Package ‘DEGseq’

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**Title**  Identify Differentially Expressed Genes from RNA-seq data

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**Description**  DEGseq is an R package to identify differentially expressed genes from RNA-Seq data.

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DEGexp | **DEGexp: Identifying Differentially Expressed Genes from gene expression data**

**Description**

This function is used to identify differentially expressed genes when users already have the gene expression values (or the number of reads mapped to each gene).

**Usage**

```r
DEGexp(geneExpMatrix1, geneCol1=1, expCol1=2, depth1=rep(0, length(expCol1)), groupLabel1="group1", geneExpMatrix2, geneCol2=1, expCol2=2, depth2=rep(0, length(expCol2)), groupLabel2="group2", method=c("LRT", "CTR", "FET", "MARS", "MATR", "FC"), pValue=1e-3, zScore=4, qValue=1e-3, foldChange=4, thresholdKind=1, outputDir="none", normalMethod=c("none", "loess", "median"), replicateExpMatrix1=NULL, geneColR1=1, expColR1=2, depthR1=rep(0, length(expColR1)), replicateLabel1=NULL, replicateExpMatrix2=NULL, geneColR2=1, expColR2=2, depthR2=rep(0, length(expColR2)), replicateLabel2=NULL)
```

**Arguments**

- `geneExpMatrix1` gene expression matrix for replicates of sample1 (or replicate1 when method="CTR").
- `geneCol1` gene id column in geneExpMatrix1.
- `expCol1` expression value columns in geneExpMatrix1 for replicates of sample1 (numeric vector).
  
  *Note:* Each column corresponds to a replicate of sample1.
- `depth1` the total number of reads uniquely mapped to genome for each replicate of sample1 (numeric vector).
  
  *Note:* Each column corresponds to a replicate of sample1.
- `groupLabel1` label of group1 on the plots.
- `geneExpMatrix2` gene expression matrix for replicates of sample2 (or replicate2 when method="CTR").
- `expCol2` expression value columns in geneExpMatrix2 for replicates of sample2 (numeric vector).
  
  *Note:* Each column corresponds to a replicate of sample2.
**DEGexp**

depth2  the total number of reads uniquely mapped to genome for each replicate of sample2 (numeric vector),
default: take the total number of reads mapped to all annotated genes as the depth for each replicate.

groupLabel2 label of group2 on the plots.

method  method to identify differentially expressed genes. Possible methods are:
- "LRT": Likelihood Ratio Test (Marioni et al. 2008),
- "CTR": Check whether the variation between Technical Replicates can be explained by the random sampling model (Wang et al. 2009),
- "FET": Fisher’s Exact Test (Joshua et al. 2009),
- "MARS": MA-plot-based method with Random Sampling model (Wang et al. 2009),
- "MATR": MA-plot-based method with Technical Replicates (Wang et al. 2009),
- "FC": Fold-Change threshold on MA-plot.

pValue  pValue threshold (for the methods: LRT, FET, MARS, MATR).
only used when thresholdKind=1.

zScore  zScore threshold (for the methods: MARS, MATR).
only used when thresholdKind=2.

qValue  qValue threshold (for the methods: LRT, FET, MARS, MATR).
only used when thresholdKind=3 or thresholdKind=4.

thresholdKind  the kind of threshold. Possible kinds are:
- 1: pValue threshold,
- 2: zScore threshold,
- 3: qValue threshold (Benjamini et al. 1995),
- 4: qValue threshold (Storey et al. 2003),
- 5: qValue threshold (Storey et al. 2003) and Fold-Change threshold on MA-plot are both required (can be used only when method="MARS").

foldChange  fold change threshold on MA-plot (for the method: FC).

outputDir  the output directory.

normalMethod  the normalization method: "none", "loess", "median" (Yang et al. 2002).
recommend: "none".

replicateExpMatrix1  matrix containing gene expression values for replicate batch1 (only used when method="MATR").
*Note: replicate1 and replicate2 are two (groups of) technical replicates of a sample.*

geneColR1  gene id column in the expression matrix for replicate batch1 (only used when method="MATR").

expColR1  expression value columns in the expression matrix for replicate batch1 (numeric vector) (only used when method="MATR").
depthR1  
the total number of reads uniquely mapped to genome for each replicate in replicate batch1 (numeric vector),
default: take the total number of reads mapped to all annotated genes as the depth for each replicate (only used when method="MATR").

replicateLabel1  
label of replicate batch1 on the plots (only used when method="MATR").

replicateExpMatrix2  
matrix containing gene expression values for replicate batch2 (only used when method="MATR").
Note: replicate1 and replicate2 are two (groups of) technical replicates of a sample.

geneColR2  
gene id column in the expression matrix for replicate batch2 (only used when method="MATR").

expColR2  
expression value columns in the expression matrix for replicate batch2 (numeric vector) (only used when method="MATR").

depthR2  
the total number of reads uniquely mapped to genome for each replicate in replicate batch2 (numeric vector),
default: take the total number of reads mapped to all annotated genes as the depth for each replicate (only used when method="MATR").

replicateLabel2  
label of replicate batch2 on the plots (only used when method="MATR").

rawCount  
a logical value indicating the gene expression values are based on raw read counts or normalized values.

References


See Also

DEGexp2, DEGseq, getGeneExp, readGeneExp, GeneExpExample1000, GeneExpExample5000.
### Examples

```r
## kidney: R1L1Kidney, R1L3Kidney, R1L7Kidney, R2L2Kidney, R2L6Kidney
## liver: R1L2Liver, R1L4Liver, R1L6Liver, R1L8Liver, R2L3Liver

geneExpFile <- system.file("extdata", "GeneExpExample5000.txt", package="DEGseq")
cat("geneExpFile: ", geneExpFile, "\n")
outputDir <- file.path(tempdir(), "DEGexpExample")
gegeneExpMatrix1 <- readGeneExp(file=geneExpFile, geneCol=1, valCol=c(7,9,12,15,18))
gegeneExpMatrix2 <- readGeneExp(file=geneExpFile, geneCol=1, valCol=c(8,10,11,13,16))
gegeneExpMatrix1[30:32,]
gegeneExpMatrix2[30:32,]
DEGexp(geneExpMatrix1=geneExpMatrix1, geneCol1=1, expCol1=c(2,3,4,5,6), groupLabel1="kidney",
genExpMatrix2=geneExpMatrix2, geneCol2=1, expCol2=c(2,3,4,5,6), groupLabel2="liver",
method="LRT", outputDir=outputDir)
cat("outputDir: ", outputDir, "\n")
```

### Description

This function is another (old) version of DEGexp. It takes the gene expression files as input instead of gene expression matrices.

### Usage

```r
DEGexp2(geneExpFile1, geneCol1=1, expCol1=2, depth1=rep(0, length(expCol1)), groupLabel1="group1",
geneExpFile2, geneCol2=1, expCol2=2, depth2=rep(0, length(expCol2)), groupLabel2="group2",
header=TRUE, sep="", method=c("LRT", "CTR", "FET", "MARS", "MATR", "FC"),
pValue=1e-3, zScore=4, qValue=1e-3, foldChange=4,
thresholdKind=1, outputDir="none", normalMethod=c("none", "loess", "median"),
replicate1="none", geneColR1=1, expColR1=2, depthR1=rep(0, length(expColR1)), replicateLabel1="r",
replicate2="none", geneColR2=1, expColR2=2, depthR2=rep(0, length(expColR2)), replicateLabel2="r")
```

### Arguments

- **geneExpFile1**: file containing gene expression values for replicates of sample1 (or replicate1 when method="CTR").
- **geneCol1**: gene id column in geneExpFile1.
- **expCol1**: expression value columns in geneExpFile1 for replicates of sample1 (numeric vector).
  
  **Note**: Each column corresponds to a replicate of sample1.
- **depth1**: the total number of reads uniquely mapped to genome for each replicate of sample1 (numeric vector),
  default: take the total number of reads mapped to all annotated genes as the depth for each replicate.
groupLabel1  label of group1 on the plots.
geneExpFile2  file containing gene expression values for replicates of sample2 (or replicate2 when method="CTR").
geneCol2  gene id column in geneExpFile2.
expCol2  expression value columns in geneExpFile2 for replicates of sample2 (numeric vector).
Note: Each column corresponds to a replicate of sample2.
depth2  the total number of reads uniquely mapped to genome for each replicate of sample2 (numeric vector),
default: take the total number of reads mapped to all annotated genes as the depth for each replicate.
groupLabel2  label of group2 on the plots.
header  a logical value indicating whether geneExpFile1 and geneExpFile2 contain the names of the variables as its first line. See \texttt{read.table}.
sep  the field separator character. If sep = " " (the default for read.table) the separator is white space, that is one or more spaces, tabs, newlines or carriage returns. See \texttt{read.table}.
method  method to identify differentially expressed genes. Possible methods are:
\begin{itemize}
  \item "LRT": Likelihood Ratio Test (Marioni et al. 2008),
  \item "CTR": Check whether the variation between Technical Replicates can be explained by the random sampling model (Wang et al. 2009),
  \item "FET": Fisher's Exact Test (Joshua et al. 2009),
  \item "MARS": MA-plot-based method with Random Sampling model (Wang et al. 2009),
  \item "MATR": MA-plot-based method with Technical Replicates (Wang et al. 2009),
  \item "FC": Fold-Change threshold on MA-plot.
\end{itemize}
pValue  pValue threshold (for the methods: LRT, FET, MARS, MATR).
only used when thresholdKind=1.
zScore  zScore threshold (for the methods: MARS, MATR).
only used when thresholdKind=2.
qValue  qValue threshold (for the methods: LRT, FET, MARS, MATR).
only used when thresholdKind=3 or thresholdKind=4.
thresholdKind  the kind of threshold. Possible kinds are:
\begin{itemize}
  \item 1: pValue threshold,
  \item 2: zScore threshold,
  \item 3: qValue threshold (Benjamini et al. 1995),
  \item 4: qValue threshold (Storey et al. 2003),
  \item 5: qValue threshold (Storey et al. 2003) and Fold-Change threshold on MA-plot are both required (can be used only when method="MARS").
\end{itemize}
foldChange  fold change threshold on MA-plot (for the method: FC).
outputDir  the output directory.
normalMethod: the normalization method: "none", "loess", "median" (Yang et al. 2002). recommend: "none".

replicate1: file containing gene expression values for replicate batch1 (only used when method="MATR").
Note: replicate1 and replicate2 are two (groups of) technical replicates of a sample.

geneColR1: gene id column in the expression file for replicate batch1 (only used when method="MATR").
expColR1: expression value columns in the expression file for replicate batch1 (numeric vector) (only used when method="MATR").
depthR1: the total number of reads uniquely mapped to genome for each replicate in replicate batch1 (numeric vector), default: take the total number of reads mapped to all annotated genes as the depth for each replicate (only used when method="MATR").
replicateLabel1: label of replicate batch1 on the plots (only used when method="MATR").

replicate2: file containing gene expression values for replicate batch2 (only used when method="MATR").
Note: replicate1 and replicate2 are two (groups of) technical replicates of a sample.

geneColR2: gene id column in the expression file for replicate batch2 (only used when method="MATR").
expColR2: expression value columns in the expression file for replicate batch2 (numeric vector) (only used when method="MATR").
depthR2: the total number of reads uniquely mapped to genome for each replicate in replicate batch2 (numeric vector), default: take the total number of reads mapped to all annotated genes as the depth for each replicate (only used when method="MATR").
replicateLabel2: label of replicate batch2 on the plots (only used when method="MATR").

rawCount: a logical value indicating the gene expression values are based on raw read counts or normalized values.

References


**See Also**

DEGexp, DEGseq, getGeneExp, readGeneExp, GeneExpExample1000, GeneExpExample5000.

**Examples**

```r
## kidney: R1L1Liver, R1L3Liver, R1L7Liver, R2L2Liver, R2L6Liver
## liver: R1L2Liver, R1L4Liver, R1L6Liver, R1L8Liver, R2L3Liver
geneExpFile <- system.file("extdata", "GeneExpExample5000.txt", package="DEGseq")
outputDir <- file.path(tempdir(), "DEGexpExample")
exp <- readGeneExp(file=geneExpFile, geneCol=1, valCol=c(7,9,12,15,18))
exp[30:35,]

DEGexp2(geneExpFile1=geneExpFile, geneCol1=1, expCol1=c(7,9,12,15,18), groupLabel1="kidney",
geneExpFile2=geneExpFile, geneCol2=1, expCol2=c(8,10,11,13,16), groupLabel2="liver",
method="MARS", outputDir=outputDir)
cat("outputDir:", outputDir, 
"
")
```

---

**DEGseq**

**DEGseq: Identify Differentially Expressed Genes from RNA-seq data**

**Description**

This function is used to identify differentially expressed genes from RNA-seq data. It takes uniquely mapped reads from RNA-seq data for the two samples with a gene annotation as input. So users should map the reads (obtained from sequencing libraries of the samples) to the corresponding genome in advance.

**Usage**

```r
DEGseq(mapResultBatch1, mapResultBatch2, fileFormat="bed", readLength=32,
strandInfo=FALSE, refFlat, groupLabel1="group1", groupLabel2="group2",
method=c("LRT", "CTR", "FET", "MARS", "MATR", "FC"),
pValue=1e-3, zScore=4, qValue=1e-3, foldChange=4, thresholdKind=1,
outputDir="none", normalMethod=c("none", "loess", "median"),
depthKind=1, replicate1="none", replicate2="none",
replicateLabel1="replicate1", replicateLabel2="replicate2")
```
Arguments

mapResultBatch1
vector containing uniquely mapping result files for technical replicates of sample1 (or replicate1 when method="CTR").

mapResultBatch2
vector containing uniquely mapping result files for technical replicates of sample2 (or replicate2 when method="CTR").

fileFormat
file format: "bed" or "eland".
example of "bed" format: chr12 7 38 readID 2 +
example of "eland" format: readID chr12.fa 7 U2 F

Note: The field separator character is TAB. And the files must follow the format as one of the examples.

readLength
the length of the reads (only used if fileFormat="eland").

strandInfo
whether the strand information was retained during the cloning of the cDNAs.

• "TRUE": retained,
• "FALSE": not retained.

refFlat
gene annotation file in UCSC refFlat format.
See http://genome.ucsc.edu/goldenPath/gbdDescriptionsOld.html#RefFlat.

groupLabel1
label of group1 on the plots.

groupLabel2
label of group2 on the plots.

method
method to identify differentially expressed genes. Possible methods are:

• "LRT": Likelihood Ratio Test (Marioni et al. 2008),
• "CTR": Check whether the variation between two Technical Replicates can be explained by the random sampling model (Wang et al. 2009),
• "FET": Fisher’s Exact Test (Joshua et al. 2009),
• "MARS": MA-plot-based method with Random Sampling model (Wang et al. 2009),
• "MATR": MA-plot-based method with Technical Replicates (Wang et al. 2009),
• "FC": Fold-Change threshold on MA-plot.

pValue
pValue threshold (for the methods: LRT, FET, MARS, MATR).
only used when thresholdKind=1.

zScore
zScore threshold (for the methods: MARS, MATR).
only used when thresholdKind=2.

qValue
qValue threshold (for the methods: LRT, FET, MARS, MATR).
only used when thresholdKind=3 or thresholdKind=4.

thresholdKind
the kind of threshold. Possible kinds are:

• 1: pValue threshold,
• 2: zScore threshold,
• 3: qValue threshold (Benjamini et al. 1995),
• 4: qValue threshold (Storey et al. 2003),
5: qValue threshold (Storey et al. 2003) and Fold-Change threshold on MA-plot are both required (can be used only when method="MARS").

**foldChange**
fold change threshold on MA-plot (for the method: FC).

**outputDir**
the output directory.

**normalMethod**
the normalization method: "none", "loess", "median" (Yang,Y.H. et al. 2002). recommend: "none".

**depthKind**
1: take the total number of reads uniquely mapped to genome as the depth for each replicate,
0: take the total number of reads uniquely mapped to all annotated genes as the depth for each replicate.
We recommend taking depthKind=1, especially when the genes in annotation file are part of all genes.

**replicate1**
files containing uniquely mapped reads obtained from replicate batch1 (only used when method="MTR").

**replicate2**
files containing uniquely mapped reads obtained from replicate batch2 (only used when method="MTR").

**replicateLabel1**
label of replicate batch1 on the plots (only used when method="MTR").

**replicateLabel2**
label of replicate batch2 on the plots (only used when method="MTR").

**References**


**See Also**

DEGexp, getGeneExp, readGeneExp, kidneyChr21.bed, liverChr21.bed, refFlatChr21.
Examples

```r
kidneyR1L1 <- system.file("extdata", "kidneyChr21.bed.txt", package="DEGseq")
liverR1L2 <- system.file("extdata", "liverChr21.bed.txt", package="DEGseq")
refFlat <- system.file("extdata", "refFlatChr21.txt", package="DEGseq")
mapResultBatch1 <- c(kidneyR1L1)  ## only use the data from kidneyR1L1 and liverR1L2
mapResultBatch2 <- c(liverR1L2)
outputDir <- file.path(tempdir(), "DEGseqExample")
DEGseq(mapResultBatch1, mapResultBatch2, fileFormat="bed", refFlat=refFlat,
       outputDir=outputDir, method="LRT")
cat("outputDir:", outputDir, "\n")
```

Description

GeneExpExample1000.txt includes the first 1000 lines in SupplementaryTable2.txt which is a supplementary file for Marioni, J.C. et al. (2008) (http://genome.cshlp.org/content/18/9/1509/suppl/DC1).

References


See Also

DEGexp, getGeneExp, readGeneExp, GeneExpExample5000.

Description

GeneExpExample5000.txt includes the first 5000 lines in SupplementaryTable2.txt which is a supplementary file for Marioni, J.C. et al. (2008) (http://genome.cshlp.org/content/18/9/1509/suppl/DC1).

References


See Also

DEGexp, getGeneExp, readGeneExp, GeneExpExample1000.
getGeneExp

getGeneExp: Count the number of reads and calculate the RPKM for each gene

Description

This function is used to count the number of reads and calculate the RPKM for each gene. It takes uniquely mapped reads from RNA-seq data for a sample with a gene annotation file as input. So users should map the reads (obtained from sequencing library of the sample) to the corresponding genome in advance.

Usage

getGeneExp(mapResultBatch, fileFormat="bed", readLength=32, strandInfo=FALSE, refFlat, output=paste(mapResultBatch[1],".exp",sep=""), min.overlapPercent=1)

Arguments

mapResultBatch vector containing uniquely mapping result files for a sample. 
   Note: The sample can have multiple technical replicates.

fileFormat file format: "bed" or "eland".
   example of "bed" format: chr12 7 38 readID 2 +
   example of "eland" format: readID chr12.fa 7 U2 F
   Note: The field separator character is TAB. And the files must follow the format as one of the examples.

readLength the length of the reads (only used if fileFormat="eland").

strandInfo whether the strand information was retained during the cloning of the cDNAs.
   • "TRUE": retained,
   • "FALSE": not retained.

refFlat gene annotation file in UCSC refFlat format.
   See http://genome.ucsc.edu/goldenPath/gbdDescriptionsOld.html#RefFlat.

output the output file.

min.overlapPercent the minimum percentage of the overlapping length for a read and an exon over the length of the read itself, for counting this read from the exon. should be <=1.
   0: at least 1 bp overlap between a read and an exon.

Note

This function sums up the numbers of reads coming from all exons of a specific gene (according to the known gene annotation) as the gene expression value. The exons may include the 5'-UTR, protein coding region, and 3'-UTR of a gene. All introns are ignored for a gene for the sequenced reads are from the spliced transcript library. If a read falls in an exon (usually, a read is shorter than an exon), the read count for this exon plus 1. If a read is crossing the boundary of an exon, users can tune the parameter min.overlapPercent, which is the minimum percentage of the overlapping length for a read and an exon over the length of the read itself, for counting this read from the exon. The method use the union of all possible exons for calculating the length for each gene.
References


See Also

DEGexp, DEGseq, readGeneExp, kidneyChr21.bed, liverChr21.bed, refFlatChr21.

Examples

kidneyR1L1 <- system.file("extdata", "kidneyChr21.bed.txt", package="DEGseq")
refFlat <- system.file("extdata", "refFlatChr21.txt", package="DEGseq")
mapResultBatch <- list(kidneyR1L1)
output <- file.path(tempdir(), "kidneyChr21.bed.exp")
exp <- getGeneExp(mapResultBatch, refFlat=refFlat, output=output)
write.table(exp[30:35,], row.names=FALSE)
cat("output: ", output, "\n")

Description

The reads uniquely mapped to human chromosome 21 obtained from the kidney sample sequenced in Run 1, Lane 1.

References


See Also

DEGexp, DEGseq, getGeneExp, readGeneExp, liverChr21.bed, refFlatChr21.

Description

The reads uniquely mapped to human chromosome 21 obtained from the kidney sample sequenced in Run 1, Lane 1.

References

See Also

DEGexp, DEGseq, getGeneExp, readGeneExp, liverChr21.bed, refFlatChr21.

liverChr21.bed

Description

The reads uniquely mapped to human chromosome 21 obtained from the liver sample sequenced in Run 1, Lane 2.

References


See Also

DEGexp, DEGseq, getGeneExp, readGeneExp, kidneyChr21.bed, refFlatChr21.

liverChr21Bowtie

Description

The reads uniquely mapped to human chromosome 21 obtained from the liver sample sequenced in Run 1, Lane 2.

References


See Also

DEGexp, DEGseq, getGeneExp, readGeneExp, kidneyChr21.bed, refFlatChr21.
readGeneExp

**Description**

This method is used to read gene expression values from a file to a matrix in R workspace. So that the matrix can be used as input of other packages, such as `edgeR`. The input of the method is a file that contains gene expression values.

**Usage**

```r
readGeneExp(file, geneCol=1, valCol=2, label = NULL, header=TRUE, sep="")
```

**Arguments**

- **file**: file containing gene expression values.
- **geneCol**: gene id column in file.
- **valCol**: expression value columns to be read in the file.
- **label**: label for the columns.
- **header**: a logical value indicating whether the file contains the names of the variables as its first line. See ?read.table.
- **sep**: the field separator character. If sep = "" (the default for read.table) the separator is white space, that is one or more spaces, tabs, newlines or carriage returns. See ?read.table.

**See Also**

`getGeneExp`, `GeneExpExample1000`, `GeneExpExample5000`.

**Examples**

```r
## If the data files are collected in a zip archive, the following
## commands will first extract them to the temporary directory.

geneExpFile <- system.file("extdata", "GeneExpExample1000.txt", package="DEGseq")
exp <- readGeneExp(file=geneExpFile, geneCol=1, valCol=c(7,9,12,15,18,8,10,11,13,16))
exp[30:35,]
```
Description

The gene annotation file includes the annotations of genes on chromosome 21, and is in UCSC refFlat format. See http://genome.ucsc.edu/goldenPath/gbdDescriptionsOld.html#RefFlat.

See Also

DEGseq, DEGexp, kidneyChr21.bed, liverChr21.bed.
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