

# 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>

**VignetteBuilder** knitr

**License** LGPL-3

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## Contents

APABox . . . . .	2
APAdiff . . . . .	3
APAVolcano . . . . .	4
download_testbam . . . . .	6
GENEXP_CDS . . . . .	6
PAS2GEF . . . . .	7
PASEXP_3UTR . . . . .	8
PASEXP_IPA . . . . .	9
REF3UTR . . . . .	10
REF4PAS . . . . .	10
REFCDS . . . . .	11
ThreeMostPairBam . . . . .	12
<b>Index</b>	<b>13</b>

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APABox	<i>APABox, APA RED Box plotting</i>
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### 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="APALyzer")
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 multi-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")
```

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APAVolcano

*APAVolcano, APA Volcano plotting*

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**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="APAlzyer")
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')

```

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download_testbam	<i>download_testbam, download bam files of mouse testis and heart</i>
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**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()
```

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GENEXP_CDS	<i>GENEXP_CDS, count reads mapped to CDS regions and calculate TPM for coding gene</i>
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**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()
expath = system.file("extdata", "mm9.chr19.refGene.R.DB", package="APalyzer")
txdb = loadDb(expath, 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
```

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PASEXP_3UTR	<i>PASEXP_3UTR, calculate relative expression of aUTR and cUTR regions</i>
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### 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 <a href="#">featureCounts</a> , nthread option, Default is 1
minMQS	minimum mapping quality score of counted reads, parameter used by <a href="#">featureCounts</a> , 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)
```

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

**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)
```

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

**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')
```

# Index

[APABox](#), [2](#)

[APAdiff](#), [3](#)

[APAVolcano](#), [4](#)

[download\\_testbam](#), [6](#)

[featureCounts](#), [9](#)

[GENEXP\\_CDS](#), [6](#)

[PAS2GEF](#), [7](#)

[PASEXP\\_3UTR](#), [8](#)

[PASEXP\\_IPA](#), [9](#)

[REF3UTR](#), [10](#)

[REF4PAS](#), [10](#)

[REFCDS](#), [11](#)

[ThreeMostPairBam](#), [12](#)