

# Package ‘BitSeq’

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**Type** Package

**Title** Transcript expression inference and differential expression analysis for RNA-seq data

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**Description** The BitSeq package is targeted for transcript expression analysis and differential expression analysis of RNA-seq data in two stage process. In the first stage it uses Bayesian inference methodology to infer expression of individual transcripts from individual RNA-seq experiments. The second stage of BitSeq embraces the differential expression analysis of transcript expression. Providing expression estimates from replicates of multiple conditions, Log-Normal model of the estimates is used for inferring the condition mean transcript expression and ranking the transcripts based on the likelihood of differential expression.

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**biocViews** ImmunoOncology, GeneExpression, DifferentialExpression, Sequencing, RNASeq, Bayesian, AlternativeSplicing, DifferentialSplicing, Transcription

**Depends** Rsamtools (>= 1.99.3)

**Imports** S4Vectors, IRanges, methods, utils

**LinkingTo** Rhtslib (>= 1.15.5)

**SystemRequirements** GNU make

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## R topics documented:

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|                |   |
|----------------|---|
| BitSeq-package | <i>Bayesian Inference of Transcripts from Sequencing data</i> |
|----------------|---|

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## Description

The BitSeq package is targeted for transcript expression analysis and differential expression analysis of RNA-seq data in two stage process.

In the first stage it uses Bayesian inference methodology to infer expression of individual transcripts from individual RNA-seq experiments.

The second stage of BitSeq embraces the differential expression analysis of transcript expression. Providing expression estimates from replicates of multiple conditions, Log-Normal model of the estimates is used for inferring the condition mean transcript expression and ranking the transcripts based on the likelihood of differential expression.

## Author(s)

Peter Glaus, Antti Honkela and Magnus Rattray Maintainer: Peter Glaus <glaus@cs.man.ac.uk>

## References

Glaus, P., Honkela, A. and Rattray, M. (2012). Identifying differentially expressed transcripts from RNA-seq data with biological variation. *Bioinformatics*, 28(13), 1721-1728.

**Examples**

```
## Not run:
## basic use
res1 <- getExpression("data-c0b0.sam", "ensSelect1.fasta")
res2 <- getExpression("data-c0b1.sam", "ensSelect1.fasta")
res3 <- getExpression("data-c1b0.sam", "ensSelect1.fasta")
res4 <- getExpression("data-c1b1.sam", "ensSelect1.fasta")

deRes <- getDE( list(c(res1$fn, res2$fn),
                    c(res3$fn, res4$fn)) )
## top 10 differentially expressed
head(deRes$pplr[ order(abs(0.5-deRes$pplr$pplr), decreasing=TRUE ), ], 10)

## advanced use, keeping the intermediate files
parseAlignment( "data-c0b0.sam",
  outFile = "data-c0b0.prob",
  trSeqFile = "ensSelect1.fasta",
  trInfoFile = "data.tr",
  uniform = TRUE,
  verbose = TRUE )

estimateExpression( "data-c0b0.prob",
  outFile = "data-c0b0",
  outputType = "RPKM",
  trInfoFile = "data.tr",
  MCMC_burnIn = 200,
  MCMC_samplesN = 200,
  MCMC_samplesSave = 100,
  MCMC_scaleReduction = 1.1,
  MCMC_chainsN = 2 )

cond1Files = c("data-c0b0.rpkm", "data-c0b1.rpkm")
cond2Files = c("data-c1b1.rpkm", "data-c1b1.rpkm")
allConditions = list(cond1Files, cond2Files)

getMeanVariance( allConditions,
  outFile = "data.means",
  log = TRUE )

estimateHyperPar( allConditions,
  outFile = "data.par",
  meanFile = "data.means",
  verbose = TRUE )

estimateDE( allConditions,
  outFile = "data",
  parFile = "data.par" )

## End(Not run)
```

---

|            |   |
|------------|---|
| estimateDE | <i>Estimate condition mean expression and calculate Probability of Positive Log Ratio(PPLR)</i> |
|------------|---|

---

### Description

Estimate condition mean expression for both experimental conditions using the expression estimates obtained by [estimateExpression](#)

### Usage

```
estimateDE(conditions, outFile, parFile,
           lambda0=NULL, samples=NULL, confidencePerc=NULL,
           verbose=NULL, norm=NULL, seed=NULL, pretend=FALSE )
```

### Arguments

|                |  |
|----------------|--|
| conditions     | List of vectors, each vector containing names of files containing the expression samples from a replicate (Can be both technical and biological replicates. However, in order to get good results biological replicates for each condition are essential). |
| outFile        | Prefix for the output files.   |
| parFile        | File containing estimated hyperparameters.   |
| samples        | Produce samples of condition mean expression apart from PPLR and confidence.   |
| confidencePerc | Percentage for confidence intervals. (Default is 95%)  |
| verbose        | Verbose output. Advanced options:  |
| lambda0        | Model parameter lambda_0.  |
| norm           | Vector of (multiplicative) normalization constants for library size normalization of expression samples. Number of constants has to match the number of expression samples files.  |
| seed           | Sets the initial random seed for repeatable experiments.   |
| pretend        | Do not execute, only print out command line calls for the C++ version of the program.  |

### Details

This function takes as an input expression samples from biological replicates of two or more conditions and hyperparameters over precision distribution inferred by [estimateHyperPar](#). It uses pseudo-vectors of expression samples from all replicates to infer condition mean expression for each condition.

The condition mean expression samples are used for computation of the Probability of Positive Log Ratio (PPLR) as well as  $\log_2$  fold change of expression with confidence intervals and average

condition mean expression for each transcript. Optionally the function can produce also the samples of condition mean expression for each condition.

For more than one conditions, the comparison is done pairwise between all conditions ( $CP = \frac{C*(C-1)}{2}$  pairs), reporting: CPxPPLR CPx(log2FC ciLow ciHigh) CxMeanExpr.

### Value

.pplr file containing the PPLR, mean log2 fold change with confidence intervals, mean condition mean expressions

.est files containing samples of condition mean expressions for each condition - optional

.estVar file containing samples of inferred variance of the first condition - optional

### Author(s)

Peter Glaus

### See Also

[estimateExpression](#), [estimateHyperPar](#)

### Examples

```
## Not run:
cond1Files = c("data-c0b0.rpkm","data-c0b1.rpkm")
cond2Files = c("data-c1b0.rpkm","data-c1b1.rpkm")
estimateDE(conditions=list(cond1Files, cond2Files), outFile="data.pplr",
           parFile="data.par", norm=c(1.0, 0.999, 1.0017, 0.9998))

## End(Not run)
```

---

estimateExpression      *Estimate expression of transcripts*

---

### Description

Estimates the expression of transcripts using Markov chain Monte Carlo Algorithm

### Usage

```
estimateExpression(probFile, outFile, parFile=NULL, outputType=NULL, gibbs=NULL,
                  trInfoFile=NULL, thetaActFile=NULL, MCMC_burnIn=NULL, MCMC_samplesN=NULL,
                  MCMC_samplesSave=NULL, MCMC_chainsN=NULL, MCMC_dirAlpha=NULL, seed=NULL,
                  verbose=NULL, procN=NULL, pretend=FALSE)
estimateExpressionLegacy(probFile, outFile, parFile=NULL, outputType=NULL,
                        gibbs=NULL, trInfoFile=NULL, thetaActFile=NULL, MCMC_burnIn=NULL,
                        MCMC_samplesN=NULL, MCMC_samplesSave=NULL, MCMC_samplesNmax=NULL,
                        MCMC_chainsN=NULL, MCMC_scaleReduction=NULL, MCMC_dirAlpha=NULL,
                        seed=NULL, verbose=NULL, pretend=FALSE)
```

**Arguments**

|                     |   |
|---------------------|---|
| probFile            | File with alignment probabilities produced by parseAlignment  |
| outFile             | Prefix for the output files.  |
| outputType          | Output type, possible values: theta, RPKM, counts, tau.   |
| gibbs               | Use regular Gibbs sampling instead of Collapsed Gibbs sampling.   |
| parFile             | File containing parameters for the sampler, which can be otherwise specified by [MCMC*] options. As the file is checked after every MCMC iteration, the parameters can be adjusted while running. |
| trInfoFile          | File containing transcript information. (Necessary for RPKM)  |
| MCMC_burnIn         | Length of sampler's burn in period.   |
| MCMC_samplesN       | Initial number of samples produced. These are used either to estimate the number of necessary samples or to estimate possible scale reduction.  |
| MCMC_samplesSave    | Number of samples recorder at the end in total.   |
| MCMC_chainsN        | Number of parallel chains used. At least two chains will be used.   |
| seed                | Sets the initial random seed for repeatable experiments.  |
| verbose             | Verbose output.   |
| procN               | Maximum number of threads to be used. The program will not use more threads that there are MCMC chains. Advanced options:   |
| thetaActFile        | File for logging noise parameter thetaAct, which is only generated when regular Gibbs sampling is used.   |
| MCMC_dirAlpha       | Alpha parameter for the Dirichlet distribution.   |
| pretend             | Do not execute, only print out command line calls for the C++ version of the program.   |
| MCMC_scaleReduction | (Only for estimateExpressionLegacy.) Target scale reduction, sampler finishes after this value is met.  |
| MCMC_samplesNmax    | (Only for estimateExpressionLegacy.) Maximum number of samples produced in one iteration. After producing samplesNmax samples sampler finishes.   |

**Details**

This function runs Collapse Gibbs algorithm to sample the MCMC samples of transcript expression. The input is the .prob file containing alignment probabilities which were produced by [parseAlignment](#). Other optional input is the transcript information file specified by trInfoFile and again produced by parseAlignment.

The estimateExpression function first runs burn-in phase and initial iterations to estimate the properties of the MCMC sampling. The initial samples are used to estimate the number of samples necessary for generating MCMC\_samplesSave effective samples in the second, final, stage.

The estimateExpressionLegacy uses less efficient convergence checking via "scale reduction" estimation. After an iteration of generating MCMC\_samplesN samples, it estimates possible scale

reduction of the marginal posterior variance. While the possible scale reduction is high, it doubles the MCMC\_samplesN and starts new iteration. This process is repeated until desired value of MCMC\_scaleReduction is met, or MCMC\_samplesNmax samples are generated.

The sampling algorithm can be configured via parameters file parFile or by using the MCMC\* options. The advantage of using the file (at least an existing blank text document) is that by changing the configuration values while running, the new values do get updated after every iteration.

## Value

.thetaMeans      file containing average relative expression of transcripts  $\theta$

Either one of sample files based on output type selected:

.rpkm              for RPKM expression  
 .counts            for estimated read counts  
 .theta             for relative expression of fragments  
 .tau                for relative expression of transcripts

## Author(s)

Peter Glaus

## See Also

[parseAlignment](#)

## Examples

```
## Not run:
estimateExpression( probFile="data.prob", outFile="data", outputType="RPKM",
  trInfoFile="data.tr", seed=47, verbose=TRUE)
estimateExpression( probFile="data-c0b0.prob", outFile="data-c0b0", outputType="RPKM",
  trInfoFile="data.tr", MCMC_burnIn=200, MCMC_samplesN=200, MCMC_samplesSave=100,
  MCMC_chainsN=2 , MCMC_dirAlpha=NULL )
estimateExpression( probFile="data.prob", outFile="data-G", gibbs=TRUE,
  parFile="parameters1.txt", outputType="counts", trInfoFile="data.tr")
estimateExpressionLegacy( probFile="data-c0b0.prob", outFile="data-c0b0", outputType="RPKM",
  trInfoFile="data.tr", MCMC_burnIn=200, MCMC_samplesN=200, MCMC_samplesSave=100,
  MCMC_samplesNmax=10000, MCMC_scaleReduction=1.2, MCMC_chainsN=2 , MCMC_dirAlpha=NULL )

## End(Not run)
```

---

|                  |   |
|------------------|---|
| estimateHyperPar | <i>Estimate hyperparameters for DE model using expression samples and joint mean expression</i> |
|------------------|---|

---

### Description

Estimate hyperparameters for the Differential Expression model using expression samples and produced smoothed values of the hyperparameters depending on joint mean expression.

### Usage

```
estimateHyperPar( outFile, conditions=NULL, paramsInFile=NULL,
  meanFile=NULL, force=TRUE, exThreshold=NULL, lambda0=NULL,
  paramsAllFile=NULL, smoothOnly=NULL, lowess_f=NULL, lowess_steps=NULL,
  verbose=NULL, veryVerbose=NULL, norm=NULL, seed=NULL, pretend=FALSE )
```

### Arguments

|               |  |
|---------------|--|
| outFile       | Name of the output file.   |
| conditions    | List of vectors, each vector containing names of files containing the expression samples from a replicate (Can be both technical and biological replicates. However, in order to get good results biological replicates for each condition are essential). |
| paramsInFile  | File produced by previous run of the function using paramsAllFile flag.  |
| meanFile      | Name of the file containing joint mean and variance.   |
| exThreshold   | Threshold of lowest expression for which the estimation is done.   |
| paramsAllFile | Name of the file to which to store all parameter values generated prior to lowess smoothing(good for later, more careful re-smoothing.)  |
| smoothOnly    | Input file contains previously sampled hyperparameters which should smoothed only.   |
| verbose       | Verbose output. Advanced options:  |
| force         | Force smoothing hyperparameters, otherwise program might not produce parameters file at the end.   |
| lambda0       | Model parameter lambda0.   |
| lowess_f      | Parameter F for lowess smoothing specifying amount of smoothing.   |
| lowess_steps  | Parameter Nsteps for lowess smoothing specifying number of iterations.   |
| veryVerbose   | More verbose output.   |
| norm          | Vector of (multiplicative) normalization constants for library size normalization of expression samples. Number of constants has to match the number of expression samples files.  |
| seed          | Sets the initial random seed for repeatable experiments.   |
| pretend       | Do not execute, only print out command line calls for the C++ version of the program.  |

**Value**

.par file containing the smoothed hyperparameters  
 .ALLpar file containing all hyperparameter samples prior to smoothing - optional

**Author(s)**

Peter Glaus

**See Also**

[estimateDE](#)

**Examples**

```
## Not run:
cond1Files = c("data-c0b0.rpkm","data-c0b1.rpkm")
cond2Files = c("data-c1b0.rpkm","data-c1b1.rpkm")
estimateHyperPar( conditions=list(cond1Files, cond2Files), outFile="data.par",
  meanFile="data.means", verbose=TRUE)

estimateHyperPar( conditions=list(cond1Files, cond2Files), outFile="data.par",
  meanFile="data.means", paramsFile="data.ALLpar", force=FALSE)
estimateHyperPar( outFile="data.par", paramsInFile="data.ALLpar", smoothOnly=TRUE )

## End(Not run)
```

---

estimateVBExpression *Estimate expression of transcripts using VB*

---

**Description**

Estimates the expression of transcripts using Variational Bayes inference algorithm

**Usage**

```
estimateVBExpression (probFile, outFile, outputType=NULL, trInfoFile=NULL,
  seed=NULL, samples=NULL, optLimit=1e-5, optMethod="FR", procN=4,
  verbose=FALSE, veryVerbose=FALSE, pretend=FALSE)
```

**Arguments**

probFile File with alignment probabilities produced by parseAlignment  
 outFile Prefix for the output files.  
 outputType Output type, possible values: theta, RPKM, counts. This is only relevant when the samples option is used. Default: theta.  
 trInfoFile File containing transcript information. (Necessary for RPKM output)  
 seed Sets the initial random seed for repeatable experiments.

|             |   |
|-------------|---|
| samples     | Number of samples to be generated from the posterior distribution. Default: no samples are generated.                     |
| verbose     | Verbose output.   |
| veryVerbose | Very verbose output.  |
| procN       | Maximum number of threads to be used. The program will not use more threads than there are MCMC chains. Advanced options: |
| optLimit    | The optimisation limit in terms of minimal gradient or change of bound.   |
| optMethod   | The optimisation method, use "FR", "HR", or "steepest".   |
| pretend     | Do not execute, only print out command line calls for the C++ version of the program.                                     |

### Details

This function runs Variational Bayes algorithm to estimate the transcript expression. The input is the .prob file containing alignment probabilities which were produced by [parseAlignment](#). Other optional input is the transcript information file specified by trInfoFile and again produced by [parseAlignment](#).

It is much faster inference than MCMC which estimates mean expression equally well. However, the posterior is in form of Dirichlet distribution with underestimated variance. Use this method in cases when you are only interested in mean expression.

### Value

.m\_alphas file containing mean relative expression of transcripts  $\theta$  and parameters of the Dirichlet distribution. Please note the first line in the file corresponds to the noise transcript.

If option samples is used, the program also generates samples based of the outputType, the default would be file with extension ".VBtheta".

### Author(s)

Peter Glaus

### See Also

[parseAlignment](#), [estimateExpression](#)

### Examples

```
## Not run:
setwd(system.file("extdata",package="BitSeq"))
parseAlignment("data-c0b0.sam", outFile = "data-c0b0.prob", trSeqFile = "ensSelect1.fasta",
  trInfoFile = "data.tr", uniform = TRUE);

estimateVBExpression( probFile="data-c0b0.prob", outFile="data-c0b0-a", outputType="RPKM",
  samples=1000, trInfoFile="data.tr", seed=47, verbose=TRUE)
estimateVBExpression( probFile="data-c0b0.prob", outFile="data-c0b0-b", trInfoFile="data.tr")
estimateVBExpression( probFile="data-c0b0.prob", outFile="data-c0b0-c", trInfoFile="data.tr",
```

```

    optLimit=1e-6, optMethod = "HS", procN=12, veryVerbose=TRUE);

## End(Not run)

```

---

getDE *Estimate Probability of Positive Log Ratio*

---

### Description

Using expression samples, program estimates the probability of differential expression for each transcript.

### Usage

```

getDE(conditions, outPrefix=NULL, samples=FALSE, trInfoFile=NULL,
      norm=NULL, seed=NULL, pretend=FALSE )

```

### Arguments

|            |  |
|------------|--|
| conditions | List of vectors, each vector containing names of files containing the expression samples from a replicate (Can be both technical and biological replicates. However, in order to get good results biological replicates for each condition are essential). |
| outPrefix  | Prefix for the output files. Otherwise program creates temporary files, which are only valid for current R session.  |
| samples    | Produce samples of condition mean expression apart from PPLR and confidence.   |
| trInfoFile | Transcript information file providing the names of transcripts.  |
| norm       | Vector of (multiplicative) normalization constants for library size normalization of expression samples. Number of constants has to match the number of expression samples files.  |
| seed       | Sets the initial random seed for repeatable experiments.   |
| pretend    | Do not execute, only print out command line calls for the C++ version of the program.  |

### Details

This function uses `estimateHyperPar` function to estimate the hyperparameters for DE model and the uses `estimateDE` function to infer the condition mean expression and calculate Probability of Positive Log Ratio.

### Value

list with items:

|      |  |
|------|--|
| pplr | DataFrame with PPLR and other statistics   |
| fn   | list with file names for PPLR file, <code>fn\$pplr</code> , and condition mean expression samples, <code>fn\$samplesFiles</code> (only with option <code>samples=TRUE</code> ) |

**Author(s)**

Peter Glaus

**See Also**[getExpression](#), [estimateHyperPar](#), [estimateDE](#)**Examples**

```
## Not run:
cond1Files = c("data-c0b0.rpkm", "data-c0b1.rpkm")
cond2Files = c("data-c1b0.rpkm", "data-c1b1.rpkm")
deRes <- getDE( conditions=list(cond1Files, cond2Files))
## top 10 DE transcripts
head(deRes$pplr[ order(abs(0.5-deRes$pplr$pplr), decreasing=TRUE ), ], 10)

## End(Not run)
```

---

|                            |                                       |
|----------------------------|---------------------------------------|
| <code>getExpression</code> | <i>Estimate transcript expression</i> |
|----------------------------|---------------------------------------|

---

**Description**

Estimate expression of transcripts. Starting from alignment and reference files function handles the entire process of expression analysis resulting in transcript expression means and standard deviation together with file containing all the expression samples.

**Usage**

```
getExpression(alignFile, trSeqFile, outPrefix=NULL, uniform=TRUE, type="RPKM",
              log=FALSE, limitA=NULL, seed=NULL, pretend=FALSE, ... )
```

**Arguments**

|                        |  |
|------------------------|--|
| <code>alignFile</code> | File containing read alignments.   |
| <code>trSeqFile</code> | File containing transcript sequence in FASTA format.   |
| <code>outPrefix</code> | Prefix for the output files. Otherwise program creates temporary files, which are only valid for current R session.            |
| <code>uniform</code>   | Use uniform read distribution.   |
| <code>type</code>      | Output type, possible values: theta, RPKM, counts, tau.  |
| <code>log</code>       | Report mean and expression of logged expression samples.   |
| <code>limitA</code>    | Limit maximum number of alignments per read. Reads with more alignments than limit will be discarded.                          |
| <code>seed</code>      | Sets the initial random seed for repeatable experiments.   |
| <code>pretend</code>   | Do not execute, only print out command line calls for the C++ version of the program.  |
| <code>...</code>       | Other arguments are passed to <code>estimateExpression</code> , please see <a href="#">estimateExpression</a> for more details |

**Details**

This function uses `parseAlignment` function to compute alignment probabilities and the function `estimateExpression` to produce the expression samples.

In case of non-uniform read distribution, it first produces approximate estimates of expression using uniform read distribution with VB inference and subsequently uses these estimates to compute read distribution bias-corrected alignment probabilities, which are used in the `estimateExpression` function to produce expression estimates.

The order of transcripts in the results is always the same as in the alignment file. The transcripts can be identified by names stored in the `trInfo` part of the result.

**Value**

list with items:

|                     |   |
|---------------------|---|
| <code>exp</code>    | DataFrame with transcript expression mean and standard deviation  |
| <code>fn</code>     | name of the file containing all the expression samples  |
| <code>counts</code> | vector of estimated read counts per transcript  |
| <code>trInfo</code> | DataFrame with transcript information, contains: transcript name, possibly gene name, transcript length, and adjusted transcript length |

**Author(s)**

Peter Glaus

**See Also**

[getDE](#), [estimateExpression](#), [parseAlignment](#)

**Examples**

```
## Not run:
res1 <- getExpression("data-c0b0.sam", "ensSelect1.fasta", MCMC_chains=2,
  MCMC_samplesN=100)

## End(Not run)
```

---

`getGeneExpression`      *Calculate gene expression or relative within gene expression*

---

**Description**

Calculate either gene expression or relative within gene expression using transcript expression samples and transcript information file.

**Usage**

```

getGeneExpression(sampleFile, outFile=NULL, trInfo=NULL, trInfoFile=NULL,
  pretend=FALSE)
getWithinGeneExpression(sampleFile, outFile=NULL, trInfo=NULL, trInfoFile=NULL,
  pretend=FALSE, keepOrder=FALSE)

```

**Arguments**

|            |  |
|------------|--|
| sampleFile | File containing the transcript expression samples.   |
| outFile    | Name of the output file. If not used, function uses temporary file.  |
| trInfo     | DataFrame containing transcript information. Either trInfo or trInfoFile argument has to be provided. Otherwise function tries file with same name as sampleFile and extension tr.   |
| trInfoFile | Transcript information file. Either trInfo or trInfoFile argument has to be provided. Otherwise function tries file with same name as sampleFile and extension tr.                   |
| pretend    | Do not execute, only print out command line calls for the C++ version of the program.  |
| keepOrder  | If TRUE then transcripts will always keep same order, otherwise transcripts might be grouped by genes in the output. (The order is always same if transcripts are grouped by genes.) |

**Details**

The `getGeneExpression` function takes samples of transcript expression and produces file with expression of genes by adding up transcript expression.

The `getWithinGeneExpression` function takes samples of transcript expression and produces file with relative within gene expression samples for each transcript.

Both function need valid transcript information which contains gene transcript mapping. This can be provided either via DataFrame `trInfo` or file named `trInfoFile`.

In case of a file, it should be formatted in following manner. The first line should contain "# M <numberOfTranscripts>" and the following `numberOfTranscripts` lines have to contain "<gene-Name> <transcriptName> <transcriptLength>". Example is provided in `extdata/ensSelect1.tr`. Please note that the transcript information file automatically generated from alignment files are not sufficient because SAM/BAM files do not include gene names. We hope to provide more convenient way in future versions of BitSeq.

**Value**

Name of file containing the new expression samples.

**Author(s)**

Peter Glaus

**See Also**

[getExpression](#), [tri.load](#), [tri.file.setGeneNames](#), [tri.file.hasGeneNames](#)

**Examples**

```
setwd(system.file("extdata",package="BitSeq"))
## use transcript information as object
trinfo <- tri.load("ensSelect1.tr")
## gene expression
getGeneExpression("data-c0b1.rpkm", "data-c0b1-GE.rpkm", trInfo=trinfo)
gExpSamples <- loadSamples("data-c0b1-GE.rpkm")
gExpMeans <- rowMeans(as.data.frame(gExpSamples))
gExpMeans

## within gene expression
wgeFN <- getWithinGeneExpression("data-c0b1.rpkm", trInfoFile="ensSelect1.tr")
wgExpSamples <- loadSamples(wgeFN)
wgExpMeans <- rowMeans(as.data.frame(wgExpSamples))
head(wgExpMeans)
```

---

|                 |  |
|-----------------|--|
| getMeanVariance | <i>Calculate mean and variance of expression samples</i> |
|-----------------|--|

---

**Description**

Calculate mean and variance of expression samples or log-expression samples

**Usage**

```
getMeanVariance(sampleFiles, outFile, log=NULL, type=NULL, verbose=NULL,
                norm=NULL, pretend=FALSE)
```

**Arguments**

|             |   |
|-------------|---|
| sampleFiles | Vector of one or more files containing the expression samples.  |
| outFile     | Name of the output file.  |
| log         | Use logged values.  |
| type        | Type of variance, possible values: <code>sample,sqDif</code> for sample variance or squared difference.   |
| verbose     | Verbose output.   |
| norm        | Vector of (multiplicative) normalization constants for library size normalization of expression samples. Number of constants has to match the number of expression samples files. |
| pretend     | Do not execute, only print out command line calls for the C++ version of the program.   |

**Details**

The `getMeanVariance` function computes means and variances of MCMC expression samples. These can be computed either from single file or from multiple files using sample variance. Variance of two experiments (i.e. technical or biological replicates) can be estimated also by using `sqDif` option for `type` which specify the computation of the average square distance between the samples from two sets.

**Value**

`.means` File containing means (first column) and variance (second column) for each transcript (or row in the sample files)

**Author(s)**

Peter Glaus

**See Also**

[estimateExpression](#)

**Examples**

```
setwd(system.file("extdata", package="BitSeq"));
sampleFileNames = c("data-c1b0.rpkm", "data-c1b1.rpkm")
getMeanVariance(sampleFiles=sampleFileNames, outFile="data-c1.Lmean", log=1,
  norm=c(1.0017, 0.9998))
```

---

loadSamples

*Loading and saving expression samples*

---

**Description**

Functions for loading expression samples into `DataFrame` and saving samples from `DataFrame` into a file.

**Usage**

```
loadSamples(fileName, trInfoFile=NULL)
writeSamples(data, fileName)
```

**Arguments**

`fileName` Name of the file with samples or to which the samples are written.  
`data` `DataFrame` with samples written to the file.  
`trInfoFile` Transcript information file which can be used to name the rows.

**Details**

The `loadSamples` function load samples from the specified file into a `DataFrame`. If the transcript information file is provided, the transcript names are use as row names.

The `writeSamples` function can save samples from a `DataFrame` into a file in format which is valid for BitSeq and can be used in other functions.

**Value**

`DataFrame`      Containing the expression samples

**Author(s)**

Peter Glaus

**See Also**

[estimateExpression](#)

**Examples**

```
## Not run:
samples1<-loadSamples("data-c0b1.rpkm")
writeSamples(samples1,"new-c0b1.rpkm")

## End(Not run)
```

---

|                |  |
|----------------|--|
| parseAlignment | <i>Compute probabilities of alignments</i> |
|----------------|--|

---

**Description**

Compute probability of alignments and save them into *.prob* file.

**Usage**

```
parseAlignment( alignFile, outFile, trSeqFile, inputFormat=NULL, trInfoFile=NULL,
  expressionFile=NULL, readsN=NULL, uniform=TRUE, limitA=NULL, lenMu=NULL,
  lenSigma=NULL, excludeSingletons=NULL, mateNamesDiffer=NULL,
  verbose=NULL, veryVerbose=NULL, procN=NULL, pretend=FALSE)
```

**Arguments**

|                          |  |
|--------------------------|--|
| <code>alignFile</code>   | File containing read alignments.   |
| <code>outFile</code>     | Name of the output file.   |
| <code>inputFormat</code> | Input format: possible values SAM, BAM. (This should be detected automatically in most cases.) |
| <code>trInfoFile</code>  | File to save transcript information extracted from [BS]AM file and reference.                  |

|                   |   |
|-------------------|---|
| trSeqFile         | File containing transcript sequence in FASTA format.  |
| expressionFile    | Transcript relative expression estimates — for better non-uniform read distribution estimation.                   |
| readsN            | Total number of reads. This is usually not necessary if SAM/BAM contains also reads with no valid alignments.     |
| uniform           | Use uniform read distribution.  |
| limitA            | Limit maximum number of alignments per read. Reads with more alignments than limit will be discarded.             |
| lenMu             | Set mean of log fragment length distribution. $l_{frag} \sim \text{LogNormal}(\mu, \sigma^2)$                     |
| lenSigma          | Set $\sigma^2$ (or variance) of log fragment length distribution. $l_{frag} \sim \text{LogNormal}(\mu, \sigma^2)$ |
| excludeSingletons | Exclude single mate alignments for paired-end reads.  |
| mateNamesDiffer   | Mates from paired-end reads have different names.   |
| verbose           | Verbose output.   |
| veryVerbose       | Very verbose output.  |
| procN             | Maximum number of threads to be used.   |
| pretend           | Do not execute, only print out command line calls for the C++ version of the program.                             |

### Details

This function uses the alignments and reference file to assign probability to each alignment. It uses either bias-corrected or uniform model for the read distribution, assumes Log-Normal distribution of fragment lengths for pair-end read data and uses quality scores and mismatches to assign probability for every alignment of a read (or fragment) to a transcript.

### Value

|       |  |
|-------|--|
| .prob | file containing the alignment probabilities  |
| .tr   | file containing reference transcript names, lengths and effective lengths - optional |

### Author(s)

Peter Glaus

### See Also

[estimateExpression](#)

### Examples

```
## Not run:
parseAlignment(alignFile="data.sam", outFile="data.prob",
               trSeqFile="trReference.fa" ,trInfoFile="data.tr")

## End(Not run)
```

---

|                |  |
|----------------|--|
| transcriptInfo | <i>Manage information about transcript reference</i> |
|----------------|--|

---

## Description

Manage information about the transcript reference. These functions are used for reading, saving and updating transcript information DataFrame.

## Usage

```
tri.load(trInfoFile)
tri.save(trInfo, trInfoFile)
tri.hasGeneNames(trInfo)
tri.setGeneNames(trInfo, geneNames, transcriptNames=NULL)
```

## Arguments

|                 |   |
|-----------------|---|
| trInfoFile      | Name of the file containing transcript information or the where the information should be stored. |
| trInfo          | DataFrame containing the transcript information.  |
| geneNames       | Vector with new gene names that should be assigned to transcripts.                                |
| transcriptNames | Names of transcripts that should be associated with the gene names.                               |

## Details

If not provided with the information, BitSeq extracts information about the transcript reference from the alignment and sequence files. This information is stored in so called transcript information(trInfo) file, usually having extension `.tr`. This file contains columns with gene names (if available), transcript names, transcript lengths and optionally with adjusted lengths of transcripts. The expression of transcripts is reported in the same order as are the transcripts ordered in the trInfo file, hence it serves as identification of final results.

Other important use of trInfo file is for calculating gene expression or within gene expression, where the file is used for determining which transcripts belong to which genes. However, for this the gene names have to be properly set in the transcript info, which is not always the case.

Function `tri.load` loads transcript information from a file provided by argument `trInfoFile` into a DataFrame.

Function `tri.save` saves transcript information from a DataFrame provided by `trInfo` argument into a file name provided by argument `trInfoFile`.

Function `tri.hasGeneNames` determines whether gene names are properly set in the transcript information and returns TRUE or FALSE and a warning message identifying the problem.

Function `tri.setGeneNames` changes gene names of a transcript information `trInfo` and retruns new DataFrame with updated values. The vector `geneNames` should provide gene names of transcripts and be of the same length as is the number of transcripts. The gene names have to be either

ordered as their appropriate transcripts in `trInfo` object, or if ordered differently, vector of transcript names, ordered as gene names has to be provided by argument `transcriptNames`. The names in `transcriptNames` have to correspond to the transcript names in `trInfo` object.

### Value

Function `tri.load` returns `DataFrame` with transcript information.

Function `tri.hasGeneNames` returns boolean value.

Function `tri.setGeneNames` returns `DataFrame` with transcript information containing updated gene names (Note: the transcript names do not change.).

### Author(s)

Peter Glaus

### See Also

[getExpression](#), [getGeneExpression](#), [tri.file.setGeneNames](#)

### Examples

```
setwd(system.file("extdata", package="BitSeq"))
trinfo <- tri.load("ensSelect1.tr")
trinfo[1:10,]
## this should be true
tri.hasGeneNames(trinfo)
## reverse the gene order - this will make the information INCORRECT
rev.trinfo <- tri.setGeneNames(trinfo, rev(trinfo[,1]))
rev.trinfo[1:10,]
tri.save(rev.trinfo, "reversed-ensSelect1.tr")
```

---

`transcriptInfoFile`     *Manage file containing information about transcript reference*

---

### Description

Manage file containing information about the transcript reference. These functions are used for verifying and updating transcript information `DataFrame`.

### Usage

```
tri.file.hasGeneNames(trInfoFile)
tri.file.setGeneNames(trInfoFile, geneNames, transcriptNames=NULL)
```

## Arguments

|                              |   |
|------------------------------|---|
| <code>trInfoFile</code>      | Name of the file containing transcript information or the where the information should be stored. |
| <code>geneNames</code>       | Vector with new gene names that should be assigned to transcripts.                                |
| <code>transcriptNames</code> | Names of transcripts that should be associated with the gene names.                               |

## Details

If not provided with the information, `BitSeq` extracts information about the transcript reference from the alignment and sequence files. This information is stored in so called transcript information (`trInfo`) file, usually having extension `.tr`. This file contains columns with gene names (if available), transcript names, transcript lengths and optionally with adjusted lengths of transcripts. Important use of `trInfo` file is for calculating gene expression or within gene expression, where the file is used for determining which transcripts belong to which genes. However, for this the gene names have to be properly set in the transcript info, which is not always the case.

Function `tri.file.hasGeneNames` determines whether gene names are properly set in the transcript information file and returns `TRUE` or `FALSE` and a warning message identifying the problem.

Function `tri.file.setGeneNames` updates the gene names of a transcript information in file provided by argument `trInfoFile`. The vector `geneNames` should provide gene names of transcripts and be of the same length as is the number of transcripts. The gene names have to be either ordered as their appropriate transcripts in `trInfoFile` file, or if ordered differently, vector of transcript names, ordered as gene names has to be provided by argument `transcriptNames`. The names in `transcriptNames` have to correspond to the transcript names in actual file.

## Value

Function `tri.file.hasGeneNames` returns boolean value.

## Author(s)

Peter Glaus

## See Also

[getExpression](#), [getGeneExpression](#), [tri.load](#), [tri.save](#)

## Examples

```
setwd(system.file("extdata", package="BitSeq"))
## this should be true
tri.file.hasGeneNames("ensSelect1.tr")
```

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