Package 'RiboProfiling'

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Type Package

Title Ribosome Profiling Data Analysis: from BAM to Data Representation and Interpretation

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Description Starting with a BAM file, this package provides the necessary functions for quality assessment, read start position recalibration, the counting of reads on CDS, 3'UTR, and 5'UTR, plotting of count data: pairs, log fold-change, codon frequency and coverage assessment, principal component analysis on codon coverage.

biocViews RiboSeq, Sequencing, Coverage, Alignment, QualityControl, Software, PrincipalComponent

Depends R (>= 3.2.2), Biostrings

Imports BiocGenerics, GenomeInfoDb, GenomicRanges, IRanges, reshape2, GenomicFeatures, grid, plyr, S4Vectors, GenomicAlignments, ggplot2, ggbio, Rsamtools, rtracklayer, data.table, sqldf

Suggests knitr, BiocStyle, TxDb.Hsapiens.UCSC.hg19.knownGene, BSgenome.Hsapiens.UCSC.hg19, testthat, SummarizedExperiment

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Contents

`aroundPromoter` *Returns the flank size around the TSS for the x % CDSs*

Description

Returns the flank size around the TSS for the x % CDSs

Usage

```
aroundPromoter(txdb, alnGRanges, percBestExpressed, flankSize)
```

Arguments

`txdb`	a TxDb object containing the annotations info to intersect with the alignment files.
`alnGRanges`	A GRanges object containing the alignment information. In order to improve the performance of this function the GAlignments BAM object should be transformed into a GRanges object containing the cigar match size information as metadata.

percBestExpressed
: a numeric [between 0 and 1]. The percentage of the best expressed CDSs on which to plot the coverage around the TSS. Necessary if the shiftValue parameter must be estimated. Default value 0.03 (3%).

flankSize
: a numeric positive integer. How many bp left and right of the TSS should the coverage be performed? Ex. flankSize <- 20

Value

A GRanges object containing the 1 bp ranges for the selected CDSs in the TSS defined flanking region.

Examples

```
#read the BAM into a GAlignments object using
#GenomicAlignments::readGAlignments
#the GAlignments object should be similar to ctrlGAlignments
data(ctrlGAlignments)
aln <- ctrlGAlignments
#transform the GAlignments object into a GRanges object (faster processing)
alnGRanges <- readsToStartOrEnd(aln, what="start")
#make a txdb object containing the annotations for the specified species.
#In this case hg19.
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene
#Please make sure that seqnames of txdb correspond to
#the seqnames of the alignment files ("chr" particle)
#if not rename the txdb seqlevels
#renameSeqlevels(txdb, sub("chr", "",seqlevels(txdb)))
#get the flanking region around the promoter of the best expressed CDSs
oneBinRanges <- aroundPromoter(txdb, alnGRanges)
```

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cdsPosTransc *Per transcript relative position of start and end codons for dataset ctrlGAlignments*

Description

A list of start and end codons relative to transcript

Usage

```
data(cdsPosTransc)
```

Format

A list

Value

A list

codonDataCtrl *Codon frequency and coverage in ORFs on chromosome 1, for dataset ctrlGAlignments*

Description

A list of 2 data.frame objects: one with the number of times each codon type is found in each ORF and one with the number of reads for each codon type in each ORF.

Usage

```
data(codonDataCtrl)
```

Format

A list of 2 lists.

Value

A list of 2 lists.

codonIndexCovCtrl *The read coverage for each codon in ORFs on chromosome 1, for dataset ctrlGAlignments*

Description

A list containing the number of reads for each codon in each ORF. Codons are reported on their index in the ORF and no information is available about their type/sequence.

Usage

```
data(codonIndexCovCtrl)
```

Format

A list of 2 columns dataframes.

Value

A list of 2 columns dataframes.

codonInfo *Associates the read counts on codons with the codon type for each ORF.*

Description

Associates the read counts on codons with the codon type for each ORF.

Usage

```
codonInfo(listReadsCodon, genomeSeq, orfCoord, motifSize)
```

Arguments

listReadsCodon	a list of data.frame objects. It contains the number of reads per codon in a CDS.
genomeSeq	a BSgenome object. It contains the full genome sequences for the organism.
orfCoord	a GRangesList. The coordinates of the ORFs on the genome.
motifSize	an integer. The number of nucleotides in each motif on which to compute coverage and usage. Either 3, 6, or 9. Default 3 nucleotides (codon). Attention! For long motifs, the function can be quite slow!!

Value

a list of 2 data.frame objects: one with the number of times each codon type is found in each ORF and one with the number of reads for each codon type in each ORF.

Examples

```
#for each codon in each ORF get the read coverage
#parameter listReadsCodon can be returned by the riboSeqFromBam function
#it corresponts to the 2nd element in the list returned by riboSeqFromBam
data(codonIndexCovCtrl)
listReadsCodon <- codonIndexCovCtrl

txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene

#get the names of the ORFs
#grouped by transcript
cds <- GenomicFeatures::cdsBy(txdb, use.names=TRUE)
orfCoord <- cds[names(cds) %in% names(listReadsCodon)]

#get the genome, please check that the genome has the same seqlevels
genomeSeq <- BSgenome.Hsapiens.UCSC.hg19::BSgenome.Hsapiens.UCSC.hg19
#if not rename it
#gSeq <- GenomeInfoDb::renameSeqlevels(genomeSeq,
#sub("chr", "", GenomeInfoDb::seqlevels(genomeSeq)))

#codon frequency, coverage, and annotation
codonData <- codonInfo(listReadsCodon, genomeSeq, orfCoord)
```

codonPCA *PCA graphs on codon coverage*

Description

PCA graphs on codon coverage

Usage

```
codonPCA(data, typeData)
```

Arguments

data	a list of 2 data.frames: one with the number of times each codon type is found in each ORF and one with the number of reads for each codon in each ORF.
typeData	a character string. It is used as title for the PCA. Ex. typeData="codonCoverage"

Value

a list of length 2: PCA_scores - matrix of the scores on the first 4 principal components. PCA_plots - a list of 5 PCA scatterplots.

Examples

```
#How to perform a PCA analysis based on codon coverage
#adapted from
#http://stackoverflow.com/questions/20260434/test-significance-of-clusters-on-a-pca-plot
#either get the codon frequency, coverage, and annotation using a function
#such as codonInfo in this package
#or create a list of matrices with the above information
data(codonDataCtrl)
codonData <- codonDataCtrl
codonUsage <- codonData[[1]]
codonCovMatrix <- codonData[[2]]

#keep only genes with a minimum number of reads
nbrReadsGene <- apply(codonCovMatrix, 1, sum)
ixExpGenes <- which(nbrReadsGene >= 50)
codonCovMatrix <- codonCovMatrix[ixExpGenes, ]

#get the PCA on the codon coverage
codonCovMatrixTransp <- t(codonCovMatrix)
rownames(codonCovMatrixTransp) <- colnames(codonCovMatrix)
colnames(codonCovMatrixTransp) <- rownames(codonCovMatrix)

listPCACodonCoverage <- codonPCA(codonCovMatrixTransp,"codonCoverage")
print(listPCACodonCoverage[[2]])
#See aditional examples in the pdf manual
```

countShiftReads *Apply an offset on the read start along the transcript and returns the coverage on the 5pUTR, CDS, 3pUTR, as well as a matrix of codon coverage per ORF.*

Description

Apply an offset on the read start along the transcript and returns the coverage on the 5pUTR, CDS, 3pUTR, as well as a matrix of codon coverage per ORF.

Usage

```
countShiftReads(exonGRanges, cdsPosTransc, alnGRanges, shiftValue, motifSize)
```

Arguments

exonGRanges	a GRangesList. It contains the exon coordinates grouped by transcript.
cdsPosTransc	a list. It contains the relative positions of the start and end of the ORFs. The transcript names in exonGRanges and cdsPosTransc should be the same.
alnGRanges	A GRanges object containing the alignment information. In order to improve the performance the GAlignments BAM object should be transformed into a GRanges object with cigar match size metadata.
shiftValue	integer. The offset for recalibrating reads on transcripts when computing coverage. The default value for this parameter is 0, no offset should be performed.
motifSize	an integer. The number of nucleotides in each motif on which to compute coverage and usage. Either 3, 6, or 9. Default 3 nucleotides (codon).

Value

a list with 2 objects. The first object in the list is a data.frame containing: information on ORFs (names, chromosomal position, length) as well as the counts on the 5pUTR, CDS and 3pUTR once the offset is applied. The second object in the list is a list in itself. It contains: for each ORF in the cdsPosTransc, for each codon the sum of read starts covering the 3 codon nucleotides. For motifs of size 6 nucleotides, the motif coverage is computed only for the first codon in the motif, considered as the codon in the P-site. For motifs of size 9 nucleotides, the motif coverage is computed only for the second codon in the motif, considered as the codon in the P-site. This per codon coverage does not contain information on the codon type, just its position in the ORF and its coverage.

Examples

```
#read the BAM file into a GAlignments object using
#GenomicAlignments::readGAlignments
#the GAlignments object should be similar to ctrlGAlignments
data(ctrlGAlignments)
aln <- ctrlGAlignments

#transform the GAlignments object into a GRanges object (faster processing)
```

```
alnGRanges <- readsToStartOrEnd(aln, what="start")

#make a txdb object containing the annotations for the specified species.
#In this case hg19.
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene
#Please make sure that seqnames of txdb correspond to
#the seqnames of the alignment files ("chr" particle)
#if not rename the txdb seqlevels
#renameSeqlevels(txdb, sub("chr", "", seqlevels(txdb)))

#get all CDSs by transcript
cds <- GenomicFeatures::cdsBy(txdb, by="tx", use.names=TRUE)

#get all exons by transcript
exonGRanges <- GenomicFeatures::exonsBy(txdb, by="tx", use.names=TRUE)
#get the per transcript relative position of start and end codons
#cdsPosTransc <- orfRelativePos(cds, exonGRanges)
data(cdsPosTransc)

#compute the counts on the different features after applying
#the specified shift value on the read start along the transcript
countsData <- countShiftReads(exonGRanges[names(cdsPosTransc)], cdsPosTransc,
           alnGRanges, -14)
```

`countsPlot` *Graphs of sample read counts (quality assesment)*

Description

Graphs of sample read counts (quality assesment)

Usage

```
countsPlot(listCounts, ixCounts, log2Bool)
```

Arguments

`listCounts`	a list of data.frame objects. It contains the counts on the genomic features. Each data.frame in the list should have the same number of columns.
`ixCounts`	a numeric (a vector of integers). It contains the index of the columns containing counts in the dataFrame.
`log2Bool`	a numeric, either 0 or 1. 0 (default) for no log2 transformation and 1 for log2 transformation.

Value

A list of pairs and boxplots between the counts data in each data.frame.

Examples

```
#read the BAM file into a GAlignments object using
#GenomicAlignments::readGAlignments
#the GAlignments object should be similar to ctrlGAlignments
data(ctrlGAlignments)
aln <- ctrlGAlignments

#transform the GAlignments object into a GRanges object (faster processing)
alnGRanges <- readsToStartOrEnd(aln, what="start")

#make a txdb object containing the annotations for the specified species.
#In this case hg19.
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene
#Please make sure that seqnames of txdb correspond to
#the seqnames of the alignment files ("chr" particle)
#if not rename the txdb seqlevels
#renameSeqlevels(txdb, sub("chr", "",seqlevels(txdb)))
#get the flanking region around the promoter of the best expressed CDSs

#get all CDSs by transcript
cds <- GenomicFeatures::cdsBy(txdb,by="tx",use.names=TRUE)

#get all exons by transcript
exonGRanges <- GenomicFeatures::exonsBy(txdb,by="tx",use.names=TRUE)

#get the per transcript relative position of start and end codons
cdsPosTransc <- orfRelativePos(cds, exonGRanges)

#compute the counts on the different features after applying
#the specified shift value on the read start along the transcript
countsData <-
   countShiftReads(
         exonGRanges[names(cdsPosTransc)],
         cdsPosTransc,
         alnGRanges,
         -14
     )

#now make the plots
listCountsPlots <- countsPlot(
   list(countsData[[1]]),
   grep("_counts$", colnames(countsData[[1]])),
   1
)
listCountsPlots
```

`ctrlGAlignments` *Ribosome profiling data on chr1 in human primary BJ fibroblasts control data: PMID: 23594524.*

Description

A dataset containing the alignment information on chromosome 1 from the control BAM. The data object is a GAlignments object containing 3,504,859 hg19 mapped reads.

Usage

```
data(ctrlGAlignments)
```

Format

A GAlignments object with 3,504,859 reads.

Value

the GAlignments object of reads on chr 1

`histMatchLength` *Histogram of match length distribution of reads.*

Description

Histogram of match length distribution of reads.

Usage

```
histMatchLength(aln, log10Transf = 0, titleHist)
```

Arguments

`aln`	A GAlignments object of the BAM mapping file.
`log10Transf`	A boolean. Either 0 (default) or 1 (log10).
`titleHist`	a character. The main title for the histogram. Default - none.

Value

A list with 2 elements. The first element: a data.frame of the number of counts per match length distribution. The second element in the list: a ggplot2 histogram of the match length distribution.

Examples

```
#starting from a GAlignment object
data(ctrlGAlignments)
aln <- ctrlGAlignments
#no log10 scaling
matchLenDistr <- histMatchLength(aln, 0)
#to plot the histogram
matchLenDistr[[2]]
```

orfRelativePos — *Relative position of the start and stop codon along the transcript*

Description

Relative position of the start and stop codon along the transcript

Usage

```
orfRelativePos(cdsTransc, exonGRanges)
```

Arguments

cdsTransc	a GRangesList. It contains the CDS coordinates grouped by transcript.
exonGRanges	a GRangesList. It contains the exon coordinates grouped by transcript.

Value

a list. A list of relative positions of the start and end of ORFs.

Examples

```
#make a txdb object containing the annotations for the specified species.
#In this case hg19.
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene

#get all CDSs by transcript
cds <- GenomicFeatures::cdsBy(txdb, by="tx", use.names=TRUE)

#get all exons by transcript
exonGRanges <- GenomicFeatures::exonsBy(txdb, by="tx", use.names=TRUE)

#retrieve the positions of start and end codons relative to the transcript
cdsPosTransc <- orfRelativePos(cds, exonGRanges)
```

plotSummarizedCov — *Plots the summarized coverage in a specified range (e.g. around TSS) for the specified match sizes*

Description

Plots the summarized coverage in a specified range (e.g. around TSS) for the specified match sizes

Usage

```
plotSummarizedCov(covSummarized)
```

Arguments

covSummarized	a list of GRanges objects. For each matchSize a GRanges object of the summarized coverage.

Value

a ggplot2 plot of read coverage in interval

Examples

```
#read the BAM file into a GAlignments object using
#GenomicAlignments::readGAlignments
#the GAlignments object should be similar to ctrlGAlignments
data(ctrlGAlignments)
aln <- ctrlGAlignments
#transform the GAlignments object into a GRanges object (faster processing)
alnGRanges <- readsToStartOrEnd(aln, what="start")
#make a txdb object containing the annotations for the specified species.
#In this case hg19.
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene
#Please make sure that seqnames of txdb correspond to
#the seqnames of the alignment files ("chr" particle)
#if not rename the txdb seqlevels
#renameSeqlevels(txdb, sub("chr", "",seqlevels(txdb)))
#get the flanking region around the promoter of the best expressed CDSs
oneBinRanges <- aroundPromoter(txdb, alnGRanges)
#the read start coverage around the TSS as a percentage for all match sizes.
covSummarized <- readStartCov(alnGRanges, oneBinRanges, matchSize="all",
c(-20,20), "aroundTSS", charPerc="perc")
trackPlotTSS <- plotSummarizedCov(covSummarized)
print(trackPlotTSS)
```

---

printPCA *Plots the PCA scatterplots produced by codonPCA function.*

Description

Plots the PCA scatterplots produced by codonPCA function.

Usage

```
printPCA(listPCAGraphs)
```

Arguments

listPCAGraphs	a list of 5 PCA ggplot scatterplots.

Value

a unique plot with the 5 PCA scatterplots.

Examples

```
#How to perform a PCA analysis based on codon coverage
data(codonDataCtrl)
codonData <- codonDataCtrl
codonUsage <- codonData[[1]]
codonCovMatrix <- codonData[[2]]

#keep only genes with a minimum number of reads
nbrReadsGene <- apply(codonCovMatrix, 1, sum)
ixExpGenes <- which(nbrReadsGene >= 50)
codonCovMatrix <- codonCovMatrix[ixExpGenes, ]

#get the PCA on the codon coverage
codonCovMatrixTransp <- t(codonCovMatrix)
rownames(codonCovMatrixTransp) <- colnames(codonCovMatrix)
colnames(codonCovMatrixTransp) <- rownames(codonCovMatrix)

listPCACodonCoverage <- codonPCA(codonCovMatrixTransp,"codonCoverage")
printPCA(listPCACodonCoverage[[2]])
```

`readStartCov` *Read start coverage around the TSS on the predifined CDSs*

Description

Read start coverage around the TSS on the predifined CDSs

Usage

```
readStartCov(alnGRanges, oneBinRanges, matchSize = "all", fixedInterval,
  renameChr, charPerc = "perc")
```

Arguments

`alnGRanges`	A GRanges object containing the alignment information. In order to improve the performance transform the GAlignments BAM object into a GRanges object containing cigar match size as metadata.
`oneBinRanges`	A GRanges object. Transform the gene GRangesList into one big GRanges object. Add the info on the cds_id.
`matchSize`	either "all" or a vector of read match sizes. If matchSize <- "all", then all the reads are used to compute the coverage. If the matchSize is a vector of read match sizes, the summarized coverage is reported per match size and for the sum up.
`fixedInterval`	a numeric vector with the extremities of the interval. Ex. fixedInterval <- c(-20,20) or fixedInterval <- c(0,40) ...
`renameChr`	a character object. It contains the name to be given to the new summarized coverage interval. Ex. renameChr <- "aroundTSS" the summarized region around the TSS.

charPerc — a character object. Either "perc" (the default) or "sum" for percentage of counts per position or the sum of counts per position.

Value

a list of GRanges objects (for each matchSize chosen). It contains the summarized coverage for the specified read match sizes.

Examples

```
#read the BAM into a GAlignments object using
#GenomicAlignments::readGAlignments
#the GAlignments object should be similar to ctrlGAlignments
data(ctrlGAlignments)
aln <- ctrlGAlignments

#transform the GAlignments object into a GRanges object (faster processing)
alnGRanges <- readsToStartOrEnd(aln, what="start")

#make a txdb object containing the annotations for the specified species.
#In this case hg19.
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene
#Please make sure that seqnames of txdb correspond to
#the seqnames of the alignment files ("chr" particle)
#if not rename the txdb seqlevels
#renameSeqlevels(txdb, sub("chr", "", seqlevels(txdb)))

#get the flanking region around the promoter of the best expressed CDSs
oneBinRanges <- aroundPromoter(txdb, alnGRanges, percBestExpressed=0.01)

#the coverage in the TSS flanking region for the summarized read match sizes
listPromoterCov <- readStartCov(
     alnGRanges,
     oneBinRanges,
     matchSize="all",
     fixedInterval=c(-20, 20),
     renameChr="aroundTSS",
     charPerc="perc"
)
```

---

readsToStartOrEnd — *Reads in GAlignments converted to either Read Start (5') or End (3') Positions*

Description

Reads in GAlignments converted to either Read Start (5') or End (3') Positions

Usage

```
readsToStartOrEnd(aln, what)
```

Arguments

aln	A GAlignments object of the BAM mapping file.
what	A character object. Either "start" (the default) or "end" for read start or read end.

Value

A GRanges object containing either the read start or end genomic positions.

Examples

```
#read the BAM file into a GAlignments object using
#GenomicAlignments::readGAlignments
#the GAlignments object should be similar to ctrlGAlignments object
data(ctrlGAlignments)
aln <- ctrlGAlignments
#transform the GAlignments object into a GRanges object (faster processing)
alnGRanges <- readsToStartOrEnd(aln, what = "end")
```

RiboProfiling *RiboProfiling.*

Description

RiboProfiling.

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riboSeqFromBAM *Starting from a BAM file path: quality plots, shift ribosome position, coverage on multiple transcript features and on codons.*

Description

Starting from a BAM file path: quality plots, shift ribosome position, coverage on multiple transcript features and on codons.

Usage

```
riboSeqFromBAM(listeInputBamFile, paramScanBAM, genomeName, txdb,
  percBestExpressed, flankSize, offsetStartEnd, listShiftValue)
```

Arguments

listeInputBamFile	A character path or a vector of paths to the ribo-seq BAM file(s). If multiple BAM files are provided, they should come from the same genome alignment.
paramScanBAM	NULL or ScanBamParam object specifying what fields and which records are imported. Default value is NULL.
genomeName	a character object containing the name of the genome used for the alignment BAM file. The name should be one of the UCSC ensGene list: ucscGenomes()[, "db"]. Ex. "hg19" or "mm10". This parameter is used to build the TxDb object.
txdb	a TxDb object containing the annotations info to comfront with the alignment files. Either genomeName or txdb parameters should be provided.
percBestExpressed	numeric [between 0 and 1]. The percentage of best expressed CDSs on which to plot the coverage around the TSS. Necessary if the shiftValue parameter must be estimated. Default value 0.03 (3%).
flankSize	an integer. How many bp left and right of the TSS should the coverage be performed?
offsetStartEnd	a character object. Either "start" (the default) or "end" for read start or read end to define the offset.
listShiftValue	a vector of integer. It should have the same length as the inputBamFile vector. The numeric value for shifting ranges of reads on genomic features when computing coverage. Set this parameter to 0 if no shift should be performed. If this parameter is missing, the shiftValue is computed based on the maximum peak of read start coverage around the TSS. A plot is produced to illustrate this estimation.

Value

A list of list for each BAM file in the inputBamFile list. For each BAM file 2 objects are returned: one data.frame with info on the genomic features and the corresponding coverage column, and one list of per ORF codon coverage.

Examples

```
#the txdb object can be given as parameter or not.
#If it is not specified, a txdb object is build from UCSC.
txdb <- TxDb.Hsapiens.UCSC.hg19.knownGene::TxDb.Hsapiens.UCSC.hg19.knownGene

#in this example only one BAM file is treated.
#However, multiple BAM files can be analyzed together.
myFile <- system.file("extdata", "ctrl_sample.bam", package="RiboProfiling")
listeInputBam <- c(myFile)

#when running this function it is important that chromosome names
#in UCSC and your BAM correspond: the "chr" particle
covData <- riboSeqFromBAM(listeInputBam, txdb=txdb, listShiftValue=c(-14))
```

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