

Package ‘InPAS’

October 12, 2016

Type Package

Title Identification of Novel alternative PolyAdenylation Sites (PAS)

Version 1.4.4

Date 2016-06-20

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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 from RNAseq data. It leverages cleanUpdTSeq to fine tune identified APA sites.

biocViews RNASeq, Sequencing, AlternativeSplicing, Coverage, DifferentialSplicing, GeneRegulation, Transcription

License GPL (>= 2)

Lazyload yes

Imports AnnotationDbi, BSgenome, cleanUpdTSeq, Gviz, seqinr, preprocessCore, IRanges, GenomeInfoDb, depmixS4, limma, BiocParallel

Depends R (>= 3.1), methods, Biobase, GenomicRanges, GenomicFeatures, S4Vectors

Suggests RUnit, BiocGenerics, BiocStyle, BSgenome.Hsapiens.UCSC.hg19, BSgenome.Mmusculus.UCSC.mm10, org.Hs.eg.db, org.Mm.eg.db, TxDb.Hsapiens.UCSC.hg19.knownGene, TxDb.Mmusculus.UCSC.mm10.knownGene, rtracklayer, knitr

VignetteBuilder knitr

NeedsCompilation no

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Arguments

bedgraphs	The file names of bedgraphs generated by bedtools. eg: bedtools genomecov -bg -split -ibam \$bam -g mm10.size.txt > \$bedgraph
tags	the names for each input bedgraphs
genome	an object of BSgenome
hugeData	is this dataset consume too much memory? if it is TRUE, the coverage will be saved into tempfiles.
BPPARAM	An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation, or a list of BiocParallelParam instances, to be applied in sequence for nested calls to bplapply .
...	parameters can be passed into tempfile. This is useful when you submit huge dataset to cluster.

Value

return a list of coverage for each bedgraph files. For each item in the list, it is a list of coverage for each chromosome. And the chromosome must start from "chr".

Author(s)

Jianhong Ou

Examples

```
if(interactive()){
  library(BSgenome.Mmusculus.UCSC.mm10)
  path <- file.path(find.package("InPAS"), "extdata")
  bedgraphs <- file.path(path, "Baf3.extract.bedgraph")
  data(utr3.mm10)
  tags <- "Baf3"
  genome <- BSgenome.Mmusculus.UCSC.mm10
  coverage <-
    coverageFromBedGraph(bedgraphs, tags, genome, hugeData=FALSE)
}
```

covThreshold	<i>calculate the cutoff threshold of coverage</i>
--------------	---

Description

calculate the cutoff threshold of coverage for long form and short form

Usage

```
covThreshold(coverage, genome, txdb, utr3,
             chr="chr1", hugeData, groupList)
```

Arguments

coverage	coverage for each sample, output of coverageFromBedGraph
genome	an object of BSgenome
txdb	an object of TxDb
utr3	output of utr3Annotation
chr	chromosome to be used for calculation, default is "chr1"
hugeData	is this dataset consume too much memory? if it is TRUE, the coverage will be saved into tempfiles.
groupList	group list of tag names

Value

a numeric vector

Author(s)

Jianhong Ou

See Also

[CPsite_estimation](#)

CPsites	<i>predict the cleavage and polyadenylation(CP) site</i>
---------	--

Description

predict the alternative cleavage and polyadenylation (CP or APA) site.

Usage

```
CPsites(coverage, groupList=NULL, genome, utr3,
        window_size=100, search_point_START=50, search_point_END=NA,
        cutStart=window_size, cutEnd=0, adjust_distal_polyA_end=TRUE,
        coverage_threshold=5, long_coverage_threshold=2,
        background=c("same_as_long_coverage_threshold",
                    "1K", "5K", "10K", "50K"),
        txdb=NA,
        gcCompensation=NA, mappabilityCompensation=NA,
        FFT=FALSE, fft.sm.power=20,
        PolyA_PWM=NA, classifier=NA, classifier_cutoff=.8, shift_range=window_size,
        BPPARAM=NULL, tmpfolder=NULL, silence=TRUE)
```

Arguments

coverage	coverage for each sample, output of coverageFromBedGraph
groupList	group list of tag names
genome	an object of BSgenome
utr3	output of utr3Annotation
window_size	window size for noval distal position searching and adjusted polyA searching, default: 100
search_point_START	start point for searching
search_point_END	end point for searching
cutStart	how many nucleotides should be removed from the start before search, 0.1 means 10 percent, 25 means cut first 25.
cutEnd	how many nucleotides should be removed from the end before search, 0.1 means 10 percent.
adjust_distal_polyA_end	If true, adjust distal polyA end by cleanUpdTSeq
coverage_threshold	cutoff coverage threshold for first 100 nucleotides. If the coverage of first 100 nucleotides is lower than coverage_threshold, that transcript will be dropped.
long_coverage_threshold	cutoff threshold for coverage in the region of long form. If the coverage in the region of long form is less than long_coverage_threshold, that transcript will be dropped.
background	the range for calculating cutoff threshold of local background
txdb	an object of TxDb
gcCompensation	GC content compensation vector
mappabilityCompensation	mappability compensation vector
FFT	use Fast Fourier Transform Algorithm to smooth the data or not. default: FALSE
fft.sm.power	if FFT is TRUE, the frequency should be removed
PolyA_PWM	Position Weight Matrix of polyA
classifier	An object of class " PASclassifier "
classifier_cutoff	This is the cutoff used to assign whether a putative pA is true or false. This can be any floating point number between 0 and 1. For example, classifier_cutoff = 0.5 will assign an putative pA site with prob.1 > 0.5 to the True class (1), and any putative pA site with prob.1 <= 0.5 as False (0).
shift_range	the shift range for polyA site searching
BPPARAM	An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation, or a list of BiocParallelParam instances, to be applied in sequence for nested calls to bplapply .
tmpfolder	temp folder could save and reload the analysis data for resume analysis.
silence	report progress or not. default not report.

Value

return an object of GRanges contain the estimated CP sites.

Author(s)

Jianhong Ou

References

ref: Cheung MS, Down TA, Latorre I, Ahringer J. Systematic bias in high-throughput sequencing data and its correction by BEADS. *Nucleic Acids Res.* 2011 Aug;39(15):e103. doi: 10.1093/nar/gkr425. Epub 2011 Jun 6. PubMed PMID: 21646344; PubMed Central PMCID: PMC3159482.

mappability could be calculated by [GEM](<http://algorithms.cnag.cat/wiki/Man:gem-mappability>)

ref: Derrien T, Estelle J, Marco Sola S, Knowles DG, Raineri E, Guigo R, Ribeca P. Fast computation and applications of genome mappability. *PLoS One.* 2012;7(1):e30377. doi: 10.1371/journal.pone.0030377. Epub 2012 Jan 19. PubMed PMID: 22276185; PubMed Central PMCID: PMC3261895.

Examples

```
if(interactive()){
  library(BSgenome.Mmusculus.UCSC.mm10)
  path <- file.path(find.package("InPAS"), "extdata")
  bedgraphs <- file.path(path, "Baf3.extract.bedgraph")
  data(utr3.mm10)
  tags <- "Baf3"
  genome <- BSgenome.Mmusculus.UCSC.mm10
  coverage <-
    coverageFromBedGraph(bedgraphs, tags, genome, hugeData=FALSE)
  CP <- CPSites(coverage=coverage, gp1=tags, gp2=NULL, genome=genome,
    utr3=utr3.mm10, coverage_threshold=5, long_coverage_threshold=5)
}
```

CPsite_estimation *estimate the cpsites*

Description

estimate the cpsites for a giving chromosome

Usage

```
CPsite_estimation(chr.cov, utr3, MINSIZE, window_size, search_point_START,
  search_point_END, cutStart, cutEnd, adjust_distal_polyA_end,
  background, z2s, coverage_threshold, long_coverage_threshold,
  PolyA_PWM, classifier, classifier_cutoff, shift_range,
  depth.weight, genome,
  tmpfolder=NULL, silence=TRUE)
```

Arguments

chr.cov	coverage list for one chromosome
utr3	output of utr3Annotaion
MINSIZE	min size of short form
window_size	window size
search_point_START	search start point
search_point_END	search end point
cutStart	cut from start
cutEnd	cut from end
adjust_distal_polyA_end	adjust distal site or not
background	how to get the local background
z2s	output of zScoreThreshold
coverage_threshold	cutoff value for coverage
long_coverage_threshold	cutoff value for long form
PolyA_PWM	polyA PWM
classifier	classifier
classifier_cutoff	classifier cutoff
shift_range	shift range
depth.weight	output of depthWeight
genome	a BSgenome object
tmpfolder	temp folder could save and reload the analysis data for resume analysis.
silence	report progress or not. default not report.

Value

a data.frame

Author(s)

Jianhong Ou

See Also

[CPsites](#), [searchProximalCPs](#), [proximalAdj](#), [proximalAdjByPWM](#), [proximalAdjByCleanUpdTSeq](#), [PAScore](#), [PAScore2](#)

depthWeight	<i>calculate the depth weight for each example</i>
-------------	--

Description

calculate the depth weight for each example

Usage

```
depthWeight(coverage, hugeData, groupList=NULL)
```

Arguments

coverage	a list. output of coverageFromBedGraph
hugeData	is it a huge dataset?
groupList	group list for huge dataset

Value

a numeric vector with depth weight

Author(s)

Jianhong Ou

distalAdj	<i>adjust distal CP sites by cleanUpdTSeq</i>
-----------	---

Description

adjust distal CP sites by cleanUpdTSeq

Usage

```
distalAdj(distalCPs, classifier, classifier_cutoff, shift_range, genome)
```

Arguments

distalCPs	the output of searchDistalCPs
classifier	cleanUpdTSeq classifier
classifier_cutoff	cutoff value of the classifier
shift_range	the searching range for the better CP sites
genome	a BSgenome object

Value

a list could be input of [searchProximalCPs](#)

Author(s)

Jianhong Ou

See Also

[searchDistalCPs](#), [PAscore2](#)

filterRes

filter results

Description

filter results of [testUsage](#)

Usage

```
filterRes(res,
          gp1, gp2,
          background_coverage_threshold=2,
          P.Value_cutoff=0.05,
          adj.P.Val_cutoff=0.05,
          dPDUI_cutoff=0.3,
          PDUI_logFC_cutoff)
```

Arguments

res	output of testUsage
gp1	tag names involved in group 1
gp2	tag names involved in group 2
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

Author(s)

Jianhong Ou

See Also

[testUsage](#)

Examples

```
path <- file.path(find.package("InPAS"), "extdata")
load(file.path(path, "CPs.MAQC.rda"))
load(file.path(path, "coverage.MAQC.rda"))
library(BSgenome.Hsapiens.UCSC.hg19)
data(utr3.hg19)
res <- testUsage(CPsites=CPs,
                 coverage=coverage,
                 genome=BSgenome.Hsapiens.UCSC.hg19,
                 utr3=utr3.hg19,
                 method="fisher.exact",
                 gp1=c("Brain.auto", "Brain.phiX"),
                 gp2=c("UHR.auto", "UHR.phiX"))
filterRes(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)
```

fisher.exact.test *do fisher exact test for two group datasets*

Description

do fisher exact test for two group datasets

Usage

```
fisher.exact.test(UTR3eset, gp1, gp2)
```

Arguments

UTR3eset	output of getUTR3eSet
gp1	tag names of group 1
gp2	tag names of group 2

Value

a matrix of test results

Author(s)

Jianhong Ou

See Also

[singleSampleAnalyze](#), [singleGroupAnalyze](#), [limmaAnalyze](#)

Examples

```
path <- file.path(find.package("InPAS"), "extdata")
load(file.path(path, "eset.MAQC.rda"))
tags <- colnames(eset$PDUI.log2)
res <- fisher.exact.test(eset, gp1=tags[1:2], gp2=tags[3:4])
```

get.regions.coverage *claculate coverage for giving region*

Description

claculate coverage for giving region

Usage

```
get.regions.coverage(chr, utr3.regions.chr,
                     hugeData, coverage, phmm=FALSE)
```

Arguments

chr	chromosome
utr3.regions.chr	the GRanges of region to be extracted
hugeData	is it a huge dataset?
coverage	output of coverageFromBedGraph
phmm	prepare data for singleSample analysis?

Value

GRanges with coverage data

Author(s)

Jianhong Ou

getCov	<i>extract coverage from bedgraph file</i>
--------	--

Description

extract coverage from bedgraph file

Usage

```
getCov(bedgraph, genome, seqLen)
```

Arguments

bedgraph	bedGraph file names
genome	an object BSgenome
seqLen	lengthes of each chromosome

Value

a Rle object for a sample coverage

Author(s)

Jianhong Ou

See Also

[coverageFromBedGraph](#)

getUTR3eSet	<i>prepare dataset for test</i>
-------------	---------------------------------

Description

Generate a UTR3eSet object with PDUI infomation for statistic test

Usage

```
getUTR3eSet(CPsites, coverage, genome, utr3,  
            normalize=c("none", "quantiles", "quantiles.robust",  
                        "mean", "median"),  
            ...,  
            BPPARAM=NULL, singleSample=FALSE)
```

Arguments

CPsites	outputs of CPsites
coverage	coverage for each sample, outputs of coverageFromBedGraph
genome	an object of BSgenome
utr3	output of utr3Annotation
normalize	normalization method
...	parameter can be passed into normalize.quantiles.robust
BPPARAM	An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation, or a list of BiocParallelParam instances, to be applied in sequence for nested calls to bply .
singleSample	prepare data for singleSample analysis? default is FALSE

Value

An object of [UTR3eSet](#) which contains following elements:

usage: an 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

Examples

```
path <- file.path(find.package("InPAS"), "extdata")
load(file.path(path, "CPs.MAQC.rda"))
load(file.path(path, "coverage.MAQC.rda"))
library(BSgenome.Hsapiens.UCSC.hg19)
data(utr3.hg19)
getUTR3eSet(CPsites=CPs,
            coverage=coverage,
            genome=BSgenome.Hsapiens.UCSC.hg19,
            utr3=utr3.hg19)
```

getUTR3region	<i>extract long and short 3UTR region</i>
---------------	---

Description

extract long and short 3UTR region

Usage

```
getUTR3region(.grs)
```

Arguments

.grs output of CPsites

Value

GRanges with short form and long form

Author(s)

Jianhong Ou

inPAS	<i>do estimation of alternative polyadenylation and cleavage site in one step</i>
-------	---

Description

do estimation of alternative polyadenylation and cleavage site in one step

Usage

```
inPAS(bedgraphs, genome, utr3, txdb=NA,
      tags, hugeData=FALSE, ...,
      gp1, gp2,
      window_size=100,
      search_point_START=50, search_point_END=NA,
      cutStart=window_size, cutEnd=0,
      coverage_threshold=5, long_coverage_threshold=2,
      background=c("same_as_long_coverage_threshold",
                  "1K", "5K", "10K", "50K"),
      gcCompensation=NA, mappabilityCompensation=NA,
      FFT=FALSE, fft.sm.power=20,
```

```

adjust_distal_polyA_end=TRUE,
PolyA_PWM=NA, classifier=NA, classifier_cutoff=.8,
shift_range=window_size,

method=c("limma", "fisher.exact",
         "singleSample", "singleGroup"),
normalize=c("none", "quantiles", "quantiles.robust",
           "mean", "median"),
design, contrast.matrix, coef=1,

P.Value_cutoff=0.05,
adj.P.Val_cutoff=0.05,
dPDUI_cutoff=0.3,
PDUI_logFC_cutoff=0.59,

BPPARAM=NULL)

```

Arguments

bedgraphs	The file names of bedgraphs generated by bedtools. eg: bedtools genomecov -bg -split -ibam \$bam -g mm10.size.txt > \$bedgraph
genome	an object of BSgenome
utr3	output of utr3Annotation
txdb	an object of TxDb
tags	the names for each input bedgraphs
hugeData	is this dataset consume too much memory? if it is TRUE, the coverage will be saved into tempfiles.
...	parameters can be passed into tempfile. This is useful when you submit huge dataset to cluster.
gp1	tag names involved in group 1
gp2	tag names involved in group 2
window_size	window size for noval distal position searching and adjusted polyA searching, default: 100
search_point_START	start point for searching
search_point_END	end point for searching
cutStart	how many nucleotides should be removed from the start before search, 0.1 means 10 percent.
cutEnd	how many nucleotides should be removed from the end before search, 0.1 means 10 percent.
coverage_threshold	cutoff threshold for coverage in the region of short form
long_coverage_threshold	cutoff threshold for coverage in thre region of long form

background	the range for calculating cutoff threshold of local background
gcCompensation	GC content compensation vector
mappabilityCompensation	mappability compensation vector
FFT	use Fast Fourier Transform Algorithm to smooth the data or not. default: FALSE
fft.sm.power	if FFT is TRUE, the frequency should be removed
adjust_distal_polyA_end	If true, adjust distal polyA end by cleanUpdTSeq
PolyA_PWM	Position Weight Matrix of polyA
classifier	An object of class " PASclassifier "
classifier_cutoff	This is the cutoff used to assign whether a putative pA is true or false. This can be any floating point number between 0 and 1. For example, classifier_cutoff = 0.5 will assign an putative pA site with prob.1 > 0.5 to the True class (1), and any putative pA site with prob.1 <= 0.5 as False (0).
shift_range	the shift range for polyA site searching
method	test method. see singleSampleAnalyze , singleGroupAnalyze , fisher.exact.test , limmaAnalyze
normalize	normalization method
design	the design matrix of the experiment, with rows corresponding to arrays and columns to coefficients to be estimated. Defaults to the unit vector meaning that the arrays are treated as replicates. see model.matrix
contrast.matrix	numeric matrix with rows corresponding to coefficients in fit and columns containing contrasts. May be a vector if there is only one contrast. see makeContrasts
coef	column number or column name specifying which coefficient or contrast of the linear model is of interest. see more topTable . default value: 1
P.Value_cutoff	cutoff of P value
adj.P.Val_cutoff	cutoff value for adjusted p.value
dPDUI_cutoff	cutoff value for differential PAS(polyadenylation signal) usage index
PDUI_logFC_cutoff	cutoff value for log2 fold change of PAS(polyadenylation signal) usage index
BPPARAM	An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation, or a list of BiocParallelParam instances, to be applied in sequence for nested calls to bplapply .

Value

return an object of GRanges

Author(s)

Jianhong Ou

Examples

```

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

  path <- file.path(find.package("InPAS"), "extdata")
  bedgraphs <- file.path(path, "Baf3.extract.bedgraph")
  data(utr3.mm10)
  res <- inPAS(bedgraphs=bedgraphs, tags=c("Baf3"),
              genome=BSgenome.Mmusculus.UCSC.mm10,
              utr3=utr3.mm10, gp1="Baf3", gp2=NULL,
              txdb=TxDb.Mmusculus.UCSC.mm10.knownGene,
              search_point_START=200,
              short_coverage_threshold=15,
              long_coverage_threshold=3,
              cutStart=0, cutEnd=.2,
              hugeData=FALSE)
  res
}

```

lastCDSusage

extract coverage of last CDS exon region

Description

extract coverage of last CDS exon region

Usage

```
lastCDSusage(CDS, coverage, hugeData, BPPARAM=NULL, phmm=FALSE)
```

Arguments

CDS	GRanges object of CDS
coverage	output of coverageFromBedGraph
hugeData	is it a huge dataset?
BPPARAM	An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation, or a list of BiocParallelParam instances, to be applied in sequence for nested calls to bplapply .
phmm	prepare data for singleSample analysis?

Value

the average coverage of last CDS for each transcript

Author(s)

Jianhong Ou

limmaAnalyze	<i>use limma to analyze the PDUI</i>
--------------	--------------------------------------

Description

use limma to analyze the PDUI

Usage

```
limmaAnalyze(UTR3eset, design, contrast.matrix, coef=1, robust=FALSE, ...)
```

Arguments

UTR3eset	an UTR3eSet object
design	the design matrix of the experiment, with rows corresponding to arrays and columns to coefficients to be estimated. Defaults to the unit vector meaning that the arrays are treated as replicates. see model.matrix
contrast.matrix	numeric matrix with rows corresponding to coefficients in fit and columns containing contrasts. May be a vector if there is only one contrast. see makeContrasts
coef	column number or column name specifying which coefficient or contrast of the linear model is of interest. see more topTable . default value: 1
robust	logical, should the estimation of the empirical Bayes prior parameters be robustified against outlier sample variances?
...	other arguments are passed to <code>lmFit</code> .

Value

fit results of eBayes by limma. It is an object of class `MArrayLM` containing everything found in fit. see [eBayes](#)

Author(s)

Jianhong Ou

See Also

[singleSampleAnalyze](#), [singleGroupAnalyze](#), [fisher.exact.test](#)

Examples

```
library(limma)
path <- file.path(find.package("InPAS"), "extdata")
load(file.path(path, "eset.MAQC.rda"))
tags <- colnames(eset$PDUI.log2)
g <- factor(gsub("\\.*$", "", tags))
design <- model.matrix(~-1+g)
colnames(design) <- c("Brain", "UHR")
contrast.matrix <- makeContrasts(contrasts="Brain-UHR",levels=design)
res <- limmaAnalyze(eset, design, contrast.matrix)
head(res)
```

optimalSegmentation *calculate SSE*

Description

calculate SSE values

Usage

```
optimalSegmentation(.ele, search_point_START, search_point_END, n = 1, savedID = NA)
```

Arguments

.ele	3UTR coverage
search_point_START	start position to calculate
search_point_END	end position to calculate
n	the length of output
savedID	the proximal CPsites for noval distal events

Value

a list of SSE and idx

Author(s)

Jianhong Ou

PAscore	<i>calculate the CP score</i>
---------	-------------------------------

Description

calculate the CP score by PWM

Usage

```
PAscore(seqname, pos, str, idx, PWM, genome, ups = 50, dws = 50)
```

Arguments

seqname	sequence names
pos	genomic positions
str	strands
idx	offset position
PWM	polyA position weight matrix
genome	an object of BSgenome
ups	upstream base
dws	downstream base

Value

idx list after filter

Author(s)

Jianhong Ou

See Also

[PAscore2](#)

PAscore2	<i>calculate the CP score</i>
----------	-------------------------------

Description

calculate CP score by cleanUpdTSeq

Usage

```
PAscore2(seqname, pos, str, idx, idx.gp, genome, classifier, classifier_cutoff)
```

Arguments

seqname	sequence names
pos	genomic positions
str	strands
idx	offset postion
idx.gp	group number of the offset postion
genome	an object of BSgenome
classifier	a cleanUpdTSeq classifier
classifier_cutoff	classifier cutoff value

Value

a data.frame

Author(s)

Jianhong Ou

See Also

[PAscore](#)

polishCPs

polish the searching results of CP sites

Description

remove the multiple positions of CP sites for same 3UTRs and only keep the best CP sites for proximal and distal.

Usage

```
polishCPs(CPs)
```

Arguments

CPs output of [searchProximalCPs](#) or [proximalAdj](#)

Value

a matrix with columns: "fit_value", "Predicted_Proximal_APA", "Predicted_Distal_APA", "utr3start", "utr3end", "type"

Author(s)

Jianhong Ou

See Also

[CPsite_estimation](#), [searchProximalCPs](#), [proximalAdj](#), [proximalAdjByPWM](#), [proximalAdjByCleanUpdTSeq](#), [PAScore](#), [PAScore2](#)

prepare4GSEA

prepare the files for GSEA analysis

Description

output the log2 transformed delta PDUI txt file and chip file for GSEA analysis

Usage

```
prepare4GSEA(eset, groupList, Preranked=TRUE,  
             folder=".",  
             rnkFilename="InPAS.rnk",  
             chipFilename="InPAS.chip",  
             dataFilename="dPDUI.txt",  
             PhenFilename="group.cls")
```

Arguments

eset	a UTR3eSet object
groupList	group list of tag names
Preranked	logical value, out preranked or not
folder	output folder
rnkFilename	filename of preranked file
chipFilename	filename of chip
dataFilename	filename of dataset
PhenFilename	filename of Phenotype labels

Value

None

Author(s)

Jianhong Ou

Examples

```
file <- system.file("extdata", "eset.MAQC.rda", package="InPAS")
load(file)
gp1=c("Brain.auto", "Brain.phiX")
gp2=c("UHR.auto", "UHR.phiX")
groupList <- list(Brain=gp1, UHR=gp2)
prepare4GSEA(eset, groupList=groupList, Preranked=FALSE)
```

proximalAdj

adjust the proximal CP sites

Description

adjust the proximal CP sites by PolyA PWM and cleanUpdTSeq

Usage

```
proximalAdj(CPs, MINSIZE, PolyA_PWM, genome, classifier, classifier_cutoff,
            shift_range, search_point_START)
```


Arguments

<code>idx.list</code>	the offset of positions of CP sites
<code>cov_diff.list</code>	the SSE values
<code>seqnames</code>	sequence names
<code>starts</code>	starts
<code>strands</code>	strands
<code>genome</code>	a BSgenome object
<code>classifier</code>	cleanUpdTSeq classifier
<code>classifier_cutoff</code>	cutoff value of the classifier
<code>shift_range</code>	the searching range for the better CP sites
<code>search_point_START</code>	just in case there is no better CP sites

Details

the step for calculating is 10, can not do every base base it is really very slow.

Value

the offset of positions of CP sites after filter

Author(s)

Jianhong Ou

See Also

[proximalAdjByPWM](#), [proximalAdj](#), [PAscore2](#)

proximalAdjByPWM

adjust the proximal CP sites by PWM

Description

adjust the proximal CP sites by polyA Position Weight Metrix. It only need the PWM get match in upstream or downstream `shift_range` nr.

Usage

```
proximalAdjByPWM(idx, PolyA_PWM, seqnames, starts, strands, genome,  
                 shift_range, search_point_START)
```

Arguments

idx	the offset of positions of CP sites
PolyA_PWM	polyA PWM
seqnames	sequence names
starts	start position in the genome
strands	strands
genome	an BSgenome object
shift_range	the shift range of PWM hits
search_point_START	Not use

Details

the hits is searched by [matchPWM](#) and the cutoff is 70%

Value

the offset of positions of CP sites after filter

Author(s)

Jianhong Ou

See Also

[proximalAdjByCleanUpdTSeq](#), [proximalAdj](#), [PAscore](#)

removeUTR3__UTR3 *remove the candidates LIKE UTR3__UTR3*

Description

some of the results is from connected two UTR3. We want to remove them. However, the algorithm need to be improved.

Usage

```
removeUTR3__UTR3(x)
```

Arguments

x	the distal 3UTR coverage
---	--------------------------

Value

the 3UTR coverage after removing the next 3UTR

Author(s)

Jianhong Ou

searchDistalCPs	<i>search distal CP sites</i>
-----------------	-------------------------------

Description

search distal CP sites

Usage

```
searchDistalCPs(chr.cov.merge, conn_next_utr3,  
                curr_UTR, window_size,  
                depth.weight,  
                long_coverage_threshold,  
                background, z2s)
```

Arguments

chr.cov.merge	coverage of current chromosome
conn_next_utr3	joint to next 3UTR or not (used for removeUTR3__UTR3)
curr_UTR	GRanges of current 3UTR
window_size	window size
depth.weight	output of depthWeight
long_coverage_threshold	cutoff value for coverage of long form 3UTR
background	local background range
z2s	cut off background scores. see zScoreThrethold

Value

a list

Author(s)

Jianhong Ou

See Also[distalAdj](#), [PAscore2](#)

searchProximalCPs *search proximal CPsites*

Description

search proximal CPsites

Usage

```
searchProximalCPs(CPs, curr_UTR, window_size,  
                  MINSIZE, cutEnd,  
                  search_point_START,  
                  search_point_END)
```

Arguments

CPs	output of searchDistalCPs or distalAdj
curr_UTR	GRanges of current 3UTR
window_size	window size
MINSIZE	MINSIZE for short form
cutEnd	how many nucleotides should be removed from the end before search, 0.1 means 10 percent.
search_point_START	start point for searching
search_point_END	end point for searching

Value

a list

Author(s)

Jianhong Ou

See Also

[proximalAdj](#), [polishCPs](#), [proximalAdjByPWM](#), [proximalAdjByCleanUpdTSeq](#), [PAscore](#), [PAscore2](#)

seqLen *get sequence lengths*

Description

get sequence lengths from a BSgenome object

Usage

```
seqLen(genome)
```

Arguments

genome an object of [BSgenome](#)

Value

a numeric vector

Author(s)

Jianhong Ou

See Also

[seqlengths](#)

singleGroupAnalyze *do analysis for single group samples*

Description

do analysis for single group samples by anova test

Usage

```
singleGroupAnalyze(UTR3eset)
```

Arguments

UTR3eset must be the output of [getUTR3eSet](#)

Value

a matrix of test results

Author(s)

Jianhong Ou

See Also

[UTR3eSet](#), [getUTR3eSet](#)

Examples

```
path <- file.path(find.package("InPAS"), "extdata")
load(file.path(path, "eset.MAQC.rda"))
res <- singleGroupAnalyze(eset)
```

singleSampleAnalyze *do analysis for single sample*

Description

do analysis for single sample by a hidden Markov model

Usage

```
singleSampleAnalyze(UTR3eset)
```

Arguments

UTR3eset must be the output of [getUTR3eSet](#)

Details

the test will be performed by a two states hidden Markov model.

Value

a matrix of test results

Author(s)

Jianhong Ou

See Also

[UTR3eSet](#), [getUTR3eSet](#), [depmix](#)

Examples

```
path <- file.path(find.package("InPAS"), "extdata")
load(file.path(path, "eset.MAQC.rda"))
res <- singleSampleAnalyze(eset)
```

sortGR	<i>sort GRanges</i>
--------	---------------------

Description

sort a GRanges by chromosome and start position

Usage

```
sortGR(.ele)
```

Arguments

.ele an object of GRanges

Value

an sorted object of GRanges

Author(s)

Jianhong Ou

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

Description

do test for dPDUI

Usage

```
testUsage(CPsites, coverage, genome, utr3, BPPARAM=NULL,
          method=c("limma", "fisher.exact",
                  "singleSample", "singleGroup"),
          normalize=c("none", "quantiles", "quantiles.robust",
                    "mean", "median"),
          design, contrast.matrix, coef=1, robust=FALSE, ...,
          gp1, gp2)
```


Arguments

CPsites	outputs of CPsites
coverage	coverage for each sample, outputs of coverageFromBedGraph
genome	an object of BSgenome
utr3	output of utr3Annotation
BPPARAM	An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation, or a list of BiocParallelParam instances, to be applied in sequence for nested calls to bplapply .
method	test method. see singleSampleAnalyze , singleGroupAnalyze , fisher.exact.test , limmaAnalyze
normalize	normalization method
design	the design matrix of the experiment, with rows corresponding to arrays and columns to coefficients to be estimated. Defaults to the unit vector meaning that the arrays are treated as replicates. see model.matrix
contrast.matrix	numeric matrix with rows corresponding to coefficients in fit and columns containing contrasts. May be a vector if there is only one contrast. see makeContrasts
coef	column number or column name specifying which coefficient or contrast of the linear model is of interest. see more topTable . default value: 1
robust	logical, should the estimation of the empirical Bayes prior parameters be robustified against outlier sample variances?
...	other arguments are passed to lmFit .
gp1	tag names involved in group 1
gp2	tag names involved in group 2

Details

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

Value

a list with test results. the output of test results is a matrix.

Author(s)

Jianhong Ou

See Also

[singleSampleAnalyze](#), [singleGroupAnalyze](#), [fisher.exact.test](#), [limmaAnalyze](#)

Examples

```

library(limma)
path <- file.path(find.package("InPAS"), "extdata")
load(file.path(path, "CPs.MAQC.rda"))
load(file.path(path, "coverage.MAQC.rda"))
library(BSgenome.Hsapiens.UCSC.hg19)
data(utr3.hg19)
tags <- names(coverage)
g <- factor(gsub("\\..*$", "", tags))
design <- model.matrix(~-1+g)
colnames(design) <- c("Brain", "UHR")
contrast.matrix<-makeContrasts(contrasts="Brain-UHR", levels=design)
res <- testUsage(CPsites=CPs,
                 coverage=coverage,
                 genome=BSgenome.Hsapiens.UCSC.hg19,
                 utr3=utr3.hg19,
                 method="limma",
                 design=design,
                 contrast.matrix=contrast.matrix)

```

totalCoverage

total coverage

Description

for huge dataset, it will read in the coverage from tmp files and merge them by groups

Usage

```
totalCoverage(coverage, genome, hugeData, groupList=NULL)
```

Arguments

coverage	coverage for each sample, outputs of coverageFromBedGraph
genome	an object of BSgenome
hugeData	hugeData or not
groupList	tag names involved in each groups

Value

a coverage list

Author(s)

Jianhong Ou

trimSeqnames	<i>trim the sequence names</i>
--------------	--------------------------------

Description

only `^chr[0-9XY]+$` is OK.

Usage

```
trimSeqnames(genome)
```

Arguments

genome an BSgenome object

Value

an character vector with trimmed seqnames

Author(s)

Jianhong Ou

usage4plot	<i>prepare coverage data and fitting data for plot</i>
------------	--

Description

prepare coverage data and fitting data for plot

Usage

```
usage4plot(gr, coverage, proximalSites, genome, groupList,
           gcCompensation=NA, mappabilityCompensation=NA,
           FFT=FALSE, fft.sm.power=20)
```

Arguments

gr an object of GRanges
coverage coverage for each sample
proximalSites proximal sites
genome an object of [BSgenome](#)
groupList the list of sample names
gcCompensation GC content compensation vector

mappabilityCompensation
 mappability compensation vector

FFT use FFT to smooth the data or not. default: FALSE

fft.sm.power if FFT is TRUE, the frequency should be removed

Value

Formal class 'GRanges' [package "GenomicRanges"] with metadata:

dat matrix, first column is the fit data, the other columns are coverage data for each sample

offset offset from the start of 3UTR

Author(s)

Jianhong Ou

Examples

```
library(BSgenome.Mmusculus.UCSC.mm10)
path <- file.path(find.package("InPAS"), "extdata")
bedgraphs <- c(file.path(path, "Baf3.extract.bedgraph"),
               file.path(path, "UM15.extract.bedgraph"))
coverage <- coverageFromBedGraph(bedgraphs, tags=c("Baf3", "UM15"),
                                 genome=Mmusculus, hugeData=FALSE)
gr <- GRanges("chr6", IRanges(128846245, 128850081), strand="-")
dat <- usage4plot(gr, coverage, proximalSites=128849148, Mmusculus)
data <- dat$dat[[1]]
op <- par(mfrow=c(3, 1))
plot(data[,1], type="l", xlab="", ylab="The fitted value")
abline(v=dat$offset)
plot(data[,2], type="l", xlab="", ylab="Baf3")
plot(data[,3], type="l", xlab="", ylab="UM15")
par(op)
```

utr3.hg19

3'UTR annotation for hg19 obtained from utr3Annotation

Description

3'UTR annotation obtained from utr3Annotation by TxDb.Hsapiens.UCSC.hg19.knownGene and org.Hs.eg.db

Usage

```
data(utr3.hg19)
```

Format

GRanges with slot start holding the start position of the 3'UTR, slot end holding the end position of the 3'UTR, slot names holding transcripts and gene names of 3'UTR, slot seqnames holding the chromosome location where the 3'UTR is located and slot strand for strand of 3'UTR. In addition, the following variables are included.

feature should be unknown or proximalCP_XXXXXXXX

id should be utr3 or next.exon.gap

exon exon id

transcript transcript id

gene entriz gene id

symbol gene symbol

Details

used in the examples Annotation data obtained by: library(TxDb.Hsapiens.UCSC.hg19.knownGene)

library(org.Hs.eg.db)

utr3Annotation(TxDb.Hsapiens.UCSC.hg19.knownGene, org.Hs.egSYMBOL)

Value

an object of GRanges.

Examples

```
data(utr3.hg19)
```

```
head(utr3.hg19)
```

```
utr3.mm10
```

```
3'UTR annotation for mm10 obtained from utr3Annotation
```

Description

3'UTR annotation obtained from utr3Annotation by TxDb.Mmusculus.UCSC.mm10.knownGene and org.Mm.eg.db

Usage

```
data(utr3.mm10)
```

Format

GRanges with slot start holding the start position of the 3'UTR, slot end holding the end position of the 3'UTR, slot names holding transcripts and gene names of 3'UTR, slot seqnames holding the chromosome location where the 3'UTR is located and slot strand for strand of 3'UTR. In addition, the following variables are included.

feature should be unknown or proximalCP_XXXXXXXX

id should be utr3 or next.exon.gap

exon exon id

transcript transcript id

gene entriz gene id

symbol gene symbol

Details

used in the examples Annotation data obtained by: library(TxDb.Mmusculus.UCSC.mm10.knownGene)

library(org.Mm.eg.db)

utr3Annotation(TxDb.Mmusculus.UCSC.mm10.knownGene, org.Mm.egSYMBOL)

Value

an object of GRanges.

Examples

```
data(utr3.mm10)
head(utr3.mm10)
```

utr3Annotation	<i>extract 3'UTR from TxDb object</i>
----------------	---------------------------------------

Description

extract 3'UTR from a [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

```
utr3Annotation(txdb, orgDbSYMBOL, MAX_EXONS_GAP = 10000)
```

Arguments

txdb	an object of TxDb
orgDbSYMBOL	a string indicates org SYMBOL to entriz id map
MAX_EXONS_GAP	maximul exon gap for distal CP site

Value

return an object of GRanges

Author(s)

Jianhong Ou

Examples

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

  library(org.Mm.eg.db)

  utr3Annotation(TxDb.Mmusculus.UCSC.mm10.knownGene, "org.Mm.egSYMBOL")
}
```

 UTR3eSet-class

 Class UTR3eSet

Description

An object of class UTR3eSet represents the results of 3UTR usage

Objects from the Class

Objects can be created by calls of the form `new("UTR3eSet", usage, PDUI, PDUI.log2, short, long, signals, testRes)`

Slots

`usage` an [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

`signals` signals used for single sample

`testRes` a matrix of test results of [testUsage](#)

Methods

`$, $<-` Get or set the slot of [UTR3eSet](#)

`as("UTR3eSet", "ExpressionSet")` Convert a UTR3eSet to an [ExpressionSet](#).

`as("UTR3eSet", "GRanges")` Convert a UTR3eSet to an [GRanges](#).

Author(s)

Jianhong Ou

UTR3TotalCoverage *extract coverage of 3UTR for CP sites prediction*

Description

extract 3UTR coverage from totalCov according and GRanges object utr3.

Usage

```
UTR3TotalCoverage(utr3, totalCov, gcCompensation = NA,
                  mappabilityCompensation = NA,
                  FFT = FALSE, fft.sm.power = 20)
```

Arguments

utr3 an GRanges object. must be the output of [utr3Annotation](#)
totalCov total coverage of each sample. must be the output of [totalCoverage](#)
gcCompensation GC compensation vector. Not support yet.
mappabilityCompensation
 mappability compensation vector. Not support yet.
FFT Use FFT smooth or not.
fft.sm.power the cut-off frequency of FFT smooth.

Value

a list. level 1: chromosome; level 2: each transcripts; level3: data matrix

Author(s)

Jianhong Ou

UTR3usage *calculate the usage of long and short form of UTR3*

Description

calculate the usage of long and short form of UTR3 for the results of [CPsites](#)

Usage

```
UTR3usage(CPsites, coverage, hugeData, BPPARAM = NULL, phmm = FALSE)
```


Arguments

CPsites	outputs of CPsites
coverage	coverage for each sample, outputs of coverageFromBedGraph
hugeData	is this dataset consume too much memory? if it is TRUE, the coverage will be saved into tempfiles.
BPPARAM	An optional BiocParallelParam instance determining the parallel back-end to be used during evaluation, or a list of BiocParallelParam instances, to be applied in sequence for nested calls to bplapply .
phmm	prepare data for singleSample analysis? default is FALSE

Value

GRanges object

Author(s)

Jianhong Ou

See Also

[CPsites](#)

utr3UsageEstimation *estimation of 3'UTR usage for each region*

Description

estimation of 3'UTR usage for short form and long form

Usage

```
utr3UsageEstimation(CPsites, coverage, genome, utr3,
  gp1, gp2=NULL,
  short_coverage_threshold = 10,
  long_coverage_threshold = 2,
  adjusted.P_val.cutoff = 0.05,
  dPDUI_cutoff = 0.3,
  PDUI_logFC_cutoff=0.59, BPPARAM=NULL)
```

Arguments

CPsites	outputs of CPsites
coverage	coverage for each sample, outputs of coverageFromBedGraph
genome	an object of BSgenome
utr3	output of utr3Annotation

gp1 tag names involved in group 1
 gp2 tag names involved in group 2
 short_coverage_threshold
 cutoff threshold for coverage in thre region of short form
 long_coverage_threshold
 cutoff threshold for coverage in thre region of long form
 adjusted.P_val.cutoff
 cutoff value for adjusted p.value
 dPDUI_cutoff cutoff value for differential PAS(polyadenylation signal) usage index
 PDUI_logFC_cutoff
 cutoff value for log2 fold change of PAS(polyadenylation signal) usage index
 BPPARAM An optional [BiocParallelParam](#) instance determining the parallel back-end to be used during evaluation, or a list of [BiocParallelParam](#) instances, to be applied in sequence for nested calls to `bplapply`.

Value

return an object of GRanges

Author(s)

Jianhong Ou

Examples

```
if(interactive()){
  library(BSgenome.Mmusculus.UCSC.mm10)
  path <- file.path(find.package("InPAS"), "extdata")
  bedgraphs <- file.path(path, "Baf3.extract.bedgraph")
  data(utr3.mm10)
  tags <- "Baf3"
  genome <- BSgenome.Mmusculus.UCSC.mm10
  coverage <-
    coverageFromBedGraph(bedgraphs, tags, genome, hugeData=FALSE)
  CP <- CPsites(coverage=coverage, gp1=tags, gp2=NULL, genome=genome,
    utr3=utr3.mm10, coverage_threshold=5, long_coverage_threshold=5)
  res <- utr3UsageEstimation(CP, coverage,
    utr3.mm10, genome, gp1=tags, gp2=NULL)
}
```

valley

get the local minimal square standard error (SSE)

Description

For a giving numeric vectors, calculate the top N local minimal square standard error. It will also include the saved ID if it is in the range of (ss, se)

Usage

```
valley(x, ss, se, n = 1, savedID = NA, filterByPval = TRUE)
```

Arguments

x	numeric vector
ss	start searching position
se	end searching position
n	the length of output. If n=-1, output all the local minimal SSE positions.
savedID	saved positions
filterByPval	logical. Filter the positions by p value or not.

Value

a numeric vector, position list.

Author(s)

Jianhong Ou

zScoreThrethold	<i>calculate local background cutoff value</i>
-----------------	--

Description

calculate local background cutoff value based on z-score

Usage

```
zScoreThrethold(background, introns, totalCov, utr3, z = 2)
```

Arguments

background	background range
introns	GRanges of introns
totalCov	total coverage of output of totalCoverage
utr3	output of utr3Annotation
z	z score cut off value

Value

a numeric vector

Author(s)

Jianhong Ou

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