Package ‘M3Drop’

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Description This package fits a Michaelis-Menten model to the pattern of dropouts in single-cell RNASeq data. This model is used as a null to identify significantly variable (i.e. differentially expressed) genes for use in downstream analysis, such as clustering cells.
URL https://github.com/tallulandrews/M3Drop
BugReports https://github.com/tallulandrews/M3Drop/issues
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R topics documented:

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Identify Highly Variable Genes

Description

Implements the method of Brennecke et al. (2013) to identify highly variable genes.

Usage

BrenneckeGetVariableGenes(expr_mat, spikes=NA, suppress.plot=FALSE, fdr=0.1, minBiolDisp=0.5)

Arguments

expr_mat  
a numeric matrix of normalized or raw (not log-transformed) expression values, columns = samples, rows = genes.

spikes  
a vector of gene names of row numbers of spike-in genes which are subject to only technical variance.

suppress.plot  
Whether to make the plot or just calculate the requisite values.

fdr  
Use FDR to identify significantly highly variable genes.

minBiolDisp  
Minimum percentage of variance due to biological factors.

Details

Identifies significantly highly variable genes as detailed in Brennecke et al [1]. If spike-ins are provided they are used fit a function to the relationship between gene expression and variance due to technical factors. If spike-ins are not provided then all genes are used in the fitting.

Value

Vector of names of highly variable genes.

References


Examples

library(M3DExampleData)
HVG <- BrenneckeGetVariableGenes(Mmus_example_list$data)
HVG_spike <- BrenneckeGetVariableGenes(Mmus_example_list$data, spikes=5550:5600)
Fitting Dropout Models

*Fit functions to the dropouts vs expression distribution.*

**Description**

Fits the modified Michaelis-Menten equation (MM), a logistic regression (logistic), or a double exponential (ZIFA) function to the relationship between mean expression and dropout-rate (proportion of zero values).

**Usage**

```r
bg__fit_MM(p, s)
bg__fit_logistic(p, s)
bg__fit_ZIFA(p, s)
```

**Arguments**

- `p`: a vector of dropout rates for each gene.
- `s`: a vector of mean expression values for each gene. Must be the same order & length as `p`.

**Details**

Fits one of different models to the relationship between dropout rate and mean expression. The three models are:

- `bg__fit_MM`: the Michaelis-Menten function
  \[
  P = 1 - \frac{S}{S + K}
  \]
  (see: [1]). Fit using `mle2` using normally distributed error.

- `bg__fit_logistic`: a logistic regression between `P` and log base 10 of `S` (used by [2]). Fit using `glm` (excludes genes where `S == 0`).

- `bg__fit_ZIFA`: a double exponential
  \[
  P = e^{\lambda S^2}
  \]
  (used by [3]). Fit using `lm` after log-transformation (genes were `P == 0` are assigned a value of one tenth of the smallest `P` which is not 0).

**Value**

Named list including:
- `K`, `fitted_err/B0,B1/lambda`, `fitted_err`
- `predictions`: predicted values of `p` for each gene
- `SSr/SAr`: sum of squared/absolute residuals
- `model`: vector of string descriptors of the fit

**References**


M3DropCleanData

**Filter Expression Data**

Filters and normalizes a given expression matrix. Removes low quality cells and undetected genes, and normalizes counts to counts per million.

**Usage**

```r
M3DropCleanData(expr_mat, labels = NA, is.counts = TRUE, suppress.plot = FALSE, pseudo_genes = NA, min_detected_genes = NA)
```

**Arguments**

- `expr_mat`: a numeric matrix of raw or normalized (not log-transformed) expression values, columns = samples/cells, rows = genes.
- `labels`: a vector of length equal to the number of columns of `expr_mat` with names or group IDs for each cell.
- `is.counts`: logical, whether the provided data is unnormalized read/fragment counts.
- `suppress.plot`: logical, whether to plot the distribution of number of detected genes per cell.
- `pseudo_genes`: a vector of gene names of known pseudogenes which will be removed from the cleaned data.
- `min_detected_genes`: minimum number of genes/cell for a cell to be included in the cleaned data.

**Details**

Retains genes detected (expression$>0$) in more than 3 cells and with mean normalized expression $\geq 10^{-5}$. If `min_detected_genes` is defined all cells not reaching the threshold are removed. Otherwise, fits a normal distribution to the distribution of detected genes/cell and removes those cells with significantly few detected genes (FDR 5%). This fit is plotted for visual inspection. If `is.counts`=TRUE then each column is converted to counts per million (ignoring ERCC spike-ins if present).

**Value**

list with elements: data, the normalized filtered expression matrix; and `labels`, labels of the remaining cells.
Examples

library(M3DEexampleData)
# Remove all cells with < 2000 detected genes and convert to cpm
cpm <- M3DropCleanData(Mmus_example_list$data, Mmus_example_list$labels,
is.counts=TRUE, min_detected_genes=2000)
# Removes cells with significantly few detected genes (FDR=5%)
filtered_only <- M3DropCleanData(Mmus_example_list$data, Mmus_example_list$labels,
is.counts=FALSE)

M3DropDifferentialExpression

Differentially Expressed Genes.

Description

Use Michaelis-Menten curve to find differentially expressed (DE) genes.

Usage

M3DropDifferentialExpression(expr_mat, mt_method="bon", mt_threshold=0.05, suppress.plot=FALSE)

Arguments

expr_mat a numeric matrix of normalized (not log-transformed) expression values, columns = samples, rows = genes.
mt_method the multiple testing method used in p.adjust
mt_threshold the threshold for identifying significantly DE genes.
suppress.plot logical, whether to plot the fitted curve and highlight DE genes.

Details

Fits a Michaelis-Menten function to the dropout-rate (if not provided) of the provided expression matrix. Identifies genes where the gene-specific $K$ calculated as ($S$ = mean expression, $P$ = dropout rate):

$$K = \frac{S * P}{1 - P}$$

is significantly larger than the $K$ fitted to the entire dataset. Combines standard errors of the fitted $K$, the gene-specific dropout rate and the gene-specific average expression using error propagation rules. Determines the significance of the gene-specific $K$ using a Z-test of the log-transformed $K$s with the propagated error then applies the specified multiple testing correction to identify DE genes. Plots the dropout rate vs gene expression with the fitted MM curve and highlights in purple the significantly DE genes.

Value

M3Drop_Differential Expression: a data.frame of significantly differentially expressed genes with columns: Gene, p.value, q.value
library(M3DExampleData)
Normalized_data <- M3DropCleanData(Mmus_example_list$data, 
labels = Mmus_example_list$labels, 
is.counts=TRUE, min_detected_genes=2000)
DE_genes <- M3DropDifferentialExpression(Normalized_data$data, 
mt_method="fdr", mt_threshold=0.01)

M3DropDropoutModels

Fit functions to the dropouts vs expression distribution.

Description
Fits the modified Michaelis-Menten equation (MM), a logistic regression (logistic), or a double exponential (ZIFA) function to the relationship between mean expression and dropout-rate (proportion of zero values).

Usage
M3DropDropoutModels(expr_mat, xlim=NA, suppress.plot=FALSE)

Arguments
expr_mat     a numeric matrix of normalized (not log-transformed) expression values, columns = samples, rows = genes.
xlim         limits for x-axis of plot.
suppress.plot logical, whether to plot fit curves or not.

Details
Plots the dropout-rate (P) vs average gene expression (S) for all genes. Fits three different models and adds the fitted curves to the plot. The three models are: MMfit : the Michaelis-Menten function

\[ P = \frac{S}{S + K} \]

(see: [1]). LogiFit : a logistic regression between P and log base 10 of S (used by [2]). ExpoFit : a double exponential

\[ P = e^{\lambda S^2} \]

(used by [3]).

Value
Invisibly, a list of output from each fit (MMfit, LogiFit, ExpoFit).

References
Examples

```r
library(M3DExampleData)
M3DropDropoutModels(Mmus_example_list$data)
```

---

**M3DropExpressionHeatmap**

*Plot Heatmap of Gene Expression*

**Description**

Plots a customized heatmap of scaled log expression values.

**Usage**

```r
M3DropExpressionHeatmap(genes, expr_mat, cell_labels=NA, interesting_genes=NA, key_genes=genes, key_cells=NA)
```

**Arguments**

- `genes` a character vector of gene names to be plot.
- `expr_mat` a numeric matrix of normalized (not log-transformed) expression values, columns = samples, rows = genes.
- `cell_labels` factor of labels for each cell in the expression matrix that will be used to coloured in a top bar of the heatmap.
- `interesting_genes` list of vectors of gene names that will be used to colour the bar to the left of the heatmap.
- `key_genes` a character vector of gene names to be labelled on the heatmap.
- `key_cells` a character vector of cells to be labelled on the heatmap. Unlabelled cells will be assigned a numerical index

**Details**

Modifies the gplots function heatmap.2 to replace the row dendrogram with a legend of the colours used in the columns colour bar (cell_labels) and use a custom colour scalling. Expression is displayed as Z-scores of log transformed expression (adding a pseudocount of 1) coloured blue-white-red centered at 0 and binned in the range \([-2,2]\).

**Value**

Invisibly, output from heatmap.2 call.

**Examples**

```r
library(M3DExampleData)
M3DropExpressionHeatmap(head(rownames(Mmus_example_list$data),20), Mmus_example_list$data, cell_labels = Mmus_example_list$labels)
```
M3DropGetExtremes

Get outliers from MM curve.

Description

Identifies outliers left and right of a fitted Michaelis-Menten curve.

Usage

\[
\text{M3DropGetExtremes}(\text{expr\_mat}, \text{fdr\_threshold}=0.1, \text{percent}=\text{NA}, \text{v\_threshold}=\text{c}(0.05, 0.95), \text{suppress.plot}=\text{FALSE})
\]

Arguments

- **expr\_mat**: a numeric matrix of normalized (not log-transformed) expression values, columns = samples, rows = genes.
- **fdr\_threshold**: the threshold for identifying significant outliers after multiple testing correction.
- **percent**: identify this percentage of data that is most extreme in each direction.
- **v\_threshold**: restrict to this range of dropout rates to avoid poorly fit regions of the data.
- **suppress.plot**: logical, whether to plot the fitted Michaelis-Menten curve and highlight identified most extreme outliers.

Details

Fits a Michaelis-Menten function to the dropout-rate of the provided data, then identifies the most extreme left and/or right outliers from the curve. Horizontal residuals are calculated as:

\[
\log_{10} S - \log_{10} \left(\frac{K \cdot (1 - P)}{P}\right)
\]

. Extreme left[right] outliers are identified either as the percent smallest[largest] horizontal residuals. If percent is undefined (default) a normal distribution is fitted to the horizontal residuals and a Z-test is used to identify significant outliers after FDR multiple testing correction.

If only genes with dropout rates within v\_threshold will be considered to avoid the skewing of residuals due to the exponential parts of the MM curve near \(P = 0\) & \(P = 1\).

M3DropGetExtremes identifies both left and right residuals using the provided thresholds in each direction. Eg. will return the percent smallest and percent largest residuals. It also plots the fitted MM curve and highlights the left and right extreme outliers unless suppress.plot=TRUE.

Value

M3DropGetExtremes List containing elements left and right, vectors of the names of the extreme genes to the left and right of the curve respectively.

Examples

\[
\text{library(M3DExampleData)}
\]

\[
\text{extreme\_gene\_lists} \leftarrow \text{M3DropGetExtremes(Mmus\_example\_list\$data, fdr\_threshold=0.1)}
\]

\[
\text{extreme\_gene\_lists} \leftarrow \text{M3DropGetExtremes(Mmus\_example\_list\$data, percent=0.01)}
\]
M3DropGetHeatmapCellClusters

Extracts cell clusters from heatmap output

Description
Extracts the clustering corresponding to the given number of clusters from heatmap output.

Usage
M3DropGetHeatmapCellClusters(heatout, k)

Arguments
heatout Output from a gene-expression heatmap.
k Number of clusters.

Details
Traverses down the dendrogram and cuts at the first point where there are at least k clusters.

Value
A vector of cluster labels for each cell.

Examples
library(M3DExampleData)
genes <- rownames(Mmus_example_list$data)[1:20]
heatmap_out <- M3DropExpressionHeatmap(genes, Mmus_example_list$data)
clusters <- M3DropGetHeatmapCellClusters(heatmap_out, k=5)

M3DropGetMarkers
Identify marker genes

Description
Calculates area under the ROC curve for each gene to predict the best group of cells from all other cells.

Usage
M3DropGetMarkers(expr_mat, labels)

Arguments
expr_mat a numeric matrix of normalized expression values, columns = samples, rows = genes.
labels a vector of group ids for each cell/sample.
Details

Uses the ROCR package to calculate the AUC for each gene for the group with the highest average rank. Significant is calculated using a Wilcoxon rank-sum test.

Value

A dataframe with a row for each gene and columns: AUC, Group (which label this gene had the highest average rank for), and pval (uncorrected p-value of prediction).

Examples

```r
library(M3DExampleData)
marker_gene_table <- M3DropGetMarkers(Mmus_example_list$data, Mmus_example_list$labels)
```

Description

Tests whether a given set of genes are significantly shifted to the left or right of the Michaelis-Menten curve.

Usage

```r
M3DropTestShift(expr_mat, genes_to_test, name="", background=rownames(expr_mat), suppress.plot=FALSE)
```

Arguments

- `expr_mat`: a numeric matrix of normalized (not log-transformed) expression values, columns = samples, rows = genes.
- `genes_to_test`: vector of gene names to test.
- `name`: string used to title the plot.
- `background`: vector of gene names to test against. (default = all genes)
- `suppress.plot`: logical, whether to fitted Michaelis-Menten curve and highlight the given set of genes to test.

Details

Fits a Michaelis-Menten function to the dropout-rate of the provided data, then tests whether a given set of genes (eg. pseudogenes) is significantly shifted left or right of the curve. Horizontal residuals are calculated as:

\[
\log_{10} S - \log_{10} \left( \frac{K \cdot (1 - P)}{P} \right)
\]

Uses a Wilcoxon rank-sum test/Mann-Whitney U test to compare the residuals for the given genes to the residuals for all genes.

Value

A one row dataframe with columns: sample (median horizontal residual of genes in the test set), pop (median horizontal residual of genes in the background set), p.value
Examples

```r
library(M3DExampleData)
gene_set <- c("Dppa2","Tdgf1","Rnf130","Tet1","Uhrf1","Pttg1","Zfp600","Stat1")
shift_output <- M3DropTestShift(Mmus_example_list$data, gene_set)
```

Description

Plot an area-proportional three-set Venn Diagram with labels.

Usage

```r
M3DropThreeSetVenn(set1, set2, set3, names)
```

Arguments

- **set1**: a vector of items in the first set.
- **set2**: a vector of items in the second set.
- **set3**: a vector of items in the third set.
- **names**: a vector of names of each set

Details


Value

None

Examples

```r
SetA <- c(1:20)
SetB <- c(15:30)
SetC <- c(5,10,15,20,25,30,35,40,45,50,55,60)
M3DropThreeSetVenn(SetA, SetB, SetC, names=c("A","B","C"))
```
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