Package 'DEGseq'

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Title Identify Differentially Expressed Genes from RNA-seq data

Version 1.24.0

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

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Depends R (>= 2.8.0), qvalue, samr, methods

Imports graphics, grDevices, methods, stats, utils

License LGPL (>=2)

Collate AllClasses.R AllGenerics.R Bind.R methodPlots.R NormMethods.R
   functions.R IdentifyDiffExpGenes.R MainFunction.R
   MainFunctionWrap.R samWrapper.R MainFunction2.R

LazyLoad yes

biocViews RNASeq, Preprocessing, GeneExpression,
   DifferentialExpression

NeedsCompilation yes

R topics documented:

DEGexp   *DEGexp: Identifying Differentially Expressed Genes from gene ex-*
                                pression 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

```
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)), replicateL
      replicateExpMatrix2=NULL, geneColR2=1, expColR2=2, depthR2=rep(0, length(expColR2)), replicateL
```

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), 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.
geneExpMatrix2	gene expression matrix for replicates of sample2 (or replicate2 when method="CTR").
geneCol2	gene id column in geneExpMatrix2.
expCol2	expression value *columns* in geneExpMatrix2 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.
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 repli-
                cate 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

Benjamini,Y. and Hochberg,Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* **57**, 289-300.

Jiang,H. and Wong,W.H. (2008) Statistical inferences for isoform expression in RNA-seq. *Bioinformatics*, **25**, 1026-1032.

Bloom,J.S. et al. (2009) Measuring differential gene expression by short read sequencing: quantitative comparison to 2-channel gene expression microarrays. *BMC Genomics*, **10**, 221.

Marioni,J.C. et al. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.*, **18**, 1509-1517.

Storey,J.D. and Tibshirani,R. (2003) Statistical significance for genomewide studies. *Proc. Natl. Acad. Sci.* **100**, 9440-9445.

Wang,L.K. and et al. (2010) DEGseq: an R package for identifying differentially expressed genes from RNA-seq data, *Bioinformatics* **26**, 136 - 138.

Yang,Y.H. et al. (2002) Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Research*, **30**, e15.

See Also

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

Examples

```
## 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")
geneExpMatrix1 <- readGeneExp(file=geneExpFile, geneCol=1, valCol=c(7,9,12,15,18))
geneExpMatrix2 <- readGeneExp(file=geneExpFile, geneCol=1, valCol=c(8,10,11,13,16))
```

```
geneExpMatrix1[30:32,]
geneExpMatrix2[30:32,]
DEGexp(geneExpMatrix1=geneExpMatrix1, geneCol1=1, expCol1=c(2,3,4,5,6), groupLabel1="kidney",
       geneExpMatrix2=geneExpMatrix2, geneCol2=1, expCol2=c(2,3,4,5,6), groupLabel2="liver",
         method="LRT", outputDir=outputDir)
cat("outputDir:", outputDir, "\n")
```

DEGexp2	*DEGexp2: Identifying Differentially Expressed Genes from gene expression data*

Description

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

Usage

```
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=
        replicate2="none", geneColR2=1, expColR2=2, depthR2=rep(0, length(expColR2)), replicateLabel2=
```

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 ?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.
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".
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

Benjamini,Y. and Hochberg,Y (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* **57**, 289-300.

Jiang,H. and Wong,W.H. (2008) Statistical inferences for isoform expression in RNA-seq. *Bioinformatics*, **25**, 1026-1032.

Bloom,J.S. et al. (2009) Measuring differential gene expression by short read sequencing: quantitative comparison to 2-channel gene expression microarrays. *BMC Genomics*, **10**, 221.

Marioni,J.C. et al. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.*, **18**, 1509-1517.

Storey,J.D. and Tibshirani,R. (2003) Statistical significance for genomewide studies. *Proc. Natl. Acad. Sci.* **100**, 9440-9445.

Wang,L.K. and et al. (2010) DEGseq: an R package for identifying differentially expressed genes from RNA-seq data, *Bioinformatics* **26**, 136 - 138.

Yang,Y.H. et al. (2002) Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Research*, **30**, e15.

See Also

[DEGexp](), [DEGseq](), [getGeneExp](), [readGeneExp](), [GeneExpExample1000](), [GeneExpExample5000]().

Examples

```
## kidney: R1L1Kidney, R1L3Kidney, R1L7Kidney, R2L2Kidney, R2L6Kidney
## 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,]
exp <- readGeneExp(file=geneExpFile, geneCol=1, valCol=c(8,10,11,13,16))
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, "\n")
```

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

```
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="MATR"`).

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

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

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

References

Benjamini,Y. and Hochberg,Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. R. Stat. Soc. Ser. B* **57**, 289-300.

Jiang,H. and Wong,W.H. (2009) Statistical inferences for isoform expression in RNA-seq. *Bioinformatics*, **25**, 1026-1032.

Bloom,J.S. et al. (2009) Measuring differential gene expression by short read sequencing: quantitative comparison to 2-channel gene expression microarrays. *BMC Genomics*, **10**, 221.

Marioni,J.C. et al. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.*, **18**, 1509-1517.

Storey,J.D. and Tibshirani,R. (2003) Statistical significance for genomewide studies. *Proc. Natl. Acad. Sci.* **100**, 9440-9445.

Wang,L.K. and et al. (2010) DEGseq: an R package for identifying differentially expressed genes from RNA-seq data, *Bioinformatics* **26**, 136 - 138.

Yang,Y.H. et al. (2002) Normalization for cDNA microarray data: a robust composite method addressing single and multiple slide systematic variation. *Nucleic Acids Research*, **30**, e15.

See Also

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

Examples

```
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")
```

GeneExpExample1000 *GeneExpExample1000*

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

Marioni,J.C. et al. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.*, **18**, 1509-1517.

See Also

DEGexp, getGeneExp, readGeneExp, samWrapper, GeneExpExample5000.

GeneExpExample5000 *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

Marioni,J.C. et al. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.*, **18**, 1509-1517.

See Also

DEGexp, getGeneExp, readGeneExp, samWrapper, 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

Mortazavi,A. et al. (2008) Mapping and quantifying mammalian transcriptomes by RNA-seq. *Nat. Methods*, **5**, 621-628.

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")
```

---

kidneyChr21.bed   *kidneyChr21.bed*

Description

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

References

Marioni,J.C. et al. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.*, **18**, 1509-1517.

See Also

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

---

kidneyChr21Bowtie   *kidneyChr21Bowtie*

Description

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

References

Marioni,J.C. et al. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.*, **18**, 1509-1517.

See Also

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

---

liverChr21.bed   *liverChr21.bed*

Description

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

References

Marioni,J.C. et al. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.*, **18**, 1509-1517.

See Also

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

---

liverChr21Bowtie   *liverChr21Bowtie*

Description

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

References

Marioni,J.C. et al. (2008) RNA-seq: an assessment of technical reproducibility and comparison with gene expression arrays. *Genome Res.*, **18**, 1509-1517.

See Also

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

---

readGeneExp   *readGeneExp: read gene expression values to a matrix*

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

```
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

```
## 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,]
```

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refFlatChr21 *refFlatChr21*

Description

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

See Also

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

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samWrapper *samWrapper: A Wrapper (with some modification) of the functions in the package samr to identify differentially expressed genes for the RNA-seq data from two groups of paired or unpaired samples.*

Description

This function is a wrapper of the functions in *samr*. It is used to identify differentially expressed genes for two sets of samples with multiple replicates or two groups of samples from different individuals (e.g. disease samples vs. control samples). For the advanced users, please see *samr* http://cran.r-project.org/web/packages/samr/index.html for detail.

Usage

```
samWrapper(geneExpFile1, geneCol1=1, expCol1=2, measure1=rep(1, length(expCol1)),
          geneExpFile2, geneCol2=1, expCol2=2, measure2=rep(2, length(expCol2)),
           header=TRUE, sep="", paired=FALSE, s0=NULL, s0.perc=NULL, nperms=100,
         testStatistic=c("standard","wilcoxon"), max.qValue=1e-3, min.foldchange=0,
            logged2=FALSE, output)
```

Arguments

geneExpFile1	file containing gene expression values for group1.
geneCol1	gene id column in geneExpFile1.
expCol1	expression value *columns* in geneExpFile1. See the example.
measure1	numeric vector of outcome measurements for group1. like c(1,1,1...) when paired=FALSE, or like c(-1,-2,-3,...) when paired=TRUE.
geneExpFile2	file containing gene expression values for group2.
geneCol2	gene id column in geneExpFile2.
expCol2	expression value *columns* in geneExpFile2. See the example.
measure2	numeric vector of outcome measurements for group2. like c(2,2,2...) when paired=FALSE, or like c(1,2,3,...) when paired=TRUE.
header	a logical value indicating whether geneExpFile1 and geneExpFile2 contain 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.
paired	a logical value indicating whether the samples are paired.
s0	exchangeability factor for denominator of test statistic; Default is automatic choice.
s0.perc	percentile of standard deviation values to use for s0; default is automatic choice; -1 means s0=0 (different from s0.perc=0, meaning s0=zeroeth percentile of standard deviation values= min of sd values.
nperms	number of permutations used to estimate false discovery rates.
testStatistic	test statistic to use in two class unpaired case. Either "standard" (t-statistic) or "wilcoxon" (Two-sample wilcoxon or Mann-Whitney test). recommend "standard".
max.qValue	the max qValue desired; shoube be <1.
min.foldchange	the minimum fold change desired; should be >1. default is zero, meaning no fold change criterion is applied.
logged2	a logical value indicating whether the expression values are logged2.
output	the output file.

References

Tusher,V., and et al. (2001): Significance analysis of microarrays applied to the ionizing radiation response *PNAS* **98**, 5116-5121.

Tibshirani,R, and et al.: samr http://cran.r-project.org/web/packages/samr/index.html.

A more complete description is given in the SAM manual at http://www-stat.stanford.edu/~tibs/SAM.

See Also

DEGexp, DEGseq, GeneExpExample1000, GeneExpExample5000.

Examples

```
## 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")
set.seed(100)
geneExpFile1 <- geneExpFile
geneExpFile2 <- geneExpFile
output <- file.path(tempdir(), "samWrapperOut.txt")
exp <- readGeneExp(file=geneExpFile, geneCol=1, valCol=c(7,9,12,15,18))
exp[30:35,]
exp <- readGeneExp(file=geneExpFile, geneCol=1, valCol=c(8,10,11,13,16))
exp[30:35,]
samWrapper(geneExpFile1=geneExpFile1, geneCol1=1, expCol1=c(7,9,12,15,18), measure1=c(-1,-2,-3,-4,-5),
           geneExpFile2=geneExpFile2, geneCol2=1, expCol2=c(8,10,11,13,16), measure2=c(1,2,3,4,5),
              nperms=100, min.foldchange=2, max.qValue=1e-4, output=output, paired=TRUE)
cat("output:", output, "\n")
```

Index