Package 'RNAModR'

October 28, 2019

Type Package
Title Functional Analysis of RNA Modifications
Version 0.2.3
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Description Transcriptome-wide analysis of RNA modifications.
Imports AnnotationDbi, Biostrings, beanplot, GenomeInfoDb, gplots, graphics, graphics, grDevices, magrittr, methods, RSQLite, S4Vectors, stats, utils
Depends GenomicRanges, GenomicFeatures, IRanges, R (>= 3.5.0)
Suggests knitr, testthat, rmarkdown
License GPL-3 file LICENSE
LazyData false
RoxygenNote 6.1.1
Encoding UTF-8
VignetteBuilder knitr
R topics documented: BuildTx

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BuildTx

Build a custom transcriptome.

Description

Build a custom, organism-specific transcriptome. See 'Details'.

Usage

```
BuildTx(genomeVersion = c("hg38", "hg19", "hg18", "mm10", "mm9", "mm8",
   "dm6", "dm3", "dm2", "sacCer3", "sacCer2", "sacCer1"), force = FALSE)
```

Arguments

genomeVersion A character string; refers to a specific reference genome assembly version; de-

fault is "hg38".

force A logical scalar; if TRUE force rebuild of transcriptome; this will overwrite ex-

isting data.

Datasets 3

Details

The function builds an organism-specific transcriptome containing one transcript per unique Entrez ID; the transcript is selected from all UCSC RefSeq annotation-based isoforms as the transcript with the longest CDS, and longest upstream/downstream adjoining UTRs. Transcript segments are stored per transcript section, and written into a .RData file. For most operations, the user will run this function once, and continue with further downstream analyses. Various RNAModR routines will automatically load the transcriptome data to e.g. map sites to and from the transcriptome. Currently, RNAModR supports analyses of human, mouse, fruitfly and yeast data, based on different reference genome versions:

Homo sapiens: hg38, hg19, hg18Mus musculus: mm10, mm9, mm8

• Drosophila melanogaster: dm6, dm3, dm2

• Cerevisiae saccharomyces: sacCer3, sacCer2, sacCer1

Reconstruction of existing transcriptome data can be achieved by running BuildTx with force = TRUE. Note that this will overwrite the existing RData file. Running BuildTx with sanityCheck = TRUE performs additional checks of the various transcriptome components, and is intended for debugging purposes only. It is usually safe to run with the default sanityCheck = FALSE.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

Examples

```
## Not run:
# Build the human hg38-based reference transcriptome
BuildTx("hg38");
## End(Not run)
```

Datasets

Internal raw datasets.

Description

Internal raw datasets in BED6 format. All genome positions where converted to the most recent genome assembly version; i.e. for human data, all positions are given in GRCh38/hg38 coordinates. For mouse data, positions are given in mm10 coordinates.

Details

```
bsRNAseq_m5C_Squires2012_hg38.bed bsRNA-seq 5-methylcytosine (m5C) sites. Reference: https://www.ncbi.nlm.nih.gov/pubmed/22344696

HITSCLIP_eIF4A3_Sauliere2012_hg38.bed HITSCLIP eIF4AIII binding sites. Reference: https://www.ncbi.nlm.nih.gov/pubmed/23085716
```

MeRIPseq_m1A_Dominissini2016_hg38.bed MeRIP-seq N(1)-methyladenosine (m1A) sites. Reference: https://www.ncbi.nlm.nih.gov/pubmed/26863196

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```
miCLIP_m6A_Linder2015_hg38.bed miCLIP N(6)-methyladenosine (m6A) sites. Reference: https:
    //www.ncbi.nlm.nih.gov/pubmed/26121403

PARCLIP_AGO1234_Hafner2010_hg38.bed PAR-CLIP AGO1-4 binding sites. Reference: https:
    //www.ncbi.nlm.nih.gov/pubmed/20371350

PARCLIP_eIF3_Meyer2015_hg38.bed PAR-CLIP eIF3 binding sites. Reference: https://www.ncbi.nlm.nih.gov/pubmed/26593424

PARCLIP_eIF3_Lee2015_hg38.bed PAR-CLIP eIF3 binding sites. Reference: https://www.ncbi.nlm.nih.gov/pubmed/25849773

PARCLIP_YTHDF2_Wang2014_hg38.bed PAR-CLIP YTHDF2 binding sites. Reference: https:
    //www.ncbi.nlm.nih.gov/pubmed/24284625

PseudoU_Schwartz2014_Li2015_hg38.bed Merged Psi-seq and CeU-seq pseudouridine (Psi) sites.
    References: https://www.ncbi.nlm.nih.gov/pubmed/25219674, https://www.ncbi.nlm.nih.gov/pubmed/26075521

TargetScan_miRNAtargets_hg38.bed TargetScan-based miRNA target sites. Reference: http:
    //www.targetscan.org/vert_72/
```

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

Examples

```
## Not run:
bedFile <- ReadBED(system.file(
    "extdata",
    "miCLIP_m6A_Linder2015_hg38.bed",
    package = "RNAModR")
## End(Not run)</pre>
```

DownsampleTxLoc

Downsample a txLoc object.

Description

Downsample a txLoc1 object based on the number of sites per region from a txLoc2 object.

Usage

```
DownsampleTxLoc(txLoc1, txLoc2, seed = NULL)
```

Arguments

txLoc1	A txLoc object; this is the txLoc object that will be downsampled.
txLoc2	A txLoc object; this is the txLoc object that will be used as a target for the downsampling.
seed	A single value, interpreted as an integer, or NULL; this is to ensure reproducibility when subsampling txLoc2 sites; default is NULL.

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Value

A txLoc object.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

FilterTxLoc

Filter sections of a txLoc object.

Description

Filter sections of a txLoc object.

Usage

```
FilterTxLoc(txLoc, filter = NULL)
```

Arguments

txLoc A txLoc object.

filter A character vector; only keep transcript regions that match entries from filter;

if NULL, return the original txLoc object.

Value

A txLoc object.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

GenerateNull

Generate a list of null sites.

Description

Generate a list of null sites. See 'Details'.

```
GenerateNull(txLoc, id = NULL, method = c("ntAbund", "perm"),
  nt = "C", showPb = TRUE)
```

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Arguments

Details

showPb

The function generates a null distribution of single-nucleotide sites across different transcript sections, and returns a txLoc object. Two different methods can be employed:

A logical scalar; if TRUE show a progress bar; default is TRUE.

- 1. method = "ntAbund": Null sites are generated based on the position of all non-modified nucleotides of type nt in those transcript sections that also contain a modified site of the same type and as specified in locus. For example, if locus contains a list of m\$^6\$A sites, the list of null sites consists of all non-methylated adenosines in those transcripts that contain at least one m\$^6\$A site.
- 2. method = "perm": Null sites are generated by randomly permuting the position of sites from locus uniformly within the corresponding transcript section. Note that this will generate a list of null sites with the same abundance ratios across transcript sections as the list of sites from locus. It is therefore not useful for assessing an enrichment of sites within a particular transcript section. In fact, this method should not be used and is included purely for paedagogical purposes (to demonstrate the importance of a sensible null distribution). It is likely that this method will be removed from future RNAModR versions.

It is import to emphasise that any downstream enrichment analysis may depend critically on the choice of the null distribution. For example, a position permution-based null distribution may not be a valid null distribution, if the distribution of nucleotides is highly non-uniform across a transcript section. This is the case e.g. for the spatial distribution of cytosines within and across the 5'UTR, CDS and/or 3'UTR. In this case, a permutation- based distribution of cytosines will not give a sensible null distribution. Instead, a sensible null distribution can be derived from the position of all cytosines in the relevant transcript region containing the methylated cytosine site in locus. method = "ntAbund" generates a list of null sites using this approach.

Value

A txLoc object. See 'Details'.

Author(s)

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Examples

GetEEJunct

Get exon-exon boundary sites from reference transcriptome

Description

Get exon-exon boundary sites from transcriptome. See 'Details'.

Usage

```
GetEEJunct(refGenome = "hg38", filter = c("CDS", "5'UTR"))
```

Arguments

refGenome A character string; specifies a specific reference genome assembly version based

on which the matching transcriptome is loaded; default is "hg38".

filter A character vector; only consider transcript sections specified in filter; default

is c("CDS", "5'UTR").

Details

The function extracts exon-exon boundary sites (EEBS) from a reference trancriptome specified by refGenome, and returns a GRanges object. Boundary sites are defined as the location of those exonic single nucleotides that are closest upstream to the intronic donor site.

Value

A GRanges object. See 'Details'.

Author(s)

8 GetMotifLoc

GetMotifLoc	Get loci of motif(s).
-------------	-----------------------

Description

Get loci of motif(s) from transcriptome. See 'Details'.

Usage

```
GetMotifLoc(motif = NULL, refGenome = "hg38", id = NULL, maxMM = 0,
    showPb = TRUE)
```

Arguments

Motif A character vector; specifies the query sequences that will be matched against the reference transcriptome.

refGenome A character string; specifies the reference genome version; default is "hg38".

id A character string; specifies an identifier for motif; default is NULL

maxMM An integer scalar; specifies the maximum number of mismatches that are al-

lowed during the motif matching; default is 0.

showPb A logical scalar; if TRUE show a progress bar; default is TRUE.

Details

Based on a set of query sequences motif, extract the loci of matching sequences within different regions of a reference transcriptome refGenome. The function returns a txLoc object including transcriptomic and genomic loci of the query motif(s). The maximum number of mismatches allowed in the motif search can be adjusted through maxMM. Note that the motif search may take a few minutes, depending on the number of motif(s), hardware, and size of the transcriptome.

Value

A txLoc object.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

Examples

```
## Not run:
PAS <- GetMotifLoc(id = "PAS")
PAS <- FilterTxLoc(PAS, c("3'UTR", "CDS", "5'UTR"))
PlotSpatialDistribution(PAS, absolute = FALSE)
## End(Not run)</pre>
```

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GetSplicingSites	Get splicing sites from reference transcriptome

Description

Get splicing sites from transcriptome. See 'Details'.

Usage

```
GetSplicingSites(refGenome = "hg38", filter = c("CDS", "5'UTR"))
```

Arguments

refGenome A character string; specifies a specific reference genome assembly version based

on which the matching transcriptome is loaded; default is "hg38".

filter A character vector; only consider transcript sections specified in filter; default

is c("CDS", "5'UTR").

Details

The function extracts splicing sites from a reference trancriptome specified by refGenome, and returns a GRanges object. Splicing sites are identified as either donor (splicing site at the 5' end of the intron) or acceptor (splicing site at the 3' end of the intron) site.

Value

A GRanges object. See 'Details'.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

PlotGC Plot GC content.

Description

Plot and assess the difference in the distributions of GC content within a window around sites from two txLoc objects. See 'Details'.

```
PlotGC(txLoc1, txLoc2, flank = 10, norm_region = FALSE,
  downsample = TRUE, seed = NULL)
```

10 PlotOverlap

Arguments

txLoc1 A txLoc object. txLoc2 A txLoc object.

flank An integer scalar; see 'Details'.

norm_region A logical scalar; if TRUE normalise the GC content in the window to the GC

content of the corresponding transcript region; default is FALSE.

downsample A logical scalar; if TRUE, subsample sites from txLoc2 to match the number

of sites per transcript region from txLoc1; default is TRUE.

seed A single value, interpreted as an integer, or NULL; this is to ensure reproducibil-

ity when subsampling txLoc2 sites; ignored when downsample == FALSE; de-

fault is NULL.

Details

The function calculates the GC content within a window around every site from two txLoc objects. The window is defined by extending the position of every txLoc site upstream and downstream by flank nucleotides (if possible). The means of the resulting GC content distributions are assessed using a two-sample two-tailed t-test. If norm_region = TRUE, the GC content in the window is normalised to the GC content of the entire transcript region. If downsample = TRUE, the number of windows/sites from the *second* txLoc2 object is downsampled to match the number of windows/sites from the txLoc1 object. This is useful (and therefore the default setting) when comparing the GC distribution around positive and null sites, as the list of null sites is often significantly larger than that of the positive sites. Note that this function calls GetGC, which performs the sequence extraction and GC calculation. See ?GetGC for details.

Value

NULL.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

PlotOverlap Plot overlap of sites.

Description

Plot overlap of sites from two txLoc objects. See 'Details'.

Usage

PlotOverlap(txLoc1, txLoc2)

Arguments

txLoc1 A txLoc object. txLoc2 A txLoc object. PlotRelDistDistribution 11

Details

The function plots one or multiple Venn diagrams showing the spatial overlap between entries from two txLoc objects. Two features are defined as overlapping, if they overlap by at least one nucleotide. Overlaps are determined using the function GenomicRanges::countOverlaps.

Value

NULL.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

PlotRelDistDistribution

Plot distribution of relative distances.

Description

Plot distribution of relative distances between sites from two txLoc objects. See 'Details'.

Usage

```
PlotRelDistDistribution(txLoc, txLocRef, flank = 1000, binWidth = 20,
  doBootstrap = TRUE)
```

Arguments

txLoc A txLoc object. txLocRef A txLoc object.

flank An integer scalar; specifies the absolute maximum relative distance used as a

cutoff; default is 1000.

binWidth An integer scalar; specifies the spatial width by which distances will be binned;

default is 20.

doBootstrap A logical scalar; if YES calculate 95 transcript region; default is TRUE.

Details

The function calculates minimum distances per transcript region, between entries from txLoc relative to txLocRef. Relative distances are shown within a window (-flank, flank), where negative distances correspond to a feature from txLoc that is upstream of a site from txLocRef, and positive distances indicate a feature from txLoc that is downstream of a site from txLocRef. Relative distances are binned in bins of binWidth nt, and shown as an abundance histogram. If doBootstrap = TRUE, 95 are calculated and shown, based on an empirical bootstrap of relative distances.

Value

NULL.

Author(s)

12 PlotRelDistEnrichment

PlotRelDistEnrichment Perform enrichment analysis of relative distances.

Description

Perform enrichment analysis and plot results of two relative distance distributions. See 'Details'.

Usage

```
PlotRelDistEnrichment(txLoc1, txLoc2, txLocRef, flank = 1000,
    binWidth = 20)
```

Arguments

txLoc1 A txLoc object.

txLoc2 A txLoc object.

txLocRef A txLoc object.

flank An integer scalar; specifies the absolute maximum relative distance used as a

cutoff; default is 1000.

binWidth An integer scalar; specifies the spatial width by which distances will be binned;

default is 20.

Details

The function calculates minimum distances per transcript region, between entries from txLoc1 relative to txLocRef, and txLoc2 relative to txLocRef. Enrichment/depletion is assessed using multiple Fisher's exact tests on the counts per distance bin relative to the counts in all other bins within the window defined by (-flank, flank). Resulting enrichment plots show odds-ratios (including 95% confidence intervals) and associated p-values as a function of relative distance bins. Negative distances indicate sites from txLoc1 and txLoc2 that are *upstream* of sites from txLocRef; positive distances correspond to sites from txLoc1 and txLoc2 that are *downstream* of txLocRef. The bin width and window size can be adjusted with flank and binWidth.

Value

NULL.

Author(s)

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PlotSectionDistribution

Plot piechart of the number of loci in every transcript region

Description

Plot piechart of the number of loci in every transcript region.

Usage

```
PlotSectionDistribution(txLoc)
```

Arguments

txLoc

A txLoc object.

Value

NULL.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

Examples

PlotSectionEnrichment Perform enrichment analysis of sites per transcript region and plot results.

Description

Perform enrichment analysis of the number of positive sites in txLoc.pos relative to the number of null sites in txLoc.neg per transript region and plot results. Enrichment/depletion is evaluated using (multiple) Fisher's exact test(s). Multiple hypothesis testing correction is applied following the method of Bejamini and Hochberg.

```
PlotSectionEnrichment(txLoc.pos, txLoc.neg, xAxisLblFmt = 2)
```

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Arguments

txLoc.pos A txLoc object. These correspond to the positive sites.

txLoc.neg A txLoc object. These correspond to the negative (null) sites.

xAxisLblFmt Plot extended axis labels. Default is 2. See ??? for details.

Value

NULL.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

Examples

PlotSeqLogo

Plot sequence logo.

Description

Plot sequence logo.

Usage

```
PlotSeqLogo(txLoc, flank = 5, ylim = c(0, 2))
```

Arguments

txLoc A txLoc object.

flank An integer scalar; see 'Details'.

ylim An integer vector; specifies limits for the y-axis; automatically determined if

ymin = NULL; default is c(0,2).

Details

The function determines the sequence logo within a window defined by extending sites from txLoc upstream and downstream by flank nucleotides.

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Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

PlotSpatialDistribution

Plot spatial distribution of loci from a txLoc object.

Description

Plot spatial distribution of loci from a txLoc object within every transcript region.

Usage

```
PlotSpatialDistribution(txLoc, nbreaks = 100, absolute = FALSE,
  binWidth = NULL, posMax = 1000, doBootstrap = TRUE, ...)
```

Arguments

txLoc A txLoc object.

Number of spatial bins. Default is 100.

absolute Plot spatial distribution in absolute coordinates. Default is FALSE.

binWidth Spatial bin width. Overrides nbreaks if not NULL.

posMax If absolute == TRUE, show spatial distribution within a window given by posMax from the 5'/3' position of the transcript feature. Default is 1000 nt.

doBootstrap Calculate 95 sites within transcript region. Default is TRUE.

... Additional parameters passed to plot.

Value

NULL.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

Examples

PlotSpatialEnrichment

PlotSpatialEnrichment Perform spatial enrichment analysis and plot results.

Description

Perform enrichment analysis of the spatial distribution of positive sites in txLoc.pos relative to the distribution of null sites in txLoc.neg per transcript region and plot results. Enrichment/depletion is evaluated using (multiple) Fisher's exact test(s). Multiple hypothesis testing correction is applied following the method of Bejamini and Hochberg.

Usage

```
PlotSpatialEnrichment(txLoc.pos, txLoc.neg, binWidth = 20,
    posMax = 1000)
```

Arguments

txLoc.pos A txLoc object. These correspond to the positive sites.txLoc.neg A txLoc object. These correspond to the negative (null) sites.

binWidth Spatial bin width. Default is 20 nt.

posMax Evaluate enrichment within a window given by posMax. Default is 1000 nt.

Value

NULL.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

Examples

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ReadBED

Read BED-formatted file.

Description

Read BED-formatted file. See 'Details'.

Usage

```
ReadBED(file, collapseRange = TRUE)
```

Arguments

file A character string; specifies the input BED file.

collapseRange A logical scalar; should TRUE loci spanning more than one nucleotide be col-

lapsed to a single nucleotide locus corresponding to the midpoint of the range;

default is TRUE.

Details

The function opens and reads in a BED6-formatted file, and stores the annotation features in a GRanges object. Chromosome names must follow either UCSC (chr1, ..., chrM, chrX) or Ensembl naming conventions (1, ..., MT, X).

Value

A GRanges object. See 'Details'.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

Examples

SmartMap

Map genome coordinates to transcript coordinates.

Description

Map genome coordinates to transcript coordinates.

```
SmartMap(gr, id = NULL, refGenome = "hg38", ignore.strand = FALSE,
    showPb = TRUE)
```

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Arguments

gr A GRanges object; specifies the list of of genomic features to be mapped.

id A character string; specifies a name for loci from gr; if NULL then id = "";

default is NULL.

refGenome A character string; specifies a specific reference genome assembly version

based on which the matching transcriptome is loaded; default is "hg38".

ignore.strand A logical scalar; if TRUE strand information is ignored when mapping genome

coordinates to transcript coordinates; default is FALSE.

showPb A logical scalar; if TRUE show a progress bar; default is TRUE.

Details

The function maps genomic coordinates from locus to transcript section coordinates. The function automatically loads a reference transcriptome based on refGenome. An error is produced if a reference transcriptome could not be found. This usually means that BuildTx was not yet run successfully. The function returns a txLoc object of mapped positions.

Value

```
A txLoc object. See 'Details'.
```

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

Examples

SubsampleTxLoc

Subsample a txLoc object.

Description

Subsample a txLoc1 object based on a vector of fractions for every transcript region.

```
SubsampleTxLoc(txLoc, fractions, seed = NULL)
```

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Arguments

txLoc A txLoc object; this is the txLoc object from which the subsampled txLoc will

be created.

fractions A numeric vector, specifying the fraction of sites that will be sampled from

every transcript region. Note that the length of fractions has to match the

length of GetRegions(txLoc).

seed A single value, interpreted as an integer, or NULL; this is to ensure reproducibil-

ity when subsampling txLoc2 sites; default is NULL.

Value

A txLoc object.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

txLoc-accessors

txLoc accessors.

Description

Various txLoc accessors.

Usage

```
## S4 method for signature 'txLoc'
show(object)

## S4 method for signature 'txLoc'
GetLoci(object)

## S4 method for signature 'txLoc'
GetId(object)

## S4 method for signature 'txLoc'
GetRef(object)

## S4 method for signature 'txLoc'
GetVersion(object)

## S4 method for signature 'txLoc'
GetNumberOfLoci(object)

## S4 method for signature 'txLoc'
GetRegions(object)
```

Arguments

object A txLoc object.

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Details

- show(object): Print summary information about the txLoc object.
- GetLoci(object): Get a list of DataFrame objects of the loci of the txLoc object.
- GetId(object): Get the identifier string of the txLoc object.
- GetRef(object): Get the reference genome string of the txLoc object.
- GetVersion(object): Get the version string of a txLoc object.
- GetNumberOfLoci(object): Get the number of loci of a txLoc object in every transcript section.
- GetRegions(objects): Character vector of the transcript regions of a txLoc object.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

txLoc-class

txLoc object.

Description

The S4 object stores information about mapped loci per transcript region.

Details

The txLoc S4 object contains the following four slots:

- 1. loci: A list of DataFrame objects, each with the following columns:
 - locus_in_txx_region: A GRanges object
 - locus_in_genome: A GRanges object
 - score: A numeric vector
 - id: A character vector
 - ullet tx_region: A character vector
 - tx_region_width: An integer vector
 - tx_region_sequence: A DNAStringSet object
 - tx_refseq: A character vector
 - gene_entrez: A character vector
 - gene_symbol: A character vector
 - gene_ensembl: A character vector
 - gene_name: A character vector

The loci slot can be accessed using GetLoci(txLoc).

- 2. id: An identifier specified by the user. The id slot can be accessed using GetId(txLoc).
- 3. refGenome: The reference genome version (e.g. "hg38"), which determines the mapping between genomic and transcriptomic coordinates. The refGenome slot can be accessed using GetRef(txLoc).
- 4. 'version': A version identifier; currently this slot is used to to store the current system time & date. The version slot can be accessed using GetVersion(txLoc).

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Slots

```
loci A list of DataFrame objects; see 'Details'.
id A character string; see 'Details.
refGenome A character string; see 'Details'.
version A character string; see 'Details'.
```

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

WriteBED

Write txLoc object to a BED file.

Description

Write txLoc object to a BED file. See 'Details'.

Usage

```
WriteBED(txLoc, file = NULL)
```

Arguments

txLoc A txLoc object.

file A character string; specifies the filename of the output BED file. If NULL, then

file = "sites.bed"; default is NULL.

Details

The function writes entries from a txLoc object to a 6-column BED file (BED6). Note that this process is not "splice-aware", i.e. if an entry spans an intron the BED entry gives the left and right-most genomic coordinate of the feature. If file = NULL, entries will be written to sites.bed.

Author(s)

Maurits Evers, <maurits.evers@anu.edu.au>

Examples

22 WriteCSV

WriteCSV	Write txLoc object to CSV file.	

Description

Write a txLoc object to a comma-separated-values file.

Usage

```
WriteCSV(txLoc, file = NULL, withSeq = FALSE, withGC = FALSE)
```

Arguments

txLoc A txLoc object.

file Filename of output CSV file. If NULL then file = "sites.csv".

withSeq If TRUE then include full sequence. Default is FALSE.

withGC If TRUE then include GC content. Default is FALSE. Unused.

Author(s)

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