

Package: CIARA (via r-universe)

August 30, 2024

Type Package

Title Cluster Independent Algorithm for Rare Cell Types Identification

Version 0.1.0

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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

Suggests circlize, clustree, ComplexHeatmap, plotly, Seurat (>= 4.0), testthat, knitr, rmarkdown

biocViews software

Config/testthat/edition 3

Encoding UTF-8

RoxygenNote 7.1.1

VignetteBuilder knitr

NeedsCompilation no

Date/Publication 2022-02-22 20:00:02 UTC

Repository <https://gabrielelubatti.r-universe.dev>

RemoteUrl <https://github.com/cran/CIARA>

RemoteRef HEAD

RemoteSha fdba9cd1aa1d41a784a882c0639d08710c2037ac

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| | |
|-------|--------------|
| CIARA | <i>CIARA</i> |
|-------|--------------|

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

| | |
|---------------------------|--|
| <code>norm_matrix</code> | Norm count matrix (n_genes X n_cells). |
| <code>knn_matrix</code> | K-nearest neighbors matrix (n_cells X n_cells). |
| <code>background</code> | Vector of genes for which the function <i>CIARA_gene</i> is run. |
| <code>cores_number</code> | Integer.Number of cores to use. |
| <code>p_value</code> | p value returned by the function <i>fisher.test</i> with parameter <code>alternative = "g"</code> |
| <code>odds_ratio</code> | odds_ratio returned by the function <i>fisher.test</i> with parameter <code>alternative = "g"</code> |

`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>

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 <i>resolution</i> used in the Seurat function <i>FindClusters</i> . |
| neighbors | Numeric value specifying the parameter <i>k.param</i> in the Seurat function <i>FindNeighbors</i> . |
| max_dimension | Numeric value specifying the maximum number of the PCA dimensions used in the parameter <i>dims</i> for the Seurat function <i>FindNeighbors</i> . |
| feature_genes | vector of features specifying the argument <i>features</i> in the Seurat function <i>RunPCA</i> . |

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 <i>resolution</i> used in the Seurat function <i>FindClusters</i> . |
| neighbors | Numeric value specifying the parameter <i>k.param</i> in the Seurat function <i>FindNeighbors</i> |
| max_dimension | Numeric value specifying the maximum number of the PCA dimensions used in the parameter <i>dims</i> for the Seurat function <i>FindNeighbors</i> |
| 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>

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 <i>cluster_analysis_sub</i> or by <i>cluster_analysis_integrate_rare</i> . |
| 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>

| | |
|---------------|----------------------|
| merge_cluster | <i>merge_cluster</i> |
|---------------|----------------------|

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 <i>old_cluster</i> . |
| max_number | Threshold in size for clusters in <i>new_cluster</i> . Only cluster with number of cells smaller than <i>max_number</i> will be integrated in <i>old cluster</i> . If <i>max_number</i> is NULL, then all the clusters in <i>new_cluster</i> are integrated in <i>old cluster</i> . |

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 <i>markers_cluster_seurat</i> |
| 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>

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 <i>markers_cluster_seurat</i> |
| marker_all_cluster | Second element returned by <i>markers_cluster_seurat</i> |
| 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>

| | |
|------------------|-------------------------|
| plot_interactive | <i>plot_interactive</i> |
|------------------|-------------------------|

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 <i>selection_localized_genes</i> . |
| 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 | <i>test_hvg</i> |
|----------|-----------------|

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

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

Value

A list with two elements.

| | |
|-----------------------------|---|
| <code>first element</code> | 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 <i>localized_genes</i> and in top <i>number_hvg</i> HVGs . |
| <code>second element</code> | a character vector with the name of the cluster that have a p value smaller than <i>min_p_value</i> . |

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 <i>markers_cluster_seurat</i> |
| threshold | Numeric. The median of the genes across cells belong to <i>single_cluster</i> has to be greater than <i>threshold</i> in order to be consider as a white-black marker for <i>single_cluster</i> |

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)

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

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