Package 'CIARA'

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Type Package

Title Cluster Independent Algorithm for Rare Cell Types Identification

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Description Identification of markers of rare cell types by looking at genes whose expression is confined in small regions of the expression space <https://github.com/ScialdoneLab>.

License Artistic-2.0

Depends R (>= 4.0)

Imports Biobase, ggplot2, ggraph, magrittr

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Contents

CIARA *CIARA*

Description

It selects highly localized genes as specified in *CIARA_gene*, starting from genes in *background*

Usage

```
CIARA(
  norm_matrix,
  knn_matrix,
  background,
  cores_number = 1,
  p_value = 0.001,
  odds_ratio = 2,
  local_region = 1,
  approximation = FALSE
)
```

Arguments

norm_matrix	Norm count matrix (n_genes X n_cells).
knn_matrix	K-nearest neighbors matrix (n_cells X n_cells).
background	Vector of genes for which the function *CIARA_gene* is run.
cores_number	Integer.Number of cores to use.
p_value	p value returned by the function *fisher.test* with parameter alternative = "g"
odds_ratio	odds_ratio returned by the function *fisher.test* with parameter alternative = "g"
local_region	Integer. Minimum number of local regions (cell with its knn neighbours) where the binarized gene expression is enriched in 1.
approximation	Logical.For a given gene, the fisher test is run in the local regions of only the cells where the binarized gene expression is 1.

Value

Dataframe with n_rows equal to the length of *background* . Each row is the output from *CIARA_gene*.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

CIARA_gene *CIARA_gene*

Description

The gene expression is binarized (1/0) if the value in a given cell is above/below the median. Each of cell with its first K nearest neighbors defined a local region. If there are at least *local_region* enriched in 1 according to *fisher.test*, then the gene is defined as highly localized and a final p value is assigned to it. The final p value is the minimum of the p values from all the enriched local regions. If there are no enriched local regions, then the p value by default is set to 1

Usage

```
CIARA_gene(
  norm_matrix,
  knn_matrix,
  gene_expression,
  p_value = 0.001,
  odds_ratio = 2,
  local_region = 1,
  approximation = FALSE
)
```

Arguments

norm_matrix	Norm count matrix (n_genes X n_cells).
knn_matrix	K-nearest neighbors matrix (n_cells X n_cells).
gene_expression	numeric vector with the gene expression (length equal to n_cells). The gene expression is binarized (equal to 0/1 in the cells where the value is below/above the median)
p_value	p value returned by the function *fisher.test* with parameter alternative = "g"
odds_ratio	odds_ratio returned by the function *fisher.test* with parameter alternative = "g"
local_region	Integer. Minimum number of local regions (cell with its knn neighbours) where the binarized gene expression is enriched in 1.
approximation	Logical.For a given gene, the fisher test is run in the local regions of only the cells where the binarized gene expression is 1.

Value

List with one element corresponding to the p value of the gene.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/fisher.test

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`cluster_analysis_integrate_rare`
cluster_analysis_integrate_rare

Description

cluster_analysis_integrate_rare

Usage

```
cluster_analysis_integrate_rare(
  raw_counts,
  project_name,
  resolution,
  neighbors,
  max_dimension,
  feature_genes = NULL
)
```

Arguments

`raw_counts`	Raw count matrix (n_genes X n_cells).
`project_name`	Character name of the Seurat project.
`resolution`	Numeric value specifying the parameter *resolution* used in the Seurat function *FindClusters*.
`neighbors`	Numeric value specifying the parameter *k.param* in the Seurat function *FindNeighbors*
`max_dimension`	Numeric value specifying the maximum number of the PCA dimensions used in the parameter *dims* for the Seurat function *FindNeighbors*
`feature_genes`	vector of features specifying the argument *features* in the Seurat function *RunPCA*.

Value

Seurat object including raw and normalized counts matrices, UMAP coordinates and cluster result.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://www.rdocumentation.org/packages/Seurat/versions/4.0.1/topics/FindClusters
https://www.rdocumentation.org/packages/Seurat/versions/4.0.1/topics/FindNeighbors
https://www.rdocumentation.org/packages/Seurat/versions/4.0.1/topics/RunPCA

`cluster_analysis_sub` *cluster_analysis_sub*

Description

cluster_analysis_sub

Usage

```
cluster_analysis_sub(
  raw_counts,
  resolution,
  neighbors,
  max_dimension,
  name_cluster
)
```

Arguments

`raw_counts`	Raw count matrix (n_genes X n_cells).
`resolution`	Numeric value specifying the parameter *resolution* used in the Seurat function *FindClusters*.
`neighbors`	Numeric value specifying the parameter *k.param* in the Seurat function *FindNeighbors*
`max_dimension`	Numeric value specifying the maximum number of the PCA dimensions used in the parameter *dims* for the Seurat function *FindNeighbors*
`name_cluster`	Character.Name of the original cluster for which the sub clustering is done.

Value

Seurat object including raw and normalized counts matrices and cluster result.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://www.rdocumentation.org/packages/Seurat/versions/4.0.1/topics/RunPCA https://www.rdocumentation.org/packages/Seurat/versions/4.0.1/topics/FindVariableFeatures

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`find_resolution` *find_resolution*

Description

find_resolution

Usage

```
find_resolution(seurat_object, resolution_vector)
```

Arguments

`seurat_object`	Seurat object as returned by *cluster_analysis_integrate_rare*
`resolution_vector`	vector with all values of resolution for which the Seurat function *FindClusters* is run

Value

Clustree object showing the connection between clusters obtained at different level of resolution as specified in *resolution_vector*.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://CRAN.R-project.org/package=clustree

`get_background_full` *get_background_full*

Description

get_background_full

Usage

```
get_background_full(
  norm_matrix,
  threshold = 1,
  n_cells_low = 3,
  n_cells_high = 20
)
```

Arguments

`norm_matrix`	Norm count matrix (n_genes X n_cells).
`threshold`	threshold in expression for a given gene
`n_cells_low`	minimum number of cells where a gene is expressed at a level above threshold
`n_cells_high`	maximum number of cells where a gene is expressed at a level above threshold

Value

Character vector with all genes expressed at a level higher than *threshold* in a number of cells between *n_cells* and *n_cells_high*.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

`markers_cluster_seurat` *markers_cluster_seurat*

Description

The Seurat function *FindMarkers* is used to identify general marker for each cluster (specific cluster vs all other cluster). This list of markers is then filtered keeping only the genes that appear as markers in a unique cluster.

Usage

```
markers_cluster_seurat(seurat_object, cluster, cell_names, number_top)
```

Arguments

seurat_object	Seurat object as returned by *cluster_analysis_sub* or by *cluster_analysis_integrate_rare*.
cluster	Vector of length equal to the number of cells, with cluster assignment.
cell_names	Vector of length equal to the number of cells, with cell names.
number_top	Integer. Number of top marker genes to keep for each cluster.

Value

List of three elements. The first is a vector with *number_top* marker genes for each cluster. The second is a vector with *number_top* marker genes and corresponding cluster. The third element is a vector with all marker genes for each cluster.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://www.rdocumentation.org/packages/Seurat/versions/4.0.1/topics/FindMarkers

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merge_cluster *merge_cluster*

Description

merge_cluster

Usage

```
merge_cluster(old_cluster, new_cluster, max_number = NULL)
```

Arguments

old_cluster	original cluster assignment that need to be updated
new_cluster	new cluster assignment that need to be integrated with *old_cluster*.
max_number	Threshold in size for clusters in *new_cluster*. Only cluster with number of cells smaller than *max_number* will be integrated in *old cluster*. If *max_number* is NULL, then all the clusters in *new_cluster* are integrated in *old cluster*.

Value

Numeric vector of length equal to *old_cluster* showing the merged cluster assignment between *old cluster* and *new_cluster*.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

plot_balloon_marker *plot_balloon_marker*

Description

plot_balloon_marker

Usage

```
plot_balloon_marker(
  norm_counts,
  cluster,
  marker_complete,
  max_number,
  max_size = 5,
  text_size = 7
)
```

Arguments

norm_counts	Norm count matrix (genes X cells).
cluster	Vector of length equal to the number of cells, with cluster assignment.
marker_complete	Third element of the output list as returned by the function *markers_cluster_seurat*
max_number	Integer. Maximum number of markers for each cluster for which we want to plot the expression.
max_size	Integer. Size of the dots to be plotted.
text_size	Size of the text in the heatmap plot.

Value

ggplot2 object showing balloon plot.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

plot_gene *plot_gene*

Description

Cells are coloured according to the expression of *gene_id* and plotted according to *coordinate_umap*.

Usage

```
plot_gene(norm_counts, coordinate_umap, gene_id, title_name)
```

Arguments

norm_counts	Norm count matrix (genes X cells).
coordinate_umap	Data frame with dimensionality reduction coordinates. Number of rows must be equal to the number of cells
gene_id	Character name of the gene.
title_name	Character name.

Value

ggplot2 object.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://CRAN.R-project.org/package=ggplot2

plot_genes_sum *plot_genes_sum*

Description

The sum of each gene in *genes_relevant* across all cells is first normalized to 1. Then for each cell, the sum from the (normalized) genes expression is computed and shown in the output plot.

Usage

```
plot_genes_sum(coordinate_umap, norm_counts, genes_relevant, name_title)
```

Arguments

coordinate_umap	Data frame with dimensionality reduction coordinates. Number of rows must be equal to the number of cells
norm_counts	Norm count matrix (genes X cells).
genes_relevant	Vector with gene names for which we want to visualize the sum in each cell.
name_title	Character value.

Value

ggplot2 object.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://CRAN.R-project.org/package=ggplot2

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plot_heatmap_marker *plot_heatmap_marker*

Description

plot_heatmap_marker

Usage

```
plot_heatmap_marker(
  marker_top,
  marker_all_cluster,
  cluster,
  condition,
  norm_counts,
  text_size
)
```

Arguments

marker_top	First element returned by *markers_cluster_seurat*
marker_all_cluster	Second element returned by *markers_cluster_seurat*
cluster	Vector of length equal to the number of cells, with cluster assignment.
condition	Vector or length equal to the number of cells, specifying the condition of the cells (i.e. batch, dataset of origin..)
norm_counts	Norm count matrix (genes X cells).
text_size	Size of the text in the heatmap plot.

Value

Heatmap class object.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://www.rdocumentation.org/packages/ComplexHeatmap/versions/1.10.2/topics/Heatmap

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plot_interactive *plot_interactive*

Description

It shows in an interactive plot which are the highly localized genes in each cell. It is based on plotly library

Usage

```
plot_interactive(
  coordinate_umap,
  color,
  text,
  min_x = NULL,
  max_x = NULL,
  min_y = NULL,
  max_y = NULL
)
```

Arguments

coordinate_umap	Data frame with dimensionality reduction coordinates. Number of rows must be equal to the number of cells
color	vector of length equal to n_rows in coordinate_umap.Each cell will be coloured following a gradient according to the corresponding value of this vector.
text	Character vector specifying the highly localized genes in each cell. It is the output from *selection_localized_genes*.
min_x	Set the min limit on the x axis.
max_x	Set the max limit on the x axis.
min_y	Set the min limit on the y axis.
max_y	Set the min limit on the y axis.

Value

plotly object given by *plot_ly function* (from library *plotly*).

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://plotly.com/r/

`plot_umap` *plot_umap*

Description

plot_umap

Usage

```
plot_umap(coordinate_umap, cluster)
```

Arguments

`coordinate_umap`	Data frame with dimensionality reduction coordinates. Number of rows must be equal to the number of cells
`cluster`	Vector of length equal to the number of cells, with cluster assignment.

Value

ggplot2 object.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://CRAN.R-project.org/package=ggplot2

selection_localized_genes *selection_localized_genes*

Description

selection_localized_genes

Usage

```
selection_localized_genes(
  norm_counts,
  localized_genes,
  min_number_cells = 4,
  max_number_genes = 10
)
```

Arguments

norm_counts	Norm count matrix (genes X cells).
localized_genes	vector of highly localized genes as provided by the last element of the list given as output from *CIARA_mixing_final*.
min_number_cells	Minimum number of cells where a genes must be expressed (> 0).
max_number_genes	Maximum number of genes to show for each cell in the interactive plot from *plot_interactive*.

Value

Character vector where each entry contains the name of the top *max_number_genes* for the corresponding cell.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

test_hvg *test_hvg*

Description

For each cluster in *cluster*, HVGs are defined with Seurat function *FindVariableFeatures*. A Fisher test is performed to see if there is a statistically significant enrichment between the top *number_hvg* and the *localized_genes*

Usage

```
test_hvg(
  raw_counts,
  cluster,
  localized_genes,
  background,
  number_hvg,
  min_p_value
)
```

Arguments

raw_counts	Raw count matrix (n_genes X n_cells).
cluster	Vector of length equal to the number of cells, with cluster assignment.
localized_genes	Character vector with localized genes detected by CIARA.
background	Character vector with all the genes names to use as background for the Fisher test.
number_hvg	Integer value. Number of top HVGs provided by the Seurat function *FindVariableFeatures*.
min_p_value	Threshold on p values provided by Fisher test.

Value

A list with two elements.

first element	The first one is a list with length equal to the number of clusters. Each entry is list of three elements. The first two elements contain the p value and the odds ration given by the Fisher test The third is a vector with genes names that are present both in *localized_genes* and in top *number_hvg* HVGs .
second element	a character vector with the name of the cluster that have a p value smaller than *min_p_value*.

Author(s)

Gabriele Lubatti <gabriele.lubatti@helmholtz-muenchen.de>

See Also

https://www.rdocumentation.org/packages/stats/versions/3.6.2/topics/fisher.test

---

`white_black_markers` *white_black_markers*

Description

A white-marker is a gene whose median expression across cells belong to *single_cluster* is greater than *threshold* and in all the other clusters is equal to zero.

Usage

```
white_black_markers(
  cluster,
  single_cluster,
  norm_counts,
  marker_list,
  threshold = 0
)
```

Arguments

`cluster`	Vector of length equal to the number of cells, with cluster assignment.
`single_cluster`	Character. Label of one specify cluster
`norm_counts`	Norm count matrix (genes X cells).
`marker_list`	Third element of the output list as returned by the function *markers_cluster_seurat*
`threshold`	Numeric. The median of the genes across cells belong to *single_cluster* has to be greater than *threshold* in order to be consider as a white-black marker for *single_cluster*

Value

Logical vector of length equal to *marker_list*, with TRUE/FALSE if the gene is/is not a white-black marker for *single_cluster*.

Author(s)

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