

Package ‘VaSP’

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Type Package

Version 1.6.0

Title Quantification and Visualization of Variations of Splicing in Population

Description Discovery of genome-wide variable alternative splicing events from short-read RNA-seq data and visualizations of gene splicing information for publication-quality multi-panel figures in a population.

URL <https://github.com/yuhuihui2011/VaSP>

BugReports <https://github.com/yuhuihui2011/VaSP/issues>

License GPL (>= 2.0)

Depends R (>= 4.0), ballgown

Imports IRanges, GenomicRanges, S4Vectors, Sushi, parallel, matrixStats, GenomicAlignments, GenomeInfoDb, Rsamtools, cluster, stats, graphics, methods

Suggests knitr, rmarkdown

VignetteBuilder knitr

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BMfinder	<i>Discover bimodal distrubition features</i>
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Description

Find bimodal distrubition features and divide the samples into 2 groups by k-means clustering.

Usage

```
BMfinder(x, p.value = 0.01, maf = 0.05, miss = 0.05, fold = 2, log = FALSE,
        cores = detectCores() - 1)
```

Arguments

x	a numeric matrix with feature rows and sample columns, e.g., splicing score matrix from spliceGenome or spliceGene function.
p.value	p.value threshold for bimodal distrubition test
maf	minor allele frequency threshold in k-means clustering
miss	missing grouping rate threshold in k-means clustering
fold	fold change threshold between the two groups
log	whether the scores are to be logarithmic. If TRUE, all the scores are log2 transformed before k-means clustering: $x = \log_2(x+1)$.
cores	threads to be used. This value is passed to ?mclapply in parallel package

Details

The matrix contains 1, 2 and NA, and values of 'x' in group 2 are larger than group 1.

Value

a matrix with feature rows and sample columns.

Examples

```
data(rice.bg)
score<-spliceGene(rice.bg, 'MSTRG.183',junc.type='score')
score<-round(score,2)
as<-BMfinder(score,cores=1) # 4 bimodal distrubition features found

##compare
as
score[rownames(score)%in%rownames(as),]
```

getDepth

*Get Read Depth***Description**

Get read depth from a BAM file (in bedgraph format)

Usage

```
getDepth(x, chrom, start, end)
```

Arguments

x	path to a BAM file
chrom	chromosome of a region to be searched
start	start position
end	end position

Value

a data.frame in bedgraph file format which can be used as input for [plotBedgraph](#) in the **SuShi** package.

See Also

[splicePlot](#)

Examples

```
path <- system.file('extdata',package='VaSP')
bam_files<-list.files(path, 'bam$')
bam_files

depth<-getDepth(file.path(path, bam_files[1]), 'Chr1',
                 start=1171800, end=1179400)
head(depth)

library(Sushi)
```

```
plotBedgraph(depth,'Chr1',chromstart=1171800, chromend=1179400,yaxt='s')
mtext('Depth',side=2,line=2.5,cex=1.2,font=2)
labelgenome('Chr1',1171800,1179400,side=1,scipen=20,n=5,scale='Kb')
```

getGeneinfo

Get Gene Informaton from a ballgown object

Description

Get gene informaton from a ballgown object by genes or by genomic regions

Usage

```
getGeneinfo(genes = NA, bg, chrom, start, end, samples = sampleNames(bg),
            trans.select = NA)
```

Arguments

genes	a character vector specifying gene IDs in 'bg'. Any values other than NA override genomic region (chrom, start, stop)
bg	ballgown object
chrom	chromosome of a region
start	start postion
end	stop postion
samples	names of samples. The transcripts in these samples are subjected to 'trans.select'
trans.select	logical expression-like string, indicating transcript rows to select from a matrix of transcript coverages: NA value keeps all transcripts.

Value

a data.frame in bed-like file format that can be used as input for [plotGenes](#) in the **SuShi** package

See Also

[splicePlot](#); [plotGenes](#) in **Sushi** package

Examples

```
data(rice.bg)
unique(geneIDs(rice.bg))

gene_id <- c('MSTRG.181', 'MSTRG.182', 'MSTRG.183')
geneinfo <- getGeneinfo(genes=gene_id, rice.bg)
trans <- table(geneinfo$name) # show how many exons each transcript has
trans

library(Sushi)
```

```

chrom = geneinfo$chrom[1]
chromstart = min(geneinfo$start) - 1e3
chromend = max(geneinfo$stop) + 1e3
color = rep(SushiColors(2)(length(trans)), trans)

par(mar=c(3,1,1,1))
plotGenes(geneinfo, chrom, chromstart, chromend, col = color, bheight = 0.2,
          bentline = FALSE, plotgenotype = 'arrow', labeloffset = 0.5)
labelgenome(chrom, chromstart, chromend, side = 1, n = 5, scale = 'Kb')

```

rice.bg

*Rice ballgown object***Description**

Small ballgown object created with a subset of rice RNAseq data, for demonstration purposes

Format

a ballgown object with 33 transcripts and 6 samples

Details

The raw RNA-seq data were screened and trimmed using Trimmomatic (Bolger et al., 2014) and RNA-seq mapping, transcript assembly, and quantification were conducted with HISAT, StringTie, and Ballgown by following the method described by Perteau et al. (Perteau et al., 2016). The rice.bg is a subset ballgown object with 33 transcripts and 6 samples (Yu et al., 2021).

Source

The raw RNA-seq data were from the project of variation in transcriptional responses to salt stress in rice (SRA Accession: [SRP106054](#))

References

Yu, H., Du, Q., Campbell, M., Yu, B., Walia, H. and Zhang, C. (2021), Genome-wide discovery of natural variation in pre-mRNA splicing and prioritising causal alternative splicing to salt stress response in rice. *New Phytol.* <https://doi.org/10.1111/nph.17189>

Bolger, A.M., Lohse, M., and Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114-2120.

Perteau, M., Kim, D., Perteau, G.M., Leek, J.T., and Salzberg, S.L. (2016). Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat Protoc* 11, 1650-1667.

Examples

```

data(rice.bg)
rice.bg
# ballgown instance with 33 transcripts and 6 samples

```

spliceGene

*Calculate Splicing Scores for One Gene***Description**

Calculate splicing Scores from ballgown object for a given gene. This function can only calculate one gene. Please use function `spliceGenome` to obtain genome-wide splicing scores.

Usage

```
spliceGene(bg, gene, samples = sampleNames(bg), junc.type = c("score", "count"),
           trans.select = "rowMaxs(x)>=1", junc.select = "rowMaxs(x)>=5")
```

Arguments

<code>bg</code>	ballgown object
<code>gene</code>	a character string specifying gene id
<code>samples</code>	names of samples
<code>junc.type</code>	type of junction estimate ('score' for junction score; 'count' for junction read count)
<code>trans.select</code>	logical expression-like string, indicating transcript rows to select from a matrix of transcript coverages: NA value keeps all transcripts. e.g. use <code>trans.select='rowMaxs(x)>=1'</code> to filter the transcripts with the maximum coverage among all the samples less than 1.
<code>junc.select</code>	logical expression-like string, indicating junction rows to select from a matrix of junction counts: NA value keeps all junctions. e.g. use <code>junc.select='rowMaxs(x)>=5'</code> to filter the junctions with the maximum read count among all the samples less than 5.

Details

`score` = junction count/gene-level per base read coverage. Row functions for matrices are useful to select transcripts and junctions. See `matrixStats` package.

Value

a matrix of junction scores with intron rows and sample columns.

References

Yu, H., Du, Q., Campbell, M., Yu, B., Walia, H. and Zhang, C. (2021), Genome-wide discovery of natural variation in pre-mRNA splicing and prioritising causal alternative splicing to salt stress response in rice. *New Phytol.* <https://doi.org/10.1111/nph.17189>

See Also

`spliceGenome`, which calculates splicing scores in whole genome.

Examples

```
data(rice.bg)
rice.bg
head(geneIDs(rice.bg))

score<-spliceGene(rice.bg, 'MSTRG.183',junc.type='score')
count<-spliceGene(rice.bg, 'MSTRG.183',junc.type='count')

## compare
tail(score)
tail(count)

## get intron structure
intron<-structure(rice.bg)$intron
intron[intron$id%in%rownames(score)]
```

spliceGenome	<i>Calculate Genome-wide Splicing Scores</i>
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Description

Calculate splicing scores from ballgown objects for all genes.

Usage

```
spliceGenome(bg, gene.select = "rowQuantiles(x,probs = 0.05)>=1",
             intron.select = "rowQuantiles(x,probs = 0.95)>=5")
```

Arguments

bg	ballgown object
gene.select	logical expression-like string, indicating genes to select from a matrix of gene-level coverages: NA value keeps all genes. e.g. gene.select = 'rowQuantiles(x,probs = 0.05)>=1' keeps the genes with the read coverage greater than or equal to 1 in at least 95 (0.05 quantile). Used to filter low expressed genes.
intron.select	logical expression-like string, indicating introns to select from a matrix of junction counts: NA value keeps all introns. e.g. intron.select = 'rowQuantiles(x,probs = 0.95)>=5' keeps the introns with the read count greater than or euqal to 5 in at least 5 (0.95 quantile). Used to filter introns with very few junction reads supporting.

Details

score = junction count/gene-level per base read coverage. Row functions for matrices in [matrixStats](#) package are useful to select genes and introns.

Value

a list of two elements: 'score' is matrix of intron splicing scores with intron rows and sample columns and 'intron' is a [GRanges](#) object of intron structure. See [structure](#) in **ballgown** package

References

Yu, H., Du, Q., Campbell, M., Yu, B., Walia, H. and Zhang, C. (2021), Genome-wide discovery of natural variation in pre-mRNA splicing and prioritising causal alternative splicing to salt stress response in rice. *New Phytol.* <https://doi.org/10.1111/nph.17189>

See Also

[spliceGene](#), which calculates splicing scores in one gene.

Examples

```
data(rice.bg)
rice.bg

splice<-spliceGenome(rice.bg, gene.select=NA, intron.select=NA)
names(splice)

head(splice$score)
splice$intron
```

splicePlot

Gene Splicing Plot

Description

Visualization of read coverage, splicing information and gene information in a gene region. This function is a wrapper of [getDepth](#), [getGeneinfo](#), [spliceGene](#), [plotBedgraph](#) and [plotGenes](#).

Usage

```
splicePlot(bg, gene, samples, bam.dir = NA, start = NA, end = NA,
           labels = samples, junc.type = c("score", "count"), junc.text = TRUE,
           trans.select = "rowMaxs(x)>=1", junc.select = "rowMaxs(x)>=5",
           col = SushiColors(2)(length(samples) + 1)[-1], transparency = 0.5,
           scale = "Kb", plotgenetype = "arrow", ...)
```

Arguments

bg	ballgown object. See ballgown .
gene	string indicating a gene ID (must be in the 'bg')
samples	names of the samples to be shown (must be in the 'bg' and have bam files in the 'bam.dir')

<code>bam.dir</code>	bam file directory of the samples. If NA, instead of read depth, conserved exons are drawn.
<code>start</code>	start position to be shown. If NA, start position of the gene will be used.
<code>end</code>	stop position to be shown. If NA, end position of the gene will be used.
<code>labels</code>	labels for samples (default: sample names). If it is NA, neither sample names nor gene names will be labeled
<code>junc.type</code>	type of junction estimates to be shown ('score' for junction score; 'count' for junction read count)
<code>junc.text</code>	TRUE/FALSE indicating whether junction estimates should be labeled
<code>trans.select</code>	logical expression-like string, indicating transcript rows to select from a matrix of transcript coverages: NA value keeps all transcripts. See spliceGene
<code>junc.select</code>	logical expression-like string, indicating junction rows to select from a matrix of junction counts: NA value keeps all junctions. See spliceGene
<code>col</code>	a vector of length(samples) specifying colors of read depths.
<code>transparency</code>	value between 0 and 1 indicating the degree of transparency of read depths.
<code>scale</code>	scale of the labelgenome ('bp', 'Kb', 'Mb')
<code>plotgenetype</code>	string specifying whether the genes should resemble a 'box' or a 'arrow'. See plotGenes .
<code>...</code>	values to be passed to plotGenes .

Value

see [plotGenes](#).

Examples

```
data(rice.bg)
rice.bg

samples <- paste('Sample', c('027', '102', '237'), sep='_')
bam.dir <- system.file('extdata', package = 'VaSP')

## plot the whole gene region
splicePlot(rice.bg, samples, bam.dir, gene='MSTRG.183', bheight=0.2)

## plot the alternative splicing region
splicePlot(rice.bg, samples, bam.dir, gene='MSTRG.183', start=1179000)
```

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