Package ‘scFeatureFilter’

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Type Package
Title A correlation-based method for quality filtering of single-cell RNAseq data
Version 1.1.0
Description An R implementation of the correlation-based method developed in the Joshi laboratory to analyse and filter processed single-cell RNAseq data. It returns a filtered version of the data containing only genes expression values unaffected by systematic noise.
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Author Angeles Arzalluz-Luque [aut], Guillaume Devailly [aut, cre], Anagha Joshi [aut]
Maintainer Guillaume Devailly <gdevailly@hotmail.com>

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Bin genes by mean expression.

**Description**

Divides the genes that were not included in the top window in windows of the same size with decreasing mean expression levels.

**Usage**

```r
bin_scdata(dataset, window_number = NULL, window_size = NULL,
            verbose = TRUE)
```

**Arguments**

- `dataset`: A list, containing the top window generated by `extract_top_genes` as the first element, and the rest of undivided genes as the second. Usually the output of `define_top_genes`.
- `window_number`: An integer, indicating the number of bins to be used.
- `window_size`: An integer, indicating the number of genes to be included in each window. Ignored if `window_size` is defined.
- `verbose`: A boolean. Should the function print a message about window size or the number of windows created?

**Details**

Two binning methods are available:

- `window_number`: Divides the genes into the number of windows specified.
- `window_size`: Divides the genes into windows of the size specified.

This function adds a bin number column to the data frame.

This function is designed to take the list output by the `extract_top_window` function as an argument, operating only on the second element of it. Once the genes in it have been binned, both elements of the list are bound together in a data frame and returned. The output contains a new column `bin`, which indicates the window number assigned to each gene.
**calculate_cvs**

Value

A data frame containing the binned genes.

Examples

```r
library(magrittr)
expMat <- matrix(
  c(1, 1, 1,
    1, 2, 3,
    0, 1, 2,
    0, 0, 2),
  ncol = 3, byrow = TRUE, dimnames = list(paste("gene", 1:4), paste("cell", 1:3)))

calculate_cvs(expMat) %>%
  define_top_genes(window_size = 1) %>%
  bin_scdata(window_number = 2)
```

```r
calculate_cvs(expMat) %>%
  define_top_genes(window_size = 1) %>%
  bin_scdata(window_size = 1)
```

**calculate_cvs**

*Compute mean expression level, standard deviation and coefficient of variation of each feature.*

Description

Compute mean expression level, standard deviation and coefficient of variation (CV) of each feature (i.e. gene or transcript) in the supplied data. Filter features with high proportion of 0 expression.

Usage

```r
calculate_cvs(data, max_zeros = 0.75, sce_assay = NULL)
```

Arguments

- **data**: A data frame, a matrix or a SingleCellExperiment object. If data frame or matrix, it should contain expression values for each gene as rows, and expression values for the cells as columns.
- **max_zeros**: A number between 0 and 1 indicating the maximum proportion of zero expression values allowed per row. Features with a higher proportion of 0 will be discarded.
- **sce_assay**: If data is a SingleCellExperiment object, sce_assay should be one of names(assays(<SingleCellExperiment>)).

Details

Before CV computation, the function removes all rows that have a proportion of zeros above the specified threshold. Genes with many 0s are poorly informative, and would bias the later correlations. Removing them also prevents division by zero when calculating CVs.
The data provided must cell/sample names as column names. Feature name can be given either in the first column or as row names.

In the output, mean, standard deviation and CV are incorporated as new columns in the data frame, named `mean`, `sd` and `c_v`.

**Value**

A data frame, containing the filtered data with additional columns: mean, standard deviation and CV values for each row.

**Examples**

```r
expMat <- matrix(c(1, 1, 1,
                   1, 2, 3,
                   0, 1, 2,
                   0, 0, 2),
                ncol = 3, byrow = TRUE, dimnames = list(paste("gene", 1:4), paste("cell", 1:3)))
calculate_cvs(expMat)
calculate_cvs(expMat, max_zeros = 0.5)
```

**correlate_windows**

*Calculate correlations against top window.*

**Description**

Calculates pairwise correlations between all features each window against all features in the reference window.

**Usage**

```r
correlate_windows(dataset, n_random = 3, ...)
```

**Arguments**

- `dataset` A data frame containing all the binned genes. Usually the output of `bin_scdata`.
- `n_random` Number of top window randomization to serve as a negative control. Default to 3.
- `...` Additional arguments to be passed to `cor`. Default method is `pearson` which is the fastest.

**Details**

This function:

- correlates each feature in each window to each feature in the top window.
- randomize the top window by shuffling expression value, and correlate each gene in each window to the randomized top window. This negative control is repeated as many time as specified by the `n_random` parameter.

The input of this function is usually the output of the `bin_scdata` function.
correlations_to_densities

Value

A tibble containing correlation values.

Examples

library(magrittr)
exMat <- matrix(
c(1, 1, 5,
 1, 2, 3,
 0, 1, 4,
 0, 0, 2),
ncol = 3, byrow = TRUE, dimnames = list(paste("gene", 1:4), paste("cell", 1:3))
)
calculate_cvs(expMat) %>%
define_top_genes(window_size = 2) %>%
bin_scdata(window_number = 1) %>%
correlate_windows

correlations_to_densities

Transform the correlation table to density distributions of correlation values

Description

Takes the output of correlate_windows and computes density curves of correlation coefficient for each window comparison.

Usage

correlations_to_densities(df, n = 64, absolute_cc = TRUE)

Arguments

df A data frame, usually the output of correlate_windows.
n Resolution of the correlation density curve. Default to 64.
absolute_cc Should the function use the absolute value of correlation coefficients? Default to TRUE to simplify plots and avoid annoying, non-symmetrical, near 0, shifts of distributions.

Value

A tibble with columns bin, window, cor_coef and density.
Examples

```r
library(magrittr)
expMat <- matrix(
  c(1, 1, 5,
    1, 2, 3,
    0, 1, 4,
    0, 0, 2),
  ncol = 3, byrow = TRUE, dimnames = list(paste("gene", 1:4), paste("cell", 1:3))
)

calculate_cvs(expMat) %>%
define_top_genes(window_size = 2) %>%
bin_scdata(window_number = 1) %>%
correlate_windows %>%
correlations_to_densities
```

**define_top_genes**

Define the reference window using the most highly expressed features.

**Description**

Define the group of features in the dataset that will be considered as reference, the top window, by specifying either a number of features or an expression threshold.

**Usage**

```r
define_top_genes(dataset, window_size = NULL, mean_expression = NULL, min_expression = NULL)
```

**Arguments**

- **dataset**: A data frame, containing features as rows and cells as columns, and where the mean expression value for each gene has been added as a column. Usually the output of `calculate_cvs`.
- **window_size**: Number of features in the defined top window. Recommended to 100 features.
- **mean_expression**: A number. Genes with a mean expression across cells higher than the value will be selected. Ignored if `window_size` is defined.
- **min_expression**: A number. Genes with a minimum expression across all cells higher than the value will be selected. Ignored if `window_size` or `mean_expression` is defined.

**Details**

There are three selection methods available:

- **window_size**: features are ranked by mean expression across cells, and the top slice of the specified size is selected.
- **mean_expression**: the mean column is checked, and all features with mean expression above the threshold indicated are selected.
• min_expression: features where all expression values are above the expression threshold indicated are selected.

In general, it is advisable to avoid generating top windows larger than 250 features (100 features is the recommended value), to prevent excessively long computation time as well as to preserve the quality of the analysis, as the top window should only include a subset of reliable values.

Value
A list with two elements, both data frames: the defined top window, and the rest of the genes.

Examples
library(magrittr)
expMat <- matrix(
  c(1, 1, 1, 1, 2, 3, 0, 1, 2, 0, 0, 2),
  ncol = 3, byrow = TRUE, dimnames = list(paste("gene", 1:4), paste("cell", 1:3))
)

calculate_cvs(expMat) %>%
define_top_genes(window_size = 2)

calculate_cvs(expMat) %>%
define_top_genes(mean_expression = 1.5)

determine_bin_cutoff

Determine a threshold for selecting bins of features based on the metric table

Description
Takes the output of get_mean_median and decide until which window to keep based on background level and a threshold.

Usage
determine_bin_cutoff(metric_table, threshold = 2,
  selected_metric = c("mean", "median", "score"),
  random_function_summarisation = mean)

Arguments
metric_table A data frame, usually the output of get_mean_median.
threshold How many time higher than the background should the last bin be? Default to 2.
selected_metric Which metric to use (i.e. which column from metric_table to work with). Default to mean.
random_function_summarisation A function used to aggregate the randomised control across bin. Default to mean.
filter_expression_table

Details

Background level is estimated by averaging correlation coefficient obtained from the top window randomisations.
Bins (or windows) of features are kept until the mean (or median) correlation coefficient falls under a threshold value threshold x background level.

Value

A number, the first bin of features to discard.

See Also

get_mean_median, plot_metric

Examples

myData <- tibble::tibble(
  bin = rep(c(1, 2, 3), each = 3),
  window = rep(c("top_window", "shuffled_top_window_1", "shuffled_top_window_2"), 3),
  mean = c(0.8, 0.1, 0.11, 0.14, 0.12, 0.09, 0.10, 0.13, 0.08)
)
determine_bin_cutoff(myData)

filter_expression_table

Filter binned expression matrix

Description

Takes a binned expression table (the output of bin_scdata), a bin number (usually the output of determine_bin_cutoff) and returned a filtered expression table or matrix.

Usage

filter_expression_table(bined_table, bin_cutoff, as_matrix = FALSE)

Arguments

bined_table  A tibble, usually the output of bin_scdata.
bin_cutoff   the number of the first bin to be filtered out. Can be the output of determine_bin_cutoff.
as_matrix    A boolean. Should the return be a tibble (FALSE, the default) or a matrix (TRUE).

Value

A tibble or a matrix depending on the value of as_matrix

See Also

bin_scdata, determine_bin_cutoff
**get_mean_median**

**Extract mean and median correlation coefficient values**

**Description**

Takes the output of `correlate_windows` and extract the mean and the median correlation value for each window comparison.

**Usage**

```r
get_mean_median(df, absolute_cc = TRUE)
```

**Arguments**

- **df**
  - A data frame, usually the output of `correlate_windows`.
- **absolute_cc**
  - Should the function work of absolute value of correlation coefficients? Default to TRUE to simplify plots and avoid annoying, non-symmetrical, near 0, shifts of distributions.

**Value**

A data.frame with columns `bin`, `window`, `mean` and `median`.

**Examples**

```r
library(magrittr)
expMat <- matrix(
  c(1, 1, 5,
    1, 2, 3,
    0, 1, 4,
    0, 0, 2),
  ncol = 3, byrow = TRUE, dimnames = list(paste("gene", 1:4), paste("cell", 1:3))
)
calculate_cvs(expMat) %>%
  define_top_genes(window_size = 2) %>%
  bin_scdata(window_number = 1) %>%
  correlate_windows(n_random = 2) %>%
  get_mean_median
```
plot_correlations_distributions

Produce a density plot of correlation values for each window of feature

Description
Feature by feature correlation values between every windows and the reference to window of features are visualized as density lines, one facet per comparison. Two density lines are drawn in each facets:

- A thin colored line, the correlations between the bin and the reference top bin of features
- A thicker blue line with grey error area, the correlations between the bin and the randomized top bin of features. The lines are not shown if \( n_{\text{random}} = 0 \) in `correlate_windows`.

Usage

`plot_correlations_distributions(df, metrics = NULL, vlines = c("mean", "median"), facet_ncol = 4)`

Arguments

- `df`: A tibble, usually the output of `correlations_to_densities`.
- `metrics`: Optional. The output of `get_mean_median`. Dashed line will represent mean or median of the correlation coefficient distributions.
- `vlines`: A string, either "mean" or "median". Should the dashed line represent the mean or the median of the correlation coefficient distributions? Ignored if `metrics` is NULL.
- `facet_ncol`: The number of columns to arrange the plots.

Value
A ggplot2 plot.

See Also
`correlations_to_densities, get_mean_median`

Examples

```r
library(magrittr)
myData <- scData_hESC %>%
calculate_cvs %>%
define_top_genes(window_size = 100) %>%
bin_scdata(window_size = 1000)
corDistrib <- correlate_windows(myData, n_random = 3)
corDens <- correlations_to_densities(corDistrib)
plot_correlations_distributions(corDens)
metrics <- get_mean_median(corDistrib)
```
plot\_mean\_variance

plot\_correlations\_distributions(corDens, metrics = metrics)

---

**plot\_mean\_variance**  
*Produce a mean expression x coefficient of variation scatter plot.*

**Description**

Use the output of `calculate\_cvs` or `bin\_scdata` and plot a feature mean expression x coefficient of variation scatter plot. Mean expression is represented as \( \log_{10}(\text{mean} + 1) \). Each dot represents a feature. Means and coefficient of variations were obtained across single cells. Optionally, colours each dot according to the defined bins of features. Optionally, adds a density2d geom.

**Usage**

```r
plot\_mean\_variance(df, density = TRUE, colourByBin = TRUE, density\_color = "blue", ...)
```

**Arguments**

- `df`: A tibble, usually the output of `calculate\_cvs` or `bin\_scdata`.
- `density`: A boolean. Should a density2d geom be added to the plot?
- `colourByBin`: A boolean. Should feature be coloured by bin? Need a bin column in `df` (i.e. the output of `bin\_scdata`).
- `density\_color`: Colour of the density2d curves.
- `...`: Further arguments are passed to `geom\_point` such as `size`.

**Value**

A ggplot2 plot.

**See Also**

- `calculate\_cvs`, `bin\_scdata`

**Examples**

```r
library(magrittr)
scData\_hESC \%>%
calculate\_cvs \%>%
plot\_mean\_variance(colourByBin = FALSE)

scData\_hESC \%>%
calculate\_cvs \%>%
define\_top\_genes(window\_size = 100) \%>%
bin\_scdata(window\_size = 1000) \%>%
plot\_mean\_variance
```
plot_metric

Produce a bar chart of mean (or median) correlation coefficient per bin of feature.

Description
Use the output of get_mean_median and produce a bar chart of mean (or median) correlation coefficient per bin of features. Correlations against the randomised top window are shown as dot-and-whiskers, and are used to estimate a background level.

Usage
plot_metric(metric_table, selected_metric = c("mean", "median", "score"), show_ctrl = TRUE, control_color = "blue", show_threshold = TRUE, threshold = 2, threshold_color = "red", line_size = 1, annotate_lines = TRUE)

Arguments
metric_table A tibble, usually the output of get_mean_median.
selected_metric Which column in metricsTable to use? Default to mean.
show_ctrl A boolean. Should a dashed line indicate the estimated background level?
control_color The colour of the background dashed line (default to blue).
show_threshold A boolean. Should a dashed line indicate the estimated threshold level?
threshold How many times the background level should be multiplies do determine a threshold? Default to 2. The higher the more stringent.
threshold_color The colour of the threshold dashed line (default to blue).
line_size Thickness of the dashed lines.
annotate_lines A boolean. Should the dashed lines be annotated?

Value
A ggplot2 plot.

See Also
get_mean_median

Examples
library(magrittr)
scData_hESC %>%
calculate_cvs %>%
define_top_genes(window_size = 100) %>%
bin_scdata(window_size = 1000) %>%
correlate_windows(n_random = 3) %>%
get_mean_median %>%
plot_metric
plot_top_window_autocor

Utility plot to choose a top_window size

Description

Plot mean autocorrelation value of the features of the top window depending on increasing top window size.

Usage

plot_top_window_autocor(sc_data, from = 10, to = 400, by = 2, ...)

Arguments

- sc_data: A tibble, usually the output of `calculate_cvs`.
- from: Minimum size of the top window.
- to: Maximum size of the top window.
- by: Size of the steps to walk from from to to. See seq.
- ...: Arguments to be passed to cor, for example method = "spearman"

Value

A ggplot2 plot.

Examples

plot_top_window_autocor(calculate_cvs(scData_hESC))

scData_hESC

Expression data from 32 human embryonic stem cells

Description

Expression of 60,468 Gencode gene (in FPKM) from 32 single cell RNAseq of human embryonic stem cells

Usage

scData_hESC

Format

A tibble with 60,468 rows (genes) and 33 columns (cells):

- tracking_id: The Gencode human encode gene id
- next 33 columns: Single embryonic stem cells
sc_feature_filter

Value
A tibble.

Source

sc_feature_filter  Filter scRNA-seq expression matrix to keep only highly informative features. Integrated pipeline.

Description
This pipeline function takes an expression matrix as an input and select the features (genes, transcripts) with an estimated technical noise level lower that biological variation in the data. This is achieved by binning the data and calculating the correlation for each bin with highly expressed (lowest noise) gene set (see the vignette for details on the method).

Usage
```
sc_feature_filter(sc_data, print_plots = FALSE, max_zeros = 0.75, threshold = 2, top_window_size = 100, other_window_size = 1000, n_random = 3, sce_assay = NULL)
```

Arguments
- **sc_data**: A data frame, a matrix or a SingleCellExperiment object. If data frame or matrix, it should contain expression values for each gene as rows, and expression values for the cells as columns.
- **print_plots**: A boolean. Should the function produce three plots as a side effect? Plots are the output of `plot_mean_variance`, `plot_correlations_distributions` and `plot_metric`.
- **max_zeros**: A number between 0 and 1. Maximum proportion of cells with 0 expression for a feature to be kept.
- **threshold**: A number higher than 1. The higher the more stringent the feature selection will be. See `determine_bin_cutoff`.
- **top_window_size**: Size of the reference bin. See `define_top_genes`.
- **other_window_size**: Size of the other bins of feature. See `bin_scdata`.
- **n_random**: Number of control windows generated by shuffling the top bin of features.
- **sce_assay**: If `sc_data` is a SingleCellExperiment object, `sce_assay` should be one of names(assays(<SingleCellExperiment>)).

Details
The function can optionally produce three plots of `print_plots` is TRUE. It is recommended to open a graphical device (i.e. through `pdf` or `png`), to call `scFeatureFilter`, and then to close the device with `dev.off`.
Value

A matrix or a tibble, depending on the type of sc_data, containing only the top expressed features.

Examples

```r
sc_feature_filter(scData_hESC)

# with plots
## Not run:
pdf("diagnostic.pdf")
sc_feature_filter(sc_data, print_plots = TRUE)
dev.off()

## End(Not run)
```
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