Package ‘cellpypes’

May 19, 2022

Title  Cell Type Pipes for Single-Cell RNA Sequencing Data
Version  0.1.3
Description  Annotate single-cell RNA sequencing data manually based on
marker gene thresholds.
Find cell type rules (gene+threshold) through exploration,
use the popular piping operator '%>%' to reconstruct complex
cell type hierarchies.
'cellpypes' models technical noise to find positive and negative cells for
a given expression threshold and returns cell type labels or pseudobulks.
Cite this package as Frauhammer (2022) doi:10.5281/zenodo.6555728 and
visit https://github.com/FelixTheStudent/cellpypes for tutorials and newest
features.

URL  https://github.com/FelixTheStudent/cellpypes
BugReports  https://github.com/FelixTheStudent/cellpypes/issues
License  GPL (>= 3)
Encoding  UTF-8
LazyData  true
RoxygenNote  7.1.1
Suggests  testthat (>= 3.0.0), knitr, rmarkdown, Seurat, DESeq2,
         RcppAnnoy, tibble, SeuratObject

Config/testthat/edition  3
Imports  scUtils, ggplot2, Matrix, rlang, viridis, cowplot, dplyr,
         scales, methods, scattermore
Depends  R (>= 2.10)
NeedsCompilation  no
Author  Felix Frauhammer [aut, cre]
Maintainer  Felix Frauhammer <felixfrauhammer@gmail.com>
Repository  CRAN
Date/Publication  2022-05-19 07:00:08 UTC
R topics documented:

classify ................................................................. 2
class_to_deseq2 ...................................................... 3
feat ................................................................. 5
find_knn ............................................................. 6
is_classes ............................................................ 7
is_rules .............................................................. 7
plot_classes .......................................................... 8
plot_last ............................................................. 9
pool_across_neighbors .............................................. 11
pseudobulk .......................................................... 11
pseudobulk_id ....................................................... 12
pype_code_template ................................................ 13
pype_from_seurat .................................................... 14
rule ................................................................. 15
simulated_umis ....................................................... 16

Index

classify .............................. Classify cells on previously defined rules

Description

Classify cells on previously defined rules

Usage

classify(
  obj,
  classes = NULL,
  replace_overlap_with = "Unassigned",
  return_logical_matrix = FALSE
)

Arguments

obj  A cellpypes object, see section cellpypes Objects below.

classes  Character vector with one or more class names. If NULL (the default), plots finest available cell types (all classes that are not parent of any other class).

replace_overlap_with  Character string, by default: "Unassigned". See section Handling overlap.

return_logical_matrix  logical. If TRUE, a logical matrix with classes in columns and cells in rows is returned instead of resolving overlaps with replace_overlap_with. If a single class is supplied, the matrix has exactly one
class_to_deseq2

Value
A factor with cell type labels.

celltypes Objects
A celltypes object is a list with four slots:

- raw (sparse) matrix with genes in rows, cells in columns
- totalUMI the colSums of obj$raw
- embed two-dimensional embedding of the cells, provided as data.frame or tibble with two columns and one row per cell.
- neighbors index matrix with one row per cell and k columns, where k is the number of nearest neighbors (we recommend 15<k<100, e.g. k=50). Here are two ways to get the neighbors index matrix:
  - Use find_knn(featureMatrix)$idx, where featureMatrix could be principal components, latent variables or normalized genes (features in rows, cells in columns).
  - use as(seurat@graphs[["RNA_nn"]], "dgCMatrix")> .1 to extract the kNN graph computed on RNA. The > .1 ensures this also works with RNA_snn, wknnsn or any other available graph – check with names(seurat@graphs).

Handling overlap
Overlap denotes all cells for which rules from multiple classes apply, and these cells will be labeled as Unassigned by default. If you are in fact interested in where the overlap is, set return_logical_matrix=TRUE and inspect the result. Note that it matters whether you call classify("Tcell") or classify(c("Tcell","Bcell") – any existing overlap between T and B cells is labelled as Unassigned in this second call, but not in the first.

Replacing overlap happens only between mutually exclusive labels (such as Tcell and Bcell), but not within a lineage. To make an example, overlap is NOT replaced between child (PD1+Ttox) and parent (Ttox) or any other ancestor (Tcell), but instead the most detailed cell type (PD1+Ttox) is returned.

All of the above is also true for plot_classes, as it wraps classify.

Examples

classify(rule(simulated_umis, "Tcell", "CD3E", ">", 1))

class_to_deseq2 Create DESeq2 object for a given cell type

Description
Create a DESeq2 data set (‘dds’ in the DESeq2 vignette) for the specified class (cell type).
Usage

class_to_deseq2(obj, meta_df, class, design = ~condition)

Arguments

obj
A cellpypes object, see section cellpypes Objects below.

meta_df
Data frame where each column helps to identify a pseudobulk. Typical columns of meta_df are for example patient, treatment and cell type – anything that uniquely identifies a replicate / batch / 10x run. Each row in meta_df corresponds to a single cell in your raw count matrix.

class
The name of cellpypes class for which you want to test for differential expression.

design
A formula based on columns in meta_df. To test differential expression between two groups in meta_df$condition, use formula ~ condition. More complex formulas (e.g. with interactions) are possible, for example ~ genotype + treatment + genotype:treatment.

Value

A DESeq2 object (e.g. dds)

cellpypes Objects

A cellpypes object is a list with four slots:

- **raw** (sparse) matrix with genes in rows, cells in columns
- **totalUMI** the colSums of obj$raw
- **embed** two-dimensional embedding of the cells, provided as data.frame or tibble with two columns and one row per cell.
- **neighbors** index matrix with one row per cell and k columns, where k is the number of nearest neighbors (we recommend 15<k<100, e.g. k=50). Here are two ways to get the neighbors index matrix:
  - Use find_knn(featureMatrix)$idx, where featureMatrix could be principal components, latent variables or normalized genes (features in rows, cells in columns).
  - use as(seurat@graphs[["RNA.nn"]], "dgCMatrix">.1 to extract the kNN graph computed on RNA. The > .1 ensures this also works with RNA_snn, wknn/wsnn or any other available graph – check with names(seurat@graphs).

Examples

data("simulated_umis")
# Meta data
ncells <- ncol(simulated_umis$raw)
dummy_variable <- function(x) factor(sample(x, ncells, replace=TRUE))
meta_data <- data.frame(patient=dummy_variable(paste0("patient", 1:6)),
treatment=dummy_variable(c("control", "treated")))
obj <- rule(simulated_umis, "T", "CD3E",">", 1e-4)
# > 5 s in CRAN check
dds <- class_to_deseq2(obj, meta_data, "T", ~ treatment)

feat <- rule(simulated_umis, "T", "CD3E",">", 1e-4)
# > 5 s in CRAN check
dds <- class_to_deseq2(obj, meta_data, "T", ~ treatment)

**Description**

Highlight gene expression in UMAP embeddings, for example.

**Usage**

feat(obj, features, fast = NULL, verbose = TRUE, ...)

**Arguments**

- **obj**: A cellptypes object, see section **cellptypes Objects** below.
- **features**: A vector of genes (features) to colour by.
- **fast**: Set this to TRUE if you want fast plotting in spite of many cells (using the scattermore package). If NULL (default), cellptypes decides automatically and fast plotting is done for more than 10k cells.
- **verbose**: feat ignores gene names not present in your object and warns you about them by default. verbose=FALSE will suppress the warning (not recommended in interactive use).
- **...**: Arguments passed to cowplot’s `plot_grid` function, for example ncol or rel_widths.

**Value**

A ggplot object (assembled by cowplot).

**cellptypes Objects**

A cellptypes object is a list with four slots:

- **raw** (sparse) matrix with genes in rows, cells in columns
- **totalUMI** the colSums of obj$raw
- **embed** two-dimensional embedding of the cells, provided as data.frame or tibble with two columns and one row per cell.
- **neighbors** index matrix with one row per cell and k columns, where k is the number of nearest neighbors (we recommend 15<k<100, e.g. k=50). Here are two ways to get the neighbors index matrix:
  - Use `find_knn(featureMatrix)$idx`, where featureMatrix could be principal components, latent variables or normalized genes (features in rows, cells in columns).
  - `as(seurat@graphs[["RNA_nn"]], "dgCMatrix") > .1` to extract the kNN graph computed on RNA. The > .1 ensures this also works with RNA_snn, wknn/wssnn or any other available graph – check with `names(seurat@graphs)`. 
Examples

```r
feat(simulated_umis, "CD3E")
```

---

**find_knn**

*Find approximate k-nearest neighbors*

**Description**

Implements RcppAnnoy’s approximate nearest neighbor search (much faster than precise neighbors). Random search is made reproducible using `set.seed(seed)`. Hint: If you pass `find_knn`’s output directly to `uwot::umap` via the `nn_method` argument, make sure to set `umap`’s argument `n_sgd_threads` to <=1 to ensure the UMAP embedding is reproducible.

**Usage**

```r
find_knn(featureMatrix, k = 50, n_trees = 50, seed = 42)
```

**Arguments**

- `featureMatrix` Numeric matrix with features in rows, cells in columns. Rows could be normalized genes or latent dimensions such as principal components.
- `k` Number of neighbors to find.
- `seed` Random seed for neighbor search, default: 42.

**Value**

List with two slots:

- `idx` A NxK matrix (N cells, K neighbors) containing the integer indexes of the approximate nearest neighbors in `featureMatrix`. Each cell is considered to be its own nearest neighbor, next to K-1 other neighbors.
- `dist` A NxK matrix containing the distances of the nearest neighbors.

Inspired by `uwot::umap`’s return value when setting `ret_nn=TRUE`.

**Examples**

```r
# Imagine we have 30 cells and 100 features:
fmat <- matrix(rnorm(3000), ncol=30)
nn <- find_knn(fmat,k=15)
# nn$idx has 30 rows and 15 columns.
```
is_classes

Check if obj$classes looks as expected. is_class returns FALSE for example in these cases: is_classes(NULL) is_classes(data.frame()) is_classes(data.frame(class=c("T", "T"), parent=c("..root..", "..root..")))

Description

Check if obj$classes looks as expected. is_class returns FALSE for example in these cases: is_classes(NULL) is_classes(data.frame()) is_classes(data.frame(class=c("T", "T"), parent=c("..root..", "..root..")))

Usage

is_classes(classes)

Arguments

classes The obj$classes you want to check.

Value

logical scalar.

is_rules Check if obj$rules looks as expected.

Description

Check if obj$rules looks as expected.

Usage

is_rules(rules)

Arguments

rules The obj$rules slot of a cellpypes object.

Value

logical scalar
plot_classes

Call and visualize 'classify' function

Description

Call and visualize 'classify' function

Usage

plot_classes(
  obj,
  classes = NULL,
  replace_overlap_with = "Unassigned",
  return_logical_matrix = FALSE,
  fast = NULL,
  point_size = 0.4,
  point_size_legend = 2,
  base_size = 15
)

Arguments

obj  A cellpypes object, see section cellpypes Objects below.
classes  Character vector with one or more class names. If NULL (the default), plots finest available cell types (all classes that are not parent of any other class).
replace_overlap_with  Character string, by default: "Unassigned". See section Handling overlap.
return_logical_matrix  logical. If TRUE, a logical matrix with classes in columns and cells in rows is returned instead of resolving overlaps with replace_overlap_with. If a single class is supplied, the matrix has exactly one
fast  Set this to TRUE if you want fast plotting in spite of many cells (using the scattermore package). If NULL (default), cellpypes decides automatically and fast plotting is done for more than 10k cells.
point_size  Dot size used by geom_point.
point_size_legend  Dot size displayed in legend. Legend colors are easier to read with larger points.
base_size  The base_size of theme_bw, i.e. how large text is displayed. Default: 15.

Value

A ggplot2 object.
**cellpypes Objects**

A cellpypes object is a list with four slots:

- **raw** (sparse) matrix with genes in rows, cells in columns
- **totalUMI** the colSums of obj$raw
- **embed** two-dimensional embedding of the cells, provided as data.frame or tibble with two columns and one row per cell.
- **neighbors** index matrix with one row per cell and k columns, where k is the number of nearest neighbors (we recommend 15<k<100, e.g. k=50). Here are two ways to get the neighbors index matrix:
  - Use `find_knn(featureMatrix)$idx`, where featureMatrix could be principal components, latent variables or normalized genes (features in rows, cells in columns).
  - use `as(seurat@graphs[['RNA_nn']], "dgCMatrix"> .1` to extract the kNN graph computed on RNA. The > .1 ensures this also works with RNA_snn, wknn/wsnn or any other available graph – check with `names(seurat@graphs)`.

**Handling overlap**

Overlap denotes all cells for which rules from multiple classes apply, and these cells will be labeled as Unassigned by default. If you are in fact interested in where the overlap is, set `return_logical_matrix=TRUE` and inspect the result. Note that it matters whether you call `classify("Tcell")` or `classify(c("Tcell","Bcell"))` – any existing overlap between T and B cells is labelled as Unassigned in this second call, but not in the first.

Replacing overlap happens only between mutually exclusive labels (such as Tcell and Bcell), but not within a lineage. To make an example, overlap is NOT replaced between child (PD1+Ttox) and parent (Ttox) or any other ancestor (Tcell), but instead the most detailed cell type (PD1+Ttox) is returned.

All of the above is also true for `plot_classes`, as it wraps `classify`.

**Examples**

```r
plot_classes(rule(simulated_umis, "T", "CD3E",">", 1))
```

---

**plot_last**

*Plot the last modified rule or class*

**Description**

Plot the last modified rule or class
Usage

plot_last(
  obj,
  show_feat = TRUE,
  what = "rule",
  fast = NULL,
  legend_rel_width = 0.3
)

Arguments

obj A cellpypes object, see section cellpypes Objects below.
show_feat If TRUE (default), a second panel shows the feature plot of the relevant gene.
what Either "rule" or "class".
fast Set this to TRUE if you want fast plotting in spite of many cells (using the scattermore package). If NULL (default), cellpypes decides automatically and fast plotting is done for more than 10k cells.
legend_rel_width Relative width compared to the other two plots (only relevant if show_feat=TRUE).

Value

Returns a ggplot2 object with the plot.

cellpypes Objects

A cellpypes object is a list with four slots:

- raw (sparse) matrix with genes in rows, cells in columns
- totalUMI the colSums of obj$raw
- embed two-dimensional embedding of the cells, provided as data.frame or tibble with two columns and one row per cell.
- neighbors index matrix with one row per cell and k columns, where k is the number of nearest neighbors (we recommend 15<k<100, e.g. k=50). Here are two ways to get the neighbors index matrix:
  - Use find_knn(featureMatrix)$idx, where featureMatrix could be principal components, latent variables or normalized genes (features in rows, cells in columns).
  - use as(seurat@graphs[["RNA_nn"]], "dgCMatrix")>.1 to extract the kNN graph computed on RNA. The >.1 ensures this also works with RNA_snn, wknn/wsnn or any other available graph – check with names(seurat@graphs).

Examples

plot_last(rule(simulated_umis, "T", "CD3E",">", 1))
**pool_across_neighbors**

*Sum up x across neighbors in a nearest neighbor graph.*

**Description**

Neighbor pooling means that x is summed across the nearest neighbors.

**Usage**

```r
pool_across_neighbors(x, neighbors)
```

**Arguments**

- `x` Numeric vector.
- `neighbors` Nearest neighbor graph provided as NxK index matrix (N observations, K neighbors) or NxN adjacency matrix. Index matrices can be obtained with `find_knn` (specifically the slot `idx` in the list it returns).

**Value**

Numeric vector of length x.

**Examples**

```r
set.seed(42)
# simulate 30 cells without biological signal:
dummy_dat <- matrix(rpois(3000, .1), ncol=30)
# find 15 approximate nearest neighbors
eighbors <- find_knn(dummy_dat, k = 15)
# pool gene1 counts across neighbors:
neighbor_sum_gene1 <- pool_across_neighbors(dummy_dat[,1], neighbors$idx)
```

---

**pseudobulk**

*Form pseudobulks from single cells.*

**Description**

Sum up cells in count matrix raw for bulk RNA methods such as DESeq2.

**Usage**

```r
pseudobulk(raw, pseudobulk_id)
```
pseudobulk_id

Arguments

- **raw**: A matrix with raw UMI counts, cells in columns.
- **pseudobulk_id**: A factor that identifies which cells should go to which pseudobulk. Generate pseudobulk_ids with the `pseudobulk_id` function!

Value

A matrix where each column is a pseudobulk and each row a gene.

Examples

```r
# Create pseudobulk counts and coldata for DESeq2:
coldata <- data.frame(
  celltype = rep(c("X+Y-", "X+Y+", "X-Y+", "X-Y-"), each = nrow(simulated_umis$embed)/4), # 4 cell types
  patient = c("3", "500.", "*5", "/")
)
coldata$pseudobulk_id <- pseudobulk_id(coldata)
counts <- pseudobulk(simulated_umis$raw, coldata$pseudobulk_id)
# Use counts/coldata as input for DESeqDataSetFromMatrix (DESeq2).
```

Description

This function generates unique IDs that are valid colnames as well. Use these IDs in function pseudobulk.

Usage

```r
pseudobulk_id(factor_df)
```

Arguments

- **factor_df**: Data frame where each column helps to identify a pseudobulk. Each row in `factor_df` corresponds to a single cell in your raw count matrix. Typical columns of `factor_df` are for example patient, treatment and cell type – anything that uniquely identifies a replicate.

Details

Wraps `make.names` to generate syntactically valid IDs. Use these IDs in the `pseudobulk` function. Note that this function combines all columns in `factor_df`, so only include the columns that uniquely identify replicates. Cells from the same experimental unit
Value

Factor with syntactically valid and unique IDs.

Examples

# Create pseudobulk counts and coldata for DESeq2:
coldata <- data.frame(
celltype = rep(c("X+Y-", "X+Y+", "X-Y+", "X-Y-"),
    each = nrow(simulated_umis$embed)/4), # 4 cell types
  patient = c("3", "500.", "*5", "/"))
coldata$pseudobulk_id <- pseudobulk_id(coldata)
counts <- pseudobulk(simulated_umis$raw, coldata$pseudobulk_id)
# Use counts/coldata as input for DESeqDataSetFromMatrix (DESeq2).

pype_code_template  Generate code template for cellpype rules

Description

This function rule code snippet with neat text alignment to the console. Paste this into your script and start changing the rules.

Usage

pype_code_template(n_rules = 3)

Arguments

n_rules Number of lines (rules) to generate

Value

Prints rules to the consoles.

Examples

pype_code_template()
pype_from_seurat  
Convert Seurat to cellpypes object.

Description

Start cellpyping a Seurat object. This function saves the user from building his own cellpypes object, which is done with list(umi, neighbors, embed, totalUMI).

Usage

pype_from_seurat(seurat)

Arguments

seurat  
A Seurat object.

Value

A cellpypes object.

cellpypes Objects

A cellpypes object is a list with four slots:

- raw (sparse) matrix with genes in rows, cells in columns
- totalUMI the colSums of obj$raw
- embed two-dimensional embedding of the cells, provided as data.frame or tibble with two columns and one row per cell.
- neighbors index matrix with one row per cell and k columns, where k is the number of nearest neighbors (we recommend 15<k<100, e.g. k=50). Here are two ways to get the neighbors index matrix:
  - Use find_knn(featureMatrix)$idx, where featureMatrix could be principal components, latent variables or normalized genes (features in rows, cells in columns).
  - use as(seurat@graphs[['RNA_nn']], "dgCMatrix") > .1 to extract the kNN graph computed on RNA. The > .1 ensures this also works with RNA_snn, wknn/wsnn or any other available graph – check with names(seurat@graphs).
**rule**

*Add a cell type rule.*

---

**Description**

This is the heart of cellpypes and best used by piping from one rule into the next with `magrittr::%>%`. Check out examples at [GitHub](https://github.com).

**Usage**

```r
rule(
  obj, 
  class, 
  feature, 
  operator = ">", 
  threshold, 
  parent = NULL, 
  use_CP10K = TRUE
)
```

**Arguments**

- `obj` A cellpypes object, see section **cellpypes Objects** below.
- `class` Character scalar with the class name. Typically, cellpypes classes are literature cell types ("T cell") or any subpopulation of interest ("CD3E+TNF+LAG3-.").
- `feature` Character scalar naming the gene you’d like to threshold. Must be a row name in `obj$raw`.
- `operator` One of `c(">","<")`. Use ">" for positive (CD3E+) and "<" for negative markers (MS4A1-).
- `threshold` Numeric scalar with the expression threshold separating positive from negative cells. Experiment with this value, until expression and selected cells agree well in UMAP (see examples on [GitHub](https://github.com)).
- `parent` Character scalar with the parent class (e.g. "T cell" for "Cytotoxic T cells"). Only has to be specified once per class (else most recent one is taken), and defaults to ".root." if NULL is passed in all rules.
- `use_CP10K` If TRUE, threshold is taken to be counts per 10 thousand UMI counts, a measure for RNA molecule fractions. We recommend CP10K for human intuition (1 CP10K is roughly 1 UMI per cell), but the results are the exact same whether you use `threshold=1,CP10K=TRUE` or `threshold=1e-4,CP10K=FALSE`.

**Details**

Calling `rule` is computationally cheap because it only stores the cell type rule while all computations happen in `classify`. If you have classes with multiple rules, the most recent parent and feature-threshold combination counts. It is ok to mix rules with and without `use_CP10K=TRUE`. 

Value

obj is returned, but with the rule and class stored in obj$rules and obj$classes, to be used by classify.

cellpypes Objects

A cellpypes object is a list with four slots:

- `raw` (sparse) matrix with genes in rows, cells in columns
- `totalUMI` the colSums of obj$raw
- `embed` two-dimensional embedding of the cells, provided as data.frame or tibble with two columns and one row per cell.
- `neighbors` index matrix with one row per cell and k columns, where k is the number of nearest neighbors (we recommend 15<k<100, e.g. k=50). Here are two ways to get the neighbors index matrix:
  - Use find_knn(featureMatrix)$idx, where featureMatrix could be principal components, latent variables or normalized genes (features in rows, cells in columns).
  - use as(seurat@graphs[["RNA_nn"]], "dgCMatrix")> .1 to extract the kNN graph computed on RNA. The > .1 ensures this also works with RNA_snn, wknn/wsnn or any other available graph – check with names(seurat@graphs).

See Also

To have nicely formatted code in the end, copy the output of pype_code_template() to your script and start editing.

Examples

# T cells are CD3E+
obj <- rule(simulated_umis, "T", "CD3E", ">", .1)
# T cells are MS4A1-
obj <- rule(obj, "T", "MS4A1", "<", 1)
# Tregs are a subset of T cells:
obj <- rule(obj, "Treg", "FOXP3", ">", .1, parent="T")

simulated_umis  Simulated scRNAseq data

Description

This data serves to develop cellpypes and to illustrate its functionality. I made it up entirely.

Usage

simulated_umis
**simulated_umis**

**Format**

A list with 4 entries:

- **raw**  Raw (unnormalized) UMI counts for a handful of genes, last row are totalUMI.
- **neighbors**  Indices of each cell’s 50 nearest neighbors.
- **embed**  Simulated UMAP embedding.
- **celltype**  Cell type label that I used to simulate the data.

**Source**

Very simple simulation (c.f. data-raw/simulated_umis.R in source code).
Index

* datasets
  simulated_umis, 16

class_to_deseq2, 3
classify, 2, 13, 16

feat, 5
find_knn, 6, 11

geom_point, 8

is_classes, 7
is_rules, 7

list, 3–5, 9, 10, 14, 16

make.names, 12

plot_classes, 8
plot_grid, 5
plot_last, 9
pool_across_neighbors, 11
pseudobulk, 11, 12
pseudobulk_id, 12, 12
pype_code_template, 13
pype_from_seurat, 14

rule, 13, 15

simulated_umis, 16

theme_bw, 8