# Package 'atena'

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Type Package

Title Analysis of Transposable Elements

**Version** 1.17.0

**Description** Quantify expression of transposable elements (TEs) from RNA-seq data through different methods, including ERVmap, TEtranscripts and Telescope. A common interface is provided to use each of these methods, which consists of building a parameter object, calling the quantification function with this object and getting a SummarizedExperiment object as output container of the quantified expression profiles. The implementation allows one to quantify TEs and gene transcripts in an integrated manner.

**License** Artistic-2.0 **Encoding** UTF-8

**Depends** R (>= 4.3.0), SummarizedExperiment

Imports methods, stats, Matrix, BiocGenerics, MatrixGenerics, BiocParallel, S4Vectors, IRanges, Seqinfo, GenomicFeatures, GenomicRanges, GenomicAlignments, Rsamtools, GenomeInfoDb, SQUAREM, sparseMatrixStats, AnnotationHub, matrixStats, cli

**Suggests** covr, BiocStyle, knitr, rmarkdown, RUnit, TxDb.Dmelanogaster.UCSC.dm6.ensGene, RColorBrewer

**biocViews** Transcription, Transcriptomics, RNASeq, Sequencing, Preprocessing, Software, GeneExpression, Coverage, DifferentialExpression, FunctionalGenomics

VignetteBuilder knitr

URL https://github.com/rcastelo/atena

BugReports https://github.com/rcastelo/atena/issues

RoxygenNote 7.3.2

Collate 'AllGenerics.R' 'AllClasses.R' 'ERVmap.R' 'TEtranscripts.R' 'Telescope.R' 'annotations.R' 'atena.R' 'atenaMethod.R' 'overlappingModes.R' 'qtex.R' 'utils.R' 'zzz.R'

git\_url https://git.bioconductor.org/packages/atena

2 atena-package

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atena	package atena: analysis of transposable elements in R and Bioconductor	

# Description

The atena package provides a complete re-implementation in R of three existing methods for the quantification of transposable element (TE) expression in order to facilitate its integration into Bioconductor workflows for the analysis of RNA-seq data. The three methods are TEtranscripts (Jin et al. (2015)), ERVmap (Tokuyama et al. (2018)) and Telescope (Bendall et al. (2019)).

# **Details**

The main functions are:

• TEtranscriptsParam - build parameter objects of the class TEtranscriptsParam-class for the TEtranscripts expression quantification method

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• ERVmapParam - build parameter objects of the class ERVmapParam-class for the ERVmap expression quantification method

- TelescopeParam build parameter objects of the class TelescopeParam-class for the Telescope expression quantification method
- qtex call the TE expression quantification method using a previously built parameter object

For detailed information on usage, see the package vignette, by typing vignette("atena").

All questions and bug reports should be posted to the Bioconductor Support Site:

```
https://support.bioconductor.org
```

The code of the development version of the package is available at the GitHub repository:

```
https://github.com/functionalgenomics/atena
```

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# References

Jin Y et al. TEtranscripts: a package for including transposable elements in differential expression analysis of RNA-seq datasets. Bioinformatics. 2015;31(22):3593-3599. DOI: https://doi.org/10.1093/bioinformatics/btv422

Tokuyama M et al. ERVmap analysis reveals genome-wide transcription of human endogenous retroviruses. PNAS. 2018;115(50):12565-12572. DOI: https://doi.org/10.1073/pnas.1814589115

Bendall et al. Telescope: characterization of the retrotranscriptome by accurate estimation of transposable element expression. PLOS Comp. Biol. 2019;15(9):e1006453. DOI: https://doi.org/10.1371/journal.pcbi.1006453

## See Also

Useful links:

- https://github.com/rcastelo/atena
- Report bugs at https://github.com/rcastelo/atena/issues

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annotaTEs

Get RepeatMasker UCSC annotations

#### **Description**

The annotaTEs() function fetches RepeatMasker UCSC transposable element (TE) annotations using AnnotationHub and parses them.

# Usage

```
annotaTEs(
  genome = "hg38",
  parsefun = rmskidentity,
  verbose = TRUE,
  AHid = NULL,
  ...
)
```

# **Arguments**

genome parsefun The genome version of the desired RepeatMasker annotations (e.g. "hg38").

A function to parse the annotations:

- Function rmskidentity returns RepeatMasker annotations as present in AnnotationHub, without processing them.
- Function rmskbasicparser parses annotations by removing low complexity regions, simple repeats, satellites, rRNA, scRNA, snRNA, srpRNA and tRNA. Also removes TEs with a strand different than "+" or "-". Modifies "repFamily" and "repClass" columns when a "?" is present or when they are defined as "Unknown" or "Other". Finally, assigns a unique id to each TE instance by adding the suffix "\_dup" plus a number at the end of the "repName".
- Function rmskatenaparser parses RepeatMasker annotations reconstructing fragmented TEs by assembling together fragments from the same TE that are close enough. For LTR class TEs, it tries to reconstruct full-length and partial TEs following the LTR internal region LTR structure. Input is a GRanges object and output is a GRangesList object.
- Function OneCodeToFindThemAll parses annotations following the 'One code to find them all' method by (Bailly-Bechet et al. 2014). Input is a GRanges object and output is a GRangesList object.
- User-defined function. Input and output should be GRanges objects.

verbose AHid (Default TRUE) Logical value indicating whether to report progress.

AnnotationHub unique identifier, of the form AH12345, of an object with TE annotations. This is an optional argument to specify a concrete AnnotationHub resource, for instance when more there is more than one RepeatMasker annotation available for a specific genome version. If AHid is not specified, the latest RepeatMasker annotation is be used.

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... Arguments passed to parsefun.

#### **Details**

Given a specific genome version, the annotaTEs() function fetches RepeatMasker annotations from UCSC Genome Browser using the AnnotationHub package. Since RepeatMasker not only provides TE annotations but also low complexity DNA sequences and other types of repeats, a specific parsefun can be set to parse these annotations (e.g. rmskbasicparser or a user-defined function). If no parsing is required, parsefun can be set to rmskidentity.

#### Value

A ['GRanges'][GenomicRanges::GRanges-class] object with transposable element annotations.

#### See Also

AnnotationHub

# **Examples**

```
rmskid <- annotaTEs(genome="hg19", parsefun=rmskidentity)
rmskid</pre>
```

annotateTEsGetters

Getter functions of TE classes from parsed RepeatMasker annotations.

# **Description**

Getter functions of TE classes from parsed RepeatMasker annotations.

# Usage

```
getLTRs(
   annot,
   relLength = 0.9,
   fullLength = TRUE,
   partial = FALSE,
   soloLTR = FALSE,
   otherLTR = FALSE,
   returnMask = FALSE
)

getLINEs(annot, relLength = 0.9, returnMask = FALSE)

getSINEs(annot, relLength = 0.9, returnMask = FALSE)

getDNAtransposons(annot, relLength = 0.9, returnMask = FALSE)
```

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#### **Arguments**

 $A \ [GRanges'] \ [Genomic Ranges::GRanges-class] \ or \ [GRanges List'] \ [Genomic Ranges::GRanges List'] \ [Genomic Ranges List'] \ [Genomic Ranges::GRanges List'] \ [Genomic Ranges::GRanges List'] \ [Genomic Ranges::GRanges List'] \ [Genomic Ranges Li$ 

class] object obtained with the function 'annotaTES()', using either ['OneCodeToFind-ThemAll'] or ['rmskatenaparser'] as RepeatMasker parser functions. Alterna-

tively, if 'annot' is a ['QuantifyParam'] or a ['SummarizedExperiment'][SummarizedExperiment::SummarizedExperiment']

class] object produced by the 'qtex()' function, this function will attempt to ex-

tract the corresponding annotations from inside those objects.

relLength (Default 0.9) Numeric value that can take values between 0 to 1. Sets the mini-

mum relative length required for features. Elements with a lower relative length than relLength will be filtered. The relative length used is the one obtained by OneCodeToFindThemAll() or rmskatenaparser(). (length of the recon-

structed TE / length of the reference).

fullLength (Default TRUE) Logical value on whether reconstructed full-length LTR TEs

(elements with structure LTR - internal region - LTR) should be selected.

partial (Default FALSE) Logical value on whether partially reconstructed LTR TEs

should be selected (structure LTR - internal region or internal region - LTR).

soloLTR (Default FALSE) Logical value on whether solo LTRs should be selected. Note

that only fragments unambiguously identified as LTRs thanks to the identifica-

tion of their equivalent internal region are considered as LTRs.

otherLTR (Default FALSE) Logical value on whether other TEs from the LTR class, not in-

cluded in any of the previous three categories, should be selected. These include TEs from LTR class that cannot be unambiguously identified as LTR o internal region, and thus cannot be reconstructed into partial or full-length elements; as

well as solo internal regions.

returnMask (Default FALSE) Logical value indicating whether a subset of the input anno-

tations should be returned (default) or a logical mask of the same length as the input annotations where TRUE values indicate what annotations belong to the  ${\rm TE}$ 

class we want to obtain with the getter function.

#### **Details**

Retrieves annotations from the TE class corresponding to the getter function, using RepeatMasker annotations after parsing them with the OneCodeToFindThemAll() or rmskatenaparser() function. The relLength parameter can be used to filter out elements with a lower relative length. Further parameters can be used to fine-tune the type of elements to be reported.

## Value

A ['GRangesList'][GenomicRanges::GRangesList-class] object with annotations from class corresponding to the getter function (LTRs, LINEs, SINEs or DNA transposons).

```
partial=TRUE)
rmskatLTR

rmskat_line <- getLINEs(rmskat, relLength=0.95)

rmskat_sine <- getSINEs(rmskat, relLength=0.95)

rmskat_DNAtrans <- getDNAtransposons(rmskat, relLength=0.95)</pre>
```

atenaParam-class

atena parameter class

# Description

This is a class for storing parameters to quantify TE (and gene) expression using the atena method. It is a subclass of the ['QuantifyParam'].

Build an object of the class atenaParam.

# Usage

```
atenaParam(
 bfl,
  teFeatures,
  aggregateby = character(0),
  ovMode = "ovUnion",
  geneFeatures = NULL,
  singleEnd = TRUE,
  strandMode = 1L,
  ignoreStrand = FALSE,
  fragments = TRUE,
 pi_prior = 0L,
  theta_prior = 0L,
  em_epsilon = 1e-07,
 maxIter = 100L,
  reassign_mode = "exclude",
  conf_prob = 0.9,
  verbose = TRUE
)
## S4 method for signature 'atenaParam'
show(object)
```

# **Arguments**

bf1

A BamFile or BamFileList object, or a character string vector of  $BAM\ file-names.$ 

teFeatures

A GRanges or GRangesList object. Elements in this object should have names, which are used as a grouping factor for genomic ranges forming a common locus. This grouping is performed previous to TE expression quantification, unlike the aggregation of quantifications performed when the aggregateby parameter is specified, which is performed after individual TE instances are quantified.

aggregateby

Character vector with column names from the annotation to be used to aggregate quantifications. By default, this is an empty vector, which means that the names of the input GRanges or GRangesList object given in the teFeatures parameter are used to aggregate quantifications.

ovMode

Character vector indicating the overlapping mode. Available options are: "ovUnion" (default) and "ovIntersectionStrict", which implement the corresponding methods from HTSeq (https://htseq.readthedocs.io/en/release\_0.11. 1/count.html). Ambiguous alignments (alignments overlapping > 1 feature) are not counted.

geneFeatures

(Default NULL) A GRanges or GRangesList object with the gene annotated features to be quantified. Unique reads are first tallied with respect to these gene features whereas multi-mapping reads are preferentially assigned to TEs. Elements should have names indicating the gene name/id. In case that geneFeatures is a GRanges and contains a metadata column named type, only the elements with type = exon are considered for the analysis. Then, exon counts are summarized to the gene level. If NULL, gene expression is not quantified.

singleEnd

(Default TRUE) Logical value indicating if reads are single (TRUE) or paired-end (FALSE).

strandMode

(Default 1) Numeric vector which can take values 0, 1 or 2. The strand mode is a per-object switch on GalignmentPairs objects that controls the behavior of the strand getter. See GalignmentPairs class for further detail. If singleEnd = TRUE, then strandMode is ignored.

ignoreStrand

(Default FALSE) A logical which defines if the strand should be taken into consideration when computing the overlap between reads and annotated features. When ignoreStrand = FALSE, an aligned read is considered to be overlapping an annotated feature as long as they have a non-empty intersecting genomic range on the same strand, while when ignoreStrand = TRUE the strand is not considered.

fragments

(Default TRUE) A logical; applied to paired-end data only. When fragments=FALSE, the read-counting method only counts 'mated pairs' from opposite strands (non-ambiguous properly paired reads), while when fragments=TRUE same-strand pairs, singletons, reads with unmapped pairs and other ambiguous or not properly paired fragments are also counted (see "Pairing criteria" in readGAlignments()). For further details see summarizeOverlaps().

pi\_prior

(Default 0) A positive numeric object indicating the prior on pi. The same prior can be specified for all features setting pi\_prior as a scalar, or each feature can have a specific prior by setting pi\_prior as a vector with names() corresponding to all feature names. Setting a pi prior is equivalent to adding n unique reads.

theta\_prior

(Default 0) A positive numeric object indicating the prior on Q. The same prior can be specified for all features setting theta\_prior as a scalar, or each fea-

ture can have a specific prior by setting theta\_prior as a vector with names() corresponding to all feature names. Equivalent to adding n non-unique reads.

em\_epsilon (Default 1e-7) A numeric scalar indicating the EM Algorithm Epsilon cutoff.

maxIter A positive integer scalar storing the maximum number of iterations of the EM

SQUAREM algorithm (Du and Varadhan, 2020). Default is 100 and this value

is passed to the maxiter parameter of the squarem() function.

reassign\_mode (Default 'exclude') Character vector indicating reassignment mode after EM

step. Available methods are 'exclude' (reads with more than one best assignment are excluded from the final counts), 'choose' (when reads have more than one best assignment, one of them is randomly chosen), 'average' (the read count is divided evenly among the best assignments) and 'conf' (only assignments that exceed a certain threshold -defined by conf\_prob parameter- are accepted, then the read count is proportionally divided among the assignments

above conf\_prob).

conf\_prob (Default 0.9) Minimum probability for high confidence assignment. verbose (Default TRUE) Logical value indicating whether to report progress.

object A atenaParam object.

#### **Details**

This is the constructor function for objects of the class atenaParam-class. This type of object is the input to the function qtex() for quantifying expression of transposable elements, which will call the atena method with this type of object. The atena method uses a multiple '\_\_no\_feature' approach in which as many '\_\_no\_feature' features as different overlapping patterns of multimapping reads in the overlapping matrix are used to represent alignments mapping outside annotations.

#### Value

A ['atenaParam'] object.

#### **Slots**

singleEnd (Default TRUE) Logical value indicating if reads are single (TRUE) or paired-end (FALSE).

strandMode (Default 1) Numeric vector which can take values 0, 1 or 2. The strand mode is a perobject switch on GAlignmentPairs objects that controls the behavior of the strand getter. See GAlignmentPairs class for further detail. If singleEnd = TRUE, then strandMode is ignored.

ignoreStrand (Default FALSE) A logical which defines if the strand should be taken into consideration when computing the overlap between reads and annotated features. When ignoreStrand = FALSE, an aligned read is considered to be overlapping an annotated feature as long as they have a non-empty intersecting genomic range on the same strand, while when ignoreStrand = TRUE the strand is not considered.

fragments (Default TRUE) A logical; applied to paired-end data only. When fragments=FALSE, the read-counting method only counts 'mated pairs' from opposite strands (non-ambiguous properly paired reads), while when fragments=TRUE same-strand pairs, singletons, reads with unmapped pairs and other ambiguous or not properly paired fragments are also counted (see "Pairing criteria" in readGAlignments()). For further details see summarizeOverlaps().

pi\_prior (Default 0) A positive numeric object indicating the prior on pi. The same prior can be specified for all features setting pi\_prior as a scalar, or each feature can have a specific prior by setting pi\_prior as a vector with names() corresponding to all feature names. Setting a pi prior is equivalent to adding n unique reads.

theta\_prior (Default 0) A positive numeric object indicating the prior on Q. The same prior can be specified for all features setting theta\_prior as a scalar, or each feature can have a specific prior by setting theta\_prior as a vector with names() corresponding to all feature names. Equivalent to adding n non-unique reads.

em\_epsilon (Default 1e-7) A numeric scalar indicating the EM Algorithm Epsilon cutoff.

maxIter A positive integer scalar storing the maximum number of iterations of the EM SQUAREM algorithm (Du and Varadhan, 2020). Default is 100 and this value is passed to the maxiter parameter of the squarem() function.

reassign\_mode (Default 'exclude') Character vector indicating reassignment mode after EM step. Available methods are 'exclude' (reads with more than one best assignment are excluded from the final counts), 'choose' (when reads have more than one best assignment, one of them is randomly chosen), 'average' (the read count is divided evenly among the best assignments) and 'conf' (only assignments that exceed a certain threshold -defined by conf\_prob parameter- are accepted, then the read count is proportionally divided among the assignments above conf\_prob).

conf\_prob (Default 0.9) Minimum probability for high confidence assignment.

```
bamfiles <- list.files(system.file("extdata", package="atena"),</pre>
                        pattern="*.bam", full.names=TRUE)
## use the following two instructions to fetch annotations, they are here
## commented out to enable running this example quickly when building and
## checking the package
rmskat <- annotaTEs(genome="dm6", parsefun=rmskatenaparser,</pre>
                     strict=FALSE, insert=500)
rmskLTR <- getLTRs(rmskat, relLength=0.8,
                   fullLength=TRUE,
                   partial=TRUE,
                   otherLTR=TRUE)
## End(Not run)
## DO NOT TYPE THIS INSTRUCTION, WHICH JUST LOADS A PRE-COMPUTED ANNOTATION
## YOU SHOULD USE THE INSTRUCTIONS ABOVE TO FETCH ANNOTATIONS
rmskLTR <- readRDS(system.file("extdata", "rmskatLTRrlen80flenpartoth.rds",</pre>
                                package="atena"))
## build a parameter object for the atena method
atpar <- atenaParam(bfl=bamfiles,</pre>
                     teFeatures=rmskLTR,
                     singleEnd=TRUE,
                     ignoreStrand=TRUE)
atpar
```

ERVmapParam-class

ERVmap parameter class

# **Description**

This is a class for storing parameters provided to the ERVmap algorithm. It is a subclass of the ['QuantifyParam'] class.

Build an object of the class ERVmapParam

# Usage

```
ERVmapParam(
  bfl,
  teFeatures,
  aggregateby = character(0),
  ovMode = "ovUnion",
  geneFeatures = NULL,
  singleEnd = TRUE,
  ignoreStrand = TRUE,
  strandMode = 1L,
  fragments = !singleEnd,
 maxMismatchRate = 0.02,
  suboptimalAlignmentTag = "auto",
  suboptimalAlignmentCutoff = 5,
  geneCountMode = "all",
  verbose = TRUE
)
## S4 method for signature 'ERVmapParam'
show(object)
```

### **Arguments**

bf1

A BamFile or BamFileList object, or a character string vector of BAM filenames.

teFeatures

A GRanges or GRangesList object with the transposable element (TE) annotated features to be quantified. Elements in this object should have names, which are used as a grouping factor for genomic ranges forming a common locus, unless other metadata column names are specified in the aggregateby parameter.

aggregateby

Character vector with column names in the annotation to be used to aggregate quantifications. By default, this is an empty vector, which means that the names of the input GRanges or GRangesList object given in the teFeatures parameter

are used to aggregate quantifications.

ovMode

Character vector indicating the overlapping mode. Available options are: "ovUnion" (default) and "ovIntersectionStrict", which implement the corresponding methods from HTSeq (https://htseq.readthedocs.io/en/release\_0.11.

1/count.html). Ambiguous alignments (alignments overlapping > 1 feature) are addressed as in the original ERVmap algorithm.

geneFeatures

(Default NULL) A GRanges or GRangesList object with the gene annotated features to be quantified. Overlaps with unique reads are first tallied with respect to these gene features. Elements should have names indicating the gene name/id. In case that geneFeatures is a GRanges and contains a metadata column named type, only the elements with type = exon are considered for the analysis. Then, exon counts are summarized to the gene level. If NULL, gene expression is not quantified.

singleEnd

(Default TRUE) Logical value indicating if reads are single (TRUE) or paired-end (FALSE).

ignoreStrand

(Default TRUE) A logical which defines if the strand should be taken into consideration when computing the overlap between reads and annotated features. When ignore\_strand = FALSE, an aligned read is considered to be overlapping an annotated feature as long as they have a non-empty intersecting genomic range on the same strand, while when ignoreStrand = TRUE the strand is not considered.

strandMode

(Default 1) Numeric vector which can take values 0, 1 or 2. The strand mode is a per-object switch on GalignmentPairs objects that controls the behavior of the strand getter. See GalignmentPairs class for further detail. If singleEnd = TRUE, then strandMode is ignored.

fragments

(Default not singleEnd) A logical; applied to paired-end data only. When fragments=TRUE, the read-counting method in the original ERVmap algorithm is applied: each mate of a paired-end read is counted (including ambiguous and not properly paired reads). When fragments=FALSE, if the two mates of a paired-end read map to the same element, they are counted as a single hit and singletons, reads with unmapped pairs and other ambiguous or not properly paired fragments are not counted (see "Pairing criteria" in readGAlignments()).

#### maxMismatchRate

(Default 0.02) Numeric value storing the maximum mismatch rate employed by the ERVmap algorithm to discard aligned reads whose rate of sum of hard and soft clipping or whose rate of the edit distance over the genome reference to the length of the read is above this threshold.

## suboptimalAlignmentTag

(Default "auto") Character string storing the tag name in the BAM files that stores the suboptimal alignment score used in the third filter of ERVmap; see Tokuyama et al. (2018). The default, suboptimalAlignmentTag="auto", first extracts the name of the read mapper software from one or more BAM files. If BAM files were generated by BWA, the suboptimal alignment scores are obtained from a tag called XS. For other read mappers, the suboptimal alignment score is considered to be missing since, except from BWA, no other aligner provides a tag with suboptimal alignment scores. In this case, the available secondary alignments are used to implement an analogous approach to that of

the third ERVmap filter. When suboptimalAlignmentTag="none", it also performs the latter approach even when the tag XS is available. When this parameter is different from "auto" and "none", a tag with the given name is used to extract the suboptimal alignment score.

suboptimalAlignmentCutoff

(Default 5) Numeric value storing the cutoff above which the difference between the alignment score and the suboptimal alignment score is considered sufficiently large to retain the alignment. When this value is set to NA, the filtering step based on suboptimal alignment scores is skipped.

geneCountMode (Default "all") Character string indicating if the ERVmap read filters applied to

quantify TEs expression should also be applied when quantifying gene expression ("ervmap") or not ("all"), in which case all primary alignments mapping

to genes are counted.

verbose (Default TRUE) Logical value indicating whether to report progress.

object A ERVmapParam object.

#### **Details**

This is the constructor function for objects of the class ERVmapParam-class. This type of object is the input to the function qtex() for quantifying expression of transposable elements using the ERVmap method Tokuyama et al. (2018). The ERVmap algorithm processes reads following conservative filtering criteria to provide reliable raw count data for each TE.

## Value

A ERVmapParam object.

# **Slots**

readMapper The name of the software used to align reads, obtained from the BAM file header.

singleEnd (Default FALSE) Logical value indicating if reads are single (TRUE) or paired-end (FALSE).

strandMode (Default 1) Numeric vector which can take values 0, 1 or 2. The strand mode is a per-object switch on GAlignmentPairs objects that controls the behavior of the strand getter. See GAlignmentPairs class for further detail. If singleEnd = TRUE, then strandMode #' is ignored.

ignoreStrand (Default TRUE) A logical which defines if the strand should be taken into consideration when computing the overlap between reads and TEs in the annotations. When ignore\_strand = FALSE, only those reads which overlap the TE and are on the same strand are counted. On the contrary, when ignore\_strand = TRUE, any read overlapping an element in teFeatures is counted regardless of the strand.

fragments (Default not singleEnd) A logical; applied to paired-end data only. When fragments=TRUE, the read-counting method in the original ERVmap algorithm is applied: each mate of a paired-end read is counted (including ambiguous and not properly paired reads). When fragments=FALSE, if the two mates of a paired-end read map to the same element, they are counted as a single hit and singletons, reads with unmapped pairs and other ambiguous or not properly paired fragments are not counted (see "Pairing criteria" in readGAlignments()).

maxMi smatchRate (Default 0.02) Numeric value storing the maximum mismatch rate employed by the ERVmap algorithm to discard aligned reads whose rate of sum of hard and soft clipping, or of the edit distance over the genome reference, to the length of the read is above this threshold.

- suboptimalAlignmentTag (Default "auto") Character string storing the tag name in the BAM files that stores the suboptimal alignment score used in the third filter of ERVmap; see Tokuyama et al. (2018). The default, suboptimalAlignmentTag="auto", assumes that either the BAM files were generated by BWA and include a tag called XS that stores the suboptimal alignment score or, if the XS tag is not available, then it uses the available secondary alignments to implement an analogous approach to that of the third ERVmap filter. When suboptimalAlignmentTag="none", it also performs the latter approach even when the tag XS is available. When this parameter is different from "auto" and "none", a tag with the given name is used to extract the suboptimal alignment score. The absence of that tag will prompt an error.
- suboptimalAlignmentCutoff (Default 5) Numeric value storing the cutoff above which the difference between the alignment score and the suboptimal alignment score is considered sufficiently large to retain the alignment. When this value is set to NA, then the filtering step based on suboptimal alignment scores is skipped.
- geneCountMode (Default "all") Character string indicating if the ERVmap read filters applied to quantify TEs expression should also be applied when quantifying gene expression ("ervmap") or not ("all"), in which case all primary alignments mapping to genes are counted.

#### References

Tokuyama M et al. ERVmap analysis reveals genome-wide transcription of human endogenous retroviruses. PNAS. 2018;115(50):12565-12572. DOI: https://doi.org/10.1073/pnas.1814589115 Tokuyama M et al. ERVmap analysis reveals genome-wide transcription of human endogenous retroviruses. PNAS. 2018;115(50):12565-12572. DOI: https://doi.org/10.1073/pnas.1814589115

```
bamfiles <- list.files(system.file("extdata", package="atena"),</pre>
                        pattern="*.bam", full.names=TRUE)
## Not run:
rmskat <- annotaTEs(genome="dm6", parsefun=rmskatenaparser,</pre>
                     strict=FALSE, insert=500)
rmskLTR <- getLTRs(rmskat, relLength=0.8,
                    fullLength=TRUE,
                    partial=TRUE,
                    otherLTR=TRUE)
## End(Not run)
## DO NOT TYPE THIS INSTRUCTION, WHICH JUST LOADS A PRE-COMPUTED ANNOTATION
## YOU SHOULD USE THE INSTRUCTIONS ABOVE TO FETCH ANNOTATIONS
rmskLTR <- readRDS(system.file("extdata", "rmskatLTRrlen80flenpartoth.rds",</pre>
                                package="atena"))
## build a parameter object for ERVmap
empar <- ERVmapParam(bamfiles,</pre>
                      teFeatures=rmskLTR,
```

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```
singleEnd=TRUE,
ignoreStrand=TRUE,
suboptimalAlignmentCutoff=NA)
```

empar

OneCodeToFindThemAll OneCodeToFindThemAll parser of RepeatMasker annotations

# **Description**

OneCodeToFindThemAll parser of RepeatMasker annotations

# Usage

```
OneCodeToFindThemAll(
   gr,
   dictionary = NULL,
   fuzzy = FALSE,
   strict = FALSE,
   insert = -1,
   BPPARAM = SerialParam(progressbar = TRUE)
)
```

# Arguments

insert

**BPPARAM** 

gr	A ['GRanges'][GenomicRanges::GRanges-class] object with RepeatMasker annotations from AnnotationHub
dictionary	(Default NULL) When NULL, a dictionary is built based on names of repeats. If not, a data.frame with equivalences LTR - internal regions created by the user, where first column should be the name of the internal region and the second column should be the LTR(s). When more than one LTR, these should be separated by ":".
fuzzy	(Default FALSE) A logical; if TRUE, the search for equivalences between internal parts and LTRs to reconstruct LTR class transposable elements is less stringent, allowing more matches between corresponding subparts. This option can increase the proportion of false positives (incorrectly reconstructed LTR class TEs).
strict	(Default FALSE) A logical; if TRUE, the 80-80 rule is applied, i.e. only copies

(Default FALSE) A logical; if TRUE, the 80-80 rule is applied, i.e. only copies

with more than 80 and more than 80 bp long are reported.

(Default -1) An integer. When insert < 0, two fragments are assembled if the distance separating their furthest extremities is less than twice the reference length of the element. When insert > 0, fragments are assembled if the distance between their closest extremities is equal or less than insert. When insert =

0, two fragments are assembled if they are in contact next to each other.

See ?bplapply in the BiocParallel package. Can be used to run calculations in

parallel.

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#### **Details**

Implementation of One code to find them all (Bailly-Bechet et al. 2014). Parses RepeatMasker annotations from UCSC by assembling together fragments from the same transposable elemenet (TE) that are close enough (determined by the insert parameter). For TEs from the LTR class, the parser tries to reconstruct full-length, when possible, or partial TEs following the LTR - internal region - LTR structure. Equivalences between internal regions and flanking LTRs can be set by the user with the dictionary parameter or can be obtained by the parser. In this last case, the fuzzy parameter determines the level of stringency when searching for LTR - internal region equivalences.

#### Value

A ['GRangesList'][GenomicRanges::GRangesList-class] object.

#### References

Bailly-Bechet et al. "One code to find them all": a perl tool to conveniently parse RepeatMasker output files. Mobile DNA. 2014;5(1):1-15. DOI: https://doi.org/10.1186/1759-8753-5-13

# **Examples**

ovUnion

Pre-defined overlapping mode functions

# **Description**

The following functions control the way in which overlaps between aligned reads and annotated features are resolved when an aligned read overlaps more than one feature on the same locus:

# Usage

```
ovUnion(reads, features, ignoreStrand, inter.feature = TRUE)
ovIntersectionStrict(reads, features, ignoreStrand, inter.feature = TRUE)
```

#### **Arguments**

reads A GAlignments, GAlignmentList or a GAlignmentPairs object.

features A GRanges object with annotated features.

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ignoreStrand

(Default FALSE) A logical which defines if the strand should be taken into consideration when computing the overlap between reads and annotated features. When ignoreStrand = FALSE, an aligned read will be considered to be overlapping an annotated feature as long as they have a non-empty intersecting genomic ranges on the same strand, while when ignoreStrand = TRUE the strand will not be considered.

inter.feature

When TRUE, ambiguous alignments (alignments overlapping > 1 features) are removed and not counted. When inter.feature is set to FALSE, these ambiguous overlaps are taken into account and addressed differently depending on the TE quantification.

#### **Details**

- ovUnion(): (default)
- ovIntersectionStrict():
- User supplied: a function taking the same parameters as the previous three functions and returning a Hits object.

They take the following parameters:

These functions are given to the mode parameter of the <code>qtex()</code> function and are similar to the functions <code>Union()</code> and <code>IntersectionStrict()</code> from the <code>GenomicAlignments</code> package, with the difference that instead of returning counts of reads overlapping annotated features, they return the actual overlaps, because the counting is deferred to other algorithms that follow some specific strategy when a read maps to more than one feature. For this same reason, these functions lack the <code>inter.feature</code> argument found in the corresponding functions from the <code>GenomicAlignments</code> package.

# Value

A Hits object; see the Hits-class manual page.

qtex, ERVmapParam-method

Quantify transposable element expression

# Description

The qtex() method quantifies transposable element expression.

# Usage

```
## S4 method for signature 'ERVmapParam'
qtex(
  Х,
  phenodata = NULL,
 mode = ovUnion,
 yieldSize = 1000000L,
  verbose = 1,
 BPPARAM = SerialParam(progressbar = ifelse(verbose == 1, TRUE, FALSE))
## S4 method for signature 'TEtranscriptsParam'
qtex(
  Х,
  phenodata = NULL,
 mode = ovUnion,
 yieldSize = 1000000L,
 BPPARAM = SerialParam(progressbar = TRUE)
)
## S4 method for signature 'TelescopeParam'
qtex(
  Х,
  phenodata = NULL,
 mode = ovUnion,
 yieldSize = 1000000L,
  auxiliaryFeatures = FALSE,
  BPPARAM = SerialParam(progressbar = TRUE)
```

```
## S4 method for signature 'atenaParam'
qtex(
  х,
  phenodata = NULL,
 mode = ovUnion,
 yieldSize = 1000000L,
  auxiliaryFeatures = FALSE,
 BPPARAM = SerialParam(progressbar = TRUE)
)
```

## **Arguments**

Х

An QuantifyParam object of one of the following subclasses:

- A TEtranscriptsParam object built using the constructor function TEtranscriptsParam(). This object will trigger qtex() to use the quantification algorithm by Jin et al. (2015).
- A ERVmapParam object built using the constructor function ERVmapParam(). This object will trigger qtex() to use the quantification algorithm by Tokuyama et al. (2018).
- A TelescopeParam object built using the constructor function TelescopeParam(). This object will trigger qtex() to use the quantification algorithm by Bendall et al. (2019).
- An atenaParam object built using the constructor function atenaParam(). This object will trigger qtex() to use a quantification algorithm specifically developed in this package.

phenodata

A data. frame or DataFrame object storing phenotypic data to include in the resulting ['SummarizedExperiment'] [SummarizedExperiment::SummarizedExperimentclass] object. If phenodata is set, its row names will become the column names of the resulting ['SummarizedExperiment'][SummarizedExperiment::SummarizedExperimentclass] object.

mode

One of the pre-defined overlapping methods such as ovUnion(), ovIntersectionStrict or a user-supplied overlapping function. For a user-supplied overlapping function, the input parameters must match those of the pre-defined methods and the function must return a Hits object with subject hits matching the annotated features. This parameter is analogous to the mode parameter of the summarizeOverlaps() function from the GenomicAlignments package.

yieldSize

Field inherited from BamFile. The method for signature ERVmapParam() reads the BAM file by chunks. yieldSize represents the number of records (chunk size) to yield each time the file is read.

verbose

(Default 1). When verbose > 1, detailed information on the quantification steps is provided. Warnings are always present regardless of the value of verbose.

**BPPARAM** 

An object of a ['BiocParallelParam'][BiocParallel::BiocParallelParam-class] subclass to configure the parallel execution of the code. By default, ['BiocParallelParam'][BiocParallel::Seria class] object is used, which does not use any parallelization, with the flag progress=TRUE to show progress through the calculations.

auxiliaryFeatures

(Default FALSE). It only applies when 'x' is a ['TelescopeParam'] or an ['atena-Param'] object. When TRUE, auxiliary features created during expression quantification are also returned in the ['SummarizedExperiment'] ['SummarizedExperiment'] [SummarizedExperiment'] [SummarizedExperiment'] [SummarizedExperiment'] [SummarizedExperiment']

#### **Details**

Giving some AtenaParam object sub-class as input, the qtex() method quantifies the expression of transposable elements (TEs). The particular algorithm to perform the quantification will be selected depending on the specific sub-class of input AtenaParam object, see argument x above.

#### Value

A ['SummarizedExperiment'][SummarizedExperiment::SummarizedExperiment-class] object.

#### References

Jin Y et al. TEtranscripts: a package for including transposable elements in differential expression analysis of RNA-seq datasets. Bioinformatics. 2015;31(22):3593-3599. DOI: https://doi.org/10.1093/bioinformatics/btv422

Tokuyama M et al. ERVmap analysis reveals genome-wide transcription of human endogenous retroviruses. PNAS, 115(50):12565-12572, 2018. https://doi.org/10.1073/pnas.1814589115

Bendall ML et al. Telescope: characterization of the retrotranscriptome by accurate estimation of transposable element expression. PLOS Computational Biology, 15:e1006453, 2019. https://doi.org/10.1371/journal.pcbi.1006453

#### See Also

TEtranscriptsParam ERVmapParam TelescopeParam

```
bamfiles <- list.files(system.file("extdata", package="atena"),</pre>
                       pattern="*.bam", full.names=TRUE)
## Not run:
## use the following two instructions to fetch annotations, they are here
## commented out to enable running this example quickly when building and
## checking the package
rmskat <- annotaTEs(genome="dm6", parsefun=rmskatenaparser,</pre>
                    strict=FALSE, insert=500)
rmskLTR <- getLTRs(rmskat, relLength=0.8,
                   fullLength=TRUE,
                   partial=TRUE,
                   otherLTR=TRUE)
## End(Not run)
## DO NOT TYPE THIS INSTRUCTION, WHICH JUST LOADS A PRE-COMPUTED ANNOTATION
## YOU SHOULD USE THE INSTRUCTIONS ABOVE TO FETCH ANNOTATIONS
rmskLTR <- readRDS(system.file("extdata", "rmskatLTRrlen80flenpartoth.rds",</pre>
```

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QuantifyParam-class

QuantifyParam parameter class

# **Description**

This is a virtual class from which other classes are derived for storing parameters provided to quantification methods of transposable elements from RNA-seq data.

#### Usage

```
## S4 method for signature 'QuantifyParam'
path(object)
## S4 method for signature 'QuantifyParam'
features(x)
```

#### **Arguments**

object A ['QuantifyParam'] object. x A ['QuantifyParam'] object.

# Value

path(): Filesystem paths to the BAM files in the input parameter object.

features(): The GenomicRanges or GenomicRangesList object with the features in the input parameter object.

# **Slots**

bfl A ['BamFileList'][Rsamtools::BamFileList-class] object.

 $features\ A\ [`GRanges'] [Genomic Ranges:: GRanges-class]\ object.$ 

aggregateby Character vector with column names in the annotation to be used to aggregate quantifications.

ovMode Character vector indicating the overlapping mode. Available options are: "ovUnion" (default) and "ovIntersectionStrict", which implement the corresponding methods from HTSeq (https://htseq.readthedocs.io/en/release\_0.11.1/count.html). In the TEtranscripts, ERVmap and Telescope methods ambiguous alignments (alignments overlapping > 1 feature) are addressed differently depending on the method. In the atena method, those overlaps are not counted.

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# See Also

ERVmapParam-class TelescopeParam-class TEtranscriptsParam-class atenaParam-class

# **Examples**

```
bamfiles <- list.files(system.file("extdata", package="atena"),</pre>
                       pattern="*.bam", full.names=TRUE)
## Not run:
## use the following two instructions to fetch annotations, they are here
## commented out to enable running this example quickly when building and
## checking the package
rmskat <- annotaTEs(genome="dm6", parsefun=rmskatenaparser,</pre>
                    strict=FALSE, insert=500)
rmskLTR <- getLTRs(rmskat, relLength=0.8,
                   fullLength=TRUE,
                   partial=TRUE)
## End(Not run)
## DO NOT TYPE THIS INSTRUCTION, WHICH JUST LOADS A PRE-COMPUTED ANNOTATION
## YOU SHOULD USE THE INSTRUCTIONS ABOVE TO FETCH ANNOTATIONS
rmskLTR <- readRDS(system.file("extdata", "rmskatLTRrlen80flenpartoth.rds",</pre>
                                package="atena"))
## build a parameter object for TEtranscripts
ttpar <- TEtranscriptsParam(bamfiles,</pre>
                             teFeatures=rmskLTR,
                             singleEnd=TRUE,
                             ignoreStrand=TRUE)
## just check that the parameter object belongs to the expected classes
is(ttpar, "QuantifyParam")
is(ttpar, "TEtranscriptsParam")
```

rmskatenaparser

atena annotation parser of RepeatMasker annotations

# **Description**

atena annotation parser of RepeatMasker annotations

# Usage

```
rmskatenaparser(gr, strict = FALSE, insert = 1000)
```

## **Arguments**

gr

A ['GRanges'][GenomicRanges::GRanges-class] object with RepeatMasker annotations from AnnotationHub

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strict (Default FALSE) A logical; if TRUE, the 80-80 rule is applied, i.e. only copies

with more than 80 and more than 80 bp long are reported.

insert (Default 1000L) An integer > 0. Fragments are assembled together if the dis-

tance between their closest extremities is equal or less than insert. When insert = 0, two fragments are assembled if they are in contact next to each

other.

#### **Details**

atena annotation parser of RepeatMasker annotations. Parses RepeatMasker annotations from UCSC by assembling together fragments from the same transposable element (TE) that are close enough (determined by the insert parameter). For TEs from the LTR class, the parser tries to reconstruct full-length, when possible, or partial TEs following the LTR - internal region - LTR structure. Equivalences between LTR and internal regions are found by, first, identifying LTR regions (those with the "LTR" substring in their name) and internal regions (those with a suffix such as "-int", "-I", etc.). Then, LTR are assigned to internal regions for which the comparison of the two names are has a higher number of equal consecutive characters.

# Value

A ['GRangesList'][GenomicRanges::GRangesList-class] object.

# **Examples**

rmskbasicparser

Parser of RepeatMasker annotations

# **Description**

Parser of RepeatMasker annotations

# Usage

```
rmskbasicparser(gr)
```

# Arguments

gr

A ['GRanges'][GenomicRanges::GRanges-class] object with RepeatMasker annotations from AnnotationHub

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# **Details**

Parses annotations by removing low complexity regions, simple repeats, satellites, rRNA, scRNA, snRNA, srpRNA and tRNA. Also removes TEs with a strand different than "+" or "-". Modifies "repFamily" and "repClass" columns when a "?" is present or when they are defined as "Unknown" or "Other". Finally, assigns a unique id to each TE instance by adding the suffix "\_dup" plus a number at the end of the "repName".

# Value

A ['GRanges'][GenomicRanges::GRanges-class] object.

# **Examples**

```
rmskba <- annotaTEs(genome="dm6", parsefun=rmskbasicparser)
rmskba</pre>
```

rmskidentity

Identity function for parsefun

# **Description**

Identity function for parsefun

# Usage

```
rmskidentity(gr)
```

# **Arguments**

gr

A ['GRanges'][GenomicRanges::GRanges-class] object.

# **Details**

Identity function: returns the ['GRanges'][GenomicRanges::GRanges-class] object without any modification.

# Value

A ['GRanges'][GenomicRanges::GRanges-class] object.

```
rmskid <- annotaTEs(genome="dm6", parsefun=rmskidentity)
rmskid</pre>
```

TelescopeParam-class 25

TelescopeParam-class Telescope parameter class

# **Description**

This is a class for storing parameters provided to the Telescope algorithm. Build an object of the class TelescopeParam.

# Usage

```
TelescopeParam(
  bfl,
  teFeatures,
  aggregateby = character(0),
  ovMode = "ovUnion",
  geneFeatures = NULL,
  singleEnd = TRUE,
  strandMode = 1L,
  ignoreStrand = FALSE,
  fragments = FALSE,
 minOverlFract = 0.2,
  pi_prior = 0L,
  theta_prior = 0L,
  em_epsilon = 1e-07,
 maxIter = 100L,
  reassign_mode = "exclude",
  conf_prob = 0.9,
  verbose = TRUE
)
## S4 method for signature 'TelescopeParam'
show(object)
```

# **Arguments**

bfl

A BamFile or BamFileList object, or a character string vector of BAM filenames.

teFeatures

A GRanges or GRangesList object. Elements in this object should have names, which are used as a grouping factor for genomic ranges forming a common locus (equivalent to "locus" column in Telescope). This grouping is performed previous to TE expression quantification, unlike the aggregation of quantifications performed when the aggregateby parameter is specified, which is performed after individual TE instances are quantified.

aggregateby

Character vector with column names from the annotation to be used to aggregate quantifications. By default, this is an empty vector, which means that the names of the input GRanges or GRangesList object given in the teFeatures parameter are used to aggregate quantifications.

ovMode

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Character vector indicating the overlapping mode. Available options are: "ovUnion" (default) and "ovIntersectionStrict", which implement the corresponding methods from HTSeq (https://htseq.readthedocs.io/en/release\_0.11. 1/count.html). Ambiguous alignments (alignments overlapping > 1 feature) are addressed as in the original Telescope method: the overlap with the longest overlapping length is kept.

geneFeatures

(Default NULL) A GRanges or GRangesList object with the gene annotated features to be quantified. The TEtranscripts approach for gene expression quantification is used, in which overlaps with multi-mapping reads are preferentially assigned to TEs. Elements should have names indicating the gene name/id. In case that geneFeatures is a GRanges and contains a metadata column named type, only the elements with type = exon are considered for the analysis. Then, exon counts are summarized to the gene level. If NULL, gene expression is not quantified.

singleEnd

(Default TRUE) Logical value indicating if reads are single (TRUE) or paired-end (FALSE).

strandMode

(Default 1) Numeric vector which can take values 0, 1 or 2. The strand mode is a per-object switch on GalignmentPairs objects that controls the behavior of the strand getter. See GalignmentPairs class for further detail. If singleEnd = TRUE, then strandMode is ignored.

ignoreStrand

(Default FALSE) A logical which defines if the strand should be taken into consideration when computing the overlap between reads and annotated features. When ignoreStrand = FALSE, an aligned read is considered to be overlapping an annotated feature as long as they have a non-empty intersecting genomic range on the same strand, while when ignoreStrand = TRUE the strand is not considered.

fragments

(Default FALSE) A logical; applied to paired-end data only. When fragments=FALSE, the read-counting method only counts 'mated pairs' from opposite strands (non-ambiguous properly paired reads), while when fragments=TRUE same-strand pairs, singletons, reads with unmapped pairs and other ambiguous or not properly paired fragments are also counted (see "Pairing criteria" in readGAlignments()). fragments=TRUE is equivalent to the original Telescope algorithm. For further details see summarizeOverlaps().

minOverlFract

(Default 0.2) A numeric scalar. minOverlFract is multiplied by the read length and the resulting value is used to discard alignments for which the overlapping length (number of base pairs the alignment and the feature overlap) is lower. When no minimum overlap is required, set minOverlFract = 0.

pi\_prior

(Default 0) A positive integer scalar indicating the prior on pi. This is equivalent to adding n unique reads.

theta\_prior

(Default 0) A positive integer scalar storing the prior on Q. Equivalent to adding n non-unique reads.

em\_epsilon

(Default 1e-7) A numeric scalar indicating the EM Algorithm Epsilon cutoff.

maxIter

A positive integer scalar storing the maximum number of iterations of the EM SQUAREM algorithm (Du and Varadhan, 2020). Default is 100 and this value is passed to the maxiter parameter of the squarem() function.

reassign\_mode (Default 'exclude') Character vector indicating reassignment mode after EM

step. Available methods are 'exclude' (reads with more than one best assignment are excluded from the final counts), 'choose' (when reads have more than one best assignment, one of them is randomly chosen), 'average' (the read count is divided evenly among the best assignments) and 'conf' (only assignments that exceed a certain threshold -defined by conf\_prob parameter- are accepted, then the read count is proportionally divided among the assignments

above conf\_prob).

conf\_prob (Default 0.9) Minimum probability for high confidence assignment. verbose (Default TRUE) Logical value indicating whether to report progress.

object A TelescopeParam object.

#### **Details**

This is the constructor function for objects of the class TelescopeParam-class. This type of object is the input to the function qtex() for quantifying expression of transposable elements, which will call the Telescope algorithm Bendall et al. (2019) with this type of object.

#### Value

A TelescopeParam object.

#### **Slots**

singleEnd (Default TRUE) Logical value indicating if reads are single (TRUE) or paired-end (FALSE).

strandMode (Default 1) Numeric vector which can take values 0, 1 or 2. The strand mode is a perobject switch on GAlignmentPairs objects that controls the behavior of the strand getter. See GAlignmentPairs class for further detail. If singleEnd = TRUE, then strandMode is ignored.

- ignoreStrand (Default FALSE) A logical which defines if the strand should be taken into consideration when computing the overlap between reads and annotated features. When ignoreStrand = FALSE, an aligned read is considered to be overlapping an annotated feature as long as they have a non-empty intersecting genomic range on the same strand, while when ignoreStrand = TRUE the strand is not considered.
- fragments (Default FALSE) A logical; applied to paired-end data only. When fragments=FALSE, the read-counting method only counts 'mated pairs' from opposite strands (non-ambiguous properly paired reads), while when fragments=TRUE same-strand pairs, singletons, reads with unmapped pairs and other ambiguous or not properly paired fragments are also counted (see "Pairing criteria" in readGAlignments()). fragments=TRUE is equivalent to the original Telescope algorithm. For further details see summarizeOverlaps().
- minOverlFract (Default 0.2) A numeric scalar. minOverlFract is multiplied by the read length and the resulting value is used to discard alignments for which the overlapping length (number of base pairs the alignment and the feature overlap) is lower. When no minimum overlap is required, set minOverlFract = 0.
- pi\_prior (Default 0) A positive integer scalar indicating the prior on pi. This is equivalent to adding n unique reads.
- theta\_prior (Default 0) A positive integer scalar storing the prior on Q. Equivalent to adding n non-unique reads.

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em\_epsilon (Default 1e-7) A numeric scalar indicating the EM Algorithm Epsilon cutoff.

maxIter A positive integer scalar storing the maximum number of iterations of the EM SQUAREM algorithm (Du and Varadhan, 2020). Default is 100 and this value is passed to the maxiter parameter of the squarem() function.

reassign\_mode (Default 'exclude') Character vector indicating reassignment mode after EM step. Available methods are 'exclude' (reads with more than one best assignment are excluded from the final counts), 'choose' (when reads have more than one best assignment, one of them is randomly chosen), 'average' (the read count is divided evenly among the best assignments) and 'conf' (only assignments that exceed a certain threshold -defined by conf\_prob parameter- are accepted, then the read count is proportionally divided among the assignments above conf\_prob).

conf\_prob (Default 0.9) Minimum probability for high confidence assignment.

#### References

Bendall et al. Telescope: characterization of the retrotranscriptome by accurate estimation of transposable element expression. PLOS Comp. Biol. 2019;15(9):e1006453. DOI: https://doi.org/10.1371/journal.pcbi.1006453

Bendall et al. Telescope: characterization of the retrotranscriptome by accurate estimation of transposable element expression. PLOS Comp. Biol. 2019;15(9):e1006453. DOI: https://doi.org/10.1371/journal.pcbi.1006453

```
bamfiles <- list.files(system.file("extdata", package="atena"),</pre>
                       pattern="*.bam", full.names=TRUE)
## Not run:
## use the following two instructions to fetch annotations, they are here
## commented out to enable running this example quickly when building and
## checking the package
rmskat <- annotaTEs(genome="dm6", parsefun=rmskatenaparser,</pre>
                     strict=FALSE, insert=500)
rmskLTR <- getLTRs(rmskat, relLength=0.8,
                   fullLength=TRUE,
                   partial=TRUE,
                   otherLTR=TRUE)
## End(Not run)
## DO NOT TYPE THIS INSTRUCTION, WHICH JUST LOADS A PRE-COMPUTED ANNOTATION
## YOU SHOULD USE THE INSTRUCTIONS ABOVE TO FETCH ANNOTATIONS
rmskLTR <- readRDS(system.file("extdata", "rmskatLTRrlen80flenpartoth.rds",</pre>
                                package="atena"))
## build a parameter object for Telescope
tspar <- TelescopeParam(bfl=bamfiles,</pre>
                         teFeatures=rmskLTR,
                         singleEnd=TRUE,
                         ignoreStrand=TRUE)
tspar
```

TEtranscriptsParam-class

TEtranscripts parameter class

# **Description**

This is a class for storing parameters provided to the TEtranscripts algorithm. It is a subclass of the ['QuantifyParam'].

Build an object of the class TEtranscriptsParam

# Usage

```
TEtranscriptsParam(
  bfl,
  teFeatures,
  aggregateby = character(0),
  ovMode = "ovUnion",
  geneFeatures = NULL,
  singleEnd = TRUE,
  ignoreStrand = FALSE,
  strandMode = 1L,
  fragments = TRUE,
  tolerance = 1e-04,
 maxIter = 100L,
  verbose = TRUE
)
## S4 method for signature 'TEtranscriptsParam'
show(object)
```

# **Arguments**

bfl a character string vector of BAM file names.

teFeatures A GRanges or GRangesList object with the TE annotated features to be quanti-

fied. Elements in this object should have names, which are used as a grouping factor for genomic ranges forming a common locus, unless other metadata col-

umn names are specified in the aggregateby parameter.

aggregateby Character vector with column names from the annotation to be used to aggregate

quantifications. By default, this is an empty vector, which means that the names of the input GRanges or GRangesList object given in the teFeatures parameter

are used to aggregate quantifications.

ovMode Character vector indicating the overlapping mode. Available options are: "ovU-

nion" (default) and "ovIntersectionStrict", which implement the corresponding methods from HTSeq (https://htseq.readthedocs.io/en/release\_0.11.

1/count.html). Ambiguous alignments (alignments overlapping > 1 feature) are addressed as in the original TEtranscripts method.

geneFeatures (Default NULL) A GRanges or GRangesList object with the gene annotated

features to be quantified. Following the TEtranscripts algorithm, overlaps with unique reads are first tallied with respect to these gene features. Elements should have names indicating the gene name/id. In case that geneFeatures is a GRanges and contains a metadata column named type, only the elements with type = exon are considered for the analysis. Then, exon counts are summarized

to the gene level. If NULL, gene expression is not quantified.

singleEnd (Default TRUE) Logical value indicating if reads are single (TRUE) or paired-end

(FALSE).

ignoreStrand (Default FALSE) Logical value that defines if the strand should be taken into

consideration when computing the overlap between reads and annotated features. When ignoreStrand = FALSE, an aligned read is considered to be overlapping an annotated feature as long as they have a non-empty intersecting genomic range on the same strand, while when ignoreStrand = TRUE the strand

is not considered.

strandMode (Default 1) Numeric vector which can take values 0, 1 or 2. The strand mode is

a per-object switch on GAlignmentPairs objects that controls the behavior of the strand getter. See GAlignmentPairs class for further detail. If singleEnd =

TRUE, then strandMode is ignored.

fragments (Default TRUE) Logical value applied to paired-end data only. In both cases

(fragments=FALSE and fragments=TRUE), the read-counting method discards not properly paired reads. Moreover, when fragments=FALSE, only non-ambiguous properly paired reads are counted. When fragments=TRUE, ambiguous reads are also counted (see "Pairing criteria" in readGAlignments()). fragments=TRUE is equivalent to the behavior of the TEtranscripts algorithm. For further details

see summarizeOverlaps().

tolerance A positive numeric scalar storing the minimum tolerance above which the SQUAREM

algorithm (Du and Varadhan, 2020) keeps iterating. Default is 1e-4 and this

value is passed to the tol parameter of the squarem() function.

maxIter A positive integer scalar storing the maximum number of iterations of the SQUAREM

algorithm (Du and Varadhan, 2020). Default is 100 and this value is passed to

the maxiter parameter of the squarem() function.

verbose (Default TRUE) Logical value indicating whether to report progress.

object A TEtranscriptsParam object.

# **Details**

This is the constructor function for objects of the class TEtranscriptsParam-class. This type of object is the input to the function qtex() for quantifying expression of transposable elements using the TEtranscripts method Jin et al. (2015). The TEtranscripts algorithm quantifies TE expression by using an EM algorithm to optimally distribute ambiguously mapped reads.

# Value

A TEtranscriptsParam object.

#### **Slots**

- singleEnd (Default FALSE) Logical value indicating if reads are single (TRUE) or paired-end (FALSE).
- ignoreStrand (Default FALSE) A logical which defines if the strand should be taken into consideration when computing the overlap between reads and annotated features. When ignoreStrand = FALSE, an aligned read will be considered to be overlapping an annotated feature as long as they have a non-empty intersecting genomic ranges on the same strand, while when ignoreStrand = TRUE the strand will not be considered.
- strandMode (Default 1) Numeric vector which can take values 0, 1 or 2. The strand mode is a perobject switch on GAlignmentPairs objects that controls the behavior of the strand getter. See GAlignmentPairs class for further detail. If singleEnd = TRUE, then use either strandMode = NULL or do not specify the strandMode parameter.
- fragments (Default TRUE) A logical; applied to paired-end data only. In both cases (fragments=FALSE and fragments=TRUE), the read-counting method discards not properly paired reads. Moreover, when fragments=FALSE, only non-ambiguous properly paired reads are counted. When fragments=TRUE, ambiguous reads are also counted (see "Pairing criteria" in readGAlignments()). fragments=TRUE is equivalent to the behavior of the TEtranscripts algorithm. For further details see summarizeOverlaps().
- tolerance A positive numeric scalar storing the minimum tolerance above which the SQUAREM algorithm (Du and Varadhan, 2020) keeps iterating. Default is 1e-4 and this value is passed to the tol parameter of the squarem() function.
- maxIter A positive integer scalar storing the maximum number of iterations of the SQUAREM algorithm (Du and Varadhan, 2020). Default is 100 and this value is passed to the maxiter parameter of the squarem() function.

#### References

Jin Y et al. TEtranscripts: a package for including transposable elements in differential expression analysis of RNA-seq datasets. Bioinformatics. 2015;31(22):3593-3599. DOI: https://doi.org/10.1093/bioinformatics/btv422

Jin Y et al. TEtranscripts: a package for including transposable elements in differential expression analysis of RNA-seq datasets. Bioinformatics. 2015;31(22):3593-3599. DOI: https://doi.org/10.1093/bioinformatics/btv422

```
## End(Not run)
## DO NOT TYPE THIS INSTRUCTION, WHICH JUST LOADS A PRE-COMPUTED ANNOTATION
## YOU SHOULD USE THE INSTRUCTIONS ABOVE TO FETCH ANNOTATIONS
rmskLTR <- readRDS(system.file("extdata", "rmskatLTRrlen80flenpartoth.rds",</pre>
                                package="atena"))
library(TxDb.Dmelanogaster.UCSC.dm6.ensGene)
txdb <- TxDb.Dmelanogaster.UCSC.dm6.ensGene</pre>
txdb_genes <- genes(txdb)</pre>
## build a parameter object for TEtranscripts
ttpar <- TEtranscriptsParam(bamfiles,</pre>
                             teFeatures=rmskLTR,
                             geneFeatures=txdb_genes,
                             singleEnd=TRUE,
                             ignoreStrand=TRUE,
                             aggregateby="repName")
ttpar
```

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