

Package ‘SGSeq’

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Type Package

Title Splice event prediction and quantification from RNA-seq data

Version 1.40.0

Description SGSeq is a software package for analyzing splice events from RNA-seq data. Input data are RNA-seq reads mapped to a reference genome in BAM format. Genes are represented as a splice graph, which can be obtained from existing annotation or predicted from the mapped sequence reads. Splice events are identified from the graph and are quantified locally using structurally compatible reads at the start or end of each splice variant. The software includes functions for splice event prediction, quantification, visualization and interpretation.

License Artistic-2.0

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Contents

| | |
|---------------------------------------|----|
| analyzeFeatures | 3 |
| analyzeVariants | 4 |
| annotate | 5 |
| annotateSGVariants | 6 |
| assays | 7 |
| convertToSGFeatures | 8 |
| convertToTxFeatures | 9 |
| exonCompatible | 10 |
| exportFeatures | 11 |
| filterFeatures | 11 |
| findOverlapsRanges | 12 |
| findSGVariants | 13 |
| getBamInfo | 14 |
| getSGFeatureCounts | 15 |
| getSGFeatureCountsPerSample | 15 |
| getSGVariantCounts | 16 |
| gr | 17 |
| importTranscripts | 18 |
| junctionCompatible | 18 |
| makeSGFeatureCounts | 19 |
| makeVariantNames | 20 |
| mergeTxFeatures | 20 |
| plotCoverage | 21 |
| plotFeatures | 22 |
| plotSpliceGraph | 24 |
| plotVariants | 26 |
| predictCandidatesInternal | 27 |
| predictCandidatesTerminal | 28 |
| predictExonsInternal | 28 |
| predictExonsTerminal | 29 |
| predictJunctions | 30 |
| predictSpliced | 31 |
| predictTxFeatures | 32 |
| predictTxFeaturesPerSample | 33 |
| predictTxFeaturesPerStrand | 35 |
| predictVariantEffects | 36 |
| processTerminalExons | 37 |
| removeExonsIsolated | 38 |
| sgfc_ann | 38 |
| sgfc_pred | 39 |
| SGFeatureCounts | 39 |
| SGFeatures | 40 |
| sgf_ann | 41 |
| sgf_pred | 41 |
| SGSegments | 41 |
| SGVariantCounts | 42 |
| SGVariants | 42 |
| sgvc_ann | 44 |
| sgvc_ann_from_bam | 44 |
| sgvc_pred | 44 |

| | |
|------------------------------|----|
| sgvc_pred_from_bam | 45 |
| sgv_ann | 45 |
| sgv_pred | 45 |
| si | 46 |
| slots | 46 |
| splicesiteOverlap | 54 |
| tx | 55 |
| TxFeatures | 55 |
| txf_ann | 56 |
| txf_pred | 56 |
| updateObject | 57 |

Index 58

| | |
|-----------------|---|
| analyzeFeatures | <i>Analysis of splice graph features from BAM files</i> |
|-----------------|---|

Description

High-level function for the prediction and quantification of splice junctions, exon bins and splice sites from BAM files.

Usage

```
analyzeFeatures(sample_info, which = NULL, features = NULL,
  predict = is.null(features), alpha = 2, psi = 0, beta = 0.2,
  gamma = 0.2, min_junction_count = NULL, min_anchor = 1,
  min_n_sample = 1, min_overhang = NA, annotation = NULL,
  max_complexity = 20, verbose = FALSE, cores = 1)
```

Arguments

| | |
|--------------------|--|
| sample_info | Data frame with sample information. Required columns are “sample_name”, “file_bam”, “paired_end”, “read_length”, “frag_length” and “lib_size”. Library information can be obtained with function getBamInfo. |
| which | GRanges of genomic regions to be considered for feature prediction, passed to ScanBamParam |
| features | TxFeatures or SGFeatures object |
| predict | Logical indicating whether transcript features should be predicted from BAM files |
| alpha | Minimum FPKM required for a splice junction to be included |
| psi | Minimum splice frequency required for a splice junction to be included |
| beta | Minimum relative coverage required for an internal exon to be included |
| gamma | Minimum relative coverage required for a terminal exon to be included |
| min_junction_count | Minimum fragment count required for a splice junction to be included. If specified, argument alpha is ignored. |
| min_anchor | Integer specifying minimum anchor length |
| min_n_sample | Minimum number of samples a feature must be observed in to be included |

| | |
|----------------|---|
| min_overhang | Minimum overhang required to suppress filtering or trimming of predicted terminal exons (see the manual page for processTerminalExons). Use NULL to disable processing (disabling processing is useful if results are subsequently merged with other predictions and processing is postponed until after the merging step). |
| annotation | TxFeatures object used for annotation |
| max_complexity | Maximum allowed complexity. If a locus exceeds this threshold, it is skipped, resulting in a warning. Complexity is defined as the maximum number of unique predicted splice junctions overlapping a given position. High complexity regions are often due to spurious read alignments and can slow down processing. To disable this filter, set to NA. |
| verbose | If TRUE, generate messages indicating progress |
| cores | Number of cores available for parallel processing |

Details

Splice junctions and exons are predicted from BAM files with [predictTxFeatures](#).

Known features can be provided as TxFeatures or SGFeatures via argument features.

If features is not NULL and predict is TRUE, known features are augmented with predictions.

Known and/or predicted transcript features are converted to splice graph features. For details, see [convertToSGFeatures](#).

Optionally, splice graph features can be annotated with respect to a TxFeatures object provided via argument annotation. For details, see the help page for function [annotate](#).

Finally, compatible fragment counts for splice graph features are obtained from BAM files with [getSGFeatureCounts](#).

Value

SGFeatureCounts object

Author(s)

Leonard Goldstein

Examples

```
path <- system.file("extdata", package = "SGSeq")
si$file_bam <- file.path(path, "bams", si$file_bam)
sgfc <- analyzeFeatures(si, gr)
```

analyzeVariants

Analysis of splice variants

Description

High-level function for the analysis of splice variants from splice graph features. Splice variants are identified with [findSGVariants](#). Representative counts are obtained and variant frequencies estimated with [getSGVariantCounts](#).

Usage

```
analyzeVariants(object, maxnvariant = 20, include = "default",
  min_denominator = NA, min_anchor = 1, cores = 1)
```

Arguments

| | |
|-----------------|--|
| object | SGFeatureCounts object |
| maxnvariant | If more than maxnvariant variants are identified in an event, the event is skipped, resulting in a warning. Set to NA to include all events. |
| include | Character string indicating whether identified splice variants should be filtered. Possible options are "default" (only include variants for events with all variants closed), "closed" (only include closed variants) and "all" (include all variants). |
| min_denominator | Integer specifying minimum denominator when calculating variant frequencies. The total number of boundary-spanning reads must be equal to or greater than min_denominator for at least one event boundary. Otherwise estimates are set to NA. If NA, all estimates are returned. |
| min_anchor | Integer specifying minimum anchor length |
| cores | Number of cores available for parallel processing |

Value

SGVariantCounts object

Author(s)

Leonard Goldstein

Examples

```
sgvc <- analyzeVariants(sgfc_pred)
```

| | |
|----------|---|
| annotate | <i>Annotation with respect to transcript features</i> |
|----------|---|

Description

Features in query are assigned transcript names and gene names of structurally compatible features in subject (see below). If a feature in query does not match any features in subject, its geneName inherits from connected annotated features.

Usage

```
annotate(query, subject)
```

Arguments

| | |
|---------|---|
| query | SGFeatures, SGVariants, SGFeatureCounts or SGVariantCounts object |
| subject | TxFeatures object |

Details

Feature matching is performed as follows: Query splice junctions are matched with identical subject splice junctions. Query splice sites are matched with splice sites implied by subject splice junctions. Query exon bins are matched with overlapping subject exons. Spliced boundaries of query exon bins must match spliced subject exon boundaries. Query exon bins cannot extend across spliced subject exon boundaries.

Value

query with updated txName, geneName column slots

Author(s)

Leonard Goldstein

Examples

```
sgf_annotated <- annotate(sgf_pred, txf_ann)
sgv_annotated <- annotate(sgv_pred, txf_ann)
```

annotateSGVariants *Annotate splice variants in terms of canonical events*

Description

Annotate splice variants in terms of canonical events.

Usage

```
annotateSGVariants(variants)
```

Arguments

variants SGVariants object

Details

The following events are considered:

- “SE” skipped exon
- “S2E” two consecutive exons skipped
- “RI” retained intron
- “MXE” mutually exclusive exons
- “A5SS” alternative 5’ splice site
- “A3SS” alternative 3’ splice site
- “AFE” alternative first exon
- “ALE” alternative last exon
- “AS” alternative start other than “AFE”
- “AE” alternative end other than “ALE”

For events “SE” and “S2E”, suffixes “I” and “S” indicate inclusion and skipping, respectively. For event “RI” suffixes “E” and “R” indicate exclusion and retention, respectively. For events “A5SS” and “A3SS”, suffixes “P” and “D” indicate use of the proximal (intron-shortening) and distal (intron-lengthening) splice site, respectively.

All considered events are binary events defined by two alternative variants. A variant is annotated as a canonical event if it coincides with one of the two variants in the canonical event, and there is at least one variant in the same event that coincides with the second variant of the canonical event.

Value

variants with added metadata column “variantType” indicating canonical event(s)

Author(s)

Leonard Goldstein

assays

Assessing and replacing assay data

Description

Functions counts and FPKM are used to extract counts and FPKM values from SGFeatureCounts and SGVariantCounts objects. Function variantFreq is used to access relative usage estimates from SGVariantCounts objects.

Usage

```

FPKM(object, ...)

FPKM(object, ...) <- value

variantFreq(object)

variantFreq(object) <- value

## S4 method for signature 'SGFeatureCounts'
counts(object)

## S4 replacement method for signature 'SGFeatureCounts'
counts(object) <- value

## S4 method for signature 'SGFeatureCounts'
FPKM(object)

## S4 replacement method for signature 'SGFeatureCounts'
FPKM(object) <- value

## S4 method for signature 'SGVariantCounts'
counts(object, ...)

## S4 replacement method for signature 'SGVariantCounts'
```

```

counts(object, ...) <- value

## S4 method for signature 'SGVariantCounts'
FPKM(object, ...)

## S4 method for signature 'SGVariantCounts'
variantFreq(object)

## S4 replacement method for signature 'SGVariantCounts'
variantFreq(object) <- value

```

Arguments

| | |
|--------|---|
| object | Object containing assay data |
| ... | Arguments passed to method for SGVariantCounts objects. Argument option specifies whether the output should be based on the count of fragments compatible with the variant at the start (“variant5p”), end (“variant3p”) or either (“variant5pOr3p”) (the default), or whether output should be based on the count of fragments compatible with any variant belonging to the same event (“event5p” or “event3p”). Argument min_anchor specifies the minimum anchor length when computing FPKM values (defaults to 1). |
| value | Replacement value |

Value

Assay data for accessor functions or updated object for replacement functions.

Author(s)

Leonard Goldstein

Examples

```

x <- counts(sgfc_pred)
y <- FPKM(sgfc_pred)
u <- counts(sgvc_pred, option = "variant5p")
v <- FPKM(sgvc_pred, option = "variant5p")

```

convertToSGFeatures *Convert transcript features to splice graph features*

Description

Convert transcript features (predicted from RNA-seq data or extracted from transcript annotation) to splice graph features.

Usage

```
convertToSGFeatures(x, coerce = FALSE)
```


Arguments

| | |
|--------|---|
| x | TxFeatures object |
| coerce | Logical indicating whether transcript features should be coerced to splice graph features without disjoining exons and omitting splice donor and acceptor sites |

Details

Splice junctions are unaltered. Exons are disjoined into non-overlapping exon bins. Adjacent exon bins without a splice site at the shared boundary are merged.

Entries for splice donor and acceptor sites (positions immediately upstream and downstream of introns, respectively) are added.

In the returned SGFeatures object, column type takes values “J” (splice junction), “E” (exon bin), “D” (splice donor) or “A” (splice acceptor). Columns splice5p and splice3p indicate mandatory splices at the 5’ and 3’ end of exon bins, respectively (determining whether reads overlapping exon boundaries must be spliced at the boundary to be considered compatible). splice5p (splice3p) is TRUE if the first (last) position of the exon coincides with a splice acceptor (donor) and it is not adjacent to a neighboring exon bin.

Each feature is assigned a unique feature and gene identifier, stored in columns featureID and geneID, respectively. The latter indicates features that belong to the same gene, represented by a connected component in the splice graph.

Value

SGFeatures object

Author(s)

Leonard Goldstein

Examples

```
sgf <- convertToSGFeatures(txf_ann)
```

convertToTxFeatures *Convert to TxFeatures object*

Description

Convert a TxDb object or a GRangesList of exons grouped by transcripts to a TxFeatures object.

Usage

```
convertToTxFeatures(x)
```

Arguments

| | |
|---|--|
| x | TxDb object or GRangesList of exons grouped by transcript. For import from GFF format, use function importTranscripts. |
|---|--|

Details

If `x` is a `GRangesList`, transcript names and gene names can be specified as character vectors in metadata columns `txName` and `geneName`, respectively. If missing, transcript names are based on `names(x)`. For import from GFF format, use function `importTranscripts`.

In the returned `TxFeatures` object, column `type` takes values “J” (splice junction), “I” (internal exon), “F” (5'/first exon), “L” (3'/last exon) or “U” (unspliced).

Value

`TxFeatures` object

Author(s)

Leonard Goldstein

Examples

```
gr <- GRanges(c(1, 1), IRanges(c(1, 201), c(100, 300)), c("+", "+"))
grl <- split(gr, 1)
txf <- convertToTxFeatures(grl)
```

exonCompatible

Compatible fragment counts for exons

Description

Identify fragments compatible with exons.

Usage

```
exonCompatible(exons, spliceL, spliceR, frag_exonic, frag_intron,
  counts = TRUE)
```

Arguments

| | |
|--------------------------|---|
| <code>exons</code> | <code>IRanges</code> of exons |
| <code>spliceL</code> | Logical vector indicating whether LHS boundary is spliced |
| <code>spliceR</code> | Logical vector indicating whether RHS boundary is spliced |
| <code>frag_exonic</code> | <code>IRangesList</code> of exonic regions, one entry per fragment |
| <code>frag_intron</code> | <code>IRangesList</code> of introns, one entry per fragment |
| <code>counts</code> | Logical indicating whether counts or indices of compatible fragments should be returned |

Value

Counts or list of indices of compatible fragments

Author(s)

Leonard Goldstein

| | |
|----------------|-----------------------------|
| exportFeatures | <i>Export to BED format</i> |
|----------------|-----------------------------|

Description

Export features to BED format. Splice sites are not included.

Usage

```
exportFeatures(features, file)
```

Arguments

| | |
|----------|---|
| features | TxFeatures or SGFeatures object |
| file | Character string specifying output file |

Value

NULL

Author(s)

Leonard Goldstein

Examples

```
## Not run:  
exportFeatures(txf_pred, "txf.bed")  
exportFeatures(sgf_pred, "sgf.bed")  
  
## End(Not run)  
NULL
```

| | |
|----------------|----------------------------------|
| filterFeatures | <i>Filter predicted features</i> |
|----------------|----------------------------------|

Description

Filter previously predicted features using more stringent criteria.

Usage

```
filterFeatures(features, paired_end, read_length, frag_length, lib_size,  
min_junction_count = NULL, alpha, psi, beta, gamma)
```

Arguments

| | |
|--------------------|---|
| features | TxFeatures object with predicted features, including metadata columns “N”, “N_splicesite” and “coverage”. |
| paired_end | Logical, TRUE for paired-end data, FALSE for single-end data |
| read_length | Read length required for use with alpha |
| frag_length | Fragment length for paired-end data required for use with alpha |
| lib_size | Number of aligned fragments required for use with alpha |
| min_junction_count | Minimum fragment count required for a splice junction to be included. If specified, argument alpha is ignored. |
| alpha | Minimum FPKM required for a splice junction to be included. Internally, FPKMs are converted to counts, requiring arguments read_length, frag_length and lib_size. alpha is ignored if argument min_junction_count is specified. |
| psi | Minimum splice frequency required for a splice junction to be included |
| beta | Minimum relative coverage required for an internal exon to be included |
| gamma | Minimum relative coverage required for a terminal exon to be included |

Details

Initial predictions with predictTxFeatures must have been performed with include_counts = TRUE and retain_coverage = TRUE, so that predicted features contain metadata columns “N”, “N_splicesite” and “coverage”.

Value

TxFeatures object with filtered features

Author(s)

Leonard Goldstein

findOverlapsRanges *Modified findOverlaps function for IRanges, IRangesList objects*

Description

Modified findOverlaps function for IRanges, IRangesList objects that behaves analogous to findOverlaps for GRanges, GRangesList objects.

Usage

```
findOverlapsRanges(query, subject, type = "any")
```

Arguments

| | |
|---------|-------------------------------|
| query | IRanges or IRangesList object |
| subject | IRanges or IRangesList object |
| type | Passed to findOverlaps |

Value

Hits object

Author(s)

Leonard Goldstein

findSGVariants *Identify splice variants from splice graph*

Description

Identify splice variants from splice graph.

Usage

```
findSGVariants(features, maxnvariant = 20, annotate_events = TRUE,  
  include = c("default", "closed", "all"), cores = 1)
```

Arguments

| | |
|-----------------|--|
| features | SGFeatures object |
| maxnvariant | If more than maxnvariant variants are identified in an event, the event is skipped, resulting in a warning. Set to NA to include all events. |
| annotate_events | Logical indicating whether identified splice variants should be annotated in terms of canonical events. For details see help page for annotateSGVariants . |
| include | Character string indicating whether identified splice variants should be filtered. Possible options are “default” (only include variants for events with all variants closed), “closed” (only include closed variants) and “all” (include all variants). |
| cores | Number of cores available for parallel processing |

Value

SGVariants object

Author(s)

Leonard Goldstein

Examples

```
sgv <- findSGVariants(sgf_pred)
```

getBamInfo

Obtain library information from BAM files

Description

Obtain paired-end status, median aligned read length, median aligned insert size and library size from BAM files.

Usage

```
getBamInfo(sample_info, yieldSize = NULL, cores = 1)
```

Arguments

| | |
|-------------|---|
| sample_info | Data frame with sample information including mandatory columns “sample_name” and “file_bam”. Column “sample_name” must be a character vector. Column “file_bam” can be a character vector or BamFileList. |
| yieldSize | Number of records used for obtaining library information, or NULL for all records |
| cores | Number of cores available for parallel processing |

Details

BAM files must have been generated with a splice-aware alignment program that outputs the custom tag ‘XS’ for spliced reads, indicating the direction of transcription. BAM files must be indexed.

Library information can be inferred from a subset of BAM records by setting the number of records via argument yieldSize. Note that library size is only obtained if yieldSize is NULL.

Value

sample_info with additional columns “paired_end”, “read_length”, “frag_length”, and “lib_size” if yieldSize is NULL

Author(s)

Leonard Goldstein

Examples

```
path <- system.file("extdata", package = "SGSeq")
si$file_bam <- file.path(path, "bams", si$file_bam)

## data.frame as sample_info and character vector as file_bam
si <- si[, c("sample_name", "file_bam")]
si_complete <- getBamInfo(si)

## DataFrame as sample_info and BamFileList as file_bam
DF <- DataFrame(si)
DF$file_bam <- BamFileList(DF$file_bam)
DF_complete <- getBamInfo(DF)
```

getSGFeatureCounts *Compatible counts for splice graph features from BAM files*

Description

Compatible counts are obtained for each sample and combined into an SGFeatureCounts object.

Usage

```
getSGFeatureCounts(sample_info, features, min_anchor = 1,
  counts_only = FALSE, verbose = FALSE, cores = 1)
```

Arguments

| | |
|-------------|--|
| sample_info | Data frame with sample information. Required columns are "sample_name", "file_bam", "paired_end", "read_length", "frag_length" and "lib_size". Library information can be obtained with function getBamInfo. |
| features | SGFeatures object |
| min_anchor | Integer specifying minimum anchor length |
| counts_only | Logical indicating only counts should be returned |
| verbose | If TRUE, generate messages indicating progress |
| cores | Number of cores available for parallel processing |

Value

codeSGFeatureCounts object, or integer matrix of counts if counts_only = TRUE

Author(s)

Leonard Goldstein

Examples

```
path <- system.file("extdata", package = "SGSeq")
si$file_bam <- file.path(path, "bams", si$file_bam)
sgfc <- getSGFeatureCounts(si, sgf_pred)
```

getSGFeatureCountsPerSample *Compatible fragment counts for splice graph features*

Description

Obtain counts of compatible fragments for splice graph features.

Usage

```
getSGFeatureCountsPerSample(features, file_bam, paired_end, sample_name,
  min_anchor, retain_coverage, verbose, cores)
```

Arguments

| | |
|-----------------|---|
| features | SGFeatures object |
| file_bam | BAM file with genomic RNA-seq read alignments |
| paired_end | Logical, TRUE for paired-end data, FALSE for single-end data |
| sample_name | Sample name used in messages |
| min_anchor | Integer specifying minimum anchor length |
| retain_coverage | Logical indicating whether coverage for each exon should be retained as an RleList in metadata column "coverage". This allows filtering of features using more stringent criteria after the initial prediction. |
| verbose | If TRUE, generate messages indicating progress |
| cores | Number of cores available for parallel processing |

Value

Numeric vector of compatible fragment counts

Author(s)

Leonard Goldstein

getSGVariantCounts *Representative counts and frequency estimates for splice variants*

Description

For splice variants, obtain counts of compatible fragments spanning the start and/or end of each variant. Counts can be obtained from an SGFeatureCounts object or from BAM files. Only one of the two arguments feature_counts or sample_info must be specified. Local estimates of relative usage are calculated at the start and/or end of each splice variant. For splice variants with relative usage estimates at both start and end, these are combined by taking a weighted mean, where weights are proportional to the total number of reads spanning the respective boundary.

Usage

```
getSGVariantCounts(variants, feature_counts = NULL, sample_info = NULL,
  min_denominator = NA, min_anchor = 1, verbose = FALSE, cores = 1)
```

Arguments

| | |
|----------------|--|
| variants | SGVariants object |
| feature_counts | SGFeatureCounts object |
| sample_info | Data frame with sample information. Required columns are "sample_name", "file_bam", "paired_end", "read_length", "frag_length" and "lib_size". Library information can be obtained with function getBamInfo. |

| | |
|-----------------|--|
| min_denominator | Integer specifying minimum denominator when calculating variant frequencies. The total number of boundary-spanning reads must be equal to or greater than min_denominator for at least one event boundary. Otherwise estimates are set to NA. If NA, all estimates are returned. |
| min_anchor | Integer specifying minimum anchor length |
| verbose | If TRUE, generate messages indicating progress |
| cores | Number of cores available for parallel processing |

Value

SGVariantCounts object

Author(s)

Leonard Goldstein

Examples

```
sgvc_from_sgfc <- getSGVariantCounts(sgv_pred, sgfc_pred)
path <- system.file("extdata", package = "SGSeq")
si$file_bam <- file.path(path, "bams", si$file_bam)
sgvc_from_bam <- getSGVariantCounts(sgv_pred, sample_info = si)
```

gr

Example genomic region of interest

Description

FBXO31 gene locus, based on UCSC knownGene annotation.

Format

GRanges object

Author(s)

Leonard Goldstein

`importTranscripts` *Import transcripts from GFF file*

Description

Import GFF file and generate a GRangesList of transcripts suitable as input for functions `convertToTxFeatures` or `predictVariantEffects`.

Usage

```
importTranscripts(file, tag_tx = "transcript_id", tag_gene = "gene_id")
```

Arguments

| | |
|-----------------------|---|
| <code>file</code> | Character string specifying input GFF file |
| <code>tag_tx</code> | GFF attribute tag for transcript identifier |
| <code>tag_gene</code> | GFF attribute tag for gene identifier |

Value

GRangesList of exons grouped by transcripts with metadata columns `txName`, `geneName`, `cdsStart`, `cdsEnd`.

Author(s)

Leonard Goldstein

Examples

```
## Not run:
tx <- importTranscripts(file)

## End(Not run)
NULL
```

`junctionCompatible` *Compatible fragment counts for splice junctions*

Description

Identify fragments compatible with splice junctions.

Usage

```
junctionCompatible(junctions, frag_exonic, frag_intron, min_anchor,
  counts = TRUE)
```

Arguments

| | |
|-------------|---|
| junctions | IRanges of splice junctions |
| frag_exonic | IRangesList of exonic regions, one entry per fragment |
| frag_intron | IRangesList of introns, one entry per fragment |
| min_anchor | Integer specifying minimum anchor length |
| counts | Logical indicating whether counts or indices of compatible fragments should be returned |

Value

Counts or list of indices of compatible fragments

Author(s)

Leonard Goldstein

makeSGFeatureCounts *Create SGFeatureCounts object*

Description

Create SGFeatureCounts object from rowRanges, colData and counts.

Usage

```
makeSGFeatureCounts(rowRanges, colData, counts, min_anchor = 1)
```

Arguments

| | |
|------------|--|
| rowRanges | SGFeatures object |
| colData | Data frame with sample information |
| counts | Integer matrix of counts |
| min_anchor | Integer specifying minimum anchor length |

Value

SGFeatureCounts object

Author(s)

Leonard Goldstein

Examples

```
sgfc <- makeSGFeatureCounts(sgf_pred, si,
  matrix(0L, length(sgf_pred), nrow(si)))
```

makeVariantNames *Create interpretable splice variant names*

Description

Create interpretable splice variant names taking format GENE_EVENT_VARIANT/ORDER_TYPE. GENE is based on geneName if available, and geneID otherwise. EVENT and VARIANT enumerate events and variants for the same gene and event, respectively. ORDER indicates the total number of variants in the same event (e.g. 1/2 refers to the first out of two splice variants in the event). TYPE is based on variantType.

Usage

```
makeVariantNames(variants)
```

Arguments

variants SGVariants object

Value

Character vector with splice variant names

Author(s)

Leonard Goldstein

Examples

```
makeVariantNames(sgv_pred)
```

mergeTxFeatures *Merge redundant features*

Description

Merge features, typically after feature prediction in multiple samples.

Usage

```
mergeTxFeatures(..., min_n_sample = 1)
```

Arguments

... one or more TxFeatures objects, or a single list of TxFeatures objects
 min_n_sample Minimum number of samples a feature must be observed in to be included

Details

Merged features are the union of splice junctions and internal exons. For terminal exons with shared spliced boundary, the longest exon is retained.

Value

TxFeatures object with merged features

Author(s)

Leonard Goldstein

Examples

```
txf_merged <- mergeTxFeatures(txf_ann, txf_pred)
```

| | |
|--------------|---|
| plotCoverage | <i>Plot read coverage and splice junction read counts</i> |
|--------------|---|

Description

Plot read coverage and splice junction read counts for an individual sample or averaged across samples.

Usage

```
plotCoverage(x, geneID = NULL, geneName = NULL, eventID = NULL,
  which = NULL, sample_info = NULL, sizefactor = NA, toscale = c("exon",
  "none", "gene"), color = "darkblue", ylim = NULL, label = NULL,
  nbin = 200, summary = mean, curvature = 1, main = NULL,
  min_anchor = 1, cores = 1)
```

Arguments

| | |
|-------------|---|
| x | SGFeatureCounts or SGFeatures object. If x is an SGFeatureCounts object that includes multiple samples, average coverage and splice junction counts are obtained. |
| geneID | Single gene identifier used to subset x |
| geneName | Single gene name used to subset x |
| eventID | Single event identifier used to subset x |
| which | GRanges used to subset x |
| sample_info | Data frame with sample information. If x is an SGFeatureCounts object, sample information is obtained from colData(x). If sample_info includes multiple samples, average coverage and splice junction counts are obtained. |
| sizefactor | Numeric vector with length equal to the number of samples in sample_info. Used to scale coverages and splice junction counts before plotting, or before averaging across samples. Set to NA to disable scaling. If NULL, size factors are calculated as the number of bases sequenced (the product of library size and average number of bases sequenced per read or fragment), plotted coverages and splice junction counts are per 1 billion sequenced bases. |
| toscale | Controls which parts of the splice graph are drawn to scale. Possible values are "none" (exonic and intronic regions have constant length), "exon" (exonic regions are drawn to scale) and "gene" (both exonic and intronic regions are drawn to scale). |

| | |
|------------|---|
| color | Color used for plotting coverages |
| ylim | Numeric vector of length two, determining y-axis range used for plotting coverages. |
| label | Optional y-axis label |
| nbin | Number of bins for plotting coverages |
| summary | Function used to calculate per-bin coverage summaries |
| curvature | Numeric determining curvature of plotted splice junctions. |
| main | Plot title |
| min_anchor | Integer specifying minimum anchor length |
| cores | Number of cores available for parallel processing. |

Value

data.frame with information on splice junctions included in the splice graph

Author(s)

Leonard Goldstein

Examples

```
## Not run:
par(mfrow = c(4, 1))
for (j in seq_len(4)) plotCoverage(sgfc_pred[, j])

## End(Not run)
NULL
```

plotFeatures

Plot splice graph and heatmap of expression values

Description

Plot splice graph and heatmap of expression values.

Usage

```
plotFeatures(x, geneID = NULL, geneName = NULL, which = NULL,
  tx_view = FALSE, cex = 1, assay = "FPKM", include = c("junctions",
  "exons", "both"), transform = function(x) { log2(x + 1) },
  Rowv = NULL, distfun = dist, hclustfun = hclust, margin = 0.2,
  RowSideColors = NULL, square = FALSE, cexRow = 1, cexCol = 1,
  labRow = colnames(x), col = colorRampPalette(c("black", "gold"))(256),
  zlim = NULL, heightPanels = c(1, 2), ...)
```

Arguments

| | |
|---------------|---|
| x | SGFeatureCounts object |
| geneID | Single gene identifier used to subset x |
| geneName | Single gene name used to subset x |
| which | GRanges used to subset x |
| tx_view | Plot transcripts instead of splice graph (experimental) |
| cex | Scale parameter for feature labels and annotation |
| assay | Name of assay to be plotted in the heatmap |
| include | Include “exons”, “junctions” or “both” in the heatmap |
| transform | Transformation applied to assay data |
| Rowv | Determines order of rows. Either a vector of values used to reorder rows, or NA to suppress reordering, or NULL for hierarchical clustering. |
| distfun | Distance function used for hierarchical clustering of rows (samples) |
| hclustfun | Clustering function used for hierarchical clustering of rows (samples) |
| margin | Width of right-hand margin as fraction of width of the graphics device. Ignored if square is TRUE. |
| RowSideColors | Character vector (or list of character vectors) with length(s) equal to ncol(x) containing color names for horizontal side bars for sample annotation |
| square | Logical, if TRUE margins are set such that cells in the heatmap are square |
| cexRow | Scale factor for row (sample) labels |
| cexCol | Scale factor for column (feature) labels |
| labRow | Character vector of row (sample) labels |
| col | Heatmap colors |
| zlim | Range of values for which colors should be plotted, if NULL range of finite values |
| heightPanels | Numeric vector of length two indicating height of the top and bottom panels. |
| ... | further arguments passed to plotSpliceGraph |

Value

data.frame with information on exon bins and splice junctions included in the splice graph

Author(s)

Leonard Goldstein

Examples

```
## Not run:
sgfc_annotated <- annotate(sgfc_pred, txf_ann)
plotFeatures(sgfc_annotated)

## End(Not run)
NULL
```

plotSpliceGraph *Plot splice graph*

Description

Plot the splice graph implied by splice junctions and exon bins. Invisibly returns a `data.frame` with details of plotted features, including genomic coordinates.

Usage

```
plotSpliceGraph(x, geneID = NULL, geneName = NULL, eventID = NULL,
  which = NULL, toscale = c("exon", "none", "gene"), label = c("id",
  "name", "label", "none"), color = "gray", color_novel = color,
  color_alpha = 0.8, color_labels = FALSE, border = "fill",
  curvature = NULL, ypos = c(0.5, 0.1), score = NULL,
  score_color = "darkblue", score_ylim = NULL, score_ypos = c(0.3, 0.1),
  score_nbin = 200, score_summary = mean, score_label = NULL,
  ranges = NULL, ranges_color = "darkblue", ranges_ypos = c(0.1, 0.1),
  main = NULL, tx_view = FALSE, tx_dist = 0.2, short_output = TRUE)
```

Arguments

| | |
|--------------|--|
| x | SGFeatures or SGVariants object |
| geneID | Single gene identifier used to subset x |
| geneName | Single gene name used to subset x |
| eventID | Single event identifier used to subset x |
| which | GRanges used to subset x |
| toscale | Controls which parts of the splice graph are drawn to scale. Possible values are “none” (exonic and intronic regions have constant length), “exon” (exonic regions are drawn to scale) and “gene” (both exonic and intronic regions are drawn to scale). |
| label | Format of exon/splice junction labels, possible values are “id” (format E1,... J1,...), “name” (format type:chromosome:start-end:strand), “label” for labels specified in metadata column “label”, or “none” for no labels. |
| color | Color used for plotting the splice graph. Ignored if features metadata column “color” is not NULL. |
| color_novel | Features with missing annotation are highlighted in color_novel. Ignored if features metadata column “color” is not NULL. |
| color_alpha | Controls color transparency |
| color_labels | Logical indicating whether label colors should be the same as feature colors |
| border | Determines the color of exon borders, can be “fill” (same as exon color), “none” (no border), or a valid color name |
| curvature | Numeric determining curvature of plotted splice junctions. |
| ypos | Numeric vector of length two, indicating the vertical position and height of the exon bins in the splice graph, specified as fraction of the height of the plotting region (not supported for tx_view = TRUE) |
| score | RLEList containing nucleotide-level scores to be plotted with the splice graph |

| | |
|---------------|---|
| score_color | Color used for plotting scores |
| score_ylim | Numeric vector of length two, determining y-axis range for plotting scores |
| score_ypos | Numeric vector of length two, indicating the vertical position and height of the score panel, specified as fraction of the height of the plotting region |
| score_nbin | Number of bins for plotting scores |
| score_summary | Function used to calculate per-bin score summaries |
| score_label | Label used to annotate score panel |
| ranges | GRangesList to be plotted with the splice graph |
| ranges_color | Color used for plotting ranges |
| ranges_ypos | Numeric vector of length two, indicating the vertical position and height of the ranges panel, specified as fraction of the height of the plotting region |
| main | Plot title |
| tx_view | Plot transcripts instead of splice graph (experimental) |
| tx_dist | Vertical distance between transcripts as fraction of height of plotting region |
| short_output | Logical indicating whether the returned data frame should only include information that is likely useful to the user |

Details

By default, the color of features in the splice graph is determined by annotation status (see arguments `color`, `color_novel`) and feature labels are generated automatically (see argument `label`). Alternatively, colors and labels can be specified via metadata columns “color” and “label”, respectively.

Value

data.frame with information on exon bins and splice junctions included in the splice graph

Author(s)

Leonard Goldstein

Examples

```
## Not run:
sgf_annotated <- annotate(sgf_pred, txf_ann)
plotSpliceGraph(sgf_annotated)

## End(Not run)
## Not run:
sgv_annotated <- annotate(sgv_pred, txf_ann)
plotSpliceGraph(sgv_annotated)

## End(Not run)
NULL
```

plotVariants

*Plot splice graph and heatmap of splice variant frequencies***Description**

Plot splice graph and heatmap of splice variant frequencies.

Usage

```
plotVariants(x, eventID = NULL, tx_view = FALSE, cex = 1,
  transform = function(x) { x }, Rowv = NULL, distfun = dist,
  hclustfun = hclust, margin = 0.2, RowSideColors = NULL,
  square = FALSE, cexRow = 1, cexCol = 1, labRow = colnames(x),
  col = colorRampPalette(c("black", "gold"))(256), zlim = c(0, 1),
  heightPanels = c(1, 2), expand_variants = FALSE, ...)
```

Arguments

| | |
|-----------------|---|
| x | SGVariantCounts object |
| eventID | Single event identifier used to subset x |
| tx_view | Plot transcripts instead of splice graph (experimental) |
| cex | Scale parameter for feature labels and annotation |
| transform | Transformation applied to splice variant frequencies |
| Rowv | Determines order of rows. Either a vector of values used to reorder rows, or NA to suppress reordering, or NULL for hierarchical clustering. |
| distfun | Distance function used for hierarchical clustering of rows (samples) |
| hclustfun | Clustering function used for hierarchical clustering of rows (samples) |
| margin | Width of right-hand margin as fraction of width of the graphics device. Ignored if square is TRUE. |
| RowSideColors | Character vector (or list of character vectors) with length(s) equal to ncol(x) containing color names for horizontal side bars for sample annotation |
| square | Logical, if TRUE margins are set such that cells in the heatmap are square |
| cexRow | Scale factor for row (sample) labels |
| cexCol | Scale factor for column (feature) labels |
| labRow | Character vector of row (sample) labels |
| col | Heatmap colors |
| zlim | Range of values for which colors should be plotted, if NULL range of finite values |
| heightPanels | Numeric vector of length two indicating height of the top and bottom panels. |
| expand_variants | Experimental option - leave set to FALSE |
| ... | further arguments passed to plotSpliceGraph |

Value

data.frame with information on exon bins and splice junctions included in the splice graph

Author(s)

Leonard Goldstein

Examples

```
## Not run:
sgvc_annotated <- annotate(sgvc_pred, txf_ann)
plotVariants(sgvc_annotated)

## End(Not run)
NULL
```

`predictCandidatesInternal`*Identify candidate internal exons*

Description

Identify candidate internal exons based on previously identified splice sites and regions with sufficient read coverage.

Usage

```
predictCandidatesInternal(islands, splicesites, frag_coverage, relCov)
```

Arguments

| | |
|----------------------------|---|
| <code>islands</code> | IRanges of genomic regions with minimal read coverage required for internal exon prediction |
| <code>splicesites</code> | IRanges of splice sites with metadata columns “type” and “N” |
| <code>frag_coverage</code> | R1e object with fragment coverage |
| <code>relCov</code> | Minimum relative coverage required for exon prediction |

Value

IRanges of candidate internal exons

Author(s)

Leonard Goldstein

predictCandidatesTerminal

Identify candidate terminal exons

Description

Identify candidate terminal exons based on previously identified splice sites and regions with sufficient read coverage.

Usage

```
predictCandidatesTerminal(islands, splicesites, type = c("exon_L", "exon_R"))
```

Arguments

| | |
|-------------|---|
| islands | IRanges of genomic regions with minimal read coverage required for internal exon prediction |
| splicesites | IRanges of splice sites with metadata columns "type" and "N" |
| type | Character string indicating whether terminal exons should be identified to the left ("exon_L") or right ("exon_R") of provided splice sites |

Value

IRanges of candidate terminal exons

Author(s)

Leonard Goldstein

predictExonsInternal *Identify internal exons*

Description

Identify internal exons based on candidate internal exons and compatible read coverage.

Usage

```
predictExonsInternal(candidates, frag_exonic, frag_intron, relCov, min_anchor,
  include_counts, retain_coverage)
```

Arguments

| | |
|-----------------|---|
| candidates | IRanges of candidate internal exons |
| frag_exonic | IRangesList with exonic regions from alignments |
| frag_intron | IRangesList with introns implied by spliced alignments |
| relCov | Minimum relative coverage required for exon prediction |
| min_anchor | Integer specifying minimum anchor length |
| include_counts | Logical indicating whether counts of compatible fragments should be included in metadata column "N" |
| retain_coverage | Logical indicating whether coverage for each exon should be retained as an RleList in metadata column "coverage". This allows filtering of features using more stringent criteria after the initial prediction. |

Value

IRanges of internal exons with metadata column "type" and optionally "N" for include_counts = TRUE, "N_splicesite", "coverage" for retain_coverage = TRUE

Author(s)

Leonard Goldstein

predictExonsTerminal *Identify terminal exons*

Description

Identify terminal exons based on candidate terminal exons and compatible read coverage.

Usage

```
predictExonsTerminal(candidates, frag_exonic, frag_intron, relCov, min_anchor,
  type = c("exon_L", "exon_R"), include_counts, retain_coverage)
```

Arguments

| | |
|-----------------|---|
| candidates | IRanges of candidate internal exons |
| frag_exonic | IRangesList with exonic regions from alignments |
| frag_intron | IRangesList with introns implied by spliced alignments |
| relCov | Minimum relative coverage required for exon prediction |
| min_anchor | Integer specifying minimum anchor length |
| type | Character string indicating whether terminal exons should be identified to the left ("exon_L") or right ("exon_R") of provided splice sites |
| include_counts | Logical indicating whether counts of compatible fragments should be included in metadata column "N" |
| retain_coverage | Logical indicating whether coverage for each exon should be retained as an RleList in metadata column "coverage". This allows filtering of features using more stringent criteria after the initial prediction. |

Value

IRanges of terminal exons with metadata column “type” and optionally “N” for include_counts = TRUE, “N_splicesite”, “coverage” for retain_coverage = TRUE

Author(s)

Leonard Goldstein

| | |
|------------------|----------------------------------|
| predictJunctions | <i>Identify splice junctions</i> |
|------------------|----------------------------------|

Description

Identify splice junctions from genomic RNA-seq read alignments.

Usage

```
predictJunctions(frag_exonic, frag_intron, min_junction_count, psi, min_anchor,
  retain_coverage)
```

Arguments

| | |
|--------------------|---|
| frag_exonic | IRangesList with exonic regions from alignments |
| frag_intron | IRangesList with introns implied by spliced alignments |
| min_junction_count | Minimum fragment count required for a splice junction to be included. If specified, argument alpha is ignored. |
| psi | Minimum splice frequency required for a splice junction to be included |
| min_anchor | Integer specifying minimum anchor length |
| retain_coverage | Logical indicating whether coverage for each exon should be retained as an RleList in metadata column “coverage”. This allows filtering of features using more stringent criteria after the initial prediction. |

Value

IRanges of splice junctions with metadata columns “type” and “N”, and optionally “N_splicesite” for retain_coverage = TRUE

Author(s)

Leonard Goldstein

predictSpliced *Ranges-based identification of splice junctions and exons*

Description

Ranges-based identification of splice junctions and exons.

Usage

```
predictSpliced(frag_exonic, frag_intron, min_junction_count, psi, beta, gamma,
               min_anchor, include_counts, retain_coverage, junctions_only, max_complexity,
               sample_name, seqlevel, strand)
```

Arguments

| | |
|--------------------|---|
| frag_exonic | IRangesList with exonic regions from alignments |
| frag_intron | IRangesList with introns implied by spliced alignments |
| min_junction_count | Minimum fragment count required for a splice junction to be included. If specified, argument alpha is ignored. |
| psi | Minimum splice frequency required for a splice junction to be included |
| beta | Minimum relative coverage required for an internal exon to be included |
| gamma | Minimum relative coverage required for a terminal exon to be included |
| min_anchor | Integer specifying minimum anchor length |
| include_counts | Logical indicating whether counts of compatible fragments should be included in metadata column "N" |
| retain_coverage | Logical indicating whether coverage for each exon should be retained as an RleList in metadata column "coverage". This allows filtering of features using more stringent criteria after the initial prediction. |
| junctions_only | Logical indicating whether predictions should be limited to identification of splice junctions only |
| max_complexity | Maximum allowed complexity. If a locus exceeds this threshold, it is skipped, resulting in a warning. Complexity is defined as the maximum number of unique predicted splice junctions overlapping a given position. High complexity regions are often due to spurious read alignments and can slow down processing. To disable this filter, set to NA. |
| sample_name | Sample name used in messages |
| seqlevel | seqlevel to be processed |
| strand | strand to be processed |

Value

IRanges with predicted features

Author(s)

Leonard Goldstein

predictTxFeatures *Splice junction and exon prediction from BAM files*

Description

Splice junctions and exons are predicted for each sample and merged across samples. Terminal exons are filtered and trimmed, if applicable. For details, see the help pages for [predictTxFeaturesPerSample](#), [mergeTxFeatures](#), and [processTerminalExons](#).

Usage

```
predictTxFeatures(sample_info, which = NULL, alpha = 2, psi = 0,
  beta = 0.2, gamma = 0.2, min_junction_count = NULL, min_anchor = 1,
  max_complexity = 20, min_n_sample = 1, min_overhang = NA,
  verbose = FALSE, cores = 1)
```

Arguments

| | |
|--------------------|---|
| sample_info | Data frame with sample information. Required columns are “sample_name”, “file_bam”, “paired_end”, “read_length”, “frag_length” and “lib_size”. Library information can be obtained with function <code>getBamInfo</code> . |
| which | GRanges of genomic regions to be considered for feature prediction, passed to <code>ScanBamParam</code> |
| alpha | Minimum FPKM required for a splice junction to be included. Internally, FPKMs are converted to counts, requiring arguments <code>read_length</code> , <code>frag_length</code> and <code>lib_size</code> . <code>alpha</code> is ignored if argument <code>min_junction_count</code> is specified. |
| psi | Minimum splice frequency required for a splice junction to be included |
| beta | Minimum relative coverage required for an internal exon to be included |
| gamma | Minimum relative coverage required for a terminal exon to be included |
| min_junction_count | Minimum fragment count required for a splice junction to be included. If specified, argument <code>alpha</code> is ignored. |
| min_anchor | Integer specifying minimum anchor length |
| max_complexity | Maximum allowed complexity. If a locus exceeds this threshold, it is skipped, resulting in a warning. Complexity is defined as the maximum number of unique predicted splice junctions overlapping a given position. High complexity regions are often due to spurious read alignments and can slow down processing. To disable this filter, set to <code>NA</code> . |
| min_n_sample | Minimum number of samples a feature must be observed in to be included |
| min_overhang | Minimum overhang required to suppress filtering or trimming of predicted terminal exons (see the manual page for <code>processTerminalExons</code>). Use <code>NULL</code> to disable processing (disabling processing is useful if results are subsequently merged with other predictions and processing is postponed until after the merging step). |
| verbose | If <code>TRUE</code> , generate messages indicating progress |
| cores | Number of cores available for parallel processing |

Value

TxFeatures object

Author(s)

Leonard Goldstein

Examples

```
path <- system.file("extdata", package = "SGSeq")
si$file_bam <- file.path(path, "bams", si$file_bam)
txf <- predictTxFeatures(si, gr)
```

predictTxFeaturesPerSample

Identification of splice junctions and exons from BAM file

Description

Splice junctions and exons are predicted from genomic RNA-seq read alignments in BAM format.

Usage

```
predictTxFeaturesPerSample(file_bam, which, paired_end, read_length,
  frag_length, lib_size, min_junction_count, alpha, psi, beta, gamma,
  min_anchor, include_counts, retain_coverage, junctions_only, max_complexity,
  sample_name, verbose, cores)
```

Arguments

| | |
|--------------------|---|
| file_bam | BAM file with genomic RNA-seq read alignments |
| which | GRanges of genomic regions to be considered for feature prediction, passed to ScanBamParam |
| paired_end | Logical, TRUE for paired-end data, FALSE for single-end data |
| read_length | Read length required for use with alpha |
| frag_length | Fragment length for paired-end data required for use with alpha |
| lib_size | Number of aligned fragments required for use with alpha |
| min_junction_count | Minimum fragment count required for a splice junction to be included. If specified, argument alpha is ignored. |
| alpha | Minimum FPKM required for a splice junction to be included. Internally, FPKMs are converted to counts, requiring arguments read_length, frag_length and lib_size. alpha is ignored if argument min_junction_count is specified. |
| psi | Minimum splice frequency required for a splice junction to be included |
| beta | Minimum relative coverage required for an internal exon to be included |
| gamma | Minimum relative coverage required for a terminal exon to be included |
| min_anchor | Integer specifying minimum anchor length |

| | |
|------------------------------|---|
| <code>include_counts</code> | Logical indicating whether counts of compatible fragments should be included in metadata column “N” |
| <code>retain_coverage</code> | Logical indicating whether coverage for each exon should be retained as an <code>RleList</code> in metadata column “coverage”. This allows filtering of features using more stringent criteria after the initial prediction. |
| <code>junctions_only</code> | Logical indicating whether predictions should be limited to identification of splice junctions only |
| <code>max_complexity</code> | Maximum allowed complexity. If a locus exceeds this threshold, it is skipped, resulting in a warning. Complexity is defined as the maximum number of unique predicted splice junctions overlapping a given position. High complexity regions are often due to spurious read alignments and can slow down processing. To disable this filter, set to NA. |
| <code>sample_name</code> | Sample name used in messages |
| <code>verbose</code> | If TRUE, generate messages indicating progress |
| <code>cores</code> | Number of cores available for parallel processing |

Details

For spliced alignments, the direction of transcription is inferred from the XS tag in the BAM file and used to assign strand information to the read, or fragment for paired-end data.

Feature prediction is performed in two steps. First, splice junctions are identified from spliced alignments. Second, exons are identified based on regions that are flanked by splice junctions and show sufficient coverage with compatible reads.

Splice junctions implied by read alignments are filtered based on fragment count and splice frequency. The splice frequency at the splice donor (acceptor) is defined as x_J/x_D (x_J/x_A), where x_J is the number of fragments containing the splice junction, and x_D (x_A) is the number of fragments overlapping the exon/intron (intron/exon) boundary. Fragments overlapping the spliced boundary can be either spliced or extend into the intron. To be included in predicted features, splice junctions must have fragment count at least `min_junction_count` or FPKM at least `alpha`, and splice frequency at both donor and acceptor at least `psi`.

Regions between any pair of identified splice junctions with sufficient compatible read coverage are considered candidate internal exons. Read coverage for a candidate exon is computed based on compatible fragments, i.e. fragments with matching (or missing) strand information and introns consistent with the exon under consideration. Candidate exons are included in predicted features if the minimum coverage is at least `beta` * number of junction-containing fragments for either flanking junctions.

Terminal exons are regions downstream or upstream of splice junctions with compatible fragment coverage at least `gamma` * number of junction-containing fragments.

Value

`TxFeatures` object

Author(s)

Leonard Goldstein

predictTxFeaturesPerStrand

Identification of splice junctions and exons for a given chromosome and strand

Description

Identification of splice junctions and exons for a given chromosome and strand.

Usage

```
predictTxFeaturesPerStrand(file_bam, paired_end, which, min_junction_count, psi,
    beta, gamma, min_anchor, include_counts, retain_coverage, junctions_only,
    max_complexity, sample_name, verbose)
```

Arguments

| | |
|--------------------|---|
| file_bam | BAM file with genomic RNA-seq read alignments |
| paired_end | Logical, TRUE for paired-end data, FALSE for single-end data |
| which | GRanges of genomic regions to be considered for feature prediction, passed to ScanBamParam |
| min_junction_count | Minimum fragment count required for a splice junction to be included. If specified, argument alpha is ignored. |
| psi | Minimum splice frequency required for a splice junction to be included |
| beta | Minimum relative coverage required for an internal exon to be included |
| gamma | Minimum relative coverage required for a terminal exon to be included |
| min_anchor | Integer specifying minimum anchor length |
| include_counts | Logical indicating whether counts of compatible fragments should be included in metadata column "N" |
| retain_coverage | Logical indicating whether coverage for each exon should be retained as an RleList in metadata column "coverage". This allows filtering of features using more stringent criteria after the initial prediction. |
| junctions_only | Logical indicating whether predictions should be limited to identification of splice junctions only |
| max_complexity | Maximum allowed complexity. If a locus exceeds this threshold, it is skipped, resulting in a warning. Complexity is defined as the maximum number of unique predicted splice junctions overlapping a given position. High complexity regions are often due to spurious read alignments and can slow down processing. To disable this filter, set to NA. |
| sample_name | Sample name used in messages |
| verbose | If TRUE, generate messages indicating progress |

Value

GRanges of predicted features

Author(s)

Leonard Goldstein

 predictVariantEffects *Predict the effect of splice variants on protein-coding transcripts*

Description

The effect of a splice variant is predicted for individual protein-coding transcripts.

Usage

```
predictVariantEffects(sgv, tx, genome, fix_start_codon = TRUE,
  output = c("short", "full"), cores = 1)
```

Arguments

| | |
|-----------------|---|
| sgv | SGVariants object |
| tx | TxDb object, or GRangesList of exons grouped by transcript with metadata columns txName, geneName, cdsStart and cdsEnd (by convention, cdsStart < cdsEnd for both strands). For import from GFF format, use function importTranscripts. |
| genome | BSgenome object |
| fix_start_codon | Logical indicating whether the annotated start codon should be considered fixed and the variant transcript should not be scanned for alternative start codons |
| output | Character string indicating whether short results or full results (with additional columns) should be returned |
| cores | Number of cores available for parallel processing |

Value

data.frame with rows corresponding to a variant-transcript pair. The output includes columns for variant identifier, transcript name, gene name, type of alteration at the RNA and protein level, and variant description at the RNA and protein level in HGVS notation. For output = "full" additional columns are returned. These include the full-length RNA and protein sequence for the reference and variant transcript. Event start and end coordinates in the full output are 0- and 1-based, respectively (to allow for description of deletions). Coordinates for the last junction in a transcript refer to the last base of the second-to-last exon.

Author(s)

Leonard Goldstein

Examples

```
require(BSgenome.Hsapiens.UCSC.hg19)
seqlevelsStyle(Hsapiens) <- "NCBI"
predictVariantEffects(sgv_pred, tx, Hsapiens)
```

processTerminalExons *Process predicted terminal exons*

Description

Predicted terminal exons are processed as described under Details.

Usage

```
processTerminalExons(features, min_overhang = NA)
```

Arguments

| | |
|--------------|---|
| features | TxFeatures object |
| min_overhang | Minimum overhang required to suppress filtering or trimming of predicted terminal exons (see Details). Use NA to exclude all terminal exons sharing a splice with an internal exon and trim all remaining terminal exons overlapping other exons. |

Details

Processing of terminal exon predictions is done in two steps: (1) terminal exons that share a splice site with an internal exon are filtered, and (2) remaining terminal exons that overlap other exons are trimmed.

predictTxFeatures predicts flanking terminal exons for each identified splice junction. This ensures that each splice junction has a flanking exon after merging with mergeTxFeatures. This approach results in many predicted terminal exons that share a splice site with predicted internal exons (often contained within them or with a short overhang due to incorrect alignments). Most of these are not real terminal exons and are filtered before further analysis. Filtering based on the overhang is controlled with argument min_overhang.

Some of the remaining predicted terminal exons overlap other exons such that their unspliced boundary shows a short overhang with respect to a spliced boundary of the overlapping exon. Often these exon extensions into an intron are due to incorrect alignments. Terminal exons with overhang smaller than min_overhang are trimmed such that their trimmed unspliced boundary coincides with the spliced boundary of the overlapping exon.

Value

TxFeatures object with processed features

Author(s)

Leonard Goldstein

Examples

```
txf_processed <- processTerminalExons(txf_ann)
```

removeExonsIsolated *Remove exons with no flanking splice junctions*

Description

Remove exons with no flanking splice junctions.

Usage

```
removeExonsIsolated(features)
```

Arguments

features TxFeatures object

Value

TxFeatures object with filtered features

Author(s)

Leonard Goldstein

sgfc_ann *Example splice graph feature counts (annotation-based)*

Description

Compatible counts and FPKMs for FBXO31 splice graph features, based on UCSC knownGene annotation.

Format

SGFeatureCounts object

Author(s)

Leonard Goldstein

sgfc_pred

Example splice graph feature counts (predicted)

Description

Compatible counts and FPKMs for FBXO31 splice graph features, predicted from example BAM files.

Format

SGFeatureCounts object

Author(s)

Leonard Goldstein

SGFeatureCounts

Splice graph feature counts

Description

Creates an instance of S4 class SGFeatureCounts for storing compatible splice graph feature counts.

Usage

SGFeatureCounts(x)

Arguments

x RangedSummarizedExperiment with SGFeatures as rowRanges and assays “counts” and “FPKM”

Value

SGFeatureCounts object

Author(s)

Leonard Goldstein

Examples

```
sgfc <- SGFeatureCounts()
```

SGFeatures

*Splice graph features***Description**

Creates an instance of S4 class SGFeatures for storing splice graph features.

Usage

```
SGFeatures(x, type = mcols(x)$type, splice5p = mcols(x)$splice5p,
  splice3p = mcols(x)$splice3p, featureID = mcols(x)$featureID,
  geneID = mcols(x)$geneID, txName = mcols(x)$txName,
  geneName = mcols(x)$geneName)
```

Arguments

| | |
|-----------|--|
| x | GRanges with known strand (“+”, “-”) |
| type | Character vector or factor taking value J, E, D, or A |
| splice5p | Logical vector indicating a mandatory splice at the 5’ end of an exon bin (determining whether reads extending across the 5’ boundary must be spliced to be considered compatible) |
| splice3p | Logical vector indicating a mandatory splice at the 3’ end of an exon bin (determining whether reads extending across the 3’ boundary must be spliced to be considered compatible) |
| featureID | Integer vector of feature IDs |
| geneID | Integer vector of gene IDs |
| txName | CharacterList of transcript names or NULL |
| geneName | CharacterList of gene names or NULL |

Details

SGFeatures extends GRanges with column slot type specifying feature type. type is a factor with levels J (splice junction), E (exon bin), D (splice donor), A (splice acceptor).

splice5p and splice3p are logical vectors indicating mandatory splices at the 5’ and 3’ end of an exon bin, respectively. These are used to determine whether reads extending across the 5’ and 3’ boundaries of an exon bin must be spliced at the boundary to be considered compatible with the exon bin.

featureID and geneID are integer vectors representing unique identifiers for features and genes (connected components in the splice graph).

txName and geneName are CharacterLists storing transcript and gene annotation, respectively.

Value

SGFeatures object

Author(s)

Leonard Goldstein

Examples

```
sgf <- SGFeatures()
```

 sgf_ann

Example splice graph features (annotation-based)

Description

Splice graph features for FBXO31, based on UCSC knownGene annotation.

Format

SGFeatures object

Author(s)

Leonard Goldstein

 sgf_pred

Example splice graph features (predicted)

Description

Splice graph features for FBXO31, predicted from example BAM files.

Format

SGFeatures object

Author(s)

Leonard Goldstein

 SGSegments

Splice graph segments

Description

Creates an instance of S4 class SGSegments for storing splice graph segments.

Usage

```
SGSegments(x)
```

Arguments

x GRangesList of SGFeatures with appropriate outer metadata columns

Value

SGSegments object

Author(s)

Leonard Goldstein

| | |
|-----------------|------------------------------------|
| SGVariantCounts | <i>Splice graph variant counts</i> |
|-----------------|------------------------------------|

Description

Creates an instance of S4 class SGVariantCounts for storing splice variant counts.

Usage

```
SGVariantCounts(x)
```

Arguments

x RangedSummarizedExperiment with SGVariants as rowRanges and assays “variantFreq”, “countsVariant5p”, “countsVariant3p”, “countsEvent5p”, “countsEvent3p”, and optionally “countsVariant5pOr3p”

Value

SGVariantCounts object

Author(s)

Leonard Goldstein

Examples

```
sgvc <- SGVariantCounts()
```

| | |
|------------|------------------------------|
| SGVariants | <i>Splice graph variants</i> |
|------------|------------------------------|

Description

Creates an instance of S4 class SGVariants for storing splice variants.

Usage

```
SGVariants(x)
```

Arguments

x GRangesList of SGFeatures with appropriate outer metadata columns

Details

SGVariants includes columns as described below.

- `from` and `to` indicate the variant start and end, respectively. `from` nodes are splice donors (“D”) or transcript starts (“S”). `to` nodes are splice acceptors (“A”) or transcript ends (“E”).
- `type` and `featureID` describe the variant in terms of the splice graph features that make up the variant.
- `segmentID` specifies unique identifiers labelling unbranched segments of the splice graph.
- `closed5p` indicates whether nodes in the variant can be reached from nodes outside of the variant exclusively through the `from` node.
- `closed3p` indicates whether nodes in the variant can reach nodes outside of the variant exclusively through the `to` node.
- `closed5pEvent` indicates whether nodes in the event can be reached from nodes outside of the event exclusively through the `from` node.
- `closed3pEvent` indicates whether nodes in the event can reach nodes outside of the event exclusively through the `to` node.
- `geneID` has the same interpretation as for `SGFeatures`.
- `eventID` and `variantID` are unique identifiers for each event and variant, respectively.
- `featureID5p` and `featureID3p` indicate representative features used for variant quantification at the start and end of the variant, respectively.
- `featureID5pEvent` and `featureID3pEvent` indicate the ensemble of representative features at the start and end of the event, respectively.
- `txName` indicates structurally compatible transcripts.
- `geneName` behaves as for `SGFeatures`.
- `variantType` indicates whether a splice variant is consistent with a canonical splice event (for a list of possible values, see the manual page for `annotateSGVariants`).
- `variantName` provides a unique name for each splice variant (for details, see the manual page for `makeVariantNames`).

Value

SGVariants object

Author(s)

Leonard Goldstein

Examples

```
sgv <- SGVariants()
```

sgvc_ann

Example splice variant counts (annotated)

Description

Splice variant counts and frequencies for FBXO31. Splice variants are based on UCSC knownGene annotation.

Format

SGVariantCounts object

Author(s)

Leonard Goldstein

sgvc_ann_from_bam

Example splice variant counts (annotated) from BAM files

Description

Splice variant counts and frequencies for FBXO31. Splice variants are based on UCSC knownGene annotation. Counts were obtained from BAM files.

Format

SGVariantCounts object

Author(s)

Leonard Goldstein

sgvc_pred

Example splice variant counts (predicted)

Description

Splice variant counts and frequencies for FBXO31. Splice variants were predicted from example BAM files.

Format

SGVariantCounts object

Author(s)

Leonard Goldstein

| | |
|--------------------|---|
| sgvc_pred_from_bam | <i>Example splice variant counts (predicted) from BAM files</i> |
|--------------------|---|

Description

Splice variant counts and frequencies for FBXO31. Splice variants were predicted from example BAM files. Counts were obtained from BAM files.

Format

SGVariantCounts object

Author(s)

Leonard Goldstein

| | |
|---------|---|
| sgv_ann | <i>Example splice variants (annotation-based)</i> |
|---------|---|

Description

Splice variants for FBXO31, based on UCSC knownGene annotation.

Format

SGVariants object

Author(s)

Leonard Goldstein

| | |
|----------|--|
| sgv_pred | <i>Example splice variants (predicted)</i> |
|----------|--|

Description

Splice variants for FBXO31, predicted from example BAM files.

Format

SGVariants object

Author(s)

Leonard Goldstein

| | |
|----|-----------------------------------|
| si | <i>Example sample information</i> |
|----|-----------------------------------|

Description

Sample information for example BAM files included in the SGSeq package.

Format

data.frame with columns “sample_name”, “file_bam”, “paired_end”, “read_length”, “frag_length” and “lib_size”.

Author(s)

Leonard Goldstein

| | |
|-------|---|
| slots | <i>Accessing and replacing metadata columns</i> |
|-------|---|

Description

Accessor and replacement functions for metadata columns.

Usage

```
type(x) <- value
```

```
txName(x)
```

```
txName(x) <- value
```

```
geneName(x)
```

```
geneName(x) <- value
```

```
featureID(x)
```

```
featureID(x) <- value
```

```
geneID(x)
```

```
geneID(x) <- value
```

```
splice5p(x)
```

```
splice5p(x) <- value
```

```
splice3p(x)
```

```
splice3p(x) <- value
from(x) <- value
to(x) <- value
segmentID(x)
segmentID(x) <- value
variantID(x)
variantID(x) <- value
eventID(x)
eventID(x) <- value
closed5p(x)
closed5p(x) <- value
closed3p(x)
closed3p(x) <- value
closed5pEvent(x)
closed5pEvent(x) <- value
closed3pEvent(x)
closed3pEvent(x) <- value
variantType(x)
variantType(x) <- value
variantName(x)
variantName(x) <- value
featureID5p(x)
featureID5p(x) <- value
featureID3p(x)
featureID3p(x) <- value
featureID5pEvent(x)
```

```
featureID5pEvent(x) <- value

featureID3pEvent(x)

featureID3pEvent(x) <- value

## S4 method for signature 'Features'
type(x)

## S4 method for signature 'Paths'
type(x)

## S4 method for signature 'Counts'
type(x)

## S4 replacement method for signature 'Features'
type(x) <- value

## S4 replacement method for signature 'Paths'
type(x) <- value

## S4 replacement method for signature 'Counts'
type(x) <- value

## S4 method for signature 'Features'
txName(x)

## S4 method for signature 'Paths'
txName(x)

## S4 method for signature 'Counts'
txName(x)

## S4 replacement method for signature 'Features'
txName(x) <- value

## S4 replacement method for signature 'Paths'
txName(x) <- value

## S4 replacement method for signature 'Counts'
txName(x) <- value

## S4 method for signature 'Features'
geneName(x)

## S4 method for signature 'Paths'
geneName(x)

## S4 method for signature 'Counts'
geneName(x)

## S4 replacement method for signature 'Features'
```



```
geneName(x) <- value

## S4 replacement method for signature 'Paths'
geneName(x) <- value

## S4 replacement method for signature 'Counts'
geneName(x) <- value

## S4 method for signature 'SGFeatures'
featureID(x)

## S4 method for signature 'Paths'
featureID(x)

## S4 method for signature 'Counts'
featureID(x)

## S4 replacement method for signature 'SGFeatures'
featureID(x) <- value

## S4 replacement method for signature 'Paths'
featureID(x) <- value

## S4 replacement method for signature 'Counts'
featureID(x) <- value

## S4 method for signature 'SGFeatures'
geneID(x)

## S4 method for signature 'Paths'
geneID(x)

## S4 method for signature 'Counts'
geneID(x)

## S4 replacement method for signature 'SGFeatures'
geneID(x) <- value

## S4 replacement method for signature 'Paths'
geneID(x) <- value

## S4 replacement method for signature 'Counts'
geneID(x) <- value

## S4 method for signature 'SGFeatures'
splice5p(x)

## S4 method for signature 'SGSegments'
splice5p(x)

## S4 method for signature 'SGFeatureCounts'
splice5p(x)
```

```
## S4 replacement method for signature 'SGFeatures'  
splice5p(x) <- value  
  
## S4 replacement method for signature 'SGSegments'  
splice5p(x) <- value  
  
## S4 replacement method for signature 'SGFeatureCounts'  
splice5p(x) <- value  
  
## S4 method for signature 'SGFeatures'  
splice3p(x)  
  
## S4 method for signature 'SGSegments'  
splice3p(x)  
  
## S4 method for signature 'SGFeatureCounts'  
splice3p(x)  
  
## S4 replacement method for signature 'SGFeatures'  
splice3p(x) <- value  
  
## S4 replacement method for signature 'SGSegments'  
splice3p(x) <- value  
  
## S4 replacement method for signature 'SGFeatureCounts'  
splice3p(x) <- value  
  
## S4 method for signature 'Paths'  
segmentID(x)  
  
## S4 method for signature 'SGVariantCounts'  
segmentID(x)  
  
## S4 replacement method for signature 'Paths'  
segmentID(x) <- value  
  
## S4 replacement method for signature 'SGVariantCounts'  
segmentID(x) <- value  
  
## S4 method for signature 'Paths'  
from(x)  
  
## S4 method for signature 'SGVariantCounts'  
from(x)  
  
## S4 replacement method for signature 'Paths'  
from(x) <- value  
  
## S4 replacement method for signature 'SGVariantCounts'  
from(x) <- value
```

```
## S4 method for signature 'Paths'
to(x)

## S4 method for signature 'SGVariantCounts'
to(x)

## S4 replacement method for signature 'Paths'
to(x) <- value

## S4 replacement method for signature 'SGVariantCounts'
to(x) <- value

## S4 method for signature 'SGVariants'
eventID(x)

## S4 method for signature 'SGVariantCounts'
eventID(x)

## S4 replacement method for signature 'SGVariants'
eventID(x) <- value

## S4 replacement method for signature 'SGVariantCounts'
eventID(x) <- value

## S4 method for signature 'SGVariants'
variantID(x)

## S4 method for signature 'SGVariantCounts'
variantID(x)

## S4 replacement method for signature 'SGVariants'
variantID(x) <- value

## S4 replacement method for signature 'SGVariantCounts'
variantID(x) <- value

## S4 method for signature 'SGVariants'
closed5p(x)

## S4 method for signature 'SGVariantCounts'
closed5p(x)

## S4 replacement method for signature 'SGVariants'
closed5p(x) <- value

## S4 replacement method for signature 'SGVariantCounts'
closed5p(x) <- value

## S4 method for signature 'SGVariants'
closed3p(x)

## S4 method for signature 'SGVariantCounts'
```

```
closed3p(x)

## S4 replacement method for signature 'SGVariants'
closed3p(x) <- value

## S4 replacement method for signature 'SGVariantCounts'
closed3p(x) <- value

## S4 method for signature 'SGVariants'
closed5pEvent(x)

## S4 method for signature 'SGVariantCounts'
closed5pEvent(x)

## S4 replacement method for signature 'SGVariants'
closed5pEvent(x) <- value

## S4 replacement method for signature 'SGVariantCounts'
closed5pEvent(x) <- value

## S4 method for signature 'SGVariants'
closed3pEvent(x)

## S4 method for signature 'SGVariantCounts'
closed3pEvent(x)

## S4 replacement method for signature 'SGVariants'
closed3pEvent(x) <- value

## S4 replacement method for signature 'SGVariantCounts'
closed3pEvent(x) <- value

## S4 method for signature 'SGVariants'
variantName(x)

## S4 method for signature 'SGVariantCounts'
variantName(x)

## S4 replacement method for signature 'SGVariants'
variantName(x) <- value

## S4 replacement method for signature 'SGVariantCounts'
variantName(x) <- value

## S4 method for signature 'SGVariants'
variantType(x)

## S4 method for signature 'SGVariantCounts'
variantType(x)

## S4 replacement method for signature 'SGVariants'
variantType(x) <- value
```

```
## S4 replacement method for signature 'SGVariantCounts'  
variantType(x) <- value  
  
## S4 method for signature 'SGVariants'  
featureID5p(x)  
  
## S4 method for signature 'SGVariantCounts'  
featureID5p(x)  
  
## S4 replacement method for signature 'SGVariants'  
featureID5p(x) <- value  
  
## S4 replacement method for signature 'SGVariantCounts'  
featureID5p(x) <- value  
  
## S4 method for signature 'SGVariants'  
featureID3p(x)  
  
## S4 method for signature 'SGVariantCounts'  
featureID3p(x)  
  
## S4 replacement method for signature 'SGVariants'  
featureID3p(x) <- value  
  
## S4 replacement method for signature 'SGVariantCounts'  
featureID3p(x) <- value  
  
## S4 method for signature 'SGVariants'  
featureID5pEvent(x)  
  
## S4 method for signature 'SGVariantCounts'  
featureID5pEvent(x)  
  
## S4 replacement method for signature 'SGVariants'  
featureID5pEvent(x) <- value  
  
## S4 replacement method for signature 'SGVariantCounts'  
featureID5pEvent(x) <- value  
  
## S4 method for signature 'SGVariants'  
featureID3pEvent(x)  
  
## S4 method for signature 'SGVariantCounts'  
featureID3pEvent(x)  
  
## S4 replacement method for signature 'SGVariants'  
featureID3pEvent(x) <- value  
  
## S4 replacement method for signature 'SGVariantCounts'  
featureID3pEvent(x) <- value
```

Arguments

| | |
|-------|-----------------------------------|
| x | Object containing metadata column |
| value | Replacement value |

Details

S4 classes defined in the SGSeq package contain metadata columns that store information for each element in the object. For example, class TxFeatures contains a column type that indicates feature type. The specific columns contained in an object depend on its class.

Value

Content of metadata column for accessor functions or updated object for replacement functions.

Author(s)

Leonard Goldstein

Examples

```
head(type(txf_ann))
head(type(sgf_ann))
```

| | |
|-------------------|--|
| splicesiteOverlap | <i>Compatible fragment counts for splice sites</i> |
|-------------------|--|

Description

Identify fragments with alignments extending across exon/intron boundaries.

Usage

```
splicesiteOverlap(splicesites, side, frag_exonic, frag_intron, min_anchor,
  include = c("all", "spliced", "unspliced"), counts = TRUE)
```

Arguments

| | |
|-------------|---|
| splicesites | IRanges of splice sites |
| side | Character vector indicating whether the spliced boundary is to the left (“L”) or right (“R”) of the splice site |
| frag_exonic | IRangesList of exonic regions, one entry per fragment |
| frag_intron | IRangesList of introns, one entry per fragment |
| min_anchor | Integer specifying minimum anchor length |
| include | Character string indicating whether considered fragments should be all that overlap the splice site (“all”), those that are spliced at the site (“spliced”) or those that are not spliced, i.e. extend into the adjacent intron (“unspliced”) |
| counts | Logical indicating whether counts or indices of compatible fragments should be returned |

Value

Counts or list of indices of compatible fragments

Author(s)

Leonard Goldstein

| | |
|----|----------------------------|
| tx | <i>Example transcripts</i> |
|----|----------------------------|

Description

FBXO31 transcripts, based on UCSC knownGene annotation. Suitable as input for `convertToTxFeatures` and `predictVariantEffects`.

Format

GRangesList object

Author(s)

Leonard Goldstein

| | |
|------------|----------------------------|
| TxFeatures | <i>Transcript features</i> |
|------------|----------------------------|

Description

Creates an instance of S4 class TxFeatures for storing transcript features.

Usage

```
TxFeatures(x, type = mcols(x)$type, txName = mcols(x)$txName,
  geneName = mcols(x)$geneName)
```

Arguments

| | |
|----------|---|
| x | GRanges with known strand (“+”, “-”) |
| type | Character vector or factor, taking value J, I, F, L, or U |
| txName | CharacterList of transcript names or NULL |
| geneName | CharacterList of gene names or NULL |

Details

TxFeatures extends GRanges with column slot `type` specifying feature type. `type` is a factor with levels J (splice junction), I (internal exon), F (5’ terminal exon), L (3’ terminal exon), U (unspliced transcript).

`txName` and `geneName` are CharacterLists storing transcript and gene annotation, respectively.

Value

TxFeatures object

Author(s)

Leonard Goldstein

Examples

```
gr <- GRanges(1, IRanges(101, 200), "+")
txf <- TxFeatures(gr, type = "J")
```

txf_ann

Example transcript features (annotation-based)

Description

Transcript features for FBXO31, based on UCSC knownGene annotation.

Format

TxFeatures object

Author(s)

Leonard Goldstein

txf_pred

Example transcript features (predicted)

Description

Transcript features for FBXO31, predicted from example BAM files.

Format

TxFeatures object

Author(s)

Leonard Goldstein

| | |
|--------------|----------------------|
| updateObject | <i>Update object</i> |
|--------------|----------------------|

Description

Update object created with previous version of SGSeq.

Usage

```
## S4 method for signature 'SGVariants'  
updateObject(object, ..., verbose = FALSE)
```

```
## S4 method for signature 'SGVariantCounts'  
updateObject(object, ..., verbose = FALSE)
```

Arguments

| | |
|---------|---------------------------------------|
| object | Object to be updated |
| ... | Additional arguments |
| verbose | Should a warning message be generated |

Value

Updated object

Author(s)

Leonard Goldstein

Index

* internal

- annotateSGVariants, 6
 - exonCompatible, 10
 - filterFeatures, 11
 - findOverlapsRanges, 12
 - getSGFeatureCountsPerSample, 15
 - gr, 17
 - junctionCompatible, 18
 - makeVariantNames, 20
 - predictCandidatesInternal, 27
 - predictCandidatesTerminal, 28
 - predictExonsInternal, 28
 - predictExonsTerminal, 29
 - predictJunctions, 30
 - predictSpliced, 31
 - predictTxFeaturesPerSample, 33
 - predictTxFeaturesPerStrand, 35
 - removeExonsIsolated, 38
 - sgf_ann, 41
 - sgf_pred, 41
 - sgfc_ann, 38
 - sgfc_pred, 39
 - SGSegments, 41
 - sgv_ann, 45
 - sgv_pred, 45
 - sgvc_ann, 44
 - sgvc_ann_from_bam, 44
 - sgvc_pred, 44
 - sgvc_pred_from_bam, 45
 - si, 46
 - spliceSiteOverlap, 54
 - tx, 55
 - txf_ann, 56
 - txf_pred, 56
-
- analyzeFeatures, 3
 - analyzeVariants, 4
 - annotate, 4, 5
 - annotateSGVariants, 6, 13
 - assays, 7
-
- closed3p (slots), 46
 - closed3p, SGVariantCounts-method (slots), 46
 - closed3p<- (slots), 46
 - closed3p<-, SGVariantCounts-method (slots), 46
 - closed3p<-, SGVariants-method (slots), 46
 - closed3pEvent (slots), 46
 - closed3pEvent, SGVariantCounts-method (slots), 46
 - closed3pEvent, SGVariants-method (slots), 46
 - closed3pEvent<- (slots), 46
 - closed3pEvent<-, SGVariantCounts-method (slots), 46
 - closed3pEvent<-, SGVariants-method (slots), 46
 - closed5p (slots), 46
 - closed5p, SGVariantCounts-method (slots), 46
 - closed5p, SGVariants-method (slots), 46
 - closed5p<- (slots), 46
 - closed5p<-, SGVariantCounts-method (slots), 46
 - closed5p<-, SGVariants-method (slots), 46
 - closed5pEvent (slots), 46
 - closed5pEvent, SGVariantCounts-method (slots), 46
 - closed5pEvent, SGVariants-method (slots), 46
 - closed5pEvent<- (slots), 46
 - closed5pEvent<-, SGVariantCounts-method (slots), 46
 - closed5pEvent<-, SGVariants-method (slots), 46
 - convertToSGFeatures, 4, 8
 - convertToTxFeatures, 9
 - counts, SGFeatureCounts-method (assays), 7
 - counts, SGVariantCounts-method (assays), 7
 - counts<-, SGFeatureCounts-method (assays), 7
 - counts<-, SGVariantCounts-method (assays), 7

- eventID (slots), 46
- eventID, SGVariantCounts-method (slots), 46
- eventID, SGVariants-method (slots), 46
- eventID<- (slots), 46
- eventID<-, SGVariantCounts-method (slots), 46
- eventID<-, SGVariants-method (slots), 46
- exonCompatible, 10
- exportFeatures, 11
- featureID (slots), 46
- featureID, Counts-method (slots), 46
- featureID, Paths-method (slots), 46
- featureID, SGFeatures-method (slots), 46
- featureID3p (slots), 46
- featureID3p, SGVariantCounts-method (slots), 46
- featureID3p, SGVariants-method (slots), 46
- featureID3p<- (slots), 46
- featureID3p<-, SGVariantCounts-method (slots), 46
- featureID3p<-, SGVariants-method (slots), 46
- featureID3pEvent (slots), 46
- featureID3pEvent, SGVariantCounts-method (slots), 46
- featureID3pEvent, SGVariants-method (slots), 46
- featureID3pEvent<- (slots), 46
- featureID3pEvent<-, SGVariantCounts-method (slots), 46
- featureID3pEvent<-, SGVariants-method (slots), 46
- featureID5p (slots), 46
- featureID5p, SGVariantCounts-method (slots), 46
- featureID5p, SGVariants-method (slots), 46
- featureID5p<- (slots), 46
- featureID5p<-, SGVariantCounts-method (slots), 46
- featureID5p<-, SGVariants-method (slots), 46
- featureID5pEvent (slots), 46
- featureID5pEvent, SGVariantCounts-method (slots), 46
- featureID5pEvent, SGVariants-method (slots), 46
- featureID5pEvent<- (slots), 46
- featureID5pEvent<-, SGVariantCounts-method (slots), 46
- featureID5pEvent<-, SGVariants-method (slots), 46
- featureID5pEvent<-, SGVariants-method (slots), 46
- featureID<- (slots), 46
- featureID<-, Counts-method (slots), 46
- featureID<-, Paths-method (slots), 46
- featureID<-, SGFeatures-method (slots), 46
- filterFeatures, 11
- findOverlapsRanges, 12
- findSGVariants, 4, 13
- FPKM (assays), 7
- FPKM, SGFeatureCounts-method (assays), 7
- FPKM, SGVariantCounts-method (assays), 7
- FPKM<- (assays), 7
- FPKM<-, SGFeatureCounts-method (assays), 7
- from, Paths-method (slots), 46
- from, SGVariantCounts-method (slots), 46
- from<- (slots), 46
- from<-, Paths-method (slots), 46
- from<-, SGVariantCounts-method (slots), 46
- geneID (slots), 46
- geneID, Counts-method (slots), 46
- geneID, Paths-method (slots), 46
- geneID, SGFeatures-method (slots), 46
- geneID<- (slots), 46
- geneID<-, Counts-method (slots), 46
- geneID<-, Paths-method (slots), 46
- geneID<-, SGFeatures-method (slots), 46
- geneName (slots), 46
- geneName, Counts-method (slots), 46
- geneName, Features-method (slots), 46
- geneName, Paths-method (slots), 46
- geneName<- (slots), 46
- geneName<-, Counts-method (slots), 46
- geneName<-, Features-method (slots), 46
- geneName<-, Paths-method (slots), 46
- getBamInfo, 14
- getSGFeatureCounts, 4, 15
- getSGFeatureCountsPerSample, 15
- getSGVariantCounts, 4, 16
- gr, 17
- importTranscripts, 18
- junctionCompatible, 18
- makeSGFeatureCounts, 19
- makeVariantNames, 20
- mergeTxFeatures, 20, 32
- plotCoverage, 21

- plotFeatures, 22
- plotSpliceGraph, 24
- plotVariants, 26
- predictCandidatesInternal, 27
- predictCandidatesTerminal, 28
- predictExonsInternal, 28
- predictExonsTerminal, 29
- predictJunctions, 30
- predictSpliced, 31
- predictTxFeatures, 4, 32
- predictTxFeaturesPerSample, 32, 33
- predictTxFeaturesPerStrand, 35
- predictVariantEffects, 36
- processTerminalExons, 32, 37
- removeExonsIsolated, 38
- segmentID (slots), 46
- segmentID, Paths-method (slots), 46
- segmentID, SGVariantCounts-method (slots), 46
- segmentID<- (slots), 46
- segmentID<-, Paths-method (slots), 46
- segmentID<-, SGVariantCounts-method (slots), 46
- sgf_ann, 41
- sgf_pred, 41
- sgfc_ann, 38
- sgfc_pred, 39
- SGFeatureCounts, 39
- SGFeatures, 40
- SGSegments, 41
- sgv_ann, 45
- sgv_pred, 45
- SGVariantCounts, 42
- SGVariants, 42
- sgvc_ann, 44
- sgvc_ann_from_bam, 44
- sgvc_pred, 44
- sgvc_pred_from_bam, 45
- si, 46
- slots, 46
- splice3p (slots), 46
- splice3p, SGFeatureCounts-method (slots), 46
- splice3p, SGFeatures-method (slots), 46
- splice3p, SGSegments-method (slots), 46
- splice3p<- (slots), 46
- splice3p<-, SGFeatureCounts-method (slots), 46
- splice3p<-, SGFeatures-method (slots), 46
- splice3p<-, SGSegments-method (slots), 46
- splice5p (slots), 46
- splice5p, SGFeatureCounts-method (slots), 46
- splice5p, SGFeatures-method (slots), 46
- splice5p, SGSegments-method (slots), 46
- splice5p<- (slots), 46
- splice5p<-, SGFeatureCounts-method (slots), 46
- splice5p<-, SGFeatures-method (slots), 46
- splice5p<-, SGSegments-method (slots), 46
- splicesiteOverlap, 54
- to, Paths-method (slots), 46
- to, SGVariantCounts-method (slots), 46
- to<- (slots), 46
- to<-, Paths-method (slots), 46
- to<-, SGVariantCounts-method (slots), 46
- tx, 55
- txf_ann, 56
- txf_pred, 56
- TxFeatures, 55
- txName (slots), 46
- txName, Counts-method (slots), 46
- txName, Features-method (slots), 46
- txName, Paths-method (slots), 46
- txName<- (slots), 46
- txName<-, Counts-method (slots), 46
- txName<-, Features-method (slots), 46
- txName<-, Paths-method (slots), 46
- type, Counts-method (slots), 46
- type, Features-method (slots), 46
- type, Paths-method (slots), 46
- type<- (slots), 46
- type<-, Counts-method (slots), 46
- type<-, Features-method (slots), 46
- type<-, Paths-method (slots), 46
- updateObject, 57
- updateObject, SGVariantCounts-method (updateObject), 57
- updateObject, SGVariants-method (updateObject), 57
- variantFreq (assays), 7
- variantFreq, SGVariantCounts-method (assays), 7
- variantFreq<- (assays), 7
- variantFreq<-, SGVariantCounts-method (assays), 7
- variantID (slots), 46
- variantID, SGVariantCounts-method (slots), 46
- variantID, SGVariants-method (slots), 46
- variantID<- (slots), 46

variantID<- ,SGVariantCounts-method
(slots), [46](#)

variantID<- ,SGVariants-method (slots),
[46](#)

variantName (slots), [46](#)

variantName ,SGVariantCounts-method
(slots), [46](#)

variantName ,SGVariants-method (slots),
[46](#)

variantName<- (slots), [46](#)

variantName<- ,SGVariantCounts-method
(slots), [46](#)

variantName<- ,SGVariants-method
(slots), [46](#)

variantType (slots), [46](#)

variantType ,SGVariantCounts-method
(slots), [46](#)

variantType ,SGVariants-method (slots),
[46](#)

variantType<- (slots), [46](#)

variantType<- ,SGVariantCounts-method
(slots), [46](#)

variantType<- ,SGVariants-method
(slots), [46](#)