

# Package ‘InPAS’

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**Type** Package

**Title** Identification of Novel alternative PolyAdenylation Sites (PAS)

**Version** 1.14.0

**Author** Jianhong Ou, Sung Mi Park, Michael R. Green and Lihua Julie Zhu

**Maintainer** Jianhong Ou <jianhong.ou@umassmed.edu>,  
Lihua Julie Zhu <Julie.Zhu@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.eg.db, org.Mm.eg.db, TxDb.Hsapiens.UCSC.hg19.knownGene, TxDb.Mmusculus.UCSC.mm10.knownGene, rtracklayer, knitr

**VignetteBuilder** knitr

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InPAS-package

*alternative polyadenylation and cleavage estimations*

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## 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

Sheppard S, Lawson N and Zhu L (2013). Accurate identification of polyadenylation sites from 3' end deep sequencing using a naive Bayes classifier. *Bioinformatics*, 29(20), pp. 2564. ISSN 1460-2059

---

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

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 <a href="#">BiocParallelParam</a> instance determining the parallel back-end to be used during evaluation, or a list of <a href="#">BiocParallelParam</a> instances, to be applied in sequence for nested calls to <code>bplapply</code> .
...	parameters can be passed into <code>tempfile</code> . 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)
}
```

---

coverageRate	<i>coverage rate of genes and 3UTRs</i>
--------------	---

---

### 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 <a href="#">coverageFromBedGraph</a>
txdb	an object of <a href="#">TxDb</a>
genome	an object of <a href="#">BSgenome</a>
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 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 $\text{mean}(c(0.05, 0.12, 0.2, 0.17)) > \text{cutoff\_expdGene\_cvgRate}$ 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 $\text{mean}(c(0.05, 0.12, 0.07, 0.17)) > \text{cutoff\_expdGene\_cvgRate}$ $\leq \text{cutoff\_expdGene\_sampleRate}$
which	an object of <a href="#">GRanges</a> or NULL. If it is not NULL, only the exons overlapping the given ranges are used.
...	not used.

**Value**

return a datafrom with colnames : 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. 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(98013000, 140678000)))
}
```

---

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 <a href="#">coverageFromBedGraph</a>
genome	an object of <a href="#">BSgenome</a>
txdb	an object of <a href="#">TxDb</a>
utr3	output of <a href="#">utr3Annotation</a>
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,
        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 <a href="#">coverageFromBedGraph</a>
groupList	group list of tag names
genome	an object of <a href="#">BSgenome</a>
utr3	output of <a href="#">utr3Annotation</a>
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 <a href="#">cleanUpdTSeq</a>
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 <a href="#">TxDb</a>
PolyA_PWM	Position Weight Matrix of polyA
classifier	An object of class " <a href="#">PASclassifier</a> "
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 <a href="#">cleanUpdTSeq</a> .

two_way	Search the proximal site from both direction or not.
shift_range	the shift range for polyA site searching
BPPARAM	An optional <a href="#">BiocParallelParam</a> instance determining the parallel back-end to be used during evaluation, or a list of <a href="#">BiocParallelParam</a> instances, to be applied in sequence for nested calls to <code>bplapply</code> .
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, step=1, two_way=FALSE,
tmpfolder=NULL, silence=TRUE)
```

**Arguments**

chr.cov	coverage list for one chromosome
utr3	output of utr3Annotation
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 <a href="#">zScoreThreshold</a>
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 <a href="#">depthWeight</a>
genome	a <a href="#">BSgenome</a> 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 <a href="#">coverageFromBedGraph</a>
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 <a href="#">searchDistalCPs</a>
classifier	cleanUpdTSeq classifier
classifier_cutoff	cutoff value of the classifier
shift_range	the searching range for the better CP sites
genome	a <a href="#">BSgenome</a> object
step	adjust step, default 1, means adjust by each base by cleanUpdTSeq.

**Value**

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

**Author(s)**

Jianhong Ou

**See Also**

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

---

filterRes	<i>filter results</i>
-----------	-----------------------

---

**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 <a href="#">testUsage</a>
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 <a href="#">getUTR3eSet</a>
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 <a href="#">BSgenome</a>
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 <a href="#">CPSites</a>
coverage	coverage for each sample, outputs of <a href="#">coverageFromBedGraph</a>
genome	an object of <a href="#">BSgenome</a>
utr3	output of <a href="#">utr3Annotation</a>
normalize	normalization method
...	parameter can be passed into <a href="#">normalize.quantiles.robust</a>
BPPARAM	An optional <a href="#">BiocParallelParam</a> instance determining the parallel back-end to be used during evaluation, or a list of <a href="#">BiocParallelParam</a> instances, to be applied in sequence for nested calls to <code>bplapply</code> .
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                      *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

---

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"),
      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 <a href="#">BSgenome</a>
utr3	output of utr3Annotation
txdb	an object of <a href="#">TxDb</a>
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
adjust_distal_polyA_end	If true, adjust distal polyA end by <a href="#">cleanUpdTSeq</a>
PolyA_PWM	Position Weight Matrix of polyA
classifier	An object of class " <a href="#">PASclassifier</a> "
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 <a href="#">singleSampleAnalyze</a> , <a href="#">singleGroupAnalyze</a> , <a href="#">fisher.exact.test</a> , <a href="#">limmaAnalyze</a>
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 <a href="#">model.matrix</a>

<code>contrast.matrix</code>	numeric matrix with rows corresponding to coefficients in fit and columns containing contrasts. May be a vector if there is only one contrast. see <a href="#">makeContrasts</a>
<code>coef</code>	column number or column name specifying which coefficient or contrast of the linear model is of interest. see more <a href="#">topTable</a> . default value: 1
<code>P.Value_cutoff</code>	cutoff of P value
<code>adj.P.Val_cutoff</code>	cutoff value for adjusted p.value
<code>dPDUI_cutoff</code>	cutoff value for differential PAS(polyadenylation signal) usage index
<code>PDUI_logFC_cutoff</code>	cutoff value for log2 fold change of PAS(polyadenylation signal) usage index
<code>BPPARAM</code>	An optional <a href="#">BiocParallelParam</a> instance determining the parallel back-end to be used during evaluation, or a list of <a href="#">BiocParallelParam</a> instances, to be applied in sequence for nested calls to <code>bplapply</code> .

**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 <a href="#">BiocParallelParam</a> instance determining the parallel back-end to be used during evaluation, or a list of <a href="#">BiocParallelParam</a> instances, to be applied in sequence for nested calls to <code>bplapply</code> .
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 <a href="#">UTR3eSet</a> 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 <a href="#">model.matrix</a>
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 <a href="#">makeContrasts</a>
coef	column number or column name specifying which coefficient or contrast of the linear model is of interest. see more <a href="#">topTable</a> . 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 <a href="#">BSgenome</a>
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 position
idx.gp	group number of the offset position
genome	an object of <a href="#">BSgenome</a>
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 <a href="#">UTR3eSet</a> 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, step=1)
```

### Arguments

CPs	the outputs of <a href="#">searchProximalCPs</a>
MINSIZE	min size for short from
PolyA_PWM	PolyA position weight matrix
genome	a <a href="#">BSgenome</a> 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  
*adjust the proximal CP sites by cleanUpdTSeq*

---

**Description**

adjust the proximal CP sites by cleanUpdTSeq

**Usage**

```
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 <a href="#">BSgenome</a> 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 Matrix. 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 <a href="#">BSgenome</a> 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	<i>remove the candidates LIKE UTR3__UTR3</i>
------------------	--

---

**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 <a href="#">removeUTR3__UTR3</a> )
curr_UTR	GRanges of current 3UTR
window_size	window size
depth.weight	output of <a href="#">depthWeight</a>
long_coverage_threshold	cutoff value for coverage of long form 3UTR
background	local background range
z2s	cut off background scores. see <a href="#">zScoreThrethold</a>

**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 <a href="#">searchDistalCPs</a> or <a href="#">distalAdj</a>
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	<i>get sequence lengths</i>
--------	-----------------------------

---

**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	<i>do analysis for single group samples</i>
--------------------	---

---

**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 <a href="#">singleSampleAnalyze</a> , <a href="#">singleGroupAnalyze</a> , <a href="#">fisher.exact.test</a> , <a href="#">limmaAnalyze</a>
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 <a href="#">model.matrix</a>
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 <a href="#">makeContrasts</a>
coef	column number or column name specifying which coefficient or contrast of the linear model is of interest. see more <a href="#">topTable</a> . 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> .
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	<i>total coverage</i>
---------------	-----------------------

---

**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 <a href="#">coverageFromBedGraph</a>
genome	an object of <a href="#">BSgenome</a>
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 <a href="#">BSgenome</a> 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 <a href="#">BSgenome</a>
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**

```
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.danRer10`*3'UTR annotation for danRer10 obtained from utr3Annotation*

---

**Description**

3'UTR annotation obtained from utr3Annotation by TxDb.Drerio.UCSC.danRer10.refGene and org.Dr.eg.db

**Usage**

```
data(utr3.danRer10)
```

**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\_XXXXXXXXXX

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.Drerio.UCSC.danRer10.refGene)

```
library(org.Dr.eg.db)
```

```
utr3Annotation(TxDb.Drerio.UCSC.danRer10.refGene, "org.Dr.egSYMBOL")
```

**Value**

an object of GRanges.

**Examples**

```
data(utr3.danRer10)
```

```
head(utr3.danRer10)
```

---

`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\_XXXXXXXXXX

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 <a href="#">TxDb</a> 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 <a href="#">TxDb</a>
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 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.Mmusculus.UCSC.mm10.knownGene)

  library(org.Mm.eg.db)

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

---

UTR3eSet-class	<i>Class UTR3eSet</i>
----------------	-----------------------

---

**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, t`

**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 <a href="#">CPSites</a>
coverage	coverage for each sample, outputs of <a href="#">coverageFromBedGraph</a>
hugeData	is this dataset consume too much memory? if it is TRUE, the coverage will be saved into tempfiles.
BPPARAM	An optional <a href="#">BiocParallelParam</a> instance determining the parallel back-end to be used during evaluation, or a list of <a href="#">BiocParallelParam</a> instances, to be applied in sequence for nested calls to <a href="#">bplapply</a> .
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 <a href="#">CPsites</a>
coverage	coverage for each sample, outputs of <a href="#">coverageFromBedGraph</a>
genome	an object of <a href="#">BSgenome</a>
utr3	output of <a href="#">utr3Annotation</a>
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 <a href="#">BiocParallelParam</a> instance determining the parallel back-end to be used during evaluation, or a list of <a href="#">BiocParallelParam</a> instances, to be applied in sequence for nested calls to <code>bplapply</code> .

**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	<i>get the local minimal square standard error (SSE)</i>
--------	--

---

### 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 <a href="#">totalCoverage</a>
utr3	output of <a href="#">utr3Annotation</a>
z	z score cut off value

*zScoreThreshold*

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**Value**

a numeric vector

**Author(s)**

Jianhong Ou

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