# Package 'celaref'

May 10, 2024

Title Single-cell RNAseq cell cluster labelling by reference

**Version** 1.22.0

**Description** After the clustering step of a single-cell RNAseq experiment, this package aims to suggest labels/cell types for the clusters, on the basis of similarity to a reference dataset. It requires a table of read counts per cell per gene, and a list of the cells belonging to each of the clusters, (for both test and reference data).

**Depends** R (>= 3.5.0), SummarizedExperiment

**Imports** MAST, ggplot2, Matrix, dplyr, magrittr, stats, utils, rlang, BiocGenerics, S4Vectors, readr, tibble, DelayedArray

Suggests limma, parallel, knitr, rmarkdown, ExperimentHub, testthat

biocViews SingleCell

VignetteBuilder knitr

License GPL-3

**Encoding UTF-8** 

LazyData true

RoxygenNote 6.1.1

git\_url https://git.bioconductor.org/packages/celaref

git\_branch RELEASE\_3\_19

git\_last\_commit 0f17788

git\_last\_commit\_date 2024-04-30

Repository Bioconductor 3.19

Date/Publication 2024-05-10

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## Description

Produces a table of within-experiment differential expression results (for either query or reference experiment), where each group (cluster) is compared to the rest of the cells.

#### Usage

```
contrast_each_group_to_the_rest(dataset_se, dataset_name,
  groups2test = NA, num_cores = 1, n.group = Inf, n.other = n.group
  * 5)
```

#### **Arguments**

| dataset_se   | Summarised experiment object containing count data. Also requires 'ID' and 'group' to be set within the cell information (see colData())       |
|--------------|--|
| dataset_name | Short, meaningful name for this dataset/experiment.  |
| groups2test  | An optional character vector specificing specific groups to check. By default (set to NA), all groups will be tested.                          |
| num_cores    | Number of cores to use to run MAST jobs in parallel. Ignored if parallel package not available. Set to 1 to avoid parallelisation. Default = 1 |
| n.group      | How many cells to keep for each group in groupwise comparisons. Default = Inf  |
| n.other      | How many cells to keep from everything not in the group. Default = $\mathbf{n.group} * 5$  |

#### **Details**

Note that this function is *slow*, because it runs the differential expression. It only needs to be run once per dataset though (unless group labels change). Having package **parallel** installed is highly recomended.

If this function runs out of memory, consider specifying *n.group* and *n.other* to run on a subset of cells (taken from each group, and proportionally from the rest for each test). Alternatively use *subset\_cells\_by\_group* to subset **dataset\_se** for each group independently.

Both reference and query datasets should be processed with this function.

The tables produced by this function (usually named something like *de\_table.datasetname*) contain summarised results of MAST results. Each group is compared versus cells in the group, versus not in the group, (Ie. always a 2-group contrast, other groups information is ignored). As per MAST recomendataions, the proportion of genes seen in each cell is included in the model.

#### Value

A tibble the within-experiment de\_table (differential expression table). This is a core summary of the individual experiment/dataset, which is used for the cross-dataset comparisons.

The table feilds won't necessarily match across datasets, as they include cell annotations information. Important columns (used in downstream analysis) are:

**ID** Gene identifier

ci\_inner Inner (conservative) 95% confidence interval of log2 fold-change.

fdr Multiple hypothesis corrected p-value (using BH/FDR method)

**group** Cells from this group were compared to everything else

**sig\_up** Significantly differentially expressed (fdr < 0.01), with a positive fold change?

rank Rank position (within group), ranked by CI inner, highest to lowest.

**rescaled\_rank** Rank scaled 0(top most overrepresented genes in group) - 1(top most not-present genes)

dataset Name of dataset/experiment

#### **Examples**

### Description

This function loads and processes microarray data (from purified cell populations) that can be used as a reference.

#### Usage

```
contrast_each_group_to_the_rest_for_norm_ma_with_limma(norm_expression_table,
  sample_sheet_table, dataset_name, sample_name, group_name = "group",
  groups2test = NA, extra_factor_name = NA, pval_threshold = 0.01)
```

### **Arguments**

norm\_expression\_table

A logged, normalised expression table. Any filtering (removal of low-expression probes/genes)

sample\_sheet\_table

Tab-separated text file of sample information. Columns must have names. Sample/microarray ids should be listed under **sample\_name** column. The cell-type (or 'group') of each sample should be listed under a column **group\_name**.

dataset\_name Short, meaningful name for this dataset/experiment.

sample\_name Name of sample\_sheet\_table with sample ID

group\_name Name of **sample\_sheet\_table** with group/cell-type. Default = "group"

groups2test An optional character vector specificing specific groups to check. By default

(set to NA), all groups will be tested.

```
extra_factor_name
```

Optionally, an extra cross-group factor (as column name in **sample\_sheet\_table**) to include in the model used by limma. E.g. An individual/mouse id. Refer limma docs. Default = NA

pval\_threshold For reporting only, a p-value threshold. Default = 0.01

#### **Details**

Sometimes there are microarray studies measureing purified cell populations that would be measured together in a single-cell sequenicng experiment. E.g. comparing PBMC scRNA to FACs-sorted blood cell populations. This function will process microarray data with limma and format it for comparisions.

The microarray data used should consist of purified cell types from /emphone single study/experiment (due to batch effects). Ideally just those cell-types expected in the scRNAseq, but the method appears relatively robust to a few extra cell types.

Note that unlike the single-cell workflow there are no summarisedExperiment objects (they're not really comparable) - this function reads data and generates a table of within-dataset differentential expression contrasts in one step. Ie. equivalent to the output of contrast\_each\_group\_to\_the\_rest.

Also, note that while downstream functions can accept the microarray-derived data as query datasets, its not really intended and assumptions might not hold (Generally, its known what got loaded onto a microarray!)

The (otherwise optional) 'limma' package must be installed to use this function.

### Value

A tibble, the within-experiment de\_table (differential expression table)

### See Also

contrast\_each\_group\_to\_the\_rest is the function that makes comparable output on the scR-NAseq data (dataset\_se objects).

Limma Limma package for differential expression.

Other Data loading functions: load\_dataset\_10Xdata, load\_se\_from\_tables

#### **Examples**

```
contrast_each_group_to_the_rest_for_norm_ma_with_limma(
    norm_expression_table=demo_microarray_expr,
    sample_sheet_table=demo_microarray_sample_sheet,
    dataset_name="DemoSimMicroarrayRef",
    sample_name="cell_sample", group_name="group")

## Not run:
contrast_each_group_to_the_rest_for_norm_ma_with_limma(
    norm_expression_table, sample_sheet_table=samples_table,
    dataset_name="Watkins2009PBMCs", extra_factor_name='description')

## End(Not run)
```

### **Description**

Internal function to calculate differential expression within an experiment between a specified group and cells not in that group.

### Usage

```
contrast_the_group_to_the_rest(dataset_se, the_group,
   pvalue_threshold = 0.01, n.group = Inf, n.other = n.group * 5)
```

### Arguments

dataset\_se Datast summarisedExperiment object.

the\_group group to test

pvalue\_threshold

Default = 0.01

n.group How many cells to keep for each group in groupwise comparisons. Default =

Inf

n.other How many cells to keep from everything not in the group. Default = **n.group** \*

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#### **Details**

This function should only be called by contrast\_each\_group\_to\_the\_rest (which can be passed a single group name if desired). Else 'pofgenes' will not be defined.

MAST is supplied with log2(counts + 1.1), and zlm called with model '~ TvsR + pofgenes' . The p-values reported are from the hurdle model. FDR is with default fdr/BH method.

### Value

A tibble, the within-experiment de\_table (differential expression table), for the group specified.

#### See Also

```
contrast_each_group_to_the_rest
```

### **Description**

Private function used by contrast\_each\_group\_to\_the\_rest\_for\_norm\_ma\_with\_limma

#### Usage

```
contrast_the_group_to_the_rest_with_limma_for_microarray(norm_expression_table,
  sample_sheet_table, the_group, sample_name, extra_factor_name = NA,
  pval_threshold = 0.01)
```

### **Arguments**

norm\_expression\_table

A logged, normalised expression table. Any filtering (removal of low-expression probes/genes)

sample\_sheet\_table

Tab-separated text file of sample information. Columns must have names. Sample/microarray ids should be listed under **sample\_name** column. The cell-type (or 'group') of each sample should be listed under a column **group\_name**.

the\_group

Which query group is being tested.

sample\_name

Name of sample\_sheet\_table with sample ID

extra\_factor\_name

Optionally, an extra cross-group factor (as column name in **sample\_sheet\_table**) to include in the model used by limma. E.g. An individual/mouse id. Refer limma docs. Default = NA

pval\_threshold For reporting only, a p-value threshold. Default = 0.01

#### Value

A tibble, the within-experiment de\_table (differential expression table), for the group specified.

#### See Also

contrast\_each\_group\_to\_the\_rest\_for\_norm\_ma\_with\_limma public calling function Limma Limma package for differential expression. 8 convert\_se\_gene\_ids

```
convert_se_gene_ids convert_se_gene_ids
```

#### **Description**

Change the gene IDs in in the supplied datatset\_se object to some other id already present in the gene info (as seen with rowData())

#### Usage

```
convert_se_gene_ids(dataset_se, new_id, eval_col, find_max = TRUE)
```

#### **Arguments**

| dataset_se | Summarised experiment object containing count data. Also requires 'ID' and 'group' to be set within the cell information (see colData())   |
|------------|--|
| new_id     | A column within the feature information (view colData(dataset_se))) of the <b>dataset_se</b> , which will become the new ID column. Non-uniqueness of this column is handled gracefully! Any <i>NAs</i> will be dropped. |
| eval_col   | Which column to use to break ties of duplicate <b>new_id</b> . Must be a column within the feature information (view colData(dataset_se))) of the <b>dataset_se</b> . Total reads per gene feature is a good choice.     |
| find_max   | If false, this will choose the minimal <b>eval_col</b> instead of max. Default = TRUE  |

#### Value

A modified dataset\_se - ID will now be **new\_id**, and unique. It will have fewer genes if old ID to new ID was not a 1:1 mapping. The selected genes will be according to the eval col max (or min). *should* pick the alphabetical first on ties, but could change.

#### See Also

SummarizedExperiment For general doco on the SummarizedExperiment objects. load\_se\_from\_files For reading data from flat files (not 10X cellRanger output)

### **Examples**

```
# The demo dataset doesn't have other names, so make some up
# (don't do this)
dataset_se <- demo_ref_se
rowData(dataset_se)$dummyname <- toupper(rowData(dataset_se)$ID)
# If not already present, define a column to evaluate,
# typically total reads/gene.
rowData(dataset_se)$total_count <- rowSums(assay(dataset_se))
dataset_se <- convert_se_gene_ids(dataset_se, new_id='dummyname', eval_col='total_count')</pre>
```

demo\_cell\_info\_table 9

### Description

Sample sheet table listing each cell, its assignd cluster/group, and any other information that might be interesting (replicate, individual e.t.c)

### Usage

```
demo_cell_info_table
```

### **Format**

An object of class data. frame with 515 rows and 4 columns.

#### Value

An example cell info table

demo\_counts\_matrix

Demo count matrix

### Description

Counts matrix for a small, demo example datasets. Raw counts of reads per gene (row) per cell (column).

### Usage

```
demo_counts_matrix
```

#### **Format**

An object of class matrix with 200 rows and 514 columns.

#### Value

An example counts matrix.

### Description

Extra table of gene-level information for the demo example dataset. Can contain anything as long as theres a unique gene id.

### Usage

```
demo_gene_info_table
```

#### **Format**

An object of class data. frame with 200 rows and 2 columns.

#### Value

An example table of genes.

demo\_microarray\_expr Demo microarray expression table

### Description

Microarray-style expression table for the demo example dataset. Rows are genes, columns are samples, as per counts matrix.

### Usage

```
demo_microarray_expr
```

#### **Format**

An object of class matrix with 200 rows and 20 columns.

#### Value

An example table of (fake) microarray data.

demo\_microarray\_sample\_sheet

Demo microarray sample sheet table

### Description

Microarray sample sheet table for the demo example dataset. Contains array identifiers, their group and any other information that could be useful.

### Usage

```
demo_microarray_sample_sheet
```

#### **Format**

An object of class grouped\_df (inherits from tbl\_df, tbl, data.frame) with 20 rows and 2 columns.

### Value

An example microarray sample sheet

demo\_query\_se

Demo query se (summarizedExperiment)

### **Description**

A summarisedExperiment object loaded from demo info tables, for a query set.

#### Usage

```
demo_query_se
```

### **Format**

An object of class SummarizedExperiment with 200 rows and 485 columns.

### Value

An example summarised experiment (for demo query dataset)

demo\_ref\_se

Demo reference se (summarizedExperiment)

### Description

A summarisedExperiment object loaded from demo info tables, for a reference set.

### Usage

```
demo_ref_se
```

#### **Format**

An object of class SummarizedExperiment with 200 rows and 515 columns.

#### Value

An example summarised experiment (for demo reference dataset)

de\_table.demo\_query

Demo query de table

### **Description**

Small example dataset that is the output of contrast\_each\_group\_to\_the\_rest. It contains the results of each group compared to the rest of the sample (ie within sample differential expression)

### Usage

```
de_table.demo_query
```

#### **Format**

An object of class data. frame with 800 rows and 13 columns.

#### Value

An example de\_table from contrast\_each\_group\_to\_the\_rest (for demo query dataset)

de\_table.demo\_ref

### Description

Small example dataset that is the output of contrast\_each\_group\_to\_the\_rest. It contains the results of each group compared to the rest of the sample (ie within sample differential expression)

### Usage

```
de_table.demo_ref
```

#### **Format**

An object of class data. frame with 800 rows and 13 columns.

#### Value

An example de\_table from contrast\_each\_group\_to\_the\_rest (for demo ref dataset)

### Description

Internal function to find if there are significant difference between the distribitions, when there are multiple match groups.

### Usage

```
find_within_match_differences(de_table.ref.marked, matches, the_test_group,
    the_test_dataset, the_ref_dataset, the_pval)
```

#### **Arguments**

```
de_table.ref.marked
see make_ref_similarity_names_for_group
matches
see make_ref_similarity_names_for_group
the_test_group see make_ref_similarity_names_for_group
the_test_dataset
see make_ref_similarity_names_for_group
the_ref_dataset
see make_ref_similarity_names_for_group
the_pval
see make_ref_similarity_names_for_group
```

get\_inner\_or\_outer\_ci

#### **Details**

For use by make\_ref\_similarity\_names\_for\_group

#### Value

String of within match differences

#### See Also

```
make_ref_similarity_names_for_group
```

get\_counts\_index ge

get\_counts\_index

### **Description**

get\_counts\_index is an internal utility function to find out where the counts are (if anywhere.). Stops if there's no assay called 'counts', (unless there is only a single unnamed assay).

### Usage

```
get_counts_index(n_assays, assay_names)
```

#### **Arguments**

n\_assays How many assays are there? ie: length(assays(dataset\_se))
assay\_names What are the assays called? ie: names(assays(dataset\_se))

### Value

The index of an assay in assays called 'counts', or, if there's just the one unnamed assay - happily assume that that is counts.

```
get_inner_or_outer_ci
```

### **Description**

Given a fold-change, and high and low confidence interval (where lower < higher), pick the innermost/most conservative one.

```
get_inner_or_outer_ci(fc, ci.hi, ci.lo, get_inner = TRUE)
```

#### **Arguments**

| fc        | Fold-change   |
|-----------|---|
| ci.hi     | Higher fold-change CI (numerically)                                       |
| ci.lo     | smaller fold-change CI (numerically)                                      |
| get_inner | If TRUE, get the more conservative inner CI, else the bigger outside one. |

#### Value

inner or outer CI from ci.hi or ci.low

### Description

Internal function that wraps limma topTable output but also adds upper and lower confidence intervals to the logFC. Calculated according to <a href="https://support.bioconductor.org/p/36108/">https://support.bioconductor.org/p/36108/</a>

### Usage

```
get_limma_top_table_with_ci(fit2, the_coef, ci = 0.95)
```

### Arguments

| fit2     | The fit2 object after calling eBayes as per standard limma workflow. Ie object that topTable gets called on. |
|----------|--|
| the_coef | Coeffient. As passed to topTable.  |
| ci       | Confidence interval. Number between 0 and 1, default 0.95 (95%)  |

### Value

Output of topTable, but with the (95 for the logFC.

### See Also

```
contrast\_the\_group\_to\_the\_rest\_with\_limma\_for\_microarray\ Calling\ function.
```

```
{\tt get\_matched\_stepped\_mwtest\_res\_table} \\ {\tt get\_matched\_stepped\_mwtest\_res\_table}
```

### **Description**

Internal function to grab a table of the matched group(s).

### Usage

```
get_matched_stepped_mwtest_res_table(mwtest_res_table.this, the_pval)
```

### **Arguments**

```
\begin{tabular}{ll} mwtest\_res\_table.this & Combined output of get\_ranking\_and\_test\_results \\ the\_pval & Pvalue threshold \\ \end{tabular}
```

#### **Details**

For use by make\_ref\_similarity\_names\_for\_group

### Value

Stepped pvalues string

#### See Also

```
make_ref_similarity_names_for_group
```

### **Description**

Internal function to get reference group similarity contrasts for an individual query group.

```
get_ranking_and_test_results(de_table.ref.marked, the_test_group,
   the_test_dataset, the_ref_dataset, num_steps, pval = 0.01)
```

get\_rankstat\_table 17

#### **Arguments**

#### **Details**

For use by **make\_ref\_similarity\_names\_using\_marked**, see that function for parameter details. This function just runs this for a single query group **the\_test\_group** 

#### Value

Table of similarity contrast results/assigned names e.t.c for a single group. Used internally for populating mwtest\_res\_table tables.

#### See Also

make\_ref\_similarity\_names\_using\_marked which calls this.

```
get_rankstat_table get_rankstat_table
```

### **Description**

Summarise the comparison of the specified query group against in the comparison in **de\_table.ref.marked** - number of 'top' genes and their median rank in each of the reference groups, with reference group rankings.

### Usage

```
get_rankstat_table(de_table.ref.marked, the_test_group)
```

#### **Arguments**

#### Value

A tibble of query group name (test\_group), number of 'top' genes (n), reference dataset group (group) with its ranking (grouprank) and the median (rescaled 0..1) ranking of 'top' genes (median\_rank).

#### See Also

```
get_the_up_genes_for_all_possible_groups To prepare the de_table.ref.marked input.
```

#### **Examples**

```
# Make input
# de_table.demo_query <- contrast_each_group_to_the_rest(demo_query_se, "demo_query")
# de_table.demo_ref <- contrast_each_group_to_the_rest(demo_ref_se, "demo_ref")

de_table.marked.query_vs_ref <- get_the_up_genes_for_all_possible_groups(
    de_table.demo_query,
    de_table.demo_ref)

get_rankstat_table(de_table.marked.query_vs_ref, "Group3")</pre>
```

#### **Description**

Internal function to run a bionomial test of median test rank > 0.5 (random).

#### Usage

```
get_reciprocal_matches(mwtest_res_table.recip, de_table.recip.marked,
    the_pval)
```

### **Arguments**

```
mwtest_res_table.recip

Combined output of get_ranking_and_test_results for reciprocal test - ref
vs query.

de_table.recip.marked

Recriprocal ref vs query de_table.ref.marked

the_pval See make_ref_similarity_names_using_marked
```

#### **Details**

For use by make\_ref\_similarity\_names\_using\_marked

get\_stepped\_pvals\_str 19

### Value

List of table of reciprocal matches tested from reference to query.

### See Also

```
make_ref_similarity_names_using_marked
```

```
get_stepped_pvals_str get_stepped_pvals_str
```

### Description

Internal function to construct the string of stepped pvalues reported by make\_ref\_similarity\_names\_using\_marked

### Usage

```
get_stepped_pvals_str(mwtest_res_table.this)
```

### **Arguments**

### Details

For use by make\_ref\_similarity\_names\_for\_group

### Value

Stepped pvalues string

### See Also

```
make_ref_similarity_names_for_group
```

```
{\it get\_the\_up\_genes\_for\_all\_possible\_groups} \\ {\it get\_the\_up\_genes\_for\_all\_possible\_groups}
```

#### **Description**

For the most overrepresented genes of each group in the test dataset, get their rankings in all the groups of the reference dataset.

#### Usage

```
get_the_up_genes_for_all_possible_groups(de_table.test, de_table.ref,
  rankmetric = "TOP100_LOWER_CI_GTE1", n = 100)
```

#### **Arguments**

de\_table.test A differential expression table of the query experiment, as generated from contrast\_each\_group\_to\_the
de\_table.ref A differential expression table of the reference dataset, as generated from contrast\_each\_group\_to\_the
rankmetric Specifiy ranking method used to pick the 'top' genes. The default 'TOP100\_LOWER\_CI\_GTE1'
picks genes from the top 100 overrepresented genes (ranked by inner 95 work
best for distinct cell types (e.g. tissue sample.). 'TOP100\_SIG' again picks from
the top 100 ranked genes, but requires only statistical significance, 95 clusters
(e.g. PBMCs).

For tweaking maximum returned genes from different ranking methods. Will
change the p-values! Suggest leaving as default unless you're keen.

#### **Details**

This is effectively a subset of the reference data, 'marked' with the 'top' genes that represent the groups in the query data. The distribution of the *rescaled ranks* of these marked genes in each reference data group indicate how similar they are to the query group.

This function is simply a conveinent wrapper for get\_the\_up\_genes\_for\_group that merges output for each group in the query into one table.

#### Value

de\_table.marked This will alsmost be a subset of de\_table.ref, with an added column test\_group set to the query groups, and test\_dataset set to test\_dataset\_name.

If nothing passes the rankmetric criteria, a warning is thrown and NA is returned. (This can be a genuine inability to pick out the representative 'up' genes, or due to some problem in the analysis)

#### See Also

```
get_the_up_genes_for_group Function for testing a single group.
```

#### **Examples**

```
de_table.marked.query_vs_ref <- get_the_up_genes_for_all_possible_groups(
   de_table.test=de_table.demo_query ,
   de_table.ref=de_table.demo_ref )</pre>
```

```
get_the_up_genes_for_group

get_the_up_genes_for_group
```

#### **Description**

For the most overrepresented genes of the specified group in the test dataset, get their rankings in all the groups of the reference dataset.

### Usage

```
get_the_up_genes_for_group(the_group, de_table.test, de_table.ref,
  rankmetric = "TOP100_LOWER_CI_GTE1", n = 100)
```

### **Arguments**

| the_group     | The group (from the test/query experiment) to examine.   |
|---------------|--|
| de_table.test | A differential expression table of the query experiment, as generated from contrast_each_group_to_the  |
| de_table.ref  | A differential expression table of the reference dataset, as generated from contrast_each_group_to_the   |
| rankmetric    | Specifiy ranking method used to pick the 'top' genes. The default 'TOP100_LOWER_CI_GTE1' picks genes from the top 100 overrepresented genes (ranked by inner 95 work best for distinct cell types (e.g. tissue sample.). 'TOP100_SIG' again picks from the top 100 ranked genes, but requires only statistical significance, 95 clusters (e.g. PBMCs). |
| n             | For tweaking maximum returned genes from different ranking methods. Will change the p-values! Suggest leaving as default unless you're keen.   |

### **Details**

This is effectively a subset of the reference data, 'marked' with the 'top' genes that represent the group of interest in the query data. The distribution of the *rescaled ranks* of these marked genes in each reference data group indicate how similar they are to the query group.

#### Value

de\_table.marked This will be a subset of de\_table.ref, with an added column test\_group set to the\_group. If nothing passes the rankmetric criteria, NA.

22 get\_vs\_random\_pval

#### See Also

contrast\_each\_group\_to\_the\_rest For prepraring the de\_table.\* tables. get\_the\_up\_genes\_for\_all\_possible\_group For running all query groups at once.

### **Examples**

### **Description**

Internal function to run a bionomial test of median test rank > 0.5 (random).

#### Usage

```
get_vs_random_pval(de_table.ref.marked, the_group, the_test_group)
```

### **Arguments**

### **Details**

For use by make\_ref\_similarity\_names\_for\_group

#### Value

Pvalue result of a binomial test of each 'top gene' being greater than the theoretical random median rank of 0.5 (halfway).

#### See Also

```
make_ref_similarity_names_for_group
```

load\_dataset\_10Xdata

### **Description**

Convenience function to create a SummarizedExperiment object (dataset\_se) from a the output of 10X cell ranger pipeline run.

### Usage

```
load_dataset_10Xdata(dataset_path, dataset_genome, clustering_set,
  gene_id_cols_10X = c("ensembl_ID", "GeneSymbol"),
  id_to_use = gene_id_cols_10X[1])
```

#### **Arguments**

dataset\_path Path to the directory of 10X data, as generated by the cellRanger pipeline (ver-

sions 2.1.0 and 2.0.1). The directory should have subdirectires *analysis*, *filtered\_gene\_bc\_matrices* and *raw\_gene\_bc\_matrices* (only the first 2 are read).

dataset\_genome The genome that the reads were aligned against, e.g. GRCh38. Check for this

as a directory name under the *filtered\_gene\_bc\_matrices* subdirectory if unsure.

clustering\_set The 10X cellRanger pipeline produces several different cluster definitions per

dataset. Specify which one to use e.g. kmeans\_10\_clusters Find them as direc-

tory names under analysis/clustering/

gene\_id\_cols\_10X

Vector of the names of the columns in the gene description file (filtered\_gene\_bc\_matrices/GRCh38/genes

The first element of this will become the ID. Default = c("ensembl ID", "GeneSymbol")

id\_to\_use Column from **gene\_id\_cols\_10X** that defines the gene identifier to use as 'ID'

in the returned SummarisedExperiment object. Many-to-one relationships betwen the assumed unique first element of **gene\_id\_cols\_10X** and **id\_to\_use** will be handled gracefully by convert\_se\_gene\_ids. Defaults to first element of

gene id cols 10X

#### Details

This function makes a SummarizedExperiment object in a form that should work for celaref functions. Specifically, that means it will have an 'ID' feild for genes (view with rowData(dataset\_se)), and both 'cell\_sample' and 'group' feild for cells (view with colData(dataset\_se)). See parameters for detail. Additionally, the counts will be an integer matrix (not a sparse matrix), and the group feild (but not cell\_sample or ID) will be a factor.

The clustering information can be read from whichever cluster is specified, usually there will be several choices.

This function is designed to work with output of version 2.0.1 of the cellRanger pipeline, may not work with others (will not work for 1.x).

24 load\_se\_from\_tables

#### Value

A SummarisedExperiment object containing the count data, cell info and gene info.

### See Also

SummarizedExperiment For general doco on the SummarizedExperiment objects.

```
convert_se_gene_ids describes method for converting IDs.
```

```
Other Data loading functions: contrast_each_group_to_the_rest_for_norm_ma_with_limma, load_se_from_tables
```

### **Examples**

```
load_se_from_tables
```

### **Description**

Create a SummarizedExperiment object (dataset\_se) from a count matrix, cell information and optionally gene information.

load\_se\_from\_files is a wrapper for load\_se\_from\_tables that will read in tables from specified files.

```
load_se_from_tables(counts_matrix, cell_info_table, gene_info_table = NA,
   group_col_name = "group", cell_col_name = NA)

load_se_from_files(counts_file, cell_info_file, gene_info_file = NA,
   group_col_name = "group", cell_col_name = NA)
```

load\_se\_from\_tables 25

#### Arguments

counts\_matrix A tab-separated matrix of read counts for each gene (row) and each cell (column). Columns and rows should be named.

cell\_info\_table

Table of cell information. If there is a column labelled *cell\_sample*, that will be used as the unique cell identifiers. If not, the first column is assumed to be cell identifiers, and will be copied to a new feild labelled *cell\_sample*. Similarly - the clusters of these cells should be listed in one column - which can be called 'group' (case-sensitive) or specified with **group\_col\_name**. *Minimal data format: <cell\_sample> <group>* 

gene\_info\_table

Optional table of gene information. If there is a column labelled *ID*, that will be used as the gene identifiers (they must be unique!). If not, the first column is assumed to be a gene identifier, and will be copied to a new feild labelled *ID*. Must match all rownames in **counts\_matrix**. If omitted, ID wll be generated from the rownames of counts\_matrix. Default=NA

group\_col\_name Name of the column in **cell\_info\_table** containing the cluster/group that each cell belongs to. Case-sensitive. Default='group'

cell\_col\_name Name of the column in **cell\_info\_table** containing a cell id. Ignored if *cell\_sample* column is already present. If omitted, (and no *cell\_sample* column) will use first column. Case-sensitive. Default=NA

counts\_file A tab-separated file of a matrix of read counts. As per **counts\_matrix**. First column should be gene ID, and top row cell ids.

cell\_info\_file Tab-separated text file of cell information, as per **cell\_info\_table**. Columns must have names.

gene\_info\_file Optional tab-separated text file of gene information, as per **gene\_info\_file**. Columns must have names. Default=NA

#### **Details**

This function makes a SummarizedExperiment object in a form that should work for celaref functions. Specifically, that means it will have an 'ID' feild for genes (view with rowData(dataset\_se)), and both 'cell\_sample' and 'group' feild for cells (view with colData(dataset\_se)). See parameters for detail. Additionally, the counts will be an integer matrix (not a sparse matrix), and the *group* feild (but not *cell\_sample* or *ID*) will be a factor.

Note that data will be subsetted to cells present in both the counts matrix and cell info, this is handy for loading subsets of cells. However, if **gene\_info\_file** is defined, all genes must match exactly.

The load\_se\_from\_files form of this function will run the same checks, but will read everything from files in one go. The load\_se\_from\_tables form is perhaps more useful when the annotations need to be modified (e.g. programmatically adding a different gene identifier, renaming groups, removing unwanted samples).

Note that the SummarizedExperiment object can also be created without using these functions, it just needs the *cell\_sample*, *ID* and *group* feilds as described above. Since sometimes it might be easier to add these to an existing *SummarizedExperiment* from upstream analyses.

#### Value

A SummarisedExperiment object containing the count data, cell info and gene info.

#### **Functions**

• load\_se\_from\_files: To read from files

#### See Also

SummarizedExperiment For general doco on the SummarizedExperiment objects.

```
Other Data loading functions: contrast_each_group_to_the_rest_for_norm_ma_with_limma, load_dataset_10Xdata
```

#### **Examples**

### Description

Plot a panel of violin plots showing the distribution of the 'top' genes of each of query group, across the reference dataset.

```
make_ranking_violin_plot(de_table.marked = NA, de_table.test = NA,
  de_table.ref = NA, log10trans = FALSE, ...)
```

#### **Arguments**

```
The output of get_the_up_genes_for_all_possible_groups for the contrast of interest.

de_table.test A differential expression table of the query experiment, as generated from contrast_each_group_to_the de_table.ref A differential expression table of the reference dataset, as generated from contrast_each_group_to_the log10trans Plot on a log scale? Useful for distinishing multiple similar, yet distinct cell type that bunch at top of plot. Default=FALSE.

Further options to be passed to get_the_up_genes_for_all_possible_groups, e.g. rankmetric
```

#### **Details**

In the plot output, each panel correponsds to a different group/cluster in the query experiment. The x-axis has the groups in the reference dataset. The y-axis is the rescaled rank of each 'top' gene from the query group, within each reference group.

Only the 'top' genes for each query group are plotted, forming the violin plots - each individual gene is shown as a tickmark. Some groups have few top genes, and so their uncertanty can be seen on this plot.

The thick black lines reprenset the median gene rescaled ranking for each query group / reference group combination. Having this fall above the dotted median threshold marker is a quick indication of potential similarity. A complete lack of similarity would have a median rank around 0.5. Median rankings much less than 0.5 are common though (an 'anti-cell-groupA' signature), because genes overrepresented in one group in an experiment, are likely to be relatively 'underrepresented' in the other groups. Taken to an extreme, if there are only two reference groups, they'll be complete opposites.

Input can be either the precomputed de\_table.marked object for the comparison, OR both de\_table.test and de\_table.ref differential expression results to compare from contrast\_each\_group\_to\_the\_rest

#### Value

A ggplot object.

#### See Also

```
get_the_up_genes_for_all_possible_groups To make the input data.
```

#### **Examples**

#### **Description**

Construct some sensible labels or the groups/clusters in the query dataset, based on similarity the reference dataset.

This is a more low level/customisable version of make\_ref\_similarity\_names, (would usually use that instead). Suitable for rare cases to reuse an existing de\_table.ref.marked object. Or use a de\_table.ref.marked table with more than one dataset present (discoraged). Or to skip the reciprocal comparison step.

#### Usage

```
make_ref_similarity_names(de_table.test, de_table.ref, pval = 0.01,
   num_steps = 5, rankmetric = "TOP100_LOWER_CI_GTE1", n = 100)

make_ref_similarity_names_using_marked(de_table.ref.marked,
   de_table.recip.marked = NA, the_test_dataset = NA,
   the_ref_dataset = NA, pval = 0.01, num_steps = 5)
```

#### **Arguments**

pval

de\_table.test
de\_table.ref

A differential expression table of the query experiment, as generated from contrast\_each\_group\_to\_the A differential expression table of the reference dataset, as generated from contrast\_each\_group\_to\_the

Differences between the rescaled ranking distribution of 'top' genes on different reference groups are tested with a Mann-Whitney U test. If they are *significantly different*, only the top group(s) are reported. It isn't a simple cutoff threshold as it can change the number of similar groups reported. ie. A more stringent **pval** is more likely to decide that groups are similar - which would result in multiple group reporting, or no similarity at all. Unlikely that this parameter will ever

need to change. Default = 0.01.

num\_steps

After ranking reference groups according to median 'top' gene ranking, how many adjacent pairs to test for differences. Set to 1 to only compare each group to the next, or NA to perform an all-vs-all comparison. Setting too low may means it is possible to miss groups with some similarity to the reported matches (*similar\_non\_match* column)). Too high (or NA) with a large number of reference groups could be slow. Default = 5.

rankmetric

Specifiy ranking method used to pick the 'top' genes. The default 'TOP100\_LOWER\_CI\_GTE1' picks genes from the top 100 overrepresented genes (ranked by inner 95 work best for distinct cell types (e.g. tissue sample.). 'TOP100\_SIG' again picks from the top 100 ranked genes, but requires only statistical significance, 95 clusters (e.g. PBMCs).

For tweaking maximum returned genes from different ranking methods.

de\_table.ref.marked

The output of get\_the\_up\_genes\_for\_all\_possible\_groups for the contrast of interest.

de\_table.recip.marked

Optional. The (reciprocal) output of get\_the\_up\_genes\_for\_all\_possible\_groups with the test and reference datasets swapped. If omitted a reciprocal test will not be done. Default = NA.

the\_test\_dataset

Optional. A short meaningful name for the experiment. (Should match *test\_dataset* column in **de\_table.marked**). Only needed in a table of more than one dataset. Default = NA.

the\_ref\_dataset

Optional. A short meaningful name for the experiment. (Should match *dataset* column in **de\_table.marked**). Only needed in a table of more than one dataset. Default = NA.

#### **Details**

This function aims to report a) the top most similar reference group, if there's a clear frontrunner, b) A list of multiple similar groups if they have similar similarity, or c) 'No similarity', if there is none.

Each group is named according to the following rules. Testing for significant (smaller) differences with a one-directional Mann-Whitney U test on their rescaled ranks:

- 1. The first (as ranked by median rescaled rank) reference group is significantly more similar than the next: Report *first only*.
- 2. When comparing differences betwen groups stepwise ranked by median rescaled rank no group is significantly different to its neighbour: Report *no similarity*
- 3. There's no significant differences in the stepwise comparisons of the first N reference groups but there is a significant difference later on: Report *multiple group similarity*

There are some further heuristic caveats:

- 1. The distribution of top genes in the last (or only) match group is tested versus a theroetical random distribution around 0.5 (as reported in *pval\_vs\_random* column). If the distribution is not significantly above random (It is possible in edge cases where there is a skewed dataset and no/few matches), *no similarity* is reported. The significant *pval* column is left intact.
- 2. The comparison is repeated reciprocally reference groups vs the query groups. This helps sensitivity of heterogenous query groups and investigating the reciprocal matches can be informative in these cases. If a query group doens't 'match' a reference group, but the reference group does match that query group it is reported in the group label in brackets. e.g. c1:th\_lymphocytes(tc\_lympocytes). Its even possible if there was no match (and pval = NA) e.g. emphc2:(tc\_lymphocytes)

The similarity is formatted into a group label. Where there are multiple similar groups, they're listed from most to least similar by their median ranks.

For instance, a query dataset of clusters c1, c2, c3 and c4 againsts a cell-type labelled reference dataset might get names like: E.g.

- · c1:macrophage
- c2:endotheiallmesodermal
- c3:no\_similarity
- c4:mesodermal(endothelial)

Function make\_ref\_similarity\_names is a convenience wrapper function for make\_ref\_similarity\_names\_from\_marker. It accepts two 'de\_table' outputs of function contrast\_each\_group\_to\_the\_rest to compare and handles running get\_the\_up\_genes\_for\_all\_possible\_groups. Sister function make\_ref\_similarity\_names\_from\_mamay (rarely) be of use if the de\_table.marked object has already been created, or if reciprocal tests are not wanted.

#### Value

A table of automagically-generated labels for each query group, given their similarity to reference groups.

The columns in this table:

- test\_group : Query group e.g. "c1"
- shortlab: The cluster label described above e.g. "c1:macrophage"
- **pval**: If there is a similarity flagged, this is the P-value from a Mann-Whitney U test from the last 'matched' group to the adjacent 'non-matched' group. Ie. If only one label in shortlab, this will be the first of the stepped\_pvals, if there are 2, it will be the second. If there is 'no\_similarity' this will be NA (Because there is no confidence in what is the most appropriate of the all non-significant stepped pvalues.).
- **stepped\_pvals**: P-values from Mann-Whitney U tests across adjacent pairs of reference groups ordered from most to least similar (ascending median rank). ie. 1st-2nd most similar first, 2nd-3rd, 3rd-4th e.t.c. The last value will always be NA (no more reference group). e.g. refA:8.44e-10,refB:2.37e-06,refC:0.000818,refD:0.435,refE:0.245,refF:NA
- **pval\_to\_random**: P-value of test of median rank (of last matched reference group) < random, from binomial test on top gene ranks (being < 0.5).
- matches: List of all reference groups that 'match', as described, except it also includes (rare) examples where pval\_to\_random is not significant. "I" delimited.
- reciprocal\_matches: List of all reference groups that flagged test group as a match when directon of comparison is reversed. (significant pval and pval\_to\_random). "I" delimited.
- **similar\_non\_match**: This column lists any reference groups outside of shortlab that are not significantly different to a reported match group. Limited by *num\_steps*, and will never find anything if num\_steps==1. "I" delimited. Usually NA.
- similar\_non\_match\_detail: P-values for any details about similar\_non\_match results. These p-values will always be non-significant. E.g. "A > C (p=0.0214,n.s)". "I" delimited. Usually NA.
- **differences\_within**: This feild lists any pairs of reference groups in shortlab that are significantly different. "I" delimited. Usually NA.

#### **Functions**

• make\_ref\_similarity\_names\_using\_marked: Construct some sensible cluster labels, but using a premade marked table.

#### See Also

```
contrast_each_group_to_the_rest For preparing de_table input
get_the_up_genes_for_all_possible_groups To prepare the de_table.ref.marked input.
```

#### **Examples**

```
# Make input
# de_table.demo_query <- contrast_each_group_to_the_rest(demo_query_se, "demo_query")</pre>
# de_table.demo_ref <- contrast_each_group_to_the_rest(demo_ref_se,</pre>
make_ref_similarity_names(de_table.demo_query, de_table.demo_ref)
make_ref_similarity_names(de_table.demo_query, de_table.demo_ref, num_steps=3)
make_ref_similarity_names(de_table.demo_query, de_table.demo_ref, num_steps=NA)
# Make input
# de_table.demo_query <- contrast_each_group_to_the_rest(demo_query_se, "demo_query")
# de_table.demo_ref <- contrast_each_group_to_the_rest(demo_ref_se,</pre>
                                                                         "demo_ref")
de_table.marked.query_vs_ref <- get_the_up_genes_for_all_possible_groups(</pre>
     de_table.demo_query, de_table.demo_ref)
de_table.marked.reiprocal <- get_the_up_genes_for_all_possible_groups(</pre>
     de_table.demo_ref, de_table.demo_query)
make_ref_similarity_names_using_marked(de_table.marked.query_vs_ref,
                                        de_table.marked.reiprocal)
make_ref_similarity_names_using_marked(de_table.marked.query_vs_ref)
```

### Description

Internal function, called by make\_ref\_similarity\_names\_using\_marked for each group.

```
make_ref_similarity_names_for_group(the_test_group, mwtest_res_table,
  de_table.ref.marked, reciprocal_matches = NA, the_test_dataset,
  the_ref_dataset, the_pval)
```

32 run\_pair\_test\_stats

#### **Arguments**

```
the_test_group Query group to make name for
mwtest_res_table
                 Mann-whitney test results as constructed in make_ref_similarity_names_using_marked
de_table.ref.marked
                 The output of get_the_up_genes_for_all_possible_groups for the contrast
                 of interest.
reciprocal_matches
                 Simplified table of reciprocal matches prepared within make_ref_similarity_names_using_marked.
                 If omitted no reciprocal matching done. Default = NA.
the_test_dataset
                 A short meaningful name for the experiment. (Should match test_dataset col-
                 umn in de table.marked)
the_ref_dataset
                 A short meaningful name for the experiment. (Should match dataset column in
                 de_table.marked)
the_pval
                 pval as per make_ref_similarity_names_using_marked
```

#### Value

A tibble with just one group's labelling information, as per make\_ref\_similarity\_names\_using\_marked

#### See Also

make\_ref\_similarity\_names\_using\_marked Only place that uses this function, details there.

### **Description**

Internal function to compare the distribution of a query datasets 'top' genes between two different reference datasete groups with a Mann–Whitney U test. One directional test if groupA median < group B.

```
run_pair_test_stats(de_table.ref.marked, the_test_group, groupA, groupB,
  enforceAgtB = TRUE)
```

subset\_cells\_by\_group 33

### Arguments

de\_table.ref.marked

The output of get\_the\_up\_genes\_for\_all\_possible\_groups for the contrast

of interest.

the\_test\_group Name of the test group in query dataset.

groupA One of the reference group names

groupB Another of the reference group names

enforceAgtB Do a one tailed test of A 'less' B (more similar)? Or two-tailed. Default =

TRUE.

#### **Details**

For use by make\_ref\_similarity\_names\_using\_marked

#### Value

A tibble of wilcox / man-whitneyU test results for this contrast.

#### See Also

```
make_ref_similarity_names_using_marked
```

```
subset_cells_by_group
```

### **Description**

Utility function to randomly subset very large datasets (that use too much memory). Specify a maximum number of cells to keep per group and use the subsetted version to analysis.

#### Usage

```
subset_cells_by_group(dataset_se, n.group = 1000)
```

### **Arguments**

dataset\_se Summarised experiment object containing count data. Also requires 'ID' and

'group' to be set within the cell information.

n.group How many cells to keep for each group. Default = 1000

#### **Details**

The resulting differential expression table  $de\_table$  will have reduced statistical power. But as long as enough cells are left to reasonably accurately calculate differential expression between groups this should be enough for celaref to work with.

Also, this function will lose proportionality of groups (there'll be *n.groups* or less of each). Consider using the n.group/n.other parameters in *contrast\_each\_group\_to\_the\_rest* or *contrast\_the\_group\_to\_the\_rest* - which subsets non-group cells independently for each group. That may be more approriate for tissue type samples which would have similar compositions of cells.

So this function is intended for use when either; the proportionality isn't relevant (e.g. FACs purified cell populations), or, the data is just too big to work with otherwise.

Cells are randomly sampled, so set the random seed (with set.seed()) for consistant results across runs.

#### Value

dataset\_se A hopefully more managably subsetted version of the inputted dataset\_se.

#### See Also

contrast\_each\_group\_to\_the\_rest For alternative method of subsetting cells proportionally.

#### **Examples**

```
dataset_se.30pergroup <- subset_cells_by_group(demo_query_se, n.group=30)</pre>
```

```
subset_se_cells_for_group_test
subset_se_cells_for_group_test
```

### **Description**

This function for use by contrast\_each\_group\_to\_the\_rest downsamples cells from a summarizedExperiment (*dataset\_se*) - keeping **n.group** (or all if fewer) cells from the specified group, and **n.other** from the rest. This maintains the proportions of cells in the 'other' part of the differential expression comparisons.

```
subset_se_cells_for_group_test(dataset_se, the_group, n.group = Inf,
    n.other = n.group * 5)
```

#### **Arguments**

| dataset_se | Summarised experiment object containing count data. Also requires 'ID' and 'group' to be set within the cell information. |
|------------|---|
| the_group  | The group being subsetted for   |
| n.group    | How many cells to keep for each group. Default = Inf  |
| n.other    | How many cells to keep from everything not in the group. Default = $\mathbf{n.group} * 5$                                 |

#### **Details**

Cells are randomly sampled, so set the random seed (with set.seed()) for consistant results across runs

#### Value

dataset\_se A hopefully more managably subsetted version of the inputted dataset\_se

#### See Also

```
Calling function contrast_each_group_to_the_rest subset_cells_by_group Exported function for subsetting each group independently upfront. (For when this approach is still unmanageable)
```

#### **Description**

Filter and return a SummarizedExperiment object (dataset\_se) by several metrics:

- Cells with at least min lib size total reads.
- Genes expressed in at least min\_detected\_by\_min\_samples cells, at a threshold of min\_reads\_in\_sample per cell.
- Remove entire groups (clusters) of cells where there are fewer than **min\_group\_membership** cells in that group.

```
trim_small_groups_and_low_expression_genes(dataset_se,
   min_lib_size = 1000, min_group_membership = 5,
   min_reads_in_sample = 1, min_detected_by_min_samples = 5)
```

#### **Arguments**

dataset\_se Summarised experiment object containing count data. Also requires 'ID' and 'group' to be set within the cell information (see colData())

min\_lib\_size Minimum library size. Cells with fewer than this many reads removed. Default = 1000

min\_group\_membership

Throw out groups/clusters with fewer than this many cells. May change with experiment size. Default = 5

min\_reads\_in\_sample

Require this many reads to consider a gene detected in a sample. Default = 1

min\_detected\_by\_min\_samples

Keep genes detected in this many samples. May change with experiment size. Default = 5

### **Details**

If it hasn't been done already, it is highly reccomended to use this function to filter out genes with no/low total counts (especially in single cell data, there can be many) - without expression they are not useful and may reduce statistical power.

Likewise, very small groups (<5 cells) are unlikely to give useful results with this method. And cells with abnormally small library sizes may not be desireable.

Of course 'reasonable' thresholds for filtering cells/genes are subjective. Defaults are moderately sensible starting points.

### Value

A filtered dataset\_se, ready for use.

### **Examples**

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