

Package ‘ribosomeProfilingQC’

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Type Package

Title Ribosome Profiling Quality Control

Version 1.2.1

Description Ribo-Seq (also named ribosome profiling or footprinting) measures translatoome (unlike RNA-Seq, which sequences the transcriptome) by direct quantification of the ribosome-protected fragments (RPFs). This package provides the tools for quality assessment of ribosome profiling. In addition, it can preprocess Ribo-Seq data for subsequent differential analysis.

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assignReadingFrame	<i>Assign reading frame</i>
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Description

Set reading frame for each reads in CDS region to frame0, frame1 and frame2.

Usage

```
assignReadingFrame(reads, CDS, txdb)
```

Arguments

reads	Output of getPsiteCoordinates
CDS	Output of prepareCDS
txdb	A TxDb object. If it is set, assign reading frame for all reads. Default missing, only assign rading frame for reads in CDS.

Value

An GRanges object of reads with reading frame information.

Examples

```
library(Rsamtools)
bamfilename <- system.file("extdata", "RPF.WT.1.bam",
                           package="ribosomeProfilingQC")

yieldSize <- 10000000
bamfile <- BamFile(bamfilename, yieldSize = yieldSize)
pc <- getPsiteCoordinates(bamfile, bestpsite=13)
pc.sub <- pc[pc$qwidth %in% c(29, 30)]
#library(GenomicFeatures)
library(BSgenome.Drerio.UCSC.danRer10)
#txdb <- makeTxDbFromGFF(system.file("extdata",
#   "Danio_rerio.GRCz10.91.chr1.gtf.gz",
#   package="ribosomeProfilingQC"),
#   organism = "Danio rerio",
#   chrominfo = seqinfo(Drerio)["chr1"],
#   taxonomyId = 7955)
#CDS <- prepareCDS(txdb)
CDS <- readRDS(system.file("extdata", "CDS.rds",
                           package="ribosomeProfilingQC"))
pc.sub <- assignReadingFrame(pc.sub, CDS)
```

codonUsage

Start or Stop codon usage

Description

Calculate the start or stop codon usage for the identified CDSs.

Usage

```
codonUsage(reads, start = TRUE, genome)
```

Arguments

reads	Output of assignReadingFrame .
start	Calculate for start codon or stop codon.
genome	A BSgenome object.

Value

Table of codon usage.

Examples

```
pcs <- readRDS(system.file("extdata", "samplePc.rds",
                          package="ribosomeProfilingQC"))
library(BSgenome.Drerio.UCSC.danRer10)
codonUsage(pcs, genome=Drerio)
codonUsage(pcs, start=FALSE, genome=Drerio)
```

countReads

*Extract counts for RPFs and RNAs***Description**

Calculate the reads counts for gene level or transcript level.

Usage

```
countReads(
  RPFs,
  RNAs,
  gtf,
  level = c("tx", "gene"),
  bestpsite = 13,
  readsLen = c(28, 29),
  anchor = "5end",
  ...
)
```

Arguments

RPFs	Bam file names of RPFs.
RNAs	Bam file names of RNAseq.
gtf	GTF file name for annotation.
level	Transcript or gene level.
bestpsite	numeric(1). P site postion.
readsLen	numeric(1). reads length to keep.
anchor	5end or 3end. Default is 5end.
...	Parameters pass to featureCounts

Value

A list with reads counts.

Examples

```
path <- system.file("extdata", package="ribosomeProfilingQC")
RPFs <- dir(path, "RPF.*?.[12].bam$", full.names=TRUE)
gtf <- file.path(path, "Danio_rerio.GRCz10.91.chr1.gtf.gz")
cnts <- countReads(RPFs[1], gtf=gtf, level="gene", readsLen=29)
```

coverageDepth	<i>Extract coverage depth for gene level or transcript level</i>
---------------	--

Description

Calculate the coverage depth for gene level or transcript level. Coverage for RPFs will be the best P site coverage. Coverage for RNAs will be the coverage for 5' end of reads.

Usage

```
coverageDepth(
  RPFs,
  RNAs,
  gtf,
  level = c("tx", "gene"),
  bestpsite = 13,
  readsLen = c(28, 29),
  anchor = "5end",
  region = "cds",
  ext = 5000,
  ...
)
```

Arguments

RPFs	Bam file names of RPFs.
RNAs	Bam file names of RNAseq.
gtf	GTF file name for annotation or a TxDb object.
level	Transcript or gene level.
bestpsite	P site position.
readsLen	Reads length to keep.
anchor	5end or 3end. Default is 5end.
region	Annotation region. It could be "cds", "utr5", "utr3", "exon", "transcripts", "feature with extension".
ext	Extension region for "feature with extension".
...	Parameters pass to makeTxDbFromGFF

Value

A cvgd object with coverage depth.

Examples

```
path <- system.file("extdata", package="ribosomeProfilingQC")
RPFs <- dir(path, "RPF.*?\\. [12].bam$", full.names=TRUE)
gtf <- file.path(path, "Danio_rerio.GRCz10.91.chr1.gtf.gz")
cvgs <- coverageDepth(RPFs[1], gtf=gtf, level="gene")
```

coverageRates	<i>Calculate coverage rate</i>
---------------	--------------------------------

Description

Coverage is a measure as percentage of position with reads along the CDS. Coverage rate calculate coverage rate for RPFs and mRNAs in gene level. Coverage will be calculated based on best P sites for RPFs and 5'end for RNA-seq.

Usage

```
coverageRates(cvgs, RPFsampleOrder, mRNAsampleOrder)
```

Arguments

cvgs	Output of coverageDepth
RPFsampleOrder, mRNAsampleOrder	Sample order of RPFs and mRNAs. The parameters are used to make sure that the order of RPFs and mRNAs in cvgs is corresponding samples.

Value

A list with coverage rate.

Examples

```
path <- system.file("extdata", package="ribosomeProfilingQC")
RPFs <- dir(path, "RPF.*?\\. [12].bam$", full.names=TRUE)
gtf <- file.path(path, "Danio_rerio.GRCz10.91.chr1.gtf.gz")
cvgs <- coverageDepth(RPFs[1], gtf=gtf, level="gene")
cr <- coverageRates(cvgs)
```

cvgd-class	<i>Class "cvgd"</i>
------------	---------------------

Description

An object of class "cvgd" represents output of coverageDepth.

Usage

```
cvgd(...)

## S4 method for signature 'cvgd'
x$name

## S4 replacement method for signature 'cvgd'
x$name <- value

## S4 method for signature 'cvgd,ANY,ANY'
```

```
x[[i, j, ..., exact = TRUE]]

## S4 replacement method for signature 'cvgd,ANY,ANY,ANY'
x[[i, j, ...]] <- value

## S4 method for signature 'cvgd'
show(object)
```

Arguments

...	Each argument in ... becomes a slot in the new "cvgd"-class.
x	cvgd object.
name	A literal character string or a name (possibly backtick quoted).
value	value to replace.
i, j	indices specifying elements to extract or replace.
exact	see Extract
object	cvgd object.

Value

A cvgd object.

Slots

coverage "list", list of [CompressedRleList](#), specify the coverage of features of each sample.
 granges [CompressedGRangesList](#), specify the features.

Examples

```
cvgd()
```

estimatePsite	<i>Estimate P site position</i>
---------------	---------------------------------

Description

Estimate P site position from a subset reads.

Usage

```
estimatePsite(bamfile, CDS, genome, anchor = "5end")
```

Arguments

bamfile	A BamFile object.
CDS	Output of prepareCDS
genome	A BSgenome object.
anchor	5end or 3end. Default is 5end.

Value

A best P site position.

References

1: Bazzini AA, Johnstone TG, Christiano R, Mackowiak SD, Obermayer B, Fleming ES, Vejnar CE, Lee MT, Rajewsky N, Walther TC, Giraldez AJ. Identification of small ORFs in vertebrates using ribosome footprinting and evolutionary conservation. *EMBO J.* 2014 May 2;33(9):981-93. doi: 10.1002/embj.201488411. Epub 2014 Apr 4. PubMed PMID: 24705786; PubMed Central PMCID: PMC4193932.

Examples

```
library(Rsamtools)
bamfilename <- system.file("extdata", "RPF.WT.1.bam",
                           package="ribosomeProfilingQC")

yieldSize <- 10000000
bamfile <- BamFile(bamfilename, yieldSize = yieldSize)
#library(GenomicFeatures)
library(BSgenome.Drerio.UCSC.danRer10)
#txdb <- makeTxDbFromGFF(system.file("extdata",
#   "Danio_rerio.GRCz10.91.chr1.gtf.gz",
#   package="ribosomeProfilingQC"),
#   organism = "Danio rerio",
#   chrominfo = seqinfo(Drerio)["chr1"],
#   taxonomyId = 7955)
#CDS <- prepareCDS(txdb)
CDS <- readRDS(system.file("extdata", "CDS.rds",
                           package="ribosomeProfilingQC"))
estimatePsite(bamfile, CDS, Drerio)
```

filterCDS

Filter CDS by size

Description

Filter CDS by CDS size.

Usage

```
filterCDS(CDS, sizeCutoff = 100L)
```

Arguments

CDS	Output of preparedCDS
sizeCutoff	numeric(1). Cutoff size for CDS. If the size of CDS is less than the cutoff, it will be filtered out.

Value

A GRanges object with filtered CDS.

Examples

```
#library(GenomicFeatures)
library(BSgenome.Drerio.UCSC.danRer10)
#txdb <- makeTxDbFromGFF(system.file("extdata",
#      "Danio_rerio.GRCz10.91.chr1.gtf.gz",
#      package="ribosomeProfilingQC"),
#      organism = "Danio rerio",
#      chrominfo = seqinfo(Drerio)["chr1"],
#      taxonomyId = 7955)
#CDS <- prepareCDS(txdb)
CDS <- readRDS(system.file("extdata", "CDS.rds",
                          package="ribosomeProfilingQC"))

filterCDS(CDS)
```

FLOSS

*Fragment Length Organization Similarity Score (FLOSS)***Description**

The FLOSS will be calculated from a histogram of read lengths for footprints on a transcript or reading frame.

Usage

```
FLOSS(
  reads,
  ref,
  CDS,
  readLengths = c(26:34),
  level = c("tx", "gene"),
  draw = FALSE
)
```

Arguments

reads	Output of getPsiteCoordinates
ref	Reference id list. If level is set to tx, the id should be transcript names. If level is set to gene, the id should be gene id.
CDS	Output of prepareCDS
readLengths	Read length used for calculation
level	Transcript or gene level
draw	Plot FLOSS vs total reads or not.

Value

A data frame with colnames as id, FLOSS, totalReads, wilcox.test.pval, cook's distance.

References

1: Ingolia NT, Brar GA, Stern-Ginossar N, Harris MS, Talhouarne GJ, Jackson SE, Wills MR, Weissman JS. Ribosome profiling reveals pervasive translation outside of annotated protein-coding genes. *Cell Rep.* 2014 Sep 11;8(5):1365-79. doi: 10.1016/j.celrep.2014.07.045. Epub 2014 Aug 21. PubMed PMID: 25159147; PubMed Central PMCID: PMC4216110.

Examples

```
library(Rsamtools)
bamfilename <- system.file("extdata", "RPF.WT.1.bam",
                           package="ribosomeProfilingQC")

yieldSize <- 10000000
bamfile <- BamFile(bamfilename, yieldSize = yieldSize)
pc <- getPsiteCoordinates(bamfile, bestpsite=13)
#library(GenomicFeatures)
library(BSgenome.Drerio.UCSC.danRer10)
#txdb <- makeTxDbFromGFF(system.file("extdata",
#   "Danio_rerio.GRCz10.91.chr1.gtf.gz",
#   package="ribosomeProfilingQC"),
#   organism = "Danio rerio",
#   chrominfo = seqinfo(Drerio)["chr1"],
#   taxonomyId = 7955)
#CDS <- prepareCDS(txdb)
CDS <- readRDS(system.file("extdata", "CDS.rds",
                           package="ribosomeProfilingQC"))

set.seed(123)
ref <- sample(unique(CDS$gene_id), 100)
fl <- FLOSS(pc, ref, CDS, level="gene")
```

frameCounts

Extract counts for gene level or transcript level

Description

Calculate the reads counts or coverage rate for gene level or transcript level. Coverage is determined by measuring the proportion of in-frame CDS positions with ≥ 1 reads.

Usage

```
frameCounts(
  reads,
  level = c("tx", "gene"),
  frame0only = TRUE,
  coverageRate = FALSE
)
```

Arguments

reads	Output of assignReadingFrame .
level	Transcript or gene level
frame0only	Only count for reading frame 0 or not
coverageRate	Calculate for coverage or not

Value

A numeric vector with reads counts.

Examples

```
pcs <- readRDS(system.file("extdata", "samplePc.rds",
                          package="ribosomeProfilingQC"))
cnts <- frameCounts(pcs)
cnts.gene <- frameCounts(pcs, level="gene")
cvg <- frameCounts(pcs, coverageRate=TRUE)
```

getFPKM

Get FPKM values for counts

Description

Calculate Fragments Per Kilobase of transcript per Million mapped reads (FPKM) for counts.

Usage

```
getFPKM(counts, gtf, level = c("gene", "tx"))
```

Arguments

counts	Output of countReads or normByRUVs
gtf	GTF file name for annotation.
level	Transcript or gene level.

Value

A list with FPKMs

Examples

```
path <- system.file("extdata", package="ribosomeProfilingQC")
#RPFs <- dir(path, "RPF.*?.[12].bam$", full.names=TRUE)
#RNAs <- dir(path, "mRNA.*?.[12].bam$", full.names=TRUE)
#gtf <- file.path(path, "Danio_rerio.GRCz10.91.chr1.gtf.gz")
#cnts <- countReads(RPFs, RNAs, gtf, level="gene")
cnts <- readRDS(file.path(path, "cnts.rds"))
fpkm <- getFPKM(cnts)
```

getORFscore *Calculate ORFscore*

Description

To calculate the ORFscore, reads were counted at each position within the ORF.

$$ORFscore = \log_2\left(\left(\sum_{n=1}^3 \frac{(F_n - \bar{F})^2}{\bar{F}}\right) + 1\right)$$

where F_n is the number of reads in reading frame n, \bar{F} is the total number of reads across all three frames divided by 3. If F_1 is smaller than F_2 or F_3 , $ORFscore = -1 \times ORFscore$.

Usage

```
getORFscore(reads)
```

Arguments

reads Output of [getPsiteCoordinates](#)

Value

A numeric vector with ORFscore.

References

1: Bazzini AA, Johnstone TG, Christiano R, Mackowiak SD, Obermayer B, Fleming ES, Vejnar CE, Lee MT, Rajewsky N, Walther TC, Giraldez AJ. Identification of small ORFs in vertebrates using ribosome footprinting and evolutionary conservation. EMBO J. 2014 May 2;33(9):981-93. doi: 10.1002/emj.201488411. Epub 2014 Apr 4. PubMed PMID: 24705786; PubMed Central PMCID: PMC4193932.

Examples

```
pcs <- readRDS(system.file("extdata", "samplePc.rds",
                           package="ribosomeProfilingQC"))
ORFscore <- getORFscore(pcs)
```

getPsiteCoordinates *Get P site coordinates*

Description

Extract P site coordinates from a bam file to a GRanges object.

Usage

```
getPsiteCoordinates(bamfile, bestpsite, anchor = "5end")
```

Arguments

bamfile A BamFile object.
 bestpsite P site position. See [estimatePsite](#)
 anchor 5end or 3end. Default is 5end.

Value

A GRanges object with qwidth metadata which indicates the width of reads.

Examples

```
library(Rsamtools)
bamfilename <- system.file("extdata", "RPF.WT.1.bam",
                           package="ribosomeProfilingQC")
yieldSize <- 10000000
bamfile <- BamFile(bamfilename, yieldSize = yieldSize)
pc <- getPsiteCoordinates(bamfile, bestpsite=13)
```

 ggBar

barplot by ggplot2

Description

barplot with number in top.

Usage

```
ggBar(height, fill = "gray80", draw = TRUE, xlab, ylab, postfix)
```

Arguments

height data for plot
 fill, xlab, ylab parameters pass to ggplot.
 draw plot or not
 postfix Postfix of text labeled in top of bar.

Value

ggplot object.

Examples

```
ribosomeProfilingQC::ggBar(sample.int(100, 3))
```

 metaPlot

Metagene analysis plot

Description

Plot the average coverage of UTR5, CDS and UTR3.

Usage

```
metaPlot(
  UTR5coverage,
  CDScoverage,
  UTR3coverage,
  sample,
  xaxis = c("RPFs", "mRNA"),
  bins = c(UTR5 = 100, CDS = 500, UTR3 = 100),
  ...
)
```

Arguments

UTR5coverage, CDScoverage, UTR3coverage	Coverages of UTR5, CDS, and UTR3 region. Output of coverageDepth
sample	character(1). Sample name to plot.
xaxis	What to plot for x-axis.
bins	Bins for UTR5, CDS and UTR3.
...	Parameter pass to plot.

Value

A list contain the data for plot.

Examples

```
## Not run:
path <- system.file("extdata", package="ribosomeProfilingQC")
RPFs <- dir(path, "RPF.*?\\.[12].bam$", full.names=TRUE)
RNAs <- dir(path, "mRNA.*?\\.[12].bam$", full.names=TRUE)
gtf <- file.path(path, "Danio_rerio.GRCz10.91.chr1.gtf.gz")
cvgs <- coverageDepth(RPFs[1], RNAs[1], gtf)
cvgs.utr3 <- coverageDepth(RPFs[1], RNAs[1], gtf, region="utr3")
cvgs.utr5 <- coverageDepth(RPFs[1], RNAs[1], gtf, region="utr5")
metaPlot(cvgs.utr5, cvgs, cvgs.utr3, sample=1)

## End(Not run)
```

normByRUVs	<i>Normalization by RUVSeq</i>
------------	--------------------------------

Description

Normalization by RUVSeq:RUVs methods

Usage

```
normByRUVs(counts, RPFgroup, mRNAgroup = RPFgroup, k = 1)
```

Arguments

counts	Output of countReads
RPFgroup, mRNAgroup	Groups for RPF and mRNA files
k	The number of factor of unwanted variation to be estimated from the data. See RUVs

Value

Normalized counts list

Examples

```
## Not run: ##waiting for EDASeq fix the issue.
path <- system.file("extdata", package="ribosomeProfilingQC")
#RPFs <- dir(path, "RPF.*?.[12].bam$", full.names=TRUE)
#RNAs <- dir(path, "mRNA.*?.[12].bam$", full.names=TRUE)
#gtf <- file.path(path, "Danio_rerio.GRCz10.91.chr1.gtf.gz")
#cnts <- countReads(RPFs, RNAs, gtf, level="gene")
cnts <- readRDS(file.path(path, "cnts.rds"))
gp <- c("KD1", "KD1", "WT", "WT")
norm <- normByRUVs(cnts, gp, gp)

## End(Not run)
```

PAmotif	<i>Metaplot of P site distribution</i>
---------	--

Description

Metaplot of P site distribution in all the CDS aligned by the start codon or stop codon.

Usage

```
PAmotif(reads, genome, plot = TRUE)
```

Arguments

reads	Output of assignReadingFrame or shiftReadsByFrame .
genome	A BSgenome object.
plot	Plot the motif or not.

Value

A [pcm](#) object

Examples

```
pcs <- readRDS(system.file("extdata", "samplePc.rds",
                          package="ribosomeProfilingQC"))
library(BSgenome.Drerio.UCSC.danRer10)
#PAmotif(pcs, Drerio)
```

plotDistance2Codon *Metaplot of P site distribution*

Description

Metaplot of P site distribution in all the CDS aligned by the start codon or stop codon.

Usage

```
plotDistance2Codon(
  reads,
  start = TRUE,
  anchor = 50,
  col = c(Frame_0 = "#009E73", Frame_1 = "#D55E00", Frame_2 = "#0072B2")
)
```

Arguments

reads	Output of assignReadingFrame .
start	Plot for start codon or stop codon.
anchor	The maximal xlim or (min, max) position for plot.
col	Colors for different reading frame.

Value

Invisible height of the barplot.

Examples

```
pcs <- readRDS(system.file("extdata", "samplePc.rds",
                          package="ribosomeProfilingQC"))
plotDistance2Codon(pcs)
#plotDistance2Codon(pcs, start=FALSE)
#plotDistance2Codon(pcs, anchor=c(-10, 20))
```

plotFrameDensity	<i>Plot density for each reading frame</i>
------------------	--

Description

Plot density for each reading frame.

Usage

```
plotFrameDensity(  
  reads,  
  density = TRUE,  
  col = c(Frame_0 = "#009E73", Frame_1 = "#D55E00", Frame_2 = "#0072B2")  
)
```

Arguments

reads	Output of assignReadingFrame
density	Plot density or counts
col	Colors for reading frames

Value

Reading frame density

Examples

```
pcs <- readRDS(system.file("extdata", "samplePc.rds",  
  package="ribosomeProfilingQC"))  
plotFrameDensity(pcs)
```

plotSpliceEvent	<i>Plot splice event</i>
-----------------	--------------------------

Description

Plot the splice event

Usage

```
plotSpliceEvent(  
  se,  
  tx_name,  
  coverage,  
  group1,  
  group2,  
  cutoffFDR = 0.05,  
  resetIntronWidth = TRUE  
)
```

Arguments

se	Output of spliceEvent
tx_name	Transcript name.
coverage	Coverages of feature region with extensions. Output of coverageDepth
group1, group2	The sample names of group 1 and group 2
cutoffFDR	Cutoff of FDR
resetIntronWidth	logical(1). If set to true, reset the region with no read to minimal width.

Value

A ggplot object.

Examples

```
## Not run:
path <- system.file("extdata", package="ribosomeProfilingQC")
RPFs <- dir(path, "RPF.*?\\. [12].bam$", full.names=TRUE)
gtf <- file.path(path, "Danio_rerio.GRCz10.91.chr1.gtf.gz")
coverage <- coverageDepth(RPFs, gtf=gtf, level="gene",
                          region="feature with extension")
group1 <- c("RPF.KD1.1", "RPF.KD1.2")
group2 <- c("RPF.WT.1", "RPF.WT.2")
se <- spliceEvent(coverage, group1, group2)
plotSpliceEvent(se, se$feature[1], coverage, group1, group2)

## End(Not run)
```

plotTE

Plot translational efficiency

Description

Scatterplot of RNA/RPFs level compared to the translational efficiency.

Usage

```
plotTE(
  TE,
  sample,
  xaxis = c("mRNA", "RPFs"),
  removeZero = TRUE,
  log2 = TRUE,
  breaks.length = 50,
  ...
)
```

Arguments

TE	Output of translationalEfficiency
sample	character(1). Sample name to plot.
xaxis	What to plot for x-axis.
removeZero	Remove the 0 values from plots.
log2	Do log2 transform or not.
breaks.length	Length of breaks for histogram.
...	Parameters pass to plot.

Value

A invisible data.frame with x, y of points.

Examples

```
path <- system.file("extdata", package="ribosomeProfilingQC")
#RPFs <- dir(path, "RPF.*?.[12].bam$", full.names=TRUE)
#RNAs <- dir(path, "mRNA.*?.[12].bam$", full.names=TRUE)
#gtf <- file.path(path, "Danio_rerio.GRCz10.91.chr1.gtf.gz")
#cnts <- countReads(RPFs, RNAs, gtf, level="gene")
cnts <- readRDS(file.path(path, "cnts.rds"))
fpkm <- getFPKM(cnts)
te <- translationalEfficiency(fpkm)
plotTE(te, 1)
```

plotTranscript	<i>Plot reads P site abundance for a specific transcript</i>
----------------	--

Description

Plot the bundances of P site on a transcript.

Usage

```
plotTranscript(
  reads,
  tx_name,
  col = c(Frame_0 = "#009E73", Frame_1 = "#D55E00", Frame_2 = "#0072B2")
)
```

Arguments

reads	Output of assignReadingFrame
tx_name	Transcript names.
col	Colors for reading frames

Value

Invisible heights of the barplot.

Examples

```
pcs <- readRDS(system.file("extdata", "samplePc.rds",
                          package="ribosomeProfilingQC"))

plotTranscript(pcs, c("ENSDART00000152562", "ENSDART00000054987"))
```

```
prepareCDS
```

```
Prepare CDS
```

Description

Prepare CDS library from a TxDb object.

Usage

```
prepareCDS(txdb, withUTR = FALSE)
```

Arguments

txdb	A TxDb object.
withUTR	Including UTR information or not.

Value

A GRanges object with metadata which include: tx_id: transcript id; tx_name: transcript name; gene_id: gene id; isFirstExonInCDS: is first exon in CDS or not; idFirstExonInCDS: the id for the first exon; isLastExonInCDS: is last exon in CDS or not; wid.cumsu: cumulative sums of number of bases in CDS; internalPos: offset position from 1 base;

Examples

```
library(GenomicFeatures)
txdb_file <- system.file("extdata", "Biomart_Ensembl_sample.sqlite",
                        package="GenomicFeatures")
txdb <- loadDb(txdb_file)
CDS <- prepareCDS(txdb)
```

```
readsDistribution
```

```
Plot reads distribution in genomic elements
```

Description

Plot the percentage of reads in CDS, 5'UTR, 3'UTR, introns, and other elements.

Usage

```
readsDistribution(
  reads,
  txdb,
  upstreamRegion = 3000,
  downstreamRegion = 3000,
  plot = TRUE,
  ...
)
```

Arguments

reads	Output of getPsiteCoordinates
txdb	A TxDb object
upstreamRegion, downstreamRegion	The range for promoter region and downstream region.
plot	Plot the distribution or not
...	Not use.

Value

The reads with distribution assignment

Examples

```
library(Rsamtools)
bamfilename <- system.file("extdata", "RPF.WT.1.bam",
                           package="ribosomeProfilingQC")

yieldSize <- 10000000
bamfile <- BamFile(bamfilename, yieldSize = yieldSize)
pc <- getPsiteCoordinates(bamfile, bestpsite=11)
pc.sub <- pc[pc$qwidth %in% c(29, 30)]
library(GenomicFeatures)
library(BSgenome.Drerio.UCSC.danRer10)
txdb <- makeTxDbFromGFF(system.file("extdata",
                                   "Danio_rerio.GRCz10.91.chr1.gtf.gz",
                                   package="ribosomeProfilingQC"),
                      organism = "Danio rerio",
                      chrominfo = seqinfo(Drerio)["chr1"],
                      taxonomyId = 7955)
pc.sub <- readsDistribution(pc.sub, txdb, las=2)
```

readsEndPlot

Plot start/stop windows

Description

Plot the reads shifted from start/stop position of CDS.

Usage

```
readsEndPlot(
  bamfile,
  CDS,
  toStartCodon = TRUE,
  fiveEnd = TRUE,
  shift = 0,
  window = c(-29, 30),
  readLen = 25:30
)
```

Arguments

bamfile	A BamFile object.
CDS	Output of prepareCDS
toStartCodon	What to search: start or end codon
fiveEnd	Search from five or three ends of the reads.
shift	number(1). Search from 5' end or 3' end of given number. if fiveEnd set to false, please set the shift as a negative number.
window	The window of CDS region to plot
readLen	The reads length used to plot

Value

The invisible counts numbers.

Examples

```
library(Rsamtools)
bamfilename <- system.file("extdata", "RPF.WT.1.bam",
                           package="ribosomeProfilingQC")

yieldSize <- 10000000
bamfile <- BamFile(bamfilename, yieldSize = yieldSize)
#library(GenomicFeatures)
library(BSgenome.Drerio.UCSC.danRer10)
#txdb <- makeTxDbFromGFF(system.file("extdata",
#                                   "Danio_rerio.GRCz10.91.chr1.gtf.gz",
#                                   package="ribosomeProfilingQC"),
#                       organism = "Danio rerio",
#                       chrominfo = seqinfo(Drerio)["chr1"],
#                       taxonomyId = 7955)
#CDS <- prepareCDS(txdb)
CDS <- readRDS(system.file("extdata", "CDS.rds",
                           package="ribosomeProfilingQC"))

readsEndPlot(bamfile, CDS, toStartCodon=TRUE)
#readsEndPlot(bamfile, CDS, toStartCodon=TRUE, fiveEnd=FALSE)
#readsEndPlot(bamfile, CDS, toStartCodon=FALSE)
#readsEndPlot(bamfile, CDS, toStartCodon=FALSE, fiveEnd=FALSE)
readsEndPlot(bamfile, CDS, shift=13)
#readsEndPlot(bamfile, CDS, fiveEnd=FALSE, shift=-16)
```

readsLenToKeep	<i>Get reads length to keep by cutoff percentage</i>
----------------	--

Description

Set the percentage to filter the reads.

Usage

```
readsLenToKeep(readsLengthDensity, cutoff = 0.8)
```

Arguments

readsLengthDensity	Output of summaryReadsLength
cutoff	Cutoff value.

Value

Reads length to be kept.

Examples

```
reads <- GRanges("chr1", ranges=IRanges(seq.int(100), width=1),
                 qwidth=sample(25:31, size = 100, replace = TRUE,
                               prob = c(.01, .01, .05, .1, .77, .05, .01)))
readsLenToKeep(summaryReadsLength(reads, plot=FALSE))
```

ribosomeReleaseScore	<i>Ribosome Release Score (RRS)</i>
----------------------	-------------------------------------

Description

RRS is calculated as the ratio of translational efficiency in the CDS with RPFs in the 3'UTR.

Usage

```
ribosomeReleaseScore(
  cdsTE,
  utr3TE,
  CDSsampleOrder,
  UTR3sampleOrder,
  pseudocount = 0,
  log2 = FALSE
)
```

Arguments

cdsTE, utr3TE	Translational efficiency of CDS and UTR3 region. Output of translationalEfficiency
CDSsampleOrder, UTR3sampleOrder	Sample order of cdsTE and utr3TE. The parameters are used to make sure that the order of CDS and UTR3 in TE is corresponding samples.
pseudocount	The number will be add to sum of reads count to avoid X/0.
log2	Do log2 transform or not.

Value

A vector of RRS.

Examples

```
## Not run:
path <- system.file("extdata", package="ribosomeProfilingQC")
RPFs <- dir(path, "RPF.*?\\. [12].bam$", full.names=TRUE)
RNAs <- dir(path, "mRNA.*?\\. [12].bam$", full.names=TRUE)
gtf <- file.path(path, "Danio_rerio.GRCz10.91.chr1.gtf.gz")
cvgs <- coverageDepth(RPFs, RNAs, gtf)
cvgs.utr3 <- coverageDepth(RPFs, RNAs, gtf, region="utr3")
TE90 <- translationalEfficiency(cvgs, window = 90)
TE90.utr3 <- translationalEfficiency(cvgs.utr3, window = 90)
rrs <- ribosomeReleaseScore(TE90, TE90.utr3)

## End(Not run)
```

shiftReadsByFrame *Shift reads by reading frame*

Description

Shift reads P site position by reading frame. After shifting, all reading frame will be set as 0

Usage

```
shiftReadsByFrame(reads, txdb)
```

Arguments

reads	Output of getPsiteCoordinates
txdb	A TxDb object.

Value

Reads with reading frame information

Examples

```

library(Rsamtools)
bamfilename <- system.file("extdata", "RPF.WT.1.bam",
                           package="ribosomeProfilingQC")

yieldSize <- 10000000
bamfile <- BamFile(bamfilename, yieldSize = yieldSize)
pc <- getPsiteCoordinates(bamfile, bestpsite=11)
pc.sub <- pc[pc$qwidth %in% c(29, 30)]
library(GenomicFeatures)
library(BSgenome.Drerio.UCSC.danRer10)
txdb <- makeTxDbFromGFF(system.file("extdata",
                                   "Danio_rerio.GRCz10.91.chr1.gtf.gz",
                                   package="ribosomeProfilingQC"),
                       organism = "Danio rerio",
                       chrominfo = seqinfo(Drerio)["chr1"],
                       taxonomyId = 7955)
pc.sub <- shiftReadsByFrame(pc.sub, txdb)

```

simulateRPF

*Simulation function***Description**

Simulate the RPFs reads in CDS, 5'UTR and 3'UTR

Usage

```

simulateRPF(
  txdb,
  outPath,
  genome,
  samples = 6,
  group1 = c(1, 2, 3),
  group2 = c(4, 5, 6),
  readsPerSample = 1e+06,
  readsLen = 28,
  psite = 13,
  frame0 = 0.9,
  frame1 = 0.05,
  frame2 = 0.05,
  DRegions = GRanges(),
  size = 1,
  sd = 0.02,
  minDElevel = log2(2),
  includeReadsSeq = FALSE
)

```

Arguments

txdb	A TxDb object
outPath	Output folder for the bam files

genome	A BSgenome object
samples	Total samples to simulate.
group1, group2	Numeric to index the sample groups.
readsPerSample	Total reads number per sample.
readsLen	Reads length, default 100bp.
psite	P-site position. default 13.
frame0, frame1, frame2	Percentage of reads distribution in frame0, frame1 and frame2
DERegions	The regions with differential reads in exon, utr5 and utr3.
size	Dispersion parameter. Must be strictly positive.
sd	Standard deviations.
minDElevel	Minimal differential level. default: log2(2).
includeReadsSeq	logical(1). Include reads sequence or not.

Value

An invisible list of GAlignments.

Examples

```
library(GenomicFeatures)
txdb_file <- system.file("extdata", "Biomart_Ensembl_sample.sqlite",
                        package="GenomicFeatures")
txdb <- loadDb(txdb_file)
simulateRPF(txdb, samples=1, readsPerSample = 1e3)
## Not run:
cds <- prepareCDS(txdb, withUTR = TRUE)
cds <- cds[width(cds)>200]
DERegions <- cds[sample(seq_along(cds), 10)]
simulateRPF(txdb, samples=6, readsPerSample = 1e5, DERegions=DERegions)

## End(Not run)
```

spliceEvent	<i>Get splicing events</i>
-------------	----------------------------

Description

Get differential usage of alternative Translation Initiation Sites, alternative Polyadenylation Sites or alternative splicing sites

Usage

```
spliceEvent(coverage, group1, group2)
```

Arguments

coverage	Coverages of feature region with extensions. Output of coverageDepth
group1, group2	The sample names of group 1 and group 2


```

package="ribosomeProfilingQC"),
organism = "Danio rerio",
chrominfo = seqinfo(Drerio)["chr1"],
taxonomyId = 7955)
CDS <- prepareCDS(txdb)
strandPlot(pc.sub, CDS)

```

summaryReadsLength *Summary the reads lengths*

Description

Plot the reads length distribution

Usage

```
summaryReadsLength(reads, widthRange = c(20:35), plot = TRUE, ...)
```

Arguments

reads	Output of getPsiteCoordinates
widthRange	The reads range to be plot
plot	Do plot or not
...	Not use.

Value

The reads length distribution

Examples

```

reads <- GRanges("chr1", ranges=IRanges(seq.int(100), width=1),
               qwidth=sample(25:31, size = 100, replace = TRUE,
                             prob = c(.01, .01, .05, .1, .77, .05, .01)))
summaryReadsLength(reads)

```

translationalEfficiency
Translational Efficiency

Description

Calculate Translational Efficiency (TE). TE is defined as the ratios of the absolute level of ribosome occupancy divided by RNA levels for transcripts.

Usage

```
translationalEfficiency(
  x,
  window,
  RPFsampleOrder,
  mRNAsampleOrder,
  pseudocount = 1,
  log2 = FALSE,
  normByLibSize = FALSE
)
```

Arguments

x	Output of <code>getFPKM</code> or <code>normByRUVs</code> . if window is set, it must be output of <code>coverageDepth</code> .
window	numeric(1). window size for maximal counts.
RPFsampleOrder, mRNAsampleOrder	Sample order of RPFs and mRNAs. The parameters are used to make sure that the order of RPFs and mRNAs in <code>cvgs</code> is corresponding samples.
pseudocount	The number will be add to sum of reads count to avoid X/0.
log2	Do log2 transform or not.
normByLibSize	Normalization by library size or not. If window size is provied and <code>normByLibSize</code> is set to TRUE, the coverage will be normalized by library size.

Value

A list with RPFs, mRNA levels and TE as a matrix with translational efficiency

Examples

```
## Not run:
path <- system.file("extdata", package="ribosomeProfilingQC")
RPFs <- dir(path, "RPF.*?\\. [12].bam$", full.names=TRUE)
RNAs <- dir(path, "mRNA.*?\\. [12].bam$", full.names=TRUE)
gtf <- file.path(path, "Danio_rerio.GRCz10.91.chr1.gtf.gz")
cnts <- countReads(RPFs, RNAs, gtf, level="gene")
fpkm <- getFPKM(cnts)
te <- translationalEfficiency(fpkm)

## End(Not run)
```

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