

# Package ‘ILoReg’

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**Type** Package

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

**Version** 1.20.0

**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

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|                       |                                                                                    |
|-----------------------|------------------------------------------------------------------------------------|
| AnnotationScatterPlot | <i>Visualiation of a custom annotation over nonlinear dimensionality reduction</i> |
|-----------------------|------------------------------------------------------------------------------------|

---

## Description

The AnnotationScatterPlot enables visualizing arbitrary class labels over the nonliner 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**

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

**Value**

`ggplot2` object if `return.plot=TRUE`

**Examples**

```

library(SingleCellExperiment)
sce <- SingleCellExperiment(assays = list(logcounts = pbmc3k_5000))
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,
  annotation = 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

```
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

```
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)
```

---

ClusteringScatterPlot *Visualize the clustering over nonlinear dimensionality reduction*

---

## Description

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

## Usage

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

## 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 | <i>Down- and oversample data</i> |
|------------------|----------------------------------|

---

## 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

---

|                    |                                                        |
|--------------------|--------------------------------------------------------|
| FindAllGeneMarkers | <i>identification of gene markers for all clusters</i> |
|--------------------|--------------------------------------------------------|

---

## 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**

```
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
)

## 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, then the DE analysis of this cluster is skipped. Default is 3.                           |

|                                    |                                                                                                                                                                              |
|------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>max.cells.per.cluster</code> | The maximum number of cells per cluster if downsampling is performed to speed up the DE analysis. Default is NULL, i.e. no downsampling.                                     |
| <code>pseudocount.use</code>       | 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.         |
| <code>return.thresh</code>         | If <code>only.pos=TRUE</code> , 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. |
| <code>only.pos</code>              | 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_5000))
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 | <i>Identification of gene markers for a cluster or two arbitrary combinations of clusters</i> |
|-----------------|-----------------------------------------------------------------------------------------------|

---

### 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)
```

---

|             |                                                                                   |
|-------------|-----------------------------------------------------------------------------------|
| GeneHeatmap | <i>Heatmap visualization of the gene markers identified by FindAllGeneMarkers</i> |
|-------------|-----------------------------------------------------------------------------------|

---

### 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.SingleCellExperiment(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 "CalcSilhInfo" 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)
```

---

GeneScatterPlot

---

*Visualize gene expression over nonlinear dimensionality reduction*


---

**Description**

GeneScatterPlot enables visualizing gene expression of a gene over nonlinear dimensionality reduction 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
  )

```

### Arguments

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

### 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 <- RunTSNE(sce)
GeneScatterPlot(sce,"CD14",dim.reduction.type="tsne")
sce <- RunUMAP(sce)
GeneScatterPlot(sce,"CD14",dim.reduction.type="umap")

```

---

## HierarchicalClustering

*Hierarchical clustering using the Ward's method*

---

### Description

Perform Hierarchical clustering using the Ward's method.

### Usage

```

HierarchicalClustering.SingleCellExperiment(object)

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

```

**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 | <i>Clustering projection using logistic regression from the LiblineaR R package</i> |
|--------------------|-------------------------------------------------------------------------------------|

---

**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 (dgCMatrix) 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.                             |
| 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".                                                                                     |

|                                 |                                                                                                                                                                                                                                   |
|---------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <code>test.sparse.matrix</code> | A sparse matrix (dgCMatrix) containing test sample's gene expression data with genes in rows and cells in columns. Default is NULL.                                                                                               |
| <code>d</code>                  | 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

---

|               |                       |
|---------------|-----------------------|
| MergeClusters | <i>Merge clusters</i> |
|---------------|-----------------------|

---

**Description**

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

**Usage**

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

```
## S4 method for signature 'SingleCellExperiment'
```

```
MergeClusters(object, clusters.to.merge = "", new.name = "")
```

**Arguments**

|                                |                                                                                                                                           |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| <code>object</code>            | of SingleCellExperiment class                                                                                                             |
| <code>clusters.to.merge</code> | a character or numeric vector for the names of the clusters to merge                                                                      |
| <code>new.name</code>          | 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**

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)
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**

```
data(pbmc3k_500)
```

**Format**

pbmc3k\_500, dgCMatrx object

**Source**

<https://support.10xgenomics.com/single-cell-gene-expression>

**Examples**

```
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)
```

---

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**

```
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)])
```

---

RenameCluster

*Renaming one cluster*


---

**Description**

RenameCluster function enables renaming a cluster in ‘clustering.manual’ slot.

**Usage**

```
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**

```
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")
```

---

RunICP

---

*Iterative Clustering Projection (ICP) clustering*


---

**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**

```
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 $\text{ceiling}(N/k*d)$ ), 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 | <i>Run ICP runs parallelly</i> |
|----------------|--------------------------------|

---

**Description**

This functions runs in parallel  $L$  ICP runs, which is the computational bottleneck of ILoReg. With  $\sim 3,000$  cells this step should be completed in  $\sim 2$  h and  $\sim 1$  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**

|        |                                                                                                                                                                                                               |
|--------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| object | An object of SingleCellExperiment class.                                                                                                                                                                      |
| k      | 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. |

|          |                                                                                                                                                                                                                                                                                                                                                                                                          |
|----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| d        | 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.                                                                      |
| L        | 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 ( $\sim 0.01$ ) 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

```
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)
```

---

RunPCA

*PCA transformation of the joint probability matrix*

---

### Description

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

### Usage

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

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

**Arguments**

|           |                                                                                                                                  |
|-----------|----------------------------------------------------------------------------------------------------------------------------------|
| object    | 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           |

**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 <- RunTSNE(sce)
```

RunUMAP

*Uniform Manifold Approximation and Projection (UMAP)***Description**

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

**Usage**

```
RunUMAP.SingleCellExperiment(object)

## S4 method for signature 'SingleCellExperiment'
RunUMAP(object)
```

**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 <- 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**

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

**Arguments**

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

**Value**

an object of 'data.frame' 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)
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.

**Usage**

```
SilhouetteCurve.SingleCellExperiment(object, return.plot)
```

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

**Arguments**

|                          |                                                                                                  |
|--------------------------|--------------------------------------------------------------------------------------------------|
| <code>object</code>      | of <code>SingleCellExperiment</code> class                                                       |
| <code>return.plot</code> | a logical denoting whether the <code>ggplot2</code> object should be returned. 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 <- 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

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

## 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"))
```

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