

# Package ‘VaSP’

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**Type** Package

**Version** 1.4.0

**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

**biocViews** RNASeq, AlternativeSplicing, DifferentialSplicing, StatisticalMethod, Visualization, Preprocessing, Clustering, DifferentialExpression, KEGG, ImmunoOncology

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## R topics documented:

BMfinder . . . . .	2
getDepth . . . . .	3
getGeneinfo . . . . .	4
rice.bg . . . . .	5
spliceGene . . . . .	6
spliceGenome . . . . .	7
splicePlot . . . . .	8
<b>Index</b>	<b>10</b>

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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 <a href="#">spliceGenome</a> or <a href="#">spliceGene</a> 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 <b>?mclapply</b> in <b>parallel</b> 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, plotgenetype = '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](https://www.ncbi.nlm.nih.gov/sra/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)&gt;=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)&gt;=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

*Calculate Genome-wide Splicing Scores*


---

**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 <a href="#">ballgown</a> .
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')



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

**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)
```

# Index

## \* datasets

rice.bg, 5

ballgown, 8

BMfinder, 2

getDepth, 3, 8

getGeneinfo, 4, 8

GRanges, 8

labelgenome, 9

matrixStats, 6, 7

plotBedgraph, 3, 8

plotGenes, 4, 8, 9

rice.bg, 5

spliceGene, 2, 6, 8, 9

spliceGenome, 2, 6, 7

splicePlot, 3, 4, 8

structure, 8