups. Default is 0.1.

min.diff.pct Filters out genes that do not have this minimum difference in the dropout rates (fraction of cells expressing a gene) between the two comparison groups. Default is NULL.

min.cells.group The minimum number of cells in the two comparison groups to perform the DE analysis. If the number of cells is below the threshold, then the DE analysis is not performed. Default is 3.

max.cells.per.cluster The maximum number of cells per cluster if downsampling is performed to speed up the DE analysis. Default is NULL, i.e. no downsampling.

pseudocount.use A positive integer, which is added to the average gene expression values before calculating the fold-change. This makes sure that no divisions by zero occur. Default is 1.

return.thresh If only.pos=TRUE, then return only genes that have the adjusted p-value (adjusted by the Bonferroni method) below or equal to this threshold. Default is 0.01.

only.pos Whether to return only genes that have an adjusted p-value (adjusted by the Bonferroni method) below or equal to the threshold. Default is FALSE.

Value

a data frame of the results if positive results were found, else NULL

Examples

library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareIloReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce,K=5)
gene_markes_1 <- FindGeneMarkers(sce,clusters.1=1)
gene_markes_1_vs_2 <- FindGeneMarkers(sce,clusters.1=1,clusters.2=2)

---

**Description**

The GeneHeatmap function enables drawing a heatmap of the gene markers identified by FindAllGeneMarkers, where the cell are grouped by the clustering.

**Usage**

GeneHeatmap(object, clustering.type, gene.markers)

## S4 method for signature 'SingleCellExperiment'
GeneHeatmap(object, clustering.type = "manual", gene.markers = NULL)

**Arguments**

- **object**: of SingleCellExperiment class
- **clustering.type**: "manual" or "optimal". "manual" refers to the clustering formed using the SelectKClusters function and "optimal" to the clustering using the CalcSihlInfo function. Default is "manual".
- **gene.markers**: a data frame of the gene markers generated by FindAllGeneMarkers function. To accelerate the drawing, filtering the dataframe by selecting e.g. top 10 genes is recommended.

**Value**

nothing

**Examples**

library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareIloReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,r=1,k=5) # Use L=200
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce,K=5)
gene_markers <- FindAllGeneMarkers(sce,log2fc.threshold = 0.5,min.pct = 0.5)
top10_log2FC <- SelectTopGenes(gene_markers,top.N=10,
criterion.type="log2FC",inverse=FALSE)
GeneHeatmap(sce,clustering.type = "manual",
gene.markers = top10_log2FC)

Description
GeneScatterPlot enables visualizing gene expression of a gene over nonlinear dimensionality re-
duction with t-SNE or UMAP.

Usage
GeneScatterPlot.SingleCellExperiment(
  object,
  genes,
  return.plot,
  dim.reduction.type,
  point.size,
  title,
  plot.expressing.cells.last,
  nrow,
  ncol
)

## S4 method for signature 'SingleCellExperiment'
GeneScatterPlot(
  object,
  genes = "",
  return.plot = FALSE,
  dim.reduction.type = "tsne",
  point.size = 0.7,
  title = "",
  plot.expressing.cells.last = FALSE,
  nrow = NULL,
  ncol = NULL
)
Hierarchical Clustering

** Arguments **

- `object` of SingleCellExperiment class
- `genes` a character vector of the genes to be visualized
- `return.plot` whether to return the ggplot2 object or just draw it (default FALSE)
- `dim.reduction.type` "tsne" or "umap" (default "tsne")
- `point.size` point size (default 0.7)
- `title` text to write above the plot
- `plot.expressing.cells.last` whether to plot the expressing genes last to make the points more visible
- `nrow` a positive integer that specifies the number of rows in the plot grid. Default is NULL.
- `ncol` a positive integer that specifies the number of columns in the plot grid. Default is NULL.

** Value **

ggplot2 object if `return.plot=TRUE`

** Examples **

```r
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- RunTSNE(sce)
GeneScatterPlot(sce,"CD14",dim.reduction.type="tsne")
sce <- RunUMAP(sce)
GeneScatterPlot(sce,"CD14",dim.reduction.type="umap")
```

** Description **

Hierarchical clustering using the Ward’s method

** Usage **

HierarchicalClustering.SingleCellExperiment(object)

## S4 method for signature 'SingleCellExperiment'
HierarchicalClustering(object)
LogisticRegression

Arguments

object of SingleCellExperiment class

Value

object of SingleCellExperiment class

Examples

library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILOReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)

LogisticRegression

Clustering projection using logistic regression from the LiblineaR R package

Description

The function implements a script that downsamples data a dataset, trains a logistic regression classifier model and then projects its clustering onto itself using a trained L1-regularized logistic regression model.

Usage

LogisticRegression(
training.sparse.matrix = NULL,
training.ident = NULL,
C = 0.3,
reg.type = "L1",
test.sparse.matrix = NULL,
d = 0.3
)

Arguments

training.sparse.matrix
A sparse matrix (dgCMatix) containing training sample’s gene expression data with genes in rows and cells in columns. Default is NULL.

training.ident
A named factor containing sample’s cluster labels for each cell in training.sparse.matrix. Default is NULL.
**MergeClusters**

- **C**  
  Cost of constraints violation in L1-regularized logistic regression (C). Default is 0.3.

- **reg.type**  
  "L1" for LASSO and "L2" for Ridge. Default is "L1".

- **test.sparse.matrix**  
  A sparse matrix (dgCMatrix) containing test sample’s gene expression data with genes in rows and cells in columns. Default is NULL.

- **d**  
  A numeric smaller than 1 and greater than 0 that determines how many cells per cluster should be down- and oversampled (d in N/k*d), where N is the total number of cells and k the number of clusters. Default is 0.3.

**Value**

A list containing the output of the LiblineaR prediction

---

### Description

**MergeClusters** function enables merging clusters and naming the newly formed cluster.

### Usage

```r
MergeClusters.SingleCellExperiment(object, clusters.to.merge, new.name)
```

```r
## S4 method for signature 'SingleCellExperiment'
MergeClusters(object, clusters.to.merge = "", new.name = "")
```

### Arguments

- **object**  
  of SingleCellExperiment class

- **clusters.to.merge**  
  a character or numeric vector for the names of the clusters to merge

- **new.name**  
  a character for the new name of the merged cluster. If left empty, the new cluster name is formed by separating the cluster names by "_".

### Value

A list containing the output of the LiblineaR prediction
Examples

```r
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce,K=5)
sce <- MergeClusters(sce,clusters.to.merge=c(1,2),new.name="merged1")
```

### pbmc3k_500

**A toy dataset with 500 cells downsampled from the pbmc3k dataset.**

**Description**

The preprocessing was done using Cell Ranger v2.2.0 and the GRCh38.p12 human reference genome. The Normalization was done using the LogNormalize method of Seurat v3 R package. The sampling was done using the sample() function without replacement and set.seed(1) as initialization.

**Usage**

```r
data(pbmc3k_500)
```

**Format**

pbmc3k_500, dgCMatrix object

**Source**

[https://support.10xgenomics.com/single-cell-gene-expression](https://support.10xgenomics.com/single-cell-gene-expression)

**Examples**

```r
data(pbmc3k_500)
```
PCAElbowPlot

Elbow plot of the standard deviations of the principal components

Description

Draw an elbow plot of the standard deviations of the principal components to deduce an appropriate value for p.

Usage

PCAElbowPlot.SingleCellExperiment(object, return.plot)

## S4 method for signature 'SingleCellExperiment'

PCAElbowPlot(object, return.plot = FALSE)

Arguments

object object of class 'iloreg'

return.plot logical indicating if the ggplot2 object should be returned (default FALSE)

Value

ggplot2 object if return.plot=TRUE

Examples

library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
PCAElbowPlot(sce)

PrepareILoReg

Prepare SingleCellExperiment object for ILoReg analysis

Description

This function prepares the SingleCellExperiment object for ILoReg analysis. The only required input is an object of class SingleCellExperiment with at least data in the logcounts slot.
Usage

PrepareILoReg.SingleCellExperiment(object)

## S4 method for signature 'SingleCellExperiment'
PrepareILoReg(object)

Arguments

object an object of SingleCellExperiment class

Value

an object of SingleCellExperiment class

Examples

library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)

 arms

renameAllClusters  Renaming all clusters at once

Description

renameAllClusters function enables renaming all cluster at once.

Usage

renameAllClusters.SingleCellExperiment(object, new.cluster.names)

## S4 method for signature 'SingleCellExperiment'
renameAllClusters(object, new.cluster.names = "")

Arguments

object of SingleCellExperiment class
new.cluster.names object of class 'iloreg'

Value

object of SingleCellExperiment class
Examples

```r
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce,K=5)
sce <- RenameAllClusters(sce,new.cluster.names=LETTERS[seq_len(5)])
sce <- RenameCluster(sce,1,"cluster1")
```

Description

RenameCluster function enables renaming a cluster in 'clustering.manual' slot.

Usage

```r
RenameCluster.SingleCellExperiment(object, old.cluster.name, new.cluster.name)
```

## S4 method for signature 'SingleCellExperiment'
RenameCluster(object, old.cluster.name = "", new.cluster.name = "")

Arguments

- **object** of SingleCellExperiment class
- **old.cluster.name** a character variable denoting the old name of the cluster
- **new.cluster.name** a character variable the new name of the cluster

Value

object of SingleCellExperiment class

Examples

```r
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce,K=5)
sce <- RenameCluster(sce,1,"cluster1")
```
Description

The function implements Iterative Clustering Projection (ICP): a supervised learning-based clustering, which maximizes clustering similarity between the clustering and its projection by logistic regression.

Usage

```r
RunICP(normalized.data = NULL, k = 15, d = 0.3, r = 5, C = 5, reg.type = "L1", max.iter = 200)
```

Arguments

- `normalized.data` A sparse matrix (dgCMatrix) containing normalized gene expression data with genes in rows and cells in columns. Default is `NULL`.
- `k` A positive integer greater or equal to 2, denoting the number of clusters in ICP. Default is `15`.
- `d` A numeric that defines how many cells per cluster should be down- and oversampled (d in ceiling(N/k*d)), when stratified.downsampling=FALSE, or what fraction should be downsampled in the stratified approach, stratified.downsampling=TRUE. Default is `0.3`.
- `r` A positive integer that denotes the number of reiterations performed until the algorithm stops. Default is `5`.
- `C` Cost of constraints violation (C) for L1-regulatization. Default is `0.3`.
- `reg.type` "L1" for LASSO and "L2" for Ridge. Default is "L1".
- `max.iter` A positive integer that denotes the maximum number of iterations performed until the algorithm ends. Default is `200`.

Value

A list that includes the probability matrix and the clustering similarity measures: ARI, NMI, etc.
RunParallelICP

Run ICP runs parallerly

Description

This function runs in parallel L ICP runs, which is the computational bottleneck of ILoReg. With ~3,000 cells this step should be completed in ~2 h and ~1 h with 3 and 12 logical processors (threads), respectively.

Usage

RunParallelICP.SingleCellExperiment(
  object,
  k,
  d,
  L,
  r,
  C,
  reg.type,
  max.iter,
  threads
)

## S4 method for signature 'SingleCellExperiment'
RunParallelICP(
  object,
  k = 15,
  d = 0.3,
  L = 200,
  r = 5,
  C = 0.3,
  reg.type = "L1",
  max.iter = 200,
  threads = 0
)

Arguments

<table>
<thead>
<tr>
<th>object</th>
<th>An object of SingleCellExperiment class.</th>
</tr>
</thead>
<tbody>
<tr>
<td>k</td>
<td>A positive integer greater or equal to 2, denoting the number of clusters in Iterative Clustering Projection (ICP). Decreasing k leads to smaller cell populations diversity and vice versa. Default is 15.</td>
</tr>
<tr>
<td>d</td>
<td>A numeric greater than 0 and smaller than 1 that determines how many cells n are down- or oversampled from each cluster into the training data (n=N/k*d), where N is the total number of cells, k is the number of clusters in ICP. Increasing above 0.3 leads gradually to smaller cell populations diversity. Default is 0.3.</td>
</tr>
</tbody>
</table>
RunPCA

A positive integer greater than 1 denoting the number of the ICP runs to run. Default is 200. Increasing recommended with a significantly larger sample size (tens of thousands of cells). Default is 200.

**r**

A positive integer that denotes the number of reiterations performed until the ICP algorithm stops. Increasing recommended with a significantly larger sample size (tens of thousands of cells). Default is 5.

**C**

A positive real number denoting the cost of constraints violation in the L1-regularized logistic regression model from the LIBLINEAR library. Decreasing leads to more stringent feature selection, i.e. less genes are selected that are used to build the projection classifier. Decreasing to a very low value (~ 0.01) can lead to failure to identify central cell populations. Default 0.3.

**reg.type**

"L1" or "L2". L2-regularization was not investigated in the manuscript, but it leads to a more conventional outcome (less subpopulations). Default is "L1".

**max.iter**

A positive integer that denotes the maximum number of iterations performed until ICP stops. This parameter is only useful in situations where ICP converges extremely slowly, preventing the algorithm to run too long. In most cases, reaching the number of reiterations (r=5) terminates the algorithm. Default is 200.

**threads**

A positive integer that specifies how many logical processors (threads) to use in parallel computation. Set 1 to disable parallelism altogether or 0 to use all available threads except one. Default is 0.

**Value**

an object of SingleCellExperiment class

**Examples**

```r
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,r=1,k=5)
```

**Description**

Perform the PCA transformation of the joint probability matrix, which reduces the dimensionality from k*L to p

**Usage**

```r
RunPCA.SingleCellExperiment(object, p, scale, threshold)
```

```r
## S4 method for signature 'SingleCellExperiment'
RunPCA(object, p = 50, scale = FALSE, threshold = 0)
```
RunTSNE

Arguments

object of SingleCellExperiment class
p a positive integer denoting the number of principal components to calculate and select. Default is 50.
scale a logical specifying whether the probabilities should be standardized to unit-variance before running PCA. Default is FALSE.
threshold a threshold for filtering out ICP runs before PCA with the lower terminal projection accuracy below the threshold. Default is 0.

Value

object of SingleCellExperiment class

Examples

library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
Sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)

RunTSNE

Barnes-Hut implementation of t-Distributed Stochastic Neighbor Embedding (t-SNE)

Description

Run nonlinear dimensionality reduction using t-SNE with the PCA-transformed consensus matrix as input.

Usage

RunTSNE.SingleCellExperiment(object, perplexity)

## S4 method for signature 'SingleCellExperiment'
RunTSNE(object, perplexity = 30)

Arguments

object of SingleCellExperiment class
perplexity perplexity of t-SNE
**RunUMAP**

**Value**

object of `SingleCellExperiment` class

**Examples**

```r
dlibrary(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- RunTSNE(sce)
```

---

### Description

Run nonlinear dimensionality reduction using UMAP with the PCA-transformed consensus matrix as input.

### Usage

```r
RunUMAP.SingleCellExperiment(object)
```

### Arguments

- `object` of `SingleCellExperiment` class

### Value

object of `SingleCellExperiment` class

### Examples

```r
dlibrary(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- RunUMAP(sce)
```
SelectKClusters

Selecting K clusters from hierarchical clustering

Description

Selects K clusters from the dendrogram.

Usage

SelectKClusters.SingleCellExperiment(object, K)

## S4 method for signature 'SingleCellExperiment'
SelectKClusters(object, K = NULL)

Arguments

- object: of SingleCellExperiment class
- K: a positive integer denoting how many clusters to select

Value

object of SingleCellExperiment class

Examples

library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareIloReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce,K=5)

SelectTopGenes

Select top or bottom N genes based on a selection criterion

Description

The SelectTopGenes function enables selecting top or bottom N genes based on a criterion (e.g. log2FC or adj.p.value).
Usage

```r
SelectTopGenes(  
gene.markers = NULL,  
top.N = 10,  
criterion.type = "log2FC",  
inverse = FALSE
)
```

Arguments

- `gene.markers`  
  A data frame of the gene markers found by `FindAllGeneMarkers` function.
- `top.N`  
  How many top or bottom genes to select. Default is 10.
- `criterion.type`  
  Which criterion to use for selecting the genes. Default is "log2FC".
- `inverse`  
  Whether to select bottom instead of top N genes. Default is FALSE.

Value

An object of 'data.frame' class

Examples

```r
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce,K=5)
gene_markers <- FindAllGeneMarkers(sce)
## Select top 10 markers based on log2 fold-change
top10_log2FC <- SelectTopGenes(gene_markers,  
    top.N = 10,  
    criterion.type = "log2FC",  
    inverse = FALSE)
```

---

**SilhouetteCurve**

**Silhouette curve**

Description

Draw the silhouette curve: the average silhouette value across the cells for a range of different K values.
VlnPlot

**Usage**

SilhouetteCurve.SingleCellExperiment(object, return.plot)

```r
## S4 method for signature 'SingleCellExperiment'
SilhouetteCurve(object, return.plot = FALSE)
```

**Arguments**

- `object` of `SingleCellExperiment` class
- `return.plot` a logical denoting whether the ggplot2 object should be returned. Default is FALSE.

**Value**

ggplot2 object if return.plot=TRUE

**Examples**

```r
library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- CalcSilhInfo(sce)
SilhouetteCurve(sce)
```

---

VlnPlot

*Gene expression visualization using violin plots*

**Description**

The VlnPlot function enables visualizing expression levels of a gene, or multiple genes, across clusters using Violin plots.

**Usage**

```r
VlnPlot.SingleCellExperiment(
  object,
  clustering.type,
  genes,
  return.plot,
  rotate.x.axis.labels
)
```

```r
## S4 method for signature 'SingleCellExperiment'
```
VlnPlot(
    object,
    clustering.type = "manual",
    genes = NULL,
    return.plot = FALSE,
    rotate.x.axis.labels = FALSE
)

Arguments

object of SingleCellExperiment class
clustering.type "manual" or "optimal". "manual" refers to the clustering formed using the "SelectKClusters" function and "optimal" to the clustering formed using the "CalcSilhInfo" function. Default is "manual".
genes a character vector denoting the gene names that are visualized
return.plot return.plot whether to return the ggplot2 object
rotate.x.axis.labels a logical denoting whether the x-axis labels should be rotated 90 degrees. or just draw it. Default is FALSE.

Value

ggplot2 object if return.plot=TRUE

Examples

library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_500))
sce <- PrepareILoReg(sce)
## These settings are just to accelerate the example, use the defaults.
sce <- RunParallelICP(sce,L=2,threads=1,C=0.1,k=5,r=1)
sce <- RunPCA(sce,p=5)
sce <- HierarchicalClustering(sce)
sce <- SelectKClusters(sce,K=5)
VlnPlot(sce,genes=c("CD3D","CD79A","CST3"))
Index

* Approximation
  RunUMAP, 24
* Barnes-Hut
  RunTSNE, 23
* DE
  FindAllGeneMarkers, 7
  FindGeneMarkers, 9
* Embedding
  RunTSNE, 23
* ICP
  RunICP, 20
  RunParallelICP, 21
* LIBLINEAR
  RunParallelICP, 21
* LiblineaR
  LogisticRegression, 14
* Manifold
  RunUMAP, 24
* Neighbor
  RunTSNE, 23
* N
  SelectTopGenes, 25
* PCA
  PCAElbowPlot, 17
  RunPCA, 22
* Projection
  RunUMAP, 24
* Stochastic
  RunUMAP, 23
* UMAP
  RunUMAP, 24
* Uniform
  RunUMAP, 24
* all
  RenameAllClusters, 18
* analysis
  FindAllGeneMarkers, 7
  FindGeneMarkers, 9
* and
  RunUMAP, 24
* annotation
  AnnotationScatterPlot, 3
* bottom
  SelectTopGenes, 25
* clean
  PrepareILoReg, 17
* clustering
  CalcSilhInfo, 4
  ClusteringScatterPlot, 5
  HierarchicalClustering, 13
  RunICP, 20
  RunParallelICP, 21
  SilhouetteCurve, 26
* clusters
  MergeClusters, 15
  RenameAllClusters, 18
  SelectKClusters, 25
* cluster
  RenameCluster, 19
* custom
  AnnotationScatterPlot, 3
* datasets
  pbmc3k_500, 16
* data
  PrepareILoReg, 17
* differential
  FindAllGeneMarkers, 7
  FindGeneMarkers, 9
* dimensionality
  AnnotationScatterPlot, 3
  ClusteringScatterPlot, 5
* downsampling
  DownOverSampling, 6
  LogisticRegression, 14
* eigendecomposition
  RunPCA, 22
* elbow
  PCAElbowPlot, 17
* expression
  FindAllGeneMarkers, 7
  FindGeneMarkers, 9
* genes
  SelectTopGenes, 25
* gene
  FindAllGeneMarkers, 7
  FindGeneMarkers, 9
  GeneHeatmap, 11
  GeneScatterPlot, 12
* grouped
  GeneHeatmap, 11
* heatmap
  GeneHeatmap, 11
* hierarchical
  CalcSilhInfo, 4
  HierarchicalClustering, 13
  SilhouetteCurve, 26
* iloreg
  PrepareILoReg, 17
* implementation
  RunTSNE, 23
* iterative
  RunICP, 20
  RunParallelICP, 21
* logistic
  LogisticRegression, 14
  RunParallelICP, 21
* markers
  FindAllGeneMarkers, 7
  FindGeneMarkers, 9
* merge
  MergeClusters, 15
* nonlinear
  AnnotationScatterPlot, 3
  ClusteringScatterPlot, 5
* normalized
  PrepareILoReg, 17
* of
  RunTSNE, 23
* one
  RenameCluster, 19
* oversampling
  DownOverSampling, 6
  LogisticRegression, 14
* plot
  ClusteringScatterPlot, 5
  GeneScatterPlot, 12
  PCAElbowPlot, 17
  VlnPlot, 27
* prepare
  PrepareILoReg, 17
* projection
  LogisticRegression, 14
  RunICP, 20
  RunParallelICP, 21
* reduction
  AnnotationScatterPlot, 3
  ClusteringScatterPlot, 5
* regression
  LogisticRegression, 14
  RunParallelICP, 21
* rename
  RenameAllClusters, 18
  RenameCluster, 19
* scatter
  ClusteringScatterPlot, 5
  GeneScatterPlot, 12
* select
  SelectKClusters, 25
  SelectTopGenes, 25
* t-Distributed
  RunTSNE, 23
* t-SNE
  RunTSNE, 23
* t-sne
  AnnotationScatterPlot, 3
* top
  SelectTopGenes, 25
* umap
  AnnotationScatterPlot, 3
* violin
  VlnPlot, 27
* visualization
  AnnotationScatterPlot, 3
  GeneScatterPlot, 12
* ward
  CalcSilhInfo, 4
  HierarchicalClustering, 13
  SilhouetteCurve, 26

AnnotationScatterPlot, 3
AnnotationScatterPlot, SingleCellExperiment-method
(AnnotationScatterPlot), 3
AnnotationScatterPlot, SingleCellExperiment
(AnnotationScatterPlot), 3
INDEX

CalcSilhInfo, 4
CalcSilhInfo, SingleCellExperiment-method (CalcSilhInfo), 4
CalcSilhInfo.SingleCellExperiment (CalcSilhInfo), 4
ClusteringScatterPlot, 5
ClusteringScatterPlot, SingleCellExperiment-method (ClusteringScatterPlot), 5
ClusteringScatterPlot.SingleCellExperiment (ClusteringScatterPlot), 5

DownOverSampling, 6

FindAllGeneMarkers, 7
FindAllGeneMarkers, SingleCellExperiment-method (FindAllGeneMarkers), 7
FindAllGeneMarkers.SingleCellExperiment (FindAllGeneMarkers), 7
FindGeneMarkers, 9
FindGeneMarkers, SingleCellExperiment-method (FindGeneMarkers), 9
FindGeneMarkers.SingleCellExperiment (FindGeneMarkers), 9

GeneHeatmap, 11
GeneHeatmap, SingleCellExperiment-method (GeneHeatmap), 11
GeneHeatmap.SingleCellExperiment (GeneHeatmap), 11
GeneScatterPlot, 12
GeneScatterPlot, SingleCellExperiment-method (GeneScatterPlot), 12
GeneScatterPlot.SingleCellExperiment (GeneScatterPlot), 12

HierarchicalClustering, 13
HierarchicalClustering, SingleCellExperiment-method (HierarchicalClustering), 13
HierarchicalClustering.SingleCellExperiment (HierarchicalClustering), 13

LogisticRegression, 14

MergeClusters, 15
MergeClusters, SingleCellExperiment-method (MergeClusters), 15
MergeClusters.SingleCellExperiment (MergeClusters), 15

pbmc3k_500, 16

PCAElbowPlot, 17
PCAElbowPlot, SingleCellExperiment-method (PCAElbowPlot), 17
PCAElbowPlot.SingleCellExperiment (PCAElbowPlot), 17
PrepareILoReg, 17
PrepareILoReg, SingleCellExperiment-method (PrepareILoReg), 17
PrepareILoReg.SingleCellExperiment (PrepareILoReg), 17

RenameAllClusters, 18
RenameAllClusters, SingleCellExperiment-method (RenameAllClusters), 18
RenameAllClusters.SingleCellExperiment (RenameAllClusters), 18
RenameCluster, 19
RenameCluster, SingleCellExperiment-method (RenameCluster), 19
RenameCluster.SingleCellExperiment (RenameCluster), 19

RunICP, 20
RunParallelICP, 21
RunParallelICP, SingleCellExperiment-method (RunParallelICP), 21
RunParallelICP.SingleCellExperiment (RunParallelICP), 21

RunPCA, 22
RunPCA, SingleCellExperiment-method (RunPCA), 22
RunPCA.SingleCellExperiment (RunPCA), 22
RunTSNE, 23
RunTSNE, SingleCellExperiment-method (RunTSNE), 23
RunTSNE.SingleCellExperiment (RunTSNE), 23

RunUMAP, 24
RunUMAP, SingleCellExperiment-method (RunUMAP), 24
RunUMAP.SingleCellExperiment (RunUMAP), 24

SelectKClusters, 25
SelectKClusters, SingleCellExperiment-method (SelectKClusters), 25
SelectKClusters.SingleCellExperiment (SelectKClusters), 25

SelectTopGenes, 25
SilhouetteCurve, 26
SilhouetteCurve, SingleCellExperiment-method (SilhouetteCurve), 26
SilhouetteCurve.SingleCellExperiment (SilhouetteCurve), 26

VlnPlot, 27
VlnPlot, SingleCellExperiment-method (VlnPlot), 27
VlnPlot.SingleCellExperiment (VlnPlot), 27