Package 'genesorteR'

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Type Package

Title Feature Ranking in Clustered Single Cell Data

Version 0.4.0

Author Mahmoud M Ibrahim

Maintainer Mahmoud M Ibrahim <mmibrahim@pm.me>

Description The main purpose of this R extension is to select features in (possibly very large) single cell data including scRNA-Seq and scATAC-Seq.
The main idea is that the dropout rate of a gene is a good measure of its expression, and that empirical statistics calculated based on binarized expression matrices are sufficient to select marker genes in a way that is consistent with the expected definition of ``marker gene" in experimental biology research.
It can provide a ranking of genes specificity in each cell cluster, as well as select large or small sets of marker genes by a permutation test or using entropy-based feature selection.
To assess cell clustering quality, some functions can also compute cell cluster quality metrics.

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Encoding UTF-8

LazyData true

RoxygenNote 6.1.1

URL http://github.com/mahmoudibrahim/genesorteR

BugReports http://github.com/mahmoudibrahim/genesorteR/issues

Imports mclust,
parallel,
pheatmap,
methods

Depends R (>= 2.10),
Matrix

R topics documented:

getClassAUC   *getClassAUC*

Description

getClassAUC implements one way to investigate clustering quality. It processes the output of
sortGenes to obtain a curve for each cell cluster for all gene specificity scores against their ranking
in the cluster. The Area Under the Curve (AUC) can be used as a measure of clustering quality in
terms of the possibility to identify cell clusters using a few marker genes. See Details.

Usage

```
getClassAUC(gs, markers = NULL, plotCurves = TRUE, colors = NULL)
```

Arguments

gs	A list containing $specScore sparse matrix. Typically the output of sortGenes().
markers	A character vector of gene names to restrict this analysis to. See Details.
plotCurves	Should a plot be drawn? default value is TRUE.
colors	Color palette for the plot.

Details

Given the specificity score for all genes in a certain cell cluster, we can assume that a well-separated
easily-identified cell cluster will have a relatively small number of genes that have a very high
specificity score. Top marker genes for a cluster that is poorly separated from other cell clusters will
have average or low specificity scores. Sorting the genes for each cell cluster by their specificity
scores and plotting the scaled scores in order creates a curve that should be far from the diagonal
for well-separated clusters but close to the diagonal for poorly-separated clusters. The AUC of this
curve can be used to quantify this intuition and estimate a clustering quality metric.

Value

getClassAUC returns a numeric vector of length ncol($specScore) that contains the AUC for
each cell cluster.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

See Also

getMarkers returns a cell cluster Shannon index that tends to correlate well with the AUC metric returned by getClassAUC.

Examples

```
#randomly generated expression matrix and cell clusters
set.seed(1234)
exp = matrix(sample(0:20,1000,replace=TRUE), ncol = 20)
rownames(exp) = sapply(1:50, function(x) paste0("g", x))
cellType = sample(c("cell type 1","cell type 2"),20,replace=TRUE)
sg = sortGenes(exp, cellType)
classAUC = getClassAUC(sg)

#"reasonably" separated clusters
data(sim)
sg = sortGenes(sim$exp, sim$cellType)
classAUC = getClassAUC(sg)

#real data with three well separated clusters
data(kidneyTabulaMuris)
sg = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)
classAUC = getClassAUC(sg)
```

---

getMarkers   *getMarkers*

Description

getMarkers processes the output of sortGenes to select a relatively small set of marker genes.

Usage

```
getMarkers(gs, quant = 0.99, mutualInfo = FALSE, classEnt = FALSE)
```

Arguments

gs	The output of sortGenes().
quant	A number greater than zero and smaller than one. 0.99 by default. See Details.
mutualInfo	Logical. If TRUE, the mutual information between gene expression and cell clustering will be returned for each gene. FALSE by default.
classEnt	Logical. If TRUE, a "Cluster Shannon Index" is returned for each cluster. See Details. FALSE by default.

Value

getMarkers returns a list with the following components:

markers	A character vector containing the names of selected marker features.
maxScaledSpecScore	
	A numeric vector of length nrow(gs$specScore), including the maximum scaled specificity score for each gene across all cell clusters.

gene_shannon_index

A numeric vector of the same length as `maxScaledSpecScore`, including the Gene Shannon Index for each gene. See Details.

mutInfo   A numeric vector of the same length as `maxScaledSpecScore`, including the mutual information between the expression of each gene and the cell clustering.

classEntropy   A numeric vector of the same length as `ncol(specScore)`, including the Class Shannon Index for each cluster based on the selected marker genes.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

See Also

getPValues

Examples

```
#randomly generated data and cell clusters, almost no markers are found
set.seed(1234)
exp = matrix(sample(0:20,1000,replace=TRUE), ncol = 20)
rownames(exp) = sapply(1:50, function(x) paste0("g", x))
cellType = sample(c("cell type 1","cell type 2"),20,replace=TRUE)
sg = sortGenes(exp, cellType)
mm = getMarkers(sg,quant=0.95)
length(mm$markers) #only one marker gene was found


#"reasonably" separated clusters, with a few clear markers
data(sim)
gs = sortGenes(sim$exp, sim$cellType)
mm = getMarkers(gs,quant=0.95)
length(mm$markers)

#real data with three well separated clusters
data(kidneyTabulaMuris)
gs = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)
mm = getMarkers(gs, quant = 0.99)
length(mm$markers) #we found 109 candidate markers
#we want to get a more focused list:
mm = getMarkers(gs, quant = 0.999)
length(mm$markers) #11 genes that can alone descriminate between the cell types
```

---

getPValues   *getPValues*

Description

getPValues performs a permutation test on the gene-cell type specificity score to obtain a p-value value on each gene-cell type specificity score. The permutation keeps everything the same except that cell type assignments are permuted between the cells (but note that cell type proportions are also kept the same). This function is a way to select all differentially expressed genes between all cell classes globally in a dataset in one go or to define differentially expressed genes in a specific cell cluster.

Usage

```
getPValues(gs, numPerm = 5, correctMethod = "BH", testGenes = NULL,
  subsetCells = NULL, cores = 1, seed = 111)
```

Arguments

gs	The output of `sortGenes()`.
numPerm	The number of permutations to do. The default value 5 is to ensure quick running time for very large datasets but can be increased to increase the power of the permutation test, see Details.
correctMethod	The method used to correct p-values for multiple hypothesis testing. Any valid input to "method" in `p.adjust` is allowed. p-value correction is done on a gene-by-gene basis.
testGenes	A character vector of gene names to restrict the output p-values to.
subsetCells	A numeric vector of cell indeces to restrict the permutation to. Note that the selected cells should still contain at least one cell from each of the cell clusters contained in `$specScore`. Note this option is still experimental and might not give consistent results.
cores	An integer greater than zero (1 by default) that indicates how many cores to use for parallelization using mclapply.
seed	The seed for random permutations.

Value

getPValues returns a list with the following components:

permuteVal	A sparse matrix with as many rows as genes and as many columns as ncol(`$specScore`) * numPerm, containing the specificity score matrices for all permutations concatenated after each other column-wise.
startIndeces	A numeric vector of length `numPerm` that indicates the starting column for each performed permutation in `permuteVal`
pval	A matrix with as many rows as genes and columns as ncol(`$specScore`), containing the p-values for the null hypothesis that the gene is not highly specific to a cell cluster is true.
adjpval	A matrix with the same size as `pval`, containing the corrected p-values using the method specified in `correctMethod`

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

See Also

getMarkers

Examples

```
data(kidneyTabulaMuris)
gs = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)
pp = getPValues(gs)
#obtain genes that are "differentially expressed" in at least one cluster
```

```
markers = names(which(apply(pp$adjpval, 1, function(x) any(x < 0.01))))
```

---

getTable   *getTable*

Description

Summarize `sortGenes` and `getPValues` results in one data frame.

Usage

```
getTable(gs, pp, fc_cutoff = 0, adjpval_cutoff = 0.05, islog = TRUE,
  pseudocount = 1)
```

Arguments

gs	The output of `sortGenes`.
pp	The output of `getPValues`.
fc_cutoff	Default is 0, which means only genes that have an average fold-change higher than 0 for a given cluster are reported. Positive and negative numbers are allowed. Se to FALSE to switch off filtering on the average fold-change value.
adjpval_cutoff	A numeric adjusted p-value cutoff value. Default is 0.05.
islog	A logical value. TRUE (default) means the expression matrix supplied previously to `sortGenes` is in log space.
pseudocount	A numeric value. A pseudocount to add to the expression matrix before taking the log if `islog` is FALSE.

Details

`getTable` combines the results of sortGenes and getPValues in one table and also calculates the average fold-change of expression, allowing to select a fold-change cutoff and a p-value cutoff to determine variable genes.

Value

`getTable` returns a data frame containing the average log fold change, the adjusted p-value and the specificity score for all variable genes in each cluster. The data frame is sorted by the specificity score. Note that a gene may appear multiple times for multiple clusters.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

Examples

```
data(kidneyTabulaMuris)
gs = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)
pp = getPValues(gs)
tab = getTable(gs, pp)

#A quick diagnostic plot (fold change correlates with specificity score)
plot(tab$Average.Log.Fold.Change, tab$Specificity.Score, col = as.factor(tab$Cluster), pch = 20)

#all variable genes
unique(rownames(tab))

#To get all genes without any cutoffs, set adjpval_cutoff to 1 and fc_cutoff to FALSE
tab = getTable(gs, pp, fc_cutoff = FALSE, adjpval_cutoff = 1)
```

kidneyTabulaMuris	*A subset of the Tabula Muris SmartSeq2 data, restricted to three cell types from Kidney.*

Description

A subset of the Tabula Muris data with 215 cells and 23341 genes. There are three cell types, endothelial cells: 122 cells, kidney collecting duct epithelial cell: 78 cells and leukoocytes 15 cells.

Usage

```
kidneyTabulaMuris
```

Format

A list with two components:

exp Gene expression sparse matrix of class dgCMatrix, with normalized log expression values. Rows are genes, columns are cells.

cellType A character vector containing the cell types.

References

The Tabula Muris Consortium Single-cell transcriptomics of 20 mouse organs creates a Tabula Muris. Nature, 562:7727, 2018

| plotBinaryHeat | *plotBinaryHeat* |

Description

Plot a heatmap binarized values for a select set of genes or peaks across all cells. Optionally also cluster those genes.

Usage

```
plotBinaryHeat(exp, classes, markers,
  colors = colorRampPalette(c("white", "black"))(n = 100),
  newOrder = 1:length(unique(classes)), clusterGenes = FALSE,
  clusterGenesK = length(unique(classes)), averageCells = 0,
  outs = FALSE, plotheat = TRUE, gaps = TRUE, seed = 10)
```

Arguments

exp	A matrix of *binary* (0s and 1s) expression values. For example, the one found in sortGenes(...)$binary.
classes	A vector or factor of cell classes, whose length is equal to ncol(exp).
markers	A character vector of gene or peak names to plot in the heatmap.
colors	Color palette used for the heatmap.
newOrder	Reorder the clusters in the heatmap? See Examples.
clusterGenes	Cluster genes before plotting?
clusterGenesK	How many clusters should genes by clustered into? See Details.
averageCells	Plot averages of cells instead of individual cells. You can use this when you have a large number of cells. See Details.
outs	Should gene cluster output and pheatmap object be returned? FALSE by default.
plotheat	Should the heatmap be drawn? TRUE by default.
gaps	Should the heatmap have gaps between cell types and gene clusters? TRUE by default.
seed	Randomization seed used for gene clustering initialization.

Details

plotBinaryHeat is similar to plotMarkerHeat but works on the binarized matrix, plotting only 0s and 1s (for scRNA-Seq, this means ignoring expression values and just plotting whether the gene is expressed or not). If averageCells is > 1, the fraction of the averaged cells where the gene or peak was detected will be plotted (a number between 0 and 1).

clusterGenesK parameter controls the number of gene clusters when clusterGenes is TRUE. By default, it is equal to the number of cell types/clusters.

By default, the heatmap plots every single cell in one column, this might take forever if you have a lot of cells (would say >10k) or it can crash when you do not have enough RAM. If so, it might be good to set averageCells to n where n is the number of cells you want to average. For example, if averageCells = 10, every 10 cells will be averaged (without averaging across cell clusters) before plotting the heatmap. if averageCells =< 1, no averaging happens. Hints: (1) If you want one

column per cell cluster, set averageCells to a very high number (larger than the number of cells in the largest cluster). (2) Gene clustering occurs after cell averaging, so averaging will be useful in this case to prevent running k-means on binary values.

Value

If outs is TRUE, plotMarkerHeat returns a list containing:

p   The pheatmap object corresponding to the plot.

gene_class_info
                  Gene cluster assignments if gene clustering was requested. See Details.

new_class_info   The new order of cell clusters. See Examples.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

See Also

plotMarkerHeat

Examples

```
data(kidneyTabulaMuris)
gs = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)
mm = getMarkers(gs, quant = 0.999)

#this plots a heatmap without reordering genes
plotBinaryHeat(gs$binary, gs$inputClass, mm$markers)
```

---

plotCorrelationHeat   *plotCorrelationHeat*

Description

plotCorrelationHeat uses the specificity scores returned by sortGenes to correlate cell clusters with each other and plot a heatmap.

Usage

```
plotCorrelationHeat(gs, markers = NULL, corMethod = "pearson",
  colors = colorRampPalette(rev(c("orangered4", "orangered", "gray90",
  "dodgerblue", "dodgerblue4")))(n = 100), outs = FALSE,
  displayNumbers = TRUE)
```

Arguments

`gs`	The output of `sortGenes`.
`markers`	Restrict correlation analysis to those genes. A character vector.
`corMethod`	Correlation method, will passed to `method` in the function `cor`.
`colors`	Color palette for drawing the heatmap
`outs`	Should the `pheatmap` object and correlation matrix be returned? FALSE by default.
`displayNumbers`	Should correlation values be displayed on the heatmap? TRUE by default.

Value

If `outs` is TRUE, the pheatmap object and the correlation matrix will be returned.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

Examples

```
data(kidneyTabulaMuris)
gs = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)
plotCorrelationHeat(gs)

#user only marker genes and spearman correlation
mm = getMarkers(gs, quant = 0.95)
plotCorrelationHeat(gs, markers = mm$markers, corMethod = "spearman")

#do not write correlation values, useful if there are many cell clusters
plotCorrelationHeat(gs, markers = mm$markers, corMethod = "spearman", displayNumbers = FALSE)
```

---

plotMarkerHeat	*plotMarkerHeat*

Description

Plot a heatmap of expression values for a select set of genes across all cells. Optionally also cluster those genes.

Usage

```
plotMarkerHeat(exp, classes, markers,
  colors = colorRampPalette(rev(c("orangered4", "orangered", "gray90",
  "dodgerblue", "dodgerblue4")))(n = 100),
  newOrder = 1:length(unique(classes)), clusterGenes = FALSE,
  clusterGenesK = length(unique(classes)), averageCells = 0,
  outs = FALSE, plotheat = TRUE, gaps = TRUE, seed = 10)
```

Arguments

exp	A matrix of expression values. Typically the one supplied to sortGenes.
classes	A vector or factor of cell classes, whose length is equal to ncol(exp).
markers	A character vector of gene names to plot in the heatmap.
colors	Color palette used for the heatmap.
newOrder	Reorder the clusters in the heatmap? See Examples.
clusterGenes	Cluster genes before plotting?
clusterGenesK	How many clusters should genes by clustered into? See Details.
averageCells	Plot averages of cells instead of individual cells. You can use this when you have a large number of cells. See Details.
outs	Should gene cluster output and pheatmap object be returned? FALSE by default.
plotheat	Should the heatmap be drawn? TRUE by default.
gaps	Should the heatmap have gaps between cell types and gene clusters? TRUE by default.
seed	Randomization seed used for gene clustering initialization.

Details

clusterGenesK parameter controls the number of gene clusters when clusterGenes is TRUE. By default, it is equal to the number of cell types/clusters. This is usually a good initial guess, the optimal number of clusters (what does that even mean?) is typically somewhere around this value in my subjective experience, unless there are many cell clusters.

By default, the heatmap plots every single cell in one column, this might take forever if you have a lot of cells (would say >10k) or it can crash when you do not have enough RAM. If so, it might be good to set averageCells to n where n is the number of cells you want to average. For example, if averageCells = 10, every 10 cells will be averaged (without averaging across cell clusters) before plotting the heatmap. if averageCells =< 1, no averaging happens. Hints: (1) If you want one column per cell cluster, set averageCells to a very high number (larger than the number of cells in the largest cluster). (2) Gene clustering occurs after cell averaging, so averaging might be useful if the gene clusters you get do not make much sense without averaging (averaging will smooth out zeros).

Value

If outs is TRUE, plotMarkerHeat returns a list containing:

p	The pheatmap object corresponding to the plot.
gene_class_info	
	Gene cluster assignments if gene clustering was requested. See Details.
new_class_info	The new order of cell clusters. See Examples.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

See Also

plotTopMarkerHeat

Examples

```
data(kidneyTabulaMuris)
gs = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)
mm = getMarkers(gs, quant = 0.999)

#this plots a heatmap without reordering genes
plotMarkerHeat(gs$inputMat, gs$inputClass, mm$markers)

#so now cluster genes and return the clustering: a better looking plot
pp = plotMarkerHeat(gs$inputMat, gs$inputClass, mm$markers, clusterGenes=TRUE, outs=TRUE)
pp$gene_class_info #cell clusters

#reorder cell clusters in the heatmap
plotMarkerHeat(gs$inputMat, gs$inputClass, mm$markers, clusterGenes=TRUE, newOrder = c(3,1,2))

#average every 4 cells to make a less intense heatmap
plotMarkerHeat(gs$inputMat, gs$inputClass, mm$markers, clusterGenes=TRUE, newOrder = c(3,1,2),
averageCells = 4)

#only cluster genes, do not make plots
pp = plotMarkerHeat(gs$inputMat, gs$inputClass, mm$markers, clusterGenes=TRUE,
outs=TRUE, plotheat=FALSE)
```

---

plotMarkerScores   *plotMarkerScores*

Description

plotMarkerScores plots scatter plots of the specificity score and the gene specificity Shannon index to investigate `getMarkers` results.

Usage

```
plotMarkerScores(mm, gs, markers = mm$markers, colors = c("#99999920",
    "orangered4"))
```

Arguments

mm	The output of `getMarkers`
gs	The output of `sortGenes`
markers	A character vector containing the genes to highlight in the scatter plot.
colors	A color palette to use for the scatter plot.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

Examples

```
data(kidneyTabulaMuris)
gs = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)
mm = getMarkers(gs,quant=0.99)
plotMarkerScores(mm, gs)
```

plotTopBinaryHeat   *plotTopBinaryHeat*

Description

Plot a heatmap of binarized values for the top genes or peaks in each cluster, defined by `sortGenes()`.

Usage

```
plotTopBinaryHeat(sg, top_n = 10, colors = colorRampPalette(c("white",
  "black"))(n = 100), newOrder = 1:length(unique(sg$inputClass)),
  averageCells = 0, gaps = TRUE, outs = FALSE, plotheat = TRUE)
```

Arguments

`sg`	A list, typically the output of `sortGenes`.
`top_n`	The number of top genes to plot for each cluster.
`colors`	Color palette used for the heatmap.
`newOrder`	Reorder the clusters in the heatmap? See Examples.
`averageCells`	Plot averages of cells instead of individual cells. You can use this when you have a large number of cells. See Details.
`gaps`	Should the heatmap have gaps between cell types and gene clusters? TRUE by default.
`outs`	Should the top genes names be returned? FALSE by default.
`plotheat`	Should the heatmap be drawn? TRUE by default.

Details

`plotTopMarkerHeat` is similar to `plotTopMarkerHeat` but works on the binarized matrix, plotting only 0s and 1s (for scRNA-Seq, this means ignoring expression values and just plotting whether the gene is expressed or not). If `averageCells` is > 1, the fraction of the averaged cells where the gene or peak was detected will be plotted (a number between 0 and 1).

Value

If `outs` is TRUE, `plotMarkerHeat` returns a list containing the top n marker genes for each cluster.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

See Also

plotTopMarkerHeat

Examples

```
data(kidneyTabulaMuris)
gs = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)
plotTopBinaryHeat(gs) # plots the top 10 genes for each cluster

#now plot the top 20 genes and average every 5 cells
plotTopBinaryHeat(gs, top_n= 20, averageCells=5)
```

---

plotTopMarkerHeat   *plotTopMarkerHeat*

Description

Plot a heatmap of expression values for the top genes in each cluster, defined by sortGenes().

Usage

```
plotTopMarkerHeat(sg, top_n = 10,
  colors = colorRampPalette(rev(c("orangered4", "orangered", "gray90",
  "dodgerblue", "dodgerblue4")))(n = 100),
  newOrder = 1:length(unique(sg$inputClass)), averageCells = 0,
  gaps = TRUE, outs = FALSE, plotheat = TRUE)
```

Arguments

sg	A list, typically the output of sortGenes.
top_n	The number of top genes to plot for each cluster.
colors	Color palette used for the heatmap.
newOrder	Reorder the clusters in the heatmap? See Examples.
averageCells	Plot averages of cells instead of individual cells. You can use this when you have a large number of cells. See Details.
gaps	Should the heatmap have gaps between cell types and gene clusters? TRUE by default.
outs	Should the top genes names be returned? FALSE by default.
plotheat	Should the heatmap be drawn? TRUE by default.

Details

plotTopMarkerHeat is a convenience wrapper around plotMarkerHeat that plots a heatmap of the top top_n (10 by default) genes in each cell cluster. Unlike plotMarkerHeat, plotTopMarkerHeat takes the output of sortGenes as the only required input.

Value

If outs is TRUE, plotMarkerHeat returns a list containing the top n marker genes for each cluster.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

See Also

plotMarkerHeat

Examples

```
data(kidneyTabulaMuris)
gs = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)
plotTopMarkerHeat(gs) # plots the top 10 genes for each cluster

#now plot the top 20 genes and average every 5 cells
plotTopMarkerHeat(gs, top_n= 20, averageCells=5)

#just identify the top 20 genes, do not make a plot
plotTopMarkerHeat(gs, top_n= 20, averageCells=5, outs = TRUE, plotheat = FALSE)
```

---

sim	*Simulated single cell RNA-seq data with 500 cells.*

Description

A simulated dataset containing 500 cells and 3999 genes. There are two cell types, one with 245 cells and the other with 255 cells.

Usage

```
sim
```

Format

A list with two components:

exp Gene expression matrix, with raw counts. Rows are genes, columns are cells

cellType A numeric vector containing the cell types

---

sortGenes	*sortGenes*

Description

sortGenes is the main function of the genesorteR package. It takes a gene expression matrix and cell cluster assignments. It binarizes the expression matrix and calculates empirical statistics on gene expression in each cluster including a specificity score that can be used to rank genes in cell clusters.

Usage

```
sortGenes(x, classLabels, binarizeMethod = "median",
  returnInput = TRUE, cores = 1)
```

Arguments

x	A numeric (sparse) matrix. It will be coerced to a dgCMatrix sparse matrix. Rows represent genes, columns represent cells.
classLabels	A numeric or character vector or a factor of the same length as ncol(x) that represents cell cluster assignments. It will be coerced to a factor whose levels are the cell cluster names.
binarizeMethod	Either "median" (default) or "naive" or "adaptiveMedian" or a numeric cutoff. See Details.
returnInput	Return the input matrix and cell classes? TRUE by default. See Details.
cores	An integer greater than zero (1 by default) that indicates how many cores to use for parallelization using mclapply.

Details

When binarizeMethod is "median", expression matrix binarization is done by estimating a cutoff value uniformly on all values in the matrix. This is equal to the median of all non-zero entries in the matrix and is returned in cutoff. When binarizeMethod is "adaptiveMedian", expression values of genes are clustered to obtain groups of genes based on expression level, then the "median" method is applied to each group separately. This assumes the matrix supplied in x is log scaled. When binarizeMethod is "naive", all non-zero entries are kept and the minimum value of non-zero entries is returned in cutoff. When the input matrix x has already been binarized, set binarizeMethod to "naive". You can set a specific cutoff value for binarization, by setting binarizeMethod to a numeric value >= 0.

The specificity scores balance the posterior probability of observing a cell cluster given the gene (gene-cluster specificity) with its conditional probability given the cluster (a measure of gene expression). This ensures that highly specific genes are also highly expressed. The specScore matrix is considered the main output of this function, and on which many of the remaining calculations by other functions in the genesorteR package are performed. The values in this matrix can be used to rank features (genes in scRNA-Seq) in clusters.

Note that if returnInput is set to FALSE (input expression matrix will no be returned in the output), many of the other functions that accept the output of sortGenes will break.

sortGenes can in principle be applied to both a raw count matrix or a normalized log-count expression matrix.

Value

sortGenes returns a list with the following components:

binary	The binarized gene expression matrix. A sparse matrix of class dgCMatrix.
cutoff	The cutoff value used to binarize the gene expression matrix. Anything lower than this value was set to zero.
removed	A numeric vector containing the row indeces of genes that were removed because they were not expressed in any cells after binarization. If none were removed, this will be NULL.
geneProb	A numeric vector whose length is equal to nrow(binary) that lists the fraction of cells in which a gene was detected.
condGeneProb	A sparse matrix of class dgCMatrix with as many rows as nrow(binary) and as many columns as the number of cell clusters. It includes the conditional probability of observing a gene in a cluster.

postClustProb	A sparse matrix of class dgCMatrix with the same size as condGeneProb, containing the posterior probability that a cell belongs to a certain cluster or type given that the gene was observed.
specScore	A sparse matrix of class dgCMatrix with the same size as condGeneProb, containing a specificity score for each gene in each cell cluster. See Details.
classProb	A numeric vector whose length is equal to the number of cell clusters, containing the fraction of cells belonging to each cluster.
inputMat	the input x matrix, after being coerced to a sparse matrix of class dgCMatrix.
inputClass	the input classLabels after being coerced to a factor.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

Examples

```
data(kidneyTabulaMuris)
#basic functionality
gs = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType)

#the top 10 genes for each cluster by specificity scores
top_genes = apply(gs$specScore, 2, function(x) names(head(sort(x, decreasing = TRUE), n = 10)))

#the same top 10 genes but using the plotTopMarkerHeat function
plotTopMarkerHeat(gs, top_n = 10, outs = TRUE, plotheat = FALSE)

#naive binarization keeps any non-zero input in the input matrix
gs_naive = sortGenes(kidneyTabulaMuris$exp, kidneyTabulaMuris$cellType, binarizeMethod = "naive")

#different genes?
plotTopMarkerHeat(gs_naive, top_n = 10, outs=TRUE, plotheat=FALSE)
```

---

write.files   *write.files*

Description

write.files saves gene-cluster sorting information obtained from sortGenes to disk.

Usage

```
write.files(gs, prefix = "genesorteROuts", markers = NULL,
  eachCluster = FALSE)
```

Arguments

gs	The output of sortGenes().
prefix	The prefix for saving the files.
markers	Additionally, output files restricted to these genes. A character vector.
eachCluster	Also write individual files for each cluster?

Details

Three files will be saved to disk, including the specificity score matrix, the posterior cluster proba-
bility given the gene and the conditional probability of observing the gene in a cluster. If markers
is not NULL, three additional files will be created and indicated with "_markers" in their name. If
eachCluster is TRUE, additional files (one per cluster will be written which includes all genes and
their scaled specificity score for each cluster, with genes sorted by their score.

Author(s)

Mahmoud M Ibrahim <mmibrahim@pm.me>

Examples

```
data(sim)
gs = sortGenes(sim$exp, sim$cellType)
## Not run: write.files(gs) #write all files for all genes.
## Not run: write.files(gs, markers = c("g1","g2")) #additionally write files that are restricted to genes g1
```

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