Package 'clustifyr'

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Title Classifier for Single-cell RNA-seq Using Cell Clusters

Version 1.19.0

Description Package designed to aid in classifying cells from single-cell RNA sequencing data using external reference data (e.g., bulk RNA-seq, scRNA-seq, microarray, gene lists). A variety of correlation based methods and gene list enrichment methods are provided to assist cell type assignment.

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Depends R (>= 2.10)

Imports cowplot, dplyr, entropy, fgsea, ggplot2, Matrix, rlang, scales, stringr, tibble, tidyr, stats, methods, SingleCellExperiment, SummarizedExperiment, SeuratObject, matrixStats, S4Vectors, proxy, httr, utils

Suggests ComplexHeatmap, covr, knitr, rmarkdown, testthat, ggrepel, BiocStyle, BiocManager, remotes, shiny, gprofiler2, purrr, data.table, R.utils

biocViews SingleCell, Annotation, Sequencing, Microarray, GeneExpression

BugReports https://github.com/rnabioco/clustifyr/issues

URL https://github.com/rnabioco/clustifyr, https://rnabioco.github.io/clustifyr/

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Contents

`clustifyr-package` *clustifyr: Classifier for Single-cell RNA-seq Using Cell Clusters*

Description

Package designed to aid in classifying cells from single-cell RNA sequencing data using external reference data (e.g., bulk RNA-seq, scRNA-seq, microarray, gene lists). A variety of correlation based methods and gene list enrichment methods are provided to assist cell type assignment.

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See Also

Useful links:

- https://github.com/rnabioco/clustifyr
- https://rnabioco.github.io/clustifyr/
- Report bugs at https://github.com/rnabioco/clustifyr/issues

append_genes *Given a reference matrix and a list of genes, take the union of all genes in vector and genes in reference matrix and insert zero counts for all remaining genes.*

Description

Given a reference matrix and a list of genes, take the union of all genes in vector and genes in reference matrix and insert zero counts for all remaining genes.

Usage

```
append_genes(gene_vector, ref_matrix)
```

Arguments

gene_vector	char vector with gene names
ref_matrix	Reference matrix containing cell types vs. gene expression values

Value

Reference matrix with union of all genes

Examples

```
mat <- append_genes(
    gene_vector = human_genes_10x,
    ref_matrix = cbmc_ref
)
```

assess_rank_bias *Find rank bias*

Description

Find rank bias

Usage

```
assess_rank_bias(
  avg_mat,
  ref_mat,
  query_genes = NULL,
  res,
  organism,
  plot_name = NULL,
```

```
  rds_name = NULL,
  expand_unassigned = FALSE
)
```

Arguments

avg_mat	average expression matrix
ref_mat	reference expression matrix
query_genes	original vector of genes used to clustify
res	dataframe of idents, such as output of cor_to_call
organism	for GO term analysis, organism name: human - 'hsapiens', mouse - 'mmusculus'
plot_name	name for saved pdf, if NULL then no file is written (default)
rds_name	name for saved rds of rank_diff, if NULL then no file is written (default)
expand_unassigned	test all ref clusters for unassigned results

Value

pdf of ggplot object

Examples

```
## Not run:
avg <- average_clusters(
    pbmc_matrix_small,
    pbmc_meta$seurat_clusters
)
res <- clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    ref_mat = cbmc_ref,
    query_genes = pbmc_vargenes,
    cluster_col = "seurat_clusters"
)
top_call <- cor_to_call(
    res,
    metadata = pbmc_meta,
    cluster_col = "seurat_clusters",
    collapse_to_cluster = FALSE,
    threshold = 0.8
)
res_rank <- assess_rank_bias(
    avg,
    cbmc_ref,
    res = top_call
)

## End(Not run)
```

assign_ident *manually change idents as needed*

Description

manually change idents as needed

Usage

```
assign_ident(
  metadata,
  cluster_col = "cluster",
  ident_col = "type",
  clusters,
  idents
)
```

Arguments

metadata	column of ident
cluster_col	column in metadata containing cluster info
ident_col	column in metadata containing identity assignment
clusters	names of clusters to change, string or vector of strings
idents	new idents to assign, must be length of 1 or same as clusters

Value

new dataframe of metadata

average_clusters *Average expression values per cluster*

Description

Average expression values per cluster

Usage

```
average_clusters(
  mat,
  metadata,
  cluster_col = "cluster",
  if_log = TRUE,
  cell_col = NULL,
```

```
  low_threshold = 0,
  method = "mean",
  output_log = TRUE,
  subclusterpower = 0,
  cut_n = NULL
)
```

Arguments

mat	expression matrix
metadata	data.frame or vector containing cluster assignments per cell. Order must match column order in supplied matrix. If a data.frame provide the cluster_col parameters.
cluster_col	column in metadata with cluster number
if_log	input data is natural log, averaging will be done on unlogged data
cell_col	if provided, will reorder matrix first
low_threshold	option to remove clusters with too few cells
method	whether to take mean (default), median, 10% truncated mean, or trimean, max, min
output_log	whether to report log results
subclusterpower	whether to get multiple averages per original cluster
cut_n	set on a limit of genes as expressed, lower ranked genes are set to 0, considered unexpressed

Value

average expression matrix, with genes for row names, and clusters for column names

Examples

```
mat <- average_clusters(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    if_log = FALSE
)
mat[1:3, 1:3]
```

binarize_expr *Binarize scRNAseq data*

Description

Binarize scRNAseq data

Usage

```
binarize_expr(mat, n = 1000, cut = 0)
```

Arguments

mat	single-cell expression matrix
n	number of top expressing genes to keep
cut	cut off to set to 0

Value

matrix of 1s and 0s

Examples

```
pbmc_avg <- average_clusters(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified"
)

mat <- binarize_expr(pbmc_avg)
mat[1:3, 1:3]
```

build_atlas *Function to combine records into single atlas*

Description

Function to combine records into single atlas

Usage

```
build_atlas(matrix_fns = NULL, genes_fn, matrix_objs = NULL, output_fn = NULL)
```

Arguments

matrix_fns	character vector of paths to study matrices stored as .rds files. If a named character vector, then the name will be added as a suffix to the cell type name in the final matrix. If it is not named, then the filename will be used (without .rds)
genes_fn	text file with a single column containing genes and the ordering desired in the output matrix
matrix_objs	Checks to see whether .rds files will be read or R objects in a local environment. A list of environmental objects can be passed to matrx_objs, and that names will be used, otherwise defaults to numbers
output_fn	output filename for .rds file. If NULL the matrix will be returned instead of saving

Value

Combined matrix with all genes given

Examples

```
pbmc_ref_matrix <- average_clusters(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    if_log = TRUE # whether the expression matrix is already log transformed
)
references_to_combine <- list(pbmc_ref_matrix, cbmc_ref)
atlas <- build_atlas(NULL, human_genes_10x, references_to_combine, NULL)
```

---

calculate_pathway_gsea

Convert expression matrix to GSEA pathway scores (would take a similar place in workflow before average_clusters/binarize)

Description

Convert expression matrix to GSEA pathway scores (would take a similar place in workflow before average_clusters/binarize)

Usage

```
calculate_pathway_gsea(
  mat,
  pathway_list,
  n_perm = 1000,
  scale = TRUE,
  no_warnings = TRUE
)
```

Arguments

mat	expression matrix
pathway_list	a list of vectors, each named for a specific pathway, or dataframe
n_perm	Number of permutation for fgsea function. Defaults to 1000.
scale	convert expr_mat into zscores prior to running GSEA?, default = FALSE
no_warnings	suppress warnings from gsea ties

Value

matrix of GSEA NES values, cell types as row names, pathways as column names

Examples

```
gl <- list(
    "n" = c("PPBP", "LYZ", "S100A9"),
    "a" = c("IGLL5", "GNLY", "FTL")
)

pbmc_avg <- average_clusters(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified"
)

calculate_pathway_gsea(
    mat = pbmc_avg,
    pathway_list = gl
)
```

---

calc_distance *Distance calculations for spatial coord*

Description

Distance calculations for spatial coord

Usage

```
calc_distance(
  coord,
  metadata,
  cluster_col = "cluster",
  collapse_to_cluster = FALSE
)
```

Arguments

coord	dataframe or matrix of spatial coordinates, cell barcode as rownames
metadata	data.frame or vector containing cluster assignments per cell. Order must match column order in supplied matrix. If a data.frame provide the cluster_col parameters.
cluster_col	column in metadata with cluster number
collapse_to_cluster	instead of reporting min distance to cluster per cell, summarize to cluster level

Value

min distance matrix

Examples

```
cbs <- paste0("cb_", 1:100)

spatial_coords <- data.frame(
    row.names = cbs,
    X = runif(100),
    Y = runif(100)
)
group_ids <- sample(c("A", "B"), 100, replace = TRUE)
dist_res <- calc_distance(
    spatial_coords,
    group_ids
)
```

---

calc_similarity *compute similarity*

Description

compute similarity

Usage

```
calc_similarity(query_mat, ref_mat, compute_method, rm0 = FALSE, ...)
```

Arguments

query_mat	query data matrix
ref_mat	reference data matrix
compute_method	method(s) for computing similarity scores
rm0	consider 0 as missing data, recommended for per_cell
...	additional parameters

Value

matrix of numeric values

`call_consensus` *get concensus calls for a list of cor calls*

Description

get concensus calls for a list of cor calls

Usage

```
call_consensus(list_of_res)
```

Arguments

`list_of_res`	list of call dataframes from cor_to_call_rank

Value

dataframe of cluster, new ident, and mean rank

Examples

```
res <- clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    ref_mat = cbmc_ref
)

res2 <- cor_to_call_rank(res, threshold = "auto")
res3 <- cor_to_call_rank(res)
call_consensus(list(res2, res3))
```

`call_to_metadata` *Insert called ident results into metadata*

Description

Insert called ident results into metadata

Usage

```
call_to_metadata(
  res,
  metadata,
  cluster_col,
  per_cell = FALSE,
  rename_prefix = NULL
)
```

Arguments

res	dataframe of idents, such as output of cor_to_call
metadata	input metadata with tsne or umap coordinates and cluster ids
cluster_col	metadata column, can be cluster or cellid
per_cell	whether the res dataframe is listed per cell
rename_prefix	prefix to add to type and r column names

Value

new metadata with added columns

Examples

```
res <- clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    ref_mat = cbmc_ref
)

res2 <- cor_to_call(res, cluster_col = "classified")

call_to_metadata(
    res = res2,
    metadata = pbmc_meta,
    cluster_col = "classified",
    rename_prefix = "assigned"
)
```

---

cbmc_m *reference marker matrix from seurat citeseq CBMC tutorial*

Description

reference marker matrix from seurat citeseq CBMC tutorial

Usage

```
cbmc_m
```

Format

An object of class `data.frame` with 3 rows and 13 columns.

Source

https://satijalab.org/seurat/v3.0/multimodal_vignette.html#identify-differentially-expressed-protei

See Also

Other data: `cbmc_ref`, `downrefs`, `human_genes_10x`, `mouse_genes_10x`, `pbmc_markers`, `pbmc_markers_M3Drop`, `pbmc_matrix_small`, `pbmc_meta`, `pbmc_vargenes`

---

cbmc_ref *reference matrix from seurat citeseq CBMC tutorial*

Description

reference matrix from seurat citeseq CBMC tutorial

Usage

```
cbmc_ref
```

Format

An object of class `matrix` (inherits from `array`) with 2000 rows and 13 columns.

Source

https://satijalab.org/seurat/v3.0/multimodal_vignette.html#identify-differentially-expressed-protei

See Also

Other data: `cbmc_m`, `downrefs`, `human_genes_10x`, `mouse_genes_10x`, `pbmc_markers`, `pbmc_markers_M3Drop`, `pbmc_matrix_small`, `pbmc_meta`, `pbmc_vargenes`

check_raw_counts *Given a count matrix, determine if the matrix has been either log-normalized, normalized, or contains raw counts*

Description

Given a count matrix, determine if the matrix has been either log-normalized, normalized, or contains raw counts

Usage

```
check_raw_counts(counts_matrix, max_log_value = 50)
```

Arguments

counts_matrix	Count matrix containing scRNA-seq read data
max_log_value	Static value to determine if a matrix is normalized

Value

String either raw counts, log-normalized or normalized

Examples

```
check_raw_counts(pbmc_matrix_small)
```

clustify *Compare scRNA-seq data to reference data.*

Description

Compare scRNA-seq data to reference data.

Usage

```
clustify(input, ...)

## Default S3 method:
clustify(
  input,
  ref_mat,
  metadata = NULL,
  cluster_col = NULL,
  query_genes = NULL,
  n_genes = 1000,
  per_cell = FALSE,
```

```
  n_perm = 0,
  compute_method = "spearman",
  pseudobulk_method = "mean",
  verbose = TRUE,
  lookuptable = NULL,
  rm0 = FALSE,
  obj_out = TRUE,
  seurat_out = obj_out,
  vec_out = FALSE,
  rename_prefix = NULL,
  threshold = "auto",
  low_threshold_cell = 0,
  exclude_genes = c(),
  if_log = TRUE,
  organism = "hsapiens",
  plot_name = NULL,
  rds_name = NULL,
  expand_unassigned = FALSE,
  ...
)

## S3 method for class 'Seurat'
clustify(
  input,
  ref_mat,
  cluster_col = NULL,
  query_genes = NULL,
  n_genes = 1000,
  per_cell = FALSE,
  n_perm = 0,
  compute_method = "spearman",
  pseudobulk_method = "mean",
  use_var_genes = TRUE,
  dr = "umap",
  obj_out = TRUE,
  seurat_out = obj_out,
  vec_out = FALSE,
  threshold = "auto",
  verbose = TRUE,
  rm0 = FALSE,
  rename_prefix = NULL,
  exclude_genes = c(),
  metadata = NULL,
  organism = "hsapiens",
  plot_name = NULL,
  rds_name = NULL,
  expand_unassigned = FALSE,
  ...
```

```
)

## S3 method for class 'SingleCellExperiment'
clustify(
  input,
  ref_mat,
  cluster_col = NULL,
  query_genes = NULL,
  per_cell = FALSE,
  n_perm = 0,
  compute_method = "spearman",
  pseudobulk_method = "mean",
  use_var_genes = TRUE,
  dr = "umap",
  obj_out = TRUE,
  seurat_out = obj_out,
  vec_out = FALSE,
  threshold = "auto",
  verbose = TRUE,
  rm0 = FALSE,
  rename_prefix = NULL,
  exclude_genes = c(),
  metadata = NULL,
  organism = "hsapiens",
  plot_name = NULL,
  rds_name = NULL,
  expand_unassigned = FALSE,
  ...
)
```

Arguments

input	single-cell expression matrix or Seurat object
...	additional arguments to pass to compute_method function
ref_mat	reference expression matrix
metadata	cell cluster assignments, supplied as a vector or data.frame. If data.frame is supplied then cluster_col needs to be set. Not required if running correlation per cell.
cluster_col	column in metadata that contains cluster ids per cell. Will default to first column of metadata if not supplied. Not required if running correlation per cell.
query_genes	A vector of genes of interest to compare. If NULL, then common genes between the expr_mat and ref_mat will be used for comparision.
n_genes	number of genes limit for Seurat variable genes, by default 1000, set to 0 to use all variable genes (generally not recommended)
per_cell	if true run per cell, otherwise per cluster.
n_perm	number of permutations, set to 0 by default

compute_method	method(s) for computing similarity scores
pseudobulk_method	method used for summarizing clusters, options are mean (default), median, truncate (10% truncated mean), or trimean, max, min
verbose	whether to report certain variables chosen and steps
lookuptable	if not supplied, will look in built-in table for object parsing
rm0	consider 0 as missing data, recommended for per_cell
obj_out	whether to output object instead of cor matrix
seurat_out	output cor matrix or called seurat object (deprecated, use obj_out instead)
vec_out	only output a result vector in the same order as metadata
rename_prefix	prefix to add to type and r column names
threshold	identity calling minimum correlation score threshold, only used when obj_out = TRUE
low_threshold_cell	option to remove clusters with too few cells
exclude_genes	a vector of gene names to throw out of query
if_log	input data is natural log, averaging will be done on unlogged data
organism	for GO term analysis, organism name: human - 'hsapiens', mouse - 'mmusculus'
plot_name	name for saved pdf, if NULL then no file is written (default)
rds_name	name for saved rds of rank_diff, if NULL then no file is written (default)
expand_unassigned	test all ref clusters for unassigned results
use_var_genes	if providing a seurat object, use the variable genes (stored in seurat_object@var.genes) as the query_genes.
dr	stored dimension reduction

Value

single cell object with identity assigned in metadata, or matrix of correlation values, clusters from input as row names, cell types from ref_mat as column names

Examples

```
# Annotate a matrix and metadata
clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    ref_mat = cbmc_ref,
    query_genes = pbmc_vargenes,
    cluster_col = "RNA_snn_res.0.5",
    verbose = TRUE
)

# Annotate using a different method
```

```
clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    ref_mat = cbmc_ref,
    query_genes = pbmc_vargenes,
    cluster_col = "RNA_snn_res.0.5",
    compute_method = "cosine"
)

# Annotate a SingleCellExperiment object
sce <- sce_pbmc()
clustify(
    sce,
    cbmc_ref,
    cluster_col = "clusters",
    obj_out = TRUE,
    per_cell = FALSE,
    dr = "umap"
)

# Annotate a Seurat object
so <- so_pbmc()
clustify(
    so,
    cbmc_ref,
    cluster_col = "seurat_clusters",
    obj_out = TRUE,
    per_cell = FALSE,
    dr = "umap"
)

# Annotate (and return) a Seurat object per-cell
clustify(
    input = so,
    ref_mat = cbmc_ref,
    cluster_col = "seurat_clusters",
    obj_out = TRUE,
    per_cell = TRUE,
    dr = "umap"
)
```

`clustifyr_methods` *Correlation functions available in clustifyr*

Description

Correlation functions available in clustifyr

Usage

```
clustifyr_methods
```

Format

An object of class `character` of length 5.

Examples

```
clustifyr_methods
```

---

clustify_lists *Main function to compare scRNA-seq data to gene lists.*

Description

Main function to compare scRNA-seq data to gene lists.

Usage

```
clustify_lists(input, ...)

## Default S3 method:
clustify_lists(
  input,
  marker,
  marker_inmatrix = TRUE,
  metadata = NULL,
  cluster_col = NULL,
  if_log = TRUE,
  per_cell = FALSE,
  topn = 800,
  cut = 0,
  genome_n = 30000,
  metric = "hyper",
  output_high = TRUE,
  lookuptable = NULL,
  obj_out = TRUE,
  seurat_out = obj_out,
  vec_out = FALSE,
  rename_prefix = NULL,
  threshold = 0,
  low_threshold_cell = 0,
  verbose = TRUE,
  input_markers = FALSE,
  details_out = FALSE,
  ...
)

## S3 method for class 'Seurat'
clustify_lists(
```

```
  input,
  metadata = NULL,
  cluster_col = NULL,
  if_log = TRUE,
  per_cell = FALSE,
  topn = 800,
  cut = 0,
  marker,
  marker_inmatrix = TRUE,
  genome_n = 30000,
  metric = "hyper",
  output_high = TRUE,
  dr = "umap",
  obj_out = TRUE,
  seurat_out = obj_out,
  vec_out = FALSE,
  threshold = 0,
  rename_prefix = NULL,
  verbose = TRUE,
  details_out = FALSE,
  ...
)

## S3 method for class 'SingleCellExperiment'
clustify_lists(
  input,
  metadata = NULL,
  cluster_col = NULL,
  if_log = TRUE,
  per_cell = FALSE,
  topn = 800,
  cut = 0,
  marker,
  marker_inmatrix = TRUE,
  genome_n = 30000,
  metric = "hyper",
  output_high = TRUE,
  dr = "umap",
  obj_out = TRUE,
  seurat_out = obj_out,
  vec_out = FALSE,
  threshold = 0,
  rename_prefix = NULL,
  verbose = TRUE,
  details_out = FALSE,
  ...
)
```

Arguments

input	single-cell expression matrix, Seurat object, or SingleCellExperiment
...	passed to matrixize_markers
marker	matrix or dataframe of candidate genes for each cluster
marker_inmatrix	whether markers genes are already in preprocessed matrix form
metadata	cell cluster assignments, supplied as a vector or data.frame. If data.frame is supplied then cluster_col needs to be set. Not required if running correlation per cell.
cluster_col	column in metadata with cluster number
if_log	input data is natural log, averaging will be done on unlogged data
per_cell	compare per cell or per cluster
topn	number of top expressing genes to keep from input matrix
cut	expression cut off from input matrix
genome_n	number of genes in the genome
metric	adjusted p-value for hypergeometric test, or jaccard index
output_high	if true (by default to fit with rest of package), -log10 transform p-value
lookuptable	if not supplied, will look in built-in table for object parsing
obj_out	whether to output object instead of cor matrix
seurat_out	output cor matrix or called seurat object (deprecated, use obj_out instead)
vec_out	only output a result vector in the same order as metadata
rename_prefix	prefix to add to type and r column names
threshold	identity calling minimum correlation score threshold, only used when obj_out = T
low_threshold_cell	option to remove clusters with too few cells
verbose	whether to report certain variables chosen and steps
input_markers	whether input is marker data.frame of 0 and 1s (output of pos_neg_marker), and uses alternate enrichment mode
details_out	whether to also output shared gene list from jaccard
dr	stored dimension reduction

Value

matrix of numeric values, clusters from input as row names, cell types from marker_mat as column names

Examples

```
# Annotate a matrix and metadata

# Annotate using a different method
clustify_lists(
    input = pbmc_matrix_small,
    marker = cbmc_m,
    metadata = pbmc_meta,
    cluster_col = "classified",
    verbose = TRUE,
    metric = "jaccard"
)
```

---

clustify_nudge *Combined function to compare scRNA-seq data to bulk RNA-seq data and marker list*

Description

Combined function to compare scRNA-seq data to bulk RNA-seq data and marker list

Usage

```
clustify_nudge(input, ...)

## Default S3 method:
clustify_nudge(
  input,
  ref_mat,
  marker,
  metadata = NULL,
  cluster_col = NULL,
  query_genes = NULL,
  compute_method = "spearman",
  weight = 1,
  threshold = -Inf,
  dr = "umap",
  norm = "diff",
  call = TRUE,
  marker_inmatrix = TRUE,
  mode = "rank",
  obj_out = FALSE,
  seurat_out = obj_out,
  rename_prefix = NULL,
  lookuptable = NULL,
  ...
)
```

```
## S3 method for class 'Seurat'
clustify_nudge(
  input,
  ref_mat,
  marker,
  cluster_col = NULL,
  query_genes = NULL,
  compute_method = "spearman",
  weight = 1,
  obj_out = TRUE,
  seurat_out = obj_out,
  threshold = -Inf,
  dr = "umap",
  norm = "diff",
  marker_inmatrix = TRUE,
  mode = "rank",
  rename_prefix = NULL,
  ...
)
```

Arguments

input	express matrix or object
...	passed to matrixize_markers
ref_mat	reference expression matrix
marker	matrix of markers
metadata	cell cluster assignments, supplied as a vector or data.frame. If data.frame is supplied then cluster_col needs to be set.
cluster_col	column in metadata that contains cluster ids per cell. Will default to first column of metadata if not supplied. Not required if running correlation per cell.
query_genes	A vector of genes of interest to compare. If NULL, then common genes between the expr_mat and ref_mat will be used for comparision.
compute_method	method(s) for computing similarity scores
weight	relative weight for the gene list scores, when added to correlation score
threshold	identity calling minimum score threshold, only used when obj_out = T
dr	stored dimension reduction
norm	whether and how the results are normalized
call	make call or just return score matrix
marker_inmatrix	whether markers genes are already in preprocessed matrix form
mode	use marker expression pct or ranked cor score for nudging
obj_out	whether to output object instead of cor matrix
seurat_out	output cor matrix or called seurat object (deprecated, use obj_out)
rename_prefix	prefix to add to type and r column names
lookuptable	if not supplied, will look in built-in table for object parsing

Value

single cell object, or matrix of numeric values, clusters from input as row names, cell types from ref_mat as column names

Examples

```
# Seurat
so <- so_pbmc()
clustify_nudge(
    input = so,
    ref_mat = cbmc_ref,
    marker = cbmc_m,
    cluster_col = "seurat_clusters",
    threshold = 0.8,
    obj_out = FALSE,
    mode = "pct",
    dr = "umap"
)

# Matrix
clustify_nudge(
    input = pbmc_matrix_small,
    ref_mat = cbmc_ref,
    metadata = pbmc_meta,
    marker = as.matrix(cbmc_m),
    query_genes = pbmc_vargenes,
    cluster_col = "classified",
    threshold = 0.8,
    call = FALSE,
    marker_inmatrix = FALSE,
    mode = "pct"
)
```

---

collapse_to_cluster *From per-cell calls, take highest freq call in each cluster*

Description

From per-cell calls, take highest freq call in each cluster

Usage

```
collapse_to_cluster(res, metadata, cluster_col, threshold = 0)
```

Arguments

res	dataframe of idents, such as output of cor_to_call
metadata	input metadata with tsne or umap coordinates and cluster ids
cluster_col	metadata column for cluster
threshold	minimum correlation coefficent cutoff for calling clusters

Value

new metadata with added columns

Examples

```
res <- clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    ref_mat = cbmc_ref,
    per_cell = TRUE
)

res2 <- cor_to_call(res)

collapse_to_cluster(
    res2,
    metadata = pbmc_meta,
    cluster_col = "classified",
    threshold = 0
)
```

---

compare_lists *Calculate adjusted p-values for hypergeometric test of gene lists or jaccard index*

Description

Calculate adjusted p-values for hypergeometric test of gene lists or jaccard index

Usage

```
compare_lists(
  bin_mat,
  marker_mat,
  n = 30000,
  metric = "hyper",
  output_high = TRUE,
  details_out = FALSE
)
```

Arguments

bin_mat	binarized single-cell expression matrix, feed in by_cluster mat, if desired
marker_mat	matrix or dataframe of candidate genes for each cluster
n	number of genes in the genome
metric	adjusted p-value for hypergeometric test, or jaccard index
output_high	if true (by default to fit with rest of package), -log10 transform p-value
details_out	whether to also output shared gene list from jaccard

Value

matrix of numeric values, clusters from expr_mat as row names, cell types from marker_mat as column names

Examples

```
pbmc_mm <- matrixize_markers(pbmc_markers)

pbmc_avg <- average_clusters(
    pbmc_matrix_small,
    pbmc_meta,
    cluster_col = "classified"
)

pbmc_avgb <- binarize_expr(pbmc_avg)

compare_lists(
    pbmc_avgb,
    pbmc_mm,
    metric = "spearman"
)
```

---

cor_to_call *get best calls for each cluster*

Description

get best calls for each cluster

Usage

```
cor_to_call(
  cor_mat,
  metadata = NULL,
  cluster_col = "cluster",
  collapse_to_cluster = FALSE,
  threshold = 0,
  rename_prefix = NULL,
  carry_r = FALSE
)
```

Arguments

cor_mat	input similarity matrix
metadata	input metadata with tsne or umap coordinates and cluster ids
cluster_col	metadata column, can be cluster or cellid

collapse_to_cluster	if a column name is provided, takes the most frequent call of entire cluster to color in plot
threshold	minimum correlation coefficent cutoff for calling clusters
rename_prefix	prefix to add to type and r column names
carry_r	whether to include threshold in unassigned names

Value

dataframe of cluster, new ident, and r info

Examples

```
res <- clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    ref_mat = cbmc_ref
)

cor_to_call(res)
```

---

cor_to_call_rank *get ranked calls for each cluster*

Description

get ranked calls for each cluster

Usage

```
cor_to_call_rank(
  cor_mat,
  metadata = NULL,
  cluster_col = "cluster",
  collapse_to_cluster = FALSE,
  threshold = 0,
  rename_prefix = NULL,
  top_n = NULL
)
```

Arguments

cor_mat	input similarity matrix
metadata	input metadata with tsne or umap coordinates and cluster ids
cluster_col	metadata column, can be cluster or cellid

collapse_to_cluster	if a column name is provided, takes the most frequent call of entire cluster to color in plot
threshold	minimum correlation coefficent cutoff for calling clusters
rename_prefix	prefix to add to type and r column names
top_n	the number of ranks to keep, the rest will be set to 100

Value

dataframe of cluster, new ident, and r info

Examples

```
res <- clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    ref_mat = cbmc_ref
)

cor_to_call_rank(res, threshold = "auto")
```

---

cor_to_call_topn *get top calls for each cluster*

Description

get top calls for each cluster

Usage

```
cor_to_call_topn(
  cor_mat,
  metadata = NULL,
  col = "cluster",
  collapse_to_cluster = FALSE,
  threshold = 0,
  topn = 2
)
```

Arguments

cor_mat	input similarity matrix
metadata	input metadata with tsne or umap coordinates and cluster ids
col	metadata column, can be cluster or cellid

collapse_to_cluster	if a column name is provided, takes the most frequent call of entire cluster to color in plot
threshold	minimum correlation coefficent cutoff for calling clusters
topn	number of calls for each cluster

Value

dataframe of cluster, new potential ident, and r info

Examples

```
res <- clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    ref_mat = cbmc_ref,
    query_genes = pbmc_vargenes,
    cluster_col = "classified"
)

cor_to_call_topn(
    cor_mat = res,
    metadata = pbmc_meta,
    col = "classified",
    collapse_to_cluster = FALSE,
    threshold = 0.5
)
```

---

cosine *Cosine distance*

Description

Cosine distance

Usage

```
cosine(vec1, vec2)
```

Arguments

vec1	test vector
vec2	reference vector

Value

numeric value of cosine distance between the vectors

downrefs *table of references stored in clustifyrdata*

Description

table of references stored in clustifyrdata

Usage

```
downrefs
```

Format

An object of class `tbl_df` (inherits from `tbl`, `data.frame`) with 9 rows and 6 columns.

Source

various packages

See Also

Other data: `cbmc_m`, `cbmc_ref`, `human_genes_10x`, `mouse_genes_10x`, `pbmc_markers`, `pbmc_markers_M3Drop`, `pbmc_matrix_small`, `pbmc_meta`, `pbmc_vargenes`

downsample_matrix *downsample matrix by cluster or completely random*

Description

downsample matrix by cluster or completely random

Usage

```
downsample_matrix(
  mat,
  n = 1,
  keep_cluster_proportions = TRUE,
  metadata = NULL,
  cluster_col = "cluster"
)
```

Arguments

mat	expression matrix
n	number per cluster or fraction to keep
keep_cluster_proportions	whether to subsample
metadata	data.frame or vector containing cluster assignments per cell. Order must match column order in supplied matrix. If a data.frame provide the cluster_col parameters.
cluster_col	column in metadata with cluster number

Value

new smaller mat with less cell_id columns

Examples

```
set.seed(42)
mat <- downsample_matrix(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta$classified,
    n = 10,
    keep_cluster_proportions = TRUE
)
mat[1:3, 1:3]
```

---

feature_select_PCA *Returns a list of variable genes based on PCA*

Description

Extract genes, i.e. "features", based on the top loadings of principal components formed from the bulk expression data set

Usage

```
feature_select_PCA(
  mat = NULL,
  pcs = NULL,
  n_pcs = 10,
  percentile = 0.99,
  if_log = TRUE
)
```

Arguments

mat	Expression matrix. Rownames are genes, colnames are single cell cluster name, and values are average single cell expression (log transformed).
pcs	Precalculated pcs if available, will skip over processing on mat.
n_pcs	Number of PCs to selected gene loadings from. See the explore_PCA_corr.Rmd vignette for details.
percentile	Select the percentile of absolute values of PCA loadings to select genes from. E.g. 0.999 would select the top point 1 percent of genes with the largest loadings.
if_log	whether the data is already log transformed

Value

vector of genes

Examples

```
feature_select_PCA(
    cbmc_ref,
    if_log = FALSE
)
```

---

file_marker_parse *takes files with positive and negative markers, as described in garnett, and returns list of markers*

Description

takes files with positive and negative markers, as described in garnett, and returns list of markers

Usage

```
file_marker_parse(filename)
```

Arguments

filename	txt file to load

Value

list of positive and negative gene markers

Examples

```
marker_file <- system.file(
    "extdata",
    "hsPBMC_markers.txt",
    package = "clustifyr"
)

file_marker_parse(marker_file)
```

`find_rank_bias` *Find rank bias*

Description

Find rank bias

Usage

```
find_rank_bias(avg_mat, ref_mat, query_genes = NULL)
```

Arguments

`avg_mat`	average expression matrix
`ref_mat`	reference expression matrix
`query_genes`	original vector of genes used to clustify

Value

list of matrix of rank diff values

Examples

```
avg <- average_clusters(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    if_log = FALSE
)

rankdiff <- find_rank_bias(
    avg,
    cbmc_ref,
    query_genes = pbmc_vargenes
)
```

gene_pct *pct of cells in each cluster that express genelist*

Description

pct of cells in each cluster that express genelist

Usage

```
gene_pct(matrix, genelist, clusters, returning = "mean")
```

Arguments

matrix	expression matrix
genelist	vector of marker genes for one identity
clusters	vector of cluster identities
returning	whether to return mean, min, or max of the gene pct in the gene list

Value

vector of numeric values

gene_pct_markerm *pct of cells in every cluster that express a series of genelists*

Description

pct of cells in every cluster that express a series of genelists

Usage

```
gene_pct_markerm(matrix, marker_m, metadata, cluster_col = NULL, norm = NULL)
```

Arguments

matrix	expression matrix
marker_m	matrixized markers
metadata	data.frame or vector containing cluster assignments per cell. Order must match column order in supplied matrix. If a data.frame provide the cluster_col parameters.
cluster_col	column in metadata with cluster number
norm	whether and how the results are normalized

Value

matrix of numeric values, clusters from mat as row names, cell types from marker_m as column names

Examples

```
gene_pct_markerm(
    matrix = pbmc_matrix_small,
    marker_m = cbmc_m,
    metadata = pbmc_meta,
    cluster_col = "classified"
)
```

---

get_best_match_matrix *Function to make best call from correlation matrix*

Description

Function to make best call from correlation matrix

Usage

```
get_best_match_matrix(cor_mat)
```

Arguments

cor_mat	correlation matrix

Value

matrix of 1s and 0s

---

get_best_str *Function to make call and attach score*

Description

Function to make call and attach score

Usage

```
get_best_str(name, best_mat, cor_mat, carry_cor = TRUE)
```

Arguments

name	name of row to query
best_mat	binarized call matrix
cor_mat	correlation matrix
carry_cor	whether the correlation score gets reported

Value

string with ident call and possibly cor value

---

get_common_elements *Find entries shared in all vectors*

Description

return entries found in all supplied vectors. If the vector supplied is NULL or NA, then it will be excluded from the comparison.

Usage

```
get_common_elements(...)
```

Arguments

...	vectors

Value

vector of shared elements

---

get_similarity *Compute similarity of matrices*

Description

Compute similarity of matrices

Usage

```
get_similarity(
  expr_mat,
  ref_mat,
  cluster_ids,
  compute_method,
  pseudobulk_method = "mean",
  per_cell = FALSE,
  rm0 = FALSE,
  if_log = TRUE,
  low_threshold = 0,
  ...
)
```

Arguments

expr_mat	single-cell expression matrix
ref_mat	reference expression matrix
cluster_ids	vector of cluster ids for each cell
compute_method	method(s) for computing similarity scores
pseudobulk_method	method used for summarizing clusters, options are mean (default), median, truncate (10% truncated mean), or trimean, max, min
per_cell	run per cell?
rm0	consider 0 as missing data, recommended for per_cell
if_log	input data is natural log, averaging will be done on unlogged data
low_threshold	option to remove clusters with too few cells
...	additional parameters not used yet

Value

matrix of numeric values, clusters from expr_mat as row names, cell types from ref_mat as column names

---

get_ucsc_reference *Build reference atlases from external UCSC cellbrowsers*

Description

Build reference atlases from external UCSC cellbrowsers

Usage

```
get_ucsc_reference(cb_url, cluster_col, ...)
```

Arguments

cb_url	URL of cellbrowser dataset (e.g. http://cells.ucsc.edu/?ds=cortex-dev). Note that the URL must contain the ds=dataset-name suffix.
cluster_col	annotation field for summarizing gene expression (e.g. clustering, cell-type name, samples, etc.)
...	additional args passed to average_clusters

Value

reference matrix

Examples

```
## Not run:

# many datasets hosted by UCSC have UMI counts in the expression matrix
# set if_log = FALSE if the expression matrix has not been natural log transformed

get_ucsc_reference(
    cb_url = "https://cells.ucsc.edu/?ds=evocell+mus-musculus+marrow",
    cluster_col = "Clusters", if_log = FALSE
)

get_ucsc_reference(
    cb_url = "http://cells.ucsc.edu/?ds=muscle-cell-atlas",
    cluster_col = "cell_annotation",
    if_log = FALSE
)

## End(Not run)
```

---

get_unique_column *Generate a unique column id for a dataframe*

Description

Generate a unique column id for a dataframe

Usage

```
get_unique_column(df, id = NULL)
```

Arguments

df	dataframe with column names
id	desired id if unique

Value

character

`get_vargenes` *Generate variable gene list from marker matrix*

Description

Variable gene list is required for `clustify` main function. This function parses variables genes from a matrix input.

Usage

```
get_vargenes(marker_mat)
```

Arguments

`marker_mat`	matrix or dataframe of candidate genes for each cluster

Value

vector of marker gene names

Examples

```
get_vargenes(cbmc_m)
```

`gmt_to_list` *convert gmt format of pathways to list of vectors*

Description

convert gmt format of pathways to list of vectors

Usage

```
gmt_to_list(
  path,
  cutoff = 0,
  sep = "\thttp://www.broadinstitute.org/gsea/msigdb/cards/.*?\t"
)
```

Arguments

`path`	gmt file path
`cutoff`	remove pathways with less genes than this cutoff
`sep`	sep used in file to split path and genes

Value

list of genes in each pathway

Examples

```
gmt_file <- system.file(
    "extdata",
    "c2.cp.reactome.v6.2.symbols.gmt.gz",
    package = "clustifyr"
)

gene.lists <- gmt_to_list(path = gmt_file)
length(gene.lists)
```

---

human_genes_10x *Vector of human genes for 10x cellranger pipeline*

Description

Vector of human genes for 10x cellranger pipeline

Usage

```
human_genes_10x
```

Format

An object of class `character` of length 33514.

Source

https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/latest

See Also

Other data: cbmc_m, cbmc_ref, downrefs, mouse_genes_10x, pbmc_markers, pbmc_markers_M3Drop, pbmc_matrix_small, pbmc_meta, pbmc_vargenes

insert_meta_object *more flexible metadata update of single cell objects*

Description

more flexible metadata update of single cell objects

Usage

```
insert_meta_object(
  input,
  new_meta,
  type = class(input),
  meta_loc = NULL,
  lookuptable = NULL
)
```

Arguments

input	input object
new_meta	new metadata table to insert back into object
type	look up predefined slots/loc
meta_loc	metadata location
lookuptable	if not supplied, will look in built-in table for object parsing

Value

new object with new metadata inserted

Examples

```
so <- so_pbmc()
insert_meta_object(so, seurat_meta(so, dr = "umap"))
```

kl_divergence *KL divergence*

Description

Use package entropy to compute Kullback-Leibler divergence. The function first converts each vector's reads to pseudo-number of transcripts by normalizing the total reads to total_reads. The normalized read for each gene is then rounded to serve as the pseudo-number of transcripts. Function entropy::KL.shrink is called to compute the KL-divergence between the two vectors, and the maximal allowed divergence is set to max_KL. Finally, a linear transform is performed to convert the KL divergence, which is between 0 and max_KL, to a similarity score between -1 and 1.

Usage

```
kl_divergence(vec1, vec2, if_log = FALSE, total_reads = 1000, max_KL = 1)
```

Arguments

vec1	Test vector
vec2	Reference vector
if_log	Whether the vectors are log-transformed. If so, the raw count should be computed before computing KL-divergence.
total_reads	Pseudo-library size
max_KL	Maximal allowed value of KL-divergence.

Value

numeric value, with additional attributes, of kl divergence between the vectors

---

make_comb_ref *make combination ref matrix to assess intermixing*

Description

make combination ref matrix to assess intermixing

Usage

```
make_comb_ref(ref_mat, if_log = TRUE, sep = "_and_")
```

Arguments

ref_mat	reference expression matrix
if_log	whether input data is natural
sep	separator for name combinations

Value

expression matrix

Examples

```
ref <- make_comb_ref(
    cbmc_ref,
    sep = "_+_"
)
ref[1:3, 1:3]
```

marker_select *decide for one gene whether it is a marker for a certain cell type*

Description

decide for one gene whether it is a marker for a certain cell type

Usage

```
marker_select(row1, cols, cut = 1, compto = 1)
```

Arguments

row1	a numeric vector of expression values (row)
cols	a vector of cell types (column)
cut	an expression minimum cutoff
compto	compare max expression to the value of next 1 or more

Value

vector of cluster name and ratio value

Examples

```
pbmc_avg <- average_clusters(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    if_log = FALSE
)

marker_select(
    row1 = pbmc_avg["PPBP", ],
    cols = names(pbmc_avg["PPBP", ])
)
```

matrixize_markers *Convert candidate genes list into matrix*

Description

Convert candidate genes list into matrix

Usage

```
matrixize_markers(
  marker_df,
  ranked = FALSE,
  n = NULL,
  step_weight = 1,
  background_weight = 0,
  unique = FALSE,
  metadata = NULL,
  cluster_col = "classified",
  remove_rp = FALSE
)
```

Arguments

marker_df	dataframe of candidate genes, must contain "gene" and "cluster" columns, or a matrix of gene names to convert to ranked
ranked	unranked gene list feeds into hyperp, the ranked gene list feeds into regular corr_coef
n	number of genes to use
step_weight	ranked genes are tranformed into pseudo expression by descending weight
background_weight	ranked genes are tranformed into pseudo expression with added weight
unique	whether to use only unique markers to 1 cluster
metadata	vector or dataframe of cluster names, should have column named cluster
cluster_col	column for cluster names to replace original cluster, if metadata is dataframe
remove_rp	do not include rps, rpl, rp1-9 in markers

Value

matrix of unranked gene marker names, or matrix of ranked expression

Examples

```
matrixize_markers(pbmc_markers)
```

---

mouse_genes_10x *Vector of mouse genes for 10x cellranger pipeline*

Description

Vector of mouse genes for 10x cellranger pipeline

Usage

```
mouse_genes_10x
```

Format

An object of class character of length 31017.

Source

https://support.10xgenomics.com/single-cell-gene-expression/software/downloads/latest

See Also

Other data: cbmc_m, cbmc_ref, downrefs, human_genes_10x, pbmc_markers, pbmc_markers_M3Drop, pbmc_matrix_small, pbmc_meta, pbmc_vargenes

---

not_pretty_palette *black and white palette for plotting continous variables*

Description

black and white palette for plotting continous variables

Usage

```
not_pretty_palette
```

Format

An object of class character of length 9.

Value

vector of colors

---

object_data *Function to access object data*

Description

Function to access object data

Usage

```
object_data(object, ...)

## S3 method for class 'Seurat'
object_data(object, slot, n_genes = 1000, ...)

## S3 method for class 'SingleCellExperiment'
object_data(object, slot, ...)
```

Arguments

object	object after tsne or umap projections and clustering
...	additional arguments
slot	data to access
n_genes	number of genes limit for Seurat variable genes, by default 1000, set to 0 to use all variable genes (generally not recommended)

Value

expression matrix, with genes as row names, and cell types as column names

Examples

```
so <- so_pbmc()
mat <- object_data(
    object = so,
    slot = "data"
)
mat[1:3, 1:3]
sce <- sce_pbmc()
mat <- object_data(
    object = sce,
    slot = "data"
)
mat[1:3, 1:3]
```

---

object_loc_lookup *lookup table for single cell object structures*

Description

lookup table for single cell object structures

Usage

```
object_loc_lookup()
```

Value

A list populated with standardized functions to access relevant data structures in multiple single cell data formats.

object_ref *Function to convert labelled object to avg expression matrix*

Description

Function to convert labelled object to avg expression matrix

Usage

```
object_ref(input, ...)

## Default S3 method:
object_ref(
  input,
  cluster_col = NULL,
  var_genes_only = FALSE,
  assay_name = NULL,
  method = "mean",
  lookuptable = NULL,
  if_log = TRUE,
  ...
)

## S3 method for class 'Seurat'
object_ref(
  input,
  cluster_col = NULL,
  var_genes_only = FALSE,
  assay_name = NULL,
  method = "mean",
  lookuptable = NULL,
  if_log = TRUE,
  ...
)

## S3 method for class 'SingleCellExperiment'
object_ref(
  input,
  cluster_col = NULL,
  var_genes_only = FALSE,
  assay_name = NULL,
  method = "mean",
  lookuptable = NULL,
  if_log = TRUE,
  ...
)
```

Arguments

input	object after tsne or umap projections and clustering
...	additional arguments
cluster_col	column name where classified cluster names are stored in seurat meta data, cannot be "rn"
var_genes_only	whether to keep only var.genes in the final matrix output, could also look up genes used for PCA
assay_name	any additional assay data, such as ADT, to include. If more than 1, pass a vector of names
method	whether to take mean (default) or median
lookuptable	if not supplied, will look in built-in table for object parsing
if_log	input data is natural log, averaging will be done on unlogged data

Value

reference expression matrix, with genes as row names, and cell types as column names

Examples

```
so <- so_pbmc()
object_ref(
    so,
    cluster_col = "seurat_clusters"
)
```

---

overcluster *Overcluster by kmeans per cluster*

Description

Overcluster by kmeans per cluster

Usage

```
overcluster(mat, cluster_id, power = 0.15)
```

Arguments

mat	expression matrix
cluster_id	list of ids per cluster
power	decides the number of clusters for kmeans

Value

new cluster_id list of more clusters

Examples

```
res <- overcluster(
    mat = pbmc_matrix_small,
    cluster_id = split(colnames(pbmc_matrix_small), pbmc_meta$classified)
)
length(res)
```

`overcluster_test` *compare clustering parameters and classification outcomes*

Description

compare clustering parameters and classification outcomes

Usage

```
overcluster_test(
  expr,
  metadata,
  ref_mat,
  cluster_col,
  x_col = "UMAP_1",
  y_col = "UMAP_2",
  n = 5,
  ngenes = NULL,
  query_genes = NULL,
  threshold = 0,
  do_label = TRUE,
  do_legend = FALSE,
  newclustering = NULL,
  combine = TRUE
)
```

Arguments

`expr`	expression matrix
`metadata`	metadata including cluster info and dimension reduction plotting
`ref_mat`	reference matrix
`cluster_col`	column of clustering from metadata
`x_col`	column of metadata for x axis plotting
`y_col`	column of metadata for y axis plotting
`n`	expand n-fold for over/under clustering
`ngenes`	number of genes to use for feature selection, use all genes if NULL
`query_genes`	vector, otherwise genes with be recalculated

threshold	type calling threshold
do_label	whether to label each cluster at median center
do_legend	whether to draw legend
newclustering	use kmeans if NULL on dr or col name for second column of clustering
combine	if TRUE return a single plot with combined panels, if FALSE return list of plots (default: TRUE)

Value

faceted ggplot object

Examples

```
set.seed(42)
overcluster_test(
    expr = pbmc_matrix_small,
    metadata = pbmc_meta,
    ref_mat = cbmc_ref,
    cluster_col = "classified",
    x_col = "UMAP_1",
    y_col = "UMAP_2"
)
```

---

parse_loc_object *more flexible parsing of single cell objects*

Description

more flexible parsing of single cell objects

Usage

```
parse_loc_object(
  input,
  type = class(input),
  expr_loc = NULL,
  meta_loc = NULL,
  var_loc = NULL,
  cluster_col = NULL,
  lookuptable = NULL
)
```

Arguments

input	input object
type	look up predefined slots/loc
expr_loc	function that extracts expression matrix
meta_loc	function that extracts metadata
var_loc	function that extracts variable genes
cluster_col	column of clustering from metadata
lookuptable	if not supplied, will use object_loc_lookup() for parsing.

Value

list of expression, metadata, vargenes, cluster_col info from object

Examples

```
so <- so_pbmc()
obj <- parse_loc_object(so)
length(obj)
```

---

pbmc_markers *Marker genes identified by Seurat from single-cell RNA-seq PBMCs.*

Description

Dataframe of markers from Seurat FindAllMarkers function

Usage

```
pbmc_markers
```

Format

An object of class `data.frame` with 2304 rows and 7 columns.

Source

`[pbmc_matrix]` processed by Seurat

See Also

Other data: `cbmc_m`, `cbmc_ref`, `downrefs`, `human_genes_10x`, `mouse_genes_10x`, `pbmc_markers_M3Drop`, `pbmc_matrix_small`, `pbmc_meta`, `pbmc_vargenes`

pbmc_markers_M3Drop *Marker genes identified by M3Drop from single-cell RNA-seq PBMCs.*

Description

Selected features of 3k pbmcs from Seurat3 tutorial

Usage

```
pbmc_markers_M3Drop
```

Format

A data frame with 3 variables:

Source

[pbmc_matrix] processed by [M3Drop]

See Also

Other data: cbmc_m, cbmc_ref, downrefs, human_genes_10x, mouse_genes_10x, pbmc_markers, pbmc_matrix_small, pbmc_meta, pbmc_vargenes

pbmc_matrix_small *Matrix of single-cell RNA-seq PBMCs.*

Description

Count matrix of 3k pbmcs from Seurat3 tutorial, with only var.features

Usage

```
pbmc_matrix_small
```

Format

A sparseMatrix with genes as rows and cells as columns.

Source

https://satijalab.org/seurat/v3.0/pbmc3k_tutorial.html

See Also

Other data: cbmc_m, cbmc_ref, downrefs, human_genes_10x, mouse_genes_10x, pbmc_markers, pbmc_markers_M3Drop, pbmc_meta, pbmc_vargenes

pbmc_meta *Meta-data for single-cell RNA-seq PBMCs.*

Description

Metadata, including umap, of 3k pbmcs from Seurat3 tutorial

Usage

```
pbmc_meta
```

Format

An object of class `data.frame` with 2638 rows and 9 columns.

Source

`[pbmc_matrix]` processed by Seurat

See Also

Other data: `cbmc_m`, `cbmc_ref`, `downrefs`, `human_genes_10x`, `mouse_genes_10x`, `pbmc_markers`, `pbmc_markers_M3Drop`, `pbmc_matrix_small`, `pbmc_vargenes`

pbmc_vargenes *Variable genes identified by Seurat from single-cell RNA-seq PBMCs.*

Description

Top 2000 variable genes from 3k pbmcs from Seurat3 tutorial

Usage

```
pbmc_vargenes
```

Format

An object of class `character` of length 2000.

Source

`[pbmc_matrix]` processed by Seurat

See Also

Other data: `cbmc_m`, `cbmc_ref`, `downrefs`, `human_genes_10x`, `mouse_genes_10x`, `pbmc_markers`, `pbmc_markers_M3Drop`, `pbmc_matrix_small`, `pbmc_meta`

percent_clusters *Percentage detected per cluster*

Description

Percentage detected per cluster

Usage

```
percent_clusters(mat, metadata, cluster_col = "cluster", cut_num = 0.5)
```

Arguments

mat	expression matrix
metadata	data.frame with cells
cluster_col	column in metadata with cluster number
cut_num	binary cutoff for detection

Value

matrix of numeric values, with genes for row names, and clusters for column names

permute_similarity *Compute a p-value for similarity using permutation*

Description

Permute cluster labels to calculate empirical p-value

Usage

```
permute_similarity(
  expr_mat,
  ref_mat,
  cluster_ids,
  n_perm,
  per_cell = FALSE,
  compute_method,
  pseudobulk_method = "mean",
  rm0 = FALSE,
  ...
)
```

Arguments

expr_mat	single-cell expression matrix
ref_mat	reference expression matrix
cluster_ids	clustering info of single-cell data assume that genes have ALREADY BEEN filtered
n_perm	number of permutations
per_cell	run per cell?
compute_method	method(s) for computing similarity scores
pseudobulk_method	method used for summarizing clusters, options are mean (default), median, truncate (10% truncated mean), or trimean, max, min
rm0	consider 0 as missing data, recommended for per_cell
...	additional parameters

Value

matrix of numeric values

---

plot_best_call *Plot best calls for each cluster on a tSNE or umap*

Description

Plot best calls for each cluster on a tSNE or umap

Usage

```
plot_best_call(
  cor_mat,
  metadata,
  cluster_col = "cluster",
  collapse_to_cluster = FALSE,
  threshold = 0,
  x = "UMAP_1",
  y = "UMAP_2",
  plot_r = FALSE,
  per_cell = FALSE,
  ...
)
```

Arguments

cor_mat	input similarity matrix
metadata	input metadata with tsne or umap coordinates and cluster ids
cluster_col	metadata column, can be cluster or cellid
collapse_to_cluster	if a column name is provided, takes the most frequent call of entire cluster to color in plot
threshold	minimum correlation coefficent cutoff for calling clusters
x	x variable
y	y variable
plot_r	whether to include second plot of cor eff for best call
per_cell	whether the cor_mat was generate per cell or per cluster
...	passed to plot_dims

Value

ggplot object, cells projected by dr, colored by cell type classification

Examples

```
res <- clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    ref_mat = cbmc_ref,
    query_genes = pbmc_vargenes,
    cluster_col = "classified"
)

plot_best_call(
    cor_mat = res,
    metadata = pbmc_meta,
    cluster_col = "classified"
)
```

---

plot_call *Plot called clusters on a tSNE or umap, for each reference cluster given*

Description

Plot called clusters on a tSNE or umap, for each reference cluster given

Usage

```
plot_call(cor_mat, metadata, data_to_plot = colnames(cor_mat), ...)
```

Arguments

cor_mat	input similarity matrix
metadata	input metadata with tsne or umap coordinates and cluster ids
data_to_plot	colname of data to plot, defaults to all
...	passed to plot_dims

Value

list of ggplot object, cells projected by dr, colored by cell type classification

---

plot_cor *Plot similarity measures on a tSNE or umap*

Description

Plot similarity measures on a tSNE or umap

Usage

```
plot_cor(
  cor_mat,
  metadata,
  data_to_plot = colnames(cor_mat),
  cluster_col = NULL,
  x = "UMAP_1",
  y = "UMAP_2",
  scale_legends = FALSE,
  ...
)
```

Arguments

cor_mat	input similarity matrix
metadata	input metadata with per cell tsne or umap coordinates and cluster ids
data_to_plot	colname of data to plot, defaults to all
cluster_col	colname of clustering data in metadata, defaults to rownames of the metadata if not supplied.
x	metadata column name with 1st axis dimension. defaults to "UMAP_1".
y	metadata column name with 2nd axis dimension. defaults to "UMAP_2".
scale_legends	if TRUE scale all legends to maximum values in entire correlation matrix. if FALSE scale legends to maximum for each plot. A two-element numeric vector can also be passed to supply custom values i.e. c(0, 1)
...	passed to plot_dims

Value

list of ggplot objects, cells projected by dr, colored by cor values

Examples

```
res <- clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    ref_mat = cbmc_ref,
    query_genes = pbmc_vargenes,
    cluster_col = "classified"
)

plot_cor(
    cor_mat = res,
    metadata = pbmc_meta,
    data_to_plot = colnames(res)[1:2],
    cluster_col = "classified",
    x = "UMAP_1",
    y = "UMAP_2"
)
```

---

plot_cor_heatmap *Plot similarity measures on heatmap*

Description

Plot similarity measures on heatmap

Usage

```
plot_cor_heatmap(
  cor_mat,
  metadata = NULL,
  cluster_col = NULL,
  col = not_pretty_palette,
  legend_title = NULL,
  ...
)
```

Arguments

cor_mat	input similarity matrix
metadata	input metadata with per cell tsne or umap cooordinates and cluster ids
cluster_col	colname of clustering data in metadata, defaults to rownames of the metadata if not supplied.
col	color ramp to use
legend_title	legend title to pass to Heatmap
...	passed to Heatmap

Value

complexheatmap object

Examples

```
res <- clustify(
    input = pbmc_matrix_small,
    metadata = pbmc_meta,
    ref_mat = cbmc_ref,
    query_genes = pbmc_vargenes,
    cluster_col = "classified",
    per_cell = FALSE
)

plot_cor_heatmap(res)
```

---

plot_dims *Plot a tSNE or umap colored by feature.*

Description

Plot a tSNE or umap colored by feature.

Usage

```
plot_dims(
  data,
  x = "UMAP_1",
  y = "UMAP_2",
  feature = NULL,
  legend_name = "",
  c_cols = pretty_palette2,
  d_cols = NULL,
  pt_size = 0.25,
  alpha_col = NULL,
  group_col = NULL,
  scale_limits = NULL,
  do_label = FALSE,
  do_legend = TRUE,
  do_repel = TRUE
)
```

Arguments

data	input data
x	x variable
y	y variable

feature	feature to color by
legend_name	legend name to display, defaults to no name
c_cols	character vector of colors to build color gradient for continuous values, defaults to pretty_palette
d_cols	character vector of colors for discrete values. defaults to RColorBrewer paired palette
pt_size	point size
alpha_col	whether to refer to data column for alpha values
group_col	group by another column instead of feature, useful for labels
scale_limits	defaults to min = 0, max = max(data$x), otherwise a two-element numeric vector indicating min and max to plot
do_label	whether to label each cluster at median center
do_legend	whether to draw legend
do_repel	whether to use ggrepel on labels

Value

ggplot object, cells projected by dr, colored by feature

Examples

```
plot_dims(
    pbmc_meta,
    feature = "classified"
)
```

---

plot_gene *Plot gene expression on to tSNE or umap*

Description

Plot gene expression on to tSNE or umap

Usage

```
plot_gene(expr_mat, metadata, genes, cell_col = NULL, ...)
```

Arguments

expr_mat	input single cell matrix
metadata	data.frame with tSNE or umap coordinates
genes	gene(s) to color tSNE or umap
cell_col	column name in metadata containing cell ids, defaults to rownames if not supplied
...	additional arguments passed to [clustifyr::plot_dims()]

Value

list of ggplot object, cells projected by dr, colored by gene expression

Examples

```
genes <- c(
    "RP11-314N13.3",
    "ARF4"
)

plot_gene(
    expr_mat = pbmc_matrix_small,
    metadata = tibble::rownames_to_column(pbmc_meta, "rn"),
    genes = genes,
    cell_col = "rn"
)
```

---

plot_pathway_gsea *plot GSEA pathway scores as heatmap, returns a list containing results and plot.*

Description

plot GSEA pathway scores as heatmap, returns a list containing results and plot.

Usage

```
plot_pathway_gsea(
  mat,
  pathway_list,
  n_perm = 1000,
  scale = TRUE,
  topn = 5,
  returning = "both"
)
```

Arguments

mat	expression matrix
pathway_list	a list of vectors, each named for a specific pathway, or dataframe
n_perm	Number of permutation for fgsea function. Defaults to 1000.
scale	convert expr_mat into zscores prior to running GSEA?, default = TRUE
topn	number of top pathways to plot
returning	to return "both" list and plot, or either one

Value

list of matrix and plot, or just plot, matrix of GSEA NES values, cell types as row names, pathways as column names

Examples

```
gl <- list(
    "n" = c("PPBP", "LYZ", "S100A9"),
    "a" = c("IGLL5", "GNLY", "FTL")
)

pbmc_avg <- average_clusters(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified"
)

plot_pathway_gsea(
    pbmc_avg,
    gl,
    5
)
```

---

plot_rank_bias *Query rank bias results*

Description

Query rank bias results

Usage

```
plot_rank_bias(bias_df, organism = "hsapiens")
```

Arguments

bias_df	data.frame of rank diff matrix between cluster and reference cell types
organism	for GO term analysis, organism name: human - 'hsapiens', mouse - 'mmusculus'

Value

ggplot object of distribution and annotated GO terms

Examples

```
## Not run:
avg <- average_clusters(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    if_log = FALSE
)

rankdiff <- find_rank_bias(
    avg,
    cbmc_ref,
    query_genes = pbmc_vargenes
)

qres <- query_rank_bias(
    rankdiff,
    "CD14+ Mono",
    "CD14+ Mono"
)

g <- plot_rank_bias(
    qres
)

## End(Not run)
```

---

pos_neg_marker *generate pos and negative marker expression matrix from a list/dataframe of positive markers*

Description

generate pos and negative marker expression matrix from a list/dataframe of positive markers

Usage

```
pos_neg_marker(mat)
```

Arguments

mat	matrix or dataframe of markers

Value

matrix of gene expression

Examples

```
m1 <- pos_neg_marker(cbmc_m)
```

`pos_neg_select` *adapt clustify to tweak score for pos and neg markers*

Description

adapt clustify to tweak score for pos and neg markers

Usage

```
pos_neg_select(
  input,
  ref_mat,
  metadata,
  cluster_col = "cluster",
  cutoff_n = 0,
  cutoff_score = 0.5
)
```

Arguments

`input`	single-cell expression matrix
`ref_mat`	reference expression matrix with positive and negative markers(set expression at 0)
`metadata`	cell cluster assignments, supplied as a vector or data.frame. If data.frame is supplied then `cluster_col` needs to be set. Not required if running correlation per cell.
`cluster_col`	column in metadata that contains cluster ids per cell. Will default to first column of metadata if not supplied. Not required if running correlation per cell.
`cutoff_n`	expression cutoff where genes ranked below n are considered non-expressing
`cutoff_score`	positive score lower than this cutoff will be considered as 0 to not influence scores

Value

matrix of numeric values, clusters from input as row names, cell types from ref_mat as column names

Examples

```
pn_ref <- data.frame(
    "Myeloid" = c(1, 0.01, 0),
    row.names = c("CD74", "clustifyr0", "CD79A")
)

pos_neg_select(
    input = pbmc_matrix_small,
```

```
    ref_mat = pn_ref,
    metadata = pbmc_meta,
    cluster_col = "classified",
    cutoff_score = 0.8
)
```

`pretty_palette` *Color palette for plotting continous variables*

Description

Color palette for plotting continous variables

Usage

```
pretty_palette
```

Format

An object of class `character` of length 6.

Value

vector of colors

`pretty_palette2` *Color palette for plotting continous variables, starting at gray*

Description

Color palette for plotting continous variables, starting at gray

Usage

```
pretty_palette2
```

Format

An object of class `character` of length 9.

Value

vector of colors

pretty_palette_ramp_d *Expanded color palette ramp for plotting discrete variables*

Description

Expanded color palette ramp for plotting discrete variables

Usage

```
pretty_palette_ramp_d(n)
```

Arguments

n	number of colors to use

Value

color ramp

query_rank_bias *Query rank bias results*

Description

Query rank bias results

Usage

```
query_rank_bias(bias_list, id_mat, id_ref)
```

Arguments

bias_list	list of rank diff matrix between cluster and reference cell types
id_mat	name of cluster from average cluster matrix
id_ref	name of cell type in reference matrix

Value

data.frame rank diff values

Examples

```
avg <- average_clusters(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified",
    if_log = FALSE
)

rankdiff <- find_rank_bias(
    avg,
    cbmc_ref,
    query_genes = pbmc_vargenes
)

qres <- query_rank_bias(
    rankdiff,
    "CD14+ Mono",
    "CD14+ Mono"
)
```

---

ref_feature_select *feature select from reference matrix*

Description

feature select from reference matrix

Usage

```
ref_feature_select(mat, n = 3000, mode = "var", rm.lowvar = TRUE)
```

Arguments

mat	reference matrix
n	number of genes to return
mode	the method of selecting features
rm.lowvar	whether to remove lower variation genes first

Value

vector of genes

Examples

```
pbmc_avg <- average_clusters(
    mat = pbmc_matrix_small,
    metadata = pbmc_meta,
    cluster_col = "classified"
)

ref_feature_select(
    mat = pbmc_avg[1:100, ],
    n = 5
)
```

`ref_marker_select` *marker selection from reference matrix*

Description

marker selection from reference matrix

Usage

```
ref_marker_select(mat, cut = 0.5, arrange = TRUE, compto = 1)
```

Arguments

`mat`	reference matrix
`cut`	an expression minimum cutoff
`arrange`	whether to arrange (lower means better)
`compto`	compare max expression to the value of next 1 or more

Value

dataframe, with gene, cluster, ratio columns

Examples

```
ref_marker_select(
    cbmc_ref,
    cut = 2
)
```

reverse_marker_matrix *generate negative markers from a list of exclusive positive markers*

Description

generate negative markers from a list of exclusive positive markers

Usage

```
reverse_marker_matrix(mat)
```

Arguments

mat	matrix or dataframe of markers

Value

matrix of gene names

Examples

```
reverse_marker_matrix(cbmc_m)
```

run_clustifyr_app *Launch Shiny app version of clustifyr, may need to run install_clustifyr_app() at first time to install packages*

Description

Launch Shiny app version of clustifyr, may need to run install_clustifyr_app() at first time to install packages

Usage

```
run_clustifyr_app()
```

Value

instance of shiny app

Examples

```
## Not run:
run_clustifyr_app()

## End(Not run)
```

`run_gsea` *Run GSEA to compare a gene list(s) to per cell or per cluster expression data*

Description

Use fgsea algorithm to compute normalized enrichment scores and pvalues for gene set ovelap

Usage

```
run_gsea(
  expr_mat,
  query_genes,
  cluster_ids = NULL,
  n_perm = 1000,
  per_cell = FALSE,
  scale = FALSE,
  no_warnings = TRUE
)
```

Arguments

`expr_mat`	single-cell expression matrix or Seurat object
`query_genes`	A vector or named list of vectors of genesets of interest to compare via GSEA. If supplying a named list, then the gene set names will appear in the output.
`cluster_ids`	vector of cell cluster assignments, supplied as a vector with order that matches columns in `expr_mat`. Not required if running per cell.
`n_perm`	Number of permutation for fgsea function. Defaults to 1000.
`per_cell`	if true run per cell, otherwise per cluster.
`scale`	convert expr_mat into zscores prior to running GSEA?, default = FALSE
`no_warnings`	suppress warnings from gsea ties

Value

dataframe of gsea scores (pval, NES), with clusters as rownames

sce_pbmc *An example SingleCellExperiment object*

Description

An example SingleCellExperiment object

Usage

```
sce_pbmc()
```

Value

a SingleCellExperiment object populated with data from the pbmc_matrix_small scRNA-seq dataset, additionally annotated with cluster assignments.

seurat_meta *Function to convert labelled seurat object to fully prepared metadata*

Description

Function to convert labelled seurat object to fully prepared metadata

Usage

```
seurat_meta(seurat_object, ...)

## S3 method for class 'Seurat'
seurat_meta(seurat_object, dr = "umap", ...)
```

Arguments

seurat_object	seurat_object after tsne or umap projections and clustering
...	additional arguments
dr	dimension reduction method

Value

dataframe of metadata, including dimension reduction plotting info

Examples

```
so <- so_pbmc()
m <- seurat_meta(so)
```

seurat_ref *Function to convert labelled seurat object to avg expression matrix*

Description

Function to convert labelled seurat object to avg expression matrix

Usage

```
seurat_ref(seurat_object, ...)

## S3 method for class 'Seurat'
seurat_ref(
  seurat_object,
  cluster_col = "classified",
  var_genes_only = FALSE,
  assay_name = NULL,
  method = "mean",
  subclusterpower = 0,
  if_log = TRUE,
  ...
)
```

Arguments

seurat_object	seurat_object after tsne or umap projections and clustering
...	additional arguments
cluster_col	column name where classified cluster names are stored in seurat meta data, cannot be "rn"
var_genes_only	whether to keep only var_genes in the final matrix output, could also look up genes used for PCA
assay_name	any additional assay data, such as ADT, to include. If more than 1, pass a vector of names
method	whether to take mean (default) or median
subclusterpower	whether to get multiple averages per original cluster
if_log	input data is natural log, averaging will be done on unlogged data

Value

reference expression matrix, with genes as row names, and cell types as column names

Examples

```
so <- so_pbmc()
ref <- seurat_ref(so, cluster_col = "seurat_clusters")
```

so_pbmc *An example Seurat object*

Description

An example Seurat object

Usage

```
so_pbmc()
```

Value

a Seurat object populated with data from the pbmc_matrix_small scRNA-seq dataset, additionally annotated with cluster assignments.

vector_similarity *Compute similarity between two vectors*

Description

Compute the similarity score between two vectors using a customized scoring function Two vectors may be from either scRNA-seq or bulk RNA-seq data. The lengths of vec1 and vec2 must match, and must be arranged in the same order of genes. Both vectors should be provided to this function after pre-processing, feature selection and dimension reduction.

Usage

```
vector_similarity(vec1, vec2, compute_method, ...)
```

Arguments

vec1	test vector
vec2	reference vector
compute_method	method to run i.e. corr_coef
...	arguments to pass to compute_method function

Value

numeric value of desired correlation or distance measurement

write_meta *Function to write metadata to object*

Description

Function to write metadata to object

Usage

```
write_meta(object, ...)

## S3 method for class 'Seurat'
write_meta(object, meta, ...)

## S3 method for class 'SingleCellExperiment'
write_meta(object, meta, ...)
```

Arguments

object	object after tsne or umap projections and clustering
...	additional arguments
meta	new metadata dataframe

Value

object with newly inserted metadata columns

Examples

```
so <- so_pbmc()
obj <- write_meta(
    object = so,
    meta = seurat_meta(so)
)
sce <- sce_pbmc()
obj <- write_meta(
    object = sce,
    meta = object_data(sce, "meta.data")
)
```

Index