Package ‘CHETAH’

May 21, 2024

Title  Fast and accurate scRNA-seq cell type identification
Type   Package
Version 1.20.0
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Description  CHETAH (CHaracterization of cEll Types Aided by Hierarchical classification) is an ac-
accurate, selective and fast scRNA-seq classifier.
   Classification is guided by a reference dataset,
preferentially also a scRNA-seq dataset. By hierarchical clustering of the reference data, CHETAH creates
   a classification tree that enables a step-wise, top-to-bottom classification. Using a novel stopping rule,
   CHETAH classifies the input cells to the cell types of the references and to "intermedi-
at types": more general
   classifications that ended in an intermediate node of the tree.

Imports  shiny, plotly, pheatmap, bioDist, dendextend, cowplot,
corrplot, grDevices, stats, graphics, reshape2, S4Vectors,
SummarizedExperiment

Depends  R (>= 4.2), ggplot2, SingleCellExperiment
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Encoding UTF-8

biocViews Classification, RNASeq, SingleCell, Clustering,
   GeneExpression, ImmunoOncology

RoxygenNote 7.2.0
Suggests knitr, rmarkdown, Matrix, testthat, vdiff
VignetteBuilder knitr
LazyData false

BugReports https://github.com/jdekanter/CHETAH

URL https://github.com/jdekanter/CHETAH

git_url https://git.bioconductor.org/packages/CHETAH

1
CHETAHclassifier

Identification of cell types aided by hierarchical clustering

Description

CHETAH classifies an input dataset by comparing it to a reference dataset in a stepwise, top-to-bottom fashion. See 'details' for a full explanation. NOTE: We recommend to use all the default parameters

Usage

CHETAHclassifier(
  input,
  ref_cells = NULL,
  ref_profiles = NULL,
  ref_ct = "celltypes",
  input_c = NA,
  ref_c = NA,
  thresh = 0.1,
  gs_method = c("fc", "wilcox"),
  cor_method = c("spearman", "kendall", "pearson", "cosine"),
  clust_method = c("average", "single", "complete", "ward.D2", "ward.D", "mcquitty"),
)
CHETAHclassifier

```r
clust_dist = bioDist::spearman.dist,
median, centroid",
clust_dist = bioDist::spearman.dist,
n_genes = 200,
fc_thresh = 1.5,
subsample = FALSE,
fix_ngenes = TRUE,
plot.tree = FALSE,
only_pos = FALSE,
print_steps = FALSE
)
```

### Arguments

- **input** required: an input SingleCellExperiment. (see: Bioconductor, and the vignette browseVignettes("CHETAH"))
- **ref_cells** required: A reference SingleCellExperiment, with the cell types in the "cell-types" colData (or otherwise defined in ref_ct).
- **ref_profiles** optional: In case of bulk-RNA seq or micro-arrays, an expression matrix with one (average) reference expression profile per cell type in the columns. ('ref_cells' must be left empty)
- **ref_ct** the colData of ref_cells where the cell types are stored.
- **input_c** the name of the assay of the input to use. NA (default) will use the first one.
- **ref_c** same as input_c, but for the reference.
- **thresh** the initial confidence threshold, which can be changed after running by `Classify`
- **gs_method** method for gene selection. In every node of the tree: "fc" = quick method: either a fixed number (n_genes) of genes is selected with the highest fold-change (default), or genes are selected that have a fold-change higher than fc_thresh (the latter is used when fix_ngenes = FALSE). "wilcox": genes are selected based on fold-change (fc_thresh), percentage of expression (pc_thresh) and p-values (p_thresh), p-values are found by the wilcox test.
- **cor_method** the correlation measure: one of: "spearman" (default), "kendall", "pearson", "cosine"
- **clust_method** the method used for clustering the reference profiles. One of the methods from hclust
- **clust_dist** a distance measure, default: `spearman.dist`
- **n_genes** The number of genes used in every step. Only used if fix_ngenes = TRUE
- **pc_thresh** when: gs_method = "wilcox", only genes are selected for which more than a pc_tresh fraction of a reference group of cells express that gene
- **p_thresh** when: gs_method = "wilcox", only genes are selected that have a p-value < p_thresh
**CHETAHClassifier**

- **fc_thresh**
  - when: `gs_method = "wilcox" or gs_method = "fc" AND fix_ngenes = FALSE`, only genes are selected that have a log2 fold-change > `fc_thresh` between two reference groups.
  - **if this mode is selected, the reference must be in the log2 space.**

- **subsample**
  - to prevent reference types with a lot of cells to influence the gene selection, subsample types with more than `subsample` cells

- **fix_ngenes**
  - when: `gs_method = "fc"` use a fixed number of genes for all correlations. When `fix_ngenes = FALSE & gs_method = "fc"` `fc_thresh` is used to define the fold-change cut-off for gene selection.

- **plot.tree**
  - Plot the classification tree.

- **only_pos**
  - not recommended: only use genes for a reference type that are higher expressed in that type, than the others in that node.

- **print_steps**
  - whether the number of genes (positive and negative) per step per ref_cell_type should be printed

**Details**

CHETAH will hierarchically cluster reference data to produce a classification tree (ct). In each node of the ct, CHETAH will assign each input cell to one of the two branches, based on gene selections, correlations and calculation of profile and confidence scores. The assignment will only performed if the confidence score for such an assignment is higher than the Confidence Threshold. If this is not the case, classification for the cell will stop in the current node. Some input cells will reach the leaf nodes of the ct (the pre-defined cell types), these classifications are called **final types** For other cells, assignment will stop in a node. These classifications are called **intermediate types**.

**Value**

A SingleCellExperiment with added:
- `input$celltype_CHETAH` a named character vector that can directly be used in any other workflow/method.
- "hidden" ‘int_colData’ and ‘int_metadata’, not meant for direct interaction, but which can all be viewed and interacted with using: ‘PlotCHETAH’ and ‘CHETAHshiny’ A list containing the following objects is added to input$int_metadata$CHETAH
  - **classification** a named vector: the classified types with the corresponding names of the input cells
  - **tree** the hclust object of the classification tree
  - **nodetypes** A list with the cell types under each node
  - **nodecoord** the coordinates of the nodes of the classification tree
  - **genes** A list per node, containing a list per reference type with the genes used for the profile scores of that type
  - **parameters** The parameters used

A nested DataFrame is added to input$int_colData$CHETAH. It holds 3 top-levels DataFrames
- **prof_scores** A list with the profile scores
- **conf_scores** A list with the confidence scores
- **correlations** A list with the correlations of the input cells to the reference profiles
Examples

```r
# Load data
data('input_mel')
data('headneck_ref')

## Melanoma data from Tirosh et al. (2016) Science
input_mel

## Head-Neck data from Puram et al. (2017) Cancer Cell
headneck_ref

# Run CHETAHclassifier
input_mel <- CHETAHclassifier(input = input_mel, ref_cells = headneck_ref)
```

CHETAHshiny

Launch a web page to interactively go through the classification

Description

Launch a web page to interactively go through the classification

Usage

```r
CHETAHshiny(input, redD = NA, input_c = NA)
```

Arguments

- **input**: a SingleCellExperiment on which `CHETAHclassifier` has been run
- **redD**: the name of the reducedDim of the input to use for plotting
- **input_c**: the name of the assay of the input to use. NA (default) will use the first one.

Value

Opens a web page in your default browser

Classify

(Re)classify after running `CHETAHclassifier` using a confidence threshold

NOTE: In case of bulk reference profiles: only the correlations will be used, as the data does not allow for profile or confidence scores to be calculated.

Description

(Re)classify after running `CHETAHclassifier` using a confidence threshold

NOTE: In case of bulk reference profiles: only the correlations will be used, as the data does not allow for profile or confidence scores to be calculated.

Usage

```r
Classify(input, thresh = 0.1, return_clas = FALSE)
```
Arguments

- **input**: a SingleCellExperiment on which CHETAHclassifier has been run
- **thresh**: a confidence threshold between -0 and 2. Selecting 0 will classify all cells, whereas 2 will result in (almost) no cells to be classified.
  - *recommended*: between 0.1 (fairly confident) and 1 (very confident)
- **return_clas**: Instead of returning the SingleCellExperiment, only return the classification vector

Value

- a character vector of the cell types with the names of the cells

Examples

```r
data('input_mel')
data('headneck_ref')
## Classify all cells
input_mel <- Classify(input_mel, 0)

## Classify only cells with a very high confidence
input_mel <- Classify(input_mel, 1)

## Back to the default
input_mel <- Classify(input_mel)

## Return only the classification vector
celltypes <- Classify(input_mel, 1, return_clas = TRUE)
```

ClassifyReference

Use a reference dataset to classify itself. A good reference should have almost no mixture between reference cells.

Description

Use a reference dataset to classify itself. A good reference should have almost no mixture between reference cells.

Usage

```r
ClassifyReference(
  ref_cells,
  ref_ct = "celltypes",
  ref_c = "counts",
  return = FALSE,
  ...
)
```
CorrelateReference

Arguments

ref_cells  the reference, similar to CHETAHclassifier’s ref_cells
ref_ct    the colData of ref_cells where the cell types are stored.
ref_c     same as input_c, but for the reference.
return    return the matrix that was used to produce the plot
...

Other variables to pass to CHETAHclassifier

Value

A square plot. The rows are the original cell types, the columns the classification labels. The colors and sizes of the squares indicate which part of the cells of the rowname type are classified to the type of the column name. On the left of the plot, the percentage of cells that is classified to an intermediate type is plotted. A good reference would classify nearly 100

Examples

data('headneck_ref')
ClassifyReference(ref_cells = headneck_ref)

CorrelateReference  Correlate all reference profiles to each other using differentially expressed genes.

Description

Correlate all reference profiles to each other using differentially expressed genes.

Usage

CorrelateReference(
    ref_cells = NULL,
    ref_profiles = NULL,
    ref_ct = "celltypes",
    ref_c = NA,
    return = FALSE,
    n_genes = 200,
    fix_ngenes = TRUE,
    print_steps = FALSE,
    only_pos = FALSE
)
Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ref_cells</td>
<td>the reference, similar to CHETAHclassifier’s ref_cells</td>
</tr>
<tr>
<td>ref_profiles</td>
<td>similar to CHETAHclassifier’s ref_profiles</td>
</tr>
<tr>
<td>ref_ct</td>
<td>the colData of ref_cells where the cell types are stored.</td>
</tr>
<tr>
<td>ref_c</td>
<td>the assay of ref_cells to use</td>
</tr>
<tr>
<td>return</td>
<td>return the matrix that was used to produce the plot</td>
</tr>
<tr>
<td>n_genes</td>
<td>as in CHETAHclassifier</td>
</tr>
<tr>
<td>fix_ngenes</td>
<td>as in CHETAHclassifier</td>
</tr>
<tr>
<td>print_steps</td>
<td>as in CHETAHclassifier</td>
</tr>
<tr>
<td>only_pos</td>
<td>as in CHETAHclassifier</td>
</tr>
</tbody>
</table>

Value

A square plot. The values show how much two reference profiles correlate, when using the genes with the highest fold-change.

Examples

```r
data('headneck_ref')
CorrelateReference(ref_cells = headneck_ref)
```

|--------------|-------------------------------------------------------------------------------------------------------------------------------------|

Description


Usage

```r
data('headneck_ref')
```

Format

A list of expression matrices. Each object is named as the cell type of the cells in that matrix. Each matrix has the cell (names) in the columns and the genes in the rows.

Source

for the original data: GEO

References

Puram et al. (2017) Cancer Cell 171:1611-1624
input_mel

A SingleCellExperiment on which CHEATHclassifier is run using the headneck_ref. It holds subset of the Melanoma data, from Tirosh et al. (2016), Science.

**Description**

A SingleCellExperiment on which CHEATHclassifier is run using the headneck_ref. It holds subset of the Melanoma data, from Tirosh et al. (2016), Science.

**Usage**

data('input_mel')

**Format**

This is a SingleCellExperiment

**Source**

for the original data: GEO

**References**

Tirosh et al. (2016) Science 6282:189-196

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PlotCHETAH

Plot the CHETAH classification on 2D visualization like t-SNE + the corresponding classification tree, colored with the same colors

**Description**

Plot the CHETAH classification on 2D visualization like t-SNE + the corresponding classification tree, colored with the same colors

**Usage**

PlotCHETAH(
  input,
  redD = NA,
  interm = FALSE,
  return = FALSE,
  tree = TRUE,
  pt.size = 1,
  return_col = FALSE,
  col = NULL
)
Arguments

- `input`: a SingleCellExperiment on which CHETAHclassifier has been run
- `redD`: the name of the reducedDim of the input to use for plotting
- `interm`: color the intermediate instead of the final types
- `return`: return the plot instead of printing it
- `tree`: plot the tree, along with the classification
- `pt.size`: the point-size of the classification plot
- `return_col`: whether the colors that are used for the classification plot should be returned
- `col`: custom colors for the cell types. The colors should be named with the corresponding cell types

Value

- a ggplot object

Examples

data('input_mel')
## Standard plot (final types colored)
PlotCHETAH(input = input_mel)

## Intermediate types colored
PlotCHETAH(input = input_mel, interm = TRUE)

## Plot only the t-SNE plot
PlotCHETAH(input = input_mel, tree = FALSE)

PlotTree function:

Plots the chetah classification tree with nodes numbered

Description

Plots the chetah classification tree with nodes numbered

Usage

PlotTree(
  input,
  col = NULL,
  col_nodes = NULL,
  return = FALSE,
  no_bgc = FALSE,
  plot_limits = c(-0.4, 0.1),
  labelsize = 6
)
Arguments

input  
a SingleCellExperiment on which CHETAHclassifier has been run

col  
a vector of colors, with the names of the reference cell types

col_nodes  
a vector of colors, ordered for node 1 till the last node

return  
instead of printing, return the ggplot object

no_bgc  
remove the background color from the node numbers

plot_limits  
define the Decreasing the former further is useful when the labels are cut off the plot (default = c(-0.25, 0.1)).

labelszie  
the size of the intermediate and leaf node labels (default = 6)

Value

A ggplot object of the classification tree

Examples

data('input_mel')
PlotTree(input = input_mel)
Arguments

toplot the variable that should be plotted. Either a character vector or a factor, or a (continuous) numeric. If toplot is not named with the rownames of redD, it is assumed that the order of the two is the same.

input a SingleCellExperiment on which CHETAHclassifier has been run

redD the name of the reducedDim of the input to use for plotting

col a vector of colors. If toplot is a numeric, this will become a continuous scale. If toplot is a character vector, the colors should be named with the unique values (levels) of toplot

return instead of printing, return the ggplot object

limits the limits of the continuous variable to plot. When not provided the minimal and maximal value will be used

pt.size the point-size

shiny Needed for the shiny application: should always be NULL

y_limits the y-axis limits

x_limits the x-axis limits, if NULL

legend_label the label of the legend

Value

A ggplot object

Examples

data('input_mel')
CD8 <- assay(input_mel)['CD8A',]
PlotTSNE(toplot = CD8, input = input_mel)

In the CHETAH classification, replace the name of a Node and all the names of the final and intermediate types under that Node.
RenameBelowNode

Usage

RenameBelowNode(
  input,
  whichnode,
  replacement,
  nodes_exclude = NULL,
  types_exclude = NULL,
  node_only = FALSE,
  return_clas = FALSE
)

Arguments

input a SingleCellExperiment on which CHETAHclassifier has been run
whichnode the number of the Node
replacement a character vector that replaces the names under the selected Node
nodes_exclude optional the names of the types that should NOT be replaced
types_exclude optional numbers of the Nodes under the selected Node, that should NOT be replaced
node_only only rename the Node itself, without affecting the types under that Node
return_clas Instead of returning the SingleCellExperiment, only return the classification vector

Value

The SingleCellExperiment with the new classification or if 'return_clas = TRUE' the classification vector.

Examples

## In the example data replace all T-cell subtypes by "T cell"
data('input_mel')
#' input_mel <- RenameBelowNode(input = input_mel, whichnode = 7, replacement = "T cell")
Index

* datasets
  headneck_ref, 8
  input_mel, 9

CHETAHclassifier, 2, 5–8, 10–13
CHETAHshiny, 5
Classify, 3, 5
ClassifyReference, 6
CorrelateReference, 7

hclust, 3
headneck_ref, 8, 9

input_mel, 9

PlotCHETAH, 9
PlotTree, 10
PlotTSNE, 11

RenameBelowNode, 12

spearman.dist, 3