Package 'SGSeq'

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Type Package

Title Splice event prediction and quantification from RNA-seq data

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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

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analyze Features

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)
```

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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 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 spec-

ified, argument alpha is ignored.

min_anchor Integer specifiying 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 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).

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

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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)</pre>
```

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 specifiying minimum anchor length

cores Number of cores available for parallel processing

Value

 ${\tt SGVariantCounts}\ object$

Author(s)

Leonard Goldstein

Examples

```
sgvc <- analyzeVariants(sgfc_pred)</pre>
```

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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)</pre>
```

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.

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Usage

```
FPKM(object, ...)
FPKM(object, ...) <- value</pre>
variantFreq(object)
variantFreq(object) <- value</pre>
## S4 method for signature 'SGFeatureCounts'
counts(object)
## S4 replacement method for signature 'SGFeatureCounts'
counts(object) <- value</pre>
## S4 method for signature 'SGFeatureCounts'
FPKM(object)
## S4 replacement method for signature 'SGFeatureCounts'
FPKM(object) <- value</pre>
## S4 method for signature 'SGVariantCounts'
counts(object, ...)
## S4 replacement method for signature 'SGVariantCounts'
counts(object, ...) <- value</pre>
## S4 method for signature 'SGVariantCounts'
FPKM(object, ...)
## S4 method for signature 'SGVariantCounts'
variantFreq(object)
## S4 replacement method for signature 'SGVariantCounts'
variantFreq(object) <- value</pre>
```

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.

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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")</pre>
```

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

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Examples

```
sgf <- convertToSGFeatures(txf_ann)</pre>
```

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

Х

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)
```

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exportFeatures

Export to BED format

Description

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

Usage

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

Arguments

features TxFeatures or SGFeatures object file Character string specifying output file

Value

NULL

Author(s)

Leonard Goldstein

Examples

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

findSGVariants

Identify splice variants from splice graph

Description

Identify splice variants from splice graph.

Usage

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

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Arguments

features SGFeatures object

maxnvariant If more than maxnvariant variants are identified in an event, the event is skipped,

resulting in a warning. Set to NA to include all events.

annotate_events

Logical indicating whether identified splice variants should be annotated in terms

of canonical events. For details see help page for annotateSGVariants.

include Character string indicating whether identified splice variants should be filtered.

Possible options are "default" (only include variants for events with all variants closed), "closed" (only include closed variants) and "all" (include all variants).

cores Number of cores available for parallel processing

Value

SGVariants object

Author(s)

Leonard Goldstein

Examples

```
sgv <- findSGVariants(sgf_pred)</pre>
```

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.

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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)</pre>
```

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 specifiying 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

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Examples

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

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 specifiying 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)</pre>
```

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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 transcipts with metadata columns txName, geneName, cdsStart, cdsEnd.

Author(s)

Leonard Goldstein

Examples

```
## Not run:
tx <- importTranscripts(file)
## End(Not run)
NULL</pre>
```

makeSGFeatureCounts

Create SGFeatureCounts object

Description

 $Create \ \ SGFeature Counts \ object \ from \ row Ranges, \ col Data \ and \ counts.$

Usage

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

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Arguments

rowRanges SGFeatures object

colData Data frame with sample information

counts Integer matrix of counts

min_anchor Integer specifiying minimum anchor length

Value

SGFeatureCounts object

Author(s)

Leonard Goldstein

Examples

```
sgfc <- makeSGFeatureCounts(sgf_pred, si,
matrix(OL, length(sgf_pred), nrow(si)))</pre>
```

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)</pre>
```

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plotCoverage	Plot read coverage and splice junction read counts	
plotCoverage	Plot read coverage and splice junction read counts	

Description

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

Usage

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

Arguments

X	SGFeatureCounts or SGFeatures object. If x is an SGFeatureCounts object
	that includes multiple samples, average coverage and splice junction counts are

obtained.

geneID Single gene identifier used to subset x
geneName Single gene name used to subset x
eventID Single event identifier used to subset x

which GRanges used to subset x

sample_info Data frame with sample information. If x is an SGFeatureCounts object, sam-

ple information is obtained from colData(x). If sample_info includes multi-

ple 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 cover-

ages.

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 specifiying minimum anchor length

cores Number of cores available for parallel processing.

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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)

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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</pre>
```

plotSpliceGraph

Plot splice graph

Description

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

Usage

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

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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, specificed 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, specificed 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, specificed 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 infor-

mation that is likely useful to the user

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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</pre>
```

plotVariants

Plot splice graph and heatmap of splice variant frequencies

Description

Plot splice graph and heatmap of splice variant frequencies.

Usage

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

20 predictTxFeatures

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</pre>
```

 $\verb|predictTxFeatures||$

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 21

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 specifiying 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)</pre>
```

22 predictVariantEffects

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)</pre>
```

processTerminalExons 23

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 ter-

minal 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 overlang 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 trimmmed 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)</pre>

24 SGFeatures

SGFeatureCounts

Splice graph feature counts

Description

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

Usage

```
SGFeatureCounts(x)
```

Arguments

Х

 ${\tt RangedSummarizedExperiment\ with\ SGFeatures\ as\ rowRanges\ and\ assays\ ``counts''}\ and\ ``FPKM''$

Value

SGFeatureCounts object

Author(s)

Leonard Goldstein

Examples

```
sgfc <- SGFeatureCounts()</pre>
```

SGFeatures

Splice graph features

Description

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

Usage

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

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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()</pre>
```

ounts Splice graph variant counts

Description

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

Usage

```
SGVariantCounts(x)
```

26 SGVariants

Arguments

Х

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()</pre>
```

SGVariants

Splice graph variants

Description

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

Usage

SGVariants(x)

Arguments

Х

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()</pre>
```

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</pre>
```

```
splice5p(x)
splice5p(x) \leftarrow value
splice3p(x)
splice3p(x) \leftarrow value
from(x) \leftarrow value
to(x) <- value
segmentID(x)
segmentID(x) \leftarrow value
variantID(x)
variantID(x) \leftarrow value
eventID(x)
eventID(x) <- value</pre>
closed5p(x)
closed5p(x) \leftarrow value
closed3p(x)
closed3p(x) \leftarrow value
closed5pEvent(x)
closed5pEvent(x) \leftarrow value
closed3pEvent(x)
closed3pEvent(x) <- value</pre>
variantType(x)
variantType(x) <- value</pre>
variantName(x)
variantName(x) <- value</pre>
featureID5p(x)
featureID5p(x) \leftarrow value
```

```
featureID3p(x)
featureID3p(x) \leftarrow value
featureID5pEvent(x)
featureID5pEvent(x) <- value</pre>
featureID3pEvent(x)
featureID3pEvent(x) <- value</pre>
## 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</pre>
## 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) \leftarrow value
## S4 replacement method for signature 'Paths'
geneName(x) <- value</pre>
## S4 replacement method for signature 'Counts'
geneName(x) <- value</pre>
## 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) \leftarrow value
## S4 replacement method for signature 'Paths'
featureID(x) <- value</pre>
## S4 replacement method for signature 'Counts'
featureID(x) <- value</pre>
## 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) \leftarrow value
## S4 replacement method for signature 'Paths'
geneID(x) \leftarrow value
## S4 replacement method for signature 'Counts'
geneID(x) <- value</pre>
## 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) \leftarrow value
## S4 replacement method for signature 'SGSegments'
splice5p(x) \leftarrow value
## S4 replacement method for signature 'SGFeatureCounts'
splice5p(x) \leftarrow 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) \leftarrow value
## S4 replacement method for signature 'SGSegments'
splice3p(x) \leftarrow value
## S4 replacement method for signature 'SGFeatureCounts'
splice3p(x) \leftarrow value
## S4 method for signature 'Paths'
segmentID(x)
## S4 method for signature 'SGVariantCounts'
segmentID(x)
## S4 replacement method for signature 'Paths'
segmentID(x) \leftarrow value
## S4 replacement method for signature 'SGVariantCounts'
segmentID(x) \leftarrow value
## S4 method for signature 'Paths'
from(x)
## S4 method for signature 'SGVariantCounts'
from(x)
```

```
## S4 replacement method for signature 'Paths'
from(x) <- value</pre>
## S4 replacement method for signature 'SGVariantCounts'
from(x) \leftarrow value
## S4 method for signature 'Paths'
to(x)
## S4 method for signature 'SGVariantCounts'
## S4 replacement method for signature 'Paths'
to(x) \leftarrow 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</pre>
## S4 replacement method for signature 'SGVariantCounts'
eventID(x) \leftarrow value
## S4 method for signature 'SGVariants'
variantID(x)
## S4 method for signature 'SGVariantCounts'
variantID(x)
## S4 replacement method for signature 'SGVariants'
variantID(x) <- value</pre>
## S4 replacement method for signature 'SGVariantCounts'
variantID(x) \leftarrow value
## S4 method for signature 'SGVariants'
closed5p(x)
## S4 method for signature 'SGVariantCounts'
closed5p(x)
## S4 replacement method for signature 'SGVariants'
closed5p(x) \leftarrow value
```

```
## S4 replacement method for signature 'SGVariantCounts'
closed5p(x) \leftarrow value
## S4 method for signature 'SGVariants'
closed3p(x)
## S4 method for signature 'SGVariantCounts'
closed3p(x)
## S4 replacement method for signature 'SGVariants'
closed3p(x) \leftarrow value
## S4 replacement method for signature 'SGVariantCounts'
closed3p(x) \leftarrow value
## S4 method for signature 'SGVariants'
closed5pEvent(x)
## S4 method for signature 'SGVariantCounts'
closed5pEvent(x)
## S4 replacement method for signature 'SGVariants'
closed5pEvent(x) <- value</pre>
## 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</pre>
## S4 replacement method for signature 'SGVariantCounts'
closed3pEvent(x) <- value</pre>
## S4 method for signature 'SGVariants'
variantName(x)
## S4 method for signature 'SGVariantCounts'
variantName(x)
## S4 replacement method for signature 'SGVariants'
variantName(x) <- value</pre>
## S4 replacement method for signature 'SGVariantCounts'
variantName(x) <- value</pre>
## S4 method for signature 'SGVariants'
```

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

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```
## S4 replacement method for signature 'SGVariants'
featureID3pEvent(x) <- value
## S4 replacement method for signature 'SGVariantCounts'
featureID3pEvent(x) <- value</pre>
```

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

Transcript features

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 v	vith 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

36 updateObject

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")</pre>
```

updateObject

Update object

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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