Package 'CEDA'

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Type Package

Title CRISPR Screen and Gene Expression Differential Analysis

Version 1.1.1

Description Provides analytical methods for analyzing CRISPR screen data at different levels of gene expression. Multi-component normal mixture models and EM algorithms are used for modeling.

Depends R(>= 3.5.0), limma

Imports stats, mixtools, ggplot2, dplyr, ggsci, ggridges, ggprism

Suggests knitr, rmarkdown

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R topics documented:

`alphaBeta` *Calculating a significance score of a gene based on the corresponding sgRNAs' p-values of the gene.*

Description

Code was adapted from R package gscreend.

Usage

```
alphaBeta(pvec)
```

Arguments

`pvec`	A numeric vector of p-values.

Value

A min value of the kth smallest value based on the beta distribution B(k, n-k+1), where the n is the number of probabiliteis in the vector. This min value is the significance score of the gene.

`calculateGeneLFC` *Calculating gene-level log fold ratios*

Description

Log fold ratios of all sgRNAs of a gene are averaged to obtain the gene level log fold ratio.

Usage

```
calculateGeneLFC(lfcs, genes)
```

Arguments

`lfcs`	A numeric vector containing log fold change of sgRNAs.
`genes`	A character string containing gene names corresponding to sgRNAs.

Value

A numeric vector containing log fold ratio of genes.

`calculateGenePval` *Calculating gene level p-values using modified robust rank aggregation (alpha-RRA method) on sgRNAs' p-values*

Description

Code was adapted from R package gscreend. The alpha-RRA method is adapted from MAGeCK.

Usage

```
calculateGenePval(pvec, genes, alpha, nperm = 20)
```

Arguments

`pvec`	A numeric vector containing p-values of sgRNAs.
`genes`	A character string containing gene names corresponding to sgRNAs.
`alpha`	A numeric number denoting the alpha cutoff (i.e. 0.05).
`nperm`	Number of permutations, default is 20

Value

A list with four elements: 1) a list of genes with their p-values; 2) a numeric matrix of rho null, each column corresponding to a different number of sgRNAs per gene; 3)a numeric vector of rho; 4) a numeric vector of number of sgRNAs per gene.

`densityPlot` *2D density contour plot of gene log2 fold ratios against gene expression levels*

Description

This function generates a scatter plot with 2D density contour of log2 fold ratios of sgRNAs against the corresponding gene expression levels.

Usage

```
densityPlot(data, ...)
```

Arguments

`data`	A data frame from the output of preparePlotData function
`...`	Other graphical parameters

Value

No return value

`EMFit` *Fitting multi-component normal mixture models by R package mixtools*

Description

The function normalmixEM in R package mixtools is employed for fitting multi-component normal mixture models.

Usage

```
EMFit(x, k0, mean_constr, sd_constr, npara, d0)
```

Arguments

`x`	A numeric vector
`k0`	Number of components in the normal mixture model
`mean_constr`	A constrain on means of components
`sd_constr`	A constrain on standard deviations of components
`npara`	Number of parameters
`d0`	Number of times for fitting mixture model using different starting values

Value

Normal mixture model fit and BIC value of the log-likelihood

`makeRhoNull` *Generating the null distribution of the significance score of a gene.*

Description

Code was adapted from R package gscreend.

Usage

```
makeRhoNull(n, p, nperm)
```

Arguments

n	An integer representing sgRNA number of a gene.
p	A numeric vector which contains the percentiles of the p-values that meet the cut-off (alpha).
nperm	Number of permutation runs.

Value

A numric vector which contains all the significance scores (rho) of genes generated by a permutation test where the sgRNAs are randomly assigned to genes.

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mda231 *CRISPR screen data of cell line MDA-MB-231.*

Description

A dataset containing the expression data of sgRNAs in a CRISPR screen experiment of cell line MDA-MB-231.

Usage

```
mda231
```

Format

A data frame with a list of two elements:

sgRNA Raw Read counts of sgRNAs

negene A list of non-essential genes

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medianNormalization *Median normalization of sgRNA counts*

Description

This function adjusts sgRNA counts by the median ratio method. The normalized sgRNA read counts are calculated as the raw read counts devided by a size factor. The size factor is calcuated as the median of all size factors caculated from negative control sgRNAs (eg., sgRNAs corresponding to non-targeting or non-essential genes).

Usage

```
medianNormalization(data, control)
```

Arguments

data	A numeric matrix containing raw read counts of sgRNAs with rows corresponding to sgRNAs and columns correspondings to samples.
control	A numeric matrix containing raw read counts of negative control sgRNAs with rows corresponding to sgRNAs and columns corresponding to samples. Sample ordering is the same as in data.

Value

A list with two elements: 1) size factors of all samples; 2) normalized counts of sgRNAs.

Examples

```
count <- matrix(rnbinom(5000 * 6, mu=500, size=3), ncol = 6)
colnames(count) = paste0("sample", 1:6)
rownames(count) = paste0("sgRNA", 1:5000)
control <- count[1:100,]
normalizedcount <- medianNormalization(count, control)
```

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normalMM *Performing empirical Bayes modeling on limma results*

Description

This function perform an empirical Bayes modeling on log fold ratios and return the posterior log fold ratios.

Usage

```
normalMM(data, theta0, n.b = 5, d = 10)
```

Arguments

data	A numeric matrix containing limma results and log2 gene expression levels that has a column nameed 'lfc' and a column named 'exp.level.log2'
theta0	Standard deviation of log2 fold changes under permutations
n.b	Number of bins, default is 5 bins
d	Number of times for fitting mixture model using different starting values, default is 10

Value

A numeric matrix containing limma results, RNA expression levels, posterior log2 fold ratio, log p-values, and estimates of mixture model

permuteLimma *Modeling CRISPR data with a permutation test between conditions by R package limma*

Description

The lmFit function in R package limma is employed for group comparisons under permutations.

Usage

```
permuteLimma(data, design, contrast.matrix, nperm)
```

Arguments

data	A numeric matrix containing log2 expression level of sgRNAs with rows corresponding to sgRNAs and columns to samples.
design	A design matrix with rows corresponding to samples and columns to coefficients to be estimated.
contrast.matrix	A matrix with columns corresponding to contrasts.
nperm	Number of permutations

Value

A numeric matrix containing log2 fold changes with permutations

Examples

```
y <- matrix(rnorm(1000*6),1000,6)
condition <- gl(2,3,labels=c("Control","Baseline"))
design <- model.matrix(~ 0 + condition)
contrast.matrix <- makeContrasts("conditionControl-conditionBaseline",levels=design)
fit <- permuteLimma(y,design,contrast.matrix,20)
```

preparePlotData *Prepare data for density plot and ridge plot*

Description

Input a data frame with each gene one row, and geneID, geneLFC, geneFDR as columns. This function will stratify genes into five groups based on their FDR levels: <=0.001, (0.001,0.01], (0.01,0.05], (0.05,0.5], (0.5,1]

Usage

```
preparePlotData(data, gene.fdr)
```

Arguments

data	A data frame containing each gene in one row, and at least three columns with geneID, geneLFC, and geneFDR.
gene.fdr	A numeric variable (column) in the data frame, corresponding to the gene level FDR

Value

A data frame based on the original data frame, with an additional column "group" indicating which FDR group this gene belongs to.

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ridgePlot *Density ridgeline plot of gene expression levels for different FDR groups.*

Description

This function generates a density ridgeline plot of gene expression levels for different FDR groups.

Usage

```
ridgePlot(data, ...)
```

Arguments

data	A data frame from the output of preparePlotData function
...	Other graphical parameters

Value

No return value

runLimma *Modeling CRISPR screen data by R package limma*

Description

The lmFit function in R package limma is employed for group comparisons.

Usage

```
runLimma(data, design, contrast.matrix)
```

Arguments

data	A numeric matrix containing log2 expression levels of sgRNAs with rows corresponding to sgRNAs and columns corresponding to samples.
design	A design matrix with rows corresponding to samples and columns corresponding to coefficients to be estimated.
contrast.matrix	A matrix with columns corresponding to contrasts.

Value

A data frame with rows corresponding to sgRNAs and columns corresponding to limma results

Examples

```
y <- matrix(rnorm(1000*6),1000,6)
condition <- gl(2,3,labels=c("Treatment","Baseline"))
design <- model.matrix(~ 0 + condition)
contrast.matrix <- makeContrasts("conditionTreatment-conditionBaseline",levels=design)
limma.fit <- runLimma(y,design,contrast.matrix)
```

scatterPlot *Scatter plot of log2 fold ratios against gene expression levels*

Description

This function generates a scatter plot of log2 fold ratios of sgRNAs against the corresponding gene expression levels.

Usage

```
scatterPlot(data, fdr, ...)
```

Arguments

`data`	A numeric matrix from the output of normalMM function
`fdr`	A level of false discovery rate
`...`	Other graphical parameters

Value

No return value

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