Package ‘caser’

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Enhances parallel
Description Infer alternative splicing from paired-end RNA-seq data. The model is based on counting paths across exons, rather than pairwise exon connections, and estimates the fragment size and start distributions non-parametrically, which improves estimation precision.
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annotatedGenome-class

Description

The annotatedGenome class stores info about transcripts, usually created with procGenome from TxDb objects or user-provided .gtf files.

Objects from the Class

Objects are typically created with a call to procGenome (for known genomes) or to createDenovoGenome (for de novo genomes).

Slots

- **islands** GRangesList object with elements corresponding to gene islands. It indicates the start/end/name of each exon contained in the island.
- **transcripts** Each element in the list corresponds to a gene island. It indicates the exons contained in each known variant.
- **exon2island** data.frame indicating the chromosome, start and end of each exon, and its corresponding gene island.
- **exonsNI** GRanges indicating the chromosome, start/end and id of each exon.
- **aliases** data.frame indicating the aliases for each known transcript, i.e. transcripts having the exact same sequence of exons.
- **genomeVersion** Character indicating the genome version from which the object was build, e.g. "hg19".
- **dateCreated** Character indicating the date when the object was created. UCSC genomes change from time to time, so that an "hg19" genome from Jan 2012 may not be exactly the same as in Dec 2012.
- **denovo** Logical variable. FALSE indicates that the object was created using available annotation only. TRUE indicates that new exons/islands were added based on the data observed in a particular RNA-seq experiment.
- **txLength** Numeric vector storing transcript lengths.
- **knownVars** List where each element corresponds to an island, and contains a character vector with names of isoforms that should be considered as known (i.e. always included in the model).

Methods

- **show** signature(object = "annotatedGenome"): Displays general information about the object.

Author(s)

Camille Stephan-Otto Attolini

See Also

procGenome and createDenovoGenome to create annotatedGenome objects.
Examples

calcDenovo

description

calculate expression of gene splicing variants de novo.

Usage

calcDenovo(dists, targetGenomeDB, knownGenomeDB=targetGenomeDB, pc, readLength, islandid, priorq=3, mprior, minpp=0.001, selectBest=FALSE, searchMethod="submodels", niter, exactMarginal=TRUE, integrateMethod="plugin", verbose=TRUE, mc.cores=1)
Arguments

distrs | List of fragment distributions as generated by the `getDistrs` function

`targetGenomeDB` | annotatedGenome object with isoforms we wish to quantify. By default these are the same as in `knownGenomeDB`, but more typically `targetGenomeDB` is imported from a .gtf file produced by some isoform prediction software.

`knownGenomeDB` | annotatedGenome object with known isoforms, e.g. from UCSC or GENCODE annotations. Used to set the prior probability that any given isoform is expressed. `knownGenome` should be the same genome annotations used to create argument `mprior` (when provided)

`pc` | Named vector of exon path counts as returned by `pathCounts`

`readLength` | Read length in bp, e.g. in a paired-end experiment where 75bp are sequenced on each end one would set `readLength`=75.

`islandid` | Name of the gene island to be analyzed. If not specified, all gene islands are analyzed.

`priorq` | Parameter of the prior distribution on the proportion of reads coming from each variant. The prior is Dirichlet with prior sample size for each variant equal to `priorq`. We recommend `priorq=3` as this defines a non-local prior that penalizes falsely predicted isoforms that show low expression.

`mprior` | Prior on the model space returned by `modelPrior`, used to favor isoforms consistent with `knownGenomeDB`. If left missing it is estimated from `knownGenomeDB`. See details.

`minpp` | Models (i.e. splicing configurations) with posterior probability less than `minpp` are not reported. This argument can help reduce substantially the amount of required memory to store the results.

`selectBest` | If set to TRUE only the model with highest posterior probability is reported. While this can save memory, we do not recommend this option as it may ignore a substantial amount of uncertainty.

`searchMethod` | Method used to perform the model search. "allmodels" enumerates all possible models (warning: this is not feasible for genes with >5 exons). "rwmcmc" uses a random-walk MCMC scheme to focus on models with high posterior probability. "submodels" considers that some isoforms in `targetGenomeDB` may not be expressed, but does not search for new variants. "auto" uses "allmodels" for genes with up to 5 exons and "rwmcmc" for longer genes. See details.

`niter` | Number of MCMC iterations.

`exactMarginal` | Set to FALSE to estimate posterior model probabilities as the proportion of MCMC visits. Set to TRUE to use the integrated likelihoods (default). See details.

`integrateMethod` | Method to compute integrated likelihoods. The default ('plugin') evaluates likelihood*prior at the posterior mode and is the faster option. Set 'Laplace' for Laplace approximations and 'IS' for Importance Sampling. The latter increases computation cost very substantially.

`verbose` | Set to TRUE to display progress information.

`mc.cores` | Number of processors to be used for parallel computation. Can only be used if the package `multicore` is available for your system. Warning: using multiple processors substantially increases the memory requirements, so set this value carefully.
calcDenovo explores which subset of the isoforms indicated in targetGenomeDB are truly expressed. It also adds new isoforms when some reads follow an exon path that is not possible under any of the isoforms in targetGenomeDB. calcDenovo the posterior probability of each model (i.e. configuration of expressed variants) via Bayes theorem.

\[
P(\text{model}) \sim \text{m}(y|\text{model}) \times P(\text{model})
\]

where \(m(y|\text{model})\) is the integrated likelihood and \(P(\text{model})\) is the prior probability of the model. For example, a gene with 20 predicted isoforms in targetGenome gives rise \(2^{20} - 1\) possible models (configurations of expressed isoforms).

Importantly, \(P(\text{model})\) can be set by analyzing available genome annotations in knownGenomeDB. For instance, genes with 20 exons have isoforms that tend to use most of the 20 exons. They also tend to express more isoforms than genes with 5 exons. The function modelPrior analyzes knownGenomeDB to set reasonable values for \(P(\text{model})\).

An exhaustive enumeration of all possible models is not feasible unless the gene is very short (e.g. around 5 exons). For longer genes we use computational strategies to search a subset of "interesting" models. This is controlled by the argument searchMethod (see above).

In order to compute \(P(\text{model})\) one can either use the computed \(m(y|\text{model})\) \(P(\text{model})\) (option exactMarginal==TRUE) or the proportion of MCMC visits (option exactMarginal==FALSE). Unless niter is large the former option typically provides more precise estimates.

Value

A denovoGenomeExpr object.

Author(s)

Camille Stephan-Otto Attolini, Manuel Kroiss, David Rossell

References


See Also
denovoExpr to obtain expression estimates from the calcDenovo output. plotExpr to produce a plot with splicing variants and estimated expression.

Examples

```R
## See help(denovoExpr)
```
calcExp

Estimate expression of a known set of gene splicing variants.

Description

Estimate expression of gene splicing variants, assuming that the set of variants is known. When rpkm is set to TRUE, fragments per kilobase per million are returned. Otherwise relative expression estimates are returned.

Usage

calcExp(distrs, genomeDB, pc, readLength, islandid, rpkm=TRUE, priorq=2,
        priorqGeneExpr=2, ctyple="none", niter=10^3, burnin=100, mc.cores=1, verbose=FALSE)

Arguments

distrs List of fragment distributions as generated by the getDistrs function
genomeDB knownGenome object containing annotated genome, as returned by the procGenome function.
pc Named vector of exon path counts as returned by pathCounts
readLength Read length in bp, e.g. in a paired-end experiment where 75bp are sequenced on each end one would set readLength=75.
islandid Name of the gene island to be analyzed. If not specified, all gene islands are analyzed.
rpkm Set to FALSE to return relative expression levels, i.e. the proportion of reads generated from each variant per gene. These proportions add up to 1 for each gene. Set to TRUE to return fragments per kilobase per million (RPKM).
priorq Parameter of the prior distribution on the proportion of reads coming from each variant. The prior is Dirichlet with prior sample size for each variant equal to priorq. We recommend priorq=2 for estimation, as it pools the estimated expression away from 0 and 1 and returned lower estimation errors than priorq=1 in our simulated experiments.
priorqGeneExpr Parameter for prior distribution on overall gene expression. Defaults to 2, which ensures non-zero estimates for all genes
ctyle Set to "none" to return no credibility intervals. Set to "asymp" to return approximate 95% CIs (obtained via the delta method). Set to "exact" to obtain exact CIs via Monte Carlo simulation. Options "asymp" and especially "exact" can increase the computation time substantially.
niter Number of Monte Carlo iterations. Only used when ctyple="exact".
burnin Number of burnin Monte Carlo iterations. Only used when ctyple="exact".
mc.cores Number of processors to be used for parallel computation. Can only be used if the package multicore is available for your system.
verbose Set to TRUE to display progress information.

Value

Expression set with expression estimates. featureNames identify each transcript via RefSeq ids, and the featureData contains further information. If ctyple was set to a value other than "none", the featureData also contains the 95% credibility intervals (i.e. intervals that contain the true parameter value with 95% posterior probability).
Author(s)

Camille Stephan-Otto Attolini, Manuel Kroiss, David Rossell

References


See Also

relexprByGene

Examples

data(K562.r1l1)
data(hg19DB)

#Pre-process
bam0 <- rmShortInserts(K562.r1l1, isizeMin=100)
pbam0 <- procBam(bam0)
head(getReads(pbam0))

#Estimate distributions, get path counts
distrs <- getDistrs(hg19DB,bam=bam0,readLength=75)
pc <- pathCounts(pbam0, DB=hg19DB)

#Get estimates
eset <- calcExp(distrs=distrs, genomeDB=hg19DB, pc=pc, readLength=75, rpkm=FALSE)
head(exprs(eset))
head(fData(eset))

#Re-normalize relative expression to add up to 1 within gene_id rather 
# than island_id
eset <- relexprByGene(eset)

#Add fake sample by permuting and combine
eset2 <- eset[sample(1:nrow(eset),replace=FALSE),]
sampleNames(eset2) <- '2' #must have a different name
esetall <- mergeExp(eset,eset2)

#After merge samples are correctly matched
head(exprs(esetall))
head(fData(esetall))

denovoExpr

Estimate expression for de novo splicing variants.

description

Obtains expression estimates from denovoGenomeExpr objects, as returned by calcDenovo. When rpkm is set to TRUE, fragments per kilobase per million are returned. Otherwise relative expression estimates are returned.

The estimates can be obtained by Bayesian model averaging (default) or by selecting the model with highest posterior probability. See details.
denovoExpr

Usage

denovoExpr(x, pc, rpkmsummarize = "modelAvg", minProbExpr = 0.5, minExpr = 0.05)

Arguments

x       denovoGenomeExpr object returned by calcExp
pc      Named vector of exon path counts as returned by pathCounts
rpkmsummarize Set to FALSE to return relative expression levels, i.e. the proportion of reads
              generated from each variant per gene. These proportions add up to 1 for each
gene. Set to TRUE to return fragments per kilobase per million (RPKM).
minProbExpr Variants with (marginal posterior) probability of being expressed below minProbExpr
              are omitted from the results. This argument is useful to eliminate variants that
              are not at least moderately supported by the data.
minExpr  Variants with relative expression minExpr are omitted from the results. This is
          useful to eliminate variants to which few reads are assigned, e.g. due to read
          miss-alignments or biases.

Value

Expression set with expression estimates. The featureData indicates the gene island id, posterior
probability that each variant is expressed (column "probExpressed") and the number of aligned
reads per gene island (column "explCnts").

Author(s)

David Rossell

References

Rossell D, Stephan-Otto Attolini C, Kroiss M, Stocker A. Quantifying Alternative Splicing from

Examples

## NOTE: toy example with few reads & genes to illustrate code usage
## Results with complete data are much more interesting!

data(K562.r1l1)
data(hg19DB)

#Pre-process
bam0 <- rmShortInserts(K562.r1l1, isizeMin=100)
pbam0 <- procBam(bam0)

distrs <- getDistrs(hg19DB,bam=bam0,readLength=75)
pc <- pathCounts(pbam0, DB=hg19DB)

#Set prior distr on model space
mprior <- modelPrior(hg19DB, maxExons=40, smooth=FALSE)

#Fit model
denovo <- calcDenovo(distrs,targetGenomeDB=hg19DB,pc=pc,readLength=75,priorq=3,mprior=mprior,minpp=0)

head(names(denovo))
denovo[['6499']]
posprob(denovo[['6499']])

#Get estimates
eset <- denovoExpr(denovo, pc=pc, rpkm=TRUE, minProbExpr=0.5)

head(exprs(eset))
head(fData(eset))

denovoGeneExpr-class  Class “denovoGeneExpr”

Description
denovoGeneExpr stores inferred expression for de novo splicing variants for a single gene. denovoGenomeExpr stores the information for several genes (typically, the whole genome).

Objects from the Class
Objects are returned by calcDenovo. When running calcDenovo on multiple genes results are returned in a denovoGenomeExpr object. Results for a single gene can be retrieved using the [[ operator as usual, which returns a denovoGeneExpr object.

Slots
posprob data.frame containing the posterior probability of each model
expression data.frame with the estimated expression of each variant under each model
variants matrix indicating the exons contained in each variant.
integralSum Sum of the log(integrated likelihood) + log(model prior probability) across all considered models.
npathDeleted Number of paths that had 0 probability under all considered variants and had to be excluded for model fitting purposes.
priorq Input parameter to calcDenovo
txLength Length of transcripts in bp (including new isoforms found by casper)

Methods
show signature(object = “denovoGeneExpr”: Displays general information about the object.
names Show names (island ids)
"[]" Selects a subset of genes
"[i]" Selects a single gene
posprob Accesses the posterior probabilities of each model (slot posprob)
variants Accesses the variant names and their respective exons
variants<- Replaces the value of the slot variants (can be useful for renaming variants, for instance)
denovoGenomeExpr-class

Author(s)
David Rossell

See Also
calcDenovo to create objects from the class. denovoExpr to obtain expression estimates from denovoGenomeExpr objects.

Examples
showClass("denovoGeneExpr")

denovoGenomeExpr-class

Class "denovoGenomeExpr"

Description
denovoGeneExpr stores inferred expression for de novo splicing variants for a single gene. denovoGenomeExpr stores the information for several genes (typically, the whole genome).

Objects from the Class
Objects are returned by calcDenovo.

Slots
islands A list of denovoGeneExpr objects, with each element containing results for an individual gene.

Methods
show signature(object = "denovoGenomeExpr"): Displays general information about the object.
as.list Coerces the object to a list
"[" Selects a subset of genes
"[1]" Selects a single gene

Author(s)
Camille Stephan-Otto Attolini

See Also
procGenome and createDenovoGenome to create denovoGenomeExpr objects.

Examples
showClass("denovoGeneExpr")
showClass("denovoGenomeExpr")
distrsGSE37704

Estimated read start and insert size distributions from MiSeq data in GEO dataset GSE37704.

Description

We downloaded the fastq files, aligned with TopHat and processed with wrapKnown to obtain the estimated distributions for each of the 6 samples. distrsGSE37704 is a list with the 6 corresponding elements. The estimated distributions for HiSeq data were very similar, hence these distributions can be used as defaults for Illumina MiSeq and HiSeq experiments.

Usage

data(distrsGSE37704)

Format

An list with 6 elements of class readDistrs. See help(getDistrs) and help(readDistrs-class) for details.

Examples

data(distrsGSE37704)
distrsGSE37704
plot(distrsGSE37704[[1]],'readSt')
lines(distrsGSE37704[[2]],'readSt', col=2)
plot(distrsGSE37704[[1]],'fragLength')

genePlot

Plot exon structure for each transcript of a given gene.

Description

Plot exon structure for each transcript of a given gene. Optionally, aligned reads can be added to the plot.

Usage

genePlot(generanges, islandid, genomeDB, reads, exp, names.arg, xlab='', ylab='', xlim, cex=1, yaxt='n', col, ...)

Arguments

generanges Object containing the ranges with start/end of each exon.
islandid If generanges is not specified, transcripts are obtained from island islandid from the annotated genome genomeDB.
genomeDB Annotated genome produced with the "procGenome" function
reads pbam object with aligned reads. This is an optional argument.
genePlot

exp  ExpressionSet object with expression values, as returned by \texttt{calcExp}. This is an optional argument.
names.arg  Optionally, indicate the names of each transcript.
xlab  x-axis label
ylab  y-axis label
xlim  x-axis limits, defaults to start of 1st exon and end of last exon
cex  Character expansion
yaxt  The y-axis in the plot has no interpretation, hence by default it is not displayed.
col  Either single color or vector of colors to be used to draw each transcript. Defaults to rainbow colors.
...  Other arguments to be passed on to \texttt{plot}.

\textbf{Value}

A plot is produced.

\textbf{Methods}

\texttt{signature(generanges="CompressedIRangesList", islandid="ANY", genomeDB="ANY", reads="ANY", exp="ANY")}

Plots a set of transcripts. Each element in the \texttt{generanges} corresponds to a transcript. Each transcript should contain exon start/end positions.

\texttt{signature(generanges="IRanges", islandid="ANY", genomeDB="ANY", reads="ANY", exp="ANY")}

Plots a single transcript. Each range indicates the start/end of a single exon.

\texttt{signature(generanges="IRangesList", islandid="ANY", genomeDB="ANY", reads="ANY", exp="ANY")}

Plots a set of transcripts. Each element in the \texttt{generanges} corresponds to a transcript. Each transcript should contain exon start/end positions.

\texttt{signature(generanges="GRangesList", islandid="ANY", genomeDB="ANY", reads="ANY", exp="ANY")}

Plots a set of transcripts. Each element in the \texttt{generanges} corresponds to a transcript. Each transcript should contain exon start/end positions.

\texttt{signature(generanges="GRanges", islandid="ANY", genomeDB="ANY", reads="ANY", exp="ANY")}

Plots a set of transcripts. Each space in \texttt{generanges} corresponds to a transcript. Each transcript should contain exon start/end positions.

\texttt{signature(generanges="missing", islandid="character", genomeDB="annotatedGenome", reads="GRanges", exp="ANY")}

Plots all transcripts stored in \texttt{genomeDB} for island with identifier \texttt{islandid}. Individual reads are added to the plot (reads contains start/end of individual read fragments).

\texttt{signature(generanges="missing", islandid="character", genomeDB="annotatedGenome", reads="missing", exp="missing")}

Plots all transcripts stored in \texttt{genomeDB} for island with identifier \texttt{islandid}.

\texttt{signature(generanges="missing", islandid="character", genomeDB="annotatedGenome", reads="procBam", exp="missing")}

Plots all transcripts stored in \texttt{genomeDB} for island with identifier \texttt{islandid}. Individual reads and estimated expression are added to the plot (reads contains start/end of individual read fragments).

\textbf{Author(s)}

Camille Stephan-Otto Attolini, David Rossell
Examples

data(hg19DB)

#Plot an IRangesList
txs <- transcripts(txid="NM_005158", genomeDB=hg19DB)
genePlot(txs)

#Equivalently, indicate islandid
islandid <- getIsland(txid="NM_005158", genomeDB=hg19DB)
genePlot(islandid=islandid, genomeDB=hg19DB)

getDistrs

Compute fragment start and fragment length distributions

Description

Compute fragment start distributions by using reads aligned to genes with only one annotated variant. Estimate fragment length distribution using fragments aligned to long exons (>1000nt). Fragment length is defined as the distance between the start of the left-end read and the end of the right-end read.

Usage

getDistrs(DB, bam, pbam, islandid=NULL, verbose=FALSE, nreads=4*10^6, readLength, min.gt.freq = NULL, tgroups=5, mc.cores=1)

Arguments

DB
Annotated genome. Object of class knownGenome as returned by procGenome.

bam
Aligned reads, as returned by scanBam. It must be a list with elements 'qname', 'rname', 'pos' and 'mpos'. Ignored when argument pbam is specified.

pbam
Processed BAM object of class procBam, as returned by function procBam. Arguments bam and readLength are ignored when pbam is specified.

islandid
Island IDs of islands to be used in the read start distribution calculations (defaults to genes with only one annotated variant)

verbose
Set to TRUE to print progress information.

nreads
To speed up computations, only the first nreads are used to obtain the estimates. The default value of 4 millions usually gives highly precise estimates.

readLength
Read length in bp, e.g. in a paired-end experiment where 75bp are sequenced on each end one would set readLength=75.

min.gt.freq
The target distributions cannot be estimated with precision for gene types that are very unfrequent. Gene types with relative frequency below min.gt.freq are merged, e.g. min.gt.freq=0.05 means gene types making up for 5% of the genes in DB will be combined and a single read start and length distribution will be estimated for all of them.

tgroups
As an alternative to min.gt.freq you may specify the maximum number of distinct gene types to consider. A separate estimate will be obtained for the tgroups with highest frequency, all others will be combined.

mc.cores
Number of cores to use for parallel processing
getIsland

Value

An object of class readDistrs with slots:

- `lenDis` Table with number of fragments with a given length
- `stDis` Cumulative distribution function (object of type closure) for relative start position

Author(s)

Camille Stephan-Otto Attolini, David Rossell

Examples

```r
# Fragment length distribution
plot(distrs, 'fragLength')

# Fragment start distribution (relative to transcript length)
plot(distrs, 'readSt')
```

getIsland

getIsland returns the island id associated to a given entrez or transcript id in an annotatedGenome object. getChr indicates the chromosome for a given Entrez, transcript or island id.

Description

annotatedGenome objects store information regarding genes and transcripts. When there’s an overlap in exons between several genes, these genes are grouped into gene islands. getIsland retrieves the island to which each gene or transcript was assigned, while getChr indicates the chromosome.

Usage

```r
getIsland(entrezid, txid, genomeDB)
getChr(entrezid, txid, islandid, genomeDB)
```

Arguments

- `entrezid` Character indicating single Entrez identifier. Can be left missing and specify another identifier instead.
- `txid` Character indicating a single RefSeq transcript identifier. Can be left missing and specify another identifier instead.
- `islandid` Character indicating the gene island identifier. Can be left missing and specify another identifier instead.
- `genomeDB` Object of class annotatedGenome
getNreads

**Description**

getNreads returns a numeric vector with the total number of path counts in each island from a pathCounts object.

**Usage**

getNreads(pc)

**Arguments**

- pc: pathCounts object generated by pathCounts()

**Value**

Numeric vector with total number of path counts in each island of pc.
getReads

Methods

signature(pathCounts='pathCounts') Returns numeric vector with total number of path counts for each island in the pathCounts object.

Author(s)

Camille Stephan-Otto Attolini

Examples

##---- Should be DIRECTLY executable !! ----
##-- ==> Define data, use random,
##-- or do help(data=index) for the standard data sets.

getReads

getReads returns the reads stored in a procBam object.

Description

procBam objects store reads that have been split according to their CIGAR codes. getReads accesses these reads.

Usage

getReads(x)

Arguments

x Object of class procBam

Value

RangedData object with reads stored in x.

Methods

signature(x='procBam') Return reads stored in x.

Examples

#See example in calcExp
getRoc

Operating characteristics of differential expression analysis

Description
getRoc compares simulation truth and data analysis results to determine False Positives (FP), False Negatives (FN), True Positives (TP), True Negatives (TN), Positives (FP+TP), False Discovery Proportion (FP/P) and Power (TP/(TP+FN)).

Usage
getRoc(simTruth, decision)

Arguments
simTruth Binary vector or matrix indicating simulation truth (FALSE or 0 for non differential expression, TRUE or 1 for differential expression)
decision Binary vector or matrix with differential expression calls based on some data analysis.

Value
data.frame with TP, FP, TN, FN, P, FDR and Power.

Methods
signature(simTruth='logical',decision='logical') Operating characteristics are computed for a single simulation
signature(simTruth='numeric',decision='numeric') Operating characteristics are computed for a single simulation
signature(simTruth='matrix',decision='matrix') simTruth and decision contain truth and calls for several simulations (in columns). getRoc returns a data.frame with operating characteristics in each simulation.

Author(s)
David Rossell

Examples
## See help(probNonEquiv) for an example
hg19DB

Subset of human genome (UCSC hg19 version)

Description
We downloaded the human genome hg19 via procGenome and selected a few genes from chromosome 1 to use as a toy data for the vignette and examples.

Usage
data(hg19DB)

Format
An annotatedGenome object. See help(procGenome) and help(annotatedGenome-class) for details.

Examples
data(hg19DB)
hg19DB
slotNames(hg19DB)

K562.r11

Toy RNA-seq data from RGASP project.

Description
The paired-end RNA-seq data is from the RGASP project sample K562_2x75 (replicate 1, lane 1) and was obtained at ftp://ftp.sanger.ac.uk/pub/gencode/rgasp/RSASP1/inputdata/human_fastq. Reads were aligned against hg19 with tophat 2.0.2 and bowtie 0.12.5, setting the insert size at -r 200, and imported into R using scanBam from package Rsamtools. For illustration purposes, we selected reads mapping to a few genes only (namely, the genes that were also selected for the toy genome annotation in data(hg19DB)).

Usage
data(K562.r11)

Format
A list indicating read id, chromosome, start and end locations and the position of the pair, as returned by scanBam.

Source
mergeBatches

References


Examples

data(K562.r1l1)
names(K562.r1l1)

mergeBatches

Merge two ExpressionSet objects by doing quantile normalization and computing partial residuals (i.e. subtracting group mean expression in each batch). As currently implemented the method is only valid for balanced designs, e.g. each batch has the same number of samples per group.

Description

mergeBatches combines x and y into an ExpressionSet, performs quantile normalization and adjusts for batch effects by subtracting the mean expression in each batch (and then adding the grand mean so that the mean expression per gene is unaltered).

Usage

mergeBatches(x, y, mc.cores=1)

Arguments

x ExpressionSet object with data from batch 1.

y Either ExpressionSet object with data from batch 2, or simulatedSamples object with data from multiple simulations.

mc.cores Number of processors to be used (ignored when y is an ExpressionSet)

Value

When y is an ExpressionSet, mergeBatches returns an ExpressionSet with combined expressions. Its featureData contains a variable "batch" indicating the batch that each sample corresponded to.

When y is a simulatedSamples object, mergeBatches is applied to combine x with each dataset in y and a list of ExpressionSet objects is returned.

Author(s)

David Rossell
mergeExp

Examples

```r
#fake data from 2 batches
x <- matrix(rnorm(6), nrow=2)
colnames(x) <- paste("x", 1:3, sep="")
y <- matrix(1+rnorm(6), nrow=2)
colnames(y) <- paste("y", 1:3, sep="")
x <- new("ExpressionSet", exprs=x)
y <- new("ExpressionSet", exprs=y)
exprs(x)
exprs(y)

#merge & adjust
z <- mergeBatches(x,y)
exprs(z)
```

mergeExp

Merge splicing variant expression from multiple samples

Description

mergeExp combines the output of calcExp from multiple samples, i.e. multiple ExpressionSet objects, into a single ExpressionSet

Usage

```r
mergeExp(..., sampleNames, keep=c("transcript","gene_id","island_id"))
```

Arguments

- `...` ExpressionSet objects to be combined.
- `sampleNames` Character vector indicating the name of each sample. Defaults to 'Sample1', 'Sample2', etc.
- `keep` Variables in the featureData of each individual ExpressionSet to keep in the merged output.

Details

mergeExp runs some checks to ensure that object can be combined (e.g. making sure that measurements are obtained on same set of genes), then sorts and formats each input ExpressionSet.

A label with the sample name is appended to variables in the featureData that appear in multiple samples, e.g. variable 'se' reporting standard errors (obtained by setting citype='asymp' in calcExp).

Value

Object of class ExpressionSet combining the input ExpressionSets. Its featureData contains the columns indicated in the keep argument, plus a column readCount with the total number of reads mapped to each gene (or gene island, when multiple genes have overlapping exons).

Author(s)

David Rossell
modelPrior

See Also
calcExp to obtain an ExpressionSet for an individual sample.

Examples

#See example in calcExp

modelPrior  

Set prior distribution on expressed splicing variants.

Description

Set prior on expressed splicing variants using the genome annotation contained in a knownGenome object.

The prior probability of variants V1,...,Vn being expressed depends on n, on the number of exons in each variant V1,...,Vn and the number of exons in the gene. See the details section.

Usage

modelPrior(genomeDB, maxExons=40, smooth=TRUE, verbose=TRUE)

Arguments

genomeDB  

Object of class knownGenome

maxExons  
The prior distribution is estimated for genes with 1 up to maxExons exons. As there are fewer genes with many exons, the prior parameters are estimated poorly. To avoid this common estimate is used for all genes with more than maxExons exons

smooth  If set to TRUE the estimated prior distribution parameters for the number of exons in a gene are smoothed using Generalized Additive Models. This step typically improves the precision of the estimates, and is only applied to genes with 10 or more exons.

verbose  

Set to TRUE to print progress information.

Details

The goal is to set a prior that takes into account the number of annotated variants for genes with E exons, as well as the number of exons in each variant.

Suppose we have a gene with E exons. Let V_1,...,V_n be n variants of interest and let |V_1|,...,|V_n| be the corresponding number of exons in each variant. The prior probability of variants V_1,...,V_n being expressed is modeled as

\[ P(V_1,...,V_n|E) = P(n|E) P(|V_1| |E) \cdots P(|V_n| |E) \]

where \( P(n|E) = \text{NegBinom}(n; k_E, r_E) I(0 < n < 2^E) \) and \( P(|V_i| |E) = \text{BetaBinomial}(|V_i|-1; E-1, \alpha_E, \beta_E) \).

The parameters k_E, r_E, alpha_E, beta_E depend on E (the number of exons in the gene) and are estimated from the available annotation via maximum likelihood. Parameters are estimated jointly for all genes with E >= maxExons in order to improve the precision.

For smooth==TRUE, alpha_E and beta_E are modeled as a smooth function of E by calling gam and setting the smoothing parameter via cross-validation. Estimates for genes with E>=10 are substituted by their smooth versions, which typically helps improve stability in the estimates.
Value

List with 2 components.

nvarPrior  List with prior distribution on the number of expressed variants for genes with 1,2,3... exons. Each element contains the truncated Negative Binomial parameters, observed and predicted frequencies (counting the number of genes with a given number of variants).

nexonPrior  List with prior distribution on the number of exons in a variant for genes with 1,2,3... exons. Each element contains the Beta-Binomial parameters, observed and predicted frequencies (counting the number of variants with a given number of exons).

Author(s)

David Rossell, Camille Stephan-Otto Attolini

Examples

data(hg19DB)
mprior <- modelPrior(hg19DB, maxExons=10)

## Prior on number of expressed variants
## Genes with 2 exons
## mprior$nvarPrior[['2']]
## Genes with 3 exons
## mprior$nvarPrior[['3']]

## Prior on the number of exons in an expressed variant
## Genes with 2 exons
## mprior$nexonPrior[['2']]
## Genes with 3 exons
## mprior$nexonPrior[['3']]
Methods

show signature(object = "modelPriorAS"): Displays general information about the object.
"[" Selects prior parameters for genes with the specified number of exons
coef Selects a single gene

Author(s)

David Rossell

See Also

procGenome and createDenovoGenome to create modelPriorAS objects.

Examples

showClass("modelPriorAS")

Description

Compute counts for exon paths visited by aligned reads

Usage

pathCounts(reads, DB, mc.cores = 1, verbose=FALSE)

Arguments

reads Object of class procBam containing aligned reads, as returned by procBam.
DB Object of class annotatedGenome containing either a known or de novo annotated genome.
mc.cores Number of processors to be used for parallel computing. Requires having package multicore installed and loaded.
verbose Set to TRUE to print progress information.

Value

Named integer vector with counts of exon paths. Names are character strings built as ".exon1.exon2-exon3.exon4.", with dashes making the split between exons visited by left and right-end reads correspondingly.

Methods

signature(reads='list') Computes counts for exon paths from a list of procBam objects (usually reads processed and split by chromosome).
signature(reads='procBam') Compute counts for exon paths from a procBam object of processed reads.
pathCounts-class

Author(s)
Camille Stephan-Otto Attolini

See Also
procGenome to create an annotated genome object. createDenovoGenome to create a de novo annotated genome. See help(getNreads) to get number of fragments mapping to each island.

Examples
### Should be DIRECTLY executable !! ----
###-- ==> Define data, use random,
###--or do help(data=index) for the standard data sets.

pathCounts-class  Class "pathCounts"

Description
Stores exon path counts.

Objects from the Class
Objects are created with a call to pathCounts.

Slots
counts List with one element per gene island. For each island, it contains a named vector with exon path counts. The names indicate the visited exons.

For instance, consider that for gene '1' with 2 exons we observe 10 reads in which the left end falls completely in exon 1 and the right end in exon 2. Suppose that for 5 reads the left end bridges exons 1-2 and the right end falls in exon 2. Then pc[['1']] would contain c(10, 5) and names(pc[['1']]) would contain c(".1-2.",".1.2-2.").

denovo Logical variable. FALSE indicates that the counts correspond to a known genome (i.e. created with procGenome), and TRUE to a de novo annotated genome (i.e. created with createDenovoGenome).

stranded Logical variable. TRUE indicates that the path counts were obtained from an RNA-seq experiment where strand information was preserved.

Methods
show signature(object = "pathCounts"): Displays general information about the object.

Author(s)
Camille Stephan-Otto Attolini

Examples
showClass("pathCounts")
plotExpr

Plot inferred gene structure and expression.

Description

Plots variants with sufficiently large posterior probability of being expressed along with their (marginal) estimated expression.

Usage

plotExpr(gene, minProbExpr = 0.5, minExpr = 0.1, xlab = "(kb)", ylab = "", xlim, cex = 1, yaxt = "n", col, ...)
**plotPriorAS**

**Arguments**

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>gene</td>
<td>denovoGeneExpr object containing results for a single gene, as returned by calcDenovo.</td>
</tr>
<tr>
<td>minProbExpr</td>
<td>Variants with marginal posterior probability of expression below minProbExpr are not reported.</td>
</tr>
<tr>
<td>minExpr</td>
<td>Variants with (marginal) estimated expression below minExpr are not reported. Can be useful to remove sequence preference artifacts.</td>
</tr>
<tr>
<td>xlab</td>
<td>x-axis label, passed on to plot</td>
</tr>
<tr>
<td>ylab</td>
<td>y-axis label, passed on to plot</td>
</tr>
<tr>
<td>xlim</td>
<td>x-axis limits, passed on to plot</td>
</tr>
<tr>
<td>cex</td>
<td>Character expansion, passed on to plot</td>
</tr>
<tr>
<td>yaxt</td>
<td>Type of y-axis, passed on to plot</td>
</tr>
<tr>
<td>col</td>
<td>Colors for each variant, defaults to rainbow colors. It is possible to specify a single color.</td>
</tr>
<tr>
<td>...</td>
<td>Other arguments to be passed on to plot</td>
</tr>
</tbody>
</table>

**Details**

The marginal posterior probability that a variant is expressed is the sum of the posterior probabilities of all models containing that variant.

The marginal estimated expression is the average expression across all models (including those where the variant has 0 expression) weighted by the posterior probability of each model.

**Methods**

signature(gene = "denovoGeneExpr") gene contains the results from a de novo isoform expression analysis for a single gene, as returned by calcDenovo. When calcDenovo is run on multiple genes simultaneously, the desired gene can be selected using the "[["] operator as usual.

**Examples**

#See calcDenovo examples

---

**plotPriorAS**

Plot prior distribution on set of expressed variants (i.e. the model space).

**Description**

Plots the prior distribution on the number of expressed variants and the number of exons per variant in genes with exons exons (as returned by function modelPrior). The prior distribution is compared to the observed frequencies to check that the assumed distributional forms are reasonable.

**Usage**

plotPriorAS(object, type="nbVariants", exons=1:9, xlab, ylab="Probability", col=c("red","blue"))
probNonEquiv

Arguments

object  modelPriorAS object with prior distribution on model space.
type    Set to "nbVariants" to plot the prior on the number of variants per gene. Set to "nbExons" to plot the prior on the number of exons.
exons   Vector with integers. The plot is only produced with number of exons indicated in exons.
xlab    x-axis label, passed on to plot
ylab    y-axis label, passed on to plot
col     Colors for bars showing prior probabilities and frequencies in the known genome

Methods

signature(object = "modelPriorAS") object contains the prior distribution on the model space, as returned by function modelPrior

Examples

#See modelPrior examples

probNonEquiv

probNonEquiv performs a Bayesian hypothesis test for equivalence between group means. It returns the posterior probability that |mu1-mu2|>logfc.  pvalTreat is a wrapper to treat in package limma, which returns P-values for the same hypothesis test.

Description

probNonEquiv computes \(v_i=P(\theta_i > \text{logfc} \mid \text{data})\), where \(\theta_i\) is the difference between group means for gene \(i\). This posterior probability is based on the NNGCV model from package EBarrays, which has a formulation similar to limma in an empirical Bayes framework. Notice that the null hypothesis here is that \(|\theta_i|<\text{logfc}\), e.g. isoforms with small fold changes are regarded as uninteresting.

Subsequent differential expression calls are based on selecting large \(v_i\). For instance, selecting \(v_i \geq 0.95\) guarantees that the posterior expected false discovery proportion (a Bayesian FDR analog) is below 0.05.

Usage

probNonEquiv(x, groups, logfc = log(2), minCount, method = "plugin", mc.cores=1)
pvalTreat(x, groups, logfc = log(2), minCount, p.adjust.method='none', mc.cores = 1)

Arguments

x  ExpressionSet containing expression levels, or list of ExpressionSets
groups Variable in fData(x) indicating the two groups to compare (the case with more than 2 groups is not implemented).
logfc Biologically relevant threshold for the log fold change, i.e. difference between groups means in log-scale
probNonEquiv

minCount  
If specified, probabilities are only computed for rows with fData(x)$readCount >= minCount

method  
Set to 'exact' for exact posterior probabilities (slower), 'plugin' for plug-in approximation (much faster). Typically both give very similar results.

mc.cores  
Number of parallel processors to use. Ignored unless x is a list.

p.adjust.method  
P-value adjustment method, passed on to p.adjust

Value

If x is a single ExpressionSet, probNonEquiv returns a vector with posterior probabilities (NA for rows with less than minCount reads). pvalTreat returns TREAT P-values instead.

If x is a list of ExpressionSet, the function is applied to each element separately and results are returned as columns in the output matrix.

Author(s)

Victor Pena, David Rossell

References


McCarthy DJ, Smyth GK. Testing significance relative to a fold-change threshold is a TREAT. Bioinformatics, 25(6):765-771

See Also

treat in package limma, p.adjust

Examples

#Simulate toy data
p <- 50; n <- 10
x <- matrix(rnorm(p*2*n),nrow=p)
x <- new("ExpressionSet",exprs=x)
x$group <- rep(c('group1','group2'),each=n)

#Posterior probabilities
pp <- probNonEquiv(x, groups='group', logfc=0.5)
d <- rowMeans(exprs(x[,1:n])) - rowMeans(exprs(x[,1:-n]))
plot(d,pp,xlab='Observed log-FC')
abline(v=c(-.5,.5))

#Check false positives
truth <- rep(c(FALSE,TRUE),c(p-11,11))
getRoc(truth, pp>.9)
getRoc(truth, pp>.5)
procBam

Description

Process paired-end data stored in BAM object generated by scanBam. Outputs GRanges objects for reads and junctions.

Usage

procBam(bam, stranded=FALSE, seed=as.integer(1), verbose=FALSE, rname='', keep.junx=FALSE, keep.flag=FALSE, ispaired=TRUE,...)

Arguments

bam BAM object generated by scanBam
stranded Set to TRUE to indicate that the RNA-seq experiment preserved the strand information.
seed Seed for random number generator
verbose Set to TRUE to print progress information.
rname Chromosome to process be combined with the which argument in the scanBam function
keep.junx Option to store junction information. Only useful for finding denovo exons and transcripts.
keep.flag Option to store alignment flag information.
ispaired Set to TRUE is reads are paired.
... Other arguments

Details

In case of multihits with same start position for both reads but different insertions/deletions patterns only one alignment is chosen at random.

Value

An object of class procBam containing reads with both ends correctly aligned and split according to the corresponding CIGAR. Unique identifiers by fragment are stored. Junctions spanned by reads are also stored in GRanges object if the argument \( \text{keep.junx} \) is set to TRUE.

Methods

signature(bam='list',stranded='logical',seed='integer',verbose='logical',rname='character',keep

Process paired-end data stored in BAM object generated by scanBam. Outputs GRanges objects for reads and (optionally) junctions.

Author(s)

Camille Stephan-Otto Attolini
**procBam-class**

**Description**
Stores processed bam files in a RangedData format. Each read is split into disjoint ranges according to its cigar code.

**Objects from the Class**
Objects are created with a call to `procBam`.  

**Slots**
- **pbam** GRanges indicating chromosome, start and end of each disjoint range. The pair id and read id within the pair are also stored.
- **junx** GRanges indicating chromosome, start and end of junctions spanned by reads.
- **stranded** Logical variable. TRUE indicates that the reads were obtained from an RNA-seq experiment where strand information was preserved.
  In the case of stranded experiments:
- **plus** GRanges indicating chromosome, start and end of each disjoint range for fragments originated from the positive strand. The pair id and read id within the pair are also stored.
- **minus** GRanges indicating chromosome, start and end of each disjoint range for fragments originated from the negative strand. The pair id and read id within the pair are also stored.
- **pjunx** GRanges indicating chromosome, start and end of junctions spanned by reads originated from the positive strand.
- **mjunx** GRanges indicating chromosome, start and end of junctions spanned by reads originated from the negative strand.

**Methods**
- **show** signature(object = "procBam"): Displays general information about the object.
- **getReads** signature(x = "procBam"): Extracts the aligned reads stored in x.

**Author(s)**
Camille Stephan-Otto Attolini, David Rossell

**See Also**
- `getReads`

**Examples**
- `showClass("procBam")`
**procGenome**

Create an annotatedGenome object that stores information about genes and transcripts

**Description**

procGenome processes annotations for a given transcriptome, either from a TxDb object created by GenomicFeatures package (e.g. from UCSC) or from a user-provided GRanges object (e.g. by importing a gtf file).

createDenovoGenome creates a de novo annotated genome by combining UCSC annotations and observed RNA-seq data.

**Usage**

```r
procGenome(genDB, genome, mc.cores=1, verbose=TRUE)
createDenovoGenome(reads, DB, minLinks=2,
maxLinkDist=1e+05, maxDist=1000, minConn=2, minJunx=3, minLen=12, mc.cores=1)
```

**Arguments**

- `genDB`: Either a TxDb object with annotations (e.g. from UCSC or a gtf file or a GRanges object as returned by import from rtracklayer package). See details.
- `genome`: Character indicating genome version (e.g. "hg19", "dm3")
- `mc.cores`: Number of cores to use in parallel processing (multicore package required)
- `verbose`: Set to TRUE to print progress information
- `DB`: annotatedGenome object, as returned by procGenome
- `minLinks`: Minimum number of reads joining two exons to merge their corresponding genes
- `maxLinkDist`: Maximum distance between two exons to merge their corresponding genes. A value of 0 disables this option.
- `maxDist`: Maximum distance between two exons with reads joining them to merge their corresponding genes.
- `minConn`: Minimum number of fragments connecting a new exon to an annotated one to add to denovo genome.
- `minJunx`: Minimum number of junctions needed to redefine an annotated exon’s end or start.
- `minLen`: Minimum length of a junction to consider as a putative intron.
- `reads`: Processed reads stored in a RangedData, as returned by procBam

**Details**

These functions create the annotation objects that are needed for subsequent functions. Typically these objects are created only once for a set of samples.

If interested in quantifying expression for known transcripts only, one would typically use procGenome with a TxDb from the usual Bioconductor annotations, e.g. `genDB<-makeTxDbFromUCSC(genome="hg19",tablename="refGene")`, or imported from a gtf file e.g. `genDB<-makeTxDbFromGFF("transcripts.gft",format="gtf")`. GRanges
procGenome

object (e.g. genDB <- import('transcripts.gtf')). Package GenomicFeatures contains more info about how to create TxDb objects. Alternatively, one can provide annotations as a GRanges object which is returned when importing a gtf file with function import (package rtracklayer).

The output from procGenome can be used in combination with wrapKnown, which quantifies expression for a set of known transcripts, or wrapDenovo, which uses Bayesian model selection methods to assess which transcripts are truly expressed. When using wrapDenovo, you should create a single annotatedGenome object that combines information from all samples (e.g. from a gtf file produced by running your favorite isoform prediction software jointly on all samples), as this increases the power to detect new exons and isoforms.

Value

Object of class annotatedGenome.

Methods

signature(genDB = "transcriptDb") genDB is usually obtained with a call to makeTxDbFromUCSC (package GenomicFeatures), e.g. genDB<-makeTxDbFromUCSC(genome="hg19", tablename="refGene")

signature(genDB = "GRanges") genDB stores information about all transcripts and their respective exons. Chromosome, start, end and strand are stored as usual in GRanges objects. genDB must have a column named "type" taking the value "transcript" for rows corresponding to transcript and "exon" for rows corresponding to exons. It must also store transcript and gene ids. For instance, Cufflinks RABT module creates a gtf file with information formatted in this manner for known and de novo predicted isoforms.

Author(s)

Camille Stephan-Otto Attolini

See Also

See annotatedGenome-class for a description of the class. See methods transcripts to extract exons in each transcript, getIsland to obtain the island id corresponding to a given transcript id See splitGenomeByLength for splitting an annotatedGenome according to gene length.

Examples

```r
## Known transcripts from Bioconductor annotations
## library(TxDb.Hsapiens.UCSC.hg19.knownGene)
## hg19DB <- procGenome(TxDb.Hsapiens.UCSC.hg19.knownGene, genome='hg19')

## Alternative using makeTxDbFromUCSC
## genDB<-makeTxDbFromUCSC(genome="hg19", tablename="refGene")
## hg19DB <- procGenome(genDB, "hg19")

## Alternative importing .gtf file
## genDB.Cuff <- import('transcripts.gtf')
## hg19DB.Cuff <- procGenome(genDB.Cuff, genome='hg19')
```
qqnormGenomeWide

Genome-wide qq-normal and qq-gamma plots

Description

qqnormGenomeWide overlays quantile-quantile normal plots (qqnorm) for a series of genes (rows in the input matrix), to provide an overall assessment of Normality. Similarly, qqgammaGenomeWide overlays quantile-quantile gamma plots.

Note that the theoretical quantiles for z-scores under a Normal are the same for all genes, but the gamma theoretical quantiles depend on the Gamma parameter estimates for each gene and hence the theoretical quantiles are different for each gene (resulting in different x-values in each qq-plot)

Usage

qqnormGenomeWide(x, ngenes=min(1000, nrow(x)), ...)
qqgammaGenomeWide(x, ngenes=min(1000, nrow(x)), ...)

Arguments

x  ExpressionSet, matrix or data.frame with genes/isoforms in rows
ngenes  A qqnorm plot is produced for the first ngenes rows in x
...  Other arguments to be passed on to codeplot

Value

Produces a figure overlaying qq-normal or qq-gamma plots for ngenes comparing observed vs. theoretical quantiles

Author(s)

David Rossell

Examples

mu <- rnorm(100)
x <- matrix(rnorm(100*5,mu),ncol=5)
qqnormGenomeWide(x)
qqgammaGenomeWide(exp(x))
quantileNorm

Apply quantile normalization

Description
Perform quantile normalization on the columns of a matrix or ExpressionSet

Usage
quantileNorm(x)

Arguments
x ExpressionSet or matrix

Value
Returns x with quantile normalized columns

Author(s)
David Rossell

Examples
x <- cbind(rnorm(1000), rnorm(1000, 2, 4))
boxplot(x)

xnorm <- quantileNorm(x)
boxplot(xnorm)

relexprByGene Compute relative expressions within each gene

Description
Transforms relative expressions that add up to 1 within each gene island (the default output of casper) to relative expressions that add up to 1 per gene.

Usage
relexprByGene(x, normbylength=FALSE, genomeDB)

Arguments
x ExpressionSet containing relative expressions. (typically, adding up to 1 for each island_id) Column gene_id in fData(x) should contain a unique gene identifier.

normbylength If set to TRUE, isoform expressions are divided by isoform length before re-normalizing. This is useful for taking into account that longer isoforms produce more reads than shorter isoforms.

geneDB If normbylength==TRUE, genomeDB should be an annotatedGenome object containing the annotated genome (see procGenome)
Value

ExpressionSet with relative expressions adding up to one for each gene_id.

Author(s)

David Rossell

Examples

#See help(calcExp)

---

**rmShortInserts**

Remove reads with short insert sizes from imported BAM files.

Description

In paired-end experiments short inserts (i.e. the 2 ends being very close to each other), may indicate RNA degradation or that a short RNA (e.g. miRNA) is being sequenced. Typically the goal is not to study alternative splicing for such short/degraded RNA; in this case it is recommendable to remove such short inserts to avoid biasing the insert size distribution. Requiring a minimum insert size can also result in significantly faster computations when quantifying alternative splicing via calc or calcDenovo.

Usage

rmShortInserts(bam, isizeMin=100)

Arguments

- **bam**: Object with aligned reads, as returned by scanBam
- **isizeMin**: Reads with insert size smaller than isizeMin will be removed.

Value

Named list, in the same format as that returned by scanBam.

Note

The insert size is stored in objects imported with scanBam in the element named isize.

Author(s)

David Rossell

Examples

### Should be DIRECTLY executable !! ----
### ==> Define data, use random,
### or do help(data=index) for the standard data sets.
**simMAE**

*Simulate Mean Absolute Error (MAE) in estimating isoform expression under various experimental settings.*

**Description**

Simulate several future RNA-seq data under various experimental settings (sequencing depth, read length, insert sizes), estimate isoform expression and assess the MAE incurred in the estimation process. The function is a wrapper combining functions simReads and calcExp.

**Usage**

```r
simMAE(nsim, islandid, nreads, readLength, fragLength, burnin=1000, pc, distr, readLength.pilot, eset.pilot, usePilot=FALSE, retTxsError=FALSE, genomeDB, mc.cores=1, mc.cores.int=1, verbose=FALSE, writeBam=FALSE, bamFile=NULL)
```

**Arguments**

- `nsim` Number of RNA-seq datasets to generate (often as little as `nsim=10` suffice)
- `islandid` When specified this argument indicates to run the simulations only for gene islands with identifiers in `islandid`. When not specified genome-wide simulations are performed.
- `nreads` Vector indicating the target number of read pairs for each experimental setting. The actual number of reads differs from `nreads` to account for non-mappability and random read yield (see details)
- `readLength` Vector indicating the read length in each experimental setting
- `fragLength` Vector indicating the mean insert size in each experimental setting
- `burnin` Number of MCMC burn-in samples (passed on to `calcExp`)
- `pc` Observed path counts in pilot data. When not specified, these are simulated from `eset.pilot`
- `distr` Estimated read start and insert size distributions in pilot data
- `readLength.pilot` Read length in pilot data
- `eset.pilot` ExpressionSet with pilot data expression in log2-RPKM, used to simulate `pc` when not specified by the user. See details
- `usePilot` By default casper assumes that the pilot data is from a related experiment rather than the current tissue of interest (usePilot=FALSE). Hence, the pilot data is used to simulate new RNA-seq data but not to estimate its expression. However, in some cases we may be interested in re-sequencing the pilot sample at deeper length, in which case one would want to combine the pilot data with the new data to obtain more precise estimates. This can be achieved by setting `usePilot=TRUE`
- `retTxsError` If `retTxsError=TRUE`, `simMAE` returns posterior expected MAE for each individual isoform. This option is not available when `eset.pilot` is specified instead of `pc`. Else the output is a `data.frame` with overall MAE across all isoforms
- `genomeDB` annotatedGenome object, as returned by `procGenome`
- `mc.cores` Number of cores to use in the expression estimation step, passed on to `calcExp`
- `mc.cores.int` Number of cores to simulate RNA-seq datasets in parallel
verbose

Set verbose=TRUE to print progress information

writeBam

Set to TRUE to write simulated reads to a .bam file

bamFile

Name of the .bam file

Details

simMAE simulates nsim datasets under each experimental setting defined by nreads, readLength, fragLength. For each dataset the following steps are performed:

1. The number of reads is nreads * readYield * pmapped, where readYield= runif(1,0.8,1.2) accounts for deviations in read yield and pmapped= runif(1,0.6,0.9)*mappable is the proportion of mapped reads (60%-90% of the mappable reads according to the piecewise-linear power law of Li et al (2014))

2. True expression levels $pi$ are generated from their posterior distribution given the pilot data.

3. Conditional on $pi$, RNA-seq data are generated and expression estimates $pihat$ are obtained using calcExp

4. The mean absolute estimation error $\sum(abs(pihat-pi))$ across all isoforms is computed

Ideally simMAE should use pilot data from a relevant related experiment to simulate what future data may look like for the current experiment of interest. The recommended way to do this is to download a .bam file from such a related experiment and processing it in casper with function wrapKnown, as then both gene and isoform expression can be estimated accurately. The object output by wrapKnown is a list with elements named 'pc', 'distr' which can be given as input to simMAE.

As an alternative to specifying pc, simMAE allows setting eset.pilot as pilot data. Gene and isoform expression are then simulated as follows:

1. The number of reads per gene is generated from a Multinomial distribution with success probabilities proportional to $2^{exprs(eset.pilot)}$.

2. Relative isoform expression within each gene are generated from a symmetric Dirichlet distribution with parameter $1/Ig$, where Ig is the number of isoforms in gene g.

We emphasize that relative isoform expressions are not trained from the pilot data, and that while the distribution of gene expression levels resembles that in eset.pilot, no attempt is made to match gene identifiers and hence the results for individual genes should not be trusted (hence this option is only available when retTxsError==FALSE).

Value

If retTxsError==TRUE, simMAE returns posterior expected MAE for each individual isoform. Else the output is a data.frame with overall MAE across all isoforms

References

Stephan-Otto Attolini C., Pena V ., Rossell D. Bayesian designs for personalized alternative splicing RNA-seq studies (2014)


See Also

wrapKnown,simReads,calcExp
Examples

```r
## maybe str(simMAE); plot(simMAE) ...
```

simMAEcheck

Model checking for One Sample Problems.

Description

Simulates RNA-seq data under the same experimental setting as in the observed data, and compares the observed vector of number of reads per gene with the simulations.

Usage

```r
simMAEcheck(nsim, islandid, burnin=1000, pc, distr, readLength.pilot, eset.pilot, usePilot=FALSE, retTxsError=FALSE, genomeDB, mc.cores=1, mc.cores.int=1, verbose=FALSE)
```

Arguments

- `nsim`: Number of RNA-seq datasets to generate (often as little as `nsim=10` suffice)
- `islandid`: When specified this argument indicates to run the simulations only for gene islands with identifiers in `islandid`. When not specified genome-wide simulations are performed.
- `burnin`: Number of MCMC burn-in samples (passed on to `calcExp`)
- `pc`: Observed path counts in pilot data. When not specified, these are simulated from `eset.pilot`
- `distr`: Estimated read start and insert size distributions in pilot data
- `readLength.pilot`: Read length in pilot data
- `eset.pilot`: ExpressionSet with pilot data expression in log2-RPKM, used to simulate `pc` when not specified by the user. See details
- `usePilot`: By default casper assumes that the pilot data is from a related experiment rather than the current tissue of interest (`usePilot=FALSE`). Hence, the pilot data is used to simulate new RNA-seq data but not to estimate its expression. However, in some cases we may be interested in re-sequencing the pilot sample at deeper length, in which case one would want to combine the pilot data with the new data to obtain more precise estimates. This can be achieved by setting `usePilot=TRUE`
- `retTxsError`: If `retTxsError=TRUE`, `simMAE` returns posterior expected MAE for each individual isoform. This option is not available when `eset.pilot` is specified instead of `pc`. Else the output is a `data.frame` with overall MAE across all isoforms
- `genomeDB`: `annotatedGenome` object, as returned by `procGenome`
- `mc.cores`: Number of cores to use in the expression estimation step, passed on to `calcExp`
- `mc.cores.int`: Number of cores to simulate RNA-seq datasets in parallel
- `verbose`: Set `verbose=TRUE` to print progress information

Details

`simMAEcheck` simulates `nsim` datasets under the same experimental setting as in the observed data. For more details, please check the documentation for `simMAE`, which is the basis of this function.
The output is a list with 2 entries. The first entry is a data.frame with overall MAE across all isoforms in the simulations (see simMAE for details). The second entry contains the expected number of genes for which the number of reads in the data lies in the range of the posterior predictive simulations (under the hypothesis that they have the same distribution) and the actual number of genes for which the condition is satisfied.

References

Stephan-Otto Attolini C., Pena V., Rossell D. Bayesian designs for personalized alternative splicing RNA-seq studies (2014)


See Also

wrapKnown,simReads,calcExp

Examples

#Run casperDesign() to see full manual with examples

```
simMultSamples
Simulate paired end reads for multiple future samples based on pilot data, and obtain their expression estimates via casper
```

Description

Simulate true expression levels and observed data (casper expression estimates) for future samples within each group.

These simulations serve as the basis for sample size calculation: if one were to sequence nsamples new RNA-seq samples, what data would we expect to see? The simulation is posterior predictive, i.e. based on the current available data $x$.

Usage

```
simMultSamples(nsim, nsamples, nreads, readLength, fragLength, x, groups='group', distrs, genomeDB, model='LNNMV', verbose=TRUE, mc.cores=1)
```

Arguments

- `nsim`: Number of simulations to obtain
- `nsamples`: Vector indicating number of future samples per group, e.g. `nsamples=c(5,5)` to simulate 5 new samples for 2 groups.
- `nreads`: Desired number of paired-end reads per sample. The actual number of aligned reads for any given sample differs from this amount, see details.
- `readLength`: Read length, i.e. in an experiment with paired reads at 100bp each, `readLength=100`.
- `fragLength`: Desired average insert size (size of RNA molecules after fragmentation). If missing, insert sizes are as obtained from `distrs`
**simMultSamples**

- **x**: ExpressionSet containing pilot data. x[[group]] indicates groups to be compared.
- **groups**: Name of column in pData(x) indicating the groups.
- **distrs**: Fragment start and length distributions. It can be either an object or a list of objects of class readDistrs. In the latter case, an element is chosen at random for each individual sample to consider uncertainty in these distributions. If not specified, it defaults to data(distrsGSE37704).
- **genomeDB**: annotatedGenome object.
- **model**: Set to 'LNNMV' to simulate from log-normal normal with modified variance model (Yuan and Kendziorski, 2006), or to 'GaGa' to simulate from the GaGa model (Rossell, 2009). See details.
- **verbose**: Set to TRUE to print progress.
- **mc.cores**: Number of cores to use in function. mc.cores>1 requires package parallel.

**Details**

The posterior predictive simulations is based on four steps: (1) simulate true expression for each group (mean and SD), (2) simulate true expression for future samples, (3) simulate paired reads for each future sample, (4) estimate expression from the reads via Casper. Below are some more details.

1. Simulate true mean expression in each group and residual variance for each gene. If model=='LNNMV' this is based on the log-normal normal with modified variance model in package EBarrays (Yuan & Kendziorski 2006), if model=='GaGa' this is based on the GaGa model (Rossell, 2009). adapted to take into account that the expression estimates in the pilot data x are noisy (which is why simMultSamples requires the SE / posterior SD associated to exprs(x)). The simulated values are returned in component "simTruth" of the simMultSamples output.

2. Simulate true isoform expression for each of the future samples. These are independent Normal draws with mean and variance generated in step 1. True gene expression is derived from the isoform expressions.

3. Determine the number of reads to be simulated for each gene based on its true expression (generated in step 2) and a Multinomial sampling model. For each sample:
   - The number of reads yielded by the experiment is Unif(.8*nreads,1.2*nreads) - A proportion of non-mappable reads is discarded using the power law in Li et al (2014) - Amongst remaining reads, we assume that a proportion Unif(0.6,0.9) were aligned (consistenly with reports from ENCODE project)

   The final number of simulated reads is reported in component "simExpr" of the simMultSamples output.

4. Obtain expression estimates from the path counts produced in step 3 via calcExp. These are reported in component "simExpr" of the simMultSamples output.

**Value**

Object of class simulatedSamples, which extends a list of length nsim. See the class documentation for some helpful methods (e.g. coef, exprs, mergeBatches). Each element is itself a list containing an individual simulation.

- **simTruth**: data.frame indicating the mean and standard deviation of the Normal distribution used to generate data from each group.
simExpr

ExpressionSet with Casper expression estimates, as returned by `calcExp`. `pData(simExpr)` indicates group information, and `fData(simExpr)` the number of simulated reads for each sample (in columns 'explCnts') and across all samples (in column 'readCount')

Author(s)

Victor Pena, David Rossell

References


Stephan-Otto Attolini C., Pena V., Rossell D. Bayesian designs for personalized alternative splicing RNA-seq studies (2015)


Examples

# Run casperDesign() to see full manual with examples

### simReads

*Function to simulate paired end reads following given read start and fragment length distributions and gene and variant expressions.*

Description

This function generates path counts and bam files with simulated paired end reads according to given read start distribution, fragment length distribution and gene and variant expressions.

Usage

```r
simReads(islandid, nSimReads, pis, rl, seed, writeBam, distrs, genomeDB, repSims=FALSE, bamFile=NULL, stranded=FALSE, verbose=TRUE, chr=NULL, mc.cores=1)
```

Arguments

- **islandid**: Island ID’s from the genomeDB object to simulate reads
- **nSimReads**: Named numeric vector with number of fragments to simulate in each island.
- **pis**: Named numeric vector with relative expression of transcripts. Expressions add up to one for each island to simulate.
- **rl**: Read length
- **seed**: Seed of the random numbers generator
- **writeBam**: Set to 1 to generate bam files with the simulated reads
- **distrs**: Object of class ‘readDistrs’ with read start and fragment length distributions
- **genomeDB**: Object of class ‘annotated Genome’ with the genome to generate reads from
- **repSims**: Set to TRUE to return relative read starts and fragment lengths from the simulation
simulatedSamples-class

**Description**

`simulatedSamples` stores multiple simulated isoform expression datasets. Each dataset contains the (simulation) true mean expression in each group and residual variance, as well as the estimated expression in each individual sample.

**Value**

- **Nsim**: Numerical vector with the number of reads simulated for each island.
- **pc**: Object of class `pathCounts` with simulated path counts.
- **sims**: Only if 'repSims' is set to TRUE. List with vectors of length 'n' with the following elements: - 'varl': Length of variant for corresponding read - 'st': Start of fragment relative to variant start (not in genomic coordinates) - 'len': Fragment length - 'strand': Strand of gene for simulated read.

**Author(s)**

Camille Stephan-Otto Attolini

**Examples**

```r
data(hg19DB)
data(K562.r1l1)
distrs <- getDistrs(hg19DB, bam=K562.r1l1, readLength=75)

islandid <- c('10319', '463')
txs <- unlist(lapply(hg19DB@transcripts[islandid], names))
lpis <- vector(mode = 'numeric', length = length(txs))
npis <- sapply(hg19DB@transcripts[islandid], length)
lpis[1:npis[1]] <- rep(1/npis[1], npis[1])
lpis[-1:-npis[1]] <- rep(1/npis[2], npis[2])
names(lpis) <- txs

nSimReads <- c(100, 100)
names(nSimReads) <- islandid

smpc <- simReads(islandid=islandid, nSimReads=nSimReads, pis=lpis, r1=75, repSims=TRUE, seed=1, writeBam=FALSE, distrs=distrs, genomeDB=hg19DB)
```
splitGenomeByLength

Objects from the Class

Objects are returned by simMultSamples.

Slots

The class extends a list directly.

.Data A list, each element containing a different simulated dataset

Methods

show signature(object = "simulatedSamples"): Displays general information about the object.

coeff signature(object = "simulatedSamples"): Returns a matrix with difference between group means (simulation truth) in all simulated datasets

exprs signature(object = "simulatedSamples"): Returns a list of ExpressionSets containing the estimated expressions in each simulation.

mergeBatches signature(x="ExpressionSet", y="simulatedSamples"): Combines x with each element in exprs in y, and returns a list. See help(mergeBatches) for more details.

"[" x[i] selects a subset of simulations, x[,j] a subset of the samples in each simulation

Author(s)

David Rossell

See Also

mergeBatches

Examples

showClass("simulatedSamples")

splitGenomeByLength Split an annotatedGenome object into subsets according to gene length

Description

splitGenomeByLength splits an annotatedGenome according to gene length (bp), which allows estimating the fragment start and length distribution for each subset separately.

Usage

splitGenomeByLength(DB, breaks=c(0,3000,5000,Inf))

Arguments

DB Object containing annotated genome. Must be of class annotatedGenome, as returned by procGenome or createDenovoGenome.

breaks Breakpoints to define gene subgroups.
subsetGenome

Details
By default groups are <3000bp, 3000-5000bp, >5000bp, which work well for the human genome. Further sub-divisions may result in unstable estimates of fragment start and length distributions.

Value
List where each component is of class annotatedGenome.

Author(s)
David Rossell

See Also
procGenome and createDenovoGenome for creating annotatedGenome objects. getDistrs for estimating fragment start and length distribution.

Examples
```r
### Not run
## genDB<-makeTranscriptDbFromUCSC(genome="hg19", tablename="refGene")
## hg19DB <- procGenome(genDB, "hg19")
## hg19split <- splitGenomeByLength(hg19DB)
```

subsetGenome subsets an object of class annotatedGenome for a set of island IDs or chromosome names.

Description
~~ Methods for function subsetGenome in package casper ~~ Subset an annotatedGenome object by islands or chromosomes.

Usage
```
subsetGenome(islands, chr, genomeDB)
```

Arguments
- `islands` Vector of characters with the island IDs to retrieve from genome.
- `chr` Vector of characters with the names of chromosomes to retrieve from genome.
- `genomeDB` annotatedGenome object with genome to subset.

Methods
```
signature(islands = "character", chr = "missing", genomeDB = "annotatedGenome")
Subset annotatedGenome object by a set of island IDs.
signature(islands = "missing", chr = "character", genomeDB = "annotatedGenome")
Subset annotatedGenome object by chromosomes.
```
transcripts

*Extracts transcript information (exon start and ends) from an annotatedGenome object, either for all transcripts or only those corresponding to a given island or transcript.*

**Description**

annotatedGenome objects store information regarding genes and transcripts. When there's an overlap in exons between several genes, these genes are grouped into gene islands.

transcripts retrieves all stored transcripts for a given transcript or island.

matchTranscripts finds transcripts in queryDB matching a transcript in subjectDB. The best match for each transcript in subjectDB is returned, unless difference in bp is >maxbp

**Usage**

```r
transcripts(genomeDB, txid, islandid)
macthTranscripts(queryDB, subjectDB, maxbp=10)
```

**Arguments**

- `genomeDB`: Object of class annotatedGenome
- `txid`: Character indicating transcript identifier (optional)
- `islandid`: Character indicating island identifier (optional)
- `queryDB`: annotatedGenome with query transcripts
- `subjectDB`: annotatedGenome with potentially matching transcripts
- `maxbp`: Maximum difference in bp for transcripts to be matched

**Value**

IRangesList where each element in the list corresponds to a different transcript.

**Methods**

```r
signature(genomeDB = "annotatedGenome", txid="missing", islandid="missing")
  Return exons for all transcripts in genomeDB
signature(genomeDB = "annotatedGenome", txid="character", islandid="missing")
  Return exons for transcript txid
signature(genomeDB = "annotatedGenome", txid="missing", islandid="character")
  Return exons for all transcripts in island islandid
```

**See Also**

genePlot to plot the resulting transcripts

**Examples**

```r
data(hg19DB)
txs <- transcripts(txid="NM_005158", genomeDB=hg19DB)
txs
```
txLength

Description

~~ Methods for function txLength in package casper ~~

Function to retrieve transcript lengths from annotated genome (class genomeDB).

Usage

txLength(islandid, txid, genomeDB)

Arguments

islandid  Retrieve length for transcripts in island islandid.

(txid)  Retrieve length for txid transcripts.

genomeDB  Annotated genome of class genomeDB.

Details

When called for the first time lengths are calculated and stored in the object genomeDB. Subsequent calls refer to these computed values.

Value

Named numeric vector with transcript lengths.

Methods

signature(islandid = "character", txid = "missing", genomeDB = "annotatedGenome")

Retrieve lengths from genomeDB for transcripts in island islandid.

signature(islandid = "missing", txid = "character", genomeDB = "annotatedGenome")

Retrieve lengths from genomeDB for txid transcripts.

signature(islandid = "missing", txid = "missing", genomeDB = "annotatedGenome")

Retrieve or calculate lengths for all transcripts in the annotated genome genomeDB.

wrapDenovo

Run all necessary steps to get expression estimates from multiple bam files with the casper pipeline.

Description

Function to analyze bam files to generate an ExpressionSet with expression estimates for all samples, read start and fragment length distributions, path counts and optionally processed reads.
**wrapDenovo**

**Usage**

```r
wrapDenovo(bamFile, output_wrapKnown, knownGenomeDB, targetGenomeDB, readLength, rpkm=TRUE, keep.multihits=TRUE, searchMethod="submodels", exactMarginal=TRUE, integrateMethod = "plugin", maxExons=40, islandid, chroms=NULL, keep.pbam=FALSE, keepPbamInMemory=FALSE, niter=10^3, priorq=3, priorqGeneExpr=2, mc.cores.int=1, mc.cores=1, verbose=TRUE, seed=1)
```

**Arguments**

- **bamFile**: Names of bam files with the sample to analyze. These must sorted and indexed, and the index must be in the same directory.
- **output_wrapKnown**: Optional argument containing the output of an earlier call to `wrapKnown`. If provided, path counts, read start and insert size distributions are loaded from this output rather than being re-computed. Better leave this argument missing unless you know what you’re doing.
- **knownGenomeDB**: annotatedGenome object with known isoforms, e.g. from UCSC or GENCODE annotations. Used to set the prior probability that any given isoform is expressed. See `help(calcDenovo)` for details.
- **targetGenomeDB**: annotatedGenome object with isoforms we wish to quantify. By default these are the same as in `knownGenomeDB`, but more typically `targetGenomeDB` is imported from a .gtf file produced by some isoform prediction software.
- **readLength**: Read length in bp, e.g. in a paired-end experiment where 75bp are sequenced on each end one would set `readLength=75`.
- **rpkm**: Set to `TRUE` to return reads per kilobase per million (RPKM), `FALSE` for relative expression levels. Important, relative expression adds up to 1 within gene island, NOT within gene. To get relative expressions within gene run `relExprByGene` afterwards. See `help(wrapKnown)`.
- **keep.multihits**: Set to `FALSE` to discard reads aligned to multiple positions.
- **searchMethod**: Method used to perform the model search. "allmodels" enumerates all possible models (warning: this is not feasible for genes with >5 exons). "rwmcmc" uses a random-walk MCMC scheme to focus on models with high posterior probability. "submodels" considers that some isoforms in `targetGenomeDB` may not be expressed, but does not search for new variants. "auto" uses "allmodels" for genes with up to 5 exons and "rwmcmc" for longer genes. See `help(calcDenovo)`.
- **exactMarginal**: Set to `FALSE` to estimate posterior model probabilities as the proportion of MCMC visits. Set to `TRUE` to use the integrated likelihoods (default). See details.
- **integrateMethod**: Method to compute integrated likelihoods. The default (‘plugin’) evaluates likelihood*prior at the posterior mode and is the faster option. Set ‘Laplace’ for Laplace approximations and ‘IS’ for Importance Sampling. The latter increases computation cost very substantially.
- **maxExons**: Prior probabilities of isoform expression are estimated for genes with 1 up to `maxExons` exons separately, for genes with more than `maxExons` exons a combined estimate is used. See `help("modelPrior")`.
- **islandid**: Names of the gene island to be analyzed. If missing all gene islands are analyzed.
- **chroms**: Names of the chromosomes to be analyzed. If missing all chromosomes are analyzed.
wrapDenovo

keep.pbam  Set to TRUE to save processed bam object, as returned by procBam. This object can require substantial memory during execution and disk storage upon saving and is not needed for a default analysis.

keepPbamInMemory  Set to TRUE to keep processed bam objects in memory to speed up some computations.

niter  Number of MCMC iterations in the model search algorithm.

priorq  Parameter of the Dirichlet prior for the proportion of reads coming from each variant. We recommend priorq=3 as this defines a non-local prior that penalizes falsely predicted isoforms.

priorqGeneExpr  Parameter of the Dirichlet prior distribution on overall gene expression. Defaults to 2 to ensure non-zero estimates.

mc.cores  Number of cores to use in expression estimation.

mc.cores.int  Number of cores to use when loading bam files. Be careful as this is a memory intensive step.

verbose  Set to TRUE to display progress information.

seed  Set seed of random number generator.

Details

The function executes the functions procBam, getDistrs, pathCounts calcDenovo and denovoExpr and formats the output nicely. Running wrapDenovo is much more efficient in cpu speed and memory usage than running these functions separately.

When rpkm is false the function returns the estimated proportion of reads arising from each isoform within a gene island. See the details in help("wrapKnown") for more information on this.

Value

denovoGenomeDB  annotatedGenome that contains the isoforms in targetGenomeDB plus any new isoforms predicted by casper.

exp  Object of class ExpressionSet containing Bayesian model averaging expression estimates. See the fData for the posterior probability that each isoform is expressed.

distr  Object of class readDistrs

pbam  List of objects of class procBam with one element per chromosome

Author(s)

Miranda Stobbe, David Rossell

References


See Also

calcDenovo, wrapKnown, relexprByGene
### Examples
```
## not run
## Known isoforms
## library(TxDb.Hsapiens.UCSC.hg19.knownGene)
## hg19DB <- procGenome(TxDb.Hsapiens.UCSC.hg19.knownGene), genome='hg19')

## gtf with known & de novo predictions
## mygtf <- import('hg19_denovo.gtf')
## hg19denovoDB <- procGenome(mygtf, genome='hg19')

## bamFile="/path_to_bam/sorted.bam"
## ans <- wrapDenovo(bamFile=bamFile, targetGenomeDB=hg19denovoDB, knownGenomeDB=hg19DB, readLength=101)

## Estimated expression via BMA
## head(exprs(ans[['exp']]))

## Posterior probability that each isoform is expressed
## head(fData(ans[['exp']]))
```

---

**wrapKnown**

Run all necessary steps to get expression estimates from multiple bam files with the casper pipeline.

---

**Description**

Function to analyze bam files to generate an ExpressionSet with expression estimates for all samples, read start and fragment length distributions, path counts and optionally processed reads.

**Usage**

```
wrapKnown(bamFile, verbose=FALSE, seed=1, mc.cores.int=1, mc.cores=1, genomeDB, readLength, rpkm=TRUE, priorq=2, priorqGeneExpr=2, citype='none', niter=10^3, burnin=100, keep.pbam=FALSE, keep.multihits=TRUE, chroms=NULL)
```

**Arguments**

- **bamFile**: Names of bam files with the sample to analyze. These must sorted and indexed, and the index must be in the same directory.
- **verbose**: Set to TRUE to display progress information.
- **seed**: Set seed of random number generator.
- **mc.cores.int**: Number of cores to use when loading bam files. This is a memory intensive step, therefore number of cores must be chosen according to available RAM memory.
- **mc.cores**: Number of cores to use in expression estimation.
- **genomeDB**: annotatedGenome object containing annotated genome, as returned by the procGenome function.
- **readLength**: Read length in bp, e.g. in a paired-end experiment where 75bp are sequenced on each end one would set readLength=75.
wrapKnown

rpkm
Set to TRUE to return reads per kilobase per million (RPKM). Set to FALSE to return relative expression levels. Important, relative expression adds up to 1 within gene island, NOT within gene. To get relative expressions within gene run relexprByGene afterwards. See details.

priorq
Parameter of the prior distribution on the proportion of reads coming from each variant. The prior is Dirichlet with prior sample size for each variant equal to priorq. We recommend priorq=2 for estimation, as it pools the estimated expression away from 0 and 1 and returned lower estimation errors than priorq=1 in our simulated experiments.

priorqGeneExpr
Parameter for prior distribution on overall gene expression. Defaults to 2, which ensures non-zero estimates for all genes

citype
Set to "none" to return no credibility intervals. Set to "asymp" to return approximate 95% CIs (obtained via the delta method). Set to "exact" to obtain exact CIs via Monte Carlo simulation. Options "asymp" and especially "exact" can increase the computation time substantially.

niter
Number of Monte Carlo iterations. Only used when citype="exact".

burnin
Number of burnin Monte Carlo iterations. Only used when citype="exact".

keep.pbam
Set to TRUE to save processed bam object, as returned by procBam. This object can require substantial memory during execution and disk storage upon saving and is not needed for a default analysis.

keep.multihits
Set to FALSE to discard reads aligned to multiple positions.

chroms
Manually set chromosomes to be processed. By default only main chromosomes are considered (except 'chrM')

Details

The function executes the functions procBam, getDistrs and pathCounts in parallel for each chromosome, but is much more efficient in cpu speed and memory usage than running these functions separately. Data from multiple samples are then combined using mergeExp. Note that further normalization (e.g. quantileNorm) may be needed preliminary to actual data analysis.

When rpkm is false the function returns the estimated proportion of reads arising from each isoform within a gene island. casper groups two or more genes into a gene island whenever these genes share an exon (or part of an exon). Because exons are shared, isoform quantification must be done simultaneously for all those genes.

That is, the output from wrapKnown when rpkm is FALSE are proportions that add up to 1 within each island. If you would like to re-normalize these expressions so that they add up to 1 within each gene, see the help for function relexprByGene.

One last remark: casper returns the estimated proportion of reads generated by each isoform, which is not the same as relative isoform expressions. Longer isoforms tend to produce more reads than shorter isoforms. This is easily accounted for by dividing relative expressions by isoform length, see relexprByGene.

Value

distr
Object of class readDistrs

pbam
List of objects of class procBam with one element per chromosome

pc
Object of class pathCounts

exp
Object of class ExpressionSet
Author(s)
Camille Stephan-Otto Attolini, David Rossell

References

See Also
procGenome, relexprByGene, quantileNorm

Examples
```r
## genDB<-makeTranscriptDbFromUCSC(genome="hg19", tablename="refGene")
## hg19DB <- procGenome(genDB, "hg19")
## bamFile="/path_to_bam/sorted.bam"
## ans <- wrapKnown(bamFile=bamFile, mc.cores.int=4, mc.cores=3, genomeDB=hg19DB, readLength=101)
## names(ans)
## head(exprs(ans$exp))
```
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