Package ‘SGSeq’

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Type Package
Title Splice event prediction and quantification from RNA-seq data
Version 1.10.0
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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 IRanges, GenomicRanges (>= 1.23.21), Rsamtools, SummarizedExperiment, methods
Imports AnnotationDbi, BiocGenerics, Biostrings, GenomicAlignments, GenomicFeatures, GenomeInfoDb, RUnit, S4Vectors (>= 0.9.39), grDevices, graphics, igraph, parallel, rtracklayer, stats
Suggests BiocStyle, BSgenome.Hsapiens.UCSC.hg19, TxDb.Hsapiens.UCSC.hg19.knownGene, knitr, rmarkdown
VignetteBuilder knitr
biocViews AlternativeSplicing, RNASeq, Transcription
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R topics documented:

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analyzeFeatures

Analysis of splice graph features from BAM files

Description

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

Usage

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

Arguments

sample_info  Data frame with sample information. Required columns are “sample_name”, “file_bam”, “paired_end”, “read_length”, “frag_length” and “lib_size”. Library information can be obtained with function getBamInfo.

which  GRanges of genomic regions to be considered for feature prediction, passed to ScanBamParam

features  TxFeatures or SGFeatures object
analyzeFeatures

- **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**

```r
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.
convertToSGFeatures

Author(s)
Leonard Goldstein

Examples

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

Description

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

Usage

```r
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
convertToTxFeatures

Examples

sgf <- convertToSGFeatures(txf_ann)

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)
**exportFeatures**  
*Export to BED format*

**Description**

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

**Usage**

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

**Arguments**

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

**Value**

NULL

**Author(s)**

Leonard Goldstein

**Examples**

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

---

**findSGVariants**  
*Identify splice variants from splice graph*

**Description**

Identify splice variants from splice graph.

**Usage**

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

Arguments

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

Value

- SGVariants object

Author(s)

Leonard Goldstein

Examples

```r
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

```r
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`. 
getSGFeatureCounts

Compatible counts for splice graph features from BAM files

Description

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

Usage

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

Arguments

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

Value

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

Author(s)

Leonard Goldstein
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")
sgv_pred <- file.path(path, "bams", si$file_bam)
sgvc_from_bam <- getSGVariantCounts(sgv_pred, sample_info = si)
importTranscripts

**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**

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

makeSGFeatureCounts

**makeSGFeatureCounts**  
Create SGFeatureCounts object

**Description**

Create SGFeatureCounts object from rowRanges, colData and counts.

**Usage**

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

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)))

taxf_merged <- mergeTxFeatures(txf_ann, txf_pred)

mergeTxFeatures Merge redundant features

Description

Merge features, typically after feature prediction in multiple samples.

Usage

mergeTxFeatures(..., min_n_sample = 1)

Arguments

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

Details

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

Value

TxFeatures object with merged features

Author(s)

Leonard Goldstein

Examples

txf_merged <- mergeTxFeatures(txf_ann, txf_pred)
plotCoverage

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

```r
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

```r
## 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

```r
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)
plotSpliceGraph

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>margin</td>
<td>Width of right-hand margin as fraction of width of the graphics device. Ignored if square is TRUE.</td>
</tr>
<tr>
<td>RowSideColors</td>
<td>Character vector (or list of character vectors) with length(s) equal to ncol(x) containing color names for horizontal side bars for sample annotation</td>
</tr>
<tr>
<td>square</td>
<td>Logical, if TRUE margins are set such that cells in the heatmap are square</td>
</tr>
<tr>
<td>cexRow</td>
<td>Scale factor for row (sample) labels</td>
</tr>
<tr>
<td>cexCol</td>
<td>Scale factor for column (feature) labels</td>
</tr>
<tr>
<td>labRow</td>
<td>Character vector of row (sample) labels</td>
</tr>
<tr>
<td>col</td>
<td>Heatmap colors</td>
</tr>
<tr>
<td>zlim</td>
<td>Range of values for which colors should be plotted, if NULL range of finite values</td>
</tr>
<tr>
<td>heightPanels</td>
<td>Numeric vector of length two indicating height of the top and bottom panels.</td>
</tr>
<tr>
<td>...</td>
<td>further parameters passed to plotSpliceGraph</td>
</tr>
</tbody>
</table>

Value

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

Author(s)

Leonard Goldstein

Examples

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

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

```r
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 nbins = 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)
```
plotSpliceGraph

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
**plotVariants**

**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**

```r
## 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 parameters passed to `plotSpliceGraph`

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

Author(s)
Leonard Goldstein

Examples

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

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`. 
predictTxFeatures

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 getBamInfo.
which       GRanges of genomic regions to be considered for feature prediction, passed to
ScanBamParam
alpha       Minimum FPKM required for a splice junction to be included. Internally, FP-
            KMs are converted to counts, requiring arguments read_length, frag_length
            and lib_size. alpha is ignored if argument min_junction_count is specified.
psi         Minimum splice frequency required for a splice junction to be included
beta        Minimum relative coverage required for an internal exon to be included
gamma       Minimum relative coverage required for a terminal exon to be included
min_junction_count  Minimum fragment count required for a splice junction to be included. If spec-
                    ified, argument alpha 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 ter-
                 minal 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 merg-
                 ing 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)
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**

```r
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**

```r
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)
**Description**

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

**Usage**

```r
SGFeatureCounts(x)
```

**Arguments**

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

**Value**

`SGFeatureCounts` object

**Author(s)**

Leonard Goldstein

**Examples**

```r
gfc <- SGFeatureCounts()
```

---

**Description**

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

**Usage**

```r
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)
```
SGVariantCounts

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

Splice graph variant counts

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  Splice graph variants

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'
\[ \text{from}(x) \gets \text{value} \]

## S4 replacement method for signature 'SGVariantCounts'
\[ \text{from}(x) \gets \text{value} \]

## S4 method for signature 'Paths'
\[ \text{to}(x) \]

## S4 method for signature 'SGVariantCounts'
\[ \text{to}(x) \]

## S4 replacement method for signature 'Paths'
\[ \text{to}(x) \gets \text{value} \]

## S4 replacement method for signature 'SGVariantCounts'
\[ \text{to}(x) \gets \text{value} \]

## S4 method for signature 'SGVariants'
\[ \text{eventID}(x) \]

## S4 method for signature 'SGVariantCounts'
\[ \text{eventID}(x) \]

## S4 replacement method for signature 'SGVariants'
\[ \text{eventID}(x) \gets \text{value} \]

## S4 replacement method for signature 'SGVariantCounts'
\[ \text{eventID}(x) \gets \text{value} \]

## S4 method for signature 'SGVariants'
\[ \text{variantID}(x) \]

## S4 method for signature 'SGVariantCounts'
\[ \text{variantID}(x) \]

## S4 replacement method for signature 'SGVariants'
\[ \text{variantID}(x) \gets \text{value} \]

## S4 replacement method for signature 'SGVariantCounts'
\[ \text{variantID}(x) \gets \text{value} \]

## S4 method for signature 'SGVariants'
\[ \text{closed5p}(x) \]

## S4 method for signature 'SGVariantCounts'
\[ \text{closed5p}(x) \]

## S4 replacement method for signature 'SGVariants'
\[ \text{closed5p}(x) \gets \text{value} \]
## 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'
## 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 replacement method for signature 'SGVariants'
featureID5pEvent(x) <- value

## S4 method for signature 'SGVariantCounts'
featureID3pEvent(x)

## S4 method for signature 'SGVariants'
featureID3pEvent(x)
## S4 replacement method for signature 'SGVariants'

```r
featureID3pEvent(x) <- value
```

## S4 replacement method for signature 'SGVariantCounts'

```r
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

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

---

<table>
<thead>
<tr>
<th><strong>TxFeatures</strong></th>
<th><strong>Transcript features</strong></th>
</tr>
</thead>
</table>

### Description

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

### Usage

```r
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

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

updateObject  

Update object

Description

Update object created with previous version of SGSeq.

Usage

```r
## S4 method for signature 'SGVariants'
updateObject(object, verbose)

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

Arguments

- `object` Object to be updated
- `verbose` Should a warning message be generated

Value

Updated object

Author(s)

Leonard Goldstein
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