Package ‘InPAS’

March 22, 2017

Type Package
Title Identification of Novel alternative PolyAdenylation Sites (PAS)
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Maintainer Jianhong Ou <jianhong.ou@umassmed.edu>
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.es.db, org.Mm.es.db, TxDb.Hsapiens.UCSC.hg19.knownGene, TxDb.Mmusculus.UCSC.mm10.knownGene, rtracklayer, knitr

VignetteBuilder knitr

NeedsCompilation no

R topics documented:

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| InPAS-package | alternative polyadenylation and cleavage estimations |

**Description**

predict and estimate the alternative polyadenylation and cleavage site for mRNA-seq data
Details

Package: InPAS
Type: Package
Version: 1.0
Date: 2014-09-12
License: GPL (>= 2)

Author(s)
Jianhong Ou, Sung Mi Park, Michael R. Green and Lihua Julie Zhu
Maintainer: Jianhong Ou <jianhong.ou@umassmed.edu>

References

coverageFromBedGraph read coverage from bedGraph files

Description
read coverage from bedGraph files and save as a list.

Usage
coverageFromBedGraph(bedgraphs, tags, genome,
hugeData=FALSE, BPPARAM=NULL, ...)

Arguments

<table>
<thead>
<tr>
<th>Argument</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>bedgraphs</td>
<td>The file names of bedgraphs generated by bedtools. eg: bedtools genomecov -bg -split -ibam $bam -g mm10.size.txt &gt; $bedgraph</td>
</tr>
<tr>
<td>tags</td>
<td>the names for each input bedgraphs</td>
</tr>
<tr>
<td>genome</td>
<td>an object of BSgenome</td>
</tr>
<tr>
<td>hugeData</td>
<td>is this dataset consume too much memory? if it is TRUE, the coverage will be saved into tempfiles.</td>
</tr>
<tr>
<td>BPPARAM</td>
<td>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.</td>
</tr>
</tbody>
</table>

... parameters can be passed into tempfile. This is useful when you submit huge dataset to cluster.
coverageRate

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)
}

coverageRate  coverage rate of genes and 3UTRs

Description

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

Usage

coverageRate(coverage, txdb, genome,
  cutoff_readsNum=1,
  cutoff_expdGene_cvgRate=0.1,
  cutoff_expdGene_sampleRate=0.5,
  which=NULL, ...)

Arguments

coverage  coverage for each sample, output of coverageFromBedGraph
txdb     an object of TxDb
genome   an object of 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.
cutoff_expdGene_cvgRate, cutoff_expdGene_sampleRate
  cutoff_expdGene_cvgRate and cutoff_expdGene_sampleRate are the parameters used to calculate which gene is expressed in all input dataset. cutoff_expdGene_cvgRate set the cutoff value for the coverage rate of each gene; cutoff_expdGene_sampleRate set the cutoff value for ratio of numbers of expressed and all samples for each gene. for example, by default, cutoff_expdGene_cvgRate=0.1 and cutoff_expdGene_sampleRate=0.5
surpose 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 mean(c(0.05, 0.12, 0.2, 0.17) > cutoff_expdGene_cvgRate) > cutoff_expdGene_sampleRate 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 mean(c(0.05, 0.12, 0.07, 0.17) > cutoff_expdGene_cvgRate) <= cutoff_expdGene_sampleRate

which

an object of GRanges or NULL. If it is not NULL, only the exons overlapping the given ranges are used.

 Value

return a datafrom with colnames : gene.coverage.rate, expressed.gene.coverage.rate, UTR3.coverage.rate, UTR3.expressed.gene.subset.coverage.rate and rownames: the names of coverage.

 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 <- c(file.path(path, "Baf3.extract.bedgraph"),
                 file.path(path, "UM15.extract.bedgraph"))
  hugeData <- FALSE

  coverage <- coverageFromBedGraph(bedgraphs,
                                   tags=c("Baf3", "UM15"),
                                   genome=BSgenome.Mmusculus.UCSC.mm10,
                                   hugeData=hugeData)

  coverageRate(coverage,
               txdb=TxDb.Mmusculus.UCSC.mm10.knownGene,
               genome=BSgenome.Mmusculus.UCSC.mm10,
               which = GRanges("chr6", ranges=IRanges(90013000, 140678000)))
  }

 covThreshold

calculate the cutoff threshold of coverage

 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`

Description

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

Usage

```r
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,
    PolyA_PWM=NA, classifier=NA, classifier_cutoff=.8, step=1,
    two_way=FALSE,
    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`
- **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).
- **step**
  - adjust step, default 1, means adjust by each base by `cleanUpdTSeq`
- **two_way**
  - Search the proximal site from both direction or not.
- **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.
CPsite_estimation

Author(s)

Jianhong Ou

References


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


Examples

```r
if(interactive()){
    library(BSgenome.Musculus.UCSC.mm10)
    path <- file.path(find.package("InPAS"), "extdata")
    bedgraphs <- file.path(path, "Baf3.extract.bedgraph")
    data(utr3.mm10)
    tags <- "Baf3"
    genome <- BSgenome.Musculus.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

```r
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, step=1, two.way=FALSE,
                  tmpfolder=NULL, silence=TRUE)
```

Arguments

- `chr.cov`: coverage list for one chromosome
- `utr3`: output of utr3Annotatioin
- `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
step adjust step, default 1, means adjust by each base by cleanUpdTSeq.
two_way Search the proximal site from both direction or not.
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  

*calculate the depth weight for each example*

**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  

*adjust distal CP sites by cleanUpdTSeq*

**Description**

adjust distal CP sites by cleanUpdTSeq

**Usage**

distalAdj(distalCPs, classifier, classifier_cutoff, shift_range, genome, step=1)

**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
- step: adjust step, default 1, means adjust by each base by cleanUpdTSeq.

**Value**

a list could be input of `searchProximalCPs`
filterRes

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
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
get.regions.coverage

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  
 calculate coverage for giving region

Description

calculate 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
getUTR3eSet

Description

Generate a UTR3eSet object with PDI infomation for statistic test

Usage

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

getCov

Description

extract coverage from bedgraph file

Usage

getCov(bedgraph, genome, seqLen)

Arguments

- bedgraph: bedGraph file names
- genome: an object BSgenome
- seqLen: lengths of each chromosome

Value

a Rle object for a sample coverage

Author(s)

Jianhong Ou

See Also

coverageFromBedGraph
getUTR3region

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 bplapply.
- **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

```r
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  

**extract long and short 3UTR region**

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

Description

do estimation of alternative polyadenylation and cleavage site in one step

Usage

```r
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"),
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 long form

background  the range for calculating cutoff threshold of local background

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

directive 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

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 lmFit.
optimalSegmentation

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)

demand the 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

---

**PAscore**  
*calculate the CP score*

**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 postion
- `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

---

**PAscore2**  
*calculate the CP score*

**Description**  
calculate CP score by cleanUpdTSeq

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

---
**polishCPs**

**Arguments**

- **seqname**: sequence names
- **pos**: genomic positions
- **str**: strands
- **idx**: offset position
- **idx.gp**: group number of the offset position
- **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

---

**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

```r
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

```r
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

---

**proximalAdj** *adjust the proximal CP sites*

---

**Description**

adjust the proximal CP sites by PolyA PWM and cleanUpdTSeq

**Usage**

```r
proximalAdj(CPs, MINSIZE, PolyA_PWM, genome, classifier, classifier_cutoff,
            shift_range, search_point_START, step=1)
```

**Arguments**

- **CPs**: the outputs of `searchProximalCPs`
- **MINSIZE**: min size for short from
- **PolyA_PWM**: PolyA position weight metrix
- **genome**: a `BSgenome` object
- **classifier**: cleanUpdTSeq classifier
- **classifier_cutoff**: cutoff value of the classifier
- **shift_range**: the searching range for the better CP sites
- **search_point_START**: just in case there is no better CP sites
- **step**: adjust step, default 1, means adjust by each base by cleanUpdTSeq.

**Value**

keep same as `searchProximalCPs`, which can be handled by `polishCPs`.

**Author(s)**

Jianhong Ou

**See Also**

`searchProximalCPs`, `polishCPs`, `proximalAdjByPWM`, `proximalAdjByCleanUpdTSeq`, `PAscore`, `PAscore2`
proximalAdjByCleanUpdTSeq

### Description

adjust the proximal CP sites by cleanUpdTseq

### Usage

```r
proximalAdjByCleanUpdTSeq(idx.list, cov_diff.list, seqnames, starts, strands,
genome, classifier, classifier_cutoff,
shift_range, search_point_START, step=1)
```

### Arguments

- `idx.list`: the offset of positions of CP sites
- `cov_diff.list`: the SSE values
- `seqnames`: sequence names
- `starts`: starts
- `strands`: strands
- `genome`: a BSgenome object
- `classifier`: cleanUpdTSeq classifier
- `classifier_cutoff`: cutoff value of the classifier
- `shift_range`: the searching range for the better CP sites
- `search_point_START`: just in case there is no better CP sites
- `step`: adjust step, default 1, means adjust by each base by cleanUpdTSeq.

### 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**

```r
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`
**Description**

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

**Usage**

```r
removeUTR3__UTR3(x)
```

**Arguments**

- `x` the distal 3UTR coverage

**Value**

the 3UTR coverage after removing the next 3UTR

**Author(s)**

Jianhong Ou

---

**searchDistalCPs**  
*search distal CP sites*

**Description**

search distal CP sites

**Usage**

```r
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 zScoreThreshold
searchProximalCPs

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, 
two_way=FALSE)

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
two_way Search the proximal site from both direction or not.

Value
a list

Author(s)
Jianhong Ou

See Also
proximalAdj, polishCPs, proximalAdjByPWM, proximalAdjByCleanUpdTSeq, PAscore, PAscore2
**seqLen**

get sequence lengths from a BSgenome object

**Usage**

```r
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

**Usage**

```r
singleGroupAnalyze(UTR3eset)
```

**Arguments**

- **UTR3eset**
  - must be the output of getUTR3eSet

**Value**

a metrix of test results

**Author(s)**

Jianhong Ou
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 metrix 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 <- singleGroupAnalyze(eset)

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

**sort GRanges**

**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

**do test for dPDUI**

**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
normalize
design
contrast.matrix
coeff
robust
gp1
gp2

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

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

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

prepare coverage data and fitting data for plot

Description
prepare coverage data and fitting data for plot

Usage
usage4plot(gr, coverage, proximalSites, genome, groupList)

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

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

```r
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  enritz 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

extract 3' UTR from TxDb object

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**: maximal exon gap for distal CP site

Value

return an object of GRanges with 7 metadata columns: feature (utr3, next.exon.gap, CDS), annotatedProximalCP (unknown, proximalCP_<coordinate>), exon (<transcript id>_<index>), transcript, gene (entrez_id), symbol, truncated (logical).

Author(s)

Jianhong Ou

Examples

if(interactive()){
  library(TxDb.Mmuscus.UCSC.mm10.knownGene)

  library(org.Mm.eg.db)

  utr3Annotation(TxDb.Mmuscus.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, ...)
**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`
- `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**

```r
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**

```r
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)
```
utr3UsageEstimation

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
dPUI_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

zScoreThreshold

calculate local background cutoff value

Description

calculate local background cutoff value based on z-score

Usage

zScoreThreshold(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
zScoreThreshold

Value

a numeric vector

Author(s)

Jianhong Ou
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