

# Package ‘SGSeq’

September 20, 2022

**Type** Package

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

**Version** 1.30.0

**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

**LazyData** yes

**Depends** R (>= 4.0), IRanges (>= 2.13.15), GenomicRanges (>= 1.31.10), Rsamtools (>= 1.31.2), SummarizedExperiment, methods

**Imports** AnnotationDbi, BiocGenerics (>= 0.31.5), Biostrings (>= 2.47.6), GenomicAlignments (>= 1.15.7), GenomicFeatures (>= 1.31.5), GenomeInfoDb, RUnit, S4Vectors (>= 0.23.19), grDevices, graphics, igraph, parallel, rtracklayer (>= 1.39.7), stats

**Suggests** BiocStyle, BSgenome.Hsapiens.UCSC.hg19, TxDb.Hsapiens.UCSC.hg19.knownGene, knitr, rmarkdown

**VignetteBuilder** knitr

**biocViews** AlternativeSplicing, ImmunoOncology, RNASeq, Transcription

**RoxygenNote** 6.0.1

**git\_url** <https://git.bioconductor.org/packages/SGSeq>

**git\_branch** RELEASE\_3\_15

**git\_last\_commit** a19e395

**git\_last\_commit\_date** 2022-04-26

**Date/Publication** 2022-09-20

**Author** Leonard Goldstein [cre, aut]

**Maintainer** Leonard Goldstein <ldgoldstein@gmail.com>

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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 <code>processTerminalExons</code> ). 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 *Annotation with respect to transcript features*

---

**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)
```

---

assays

*Accessing 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("+", "+"))
gr1 <- split(gr, 1)
txf <- convertToTxFeatures(gr1)
```

---

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
```

---

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
maxvariant	If more than <code>maxvariant</code> 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      *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**

<code>sample_info</code>	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> .
<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)
```

---

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)
```

---

importTranscripts	<i>Import transcripts from GFF file</i>
-------------------	---

---

**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
```

---

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)))
```

---

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	<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	<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**

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
```

---

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)
```

---

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)
```

---

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()
```

---

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()
```

---

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))
```

---

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")
```

---

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



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