

# Package ‘EBSEA’

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**Type** Package

**Title** Exon Based Strategy for Expression Analysis of genes

**Version** 1.37.0

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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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**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 reference 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*


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**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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## \* **datasets**

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