Package 'IVAS'

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Type Package

Title Identification of genetic Variants affecting Alternative Splicing

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`IVAS-package` *IVAS : Identification of genomic variants affecting Alternative Splicing*

Description

The tool is to detect genomic variants affecting the alternative splicing using genotypic and gene expression data(RNA-seq).

`ASdb-class` *ASdb s4 class - a container for results from functions of the* **IVAS** *package.*

Description

This class is the main object for storing results of the present package.

Note

An ASdb object stores information of alternative splicing patterns, expression ratios between transcripts with and without alternative target exons, and significant sQTLs from the functions of the present package. This ASdb object can be populated further slots during the analysis using functions for the analysis. Typically, an ASdb object can be created when the function `Splicingfinder` completes to define alternative splicing patterns. After creation, the ASdb contains the slot labeled as "SplicingModel", and the slot includes a list object named by "ES", "ASS", and "IR" (alternative splicing exons are saved separately in each element of the list based on their splicing pattern types; "ES": Exon skipping, "ASS": Alternative splice site, and "IR": Intron retention). In the next analysis step, further result slots can be added. The function `RatioFromFPKM` can add the "Ratio" slot containing expression ratio for each alternative splicing pattern based on the "SplicingModel" slot of the present class and for each individual from a matrix of FPKM values. Then, the result of the `sQTLsFinder` function can be saved by adding the "sQTLs" slot including significance of association between the expression ratios, which is stored in the "Ratio" slot of the present class, and SNPs for each alternative splicing exon.

See Also

`Splicingfinder`, `RatioFromFPKM`, `sQTLsFinder`

Examples

```
sampleDB <- system.file("extdata", "sampleDB", package="IVAS")
sample.Txdb <- loadDb(sampleDB)
data(sampleexp)
data(samplesnp)
data(samplesnplocus)
ASdb <- Splicingfinder(sample.Txdb)
ASdb <- RatioFromFPKM(sample.Txdb,ASdb,sampleexp)
ASdb <- sQTLsFinder(ASdb,samplesnp,samplesnplocus,method="lm")
ASdb
```

---

calSignificant *Deprecated*

Description

This function is deprecated and will be made defunct. Instead, use Splicingfinder.

CalSigSNP *Calculate significance SNPs*

Description

This function performs linear regression test to identify significance associations between expression ratio and genotypes using the lm function.

Usage

```
CalSigSNP(ratio.mat=NULL,snp.mat=NULL,overlapsnp=NULL,
    each.snplocus=NULL,chr,each.gene=NULL,GroupSam=NULL,method="lm")
```

Arguments

ratio.mat	A data frame consisting of expression ratio of an alternatively spliced exon.
snp.mat	A data frame of genotype data.
overlapsnp	A data frame containing SNPs which is within an alternatively spliced exon and its flanking introns.
each.snplocus	A data frame consisting of locus information of SNP markers in the snpdata.
chr	The chromosome number that you would like to test in this function.
each.gene	The gene name that you would like to test in this function
GroupSam	A list object of a group of each sample.
method	The option for statistical models and boxplot.("lm" : analysis using linear regression model, "glm" : analysis using generalized linear mixed model, "both" : "lm" and "glm", and "boxplot" : for writing boxplot).

Value

The lm or glm method returns matrix including; SNP marker IDs, P values, information of differential median values of expression ratio among genotypes ("sig" if differential median > 0.1 and "not sig" otherwise), gene names, and methods ("lm" or "glm"). The boxplot method returns matrix with relative ratio values and genotypes of samples.

Author(s)

Seonggyun Han, Sangsoo Kim

References

Chambers, J. M. (1992) Linear models. Chapter 4 of Statistical Models in S eds J. M. Chambers and T. J. Hastie, Wadsworth & Brooks/Cole.

See Also

lm, glmer

Examples

```
sampleDB <- system.file("extdata", "sampleDB", package="IVAS")
sample.Txdb <- loadDb(sampleDB)
data(sampleexp)
data(samplesnp)
data(samplesnplocus)
ASdb <- Splicingfinder(sample.Txdb)
ASdb <- RatioFromFPKM(sample.Txdb,ASdb,sampleexp,CalIndex="ASS7")
ratio.mat <- slot(ASdb,"Ratio")$ASS
ratio.mat <- rbind(ratio.mat[,grep("NA",colnames(ratio.mat))])
each.snp <- rbind(samplesnp[rownames(samplesnp) == "rs3810232",])
each.snplocus <- rbind(samplesnplocus[samplesnplocus[,"SNP"] == "rs3810232",])
overlapsnp <- rbind(c(snp="rs3810232",locus="54704760"))
CalSigSNP(ratio.mat,as.matrix(each.snp),overlapsnp,each.snplocus,"19","ENSG00000170889",method="lm")
```

---

chrseparate *Separate a TxDb object based on a chromosome.*

Description

With the isActiveSeq method in **GenomicFeatures** package, this function filters the TxDb object in the **GenomicFeatures** package based on a single chromosome.

Usage

```
chrseparate(GTFdb = NULL, chrname = NULL)
```

Arguments

GTFdb	The TxDb object in the **GenomicFeatures** package.
chrname	The chromosome number you would like to select from TxDb

Value

This function returns the TxDb limited to the chromosome number that you want.

Author(s)

Seonggyun Han, Sangsoo Kim

References

Lawrence M, Huber W, Pages H, Aboyoun P, Carlson M, Gentleman R, Morgan M, and Carey V. Software for Computing and Annotating Genomic Ranges. PLoS Computational Biology, 9, e1003118. 2013.

See Also

isActiveSeq, seqinfo

Examples

```
sampleDB <- system.file("extdata", "sampleDB", package="IVAS")
sample.Txdb <- loadDb(sampleDB)
filtered.txdb <- chrseparate(sample.Txdb,19)
```

`findAlternative` *Find alternative exons of a gene.*

Description

Search alternative exons among transcript isoforms from a single gene.

Usage

```
findAlternative(geneid = NULL, txTable = NULL, totalExrange = NULL,
    totalInrange = NULL, one.chr = NULL)
```

Arguments

`geneid`	Ensembl gene name.
`txTable`	The matrix of transcripts including transcript IDs, Ensembl gene names, Ensembl transcript names, transcript start sites, and transcript end sites.
`totalExrange`	A list of GRanges objects including total exon ranges in each transcript resulted from the `exonsBy` function in **GenomicFeatures**.
`totalInrange`	A list of GRanges objects including total intron ranges in each transcript resulted from the `intronsByTranscript` function in **GenomicFeatures**.
`one.chr`	The chromosome number that you would like to test

Value

alterIntron	A GRanges object with flanking introns of alternative exons
tableBygene	An information table of transcripts including transcript IDs, Ensembl gene names, Ensembl transcript names, transcript start sites, and transcript end sites.
exonRange	All exons locus of a gene
intronRange	All intron locus of a gene

Author(s)

Seonggyun Han, Sangsoo Kim

References

Lawrence M, Huber W, Pages H, Aboyoun P, Carlson M, Gentleman R, Morgan M, and Carey V. Software for Computing and Annotating Genomic Ranges. PLoS Computational Biology, 9, e1003118. 2013.

See Also

GRanges, IRanges

Examples

```
  sampleDB <- system.file("extdata", "sampleDB", package="IVAS")
  sample.Txdb <- loadDb(sampleDB)
  filtered.txdb <- chrseparate(sample.Txdb,19)
  trans.exon.range <- exonsBy(filtered.txdb,by="tx")
  trans.intron.range <- intronsByTranscript(filtered.txdb)
  txTable <- select(filtered.txdb, keys=names(trans.exon.range),
                        columns=c("TXID","TXNAME","GENEID","TXSTART","TXEND"), keytype="TXID")
Altvalue <- findAlternative("ENSG00000170889",txTable,trans.exon.range,trans.intron.range,19)
```

---

findOversnp *Find SNPs which belong to alternative exons and flanking introns of them.*

Description

Find SNPs which belong to alternative exons and flanking introns of them.

Usage

```
findOversnp(altInvalue = NULL, snprange = NULL)
```

Arguments

altInvalue	A list data set from the findAlternative function.
snprange	A matrix of SNP ranges.

Value

This function returns a matrix with SNPs which are located in alternative exons and flanking introns and ranges of those SNPs.

Author(s)

Seonggyun Han, Sangsoo Kim

See Also

findOverlaps

Examples

```
 sampleDB <- system.file("extdata", "sampleDB", package="IVAS")
 sample.Txdb <- loadDb(sampleDB)
 data(samplesnplocus)
 data(samplesnp)
 filtered.txdb <- chrseparate(sample.Txdb,19)
 trans.exon.range <- exonsBy(filtered.txdb,by="tx")
 trans.intron.range <- intronsByTranscript(filtered.txdb)
 txTable <- select(filtered.txdb, keys=names(trans.exon.range),
                       columns=c("TXID","TXNAME","GENEID","TXSTART","TXEND"), keytype="TXID")
 ch.snp.locus <- as.matrix(samplesnplocus[samplesnplocus[,2] == 19,])
ch.snps <- matrix(ch.snp.locus[is.element(ch.snp.locus[,1],rownames(samplesnp)),],ncol=3,byrow=FALSE)
ch.snps.range <- GRanges(seqnames=Rle(19),ranges=IRanges(start=as.integer(ch.snps[,3]),
                                                         end=as.integer(ch.snps[,3])),
Altvalue <- findAlternative("ENSG00000170889",txTable,trans.exon.range,trans.intron.range,19)
 overlapsnp <- findOversnp(Altvalue,ch.snps.range)
```

IVAS-deprecated *Deprecated functions in package 'IVAS'*

Description

These functions are provided for compatibility with older versions of 'IVAS' only, and will be defunct at the next release.

Details

The following functions are deprecated and will be made defunct; use the replacement indicated below:

- MsqtlFinder: sQTLsFinder
- sqtlfinder: sQTLsFinder
- calSignificant: Splicingfinder

`MsqtlFinder` *Deprecated*

Description

This function is deprecated and will be made defunct. Instead, use `sQTLsFinder`.

`RatioFromFPKM` *Estimate relative expression ratio.*

Description

With the FPKM expression data set of transcripts, this function estimates relative expression ratio between transcripts with and without alternatively spliced exons based on splicing models of the ASdb object

Usage

```
RatioFromFPKM(GTFdb = NULL, ASdb = NULL,
    Total.expdata = NULL, CalIndex = NULL, Ncor = 1, out.dir = NULL)
```

Arguments

`GTFdb`	A TxDb object in the **GenomicFeatures** package.
`ASdb`	A ASdb object including "SplicingModel" slot from the `Splicingfinder` function.
`Total.expdata`	A data frame of expression data.
`CalIndex`	An index number in the ASdb object which will be tested in this function.
`Ncor`	The number of cores for multi-threads function.
`out.dir`	An output directory.

Value

ASdb with the slot (labeled by "Ratio") containing results from the the `RatioFromFPKM` function. The "Ratio" slot contains a list object and each element of the list object returns the results assigned to three elements, which is of each alternative splicing type (i.e. Exon skipping, Alternative splice site, Intron retention). Three elements are as follows;

`ES`	A data frame for the result of Exon skipping, consisting of the columns named as follows; Index (index number), EnsID (gene name), Nchr (chromosome name), 1stEX (alternatively spliced target exon), 2ndEX (second alternatively spliced target exon which is the other one of the mutually exclusive spliced exons), DownEX (downstream exon range), UpEX (upstream exon range), Types (splicing type), and names of individuals.
`ASS`	A data frame for the result of Alternative splice sites, consisting of the columns named as follows; Index (index number), EnsID (gene name), Nchr (chromosome name), ShortEX (shorter spliced target exon), LongEX (longer spliced target exon), NeighborEX (neighboring down or upstream exons), Types (splicing type), and names of individuals.

IR — A data frame for the result of Intron retention, consisting of the columns named as follows; Index (index number), EnsID (gene name), Nchr (chromosome name), RetainEX (retained intron exon), DownEX (downstream exon range), UpEX (upstream exon range), Types (splicing type), and names of individuals.

Author(s)

Seonggyun Han, Sangsoo Kim

See Also

isActiveSeq, seqinfo, Splicingfinder

Examples

```
sampleDB <- system.file("extdata", "sampleDB", package="IVAS")
sample.Txdb <- loadDb(sampleDB)
data(sampleexp)
ASdb <- Splicingfinder(sample.Txdb)
ASdb <- RatioFromFPKM(sample.Txdb,ASdb,sampleexp)
```

---

sampleexp *CEU expression data*

Description

CEU expression data including 78 individuals

Usage

```
data("sampleexp")
```

Format

A data frame with 64 transcript expressions on the 78 individuals

Value

A data frame with 64 transcript expressions on the 78 individuals

Source

The data was generated by GEUVADIS (Genetic European Variation in Health and Disease, A European Medical Sequencing Consortium) RNA sequencing project for 1000 Genomes samples (http://www.geuvadis.org/web/geuvadis/RNAseq-project).

References

Tuuli Lappalainen, et al. (2013). Transcriptome and genome sequencing uncovers functional variation in humans. Nature 501, 506-511.

Examples

```
data(sampleexp)
```

samplesnp *CEU genotype data*

Description

CEU genotype data including 78 individuals

Usage

```
data("samplesnp")
```

Format

A data frame with 11 SNPs on the 78 individuals

Value

A data frame with 11 SNPs on the 78 individuals

Source

The data has 1000 genomes Phages 1 dataset and was imputed by GEUVADIS (Genetic European Variation in Health and Disease, A European Medical Sequencing Consortium) RNA sequencing project for 1000 Genomes samples (http://www.geuvadis.org/web/geuvadis/RNAseq-project).

References

Tuuli Lappalainen, et al. (2013). Transcriptome and genome sequencing uncovers functional variation in humans. Nature 501, 506-511.

Examples

```
data(samplesnp)
```

samplesnplocus *snplocus*

Description

snplocus

Usage

```
data("samplesnplocus")
```

Format

A data frame with 11 SNPs and locus of them

Value

A data frame with 11 SNPs and locus of them

Examples

```
data(samplesnplocus)
```

`saveBplot` *Save boxplots*

Description

Save boxplots

Usage

```
saveBplot(ASdb=ASdb,Total.snpdata=NULL,Total.snplocus=NULL,
            CalIndex=NULL,out.dir=NULL)
```

Arguments

`ASdb`	A ASdb object including "sQTLs" slot from the `sQTLsFinder` function.
`Total.snpdata`	A data frame of genotype data.
`Total.snplocus`	A data frame containing locus information of SNP markers in the snpdata.
`CalIndex`	An index number in the ASdb object which will be tested in this function.
`out.dir`	An output directory.

Value

This function draws the boxplot

Author(s)

Seonggyun Han, Sangsoo Kim

See Also

`boxplot`

Examples

```
sampleDB <- system.file("extdata", "sampleDB", package="IVAS")
sample.Txdb <- loadDb(sampleDB)
data(sampleexp)
data(samplesnp)
data(samplesnplocus)
ASdb <- Splicingfinder(sample.Txdb)
ASdb <- RatioFromFPKM(sample.Txdb,ASdb,sampleexp)
ASdb <- sQTLsFinder(ASdb,samplesnp,samplesnplocus,method="lm")
saveBplot(ASdb=ASdb,Total.snpdata=samplesnp,Total.snplocus=samplesnplocus,CalIndex="ASS7",out.dir="./res
```

`Splicingfinder` *Find alternatively spliced exons*

Description

Find alternatively spliced exons based on GTF reference transcript models.

Usage

```
Splicingfinder(GTFdb = NULL , txTable = NULL , calGene = NULL , Ncor = 1 , out.dir = NULL)
```

Arguments

`GTFdb`	A TxDb object in the **GenomicFeatures** package.
`txTable`	A matrix of transcripts including transcript IDs, gene names, transcript names, transcript start sites, and transcript end sites based on a GTF reference transcript model file.
`calGene`	An interest of a gene that will be tested. If calGene is inputted by a single gene, the splicing pattern for the only gene is tested. If not, the splicing patterns for total of genes are tested
`Ncor`	The number of cores for multi-threads.
`out.dir`	An output directory.

Value

ASdb with the slot (labeled by "SplicingModel") containing results from the the `Splicingfinder` function. The "Splicingfinder" slot contains a list object and each element of the list object returns the results assigned to three elements, which is of each alternative splicing type (i.e. Exon skipping, Alternative splice site, Intron retention). Three elements are as follows;

`ES`	A data frame for the result of Exon skipping, consisting of the columns named as follows; Index (index number), EnsID (gene name), Nchr (chromosome name), 1stEX (alternatively spliced target exon), 2ndEX (second alternatively spliced target exon which is the other one of the mutually exclusive spliced exons), DownEX (downstream exon range), UpEX (upstream exon range), 1st_des (alternatively spliced target exons in a representative exon), 2nd_des (second alternatively spliced target exons in a representative exon), Do_des (downstream exons in a representative exon), Up_des (upstream exons in a representative exon), and Types (splicing type).
`ASS`	A data frame for the result of Alternative splice site, consisting of the columns named as follows; Index (index number), EnsID (gene name), Nchr (chromosome nam), ShortEX (shorter spliced target exon), LongEX (longer spliced target exon), NeighborEX (neighboring down or upstream exons), Short_des (shorter spliced target exons in a representative exon), Long_des (longer spliced target exons in a representative exon), Neighbor_des (neighboring down or upstream exons in a representative exon), and Types (splicing type).

IR — A data frame for the result of Intron retention, consisting of the columns named as follows; Index (index number), EnsID (gene name), Nchr (chromosome name), RetainEX (retained intron exon), DownEX (downstream exon range), UpEX (upstream exon range), Retain_des (retained intron exons in a representative exon), Do_des (downstream exons in a representative exon), Up_des (upstream exons in a representative exon), and Types (splicing type).

Author(s)

Seonggyun Han, Sangsoo Kim

See Also

isActiveSeq, seqinfo

Examples

```
sampleDB <- system.file("extdata", "sampleDB", package="IVAS")
sample.Txdb <- loadDb(sampleDB)
ASdb <- Splicingfinder(sample.Txdb)
```

---

sqtlfinder *Deprecated*

Description

This function is deprecated and will be made defunct. Instead, use sQTLsFinder.

---

sQTLsFinder *Find SQTLs.*

Description

Find significant SNPs using the calSignificant function.

Usage

```
sQTLsFinder(ASdb, Total.snpdata = NULL , Total.snplocus = NULL ,
  GroupSam = NULL , method = "lm" , CalIndex = NULL , Ncor = 1 , out.dir = NULL)
```

Arguments

ASdb	A ASdb object including "SplicingModel" and "Ratio" slots from the Splicingfinder and RatioFromFPKM funtions, respectively.
Total.snpdata	A data frame of genotype data.
Total.snplocus	A data frame containing locus information of SNP markers in the snpdata.
GroupSam	A list object of a conditions for each individual. If GroupSam is not NULL, the odds ratio and its confidence intervals between conditions are calculated.
method	An option for statistical models and boxplot.("lm" : analysis using linear regression model, "glm" : analysis using generalized linear mixed model, "both" : "lm" and "glm",and "boxplot" : for writing boxplot).
CalIndex	An index number in the ASdb object which will be tested in this function.
Ncor	The number of cores for multi-threads function.
out.dir	An output directory.

Value

ASdb with the slot (labeled by "sQTLs") containing results from the the sQTLsFinder function. The "Splicingfinder" slot contains a list object and each element of the list object returns the results assigned to three elements, which is of each alternative splicing type (i.e. Exon skipping, Alternative splice site, Intron retention). Three elements are as follows;

ES	A data frame for the result of Exon skipping, consisting of the columns named as follows; Index (index number), EnsID (gene name), Nchr (chromosome name), 1stEX (alternatively spliced target exon), 2ndEX (second alternatively spliced target exon which is the other one of the mutually exclusive spliced exons), DownEX (downstream exon range), UpEX (upstream exon range), Types (splicing type), pByGeno (P-values of "lm" or "glm" test for association PSI values and genotypes), FdrByGeno (pByGeno), diff ("diff" if differential median > 0.1 and "Nondiff" otherwise), pByGroups (P-values of chi-square test for association between genotypes of two groups), fdrByGroups (FDR values for the pByGroups column), OR (odds ratio), lowCI(low confidence interval), highCI(high confidence interval), and methods ("lm" or "glm").
ASS	A data frame for the result of Alternative splice sites, consisting of the columns named as follows; Index (index number), EnsID (gene name), Nchr (chromosome nam), ShortEX (shorter spliced target exon), LongEX (longer spliced target exon), NeighborEX (neighboring down or upstream exons), Types (splicing type), pByGeno (P-values of "lm" or "glm" test for association PSI values and genotypes), FdrByGeno (pByGeno), diff ("diff" if differential median > 0.1 and "Nondiff" otherwise), pByGroups (P-values of chi-square test for association between genotypes of two groups), fdrByGroups (FDR values for the pByGroups column), OR (odds ratio), lowCI(low confidence interval), highCI(high confidence interval), and methods ("lm" or "glm").
IR	A data frame for the result of Intron retention, consisting of the columns named as follows; Index (index number), EnsID (gene name), Nchr (chromosome name), RetainEX (retained intron exon), DownEX (downstream exon range), UpEX (upstream exon range), Types (splicing type), pByGeno (P-values of "lm" or "glm" test for association PSI values and genotypes), FdrByGeno (pByGeno), diff ("diff" if differential median > 0.1 and "Nondiff" otherwise), pByGroups (P-values of chi-square test for association between genotypes of two groups), fdrByGroups (FDR values for the pByGroups column), OR (odds ratio), lowCI(low

confidence interval), highCI(high confidence interval), and methods ("lm" or "glm").

The boxplot method returns matrix data with relative ratio values and genotypes of samples.

Author(s)

Seonggyun Han, Sangsoo Kim

References

Chambers, J. M. (1992) Linear models. Chapter 4 of Statistical Models in S eds J. M. Chambers and T. J. Hastie, Wadsworth & Brooks/Cole. Breslow, N.E. Clayton, D.G. (1993). Approximate Inference in Generalized Linear Mixed Models. Journal of the American Statistical Association 88.

See Also

lm, glmer

Examples

```
sampleDB <- system.file("extdata", "sampleDB", package="IVAS")
sample.Txdb <- loadDb(sampleDB)
data(sampleexp)
data(samplesnp)
data(samplesnplocus)
ASdb <- Splicingfinder(sample.Txdb)
ASdb <- RatioFromFPKM(sample.Txdb,ASdb,sampleexp)
ASdb <- sQTLsFinder(ASdb,samplesnp,samplesnplocus,method="lm")
```

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