

# Package ‘TRESS’

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**Type** Package

**Title** Toolbox for mRNA epigenetics sequencing analysis

**Version** 1.0.0

**Description** This package is devoted to analyzing MeRIP-seq data. Current functionality is for detection of transcriptome-wide m6A methylation regions. The method is based on hierarchical negative binomial models.

**Imports** utils, rtracklayer, Matrix, matrixStats, stats, methods, graphics, GenomicRanges, GenomicFeatures, IRanges, Rsamtools, AnnotationDbi

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Basal	<i>Bin-level and region-level data from basal mouse brain samples</i>
-------	---

---

### Description

A data list containing both bin-level and region-level transcriptome locations and read counts across 7 paired input and IP replicates from basal mouse brain samples. It also contains the size factor of each sample for library size normalization.

### Usage

```
data(Basal)
```

### Format

A list containing two sublists: "Bins" and "Candidates". In list "Bins", there are,

**Bins** A dataframe of 1000 obs and 5 variables, containing the transcriptome location for 1000 bins of length 50bps

**Counts** A data matrix of 1000 obs and 14 variables, containing bin-level read counts

**sf** A numerical vector, containing the size factors of 14 samples estimated from the whole transcriptome using bin-level read counts. ...

In list "Candidates", there are,

**Regions** A dataframe of 500 obs and 5 variables, containing the transcriptome location of 8011 candidates.

**Counts** A data matrix of 500 obs and 14 variables, containing region-level read counts ...

Note, bins and regions may or may not overlap with each other, as both of them are respectively randomly selected from the whole set of bins and candidate regions. However, both data share the same size factor for each sample.

---

CallCandidates

*Call candidate m6A regions or candidate differential m6A regions.*


---

### Description

This function first calls m6A bumps from each pair of input and IP sample using bin-level data. Then, bumps from all input and IP pairs are unioned together to obtain a list of candidate regions.

### Usage

```
CallCandidates(Counts, bins,
               WhichThreshold = "fdr_lfc", pval.cutoff = 1e-5,
               fdr.cutoff = 0.05, lfc.cutoff = 0.7,
               windlen = 5, lowcount = 30)
```

### Arguments

Counts	A data matrix containing bin-level (default 50bp) read counts in both IP and input samples, where the sample order is: input1, ip1, input2, ip2, ...
bins	A data frame containing the genomic coordinate of each bin of fixed length.
WhichThreshold	A character specifying a criterion to select significant bins in bump finding using an ad hoc algorithm. There are five options: "pval" (only use p-values), "fdr" (only use FDR), "lfc" (only use log fold change), "pval_lfc" (use both p-values and log fold changes) and "fdr_lfc" (use FDR and log fold changes). Default is "fdr_lfc".
pval.cutoff	A constant indicating the cutoff for p-value. Default is 1e-05.
fdr.cutoff	A constant indicating the cutoff for FDR. Default is 0.05.
lfc.cutoff	A constant indicating the cutoff for log fold change. Default is 0.7 for fold change of 2.
windlen	An integer specifying the length of consecutive bins used in simple moving average smooth of log fold change. Default is 5.
lowcount	An integer to filter out candidate regions with lower read counts in input. Default is 30.

### Details

The function involves three steps:

- Perform binomial test for each bin based bin-level counts
- Merge significant bins in each input & IP pair to form bumps using: [findBumps](#)
- Combine bumps from all input & IP pairs to construct a list of candidate regions.

**Value**

A list containing

Regions            A data frame containng genomic coordinate for each candidate region.

Counts            A data matrix containing read counts of all samples for each candidate region.

**Examples**

```
### A toy example, whose results do not have real applications.
data("Basal")
Candidates = CallCandidates(
  Counts = Basal$Bins$Counts,
  bins = Basal$Bins$Bins
)
```

---

CallPeaks.multiRep     *m6A peak calling with multiple replicates.*

---

**Description**

This function identifies and ranks significant m6A peaks, given candidate regions obtained from multiple paired of input & IP replicates.

**Usage**

```
CallPeaks.multiRep(Candidates, mu.cutoff,
  WhichThreshold = "fdr_lfc",
  pval.cutoff = 1e-5,
  fdr.cutoff = 0.05,
  lfc.cutoff = 0.7)
```

**Arguments**

Candidates        A list containing: genomic coordinates of each candidate region, read counts and log fold change between IP and input in each candidate region. It also contains the size factor of each sample.

mu.cutoff        A constant specifying the background methylation levels. This is estimated automatically based on the first step of peak calling.

WhichThreshold   A character specifying a threshold for significant peaks. There are three options: "pval" (only use p-values), "fdr" (only use FDR), "lfc" (only use log fold change), "pval\_lfc" (use both p-values and log fold changes) and "fdr\_lfc" (use FDR and log fold changes). Default is "fdr\_lfc".

pval.cutoff      A constant indicating the cutoff for p-value. Default is 1e-05.

fdr.cutoff        A constant indicating the cutoff for FDR. Default is 0.05.

lfc.cutoff        A constant indicating the cutoff for log fold change. Default is 0.7 for fold change of 2.

**Details**

This function first calls `CallPeaks.paramEsti` to conduct parameter estimation and hypothesis testing for all candidate m6A regions. Then it filters and ranks candidate regions using respective criteria to obtain a list of significant m6A peaks.

**Value**

The output is a dataframe whose columns are:

<code>chr</code>	Chromosome number of each peak.
<code>start</code>	The start of genomic position of each peak.
<code>end</code>	The end of genomic position of each peak.
<code>strand</code>	The strand of each peak.
<code>summit</code>	The summit of each peak.
<code>lg.fc</code>	Log fold change between normalized IP and normalized input read counts.
<code>mu</code>	Methylation level of each peak if there are more than one replicates.
<code>mu.var</code>	Estimated variance of methylation level for each peak, when there are more than one replicates.
<code>stats</code>	Wald test statistics of each peak, when there are more than one replicate.
<code>shrPhi</code>	Shrinkage estimation of methylation dispersion for each peak, when there are more than one replicates.
<code>shrTheta</code>	Shrinkage estimation for scale parameter theta in the gamma distribution, when there are more than one replicates.
<code>pvals</code>	P-value calculated based on the Wald-test.
<code>p.adj</code>	Adjusted p-values using Benjamini-Hochberg procedure.
<code>rSocre</code>	A score used to rank each peak. The higher the score, the higher the rank would be.

Note, there are additional columns with name `"*.bam"`. These columns contain the read counts from respective samples.

**Examples**

```
### A toy example
data("Basal")
CallPeaks.multiRep(
  Candidates = Basal$Candidates,
  mu.cutoff = 0.5
)
```

---

CallPeaks.oneRep      *m6A peak calling with only one replicate.*

---

### Description

This function conducts peak calling for data when there is only one biological replicate of input and IP sample.

### Usage

```
CallPeaks.oneRep(Counts, bins, sf = NULL,
                 WhichThreshold = "fdr_lfc",
                 pval.cutoff = 1e-05, fdr.cutoff = 0.05,
                 lfc.cutoff = 0.7, windlen = 5, lowCount = 10)
```

### Arguments

Counts	A two-column data matrix containing bin-level read counts for both IP and input samples.
sf	A numerical vector containing size factors of both IP and input samples. It can be provided by the user, or automatically estimated using "Counts". Default is NULL.
bins	A dataframe containing the genomic locations (chr, start, end, strand) of each bin.
WhichThreshold	A character specifying a criterion to select significant bins in bump finding using an ad hoc algorithm. There are five options: "pval" (only use p-values), "fdr" (only use FDR), "lfc" (only use log fold change), "pval_lfc" (use both p-values and log fold changes) and "fdr_lfc" (use FDR and log fold changes). Default is "fdr_lfc".
pval.cutoff	A constant indicating a cutoff for p-value. Default is 1e-05.
fdr.cutoff	A constant indicating a cutoff for FDR. Default is 0.05.
lfc.cutoff	A constant indicating a cutoff for log fold change. Default is 0.7 for fold change of 2.
windlen	An integer specifying the length of consecutive bins used in simple moving average smooth of log fold change. Default is 5.
lowCount	An integer to filter out m6A regions with lower read counts. Default is 10.

### Details

When there is only one replicate, TRESS assigns a p-value for each bin based on the binomial test. Then it calls candidates with the same algorithm used when there are multiple biological replicates. Binomial tests are performed one more time to select significant candidates as final list of peaks.

**Value**

It returns an excel containing the information for each peak:

chr	Chromosome number of each peak.
start	The start of genomic position of each peak.
end	The end of genomic position of each peak.
strand	The strand of each peak.
summit	The summit of each peak.
pvals	P-value for each peak calculated based on binomial test.
p.adj	Adjusted p-values using Benjamini-Hochberg procedure.
lg.fc	Log fold change between normalized IP and normalized input read counts.

Note, there are additional columns with name "\*.bam". These columns contain the read counts from IP and input samples.

**Examples**

```
## A toy example
data("Basal")
peaks = CallPeaks.oneRep(
  Counts = Basal$Bins$Counts,
  sf = Basal$Bins$sf,
  bins = Basal$Bins$Bins
)
head(peaks, 3)
```

---

CallPeaks.paramEsti     *Parameter estimation in m6A peak calling with multiple replicates.*

---

**Description**

This function estimates all involved parameters in Bayesian hierarchical negative binomial model, which is built for read counts from candidate regions generated from multiple input& IP replicates.

**Usage**

```
CallPeaks.paramEsti(mat, sf = NULL, cutoff = NULL,
  update = "Joint",
  trans = NULL,
  optM = "L-BFGS-B",
  myfscale = -1e+06)
```

**Arguments**

mat	A matrix containing read counts from all paired input & input replicates. The order of samples are: input1, IP1, input2, IP2,...
sf	A vector of size factors for each sample. It can be provided by the users or estimated automatically from the data. Default is NULL.
cutoff	Background methylation level, which can be automatically estimated based on the background read counts in IP and input samples, or provided by users. Defaults is NULL.
update	A logical value indicating whether jointly estimating the nuisance parameter theta with dispersion parameter phi listed in the proposed model. Possible options are "OnlyPhi", "Iterative" and "Joint". "OnlyPhi" means only updating phi_i using R function <code>optimize</code> while fixing parameter theta as the plug-in moment estimator; "Iterative" means iteratively updating and phi using R function <code>optimize</code> ; "Joint" means updating them together using R function <code>optim</code> . Default is "Joint".
optM	A character value to specify which optimization algorithm used in the R function <code>optim</code> . The options are: "Nelder-Mead", "BFGS", "CG", "L-BFGS-B", "SANN" and "Brent". Default is "L-BFGS-B". See more details in help pages of <code>optim</code> .
trans	Needed when <code>**optM == "Nelder-Mead"</code> . It specifies which transformation function used in the estimation of dispersion and/or theta parameter(s) which are subjected to the nonnegative constraints. Possible options are "sin()" and "exp()". Default is NULL.
myfscale	A stop criteria in <code>optim</code> . Default is <code>-1e+06</code> .

**Details**

This function mainly involves three estimation procedures:

- Estimate methylation levels
- Estimate dispersion parameters and the variance of the estimated methylation levels
- Calculate test statistics and p-values. Also, it calculates a score used for peak ranking.

**Value**

mu	Estimation of methylation levels of all peaks.
mu.var	Estimated variance for estimated methylation level.
shrkPhi	Shrinkage estimator for dispersion parameter phi_i.
shrkTheta	Shrinkage estimator for parameter theta_i if <code>update == "Joint" or "Iterative"</code> . Otherwise it would be a plug-in moment estimator.
stats	Wald-test statistics.
pvals	P-values derived from normal distribution based on the Wald-test statistics.
p.adj	Adjusted p-values using Benjamini-Hochberg procedure.
rSocre	A score used to rank each region. The higher the score, the higher the rank would be.



**Examples**

```
### A toy example using basal samples from mouse cortex
data("Basal")
res = CallPeaks.paramEsti(
  mat = as.matrix(Basal$Candidates$Counts),
  sf = Basal$Bins$sf,
  cutoff = 0.5
)
```

DivideBins

*Obtain genomic bins and bin-level read counts from BAM files.***Description**

This function first divides the whole genome into equal-sized bins and then calculates read counts in each bin for all samples. The number of bins depends on the input annotation file, bin size and whether or not including intronic regions.

**Usage**

```
DivideBins(IP.file, Input.file, Path_To_AnnoSqlite,
           InputDir, OutputDir, experimentName,
           binsize = 50, filetype = "bam",
           IncludeIntron = FALSE)
```

**Arguments**

IP.file	A vector of characters containing the name of BAM files for all IP samples.
Input.file	A vector of characters containing the name of BAM files for all input control samples.
Path_To_AnnoSqlite	A character to specify the path to a "*.Sqlite" file used for genome annotation.
experimentName	A character to specify a name for output results.
binsize	A numerical value to specify a size of window to bin the genome and get bin-level read counts. Default value is 50.
filetype	A character to specify the format of input data. Possible choices are: "bam", "bed" and "GRanges". Default is "bam".
InputDir	A character to specify the input directory of all BAM files.
OutputDir	A character to specify an output directory to save all results. Default is NA, which will not save any results.
IncludeIntron	A logical value indicating whether to include (TRUE) intronic regions or not (False). Default is FALSE.

**Value**

The value returned by this function is a list containing two components:

bins	A dataframe containing the genomic coordinates of all bins.
binCount	A M-by-N matrix containing bin-level read counts in M bins and N samples, where N is two times of the length of "IP.file" or "Input.file". The column order depends on the sample order in "Input.file" and "IP.file".

If the "OutputDir" is specified, then both genomic bins and corresponding bin-level read counts would be saved as an ".rda" file.

**Examples**

```
# use data in package datasetTRES
# available on github, which can be installed by
# install_github("https://github.com/ZhenxingGuo0015/datasetTRES")
## Not run:
library(datasetTRES)
IP.file = c("cb_ip_rep1_chr19.bam", "cb_ip_rep2_chr19.bam")
Input.file = c("cb_input_rep1_chr19.bam", "cb_input_rep2_chr19.bam")
BamDir = file.path(system.file(package = "datasetTRES"), "extdata/")
Path_sqlite = file.path(system.file(package = "datasetTRES"),
  "extdata/mm9_chr19_knownGene.sqlite")
#OutDir = "/Users/zhenxingguo/Documents/research/m6a/packagetest"
allBins = DivideBins(
  IP.file = IP.file,
  Input.file = Input.file,
  Path_To_AnnoSqlite = Path_sqlite,
  InputDir = BamDir
)

## End(Not run)
```

---

 EstiMu

*Estimation of m6A methylation.*


---

**Description**

This function calculates, for each candidate region, the averaged enrichment of normalized IP read counts versus the sum of normalized IP and input control read counts.

**Usage**

```
EstiMu(counts, sf)
```

**Arguments**

counts	A data matrix containing read counts in each region across sample input1, ip1, input2, ip2, input3, ip3, ...
sf	A numerical vector containing the size factor of each sample, which is used for sequencing depth normalization. The sample order here is the same as that in counts.

**Value**

mu	A numerical vector containing the methylation level of all candidate regions.
----	---

**Examples**

```
data("Basal")
## methylaton level
mu = EstiMu(
  counts = Basal$Candidates$Counts,
  sf = Basal$Bins$sf
)
head(mu, 3)
```

---

 EstiPhi

*Dispersion estimation.*


---

**Description**

This is a wrapper function to estimate the posterior of methylation dispersion for each candidate region.

**Usage**

```
EstiPhi(counts, sf,
  update = c("OnlyPhi", "Iterative", "Joint"),
  optM = "L-BFGS-B", myfscale = -1e+6, trans = "sin")
```

**Arguments**

counts	A data matrix containing read counts in each region across sample input1, ip1, input2, ip2, input3, ip3, ...
sf	A numerical vector containing the size factor of each sample, which is used for sequencing depth normalization. The sample order here is the same as that in counts.
update	A character specifying which strategy used for estimation of dispersion. There are three options: "OnlyPhi", "Iterative" and "Joint". "OnlyPhi" means only estimate posterior of phi with theta fixed as its moment estimate. "Iterative" means iteratively update phi and theta based on their posterior distribution. "Joint" means jointly estimate phi and theta based on their joint posterior.

optM	A character to specify which maximizing algorithm used for optimization. Default is "L-BFGS-B". See <code>optim</code> for more details.
myfscale	An overall scaling to be applied to the value of fn and gr during optimization. If negative, turns the problem into a maximization problem. Optimization is performed on fn(par)/fnscale. Default is -1e+6. See <code>optim</code> for more details.
trans	A character specifying which transformation function used for phi, in the process of obtaining its posterior mode. There are two options: "sin" and "exp" for transformation $\phi = \sin(s) + 1)/2$ and $\phi = \exp(-\exp(s))$ , where maximization is performed on s. Default is "sin". Note that, this argument only works when the value of optM is not "L-BFGS-B".

### Value

This function returns a dataframe containing: phi and theta estimates for all candidate regions.

### Examples

```
data("Basal")
## methylaton level
res = EstiPhi(counts = as.matrix(Basal$Candidates$Counts),
              sf = Basal$Bins$sf,
              update = "Joint")
head(res, 5)
```

---

findBumps

*Bump-finding from transcriptome bins.*

---

### Description

This function constructs transcriptome m6A bumps for each input & IP replicate, by merging together bins having significant enrichment of IP over input control reads.

### Usage

```
findBumps(chr, pos, strand, x, count,
          use = "pval",
          pval.cutoff,
          fdr.cutoff,
          lfc.cutoff,
          sep = 2000,
          minlen = 100,
          minCount = 3,
          dis.merge = 100,
          scorefun = mean,
          sort = TRUE)
```

**Arguments**

chr	Chromosome number of all bins.
pos	Transcriptome start position of all bins.
strand	Strand of all bins.
x	A dataframe containing the p-values, fdrs and log fold changes of all bins.
count	Read counts in each bin from paired input and IP sample.
use	A character to specify which criterion to select significant bins. It takes among "pval", "fdr", "lfc", "pval_lfc" and "fdr_lfc". "pval": The selection is only based on P-values; "fdr": The selection is only based on FDR; "lfc": The selection is only based on log fold changes between normalized IP and normalized input read counts; "pval_lfc": The selection is based on both p-values and log fold changes; "fdr_lfc": The selection is based on both FDR and log fold changes. Default is "pval".
pval.cutoff	A numerical value to specify a cutoff for p-value. Default is 1e-5.
fdr.cutoff	A numerical value to specify a cutoff for fdr. Default is 0.05.
lfc.cutoff	A numerical value to specify a cutoff for log fold change between normalized IP and input read counts. Default is 0.7 for fold change of 2.
sep	A constant used divide genome into consecutive sequenced regions. Any two bins with distance greater than sep will be grouped into different regions. Default is 2000.
minlen	A constant to select bumps who have minimum length of minlen. Default is 100.
minCount	A constant to select bumps who have at least minlen number of bins. Default is 3.
dis.merge	A constant. Any twp bumps with distance smaller than dis.merge would be merged. Default is 100.
scorefun	A character indicating a function used to assign a score for each bump base on p-values of all spanned bins. Default is "mean", meaning that the score is an average of bin-level p-values.
sort	A logical value indicating whether rank (TRUE) bumps with the score output from scorefun or not (FALSE). Default is TRUE.

**Value**

This function returns a dataframe containing the chromosome, start position, end position, length, strand, summit, total read counts (both IP and input) and score of each bump.

**Examples**

```
### Use example dataset "Basal" in TRESS
### to illustrate usage of this function
data("Basal")
bins = Basal$Bins$Bins
Counts = Basal$Bins$Counts
sf = Basal$Bins$sf
colnames(Counts)
```

```

dat = Counts[, 1:2]
thissf = sf[1:2]
### pvals based on binomial test
idx = rowSums(dat) > 0
Pvals = rep(1, nrow(dat))
Pvals[idx] = 1 - pbinom(dat[idx, 2],
                      rowSums(dat[idx, ]),
                      prob = 0.5)

### lfc
c0 = mean(as.matrix(dat), na.rm = TRUE) ### pseudocount
lfc = log((dat[, 2]/thissf[2] + c0)/(dat[, 1]/thissf[1] + c0))
x.vals = data.frame(pvals = Pvals,
                   fdr = p.adjust(Pvals, method = "fdr"),
                   lfc = lfc)

### find bumps based on pvals, fdr or lfc
Bumps = findBumps(chr = bins$chr,
                 pos = bins$start,
                 strand = bins$strand,
                 x = x.vals,
                 use = "fdr_lfc",
                 fdr.cutoff = 0.01,
                 lfc.cutoff = 0.5,
                 count = dat)

head(Bumps, 3)

```

---

ShowOnePeak

*Visualization of a single peak along the genome.*


---

## Description

This function plots the estimated methylation level (as bars) of each bin within a peak for each replicate, and the corresponding normalized input read depth (grey curve).

## Usage

```
ShowOnePeak(onePeak, allBins, binCounts,
            isDMR = FALSE, Sname = NULL,
            ext = 500, ylim = c(0, 1))
```

## Arguments

onePeak	A one-row dataframe containing the genomic position of a single peak: chr, start, end, strand.
allBins	A dataframe containing genomic position of all bins used to call peaks: chr, start, end, strand.
binCounts	A dataframe containing the read counts of all bins for each replicate. The sample order is: input1, ip1, input2, ip2, ...
isDMR	A logical value indicating whether the input region is DMR. Default is FALSE.

Sname	Sample names. If isDMR = TRUE, then it will be used as the title of each plot.
ext	An integer indicating the length of base pairs to extend the region on both sides: (start - ext, end + ext). Default is 500.
ylim	The range of y-axis to plot. Default is c(0, 1)

**Value**

It only generates a plot. No specific output.

**See Also**

ShowOneDMR from "DSS" package.

**Examples**

```
### read peaks
peaks = read.table(file.path(system.file(package = "TRESS"),
                             "extdata/examplebyBam_peaks.xls"),
                  sep = "\t", header = TRUE)
### load annotation and bin counts
load(file.path(system.file(package = "TRESS"),
                  "extdata/examplebyBam.rda"))
allBins = as.data.frame(bins$bins)
colnames(allBins)[1] = "chr"
allBins$strand = binStrand
for (i in 1:4) {
  ShowOnePeak(
    onePeak = peaks[i,],
    allBins = allBins, binCounts = allCounts
  )
}
```

---

TRESS\_peak

*Detecting m6A methylation regions from Methylated RNA Immunoprecipitation Sequencing.*


---

**Description**

This is a wrapper function to call m6A peaks transcriptome wide. When there are multiple biological replicates, it

- Divides the whole genome to obtain bin-level read counts: [DivideBins](#)
- Calls candidate m6A methylation regions: [CallCandidates](#)
- Model fitting on candidate peaks based on Negative Binomial distribution: [CallPeaks.multiRep](#)

If there is only one replicate, it calls [CallPeaks.oneRep](#) to detect m6A methylation regions.

**Usage**

```
TRESS_peak(IP.file, Input.file, Path_To_AnnoSqlite,
           binsize = 50,
           WhichThreshold = "fdr_lfc",
           pval.cutoff0 = 1e-5,
           fdr.cutoff0 = 0.05,
           lfc.cutoff0 = 0.7,
           lowcount = 30,
           InputDir,
           OutputDir = NA,
           experiment_name,
           filetype = "bam",
           IncludeIntron = FALSE)
```

**Arguments**

IP.file	A vector of characters containing the name of BAM files for all IP samples.
Input.file	A vector of characters containing the name of BAM files for all input control samples.
Path_To_AnnoSqlite	A character to specify the path to a "*.sqlite" file used for genome annotation.
binsize	A numerical value to specify the size of window to bin the genome and get bin-level read counts. Default value is 50.
WhichThreshold	A character to specify which criterion to select significant bins in the first step, and also significant m6A regions in the second step. It takes among "pval", "fdr", "lfc", "pval_lfc" and "fdr_lfc". "pval": The inference is only based on P-values; "fdr": The inference is only based on FDR; "lfc": The inference is only based on log fold changes between normalized IP and normalized input read counts; "pval_lfc": The inference is based on both p-values and log fold changes; "fdr_lfc": The inference is based on both FDR and log fold changes. Default is "fdr_lfc".
pval.cutoff0	A numerical value to specify a cutoff for p-value. Default is 1e-5.
fdr.cutoff0	A numerical value to specify a cutoff for fdr. Default is 0.05.
lfc.cutoff0	A numerical value to specify a cutoff for log fold change between normalized IP and input read counts. Default is 0.7 for fold change of 2.
lowcount	An integer to filter out regions with total input counts < lowcount. Default is 30.
InputDir	A character to specify the input directory of all BAM files.
OutputDir	A character to specify an output directory save all results. Default is NA, which will not save any results.
experiment_name	A character to specify the name of results.
filetype	A character to specify the format of input data. Possible choices are: "bam", "bed" and "GRanges". Default is "bam".
IncludeIntron	A logical value indicating whether to include (TRUE) intronic regions or not (False). Default is FALSE.



## Details

TRESS implements a two-step procedure to conduct peak calling for MeRIP-seq data with multiple biological replicates. In the first step, it quickly divide the whole genome into equal sized bins and loosely indentifies candidate peak regions using an ad hoc procedure. In the second step, it detects high confident peaks among candidate regions and ranks them with more rigorous statistical modeling based on an empirical Bayesian hierarchical model.

When there is only one biological replciate, candidate regions from the above two-step procedure will be output as the final list of peaks. P-values come from binomial test, which are further adjusted using Benjamini-Hochberg procedure.

## Value

If directory OutputDir is specified, this function will output two sets of results. One is saved as ".rda", which contains all bin-level data (genome coordinates and read counts matrix). The other one is an ".xls" file, which contains information of all peaks. The columns of the peak excel files are:

chr	Chromosome number of each peak.
start	The start of genomic position of each peak.
end	The end of genomic position of each peak.
strand	The strand of each peak.
summit	The summit of each peak.
lg.fc	Log fold change between normalized IP and normalized input read counts for each peak.
pvals	P-values calculated based on the Wald-test.
p.adj	Adjusted p-values using Benjamini-Hochberg procedure.

If there are multiple replicates, the excel will also include following columns:

mu	Estimated methylation level of each peak.
mu.var	Estimated variance for methylation level of each peak
stats	Wald test statistics of each peak
shrkJPhi	The shrinkage estimation of dispersion for mehtylation levels of each peak.
shrkJTheta	The shrinkage estimation for scale parameter theta in the gamma distribution.
rSocre	A score defined by TRESS to rank each peak. The higher the score, the higher the rank would be.

Note, there are additional columns regardless of the number of replicates. Those columns contain read counts from respective samples and have names "\*.bam".

## Author(s)

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## References

Guo, Z., Shafik, A. M., Jin, P., Wu, Z., and Wu, H. (2021) Detecting m6A methylation regions from Methylated RNA Immunoprecipitation Sequencing. *Bioinformatics*, 1-7. <https://doi-org.proxy.library.emory.edu/10.1093/bioinformatics/btab181>

## Examples

```
## Use BAM files in datasetTRES
# install_github("https://github.com/ZhenxingGuo0015/datasetTRES")
## Not run:
library(datasetTRES)
IP.file = c("cb_ip_rep1_chr19.bam", "cb_ip_rep2_chr19.bam")
Input.file = c("cb_input_rep1_chr19.bam", "cb_input_rep2_chr19.bam")
BamDir = file.path(system.file(package = "datasetTRES"), "extdata/")
annoDir = file.path(
  system.file(package = "datasetTRES"),
  "extdata/mm9_chr19_knownGene.sqlite"
)
OutDir = "/directory/to/output"
TRESS_peak(IP.file = IP.file,
           Input.file = Input.file,
           Path_To_AnnoSqlite = annoDir,
           InputDir = BamDir,
           OutputDir = OutDir,
           experiment_name = "examplebyBam",
           filetype = "bam")
peaks = read.table(paste0(OutDir, "/", "c"),
                  sep = "\t", header = TRUE)

## End(Not run)
```

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