Package 'ribosomeProfilingQC'

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Type Package

Title Ribosome Profiling Quality Control

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Description Ribo-Seq (also named ribosome profiling or footprinting) measures translatome (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 *Assign reading frame*

Description

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

Usage

```
assignReadingFrame(reads, CDS, txdb, ignore.seqlevelsStyle = FALSE)
```

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.
ignore.seqlevelsStyle	Ignore the sequence name style detection or not.

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(txdbmaker)
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)
```

codonBias *Codon usage bias*

Description

Calculate the codon usage for the reads in the identified CDSs. And then compared to the reference codon usage.

Usage

```
codonBias(
  RPFs,
  gtf,
  genome,
  bestpsite = 13,
  readsLen = c(28, 29),
  anchor = "5end",
  ignore.seqlevelsStyle = FALSE,
  summary = TRUE,
  removeDuplicates = TRUE,
  ...
)
```

Arguments

RPFs	Bam file names of RPFs.
gtf	GTF file name for annotation or a TxDb object.
genome	A BSgenome object.
bestpsite	P site postion.
readsLen	Reads length to keep.
anchor	5end or 3end. Default is 5end.
ignore.seqlevelsStyle	Ignore the sequence name style detection or not.
summary	Return the summary of codon usage bias or full list.
removeDuplicates	Remove the PCR duplicates or not. Default TRUE.
...	Parameters pass to makeTxDbFromGFF

Value

A list of data frame of codon count table if summary is TRUE. list 'reads' means the counts by raw reads. list 'reference' means the counts by sequence extracted from reference by the coordinates of mapped reads. Otherwise, return the counts (reads/reference) table for each reads.

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")
library(BSgenome.Drerio.UCSC.danRer10)
cb <- codonBias(RPFs[c(1,2)], gtf=gtf, genome=Drerio)
```

---

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",
  ignore.seqlevelsStyle = FALSE,
  ...
)
```

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.
ignore.seqlevelsStyle	Ignore the sequence name style detection or not.
...	Parameters pass to featureCounts except isGTFAnnotationFile, GTF.attrType, and annot.ext.

Value

A list with reads counts.

Examples

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

coverageDepth *Extract coverage depth for gene level or transcript level*

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,
  ignore.seqlevelsStyle = FALSE,
  ...
)
```

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 postion.
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	Extesion region for "feature with extension".
ignore.seqlevelsStyle	Ignore the sequence name style detection or not.
...	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` *Calculate coverage rate*

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 *Class* "cvgd"

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 an 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	indexes 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` *Estimate P site position*

Description

Estimate P site position from a subset reads.

Usage

```
estimatePsite(
  bamfile,
  CDS,
  genome,
  anchor = "5end",
  readLen = c(25:30),
  ignore.seqlevelsStyle = FALSE
)
```

Arguments

`bamfile`	A BamFile object.
`CDS`	Output of prepareCDS
`genome`	A BSgenome object.
`anchor`	5end or 3end. Default is 5end.
`readLen`	The reads length used to estimate.
`ignore.seqlevelsStyle`	Ignore the sequence name style detection or not.

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(txdbmaker)
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(txdbmaker)
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,
  ignore.seqlevelsStyle = FALSE
)
```

Arguments

`reads`	Output of getPsiteCoordinates
`ref`	Refercence 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.
`ignore.seqlevelsStyle`	Ignore the sequence name style detection 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(txdbmaker)
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 counnted at each position within the ORF.

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

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, $ORFscore = -1XORFscore$.

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/embj.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",
  param = ScanBamParam(what = c("qwidth"), tag = character(0), flag =
    scanBamFlag(isSecondaryAlignment = FALSE, isUnmappedQuery = FALSE,
    isNotPassingQualityControls = FALSE, isSupplementaryAlignment = FALSE))
)
```

Arguments

`bamfile`	A BamFile object.
`bestpsite`	P site postion. See estimatePsite
`anchor`	5end or 3end. Default is 5end.
`param`	A ScanBamParam object. Please note the 'qwidth' is required.

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 labled 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)
```

---

normalizeTEbyLoess *Normalize the TE by Loess*

Description

Fitting the translational efficiency values with the mRNA value by loess.

Usage

```
normalizeTEbyLoess(
  TE,
  log2 = TRUE,
  pseudocount = 0.001,
  span = 2/3,
  family.loess = "symmetric"
)
```

Arguments

TE	output of translationalEfficiency.
log2	logical(1L). Do log2 transform for TE or not. If TE value is not log2 transformed, please set it as TRUE.
pseudocount	The number will be add to sum of reads count to avoid X/0.
span, family.loess	Parameters will be passed to loess

Value

A list with RPFs, mRNA levels and TE as a matrix with log2 transformed translational efficiency.

Examples

```
path <- system.file("extdata", package="ribosomeProfilingQC")
cnts <- readRDS(file.path(path, "cnts.rds"))
fpkm <- getFPKM(cnts)
te <- translationalEfficiency(fpkm)
te1 <- normalizeTEbyLoess(te)
plotTE(te)
plotTE(te1, log2=FALSE)
```

---

normBy *Normalization by edgeR, DESeq2 or RUVSeq*

Description

Normalization by multiple known methods

Usage

```
normBy(counts, method = c("edgeR", "DESeq2", "RUVs", "fpkm", "vsn"), ...)
```

Arguments

counts	Output of countReads
method	Character(1L) to indicate the method for normalization.
...	parameters will be passed to normByRUVs or getFPKM

Value

Normalized counts list

Examples

```
path <- system.file("extdata", package="ribosomeProfilingQC")
cnts <- readRDS(file.path(path, "cnts.rds"))
norm <- normBy(cnts, method = 'edgeR')
norm2 <- normBy(cnts, method = 'DESeq2')
norm3 <- normBy(cnts, 'vsn')
```

`normByRUVs` *Normalization by RUVSeq*

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 *Metaplot of P site distribution*

Description

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

Usage

```
PAmotif(reads, genome, plot = TRUE, ignore.seqlevelsStyle = FALSE)
```

Arguments

reads	Output of assignReadingFrame or shiftReadsByFrame.
genome	A BSgenome object.
plot	Plot the motif or not.
ignore.seqlevelsStyle	Ignore the sequence name style detection 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 *Plot density for each reading frame*

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

A ggplot object.

Examples

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

plotSpliceEvent *Plot splice event*

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,
  theme = theme_classic(),
  type = "histogram",
  margins = "y",
  ...
)
```

Arguments

TE	Output of translationalEfficiency
sample	Sample names to plot.
xaxis	What to plot for x-axis.
removeZero	Remove the 0 values from plots.
log2	Do log2 transform for TE or not.
theme	Theme for ggplot2.
type, margins, ...	Parameters pass to ggMarginal

Value

A ggExtraPlot object.

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 *Plot reads P site abundance for a specific transcript*

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,
  precedence = NULL,
  ignore.seqlevelsStyle = FALSE,
  ...
)
```

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
precedence	If no precedence specified, double count will be enabled, which means that if the reads overlap with both CDS and 5'UTR, both CDS and 5'UTR will be incremented. If a precedence order is specified, for example, if promoter is specified before 5'UTR, then only promoter will be incremented for the same example. The values could be any combinations of "CDS", "UTR5", "UTR3", "OtherExon", "Intron", "upstream", "downstreama" and "InterGenic", Default=NULL

ignore.seqlevelsStyle
: Ignore the sequence name style detection 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$qwidth %in% c(29, 30)]
library(txdbmaker)
library(BSgenome.Drerio.UCSC.danRer10)
txdb <- makeTxDbFromGFF(system.file("extdata",
          "Danio_rerio.GRCz10.91.chr1.gtf.gz",
          package="ribosomeProfilingQC"),
          organism = "Danio rerio",
          chrominfo = seqinfo(Drerio)["chr1"],
          taxonomyId = 7955)
pc.sub <- readsDistribution(pc.sub, txdb, las=2)
pc.sub <- readsDistribution(pc[pc$qwidth %in% c(29, 30)],
              txdb, las=2,
              precedence=c(
              "CDS", "UTR5", "UTR3", "OtherExon",
              "Intron", "upstream", "downstream",
              "InterGenic"
              ))
```

---

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,
  ignore.seqlevelsStyle = FALSE
)
```

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
ignore.seqlevelsStyle	Ignore the sequence name style detection or not.

Value

The invisible list with counts numbers and reads in GRanges.

Examples

```
library(Rsamtools)
bamfilename <- system.file("extdata", "RPF.WT.1.bam",
                           package="ribosomeProfilingQC")
yieldSize <- 10000000
bamfile <- BamFile(bamfilename, yieldSize = yieldSize)
#library(txdbmaker)
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"))
re <- readsEndPlot(bamfile, CDS, toStartCodon=TRUE)
readsEndPlot(re$reads, CDS, toStartCodon=TRUE, fiveEnd=FALSE)
#re <- readsEndPlot(bamfile, CDS, toStartCodon=FALSE)
#readsEndPlot(re$reads, CDS, toStartCodon=FALSE, fiveEnd=FALSE)
readsEndPlot(bamfile, CDS, shift=13)
#readsEndPlot(bamfile, CDS, fiveEnd=FALSE, shift=-16)
```

readsLenToKeep *Get reads length to keep by cutoff percentage*

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 *Ribosome Release Score (RRS)*

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, ignore.seqlevelsStyle = FALSE)
```

Arguments

reads	Output of getPsiteCoordinates
txdb	A TxDb object.
ignore.seqlevelsStyle	Ignore the sequence name style detection or not.

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(txdbmaker)
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,
  DEregions = 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 *Get splicing events*

Description

Get differentical 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

Value

A GRanges object of splice events.

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)

## End(Not run)
```

strandPlot *Plot the distribution of reads in sense and antisense strand*

Description

Plot the distribution of reads in sense and antisense strand to check the mapping is correct.

Usage

```
strandPlot(
  reads,
  CDS,
  col = c("#009E73", "#D55E00"),
  ignore.seqlevelsStyle = FALSE,
  ...
)
```

Arguments

reads	Output of getPsiteCoordinates
CDS	Output of prepareCDS
col	Coloar for sense and antisense strand.
ignore.seqlevelsStyle	Ignore the sequence name style detection or not.
...	Parameter passed to barplot

Value

A ggplot object.

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(txdbmaker)
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)
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,
  shrink = FALSE,
  ...
)
```

Arguments

x	Output of getFPKM or normByRUVs. if window is set, it must be output of coverageDepth.
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 cvgs 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 provided and normByLibSize is set to TRUE, the coverage will be normalized by library size.
shrink	Shrink the TE or not.
...	Parameters will be passed to ash function from ashr.

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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