

Package ‘InPAS’

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Title Identify Novel Alternative PolyAdenylation Sites (PAS) from RNA-seq data

Version 2.4.0

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Description Alternative polyadenylation (APA) is one of the important post-transcriptional regulation mechanisms which occurs in most human genes. InPAS facilitates the discovery of novel APA sites and the differential usage of APA sites from RNA-Seq data. It leverages cleanUpdTSeq to fine tune identified APA sites by removing false sites.

biocViews Alternative Polyadenylation, Differential Polyadenylation Site Usage, RNA-seq, Gene Regulation, Transcription

License GPL (>= 2)

Imports AnnotationDbi, batchtools, Biobase, Biostrings, BSgenome, cleanUpdTSeq, depmixS4, dplyr, flock, future, future.apply, GenomeInfoDb, GenomicRanges, GenomicFeatures, ggplot2, IRanges, limma, magrittr, methods, parallelly, plyranges, preprocessCore, readr, reshape2, RSQLite, stats, S4Vectors, utils

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addChr2Exclude

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<code>addChr2Exclude</code>	<i>Add a globally-applied requirement for filtering out scaffolds from all analysis</i>
-----------------------------	-----------------------------------------------------------------------------------------

Description

This function will set the default requirement of filtering out scaffolds from all analysis.

Usage

```
addChr2Exclude(chr2exclude = c("chrM", "MT", "Pltd", "chrPltd"))
```

Arguments

<code>chr2exclude</code>	A character vector, NA or NULL, specifying chromosomes or scaffolds to be excluded for InPAS analysis. chrM and alternative scaffolds representing different haplotypes should be excluded.
--------------------------	---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

<code>addInPASEnsDb</code>	<i>Add a globally defined EnsDb to some InPAS functions.</i>
----------------------------	--------------------------------------------------------------

Description

Add a globally defined EnsDb to some InPAS functions.

Usage

```
addInPASEnsDb(EnsDb = NULL)
```

Arguments

<code>EnsDb</code>	An object of <code>ensemldb::EnsDb</code>
--------------------	-------------------------------------------

addInPASGenome	<i>Add a globally defined genome to all InPAS functions.</i>
----------------	--------------------------------------------------------------

Description

This function will set the genome across all InPAS functions.

Usage

```
addInPASGenome(genome = NULL)
```

Arguments

genome	A BSgenome object indicating the default genome to be used for all InPAS functions. This value is stored as a global environment variable. This can be overwritten on a per-function basis using the given function's genome parameter.
--------	-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------

addInPASOutputDirectory	<i>Add a globally defined output directory to some InPAS functions.</i>
-------------------------	-------------------------------------------------------------------------

Description

Add a globally defined output directory to some InPAS functions.

Usage

```
addInPASOutputDirectory(outdir = NULL)
```

Arguments

outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
--------	----------------------------------------------------------------------------------------------------------------------------------

addInPASTxDB	<i>Add a globally defined TxDb for InPAS functions.</i>
--------------	---------------------------------------------------------

Description

Add a globally defined TxDb for InPAS functions.

Usage

```
addInPASTxDB(TxDB = NULL)
```

Arguments

TxDB	An object of GenomicFeatures::TxDb
------	----------------------------------------------------

Examples

```
library("TxDb.Hsapiens.UCSC.hg19.knownGene")
addInPASTxDB(TxDB = TxDb.Hsapiens.UCSC.hg19.knownGene)
```

addLockName	<i>Add a filename for locking a SQLite database</i>
-------------	-----------------------------------------------------

Description

Add a filename for locking a SQLite database

Usage

```
addLockName(filename = NULL)
```

Arguments

filename	A character(1) vector, specifying a path to a file for locking.
----------	-----------------------------------------------------------------

assemble_allCov	<i>Assemble coverage files for a given chromosome for all samples</i>
-----------------	-----------------------------------------------------------------------

Description

Process individual sample-chromosome-specific coverage files in an experiment into a file containing a list of chromosome-specific Rle coverage of all samples

Usage

```
assemble_allCov(
  sqlite_db,
  seqname,
  outdir = getInPASOutputDirectory(),
  genome = getInPASGenome()
)
```

Arguments

sqlite_db	A path to the SQLite database for InPAS, i.e. the output of setup_sqlitedb()
seqname	A character(1) vector, the name of a chromosome/scaffold
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
genome	An object of BSgenome::BSgenome

Value

A list of paths to per-chromosome coverage files of all samples.

- seqname, chromosome/scaffold name
 - tag1, name tag for sample1
 - tag2, name tag for sample2
 - tagN, name tag for sampleN

Author(s)

Haibo Liu

Examples

```
if (interactive()) {
  library(BSgenome.Mmusculus.UCSC.mm10)
  genome <- BSgenome.Mmusculus.UCSC.mm10
  bedgraphs <- system.file("extdata", c(
    "Baf3.extract.bedgraph",
    "UM15.extract.bedgraph"
  ),
```

```

package = "InPAS"
)
tags <- c("Baf3", "UM15")
metadata <- data.frame(
  tag = tags,
  condition = c("Baf3", "UM15"),
  bedgraph_file = bedgraphs
)
outdir <- tempdir()
write.table(metadata,
  file = file.path(outdir, "metadata.txt"),
  sep = "\t", quote = FALSE, row.names = FALSE
)

sqlite_db <- setup_sqlitedb(
  metadata = file.path(
    outdir,
    "metadata.txt"
  ),
  outdir
)
coverage <- list()
addLockName(filename = tempfile())
for (i in seq_along(bedgraphs)) {
  coverage[[tags[i]]] <- get_ssRleCov(
    bedgraph = bedgraphs[i],
    tag = tags[i],
    genome = genome,
    sqlite_db = sqlite_db,
    outdir = outdir,
    chr2exclude = "chrM"
  )
}
chr_coverage <- assemble_allCov(sqlite_db,
  seqname = "chr6",
  outdir = outdir,
  genome = genome
)
}

```

extract_UTR3Anno

extract 3' UTR information from a [GenomicFeatures::TxDb](#) object

Description

extract 3' UTR information from a [GenomicFeatures::TxDb](#) object. The 3'UTR is defined as the last 3'UTR fragment for each transcript and it will be cut if there is any overlaps with other exons.

Usage

```
extract_UTR3Anno(
  sqlite_db,
  TxDb = getInPASTxDB(),
  edb = getInPASEnsDb(),
  genome = getInPASGenome(),
  outdir = getInPASOutputDirectory(),
  chr2exclude = getChr2Exclude(),
  MAX_EXONS_GAP = 10000L
)
```

Arguments

sqlite_db	A path to the SQLite database for InPAS, i.e. the output of <code>setup_sqlitedb()</code> .
TxDB	An object of <code>GenomicFeatures::TxDb</code>
edb	An object of <code>ensemldb::EnsDb</code>
genome	An object of <code>BSgenome::BSgenome</code>
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
chr2exclude	A character vector, NA or NULL, specifying chromosomes or scaffolds to be excluded for InPAS analysis. chrM and alternative scaffolds representing different haplotypes should be excluded.
MAX_EXONS_GAP	An integer(1) vector, maximal gap sizes between the last known CP sites to a nearest downstream exon. Default is 10 kb for mammalian genomes. For other species, user need to adjust this parameter.

Details

A good practice is to perform read alignment using a reference genome from Ensembl/GenCode including only the primary assembly and build a TxDb and EnsDb using the GTF/GFF files downloaded from the same source as the reference genome, such as BioMart/Ensembl/GenCode. For instruction, see Vignette of the GenomicFeatures. The UCSC reference genomes and their annotation packages can be very cumbersome.

Value

An object of `GenomicRanges::GRangesList`, containing GRanges for extracted 3' UTRs, and the corresponding last CDSs and next.exon.gap for each chromosome/scaffold. Chromosome

Author(s)

Jianhong Ou, Haibo Liu

Examples

```

library("EnsDb.Hsapiens.v86")
library("BSgenome.Hsapiens.UCSC.hg19")
library("GenomicFeatures")
## set a sqlite database
bedgraphs <- system.file("extdata", c(
  "Baf3.extract.bedgraph",
  "UM15.extract.bedgraph"
),
package = "InPAS"
)
tags <- c("Baf3", "UM15")
metadata <- data.frame(
  tag = tags,
  condition = c("Baf3", "UM15"),
  bedgraph_file = bedgraphs
)
outdir <- tempdir()

write.table(metadata,
  file = file.path(outdir, "metadata.txt"),
  sep = "\t", quote = FALSE, row.names = FALSE
)
sqlite_db <- setup_sqlitedb(
  metadata =
    file.path(outdir, "metadata.txt"),
  outdir
)

samplefile <- system.file("extdata",
  "hg19_knownGene_sample.sqlite",
  package = "GenomicFeatures"
)
TxDb <- loadDb(samplefile)
edb <- EnsDb.Hsapiens.v86
genome <- BSgenome.Hsapiens.UCSC.hg19
addInPASOutputDirectory(outdir)
seqnames <- seqnames(BSgenome.Hsapiens.UCSC.hg19)
chr2exclude <- c(
  "chrM", "chrMT",
  seqnames[grepl("_ (hap\\d+|fix|alt)$",
    seqnames,
    perl = TRUE
  )]
)
utr3 <- extract_UTR3Anno(sqlite_db, TxDb, edb,
  genome = genome,
  chr2exclude = chr2exclude,
  outdir = tempdir(),
  MAX_EXONS_GAP = 10000L
)

```

filter_testOut	<i>filter 3' UTR usage test results</i>
----------------	-----------------------------------------

Description

filter results of [test_dPDUI\(\)](#)

Usage

```
filter_testOut(
  res,
  gp1,
  gp2,
  outdir = getInPASOutputDirectory(),
  background_coverage_threshold = 2,
  P.Value_cutoff = 0.05,
  adj.P.Val_cutoff = 0.05,
  dPDUI_cutoff = 0.2,
  PDUI_logFC_cutoff = log2(1.5)
)
```

Arguments

res	a UTR3eSet object, output of test_dPDUI()
gp1	tag names involved in group 1. gp1 and gp2 are used for filtering purpose if both are specified; otherwise only other specified thresholds are used for filtering.
gp2	tag names involved in group 2
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
background_coverage_threshold	background coverage cut off value. for each group, more than half of the long form should greater than background_coverage_threshold. for both group, at least in one group, more than half of the short form should greater than background_coverage_threshold.
P.Value_cutoff	cutoff of P value
adj.P.Val_cutoff	cutoff of adjust P value
dPDUI_cutoff	cutoff of dPDUI
PDUI_logFC_cutoff	cutoff of PDUI log2 transformed fold change

Value

A data frame converted from an object of [GenomicRanges::GRanges](#).

Author(s)

Jianhong Ou, Haibo Liu

See Also

[test_dPDUI\(\)](#)

Examples

```
library(limma)
path <- system.file("extdata", package = "InPAS")
load(file.path(path, "eset.MAQC.rda"))
tags <- colnames(eset@PDUI)
g <- factor(gsub("\\..*$", "", tags))
design <- model.matrix(~ -1 + g)
colnames(design) <- c("Brain", "UHR")
contrast.matrix <- makeContrasts(
  contrasts = "Brain-UHR",
  levels = design
)
res <- test_dPDUI(
  eset = eset,
  method = "limma",
  normalize = "none",
  design = design,
  contrast.matrix = contrast.matrix
)
filter_testOut(res,
  gp1 = c("Brain.auto", "Brain.phiX"),
  gp2 = c("UHR.auto", "UHR.phiX"),
  background_coverage_threshold = 2,
  P.Value_cutoff = 0.05,
  adj.P.Val_cutoff = 0.05,
  dPDUI_cutoff = 0.3,
  PDUI_logFC_cutoff = .59
)
```

find_minMSEDist

Visualization of MSE profiles, 3' UTR coverage and minimal MSE distribution

Description

Visualization of MSE profiles, 3' UTR coverage and minimal MSE distribution

Usage

```

find_minMSEdistr(
  CPs,
  outdir = NULL,
  MSE.plot = "MSE.pdf",
  coverage.plot = "coverage.pdf",
  min.MSE.to.end.distr.plot = "min.MSE.to.end.distr.pdf"
)

```

Arguments

CPs	A list, output from search_proximalCPs() or adjust_distalCPs() or adjust_proximalCPs()
outdir	A character(1) vector, specifying the output directory
MSE.plot	A character(1) vector, specifying a PDF file name for outputting plots of MSE profiles. No directory path is allowed.
coverage.plot	A character(1) vector, specifying a PDF file name for outputting per-sample coverage profiles. No directory path is allowed.
min.MSE.to.end.distr.plot	A character(1) vector, specifying a PDF file name for outputting histograms showing minimal MSE distribution relative to longer 3' UTR end. No directory path is allowed.

getChr2Exclude	<i>Get a globally-applied requirement for filtering scaffolds.</i>
----------------	--------------------------------------------------------------------

Description

This function will get the default requirement of filtering scaffolds.

Usage

```
getChr2Exclude()
```

getInPASEnsDb	<i>Get the globally defined EnsDb.</i>
---------------	----------------------------------------

Description

Get the globally defined EnsDb.

Usage

```
getInPASEnsDb()
```

Value

An object of [ensemldb::EnsDb](#)

getInPASGenome	<i>Get the globally defined genome</i>
----------------	----------------------------------------

Description

This function will retrieve the genome that is currently in use by InPAS.

Usage

```
getInPASGenome()
```

getInPASOutputDirectory	<i>Get the path to a output directory for InPAS analysis</i>
-------------------------	--------------------------------------------------------------

Description

Get the path to a output directory for InPAS analysis

Usage

```
getInPASOutputDirectory()
```

Value

a normalized path to a output directory for InPAS analysis

getInPASSQLiteDb	<i>Get the path to an SQLite database</i>
------------------	-------------------------------------------

Description

Get the path to an SQLite database

Usage

```
getInPASSQLiteDb()
```

Value

A path to an SQLite database

getInPASTxDB	<i>Get the globally defined TxDb.</i>
--------------	---------------------------------------

Description

Get the globally defined TxDb.

Usage

```
getInPASTxDB()
```

Value

An object of [GenomicFeatures::TxDb](#)

Examples

```
library("TxDb.Hsapiens.UCSC.hg19.knownGene")
addInPASTxDB(TxDB = TxDb.Hsapiens.UCSC.hg19.knownGene)
getInPASTxDB()
```

getLockName	<i>Get the path to a file for locking the SQLite database</i>
-------------	---------------------------------------------------------------

Description

Get the path to a file for locking the SQLite database

Usage

```
getLockName()
```

Value

A path to a file for locking

get_chromosomes	<i>Identify chromosomes/scaffolds for CP site discovery</i>
-----------------	-------------------------------------------------------------

Description

Identify chromosomes/scaffolds which have both coverage and annotated 3' utr3 for CP site discovery

Usage

```
get_chromosomes(utr3, sqlite_db)
```

Arguments

utr3	An object of <code>GenomicRanges::GRangesList</code> . An output of <code>extract_UTR3Anno()</code> .
sqlite_db	A path to the SQLite database for InPAS, i.e. the output of <code>setup_sqlitedb()</code> .

Value

A vector of characters, containing names of chromosomes/scaffolds for CP site discovery

Examples

```
library(BSgenome.Mmusculus.UCSC.mm10)
genome <- BSgenome.Mmusculus.UCSC.mm10
data(utr3.mm10)
utr3 <- split(utr3.mm10, seqnames(utr3.mm10), drop = TRUE)
bedgraphs <- system.file("extdata", c(
  "Baf3.extract.bedgraph",
  "UM15.extract.bedgraph"
),
package = "InPAS"
)
tags <- c("Baf3", "UM15")
metadata <- data.frame(
  tag = tags,
  condition = c("Baf3", "UM15"),
  bedgraph_file = bedgraphs
)
outdir <- tempdir()
write.table(metadata,
  file = file.path(outdir, "metadata.txt"),
  sep = "\t", quote = FALSE, row.names = FALSE
)

sqlite_db <- setup_sqlitedb(
  metadata = file.path(
    outdir,
    "metadata.txt"
  )
)
```

```

    ),
    outdir
  )
  addLockName(filename = tempfile())
  coverage <- list()
  for (i in seq_along(bedgraphs)) {
    coverage[[tags[i]]] <- get_ssRleCov(
      bedgraph = bedgraphs[i],
      tag = tags[i],
      genome = genome,
      sqlite_db = sqlite_db,
      outdir = outdir,
      chr2exclude = "chrM"
    )
  }
  get_chromosomes(utr3, sqlite_db)

```

get_lastCDSUTR3

Extract the last unspliced region of each transcript

Description

Extract the last unspliced region of each transcript from a TxDb. These regions could be the last 3'UTR exon for transcripts whose 3' UTRs are composed of multiple exons or last CDS regions and 3'UTRs for transcripts whose 3'UTRs and last CDS regions are on the same single exon.

Usage

```

get_lastCDSUTR3(
  TxDb = getInPASTxDB(),
  genome = getInPASGenome(),
  chr2exclude = getChr2Exclude(),
  outdir = getInPASOutputDirectory(),
  MAX_EXONS_GAP = 10000
)

```

Arguments

TxDb	An object of GenomicFeatures::TxDb
genome	An object of BSgenome::BSgenome
chr2exclude	A character vector, NA or NULL, specifying chromosomes or scaffolds to be excluded for InPAS analysis. chrM and alternative scaffolds representing different haplotypes should be excluded.
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
MAX_EXONS_GAP	An integer(1) vector, maximal gap sizes between the last known CP sites to a nearest downstream exon. Default is 10 kb for mammalian genomes. For other species, user need to adjust this parameter.

Value

A BED file with 6 columns: chr, chrStart, chrEnd, name, score, and strand.

get_regionCov	<i>Get coverage for 3' UTR and last CDS regions on a single chromosome</i>
---------------	----------------------------------------------------------------------------

Description

Get coverage for 3' UTR and last CDS regions on a single chromosome

Usage

```
get_regionCov(
  chr.utr3,
  sqlite_db,
  outdir = getInPASOutputDirectory(),
  phmm = FALSE,
  min.length.diff = 200
)
```

Arguments

chr.utr3	An object of GenomicRanges::GRanges , one element of an output of extract_UTR3Anno()
sqlite_db	A path to the SQLite database for InPAS, i.e. the output of setup_sqlitedb() .
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
phmm	A logical(1) vector, indicating whether data should be prepared for singleSample analysis? By default, FALSE
min.length.diff	An integer(1) vector, specifying minimal length difference between proximal and distal APA sites which should be met to be considered for differential APA analysis. Default is 200 bp.

Value

coverage view in GRanges

Author(s)

Jianhong Ou, Haibo Liu

get_ssRleCov *Get Rle coverage from a bedgraph file for a sample*

Description

Get RLe coverage from a bedgraph file for a sample

Usage

```
get_ssRleCov(
  bedgraph,
  tag,
  genome = getInPASGenome(),
  sqlite_db,
  future.chunk.size = NULL,
  outdir = getInPASOutputDirectory(),
  chr2exclude = getChr2Exclude()
)
```

Arguments

bedgraph	A path to a bedGraph file
tag	A character(1) vector, a name tag used to label the bedgraph file. It must match the tag specified in the metadata file used to setup the SQLite database
genome	an object <code>BSgenome::BSgenome</code> . To make things easy, we suggest users creating a <code>BSgenome::BSgenome</code> instance from the reference genome used for read alignment. For details, see the documentation of <code>BSgenome::forgeBSgenomeDataPkg()</code> .
sqlite_db	A path to the SQLite database for InPAS, i.e. the output of <code>setup_sqlitedb()</code> .
future.chunk.size	The average number of elements per future ("chunk"). If Inf, then all elements are processed in a single future. If NULL, then argument <code>future.scheduling = 1</code> is used by default. Users can set <code>future.chunk.size = total number of elements/number of cores set for the backend</code> . See the <code>future.apply</code> package for details. You may adjust this number based based on the available computing resource: CPUs and RAM. This parameter affects the time for converting coverage from bedgraph to Rle.
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
chr2exclude	A character vector, NA or NULL, specifying chromosomes or scaffolds to be excluded for InPAS analysis. chrM and alternative scaffolds representing different haplotypes should be excluded.

Value

A data frame, as described below.

tag the sample tag

chr chromosome name

coverage_file path to Rle coverage files for each chromosome per sample tag

Author(s)

Jianhong Ou, Haibo Liu

Examples

```
if (interactive()) {
  library(BSgenome.Mmusculus.UCSC.mm10)
  genome <- BSgenome.Mmusculus.UCSC.mm10
  bedgraphs <- system.file("extdata", c(
    "Baf3.extract.bedgraph",
    "UM15.extract.bedgraph"
  ),
  package = "InPAS"
  )
  tags <- c("Baf3", "UM15")
  metadata <- data.frame(
    tag = tags,
    condition = c("Baf3", "UM15"),
    bedgraph_file = bedgraphs
  )
  outdir <- tempdir()
  write.table(metadata,
    file = file.path(outdir, "metadata.txt"),
    sep = "\t", quote = FALSE, row.names = FALSE
  )

  sqlite_db <- setup_sqlitedb(
    metadata = file.path(
      outdir,
      "metadata.txt"
    ),
    outdir
  )
  addLockName()
  coverage_info <- get_ssRleCov(
    bedgraph = bedgraphs[1],
    tag = tags[1],
    genome = genome,
    sqlite_db = sqlite_db,
    outdir = outdir,
    chr2exclude = "chrM"
  )
  # check read coverage depth
```

```

db_connect <- dbConnect(drv = RSQLite::SQLite(), dbname = sqlite_db)
dbReadTable(db_connect, "metadata")
dbDisconnect(db_connect)
}

```

get_usage4plot	<i>prepare coverage data and fitting data for plot</i>
----------------	--------------------------------------------------------

Description

prepare coverage data and fitting data for plot

Usage

```
get_usage4plot(gr, proximalSites, sqlite_db, hugeData)
```

Arguments

gr	An object of GenomicRanges::GRanges
proximalSites	An integer(n) vector, specifying the coordinates of proximal CP sites. Each of the proximal sites must match one entry in the GRanges object, gr.
sqlite_db	A path to the SQLite database for InPAS, i.e. the output of setup_sqlitedb() .
hugeData	A logical(1), indicating whether it is huge data

Value

An object of [GenomicRanges::GRanges](#) with metadata:

dat	A data.frame, first column is the position, the other columns are Coverage and value
offset	offset from the start of 3' UTR

Author(s)

Jianhong Ou, Haibo Liu

Examples

```

library(BSgenome.Mmusculus.UCSC.mm10)
library(TxDb.Mmusculus.UCSC.mm10.knownGene)
genome <- BSgenome.Mmusculus.UCSC.mm10
TxDb <- TxDb.Mmusculus.UCSC.mm10.knownGene

## load UTR3 annotation and convert it into a GRangesList
data(utr3.mm10)
utr3 <- split(utr3.mm10, seqnames(utr3.mm10), drop = TRUE)

bedgraphs <- system.file("extdata", c(

```

```

    "Baf3.extract.bedgraph",
    "UM15.extract.bedgraph"
  ),
  package = "InPAS"
)
tags <- c("Baf3", "UM15")
metadata <- data.frame(
  tag = tags,
  condition = c("baf", "UM15"),
  bedgraph_file = bedgraphs
)
outdir <- tempdir()
write.table(metadata,
  file = file.path(outdir, "metadata.txt"),
  sep = "\t", quote = FALSE, row.names = FALSE
)

sqlite_db <- setup_sqlitedb(
  metadata = file.path(
    outdir,
    "metadata.txt"
  ),
  outdir
)
addLockName(filename = tempfile())
coverage <- list()
for (i in seq_along(bedgraphs)) {
  coverage[[tags[i]]] <- get_ssRleCov(
    bedgraph = bedgraphs[i],
    tag = tags[i],
    genome = genome,
    sqlite_db = sqlite_db,
    outdir = outdir,
    chr2exclude = "chrM"
  )
}
data4CPsSearch <- setup_CPsSearch(sqlite_db,
  genome,
  chr.utr3 = utr3[["chr6"]],
  seqname = "chr6",
  background = "10K",
  TxDb = TxDb,
  hugeData = TRUE,
  outdir = outdir
)

gr <- GRanges("chr6", IRanges(128846245, 128850081), strand = "-")
names(gr) <- "chr6:128846245-128850081"
data4plot <- get_usage4plot(gr,
  proximalSites = 128849148,
  sqlite_db,
  hugeData = TRUE
)

```

```
plot_utr3Usage(
  usage_data = data4plot,
  vline_color = "purple",
  vline_type = "dashed"
)
```

get_UTR3eSet	<i>prepare 3' UTR coverage data for usage test</i>
--------------	----------------------------------------------------

Description

generate a UTR3eSet object with PDUI information for statistic tests

Usage

```
get_UTR3eSet(
  sqlite_db,
  normalize = c("none", "quantiles", "quantiles.robust", "mean", "median"),
  ...,
  singleSample = FALSE
)
```

Arguments

sqlite_db	A path to the SQLite database for InPAS, i.e. the output of setup_sqlitedb() .
normalize	A character(1) vector, specifying the normalization method. It can be "none", "quantiles", "quantiles.robust", "mean", or "median"
...	parameter can be passed into preprocessCore::normalize.quantiles.robust()
singleSample	A logical(1) vector, indicating whether data is prepared for analysis in a single-Sample mode? Default, FALSE

Value

An object of [UTR3eSet](#) which contains following elements: usage: an [GenomicRanges::GRanges](#) object with CP sites info. PDUI: a matrix of PDUI PDUI.log2: log2 transformed PDUI matrix short: a matrix of usage of short form long: a matrix of usage of long form if singleSample is TRUE, one more element, signals, will be included.

Author(s)

Jianhong Ou, Haibo Liu

Examples

```

if (interactive()) {
  library(BSgenome.Mmusculus.UCSC.mm10)
  library(TxDb.Mmusculus.UCSC.mm10.knownGene)
  genome <- BSgenome.Mmusculus.UCSC.mm10
  TxDb <- TxDb.Mmusculus.UCSC.mm10.knownGene

  ## load UTR3 annotation and convert it into a GRangesList
  data(utr3.mm10)
  utr3 <- split(utr3.mm10, seqnames(utr3.mm10), drop = TRUE)

  bedgraphs <- system.file("extdata", c(
    "Baf3.extract.bedgraph",
    "UM15.extract.bedgraph"
  ),
  package = "InPAS"
  )
  tags <- c("Baf3", "UM15")
  metadata <- data.frame(
    tag = tags,
    condition = c("Baf3", "UM15"),
    bedgraph_file = bedgraphs
  )
  outdir <- tempdir()
  write.table(metadata,
    file = file.path(outdir, "metadata.txt"),
    sep = "\t", quote = FALSE, row.names = FALSE
  )

  sqlite_db <- setup_sqlitedb(metadata = file.path(
    outdir,
    "metadata.txt"
  ), outdir)
  addLockName(filename = tempfile())
  coverage <- list()
  for (i in seq_along(bedgraphs)) {
    coverage[[tags[i]]] <- get_ssRleCov(
      bedgraph = bedgraphs[i],
      tag = tags[i],
      genome = genome,
      sqlite_db = sqlite_db,
      outdir = outdir,
      chr2exclude = "chrM"
    )
  }

  data4CPsSearch <- setup_CPsSearch(sqlite_db,
    genome,
    chr.utr3 = utr3[["chr6"]],
    seqname = "chr6",
    background = "10K",
    TxDb = TxDb,

```

```
hugeData = TRUE,
outdir = outdir,
minZ = 2,
cutStart = 10,
MINSIZE = 10,
coverage_threshold = 5
)
## polyA_PWM
load(system.file("extdata", "polyA.rda", package = "InPAS"))

## load the Naive Bayes classifier model from the cleanUpdTSeq package
library(cleanUpdTSeq)
data(classifier)

CPs <- search_CPs(
  seqname = "chr6",
  sqlite_db = sqlite_db,
  genome = genome,
  MINSIZE = 10,
  window_size = 100,
  search_point_START = 50,
  search_point_END = NA,
  cutEnd = 0,
  adjust_distal_polyA_end = TRUE,
  long_coverage_threshold = 2,
  PolyA_PWM = pwm,
  classifier = classifier,
  classifier_cutoff = 0.8,
  shift_range = 100,
  step = 5,
  outdir = outdir
)
utr3_cds_cov <- get_regionCov(
  chr.utr3 = utr3[["chr6"]],
  sqlite_db,
  outdir,
  phmm = FALSE
)
eSet <- get_UTR3eSet(sqlite_db,
  normalize = "none",
  singleSample = FALSE
)
test_out <- test_dPDUI(
  eset = eSet,
  method = "fisher.exact",
  normalize = "none",
  sqlite_db = sqlite_db
)
}
```

InPAS *A package for identifying novel Alternative PolyAdenylation Sites (PAS) based on RNA-seq data*

Description

The InPAS package provides three categories of important functions: parse_TxDb, extract_UTR3Anno, get_ssRleCov, assemble_allCov, get_UTR3eSet, test_dPDUI, run_singleSampleAnalysis, run_singleGroupAnalysis, run_limmaAnalysis, filter_testOut, get_usage4plot, setup_GSEA, run_coverageQC

functions for retrieving 3' UTR annotation

parse_TxDb, extract_UTR3Anno, get_lastCDSUTR3

functions for processing read coverage data

assemble_allCov, get_ssRleCov, run_coverageQC, setup_parCPsSearch

functions for alternative polyadenylation site analysis

test_dPDUI, run_singleSampleAnalysis, run_singleGroupAnalysis, run_limmaAnalysis, filter_testOut, get_usage4plot

parse_TxDb *Extract gene models from a TxDb object*

Description

Extract gene models from a TxDb object and annotate last 3' UTR exons and the last CDSs

Usage

```
parse_TxDb(  
  sqlite_db = NULL,  
  TxDb = getInPASTxDb(),  
  edb = getInPASEnsDb(),  
  genome = getInPASGenome(),  
  chr2exclude = getChr2Exclude(),  
  outdir = getInPASOutputDirectory()  
)
```

Arguments

sqlite_db	A path to the SQLite database for InPAS, i.e. the output of <code>setup_sqlitedb()</code> . It can be NULL.
TxDb	An object of <code>GenomicFeatures::TxDb</code>
edb	An object of <code>ensemldb::EnsDb</code>
genome	An object of <code>BSgenome::BSgenome</code>
chr2exclude	A character vector, NA or NULL, specifying chromosomes or scaffolds to be excluded for InPAS analysis. chrM and alternative scaffolds representing different haplotypes should be excluded.
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.

Details

A good practice is to perform read alignment using a reference genome from Ensembl/GenCode including only the primary assembly and build a TxDb using the GTF/GFF files downloaded from the same source as the reference genome, such as BioMart/Ensembl/GenCode. For instruction, see Vignette of the GenomicFeatures. The UCSC reference genomes and their annotation can be very cumbersome.

Value

A `GenomicRanges::GRanges` object for gene models

Author(s)

Haibo Liu

Examples

```
library("EnsDb.Hsapiens.v86")
library("BSgenome.Hsapiens.UCSC.hg19")
library("GenomicFeatures")

## set a sqlite database
bedgraphs <- system.file("extdata", c(
  "Baf3.extract.bedgraph",
  "UM15.extract.bedgraph"
),
package = "InPAS"
)
tags <- c("Baf3", "UM15")
metadata <- data.frame(
  tag = tags,
  condition = c("Baf3", "UM15"),
  bedgraph_file = bedgraphs
)
outdir <- tempdir()
write.table(metadata,
```

```

    file = file.path(outdir, "metadata.txt"),
    sep = "\t", quote = FALSE, row.names = FALSE
  )
  sqlite_db <- setup_sqlitedb(
    metadata =
      file.path(outdir, "metadata.txt"),
    outdir
  )

  samplefile <- system.file("extdata",
    "hg19_knownGene_sample.sqlite",
    package = "GenomicFeatures"
  )
  TxDb <- loadDb(samplefile)
  edb <- EnsDb.Hsapiens.v86
  genome <- BSgenome.Hsapiens.UCSC.hg19
  seqnames <- seqnames(BSgenome.Hsapiens.UCSC.hg19)
  chr2exclude <- c(
    "chrM", "chrMT",
    seqnames[grepl("(hap\\d+|fix|alt)$",
      seqnames,
      perl = TRUE
    )]
  )
  parsed_Txdb <- parse_Txdb(sqlite_db, TxDb, edb, genome,
    chr2exclude = chr2exclude
  )

```

plot_utr3Usage

Visualize the dPDUI events using ggplot2

Description

Visualize the dPDUI events by plotting the MSE, and total coverage per group along 3' UTR regions with dPDUI using `ggplot2::geom_line()`.

Usage

```
plot_utr3Usage(usage_data, vline_color = "purple", vline_type = "dashed")
```

Arguments

usage_data	An object of <code>GenomicRanges::GRanges</code> , an output from <code>get_usage4plot()</code> .
vline_color	color for vertical line showing position of predicated proximal CP site. Default, purple.
vline_type	line type for vertical line showing position of predicated proximal CP site. Default, dashed. See <code>ggplot2::linetype</code> .

Value

A ggplot object for refined plotting

Author(s)

Haibo Liu

See Also

For example, see [get_usage4plot\(\)](#).

run_coverageQC

Quality control on read coverage over gene bodies and 3UTRs

Description

Calculate coverage over gene bodies and 3UTRs. This function is used for quality control of the coverage. The coverage rate can show the complexity of RNA-seq library.

Usage

```
run_coverageQC(
  sqlite_db,
  TxDb = getInPASTxDb(),
  edb = getInPASEnsDb(),
  genome = getInPASGenome(),
  cutoff_readsNum = 1,
  cutoff_expdGene_cvgRate = 0.1,
  cutoff_expdGene_sampleRate = 0.5,
  chr2exclude = getChr2Exclude(),
  which = NULL,
  future.chunk.size = 1,
  ...
)
```

Arguments

sqlite_db	A path to the SQLite database for InPAS, i.e. the output of setup_sqlitedb() .
TxDb	An object of GenomicFeatures::TxDb
edb	An object of ensembladb::EnsDb
genome	An object of BSgenome::BSgenome
cutoff_readsNum	cutoff reads number. If the coverage in the location is greater than cutoff_readsNum, the location will be treated as covered by signal

<code>cutoff_expGene_cvRate</code>	<code>cutoff_expGene_cvRate</code> and <code>cutoff_expGene_sampleRate</code> are the parameters used to calculate which gene is expressed in all input dataset. <code>cutoff_expGene_cvRate</code> set the cutoff value for the coverage rate of each gene; <code>cutoff_expGene_sampleRate</code> set the cutoff value for ratio of numbers of expressed and all samples for each gene. for example, by default, <code>cutoff_expGene_cvRate=0.1</code> and <code>cutoff_expGene_sampleRate=0.5</code> , suppose there are 4 samples, for one gene, if the coverage rates by base are: 0.05, 0.12, 0.2, 0.17, this gene will be count as expressed gene because $\text{mean}(c(0.05, 0.12, 0.2, 0.17)) > \text{cutoff_expGene_cvRate}$ if the coverage rates by base are: 0.05, 0.12, 0.07, 0.17, this gene will be count as un-expressed gene because $\text{mean}(c(0.05, 0.12, 0.07, 0.17)) > \text{cutoff_expGene_cvRate}$ and $\text{mean}(c(0.05, 0.12, 0.07, 0.17)) \leq \text{cutoff_expGene_sampleRate}$
<code>cutoff_expGene_sampleRate</code>	See <code>cutoff_expGene_cvRate</code>
<code>chr2exclude</code>	A character vector, NA or NULL, specifying chromosomes or scaffolds to be excluded for InPAS analysis. chrM and alternative scaffolds representing different haplotypes should be excluded.
<code>which</code>	an object of <code>GenomicRanges::GRanges</code> or NULL. If it is not NULL, only the exons overlapping the given ranges are used. For fast data quality control, set <code>which</code> to <code>Granges</code> for one or a few large chromosomes.
<code>future.chunk.size</code>	The average number of elements per future ("chunk"). If Inf, then all elements are processed in a single future. If NULL, then argument <code>future.scheduling = 1</code> is used by default. Users can set <code>future.chunk.size = total number of elements/number of cores</code> set for the backend. See the <code>future.apply</code> package for details.
<code>...</code>	Not used yet

Value

A data frame as described below.

gene.coverage.rate coverage per base for all genes

expressed.gene.coverage.rate coverage per base for expressed genes

UTR3.coverage.rate coverage per base for all 3' UTRs

UTR3.expressed.gene.subset.coverage.rate coverage per base for 3' UTRs of expressed genes

rownames the names of coverage

Author(s)

Jianhong Ou, Haibo Liu

Examples

```
if (interactive()) {
  library("BSgenome.Mmusculus.UCSC.mm10")
  library("TxDb.Mmusculus.UCSC.mm10.knownGene")
}
```

```

library("EnsDb.Mmusculus.v79")

genome <- BSgenome.Mmusculus.UCSC.mm10
TxDb <- TxDb.Mmusculus.UCSC.mm10.knownGene
edb <- EnsDb.Mmusculus.v79

bedgraphs <- system.file("extdata", c(
  "Baf3.extract.bedgraph",
  "UM15.extract.bedgraph"
),
package = "InPAS"
)
tags <- c("Baf3", "UM15")
metadata <- data.frame(
  tag = tags,
  condition = c("Baf3", "UM15"),
  bedgraph_file = bedgraphs
)
outdir <- tempdir()
write.table(metadata,
  file = file.path(outdir, "metadata.txt"),
  sep = "\t", quote = FALSE, row.names = FALSE
)

sqlite_db <- setup_sqlitedb(
  metadata = file.path(
    outdir,
    "metadata.txt"
  ),
  outdir
)
tx <- parse_TxDb(
  sqlite_db = sqlite_db,
  TxDb = TxDb,
  edb = edb,
  genome = genome,
  outdir = outdir,
  chr2exclude = "chrM"
)
addLockName(filename = tempfile())
coverage <- list()
for (i in seq_along(bedgraphs)) {
  coverage[[tags[i]]] <- get_ssRleCov(
    bedgraph = bedgraphs[i],
    tag = tags[i],
    genome = genome,
    sqlite_db = sqlite_db,
    outdir = outdir,
    chr2exclude = "chrM"
  )
}
chr_coverage <- assemble_allCov(sqlite_db,
  seqname = "chr6",

```

```

    outdir,
    genome
  )
run_coverageQC(sqlite_db, TxDb, edb, genome,
  chr2exclude = "chrM",
  which = GRanges("chr6",
    ranges = IRanges(98013000, 140678000)
  )
)
}

```

search_CPs

Estimate the CP sites for UTRs on a given chromosome

Description

Estimate the CP sites for UTRs on a given chromosome

Usage

```

search_CPs(
  seqname,
  sqlite_db,
  genome = getInPASGenome(),
  MINSIZE = 10,
  window_size = 200,
  search_point_START = 100,
  search_point_END = NA,
  cutEnd = NA,
  filter.last = TRUE,
  adjust_distal_polyA_end = FALSE,
  long_coverage_threshold = 2,
  PolyA_PWM = NA,
  classifier = NA,
  classifier_cutoff = 0.8,
  shift_range = 100,
  step = 2,
  outdir = getInPASOutputDirectory(),
  silence = FALSE,
  cluster_type = c("interactive", "multicore", "torque", "slurm", "sge", "lsf",
    "openlava", "socket"),
  template_file = NULL,
  mc.cores = 1,
  future.chunk.size = 50,
  resources = list(walltime = 3600 * 8, ncpus = 4, mpp = 1024 * 4, queue = "long",
    memory = 4 * 4 * 1024),
  DIST2ANNOAPAP = 500,
  DIST2END = 1000,

```

```

    output.all = FALSE
)

```

Arguments

seqname	A character(1) vector, specifying a chromosome/scaffold name
sqlite_db	A path to the SQLite database for InPAS, i.e. the output of <code>setup_sqlitedb()</code> .
genome	A <code>BSgenome::BSgenome</code> object
MINSIZE	A integer(1) vector, specifying the minimal length in bp of a short/proximal 3' UTR. Default, 10
window_size	An integer(1) vector, the window size for novel distal or proximal CP site searching. default: 200.
search_point_START	A integer(1) vector, starting point relative to the 5' extremity of 3' UTRs for searching for proximal CP sites
search_point_END	A integer(1) vector, ending point relative to the 3' extremity of 3' UTRs for searching for proximal CP sites
cutEnd	An integer(1) vector a numeric(1) vector. What percentage or how many nucleotides should be removed from 5' extremities before searching for proximal CP sites? It can be a decimal between 0, and 1, or an integer greater than 1. 0.1 means 10 percent, 25 means cut first 25 bases
filter.last	A logical(1), whether to filter out the last valley, which is likely the 3' end of the longer 3' UTR if no novel distal CP site is detected and the 3' end excluded by setting cutEnd/search_point_END is small.
adjust_distal_polyA_end	A logical(1) vector. If true, distal CP sites are subject to adjustment by the Naive Bayes classifier from the cleanUpdTSeq::cleanUpdTSeq-package
long_coverage_threshold	An integer(1) vector, specifying the cutoff threshold of coverage for the terminal of long form 3' UTRs. If the coverage of first 100 nucleotides is lower than coverage_threshold, that transcript will be not considered for further analysis. Default, 2.
PolyA_PWM	An R object for a position weight matrix (PWM) for a hexamer polyadenylation signal (PAS), such as AAUAAA.
classifier	An R object for Naive Bayes classifier model, like the one in the cleanUpdTSeq package.
classifier_cutoff	A numeric(1) vector. A cutoff of probability that a site is classified as true CP sites. The value should be between 0.5 and 1. Default, 0.8.
shift_range	An integer(1) vector, specifying a shift range for adjusting the proximal and distal CP sites. Default, 50. It determines the range flanking the candidate CP sites to search the most likely real CP sites.
step	An integer (1) vector, specifying the step size used for adjusting the proximal or distal CP sites using the Naive Bayes classifier from the cleanUpdTSeq package. Default 1. It can be in the range of 1 to 10.

<code>outdir</code>	A character(1) vector, a path with write permission for storing the CP sites. If it doesn't exist, it will be created.
<code>silence</code>	A logical(1), indicating whether progress is reported or not. By default, FALSE
<code>cluster_type</code>	A character (1) vector, indicating the type of cluster job management systems. Options are "interactive", "multicore", "torque", "slurm", "sge", "lsf", "openlava", and "socket". see batchtools vignette
<code>template_file</code>	A character(1) vector, indicating the template file for job submitting scripts when <code>cluster_type</code> is set to "torque", "slurm", "sge", "lsf", or "openlava".
<code>mc.cores</code>	An integer(1), number of cores for making multicore clusters or socket clusters using batchtools , and for <code>parallel::mclapply()</code>
<code>future.chunk.size</code>	The average number of elements per future ("chunk"). If Inf, then all elements are processed in a single future. If NULL, then argument <code>future.scheduling = 1</code> is used by default. Users can set <code>future.chunk.size = total number of elements/number of cores set for the backend</code> . See the <code>future.apply</code> package for details. Default, 50. This parameter is used to split the candidate 3' UTRs for alternative SP sites search.
<code>resources</code>	A named list specifying the computing resources when <code>cluster_type</code> is set to "torque", "slurm", "sge", "lsf", or "openlava". See batchtools vignette
<code>DIST2ANNOAPAP</code>	An integer, specifying a cutoff for annotate MSE valleys with known proximal APAs in a given downstream distance. Default is 500.
<code>DIST2END</code>	An integer, specifying a cutoff of the distance between last valley and the end of the 3' UTR (where MSE of the last base is calculated). If the last valley is closer to the end than the specified distance, it will not be considered because it is very likely due to RNA coverage decay at the end of mRNA. Default is 1200. User can consider a value between 1000 and 1500, depending on the library preparation procedures: RNA fragmentation and size selection.
<code>output.all</code>	A logical(1), indicating whether to output entries with only single CP site for a 3' UTR. Default, FALSE.

Value

An object of `GenomicRanges::GRanges` containing distal and proximal CP site information for each 3' UTR if detected.

Author(s)

Jianhong Ou, Haibo Liu

See Also

[search_proximalCPs\(\)](#), [adjust_proximalCPs\(\)](#), [adjust_proximalCPsByPWM\(\)](#), [adjust_proximalCPsByNBC\(\)](#), [get_PAScore\(\)](#), [get_PAScore2\(\)](#)

Examples

```

if (interactive()) {
  library(BSgenome.Mmusculus.UCSC.mm10)
  library(TxDb.Mmusculus.UCSC.mm10.knownGene)
  genome <- BSgenome.Mmusculus.UCSC.mm10
  TxDb <- TxDb.Mmusculus.UCSC.mm10.knownGene

  ## load UTR3 annotation and convert it into a GRangesList
  data(utr3.mm10)
  utr3 <- split(utr3.mm10, seqnames(utr3.mm10), drop = TRUE)

  bedgraphs <- system.file("extdata", c(
    "Baf3.extract.bedgraph",
    "UM15.extract.bedgraph"
  ),
  package = "InPAS"
  )
  tags <- c("Baf3", "UM15")
  metadata <- data.frame(
    tag = tags,
    condition = c("Baf3", "UM15"),
    bedgraph_file = bedgraphs
  )
  outdir <- tempdir()
  write.table(metadata,
    file = file.path(outdir, "metadata.txt"),
    sep = "\t", quote = FALSE, row.names = FALSE
  )

  sqlite_db <- setup_sqlitedb(metadata = file.path(
    outdir,
    "metadata.txt"
  ), outdir)
  addLockName(filename = tempfile())
  coverage <- list()
  for (i in seq_along(bedgraphs)) {
    coverage[[tags[i]]] <- get_ssRleCov(
      bedgraph = bedgraphs[i],
      tag = tags[i],
      genome = genome,
      sqlite_db = sqlite_db,
      outdir = outdir,
      chr2exclude = "chrM"
    )
  }
}
data4CPsSearch <- setup_CPsSearch(sqlite_db,
  genome,
  chr.utr3 = utr3[["chr6"]],
  seqname = "chr6",
  background = "10K",
  TxDb = TxDb,
  hugeData = TRUE,

```

```
    outdir = outdir,
    minZ = 2,
    cutStart = 10,
    MINSIZE = 10,
    coverage_threshold = 5
  )
  ## polyA_PWM
  load(system.file("extdata", "polyA.rda", package = "InPAS"))

  ## load the Naive Bayes classifier model from the cleanUpdTSeq package
  library(cleanUpdTSeq)
  data(classifier)
  ## the following setting just for demo.
  if (.Platform$OS.type == "windows") {
    plan(multisession)
  } else {
    plan(multicore)
  }
  CPs <- search_CPs(
    seqname = "chr6",
    sqlite_db = sqlite_db,
    genome = genome,
    MINSIZE = 10,
    window_size = 100,
    search_point_START = 50,
    search_point_END = NA,
    cutEnd = 0,
    filter.last = TRUE,
    adjust_distal_polyA_end = TRUE,
    long_coverage_threshold = 2,
    PolyA_PWM = pwm,
    classifier = classifier,
    classifier_cutoff = 0.8,
    shift_range = 100,
    step = 5,
    outdir = outdir
  )
}
```

setup_CPsSearch

prepare data for predicting cleavage and polyadenylation (CP) sites

Description

prepare data for predicting cleavage and polyadenylation (CP) sites

Usage

```
setup_CPsSearch(
  sqlite_db,
```

```

genome = getInPASGenome(),
chr.utr3,
seqname,
background = c("same_as_long_coverage_threshold", "1K", "5K", "10K", "50K"),
TxDb = getInPASTxDb(),
hugeData = TRUE,
outdir = getInPASOutputDirectory(),
silence = FALSE,
minZ = 2,
cutStart = 10,
MINSIZE = 10,
coverage_threshold = 5
)

```

Arguments

sqlite_db	A path to the SQLite database for InPAS, i.e. the output of <code>setup_sqlitedb()</code> .
genome	An object of <code>BSgenome::BSgenome</code>
chr.utr3	An object of <code>GenomicRanges::GRanges</code> , an element of the output of <code>extract_UTR3Anno()</code>
seqname	A character(1), the name of a chromosome/scaffold
background	A character(1) vector, the range for calculating cutoff threshold of local background. It can be "same_as_long_coverage_threshold", "1K", "5K", "10K", or "50K".
TxDb	an object of <code>GenomicFeatures::TxDb</code>
hugeData	A logical(1) vector, indicating whether it is huge data
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
silence	report progress or not. By default it doesn't report progress.
minZ	A numeric(1), a Z score cutoff value
cutStart	An integer(1) vector a numeric(1) vector. What percentage or how many nucleotides should be removed from 5' extremities before searching for CP sites? It can be a decimal between 0, and 1, or an integer greater than 1. 0.1 means 10 percent, 25 means cut first 25 bases
MINSIZE	A integer(1) vector, specifying the minimal length in bp of a short/proximal 3' UTR. Default, 10
coverage_threshold	An integer(1) vector, specifying the cutoff threshold of coverage for first 100 nucleotides. If the coverage of first 100 nucleotides is lower than coverage_threshold, that transcript will be not considered for further analysis. Default, 5.

Value

A file storing a list as described below:

background The type of methods for background coverage calculation

z2s Z-score cutoff thresholds for each 3' UTRs

- depth.weight** A named vector containing depth weight
- chr.cov.merge** A matrix storing condition/sample-specific coverage for 3' UTR and next.exon.gap (if exist)
- conn_next_utr3** A logical vector, indicating whether a 3'UTR has a convergent 3' UTR of its downstream transcript
- chr.utr3** A GRangesList, storing extracted 3' UTR annotation of transcript on a given chr

Author(s)

Jianhong Ou, Haibo Liu

Examples

```
if (interactive()) {
  library(BSgenome.Mmusculus.UCSC.mm10)
  library("TxDb.Mmusculus.UCSC.mm10.knownGene")
  genome <- BSgenome.Mmusculus.UCSC.mm10
  TxDb <- TxDb.Mmusculus.UCSC.mm10.knownGene

  ## load UTR3 annotation and convert it into a GRangesList
  data(utr3.mm10)
  utr3 <- split(utr3.mm10, seqnames(utr3.mm10), drop = TRUE)

  bedgraphs <- system.file("extdata", c(
    "Baf3.extract.bedgraph",
    "UM15.extract.bedgraph"
  ),
  package = "InPAS"
  )
  tags <- c("Baf3", "UM15")
  metadata <- data.frame(
    tag = tags,
    condition = c("Baf3", "UM15"),
    bedgraph_file = bedgraphs
  )
  outdir <- tempdir()
  write.table(metadata,
    file = file.path(outdir, "metadata.txt"),
    sep = "\t", quote = FALSE, row.names = FALSE
  )

  sqlite_db <- setup_sqlitedb(
    metadata = file.path(
      outdir,
      "metadata.txt"
    ),
    outdir
  )
  addLockName(filename = tempfile())
  coverage <- list()
  for (i in seq_along(bedgraphs)) {
```

```

coverage[[tags[i]]] <- get_ssRleCov(
  bedgraph = bedgraphs[i],
  tag = tags[i],
  genome = genome,
  sqlite_db = sqlite_db,
  outdir = outdir,
  chr2exclude = "chrM"
)
}
data4CPsitesSearch <- setup_CPsitesSearch(sqlite_db,
  genome,
  chr.utr3 = utr3[["chr6"]],
  seqname = "chr6",
  background = "10K",
  TxDb = TxDb,
  hugeData = TRUE,
  outdir = outdir
)
}

```

setup_GSEA

prepare files for GSEA analysis

Description

output the log₂ transformed delta PDUI txt file, chip file, rank file and phynotype label file for GSEA analysis

Usage

```

setup_GSEA(
  eset,
  groupList,
  outdir = getInPASOutputDirectory(),
  preranked = TRUE,
  rankBy = c("logFC", "P.value"),
  rnkFilename = "InPAS.rnk",
  chipFilename = "InPAS.chip",
  dataFilename = "dPDUI.txt",
  PhenFilename = "group.cls"
)

```

Arguments

eset	A UTR3eSet object, output of test_dPDUI()
groupList	A list of grouped sample tag names, with the group names as the list's name, such as <code>list(groupA = c("sample_1", "sample_2", "sample_3"), groupB = c("sample_4", "sample_5", "sample_6"))</code>

outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
preranked	A logical(1) vector, out preranked or not
rankBy	A character(1) vector, indicating how the gene list is ranked. It can be "logFC" or "P.value".
rnkFilename	A character(1) vector, specifying a filename for the preranked file
chipFilename	A character(1) vector, specifying a filename for the chip file
dataFilename	A character(1) vector, specifying a filename for the dataset file
PhenFilename	A character(1) vector, specifying a filename for the file containing samples' phenotype labels

Author(s)

Jianhong Ou, Haibo Liu

See Also

data formats for GSEA. https://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/Data_formats

Examples

```
library(limma)
path <- system.file("extdata", package = "InPAS")
load(file.path(path, "eset.MAQC.rda"))
tags <- colnames(eset@PDUI)
g <- factor(gsub("\\..*$", "", tags))
design <- model.matrix(~ -1 + g)
colnames(design) <- c("Brain", "UHR")
contrast.matrix <- makeContrasts(
  contrasts = "Brain-UHR",
  levels = design
)
res <- test_dPDI(
  eset = eset,
  method = "limma",
  normalize = "none",
  design = design,
  contrast.matrix = contrast.matrix
)
gp1 <- c("Brain.auto", "Brain.phiX")
gp2 <- c("UHR.auto", "UHR.phiX")
groupList <- list(Brain = gp1, UHR = gp2)
setup_GSEA(res,
  groupList = groupList,
  outdir = tempdir(),
  preranked = TRUE,
  rankBy = "P.value"
)
```

setup_parCPsSearch	<i>Prepare data for predicting cleavage and polyadenylation (CP) sites using parallel computing</i>
--------------------	-----------------------------------------------------------------------------------------------------

Description

Prepare data for predicting cleavage and polyadenylation (CP) sites using parallel computing

Usage

```
setup_parCPsSearch(
  sqlite_db,
  genome = getInPASGenome(),
  utr3,
  seqnames,
  background = c("same_as_long_coverage_threshold", "1K", "5K", "10K", "50K"),
  TxDb = getInPASTxDb(),
  future.chunk.size = 1,
  chr2exclude = getChr2Exclude(),
  hugeData = TRUE,
  outdir = getInPASOutputDirectory(),
  silence = FALSE,
  minZ = 2,
  cutStart = 10,
  MINSIZE = 10,
  coverage_threshold = 5
)
```

Arguments

sqlite_db	A path to the SQLite database for InPAS, i.e. the output of <code>setup_sqlitedb()</code> .
genome	An object of <code>BSgenome::BSgenome</code>
utr3	An object of <code>GenomicRanges::GRangesList</code> , the output of <code>extract_UTR3Anno()</code>
seqnames	A character(1), the names of all chromosomes/scaffolds with both coverage and 3' UTR annotation. Users can get this by calling the <code>get_chromosomes()</code> .
background	A character(1) vector, the range for calculating cutoff threshold of local background. It can be "same_as_long_coverage_threshold", "1K", "5K", "10K", or "50K".
TxDb	an object of <code>GenomicFeatures::TxDb</code>
future.chunk.size	The average number of elements per future ("chunk"). If Inf, then all elements are processed in a single future. If NULL, then argument <code>future.scheduling = 1</code> is used by default. Users can set <code>future.chunk.size = total number of elements/number of cores set for the backend</code> . See the <code>future.apply</code> package for details.

chr2exclude	A character vector, NA or NULL, specifying chromosomes or scaffolds to be excluded for InPAS analysis. chrM and alternative scaffolds representing different haplotypes should be excluded.
hugeData	A logical(1) vector, indicating whether it is huge data
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
silence	report progress or not. By default it doesn't report progress.
minZ	A numeric(1), a Z score cutoff value
cutStart	An integer(1) vector a numeric(1) vector. What percentage or how many nucleotides should be removed from 5' extremities before searching for CP sites? It can be a decimal between 0, and 1, or an integer greater than 1. 0.1 means 10 percent, 25 means cut first 25 bases
MINSIZE	A integer(1) vector, specifying the minimal length in bp of a short/proximal 3' UTR. Default, 10
coverage_threshold	An integer(1) vector, specifying the cutoff threshold of coverage for first 100 nucleotides. If the coverage of first 100 nucleotides is lower than coverage_threshold, that transcript will be not considered for further analysis. Default, 5.

Value

A list of list as described below:

background The type of methods for background coverage calculation

z2s Z-score cutoff thresholds for each 3' UTRs

depth.weight A named vector containing depth weight

chr.cov.merge A list of matrice storing condition/sample- specific coverage for 3' UTR and next.exon.gap (if exist)

conn_next_utr3 A logical vector, indicating whether a 3'UTR has a convergent 3' UTR of its downstream transcript

chr.utr3 A GRangesList, storing extracted 3' UTR annotation of transcript on a given chr

Author(s)

Jianhong Ou, Haibo Liu

setup_sqllitedb *Create an SQLite database for storing metadata and paths to coverage files*

Description

Create an SQLite database with five tables, "metadata", "sample_coverage", "chromosome_coverage", "CPSites", and "utr3_coverage", for storing metadata (sample tag, condition, paths to bedgraph files, and sample total read coverage), sample-then-chromosome-oriented coverage files (sample tag, chromosome, paths to bedgraph files for each chromosome), and paths to chromosome-then-sample-oriented coverage files (chromosome, paths to bedgraph files for each chromosome), CP sites on each chromosome (chromosome, paths to cpsite files), read coverage for 3' UTR and last CDS regions on each chromosome (chromosome, paths to utr3 coverage file), respectively

Usage

```
setup_sqlitedb(metadata, outdir = getInPASOutputDirectory())
```

Arguments

metadata	A path to a tab-delimited file, with columns "tag", "condition", and "bedgraph_file", storing a unique name tag for each sample, a condition name for each sample, such as "treatment" and "control", and a path to the bedgraph file for each sample
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.

Value

A character(1) vector, the path to the SQLite database

Author(s)

Haibo Liu

Examples

```
if (interactive()) {
  bedgraphs <- system.file("extdata", c(
    "Baf3.extract.bedgraph",
    "UM15.extract.bedgraph"
  ),
  package = "InPAS"
  )
  tags <- c("Baf3", "UM15")
  metadata <- data.frame(
    tag = tags,
    condition = c("Baf3", "UM15"),
    bedgraph_file = bedgraphs
  )
  outdir <- tempdir()
  write.table(metadata,
    file = file.path(outdir, "metadata.txt"),
    sep = "\t", quote = FALSE, row.names = FALSE
  )
  sqlite_db <- setup_sqlitedb(
```

```
    metadata =
      file.path(outdir, "metadata.txt"),
    outdir
  )
}
```

set_globals*Set up global variables for an InPAS analysis*

Description

Set up global variables for an InPAS analysis

Usage

```
set_globals(
  genome = NULL,
  TxDb = NULL,
  EnsDb = NULL,
  outdir = NULL,
  chr2exclude = c("chrM", "MT", "Pltd", "chrPltd"),
  lockfile = tempfile(tmpdir = getInPASOutputDirectory())
)
```

Arguments

genome	An object BSgenome::BSgenome . To make things easy, we suggest users creating a BSgenome::BSgenome instance from the reference genome used for read alignment. For details, see the documentation of BSgenome::forgeBSgenomeDataPkg() .
TxDb	An object of GenomicFeatures::TxDb
EnsDb	An object of ensemblDb::EnsDb
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
chr2exclude	A character vector, NA or NULL, specifying chromosomes or scaffolds to be excluded for InPAS analysis. chrM and alternative scaffolds representing different haplotypes should be excluded.
lockfile	A character(1) vector, specifying a file name used for parallel writing to a SQLite database

test_dPDUI	<i>do test for dPDUI</i>
------------	--------------------------

Description

do test for dPDUI

Usage

```
test_dPDUI(
  eset,
  sqlite_db,
  outdir = getInPASOutputDirectory(),
  method = c("limma", "fisher.exact", "singleSample", "singleGroup"),
  normalize = c("none", "quantiles", "quantiles.robust", "mean", "median"),
  design,
  contrast.matrix,
  coef = 1,
  robust = FALSE,
  ...
)
```

Arguments

eset	An object of UTR3eSet . It is an output of get_UTR3eSet()
sqlite_db	A path to the SQLite database for InPAS, i.e. the output of setup_sqlitedb() .
outdir	A character(1) vector, a path with write permission for storing InPAS analysis results. If it doesn't exist, it will be created.
method	A character(1), indicating the method for testing dPDUI. It can be "limma", "fisher.exact", "singleSample", or "singleGroup"
normalize	A character(1), indicating the normalization method. It can be "none", "quantiles", "quantiles.robust", "mean", or "median"
design	a design matrix of the experiment, with rows corresponding to samples and columns to coefficients to be estimated. Defaults to the unit vector meaning that the samples are treated as replicates. see stats::model.matrix() . Required for limma-based analysis.
contrast.matrix	a numeric matrix with rows corresponding to coefficients in fit and columns containing contrasts. May be a vector if there is only one contrast. see limma::makeContrasts() . Required for limma-based analysis.
coef	column number or column name specifying which coefficient or contrast of the linear model is of interest. see more limma::topTable() . default value: 1
robust	A logical(1) vector, indicating whether the estimation of the empirical Bayes prior parameters should be robustified against outlier sample variances.
...	other arguments are passed to lmFit

Details

if method is "limma", design matrix and contrast is required. if method is "fisher.exact", gp1 and gp2 is required.

Value

An object of `UTR3eSet`, with the last element `testRes` containing the test results in a matrix.

Author(s)

Jianhong Ou, Haibo Liu

See Also

[run_singleSampleAnalysis\(\)](#), [run_singleGroupAnalysis\(\)](#), [run_fisherExactTest\(\)](#), [run_limmaAnalysis\(\)](#)

Examples

```
library(limma)
path <- system.file("extdata", package = "InPAS")
load(file.path(path, "eset.MAQC.rda"))
tags <- colnames(eset@PDUI)
g <- factor(gsub("\\..*$", "", tags))
design <- model.matrix(~ -1 + g)
colnames(design) <- c("Brain", "UHR")
contrast.matrix <- makeContrasts(
  contrasts = "Brain-UHR",
  levels = design
)
res <- test_dPDUI(
  eset = eset,
  sqlite_db,
  method = "limma",
  normalize = "none",
  design = design,
  contrast.matrix = contrast.matrix
)
```

utr3.mm10

Annotation of 3' UTRs for mouse (mm10)

Description

A dataset containing the annotation of the 3' UTRs of the mouse

Usage

utr3.mm10

Format

An object of [GenomicRanges::GRanges](#) with 7 metadata columns

feature feature type, utr3, CDS, next.exon.gap

annotatedProximalCP candidate proximal CPsites

exon exon ID

transcript transcript ID

gene gene ID

symbol gene symbol

truncated whether the 3' UTR is truncated

 UTR3eSet-class

UTR3eSet-class and its methods

Description

An object of class [UTR3eSet](#) representing the results of 3' UTR usage; methods for constructing, showing, getting and setting attributes of objects; methods for coercing object of other class to [UTR3eSet](#) objects.

Objects from the Class

Objects can be created by calls of the form `new("UTR3eSet", ...)`

Objects can be created by calls of the form `new("UTR3eSet", ...)`.

Slots

`usage` Object of class "GRanges"

`PDUI` Object of class "matrix"

`PDUI.log2` Object of class "matrix"

`short` Object of class "matrix"

`long` Object of class "matrix"

`signals` Object of class "list"

`testRes` Object of class "matrix"

UTR3eSet-class methods

`$` signature(x = "UTR3eSet"): ...

`$<-` signature(x = "UTR3eSet"): ...

`coerce` signature(from = "UTR3eSet", to = "ExpressionSet"): ...

`coerce` signature(from = "UTR3eSet", to = "GRanges"): ...

`show` signature(object = "UTR3eSet"): ...

Author(s)

Jianhong Ou

Jianhong Ou

See Also

[GRanges](#)

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