

# Package ‘SGSeq’

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**Type** Package

**Title** Splice event prediction and quantification from RNA-seq data

**Version** 1.42.1

**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

**LazyData** yes

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## Contents

analyzeFeatures . . . . .	3
analyzeVariants . . . . .	5
annotate . . . . .	6
annotateSGVariants . . . . .	7
assays . . . . .	8
convertToSGFeatures . . . . .	9
convertToTxFeatures . . . . .	10
exonCompatible . . . . .	11
exportFeatures . . . . .	12
filterFeatures . . . . .	13
findOverlapsRanges . . . . .	14
findSGVariants . . . . .	14
getBamInfo . . . . .	15
getSGFeatureCounts . . . . .	16
getSGFeatureCountsPerSample . . . . .	17
getSGVariantCounts . . . . .	18
gr . . . . .	19
importTranscripts . . . . .	19
junctionCompatible . . . . .	20
makeSGFeatureCounts . . . . .	21
makeVariantNames . . . . .	21
mergeTxFeatures . . . . .	22
plotCoverage . . . . .	23
plotFeatures . . . . .	24
plotSpliceGraph . . . . .	26
plotVariants . . . . .	28
predictCandidatesInternal . . . . .	29
predictCandidatesTerminal . . . . .	30
predictExonsInternal . . . . .	30
predictExonsTerminal . . . . .	31
predictJunctions . . . . .	32
predictSpliced . . . . .	33
predictTxFeatures . . . . .	34
predictTxFeaturesPerSample . . . . .	35
predictTxFeaturesPerStrand . . . . .	37
predictVariantEffects . . . . .	38
processTerminalExons . . . . .	39
removeExonsIsolated . . . . .	40
sgfc_ann . . . . .	41
sgfc_pred . . . . .	41
SGFeatureCounts . . . . .	41

SGFeatures	42
sgf_ann	43
sgf_pred	44
SGSegments	44
SGVariantCounts	45
SGVariants	45
sgvc_ann	47
sgvc_ann_from_bam	47
sgvc_pred	47
sgvc_pred_from_bam	48
sgv_ann	48
sgv_pred	48
si	49
slots	49
splicesiteOverlap	58
tx	59
TxFeatures	59
txf_ann	60
txf_pred	60
updateObject	61

## Index 62

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

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## 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 <code>getBamInfo</code> .
which	GRanges of genomic regions to be considered for feature prediction, passed to <code>ScanBamParam</code>
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	<i>Analysis of splice variants</i>
-----------------	------------------------------------

---

**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	<i>Annotate splice variants in terms of canonical events</i>
--------------------	--

---

## 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

**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	<i>Convert transcript features to splice graph features</i>
---------------------	---

---

**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	<i>Convert to TxFeatures object</i>
---------------------	-------------------------------------

---

## 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	<i>Compatible fragment counts for exons</i>
----------------	---

---

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

*Export to BED format*

---

**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	<i>Modified findOverlaps function for IRanges, IRangesList objects</i>
--------------------	--

---

**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	<i>Identify splice variants from splice graph</i>
----------------	---

---

**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 <a href="#">annotateSGVariants</a> .
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	<i>Compatible counts for splice graph features from BAM files</i>
--------------------	---

---

**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**

<code>sample_info</code>	Data frame with sample information. Required columns are “ <code>sample_name</code> ”, “ <code>file_bam</code> ”, “ <code>paired_end</code> ”, “ <code>read_length</code> ”, “ <code>frag_length</code> ” and “ <code>lib_size</code> ”. Library information can be obtained with function <code>getBamInfo</code> .
<code>features</code>	<code>SGFeatures</code> 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	<i>Representative counts and frequency estimates for splice variants</i>
--------------------	--

---

**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**

file	Character string specifying input GFF file
tag_tx	GFF attribute tag for transcript identifier
tag_gene	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	<i>Compatible fragment counts for splice junctions</i>
--------------------	--

---

**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	<i>Create SGFeatureCounts object</i>
---------------------	--------------------------------------

---

**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	<i>Create interpretable splice variant names</i>
------------------	--

---

**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	<i>Merge redundant features</i>
-----------------	---------------------------------

---

**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

*Plot read coverage and splice junction read counts***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	<i>Plot splice graph and heatmap of expression values</i>
--------------	---

---

**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	<i>Plot splice graph</i>
-----------------	--------------------------

---

## 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

<code>x</code>	SGFeatures or SGVariants object
<code>geneID</code>	Single gene identifier used to subset <code>x</code>
<code>geneName</code>	Single gene name used to subset <code>x</code>
<code>eventID</code>	Single event identifier used to subset <code>x</code>
<code>which</code>	GRanges used to subset <code>x</code>
<code>toscale</code>	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).
<code>label</code>	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.
<code>color</code>	Color used for plotting the splice graph. Ignored if features metadata column “color” is not NULL.
<code>color_novel</code>	Features with missing annotation are highlighted in <code>color_novel</code> . Ignored if features metadata column “color” is not NULL.
<code>color_alpha</code>	Controls color transparency
<code>color_labels</code>	Logical indicating whether label colors should be the same as feature colors
<code>border</code>	Determines the color of exon borders, can be “fill” (same as exon color), “none” (no border), or a valid color name
<code>curvature</code>	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**

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”
frag_coverage	Rle object with fragment coverage
relCov	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

---

<code>predictJunctions</code>	<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	<i>Splice junction and exon prediction from BAM files</i>
-------------------	---

---

**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 NA.

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).
verbose	If TRUE, 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

<code>lib_size</code>	Number of aligned fragments required for use with alpha
<code>min_junction_count</code>	Minimum fragment count required for a splice junction to be included. If specified, argument alpha is ignored.
<code>alpha</code>	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> . alpha is ignored if argument <code>min_junction_count</code> is specified.
<code>psi</code>	Minimum splice frequency required for a splice junction to be included
<code>beta</code>	Minimum relative coverage required for an internal exon to be included
<code>gamma</code>	Minimum relative coverage required for a terminal exon to be included
<code>min_anchor</code>	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  $\beta \times$  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  $\gamma \times$  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	<i>Process predicted terminal exons</i>
----------------------	---

---

**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)
```

---

<code>removeExonsIsolated</code>	<i>Remove exons with no flanking splice junctions</i>
----------------------------------	---

---

**Description**

Remove exons with no flanking splice junctions.

**Usage**

```
removeExonsIsolated(features)
```

**Arguments**

<code>features</code>	TxFeatures object
-----------------------	-------------------

**Value**

TxFeatures object with filtered features

**Author(s)**

Leonard Goldstein



---

sgfc_ann	<i>Example splice graph feature counts (annotation-based)</i>
----------	---

---

**Description**

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

**Format**

SGFeatureCounts object

**Author(s)**

Leonard Goldstein

---

sgfc_pred	<i>Example splice graph feature counts (predicted)</i>
-----------	--

---

**Description**

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

**Format**

SGFeatureCounts object

**Author(s)**

Leonard Goldstein

---

SGFeatureCounts	<i>Splice graph feature counts</i>
-----------------	------------------------------------

---

**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	<i>Splice graph features</i>
------------	------------------------------

---

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	<i>Example splice graph features (predicted)</i>
----------	--

---

**Description**

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

**Format**

SGFeatures object

**Author(s)**

Leonard Goldstein

---

SGSegments	<i>Splice graph segments</i>
------------	------------------------------

---

**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	<i>Example splice variant counts (annotated)</i>
----------	--

---

**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	<i>Example splice variant counts (annotated) from BAM files</i>
-------------------	---

---

**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	<i>Example splice variant counts (predicted)</i>
-----------	--

---

**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*Example sample information*

---

**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*Accessing and replacing metadata columns*

---

**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	<i>Example transcript features (annotation-based)</i>
---------	---

---

**Description**

Transcript features for FBXO31, based on UCSC knownGene annotation.

**Format**

TxFeatures object

**Author(s)**

Leonard Goldstein

---

txf_pred	<i>Example transcript features (predicted)</i>
----------	--

---

**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, 7
- exonCompatible, 11
- filterFeatures, 13
- findOverlapsRanges, 14
- getSGFeatureCountsPerSample, 17
- gr, 19
- junctionCompatible, 20
- makeVariantNames, 21
- predictCandidatesInternal, 29
- predictCandidatesTerminal, 30
- predictExonsInternal, 30
- predictExonsTerminal, 31
- predictJunctions, 32
- predictSpliced, 33
- predictTxFeaturesPerSample, 35
- predictTxFeaturesPerStrand, 37
- removeExonsIsolated, 40
- sgf\_ann, 43
- sgf\_pred, 44
- sgfc\_ann, 41
- sgfc\_pred, 41
- SGSegments, 44
- sgv\_ann, 48
- sgv\_pred, 48
- sgvc\_ann, 47
- sgvc\_ann\_from\_bam, 47
- sgvc\_pred, 47
- sgvc\_pred\_from\_bam, 48
- si, 49
- spliceSiteOverlap, 58
- tx, 59
- txf\_ann, 60
- txf\_pred, 60

- analyzeFeatures, 3
- analyzeVariants, 5
- annotate, 4, 6
- annotateSGVariants, 7, 15
- assays, 8

- closed3p (slots), 49
- closed3p, SGVariantCounts-method (slots), 49
- closed3p, SGVariants-method (slots), 49
- closed3p<- (slots), 49
- closed3p<- , SGVariantCounts-method (slots), 49
- closed3p<- , SGVariants-method (slots), 49
- closed3pEvent (slots), 49
- closed3pEvent, SGVariantCounts-method (slots), 49
- closed3pEvent, SGVariants-method (slots), 49
- closed3pEvent<- (slots), 49
- closed3pEvent<- , SGVariantCounts-method (slots), 49
- closed3pEvent<- , SGVariants-method (slots), 49
- closed5p (slots), 49
- closed5p, SGVariantCounts-method (slots), 49
- closed5p, SGVariants-method (slots), 49
- closed5p<- (slots), 49
- closed5p<- , SGVariantCounts-method (slots), 49
- closed5p<- , SGVariants-method (slots), 49
- closed5pEvent (slots), 49
- closed5pEvent, SGVariantCounts-method (slots), 49
- closed5pEvent, SGVariants-method (slots), 49
- closed5pEvent<- (slots), 49
- closed5pEvent<- , SGVariantCounts-method (slots), 49
- closed5pEvent<- , SGVariants-method (slots), 49
- convertToSGFeatures, 4, 9
- convertToTxFeatures, 10
- counts, SGFeatureCounts-method (assays),

- 8
- counts, SGVariantCounts-method (assays), 8
- counts<-, SGFeatureCounts-method (assays), 8
- counts<-, SGVariantCounts-method (assays), 8
- eventID (slots), 49
- eventID, SGVariantCounts-method (slots), 49
- eventID, SGVariants-method (slots), 49
- eventID<- (slots), 49
- eventID<-, SGVariantCounts-method (slots), 49
- eventID<-, SGVariants-method (slots), 49
- exonCompatible, 11
- exportFeatures, 12
- featureID (slots), 49
- featureID, Counts-method (slots), 49
- featureID, Paths-method (slots), 49
- featureID, SGFeatures-method (slots), 49
- featureID3p (slots), 49
- featureID3p, SGVariantCounts-method (slots), 49
- featureID3p, SGVariants-method (slots), 49
- featureID3p<- (slots), 49
- featureID3p<-, SGVariantCounts-method (slots), 49
- featureID3p<-, SGVariants-method (slots), 49
- featureID3pEvent (slots), 49
- featureID3pEvent, SGVariantCounts-method (slots), 49
- featureID3pEvent, SGVariants-method (slots), 49
- featureID3pEvent<- (slots), 49
- featureID3pEvent<-, SGVariantCounts-method (slots), 49
- featureID3pEvent<-, SGVariants-method (slots), 49
- featureID5p (slots), 49
- featureID5p, SGVariantCounts-method (slots), 49
- featureID5p, SGVariants-method (slots), 49
- featureID5p<-, SGVariantCounts-method (slots), 49
- featureID5p<-, SGVariants-method (slots), 49
- featureID5pEvent (slots), 49
- featureID5pEvent, SGVariantCounts-method (slots), 49
- featureID5pEvent, SGVariants-method (slots), 49
- featureID5pEvent<- (slots), 49
- featureID5pEvent<-, SGVariantCounts-method (slots), 49
- featureID5pEvent<-, SGVariants-method (slots), 49
- featureID<- (slots), 49
- featureID<-, Counts-method (slots), 49
- featureID<-, Paths-method (slots), 49
- featureID<-, SGFeatures-method (slots), 49
- filterFeatures, 13
- findOverlapsRanges, 14
- findSGVariants, 5, 14
- FPKM (assays), 8
- FPKM, SGFeatureCounts-method (assays), 8
- FPKM, SGVariantCounts-method (assays), 8
- FPKM<- (assays), 8
- FPKM<-, SGFeatureCounts-method (assays), 8
- from, Paths-method (slots), 49
- from, SGVariantCounts-method (slots), 49
- from<- (slots), 49
- from<-, Paths-method (slots), 49
- from<-, SGVariantCounts-method (slots), 49
- geneID (slots), 49
- geneID, Counts-method (slots), 49
- geneID, Paths-method (slots), 49
- geneID, SGFeatures-method (slots), 49
- geneID<- (slots), 49
- geneID<-, Counts-method (slots), 49
- geneID<-, Paths-method (slots), 49
- geneID<-, SGFeatures-method (slots), 49
- geneName (slots), 49
- geneName, Counts-method (slots), 49
- geneName, Features-method (slots), 49
- geneName, Paths-method (slots), 49
- geneName<- (slots), 49
- geneName<-, Counts-method (slots), 49

geneName<- ,Features-method (slots), 49  
 geneName<- ,Paths-method (slots), 49  
 getBamInfo, 15  
 getSGFeatureCounts, 4, 16  
 getSGFeatureCountsPerSample, 17  
 getSGVariantCounts, 5, 18  
 gr, 19  
  
 importTranscripts, 19  
  
 junctionCompatible, 20  
  
 makeSGFeatureCounts, 21  
 makeVariantNames, 21  
 mergeTxFeatures, 22, 34  
  
 plotCoverage, 23  
 plotFeatures, 24  
 plotSpliceGraph, 26  
 plotVariants, 28  
 predictCandidatesInternal, 29  
 predictCandidatesTerminal, 30  
 predictExonsInternal, 30  
 predictExonsTerminal, 31  
 predictJunctions, 32  
 predictSpliced, 33  
 predictTxFeatures, 4, 34  
 predictTxFeaturesPerSample, 34, 35  
 predictTxFeaturesPerStrand, 37  
 predictVariantEffects, 38  
 processTerminalExons, 34, 39  
  
 removeExonsIsolated, 40  
  
 segmentID (slots), 49  
 segmentID,Paths-method (slots), 49  
 segmentID,SGVariantCounts-method  
     (slots), 49  
 segmentID<- (slots), 49  
 segmentID<- ,Paths-method (slots), 49  
 segmentID<- ,SGVariantCounts-method  
     (slots), 49  
 sgf\_ann, 43  
 sgf\_pred, 44  
 sgfc\_ann, 41  
 sgfc\_pred, 41  
 SGFeatureCounts, 31  
 SGFeatures, 42  
 SGSegments, 44  
 sgv\_ann, 48  
 sgv\_pred, 48  
 SGVariantCounts, 45  
 SGVariants, 45  
 sgvc\_ann, 47  
 sgvc\_ann\_from\_bam, 47  
 sgvc\_pred, 47  
 sgvc\_pred\_from\_bam, 48  
 si, 49  
 slots, 49  
 splice3p (slots), 49  
 splice3p,SGFeatureCounts-method  
     (slots), 49  
 splice3p,SGFeatures-method (slots), 49  
 splice3p,SGSegments-method (slots), 49  
 splice3p<- (slots), 49  
 splice3p<- ,SGFeatureCounts-method  
     (slots), 49  
 splice3p<- ,SGFeatures-method (slots), 49  
 splice3p<- ,SGSegments-method (slots), 49  
 splice5p (slots), 49  
 splice5p,SGFeatureCounts-method  
     (slots), 49  
 splice5p,SGFeatures-method (slots), 49  
 splice5p,SGSegments-method (slots), 49  
 splice5p<- (slots), 49  
 splice5p<- ,SGFeatureCounts-method  
     (slots), 49  
 splice5p<- ,SGFeatures-method (slots), 49  
 splice5p<- ,SGSegments-method (slots), 49  
 splicesiteOverlap, 58  
  
 to,Paths-method (slots), 49  
 to,SGVariantCounts-method (slots), 49  
 to<- (slots), 49  
 to<- ,Paths-method (slots), 49  
 to<- ,SGVariantCounts-method (slots), 49  
 tx, 59  
 txf\_ann, 60  
 txf\_pred, 60  
 TxFeatures, 59  
 txName (slots), 49  
 txName,Counts-method (slots), 49  
 txName,Features-method (slots), 49  
 txName,Paths-method (slots), 49  
 txName<- (slots), 49  
 txName<- ,Counts-method (slots), 49  
 txName<- ,Features-method (slots), 49  
 txName<- ,Paths-method (slots), 49  
 type,Counts-method (slots), 49



type, Features-method (slots), 49  
type, Paths-method (slots), 49  
type<- (slots), 49  
type<-, Counts-method (slots), 49  
type<-, Features-method (slots), 49  
type<-, Paths-method (slots), 49  
  
updateObject, 61  
updateObject, SGVariantCounts-method  
    (updateObject), 61  
updateObject, SGVariants-method  
    (updateObject), 61  
  
variantFreq (assays), 8  
variantFreq, SGVariantCounts-method  
    (assays), 8  
variantFreq<- (assays), 8  
variantFreq<-, SGVariantCounts-method  
    (assays), 8  
variantID (slots), 49  
variantID, SGVariantCounts-method  
    (slots), 49  
variantID, SGVariants-method (slots), 49  
variantID<- (slots), 49  
variantID<-, SGVariantCounts-method  
    (slots), 49  
variantID<-, SGVariants-method (slots),  
    49  
variantName (slots), 49  
variantName, SGVariantCounts-method  
    (slots), 49  
variantName, SGVariants-method (slots),  
    49  
variantName<- (slots), 49  
variantName<-, SGVariantCounts-method  
    (slots), 49  
variantName<-, SGVariants-method  
    (slots), 49  
variantType (slots), 49  
variantType, SGVariantCounts-method  
    (slots), 49  
variantType, SGVariants-method (slots),  
    49  
variantType<- (slots), 49  
variantType<-, SGVariantCounts-method  
    (slots), 49  
variantType<-, SGVariants-method  
    (slots), 49