

Package ‘APalyzer’

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Type Package

Title A toolkit for APA analysis using RNA-seq data

Version 1.20.0

Description Perform 3'UTR APA, Intronic APA and gene expression analysis using RNA-seq data.

biocViews Sequencing, RNASeq, DifferentialExpression, GeneExpression, GeneRegulation, Annotation, DataImport, Software

Imports GenomicRanges, GenomicFeatures, GenomicAlignments, DESeq2, ggrepel, SummarizedExperiment, Rsubread, stats, ggplot2, methods, rtracklayer, VariantAnnotation, dplyr, tidyr, repmis, Rsamtools, HybridMTest

Suggests knitr, rmarkdown, BiocStyle, org.Mm.eg.db, AnnotationDbi, TBX20BamSubset, testthat, pasillaBamSubset

URL <https://github.com/RJWANGbioinfo/APalyzer/>

BugReports <https://github.com/RJWANGbioinfo/APalyzer/issues>

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Author Ruijia Wang [cre, aut] (<<https://orcid.org/0000-0002-4211-5207>>), Bin Tian [aut], Wei-Chun Chen [aut]

Maintainer Ruijia Wang <rjwang.bioinfo@gmail.com>

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| | |
|--------|-------------------------------------|
| APABox | <i>APABox, APA RED Box plotting</i> |
|--------|-------------------------------------|

Description

APA RED Box plotting

Usage

```
APABox (df, xlab = "APAre", ylab = "RED",
        plot_title = NULL)
```

Arguments

| | |
|------------|-------------------------------------|
| df | a dataframe of APAdiff output |
| xlab | lable of x-axis, default is 'APAre' |
| ylab | lable of y-axis, default is 'RED' |
| plot_title | Main title of plot |

Value

The function APABox return a Box plot.

Author(s)

Ruijia Wang

Examples

```

library("TBX20BamSubset")
library("Rsamtools")
flsall = getBamFileList()
exptpath = system.file("extdata",
"mm9_TBX20.APAout.RData", package="APAlzyer")
load(exptpath)
sampleTable1 = data.frame(samplename = c(names(flsall)),
condition = c(rep("NT",3),rep("KD",3)))
sampleTable2 = data.frame(samplename = c("SRR316184","SRR316187"),
condition = c("NT","KD"))
## 3'UTR APA plot
test_3UTRmuti=APAdiff(sampleTable1,DFUTRraw,
conKET='NT',trtKEY='KD',PAS='3UTR',CUTreads=0)
UTR_APA_PLOTBOX=APABox(test_3UTRmuti, plot_title='3UTR APA')

## IPA plot
test_IPAmuti=APAdiff(sampleTable1,IPA_OUTraw,
conKET='NT',trtKEY='KD',PAS='IPA',CUTreads=0)
IPA_PLOTBOX=APABox(test_IPAmuti, plot_title='IPA')

```

APAdiff

APAdiff, calculate delta relative expression (RED) and statistics significance between two sample groups

Description

Calculate delta relative expression (RED) and statistics significance between two sample groups.

Usage

```

APAdiff(sampleTable,mutiraw, conKET='NT',
trtKEY='KD', PAS='3UTR', CUTreads=0, p_adjust_methods="fdr", MultiTest='unpaired t-test')

```

Arguments

| | |
|------------------|--|
| sampleTable | a dataframe of sample table containing 8 columns for Intronic PASs: 'sample-name','condition' |
| mutiraw | a dataframe output obtained using either PASEXP_3UTR or PASEXP_IPA |
| conKET | the name of control in the sampletable, default is 'NT' |
| trtKEY | the name of control in the sampletable, default is 'KD' |
| PAS | type of PAS analyzed, either '3UTR' or 'IPA', default is '3UTR' |
| CUTreads | reads cutoff used for the analysis, default is 0 |
| p_adjust_methods | p value correction method, the method can be "holm", "hochberg", "hommel", "bonferroni", "BH", "BY", "fdr", "none", default is "fdr" |
| MultiTest | statistics testing method for muti-replicates designs, the method can be "unpaired t-test", "paired t-test", "ANOVA", default is "unpaired t-test" |

Value

The function `APAdiff` return a dataframe containing RED, pvalue and regulation pattern (UP, DN or NC) for either each gene (3'UTR APA) or each PAS (IPA).

Author(s)

Ruijia Wang

Examples

```
library("TBX20BamSubset")
library("Rsamtools")
flsall = getBamFileList()
extpath = system.file("extdata",
"mm9_TBX20.APAout.RData", package="APalyzer")
load(extpath)
sampleTable1 = data.frame(samplename = c(names(flsall)),
condition = c(rep("NT", 3), rep("KD", 3)))
sampleTable2 = data.frame(samplename = c("SRR316184", "SRR316187"),
condition = c("NT", "KD"))
## Analysis 3'UTR APA between KD and NT group using muti-replicates
test_3UTRmuti=APAdiff(sampleTable1, DFUTRraw,
conKET='NT', trtKEY='KD', PAS='3UTR', CUTreads=0, p_adjust_methods="fdr", MultiTest='unpaired t-test')

## Analysis 3'UTR APA between KD and NT group without replicates
test_3UTRsing=APAdiff(sampleTable2, DFUTRraw,
conKET='NT', trtKEY='KD', PAS='3UTR', CUTreads=0, p_adjust_methods="fdr")

## Analysis IPA between KD and NT group
test_IPAmuti=APAdiff(sampleTable1, IPA_OUTraw,
conKET='NT', trtKEY='KD', PAS='IPA', CUTreads=0, p_adjust_methods="fdr", MultiTest='unpaired t-test')

## Analysis IPA between KD and NT group without replicates
test_IPAsing=APAdiff(sampleTable2, IPA_OUTraw,
conKET='NT', trtKEY='KD', PAS='IPA', CUTreads=0, p_adjust_methods="fdr")
```

APAVolcano

APAVolcano, APA Volcano plotting

Description

APA Volcano plotting

Usage

```
APAVolcano (df, Pcol = "pvalue", PAS='3UTR',
top = -1, markergenes = NULL,
y_cutoff = 0.05, xlab = "RED", ylab = "-Log10(P-value)",
PAScolor = c("gray80", "red", "blue"),
alpha = 0.75, plot_title = NULL,
width = 4, height = 2.5)
```

Arguments

| | |
|-------------|---|
| df | a dataframe of APAdiff output |
| Pcol | p-value column used to for y-axis of volcano plot, default is 'pvalue' |
| top | number of genes/IPA to label in the plot, default is -1, which don't lable top genes, user can set it >0, e.g., top = 5 |
| markergenes | a set of genes to label in the plot |
| PAS | type of PAS analyzed, either '3UTR' or 'IPA', default is '3UTR' |
| y_cutoff | y cutoff line, default is 0.05 |
| xlab | lable of x-axis, default is 'RED' |
| ylab | lable of y-axis, default is '-Log10(P-value)' |
| PAScolor | dot color for 'NC','UP' and 'DN' gene/IPAs, default is "gray80", "red", and "blue" |
| alpha | alpha of the dot, default is 0.75 |
| plot_title | Main title of plot |
| width | width of the dot, default is 4 |
| height | height of the dot, default is 2.5 |

Value

The function APAVolcano return a Volcano plot.

Author(s)

Ruijia Wang

Examples

```

library("TBX20BamSubset")
library("Rsamtools")
flsall = getBamFileList()
extpath = system.file("extdata",
"mm9_TBX20.APAout.RData", package="APALyzer")
load(extpath)
sampleTable1 = data.frame(samplename = c(names(flsall)),
condition = c(rep("NT",3),rep("KD",3)))
sampleTable2 = data.frame(samplename = c("SRR316184","SRR316187"),
condition = c("NT","KD"))
## 3'UTR APA plot
test_3UTRmuti=APAdiff(sampleTable1,DFUTRraw,
conKET='NT',trtKEY='KD',PAS='3UTR',CUTreads=0)
UTR_APA_PLOT=APAVolcano(test_3UTRmuti, PAS='3UTR', Pcol = "pvalue", top=5, plot_title='3UTR APA')

## IPA plot
test_IPAmuti=APAdiff(sampleTable1,IPA_OUTraw,
conKET='NT',trtKEY='KD',PAS='IPA',CUTreads=0)
IPA_PLOT=APAVolcano(test_IPAmuti, PAS='IPA', Pcol = "pvalue", top=5, plot_title='IPA')

```

| | |
|------------------|---|
| download_testbam | <i>download_testbam, download bam files of mouse testis and heart</i> |
|------------------|---|

Description

download bam files of mouse testis and heart

Usage

```
download_testbam()
```

Value

The function download_testbam download test data bam files.

Author(s)

Ruijia Wang

Examples

```
download_testbam()
```

| | |
|------------|--|
| GENEXP_CDS | <i>GENEXP_CDS, count reads mapped to CDS regions and calculate TPM for coding gene</i> |
|------------|--|

Description

Map reads to CDS regions and calculate TPM for each gene.

Usage

```
GENEXP_CDS(CDSbygene, f1S, Strandtype="NONE")
```

Arguments

| | |
|------------|--|
| CDSbygene | a genomic ranges of CDS regions for each coding gene |
| f1S | bamfile lists containing the file and path of bam files |
| Strandtype | strand type of the bam file; "forward" is forward sequencing, "invert" is reverse sequencing and "NONE" is non-strand specific, Default is "NONE". |

Value

The function GENEXP_CDS() return a dataframe containing reads count, TPM for each gene

Author(s)

Ruijia Wang

Examples

```
## count reads mapped to CDS regions and calculate TPM for each gene
## using forward sequencing
library("TBX20BamSubset")
library("Rsamtools")
library("GenomicAlignments")
library("GenomicFeatures")
library("org.Mm.eg.db")
flsall = getBamFileList()
extpath = system.file("extdata", "mm9.chr19.refGene.R.DB", package="APalyzer")
txdb = loadDb(extpath, packageName='GenomicFeatures')
IDDB = org.Mm.eg.db
CDSdbraw = REFCDS(txdb,IDDB)
DFGENERaw = GENEXP_CDS(CSdbraw, flsall, Strandtype="forward")
```

PAS2GEF

*PAS2GEF, build reference regions for 3'UTR PASs***Description**

Build 3'UTR PAS and IPA (IPA and LE) Reference using GTF file.

Usage

```
PAS2GEF(GTFfile,AnnoMethod="V2")
```

Arguments

| | |
|------------|---|
| GTFfile | GTF file of gene annotation |
| AnnoMethod | annotation method used to build PAS reference, either 'legacy' or 'V2', default is 'V2' |

Value

The function PAS2GEF() returns 3 input tables of PAS references: PASREF\$refUTRraw is for 3'UTR PAS, PASREF\$dfIPA and PASREF\$dfLE are for IPA references.

Author(s)

Ruijia Wang

Examples

```
## build Reference ranges for 3'UTR PASs in mouse
download.file(url='ftp://ftp.ensembl.org/pub/release-99/gtf/mus_musculus/Mus_musculus.GRCm38.99.gtf.gz',
             destfile='Mus_musculus.GRCm38.99.gtf.gz')
GTFfile="Mus_musculus.GRCm38.99.gtf.gz"

PASREF=PAS2GEF(GTFfile, AnnoMethod="V2")
refUTRraw=PASREF$refUTRraw
dfIPA=PASREF$dfIPA
dfLE=PASREF$dfLE
```

| | |
|-------------|--|
| PASEXP_3UTR | <i>PASEXP_3UTR, calculate relative expression of aUTR and cUTR regions</i> |
|-------------|--|

Description

Map reads to 3'UTR APA regions and calculate relative expression of aUTR and cUTR regions.

Usage

```
PASEXP_3UTR(UTRdb, fls, Strandtype="NONE")
```

Arguments

| | |
|------------|--|
| UTRdb | a genomic ranges of aUTR(pPAS to dPAS) and cUTR(cdsend to pPAS) regions for each gene |
| fls | bamfile lists containing the file and path of bam files |
| Strandtype | strand type of the bam file; "forward" is forward sequencing, "invert" is reverse sequencing and "NONE" is non-strand specific, Default is "NONE". |

Value

The function PASEXP_3UTR() return a dataframe containing reads count, RPKM and relative expression of aUTR and cUTR for each gene

Author(s)

Ruijia Wang

Examples

```
## count reads mapped to 3'UTR APA regions and
## calculate relative expression of aUTR and cUTR regions
## using forward sequencing
library("TBX20BamSubset")
library("Rsamtools")
library("GenomicAlignments")
library("repmis")
flsall = getBamFileList()
URL="https://github.com/RJWANGbioinfo/PAS_reference_RData/blob/master/"
file="mm9_REF.RData"
source_data(paste0(URL,file,"?raw=True"))
refUTRraw = refUTRraw[which(refUTRraw$Chrom=='chr19'),]
UTRdbraw = REF3UTR(refUTRraw)
DFUTRraw = PASEXP_3UTR(UTRdbraw, flsall, Strandtype="forward")
```


PASEXP_IPA

*PASEXP_IPA, calculate relative expression of IPA regions***Description**

Map reads to IPA regions and calculate relative expression of aUTR and cUTR regions.

Usage

```
PASEXP_IPA(dfIPArAw, dfLErAw, fls, Strandtype="NONE", nts=1, minMQS=0, SeqType = "SingleEnd")
```

Arguments

| | |
|------------|--|
| dfIPArAw | a dataframe containing 8 columns for Intronic PASs: 'PASid', 'gene_symbol', 'Chrom', 'Strand', 'Pos', 'upstreamSS', 'downstreamSS'. 'upstreamSS' means closest 5'/3' splice site to IPA, 'downstreamSS' means closest 3' splice site |
| dfLErAw | a dataframe containing 5 columns for 3' least exon: 'gene_symbol', 'Chrom', 'Strand', 'LEstart', 'TES'. 'LEstart' means the start position of last 3' exon. |
| fls | bamfile lists containing the file and path of bam files |
| Strandtype | strand type of the bam file; "forward" is forward sequencing, "invert" is reverse sequencing and "NONE" is non-strand specific, Default is "NONE". |
| nts | number of threads used for computing, parameter used by featureCounts , nthread option, Default is 1 |
| minMQS | minimum mapping quality score of counted reads, parameter used by featureCounts , minMQS option, Default is 0 |
| SeqType | set the sequencing type of reads in bam files can be either 'SingleEnd' (default) or 'ThreeMostPairEnd'. |

Value

The function PASEXP_IPA() return a dataframe containing reads count, RPKM and relative expression of aUTR and cUTR for each gene

Author(s)

Ruijia Wang

Examples

```
## count reads mapped to IPA regions and
## calculate relative expression of aUTR and cUTR regions
## using forward sequencing
library("TBX20BamSubset")
library("Rsamtools")
library("GenomicAlignments")
library("repmis")
flsall = getBamFileList()
URL="https://github.com/RJWANGbioinfo/PAS_reference_RData/blob/master/"
file="mm9_REF.RData"
source_data(paste0(URL,file,"?raw=True"))
IPA_OUTraw=PASEXP_IPA(dfIPA, dfLE, flsall, Strandtype="forward", nts=1)
```

| | |
|---------|--|
| REF3UTR | <i>REF3UTR, build reference regions for 3'UTR PASs</i> |
|---------|--|

Description

Build 3'UTR PAS Reference for distal and proximal PAS.

Usage

```
REF3UTR(refUTR)
```

Arguments

refUTR a dataframe containing 6 colmuns for 3'UTR PASs: 'gene_symbol', 'Chrom', 'Strand', 'Proximal', 'Distal', 'cdsend'

Value

The function REF3UTR() returns a genomic ranges of aUTR(pPAS to dPAS) and cUTR(cdsend to pPAS) regions for each gene

Author(s)

Ruijia Wang

Examples

```
## build Reference ranges for 3'UTR PASs in mouse
library(repmis)
URL="https://github.com/RJWANGbioinfo/PAS_reference_RData/blob/master/"
file="mm9_REF.RData"
source_data(paste0(URL,file,"?raw=True"))
refUTRraw=refUTRraw[which(refUTRraw$Chrom=='chr19'),]
UTRdbraw=REF3UTR(refUTRraw)
```

| | |
|---------|--|
| REF4PAS | <i>REF4PAS, build reference regions for 3'UTR and Intronic PAS using dataframe formatted input</i> |
|---------|--|

Description

build reference regions for 3'UTR and Intronic PAS using dataframe formatted input

Usage

```
REF4PAS(refUTRraw, dfIPArw, dfLEraw)
```

Arguments

| | |
|-----------|--|
| refUTRraw | a dataframe containing 6 columns for 3'UTR PASs: 'gene_symbol', 'Chrom', 'Strand', 'Proximal', 'Distal', 'cdsend' |
| dfIPArw | a dataframe containing 8 columns for Intronic PASs: 'PASid', 'gene_symbol', 'Chrom', 'Strand', 'Pos', 'upstreamSS', 'downstreamSS'. 'upstreamSS' means closest 5'/3' splice site to IPA, 'downstreamSS' means closest 3' splice site |
| dfLEraw | a dataframe containing 5 columns for 3' least exon: 'gene_symbol', 'Chrom', 'Strand', 'LEstart', 'TES'. 'LEstart' means the start position of last 3' exon. |

Value

The function REF4PAS() returns list a genomic ranges of 3'UTR, Intronic PAS and last 3'exon regions for each gene

Author(s)

Ruijia Wang

Examples

```
## build Reference ranges for 3'UTR and Intronic PAS in mouse (mm9)
library(repmis)
URL="https://github.com/RJWANGbioinfo/PAS_reference_RData/blob/master/"
file="mm9_REF.RData"
source_data(paste0(URL,file,"?raw=True"))
refUTRraw=refUTRraw[which(refUTRraw$Chrom=='chr19'),]
dfIPArw=dfIPA[which(dfIPA$Chrom=='chr19'),]
dfLEraw=dfLE[which(dfLE$Chrom=='chr19'),]
PASREF=REF4PAS(refUTRraw,dfIPArw,dfLEraw)
UTRdbraw=PASREF$UTRdbraw
dfIPA=PASREF$dfIPA
dfLE=PASREF$dfLE
```

REFCDS

*REFCDS, build reference regions for CDS of protein coding genes***Description**

Build CDS reference for protein coding genes.

Usage

```
REFCDS(txdb, IDDB)
```

Arguments

| | |
|------|--|
| txdb | a TranscriptDb generate using GenomicFeatures |
| IDDB | Genome annotation of the corresponding species, e.g., "org.Hs.eg.db" |

Value

The function REFCDS() returns a genomic ranges of CDS regions for each coding gene

Author(s)

Ruijia Wang

Examples

```
## build Reference ranges for CDS in mouse coding genes
library("GenomicFeatures")
library("org.Mm.eg.db")
extpath = system.file("extdata", "mm9.chr19.refGene.R.DB", package="APALyzer")
txdb = loadDb(extpath, packageName='GenomicFeatures')
IDDB = org.Mm.eg.db
CDSdbraw = REFCDS(txdb,IDDB)
```

| | |
|------------------|--|
| ThreeMostPairBam | <i>ThreeMostPairBam, extract 3 prime most alignment of a paired-end bam file</i> |
|------------------|--|

Description

extract 3 prime most alignment of a paired-end bam file and saved into a new bam file.

Usage

```
ThreeMostPairBam(BamfilePath, OutDirPath, StrandType="NONE")
```

Arguments

| | |
|-------------|--|
| BamfilePath | file path of a bam file |
| OutDirPath | output folder path |
| StrandType | strand type of the bam file; "forward-reverse": read 1 forward but read 2 is reverse sequencing, "reverse-forward": read 2 forward but read 1 is reverse sequencing, and "NONE" is non-strand specific, Default is "NONE". |

Value

The function ThreeMostPairBam() return a single-end bam file containing 3 prime most alignment of the input paired-end file

Author(s)

Ruijia Wang

Examples

```
## Extract 3 prime most alignment of a paired-end
## bam file and saved into a new bam file
library("pasillaBamSubset")

ThreeMostPairBam (BamfilePath=untreated3_chr4(),
  OutDirPath=getwd(),
  StrandType='forward-reverse')
```

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