Package 'EBSEA'

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Type Package

Title Exon Based Strategy for Expression Analysis of genes

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Description Calculates differential expression of genes based on exon counts of genes obtained from RNA-seq sequencing data.

License GPL-2

biocViews Software, DifferentialExpression, GeneExpression, Sequencing

Imports DESeq2, graphics, stats, EmpiricalBrownsMethod

RoxygenNote 7.1.1

Encoding UTF-8

Suggests knitr, rmarkdown

VignetteBuilder knitr

Depends R (>= 4.0.0)

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EBSEA *Exon Based Startegy for Expression Analysis of genes*

Description

EBSEA takes the filtered raw exon-level read counts as input, normalizes and performs a two-group statistical comparison with DESeq2. The exon-level results are aggregated to the gene-level using empirical Brown's method. The samples in the two groups can be paired.

Usage

```
EBSEA(data, columnData, design, test = "Wald", contrasts = NULL, plot = FALSE)
```

Arguments

data	A dataframe of raw exon-counts
columnData	A dataframe indicated the groups of the samples.
design	Design matrix (see more information od design matrixes in DESeq2 reference manual)
test	The statistical test to be carried out. It can be either Wald or Likelihood Ratio Test. For further details about the methods you can look into DESeq2 refernce manual. Default: Wald
contrasts	a character vector with exactly three elements: the name of a factor in the design formula, the name of the numerator level for the fold change, and the name of the denominator level for the fold change Default: NULL
plot	A logical value indicating a volcano plot is produced. Default: FALSE

Value

The function returns a list containing containing exon and gene-level results. ExonTable is a data frame containing an average expression, log2 fold-change, p-value and adjusted p-value. GeneTable is a data frame containing the gene-level p-value, and adjusted-value. Other returned elements include the raw and normalised exon-level read counts, group information and design matrix used.

References

Laiho, A., & Elo, L. L. (2014). A note on an exon-based strategy to identify differentially expressed genes in RNA-seq experiments. PloS One, 9(12), e115964.

Examples

```
# The exon-based analysis for unpaired samples can be performed as follows:
data(exonCounts)
group <- data.frame('group' = as.factor(c('G1', 'G1', 'G1', 'G2', 'G2', 'G2', 'G2')))
row.names(group) <- colnames(exonCounts)
design <- ~group
```

```
ebsea.out <- EBSEA(exonCounts, group, design)
# The exon-based analysis for paired samples with contrast provided can be performed as follows:
data(exonCounts)
group <- data.frame('group' = as.factor(c('G1', 'G1', 'G1', 'G2', 'G2', 'G2', 'G2')),
 'paired' = as.factor(c(1,2,3,1,2,3,3)))
row.names(group) <- colnames(exonCounts)
design <- ~group
contrastInfo <- c('group', 'G2', 'G1')
ebsea.out <- EBSEA(exonCounts, group, design, contrasts = contrastInfo)
```

`exonCounts` *Subset of Pasilla Dataset*

Description

exonCounts consists of a subset of the exon counts from the pasilla dataset.

Usage

```
data("exonCounts")
```

Format

A data frame with 1000 rows and 7 variables

Source

Exoncounts from Pasilla package https://bioconductor.org/packages/release/data/experiment/html/pasilla.html

References

Huber W, Reyes A (2020). pasilla: Data package with per-exon and per-gene read counts of RNA-seq samples of Pasilla knock-down by Brooks et al., Genome Research 2011

`filterCounts` *Filter Count Data*

Description

Filtering of exons based on their expression levels

Usage

```
filterCounts(x, mean = 1, exonCount = 1)
```

Arguments

x	A numeric dataframe of exon counts across the samples. Exon number in format GeneName:Exonnumber should be indicated in the row name and sample names as column names.
mean	Exons with average count value across the dataset less than mean are filtered out. Default: 1
exonCount	After filtering the individual exons, only genes with at least the given number of exons remaining will be retained. Default: 1

Value

A dataframe of filtered counts of exons

Examples

```
data(exonCounts)
res <- filterCounts(exonCounts)
```

---

visualizeGenes *Visualize gene*

Description

Produces a visualization summarizing the normalized read count in each sample group and expression difference across the expressed exons.Top panel contains the log2 fold-change for each expressed exon. Asterisk denotes the significance level (*: < 0.05, **: < 0.01). Bottom panel shows the averaged normalized read count for each sample group. The title of the figure shows the gene name and the adjusted gene-level p-value (fdr)

Usage

```
visualizeGenes(gene, ebsea.out)
```

Arguments

gene	Gene name matching the input data.
ebsea.out	Plots the mean count and fold-change the exons of the specified gene.

Value

Plots the mean count and fold-change across the exons of the specified gene.

Examples

```
data(exonCounts)
group <- data.frame('group' = as.factor(c('G1', 'G1', 'G1', 'G2', 'G2', 'G2', 'G2')))
row.names(group) <- colnames(exonCounts)
design <- ~group
ebsea.out <- EBSEA(exonCounts, group, design)
visualizeGenes('FBgn0000017', ebsea.out)
```

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