Package: IntEREst (via r-universe)

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Title Intron-Exon Retention Estimator

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Description This package performs Intron-Exon Retention analysis on RNA-seq data (.bam files).

Depends R (>= 3.5.0), GenomicRanges, Rsamtools, SummarizedExperiment, edgeR, S4Vectors, GenomicFiles

Imports seqLogo, Biostrings, GenomicFeatures (>= 1.39.4), txdbmaker, IRanges, seqinr, graphics, grDevices, stats, utils, grid, methods, DBI, RMariaDB, GenomicAlignments, BiocParallel, BiocGenerics, DEXSeq, DESeq2

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IntEREst-package *IntEREst*

Description

Intron/Exon retention estimator quantifies and normalizes Intron retention and Exon junction read levels by analyzing mapped reads (.bam) files.

Details

Package:	IntEREst
Type:	Package
Version:	1.0
Date:	2015-11-18
License:	GPL-2

To run the pipeline use functions `interest()` or `interest.sequential()`, i.e. wrapper functions that run all the necessary functions.

Author(s)

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addAnnotation *Adding sample annotations to a* `SummarizedExperiment` *object*

Description

Adds a new sample annotation to the `SummarizedExperiment` object. In other words it adds and column with sample annotations to the colData of the `SummarizedExperiment` object.

Usage

```
addAnnotation(x, sampleAnnotationType, sampleAnnotation)
```

Arguments

`x`	Object of type `SummarizedExperiment`.
`sampleAnnotationType`	The name of the new column to be added to the `colData` table of `SummarizedExperiment` object.
`sampleAnnotation`	Vector with the same length as the row-size of the `colData` attribute of the `SummarizedExperiment` object, which includes the sample annotations.

Value

An InterestResult object.

Author(s)

Ali Oghabian

See Also

getAnnotation

Examples

```
# Check the annotation table of mdsChr22Obj data
getAnnotation(mdsChr22Obj)

# Add a new sample annotation
newMdsChr22Obj <- addAnnotation(x=mdsChr22Obj,
 sampleAnnotationType="sample_number",
 sampleAnnotation=1:16
)

# Retrieve annotations of the new object
getAnnotation(newMdsChr22Obj)
```

---

annotateU12 *Annotate the U12 (and U2) type introns*

Description

Receives coordinates, a reference genome and PWMs of splice site of U12 and U2 type introns, and returns a data.frame with 2 columns. The first column shows wheather the corresponding sequences matches U12, U2 or both (U12/U2) consensus sequences (based on their score when fitting the PWMs). The second column shows whether the match is on positive strand or negative when fitting the PWMs to the sequences.

Usage

```
annotateU12(pwmU12U2=c(), pwmSsIndex=c(), referenceChr, referenceBegin,
 referenceEnd, referenceIntronExon, intronExon='intron',
 matchWindowRelativeUpstreamPos=c() , matchWindowRelativeDownstreamPos=c(),
 minMatchScore='80%', refGenome='', setNaAs='U2', annotateU12Subtype=TRUE,
 includeMatchScores=FALSE, ignoreHybrid=TRUE, filterReference)
```

Arguments

pwmU12U2	A list containing position weight matrices of (in order): Donor site, branch point, and acceptor site of U12-type introns, and donor site and acceptor site of U2-type introns. If not provided, the information related to pwmU12db data is used.
pwmSsIndex	A list (or vector) that contains the column number in each element of pwmU12U2 that represents the 5' or 3' Splice Site; The order should be equivalent to the pwmU12U2. If not provided the information from pwmU12db data is used, i.e. pwmSsIndex=list(indexDonU12=1,indexBpU12=1, indexAccU12=3, indexDonU2=1, indexAccU2=3)
referenceChr	Chromosome names of the references (e.g. introns).
referenceBegin	A vector that corresponds to the begin coordinates of the reference (e.g. introns).
referenceEnd	A vector that corresponds to the end coordinates of the reference (e.g. introns). referenceEnd should be greater than or equal to referenceBegin.
referenceIntronExon	A vector with the same size as the referenceChr, referenceBegin and referenceEnd which contains 'intron' and 'exon' describing what (either intron or exon) each element of the 3 vectors represents.
intronExon	Should be assigned either 'intron' or 'exon' or c('intron','exon') based on whether match the PWM to the intronic, exonic, or intronic and exonic regions of the reference. By default it seeks matches in intronic regions (intronExon='intron').
matchWindowRelativeUpstreamPos	A vector the same size as the pwmU12U2 (and the same order of donor/acceptor sites' information in pwmU12U2) which consists of the upstream distance from the donor/acceptor site from which each PWM should be tested (to see if they match). If not provided, the information from pwmU12db data is used i.e. matchWindowRelativeUpstream c(NA, -29, NA, NA, NA).
matchWindowRelativeDownstreamPos	A vector the same size as the pwmU12U2 (and the same order of donor/acceptor sites' information in pwmU12U2) which consists of the downstream distance from the donor/acceptor site to which each PWM should be tested (to see if they match). If not provided, the information from pwmU12db data is used i.e. matchWindowRelativeDownstre c(NA,-9, NA, NA, NA).
minMatchScore	Min percentage match score, when scoring matching of a sequence to pwm. Different score thresholds could also be defined for the various sites (U12/U2 donors, the U12 branch point and U12/U2 acceptors); A vector with 5 elements can be assigned which each shows the match score to use for each PWM in pwmU12U2.
refGenome	The reference genome; Object of class BSgenome. Use available.genome() from the BSgenome package to see the available genomes. DNAStringSet objects (from Biostrings package) and fasta files are also accepted as input.
setNaAs	Defines that if reference (e.g. intron) did not match any of U12 or U2 type introns based on the scores obtained from PWM what should the function return. If an intron was not proven to be U12 or U2 based on PWM scores it can be considered as U2-type since the U12 type introns constitute for about 1% of

introns in human genome and they are muxh more conserved than the U2 type introns, hence the default is 'U2'; otherwise it is also possible to set it as NA or nan or 'U12/U2'.

`annotateU12Subtype`
: Whether annotate the subtypes of the U12 type Introns. The value is `TRUE` by default.

`includeMatchScores`
: If set as TRUE the final data frame result includes the PWM match scores (FALSE by default).

`ignoreHybrid`
: Whether ignore the U12 hybrid subtypes, i.e. GT-AC and AT-AG (TRUE by default).

`filterReference`
: Optional parameter that can be defined either as a GRanges or SummarizedExperiment object. If defined as the latter, the first 3 columns of the rowData must be: chr name, start and end of the coordinates. If the parameter is defined the introns/exon coordinates will be mapped against it and the intron type of all those that do not match will be set as NA.

Value

Data frame containing 3 columns representing (in order): intron type (U12, U2 or none), strand match indicating whether the PWM matches to the sequence (+ strand) or the reverese complement of the sequence (- strand) or none (NA), and the U12 subtype (GT-AG or AT-AC). If includeMatchScores is set as TRUE further columns that include the PWM match scores will also be included.

Author(s)

Ali Oghabian

See Also

`buildSsTypePwms`.

Examples

```
# Improting genome
BSgenome.Hsapiens.UCSC.hg19 <-
 BSgenome.Hsapiens.UCSC.hg19::BSgenome.Hsapiens.UCSC.hg19
#Choosing subset of rows
ind<- 69:94
# Annotate U12 introns with strong U12 donor site, branch point
# and acceptor site from the u12 data in the package
annoU12<-
 annotateU12(pwmU12U2=list(pwmU12db[[1]][,11:17],pwmU12db[[2]]
  ,pwmU12db[[3]][,38:40],pwmU12db[[4]][,11:17],
  pwmU12db[[5]][,38:40]),
 pwmSsIndex=list(indexDonU12=1, indexBpU12=1, indexAccU12=3,
  indexDonU2=1, indexAccU2=3),
```

```
 referenceChr=u12[ind,'chr'],
 referenceBegin=u12[ind,'begin'],
 referenceEnd=u12[ind,'end'],
 referenceIntronExon=u12[ind,"int_ex"],
 intronExon="intron",
 matchWindowRelativeUpstreamPos=c(NA,-29,NA,NA,NA),
 matchWindowRelativeDownstreamPos=c(NA,-9,NA,NA,NA),
 minMatchScore=c(rep(paste(80,"%",sep=""),2), "60%",
  paste(80,"%",sep=""), "60%"),
 refGenome=BSgenome.Hsapiens.UCSC.hg19,
 setNaAs="U2",
 annotateU12Subtype=TRUE)

# How many U12 and U2 type introns with strong U12 donor sites,
# acceptor sites (and branch points for U12-type) are there?
table(annoU12[,1])
```

`applyOverlap` *Apply function over counts*

Description

Runs a function on columns of the counts (assay) of a 'SummarizedExperiment' object (resulted by `interest()`, `interest.sequential()` or `readInterestResults()`) based on the overalp of its exon/intron coordinates with those of another 'SummarizedExperiment' object. The number of the rows and the dimensions of the counts of the result are equal to those of the `subject`. The function is applied on the query based on it's overlap to the subject.

Usage

```
applyOverlap(
 query,
 subject,
 type="any",
 replaceValues=FALSE,
 intExCol="int_ex",
 intronExon="intron",
 sujectGeneNamesCol,
 repeatsTableToFilter=c(),
 scaleFragment=TRUE,
 scaleLength=TRUE,
 unmapValue=0,
 FUN=mean,
 ...
)
```

Arguments

query, subject	SummarizedExperiment objects resulted by interest(), interest.sequential() or readInterestResults() functions.
type	The type of overlap. By default it considers any overlap. See findOverlaps-methods for more info.
replaceValues	Whether return a 'SummarizedExperiment' object with new counts (resulted by running function) replaced.
intExCol	Column name (or number) in the rowData of the objects that represents whether each row of the assay is "intron" or "exon".
intronExon	Should be assigned either 'intron' or 'exon' or c('intron','exon') based on whether match the PWM to the intronic, exonic, or intronic and exonic regions of the reference. By default it seeks matches in intronic regions (intronExon='intron').
sujectGeneNamesCol	The column in the row data of the subject that includes the gene names.
repeatsTableToFilter	A data.frame table that includes chr,begin and end columns. If defined, all reads mapped to the described regions will be ingnored.
scaleFragment	Logical value, indicating whether the retention levels must be scaled by (genewide) fragment levels.
scaleLength	Logical value, indicating whether the retention levels must be scaled by length of the introns/exons.
unmapValue	The value to assign to unmapped rows (i.e. introns/exons).
FUN	The function to apply.
...	Other parameter settings from aggregate() function.

Value

The returned value is a data frame if replaceValues is FALSE and it is SummarizedExperiment if replaceValues is TRUE.

Author(s)

Ali Oghabian

See Also

readInterestResults interest interest.sequential

Examples

```
mdsChr22Obj

tmp<- applyOverlap(
 query=mdsChr22Obj,
 subject=mdsChr22Obj,
```

```
 type="equal",
 replaceValues=FALSE,
 intExCol="int_ex",
 intronExon="intron",
 sujectGeneNamesCol="collapsed_transcripts",
 scaleFragment=TRUE,
 scaleLength=TRUE,
 unmapValue=0,
 FUN=head,
 n=1
)
```

`attributes` *Extracting values of useful attributes of SummarizedExperiment objects*

Description

Several functions are provided that can extract various attribnutes from an object of class `SummarizedExperiment` generated by IntEREst functions, e.g. `interest()`, `interest`, and `readInterestResults`. It is possible to extract sample annotations using `getAnnotation` function. One can also extract the scaled retention levels of the introns/exons using `scaledRetention()` function. Notes that `colData` and `rowData` methods of `SummarizedExperiment` class can also be used to extract row and column data.

Usage

```
getAnnotation(x)
scaledRetention(x)
```

Arguments

`x`	Object of type `SummarizedExperiment`.

Value

Various data types (data.frame/vector) dependent on the function used. See the "Description" for more information.

Author(s)

Ali Oghabian

See Also

`SummarizedExperiment-class` `addAnnotation` `counts-method` `plot-method`

Examples

```
# Retrieve the sample annotations from mdsChr22Obj
getAnnotation(mdsChr22Obj)
# Retrieving the scaled retention levels from mdsChr22Obj
head(scaledRetention(mdsChr22Obj))

#for row and column data SummarizedExperiment methods can be used
head(rowData(mdsChr22Obj))
colData(mdsChr22Obj)
```

`boxplot-method` *boxplot - method*

Description

boxplot method for `SummarizedExperiment` objects.

Usage

```
## S4 method for signature 'SummarizedExperiment'
boxplot(x, sampleAnnoCol=NA,
 intexTypeCol="int_type", intexType=c(), col="white", boxplotNames=c(),
 lasNames=3, outline=FALSE, addGrid=FALSE, ...)
```

Arguments

`x`	Object of type `SummarizedExperiment` generated by either `interest()`, `interest.sequential()` or `readInterestResults()`.
`sampleAnnoCol`	Which columnn of `colData` in `x` to consider for plotting.
`intexTypeCol`	Column name (or number) that represents what type of intron/exon each row of `x` assays represents.
`intexType`	A vector of characters describing types of introns/exons to be plotted. They must be elements in the `intexTypeCol` column of the rowData of `x`. rowData of `x` is a dataframe that includes various annotations of the introns/exons.
`col`	Vector showing box colours. It is either of size 1 or the same size as the number of groups to be plotted.
`boxplotNames`	Names to write under boxes. If not defined, as names, it pastes the row (intron/exon) annotation names to the sample group annotations separated by a space " ".
`lasNames`	Orientation of the box names.
`outline`	If outline is TRUE the outlier points are drawn otherwise if FALSE (default) they are not.
`addGrid`	Whether add a grid under the boxplots (FALSE by default).
`...`	Other arguments to pass to the `boxplot()` and `axis` function.

Value

Returns NULL.

Author(s)

Ali Oghabian

See Also

Class: SummarizedExperiment-class Method: counts-method plot-method

Examples

```
#Plotting U12- vs U2-type introns
par(mar=c(8,4,2,1))
boxplot(x=mdsChr22Obj, sampleAnnoCol="type", intexTypeCol="intron_type",
 intexType=c("U2", "U12"),
 col=rep(c("yellow", "orange"),3),
 boxplotNames=c(), lasNames=3, outline=FALSE,
 addGrid=TRUE)
```

---

buildSsTypePwms *Building Position Weight Matrices for Splice Sites of U12 and U2 type introns.*

Description

Builds position Weigh Matrices for the donor and acceptor sites of the U12 and U2 type introns, and the branchpoint of the U12 type introns. if pdfFileSeqLogos is defined a pdf is also produced that contains the sequence logos of the results. The result is a list that contains PWMs of the splice sites of U12 and U2 dependent introns.

Usage

```
buildSsTypePwms( cexSeqLogo=1, pdfWidth=35, pdfHeight=10, tmpDir="./",
 u12dbSpecies="Homo_sapiens",
 pwmSource="U12DB",
 u12DonorBegin, u12BranchpointBegin, u12AcceptorBegin,
 u2DonorBegin, u2AcceptorBegin, u12DonorEnd,
 u12BranchpointEnd, u12AcceptorEnd, u2DonorEnd,
 u2AcceptorEnd, pasteSites=FALSE,
 splicerackSsLinks=list(
  U12_AT_AC_donor=
   "http://katahdin.mssm.edu/splice/out/9606_logo_file.25",
  U12_AT_AC_branchpoint=
   "http://katahdin.mssm.edu/splice/out/9606_logo_file.26",
  U12_AT_AC_acceptor=
```

```
  "http://katahdin.mssm.edu/splice/out/9606_logo_file.29",
 U12_GT_AG_donor=
  "http://katahdin.mssm.edu/splice/out/9606_logo_file.22",
 U12_GT_AG_branchpoint=
  "http://katahdin.mssm.edu/splice/out/9606_logo_file.27",
 U12_GT_AG_acceptor=
  "http://katahdin.mssm.edu/splice/out/9606_logo_file.21",
 U2_GC_AG_donor="http://katahdin.mssm.edu/splice/out/9606_logo_file.24",
 U2_GC_AG_acceptor=
  "http://katahdin.mssm.edu/splice/out/9606_logo_file.30",
 U2_GT_AG_donor="http://katahdin.mssm.edu/splice/out/9606_logo_file.23",
 U2_GT_AG_acceptor=
  "http://katahdin.mssm.edu/splice/out/9606_logo_file.28"),
u12dbLink="https://genome.crg.cat/pub/software/u12/u12db_v1_0.sql.gz",
u12dbDbName="u12db", u12dbDropDb=TRUE,  pdfFileSeqLogos="",
removeTempFiles=TRUE, ...)
```

Arguments

`cexSeqLogo`	Font size of sequence logo plots; used only if `pdfFileSeqLogos` is defined.
`pdfWidth`, `pdfHeight`	The width and height of the graphics region of the pdf in inches. The default values are 35 and 10.
`tmpDir`	Path to directory used for storing temporary files.
`u12dbSpecies`	What species data to use when getting the data from the U12DB database (`pwmSource="U12DB"`).
`pwmSource`	The source used to buildSplice Sites of U12 and U2 type introns the PWM for U12 and U2 dependent introns. Default is `U12DB`; but also accepts `SpliceRack`.
`u12DonorBegin`, `u12DonorEnd`	Integer values. They correspond to the begin and end point of the donor sequences of U12-type introns to consider (optional).
`u12BranchpointBegin`, `u12BranchpointEnd`	Integer values. Begin and end points of the branch point sequences of U12-type introns (optional).
`u12AcceptorBegin`, `u12AcceptorEnd`	Integer values. Begin and end points of the acceptor sequences of U12-type introns (optional).
`u2DonorBegin`, `u2DonorEnd`	Integer values. Begin and end points of the donor sequences of U2-type introns (optional).
`u2AcceptorBegin`, `u2AcceptorEnd`	Integer values. Begin and end points of the acceptor sequences of U2-type introns (optional).
`pasteSites`	Logical. If TRUE the donor, branch point and acceptor seqs are pasted before a PWM is built; then the PWMs of each (donor, acceptor and bp) are assigned. If FALSE (default) the PWMs for each is built separately.

spliceRackSsLinks
: A list (or vector) that contains the SpliceRack URL links to the text files that contain Position Weigh Matrices of the splice sites of U12 and U2 introns. This parameter is used only when pwmSource="SpliceRack". You can get the links to PWM files from this URL (choose logo files with "File" links): http://katahdin.mssm.edu/splice/splice_matrix.cgi?database=spliceNew. The links should be defined in the following order: U12_AT_AC_donor, U12_AT_AC_branchpoint, U12_AT_AC_acceptor, U12_GT_AG_donor, U12_GT_AG_branchpoint, U12_GT_AG_acceptor, U2_GC_AG_donor, U2_GC_AG_acceptor, U2_GT_AG_donor, and U2_GT_AG_acceptor.

u12dbLink
: A character string containing the URL for downloading the zipped MySQL dump file of the U12DB. Used when pwmSource="U12DB".

u12dbDbName
: Name of the database copy of the U12DB that is build locally. Used when pwmSource="U12DB".

u12dbDropDb
: Drop (or remove) the local copy of the U12DB database at the end of the run. Used when pwmSource="U12DB".

pdfFileSeqLogos
: Path to PDF file containing the sequence logos of the results. By default it does not produce a file.

removeTempFiles
: Whether remove temporary files at the end of the run; accepts TRUE or FALSE values (default is TRUE).

...
: Authorization arguments needed by the DBMS instance. See the manual for dbConnect of the DBI package for more info.

Value

pwmDonorU12
: Matrix (with 4 rows represnting A, C, G, T and n columns representing the genomic coordinates) representing the Position Weight Matrix of donor site of U12-type introns.

pwmBpU12
: Position Weight Matrix of branchpoint of U12-type introns.

pwmAccU12
: Position Weight Matrix of acceptor site of U12-type introns.

pwmDonU2
: Position Weight Matrix of donor site of U2-type introns.

pwmAccU2
: Position Weight Matrix of acceptor site of U2-type introns.

Author(s)

Ali Oghabian

See Also

annotateU12.

Examples

```
# Time demanding function
## Not run:
#Build temp directory
```

```
tmpDir<- tempdir()


# Creating subdirectory for storing u12db temp files
dir.create(paste(tmpDir, "u12dbTmp", sep="/"))

# Extracting PWMs of Splice Sites of U12 and U2 type introns -
# based on u12db
u12dbPwm<-buildSsTypePwms(
 tmpDir=paste(tmpDir, "u12dbTmp", sep="/"),
 u12dbSpecies="Homo_sapiens",
 resource="U12DB",
 u12dbDbName="u12db",
 u12dbDropDb=TRUE,
 removeTempFiles=TRUE)


# Creating subdirectory for storing SpliceRack temp files
dir.create(paste(tmpDir, "splicerackTmp", sep="/"))

# Extracting PWMs of Splice Sites of U12 and U2 type introns -
# based on SpliceRack
spliceRackPwm<- buildSsTypePwms(
 tmpDir= paste(tmpDir, "splicerackTmp", sep="/"),
 resource="SpliceRack",
 removeTempFiles=TRUE)

## End(Not run)
```

`counts-method` *Counts - method*

Description

Returns the (row) number of reads that are mapped to introns/exons in various samples.

Usage

```
## S4 method for signature 'SummarizedExperiment'
counts(object)
```

Arguments

`object`	Object of type `SummarizedExperiment`.

Value

Returns a numeric matrix.

Author(s)

Ali Oghabian

See Also

Class: SummarizedExperiment-class

Method: plot-method.

Examples

```
#Show contents of a InterestResults object included in IntEREst
head(counts(mdsChr22Obj))

#Make a test InterestResults object
geneId<- paste("gene", c(rep(1,5), rep(2,5), rep(3,5), rep(4,5)),
 sep="_")
readCnt1<- sample(1:100, 20)
readCnt2<- sample(1:100, 20)
readCnt3<- sample(1:100, 20)
readCnt4<- sample(1:100, 20)
fpkm1<- readCnt1/(tapply(readCnt1, geneId, sum))[geneId]
fpkm2<- readCnt2/(tapply(readCnt2, geneId, sum))[geneId]
fpkm3<- readCnt3/(tapply(readCnt3, geneId, sum))[geneId]
fpkm4<- readCnt4/(tapply(readCnt4, geneId, sum))[geneId]

# Creating object using test data
interestDat<- data.frame(
  int_ex=rep(c(rep(c("exon","intron"),2),"exon"),4),
  int_ex_num= rep(c(1,1,2,2,3),4),
  gene_id= geneId,
  sam1_readCnt=readCnt1,
  sam2_readCnt=readCnt2,
  sam3_readCnt=readCnt3,
  sam4_readCnt=readCnt4,
  sam1_fpkm=fpkm1,
  sam2_fpkm=fpkm2,
  sam3_fpkm=fpkm3,
  sam4_fpkm=fpkm4
)
readFreqColIndex<- grep("_readCnt$",colnames(interestDat))
scaledRetentionColIndex<- grep("_fpkm$",colnames(interestDat))

scalRetTmp<- as.matrix(interestDat[ ,scaledRetentionColIndex])
colnames(scalRetTmp)<-gsub("_fpkm$","", colnames(scalRetTmp))

frqTmp<- as.matrix(interestDat[ ,readFreqColIndex])
colnames(frqTmp)<-gsub("_readCnt$","", colnames(frqTmp))


InterestResultObj<- InterestResult(
 resultFiles=paste("file",1:4, sep="_"),
 rowData= interestDat[ , -c(readFreqColIndex,
```

```
 scaledRetentionColIndex)],
counts= frqTmp,
scaledRetention= scalRetTmp,
scaleLength=TRUE,
scaleFragment=FALSE,
sampleAnnotation=data.frame(
 sampleName=paste("sam",1:4, sep=""),
 gender=c("M","M","F","F"),
 health=c("healthy","unhealthy","healthy","unhealthy")
 , row.names=paste("sam", 1:4, sep="")
)
)

#Show
head(counts(InterestResultObj))
```

`deseqInterest` *DESeq2 analysis for IntEREst object*

Description

Differential intron retention test adapted from the `DESeq2` package.

Usage

```
deseqInterest (x, design, pAdjustMethod = "BH",
 sizeFactor=c(), contrast, bpparam, ...)
```

Arguments

`x`	Object of type `SummarizedExperiment`.
`design`	Formula specifying the design of the experiment. It must specify an interaction term between variables from column names of `sampleData(x)`.
`pAdjustMethod`	What adjustment method to be sed on the p-values. See p.adjust for more information.
`sizeFactor`	Numeric vector with the same size as the clolumn size of the count matrix in `x`, if defined it will be used for scaling of the count matrix.
`contrast`	Argument speciftying the comparison to extract from `x`. See `results` function in the `DESeq2` package for more information.
`bpparam`	An optional `BiocParallelParam` instance defining the parallel back-end to be used. If not defined the function will run sequentially (on a single computing core).
`...`	Other parameter settings for the results function in the `DESeq2` package.

Value

a `DESeqResults` object.

Author(s)

Ali Oghabian

See Also

exactTestInterest qlfInterest, treatInterest DEXSeqIntEREst

Examples

```
mdsChr22IntObj<- mdsChr22Obj[rowData(mdsChr22Obj)$int_ex=="intron",]
deseqRes<- deseqInterest(x=mdsChr22IntObj,
 design=~test_ctrl, contrast=list("test_ctrl_test_vs_ctrl"))

# Number of U12/U2 type significantly differential retained introns in chr22
table(rowData(mdsChr22Obj)[which(deseqRes$padj<.01), "intron_type"])
```

DEXSeqIntEREst *DEXSeq test for IntEREst object*

Description

Genewise differential exon usage or intron retention test adapted from the DEXSeq package.

Usage

```
DEXSeqIntEREst (x, design, reducedModel = ~ sample + intex, fitExpToVar,
intExCol, geneIdCol, bpparam, silent=TRUE,...)
```

Arguments

x	Object of type SummarizedExperiment.
design	Formula specifying the design of the experiment. It must specify an interaction term between a variable from columns of sampleData(x) with one of the 'exon', 'intron' or 'intex' (i.e. intron and exon) variables; based on which of these variables are used (exon, intron , or 'intex') the x will be filtered reletively to include exons, introns , or introns and exons. See DEXSeqDataSet for more information.
reducedModel	The null model formula. By default it is '~ sample + intex'.
fitExpToVar	A variable name contained in the column data (i.e. column names of colData(x)). See DEXSeq for more information.
intExCol	Column name (or number) that represents whether each row is "intron" or "exon" in rowData of x.
geneIdCol	Column name (or number of column) in rowData of x, i.e. SummarizedExperiment object, that represents the gene ID of the introns and exons in x.
bpparam	An optional BiocParallelParam instance defining the parallel back-end to be used.

silent	Whether run the DEXSeq function silently (if TRUE) or allow it to print messages at each step (if FALSE).
...	Other parameter settings for the DEXSeqDataSet function in the DEXSeq package.

Details

The design and reduceModel accept formula that specify the design of the experiment. The formula must describe an interaction between variables from columns of sampleData(x) with one of the 'exon', 'intron' or 'intex' (i.e. intron and exon) variables; Based on which of these variables are used (exon, intron , or 'intex') the input object (x) will be filtered reletively to include exons, introns , or introns and exons. Hence the number of the rows of the returned value is equal to the number of the rows of the filtered object, i.e. the number of the exons, introns or both based on the design formula.

Value

A DEXSeqResults object.

Author(s)

Ali Oghabian

See Also

exactTestInterest

Examples

```
dexseqExRes<-DEXSeqIntEREst (x=mdsChr22ExObj,
 design= ~ sample + exon + test_ctrl:exon,
 reducedModel = ~ sample + exon, fitExpToVar="test_ctrl",
 intExCol="int_ex", geneIdCol="transcripts_id", silent=TRUE)
head(dexseqExRes)
```

---

exactTestInterest *Exact test*

Description

Compute genewise exact test between two groups of read counts, using the edgeR package.

Usage

```
exactTestInterest(x, sampleAnnoCol=c(), sampleAnnotation=c(),
 geneIdCol, silent=TRUE, group=c(), rejection.region="doubletail",
 big.count=900, prior.count=0.125, disp="common", ...)
```

Arguments

x	Object of type SummarizedExperiment.
sampleAnnoCol	Which columnn of colData of x to consider for the analysis.
sampleAnnotation	A vector of size 2 which cotains values from colData of SummarizedExperiment object; e.g. if getAnnotation(x)[,sampleAnnoCol]= c("test", "test", "ctrl","ctrl", ...) , and the goal is to compare "test" and "ctrl" samples, sampleAnnotation should either be c("test", "ctrl") or c("ctrl", "test").
geneIdCol	Column name (or number of column) in rowData of x, i.e. SummarizedExperiment object, that represents the gene ID of the introns and exons in x.
silent	Whether run the function silently, i.e. without printing the top differential expression tags.
group	Vector to manually define the sample groups (or annotations). It is ignored if sampleAnnopCol is defined.
rejection.region	The rejection.region parameter in exactTest from edgeR package.
big.count	The big.count parameter in exactTest from edgeR package.
prior.count	The prior.count parameter in exactTest from edgeR package.
disp	The type of estimating the dispersion in the data. Available options are: "tagwise", "trended", "common" and "genewise". It is also possible to assign a number for manually setting the disp.
...	Other parameter settings for the estimateDisp function (e.g. the design parameter) in the edgeR package.

Value

table	Data frame containing columns for the log2 fold-change (logFC), the average of log2 counts-per-million (logCPM), and the two-sided p-value (PValue).
comparison	The name of the two compared groups.
dispersionType	The name of the type of dispersion used.
dispersion	The estimated dispersion values.

Author(s)

Ali Oghabian

See Also

lfc, glmInterest, qlfInterest, treatInterest, DEXSeqIntEREst

Examples

```
geneId<- paste("gene", c(rep(1,5), rep(2,5), rep(3,5), rep(4,5)),
 sep="_")
readCnt1<- sample(1:100, 20)
readCnt2<- sample(1:100, 20)
readCnt3<- sample(1:100, 20)
readCnt4<- sample(1:100, 20)
fpkm1<- readCnt1/(tapply(readCnt1, geneId, sum))[geneId]
fpkm2<- readCnt2/(tapply(readCnt2, geneId, sum))[geneId]
fpkm3<- readCnt3/(tapply(readCnt3, geneId, sum))[geneId]
fpkm4<- readCnt4/(tapply(readCnt4, geneId, sum))[geneId]

# Creating object using test data
interestDat<- data.frame(
  int_ex=rep(c(rep(c("exon","intron"),2),"exon"),4),
  int_ex_num= rep(c(1,1,2,2,3),4),
  gene_id= geneId,
  sam1_readCnt=readCnt1,
  sam2_readCnt=readCnt2,
  sam3_readCnt=readCnt3,
  sam4_readCnt=readCnt4,
  sam1_fpkm=fpkm1,
  sam2_fpkm=fpkm2,
  sam3_fpkm=fpkm3,
  sam4_fpkm=fpkm4
)
readFreqColIndex<- grep("_readCnt$",colnames(interestDat))
scaledRetentionColIndex<- grep("_fpkm$",colnames(interestDat))

scalRetTmp<- as.matrix(interestDat[ ,scaledRetentionColIndex])
colnames(scalRetTmp)<-gsub("_fpkm$","", colnames(scalRetTmp))

frqTmp<- as.matrix(interestDat[ ,readFreqColIndex])
colnames(frqTmp)<-gsub("_readCnt$","", colnames(frqTmp))


InterestResultObj<- InterestResult(
 resultFiles=paste("file",1:4, sep="_"),
 rowData= interestDat[ , -c(readFreqColIndex,
  scaledRetentionColIndex)],
 counts= frqTmp,
 scaledRetention= scalRetTmp,
 scaleLength=TRUE,
 scaleFragment=FALSE,
 sampleAnnotation=data.frame(
  sampleName=paste("sam",1:4, sep=""),
  gender=c("M","M","F","F"), row.names=paste("sam", 1:4, sep="")
 )
)

res<- exactTestInterest(InterestResultObj, sampleAnnoCol="gender",
```

```
sampleAnnotation=c("F","M"), geneIdCol= "gene_id",
silent=TRUE, disp="common")
```

`getRepeatTable` *Get table of regions with repetetive DNA sequences*

Description

This function returns a data.frame that includes regions with repetetive DNA sequences. These sequences can bias the mapping of the reads to the genome excluding them will remove the bias.

Usage

```
getRepeatTable( dbUser="genome",
 dbHost="genome-mysql.cse.ucsc.edu", ucscGenome="hg19",
 ucscTable="rmsk", minLength=0, repFamilyFil="Alu",
 repFamilyCol="repFamily", repChrCol="genoName",
 repBegCol="genoStart", repEndCol="genoEnd",
 repStrandCol="strand", repNameCol="repName",
 repClassCol="repClass")
```

Arguments

`dbUser`	Database user name; set as "genome" by default.
`dbHost`	Database host address; set as "genome-mysql.cse.ucsc.edu" by default.
`ucscGenome`	The UCSC genome.
`ucscTable`	The UCSC table name. The table with repetetive sequences by default it is set as `"rmsk"`.
`minLength`	the minimum length criteria to consider the repetetive sequences. the default setting is `0`.
`repFamilyFil`	A vector including the repeats family to consider. By default the `"Alu"` elements are considered.
`repFamilyCol`	The name of the column of the input table (`ucscTable`) that represents the repeats family.
`repChrCol`	The column (either name or the number of the column) of the input table that represents the Chromosome names.
`repBegCol`	The column of the table that represents the start coordinates.
`repEndCol`	The column of the table that represents the end coordinates.
`repStrandCol`	The column of the table that represents the strand.
`repNameCol`	The column of the table representing the repeats' names.
`repClassCol`	The column of the table representing the repeats' classes.

Value

Data frame with columns representing coordinates and annotations of repetitive DNA elements.

Author(s)

Ali Oghabian

Examples

```
## Not run:
# Download table for Alu elemnts in the human genome
suppressWarnings(repTable<- getRepeatTable(repFamilyFil="Alu",
 ucscGenome="hg19"))

## End(Not run)
```

`glmInterest` *generalized linear model likelihood ratio tests*

Description

Compute generalized linear model likelihood ratio tests using `edgeR` package. For more information see `glmfit` and `glmLRT()` functions in `edgeR` package.

Usage

```
glmInterest(x, design=c(), silent=TRUE, disp="common",
 coef=c(), contrast=NULL, ...)
```

Arguments

`x`	Object of type `SummarizedExperiment`.
`design`	Design matrix.
`silent`	Whether run the function silently, i.e. without printing the top differential expression tags. Default is TRUE.
`disp`	The method of estimating the dispersion in the data. Available options are: "common", "trended", "tagwiseInitCommon" and "tagwiseInitTrended". It is also possible to assign a number.
`coef`	Integer or character vector indicating which coefficients of the linear model are to be tested equal to zero. See `glmLRT()` in `edgeR` for more information.
`contrast`	Numeric vector or matrix specifying contrasts of the linear model coefficients to be tested equal to zero. See `glmLRT()` in `edgeR` for more information.
`...`	Other parameter settings for the `glmLRT()` function in the `edgeR` package.

Value

All values produced by `glmLRT` in `edgeR` package plus following:

`dispersionType`	The name of the type of dispersion used.
`dispersion`	The estimated dispersion values.

Author(s)

Ali Oghabian

See Also

`exactTestInterest`, `qlfInterest`, `treatInterest`

Examples

```
#Test retention differentiation across the 3 types of sampels
group <- getAnnotation(mdsChr22Obj)[,"type"]
glmRes<- glmInterest(x=mdsChr22Obj,
 design=model.matrix(~group), silent=TRUE,
 disp="tagwiseInitTrended", coef=2:3, contrast=NULL)
```

---

interest *Wrapper function: Parallel run*

Description

A read summarization function that countsns all the reads mapping to the introns/exons based on the users detailed parameter settings. The process can be run in parallel on multiple computing cores to improve it performance.

Usage

```
interest( bamFileYieldSize=1000000, bamFile, isPaired,
 isPairedDuplicate=FALSE, isSingleReadDuplicate= NA, reference,
 referenceGeneNames, referenceIntronExon, repeatsTableToFilter=c(),
 junctionReadsOnly=FALSE, outFile, logFile="",
 returnObj= FALSE, method=c("ExEx", "IntRet", "IntSpan", "ExSkip"),
 strandSpecific,
 bpparam, appendLogFile=FALSE, sampleName="",
 scaleLength= c(TRUE,FALSE), scaleFragment= c(TRUE,TRUE),
 limitRanges=GRanges(),
 excludeFusionReads=FALSE,
 loadLimitRangesReads=FALSE, ...)
```

Arguments

`bamFileYieldSize`
: Maximum number of pair reads in the temprorary files created as the result of dividing the input .bam file.

`bamFile`
: Path of the input bam file.

`isPaired`
: Whether the bam file is the result of a paired end sequencing read mapping (TRUE) or not (FALSE).

`isPairedDuplicate`
: Whether extract only (if set TRUE), filter (FALSE) or include (if set NA) PCR dupplicates for paired mapped reads. It uses the FLAG field in the bam file to filter the duplicate read. If the mapping software does not support detection and flaging the duplicate reads `dedup` tool of `BamUtil` or `MarkDuplicates` of `Picard` tools could be used.

`isSingleReadDuplicate`
: Whether extract only (if set TRUE), filter (FALSE) or include (if set NA) PCR dupplicates for single mapped reads.

`reference`
: Dataframe to be used as reference; It should at least contain three same-size vectors with the tag names `chr`, `begin`, and `end` which describe the exons and introns genome coordinates. It also accepts a `GRanges` object. To build a new reference check the `referencePrepare` function.

`referenceGeneNames`
: A vector with the same size as the row-size of the reference which includes the gene names of the reference.

`referenceIntronExon`
: A vector with the same size as the row-size of the reference with values "intron" and "exon" describing which (intron or exon) each row of the reference represents.

`repeatsTableToFilter`
: A data.frame table with similar stucture to the `reference`. It includes `chr`, `begin`, and `end` columns. If defined, all reads mapped to the described regions would be ingnored and the Intron/exon lengths would be corrected to exclude the to exclude the regions with repetitive DNA sequences. See `getRepeatTable`.

`junctionReadsOnly`
: The parameter is considered if the `method` is set as `IntRet` or `ExEx` (NOT `IntSpan`). It declares whether only consider the Intron-Exon or Exon-Exon junction reads and ignore the reads that fully map to exons or introns. By default this argument is set as FALSE.

`outFile`
: The name or path of the result file.

`logFile`
: The log file path; if defined log information are written to the log file.

`returnObj`
: If set `TRUE` in addition to making result text files, the results would also be returned as an object of class `SummarizedExperiment`.

`method`
: A vector describing the summarization methods to use; i.e. whether count reads mapping to the introns (`IntRet`), reads mapping to the exons (`ExEx`), reads spanning the introns (`IntSpan`), or reads that skip the exons (`ExSkip`). In `IntSpan` mode the introns in the reference are taken into account only; whilst in `IntRet`

the introns and their spanning exons, and in ExEx and ExSkip mode only the exons in the reference are taken into account.

strandSpecific The description for strand specificity of the RNAseq data. The values are either "unstranded", "stranded", or "reverse".If the reads are not strand specific or directional use "unstranded". If the first read in paired-read sequencing or the reads single-read sequencing is in the same direction as the the transcript strand use "stranded". If the first read in paired-read sequencing or the reads in single-read sequencing is in the oposite direction to the transcript strand use "reverse".

bpparam An optional BiocParallelParam instance defining the parallel back-end to be used.

appendLogFile Whether log information should be appended to the logFile. It is set FALSE by default.

sampleName The name of the sample being analyzed. It will be included in the returned object if returnObj is TRUE.

scaleLength A vector constructed of TRUE/FALSE values, same size as the method argument. It indcates whether the retention levels of the intron/exons should be scaled to their lengths.

scaleFragment A vector constructed of TRUE/FALSE values, same size as the method argument. It indcates whether the retention levels of the intron/exons should be scaled to the sum of retention levels (i.e. mapped fragments) over the genes.

limitRanges A GRanges object. If defined it loads sequencing reads that fall in the defined coordinates. It is similar to which parameter in ScanBamParam.

excludeFusionReads
Only valid if limitRanges is defined. It filters the defined by limitRanges. It also filters the read pairs if each read pair maps reads pairs where one of the reads either do not fall into one of the regions to a different region defined in limitRanges. It is useful to ignore analyzing the chimeric reads and fusion reads, i.e. reads that map to fusion genes. To filter properly, limitRanges must include coordinates of all genes.

loadLimitRangesReads
Boolean (TRUE/FALSE) variable. If set as TRUE only the reads in the limitRanges are loaded from bam file (and bamFileYieldSize parameter will be ignored).

... Other parameter settings specific to BamFile-class function in the Rsamtools package. Parameters qnamePrefixEnd and qnameSuffixStart are in particular useful to modify qnames in the BAM files.

Value

If returnObj is set TRUE in addition to making result text files, dependant on whether a single or two method is defined, the results would be returned as a single object of class SummarizedExperiment or as a list of size 2 which includes 2 objects of class SummarizedExperiment one for IntRet and the other for ExEx.

Author(s)

Ali Oghabian

See Also

interest.sequential.

Examples

```
# Creating temp directory to store the results
outDir<- file.path(tempdir(),"interestFolder")
dir.create(outDir)
outDir<- normalizePath(outDir)

# Loading suitable bam file
bamF <- system.file("extdata", "small_test_SRR1691637_ZRSR2Mut_RHBDD3.bam",
 package="IntEREst", mustWork=TRUE)

# Choosing reference for the gene RHBDD3
ref= u12[u12[,"gene_name"]=="RHBDD3",]

test= interest(
 bamFileYieldSize=10000,
 bamFile=bamF,
 isPaired=TRUE,
 isPairedDuplicate=FALSE,
 isSingleReadDuplicate=NA,
 reference=ref,
 referenceGeneNames=ref[,"ens_gene_id"],
 referenceIntronExon=ref[,"int_ex"],
 repeatsTableToFilter=c(),
 outFile=paste(outDir,
     "interestRes.tsv", sep="/"),
 logFile=paste(outDir,
     "log.txt", sep="/"),
 method=c("IntRet", "IntSpan"),
 strandSpecific="unstranded",
 junctionReadsOnly=FALSE,
 returnObj=TRUE,
 scaleLength= c(TRUE,FALSE),
 scaleFragment= c(TRUE,TRUE)
)

test
```

---

interest.sequential *Wrapup function: Sequential running*

Description

A read summarization function that countsns all the reads mapping to the introns/exons based on the users detailed parameter settings. The process runs on a single computing core.

Usage

```
interest.sequential( bamFileYieldSize=1000000, bamFile, isPaired,
 isPairedDuplicate=FALSE, isSingleReadDuplicate=NA,
 reference, referenceGeneNames,
 referenceIntronExon, repeatsTableToFilter=c(),
 junctionReadsOnly=FALSE, outFile, logFile="",
 returnObj= FALSE, method=c("ExEx", "IntRet", "IntSpan", "ExSkip"),
 strandSpecific, appendLogFile=FALSE, sampleName="",
 scaleLength= c(TRUE,FALSE), scaleFragment= c(TRUE,TRUE),
 limitRanges=GRanges(),
 excludeFusionReads=FALSE,
 loadLimitRangesReads=FALSE, ...)
```

Arguments

`bamFileYieldSize`
: Maximum number of paired Reads in the temprorary files created as the result of dividing the input .bam file.

`bamFile`
: Path of the input bam file.

`isPaired`
: Whether the bam file is the result of a paired end sequencing read mapping (TRUE) or not (FALSE).

`isPairedDuplicate`
: Whether extract only (if set TRUE), filter (FALSE) or include (if set NA) PCR dupplicates for paired mapped reads. It uses the FLAG field in the bam file to filter the duplicate read. If the mapping software does not support detection and flaging the duplicate reads `dedup` tool of `BamUtil` or `MarkDuplicates` of `Picard` tools could be used.

`isSingleReadDuplicate`
: Whether extract only (if set TRUE), filter (FALSE) or include (if set NA) PCR dupplicates for single mapped reads.

`reference`
: Dataframe to be used as reference; It should at least contain three same-size vectors with the tag names `chr`, `begin`, and `end` which describe the genome coordinates of the introns and exons. It also accepts a `GRanges` object as input. To build a new reference check the `referencePrepare` function.

`referenceGeneNames`
: A vector with the same size as the row-size of the reference which include the gene names.

`referenceIntronExon`
: A vector with the same size as the row-size of the reference with values "intron" and "exon" describing which (intron or exon) each row of the reference represents.

repeatsTableToFilter
: A data frame with similar structure as the reference, i.e. includes chr, begin, and end columns. If defined, all reads mapped to the described regions would be ingnored and the Intron/exon lengths would be corrected to exclude the regions with repetetive DNA sequences. See getRepeatTable.

junctionReadsOnly
: The parameter is considered if the method is set as IntRet or ExEx (NOT IntSpan). It declares whether only consider the Intron-Exon or Exon-Exon junction reads and ignore the reads that fully map to exons or introns. By default this argument is set as FALSE.

outFile
: The name or path of the result file.

logFile
: The log file path; if defined log information are written to the log file.

returnObj
: If set TRUE in addition to producing result text files, the results would also be returned as an object of class SummarizedExperiment.

method
: A vector describing the summarization methods to use; i.e. whether count reads mapping to the introns (IntRet), reads mapping to the exons (ExEx), reads spanning the introns (IntSpan), or reads that skip the exons (ExSkip). In IntSpan mode the introns in the reference are taken into account only; whilst in IntRet the introns and their spanning exons, and in ExEx and ExSkip mode only the exons in the reference are taken into account.

strandSpecific
: The description for strand specificity of the RNAseq data. The values are either "unstranded", "stranded", or "reverse".If the reads are not strand specific or directional use "unstranded". If the first read in paired-read sequencing or the reads single-read sequencing is in the same direction as the the transcript strand use "stranded". If the first read in paired-read sequencing or the reads in single-read sequencing is in the oposite direction to the transcript strand use "reverse".

appendLogFile
: Whether log information should be appended to the logFile. It is FALSE by default.

sampleName
: The name of the sample being analyzed. It will be included in the returned object if returnObj is TRUE.

scaleLength
: A vector constructed of TRUE/FALSE values, same size as the method argument. It indcates whether the retention levels of the intron/exons should be scaled to their lengths.

scaleFragment
: A vector constructed of TRUE/FALSE values, same size as the method argument. It indcates whether the retention levels of the intron/exons should be scaled to the sum of retention levels (i.e. mapped fragments) over the genes.

limitRanges
: A GRanges object. If defined it only loads sequencing read if they fall in the defined coordinates. It is similar to which parameter in ScanBamParam.

excludeFusionReads
: Only valid if limitRanges is defined. It filters the defined by limitRanges. It also filters the read pairs if each read pair maps reads pairs where one of the reads either do not fall into one of the regions to a different region defined in limitRanges. It is useful to ignore analyzing the chimeric reads and fusion reads, i.e. reads that map to fusion genes. To filter properly, limitRanges must include coordinates of all genes.

loadLimitRangesReads
: Boolean (TRUE/FALSE) variable. If set as TRUE only the reads in the limitRanges are loaded from bam file (and bamFileYieldSize parameter will be ignored).

...
: Other parameter settings specific to BamFile-class function in the Rsamtools package. Parameters qnamePrefixEnd and qnameSuffixStart are in particular useful to modify qnames in the BAM files.

Value

If returnObj is set TRUE in addition to making result text files, dependant on whether a single or two method is defined, the results would be returned as a single object of class SummarizedExperiment or as a list of size 2 which includes 2 objects of class SummarizedExperiment one for IntRet and the other for ExEx.

Author(s)

Ali Oghabian

See Also

interest.

Examples

```
# Creating temp directory to store the results
outDir<- file.path(tempdir(),"interestFolder")
dir.create(outDir)
outDir<- normalizePath(outDir)

# Loading suitable bam file
bamF <- system.file("extdata", "small_test_SRR1691637_ZRSR2Mut_RHBDD3.bam",
 package="IntEREst", mustWork=TRUE)


# Choosing reference for the gene RHBDD3
ref=u12[u12[,"gene_name"]=="RHBDD3",]

test= interest.sequential(
 bamFileYieldSize=10000,
 bamFile=bamF,
 isPaired=TRUE,
 isPairedDuplicate=FALSE,
 isSingleReadDuplicate=NA,
 reference=ref,
 referenceGeneNames=ref[,"ens_gene_id"],
 referenceIntronExon=ref[,"int_ex"],
 repeatsTableToFilter=c(),
 outFile=paste(outDir,
     "interestRes.tsv", sep="/"),
 logFile=paste(outDir,
```

```
    "log.txt", sep="/"),
 method=c("IntRet","IntSpan"),
 strandSpecific="unstranded",
 returnObj=TRUE,
 scaleLength= c(TRUE,FALSE),
 scaleFragment= c(TRUE,TRUE)
)

test
```

`InterestResult` *Building SummarizedExperiment object from results in IntEREst.*

Description

Calls the constructors and creates a `SummarizedExperiment` object. For more information on the resulted object and the class see `SummarizedExperiment-class`.

Usage

```
InterestResult(resultFiles=c(), counts, scaledRetention,
 scaleLength, scaleFragment, sampleAnnotation, rowData)
```

Arguments

`resultFiles`	Vector of link to the result files of `interest`.
`counts`	Numeric Matrix that includes the read counts.
`scaledRetention`	Matrix that includes the scaled retention values.
`scaleLength`	Logical value, indicating whether the intron/exon retention levels are scaled to the length of the introns/exons.
`scaleFragment`	Logical value, indicating whether the intron/exon retention levels are scaled to the fragments mapped to the genes.
`sampleAnnotation`	Data frame with the row-size equal to the size of `resultFiles` and `sampleAnnotation`. Each column of the matrix represents annotations for the samples. Column name represents annotation name.
`rowData`	Data frame with Intron/Exon annotations and read count and scaled retention values for each sample.

Value

Returns an object of class `SummarizedExperiment`.

Author(s)

Ali Oghabian

See Also

SummarizedExperiment-class attributes addAnnotation counts-method plot-method

Examples

```
geneId<- paste("gene", c(rep(1,5), rep(2,5), rep(3,5), rep(4,5)),
 sep="_")
readCnt1<- sample(1:100, 20)
readCnt2<- sample(1:100, 20)
readCnt3<- sample(1:100, 20)
readCnt4<- sample(1:100, 20)
fpkm1<- readCnt1/(tapply(readCnt1, geneId, sum))[geneId]
fpkm2<- readCnt2/(tapply(readCnt2, geneId, sum))[geneId]
fpkm3<- readCnt3/(tapply(readCnt3, geneId, sum))[geneId]
fpkm4<- readCnt4/(tapply(readCnt4, geneId, sum))[geneId]

# Creating object using test data
interestDat<- data.frame(
  int_ex=rep(c(rep(c("exon","intron"),2),"exon"),4),
  int_ex_num= rep(c(1,1,2,2,3),4),
  gene_id= geneId,
  sam1_readCnt=readCnt1,
  sam2_readCnt=readCnt2,
  sam3_readCnt=readCnt3,
  sam4_readCnt=readCnt4,
  sam1_fpkm=fpkm1,
  sam2_fpkm=fpkm2,
  sam3_fpkm=fpkm3,
  sam4_fpkm=fpkm4
)
readFreqColIndex<- grep("_readCnt$",colnames(interestDat))
scaledRetentionColIndex<- grep("_fpkm$",colnames(interestDat))

scalRetTmp<- as.matrix(interestDat[ ,scaledRetentionColIndex])
colnames(scalRetTmp)<-gsub("_fpkm$","", colnames(scalRetTmp))

frqTmp<- as.matrix(interestDat[ ,readFreqColIndex])
colnames(frqTmp)<-gsub("_readCnt$","", colnames(frqTmp))


InterestResultObj<- InterestResult(
 resultFiles=paste("file",1:4, sep="_"),
 rowData= interestDat[ , -c(readFreqColIndex,
  scaledRetentionColIndex)],
 counts= frqTmp,
 scaledRetention= scalRetTmp,
 scaleLength=TRUE,
 scaleFragment=FALSE,
 sampleAnnotation=data.frame(
  sampleName=paste("sam",1:4, sep=""),
  gender=c("M","M","F","F"), row.names=paste("sam", 1:4, sep=""))
```

```
 )
)

# View object
InterestResultObj
```

`interestResultIntEx` *Building results object that contains Intron-retention and exon-exon junction information*

Description

Building `SummarizedExperiment-class` object from an intron retention and an exon-exon junction results in IntEREst. The average of the junction levels are added to the SummerizedExperiment object of the intron retentions.

Usage

```
interestResultIntEx (intObj, exObj, intExCol=c(),
 mean.na.rm=TRUE, postExName="ex_junc" )
```

Arguments

`intObj`	A `SummarizedExperiment` including intron retention information.
`exObj`	A `SummarizedExperiment` including exon-exon junction information.
`intExCol`	Column name (or number) in the `rowData` of the intron object that represents whether each row of `x` assays is "intron" or "exon".
`mean.na.rm`	Whether exclude missing values when measuring the mean.
`postExName`	The postfix to use for the column names of the exons junction values in the

Value

Returns an object of class `SummarizedExperiment`.

Author(s)

Ali Oghabian

See Also

`SummarizedExperiment-class` `attributes` `addAnnotation` `counts-method` `plot-method`

Examples

```
testIntObj<- InterestResult(
  resultFiles= paste(paste("testFile",1:3, sep="_"),"bam", sep="."),
  counts= matrix(1:15, ncol=3, nrow=5, byrow=TRUE,
   dimnames= list(c(), paste("s", 1:3, sep="_"))),
  scaledRetention= matrix(1:15, ncol=3, nrow=5, byrow=TRUE,
   dimnames= list(c(), paste("s", 1:3, sep="_"))),
  scaleLength= FALSE,
  scaleFragment= FALSE,
  sampleAnnotation= data.frame(
   files=paste(paste("testFile",1:3, sep="_"),"bam", sep="."),
   names=paste("s", 1:3, sep="_"),
   row.names=paste("s", 1:3, sep="_")),
  rowData=data.frame(id= paste("i", 1:5, sep="_"),
   chr= rep("chr1", 5),
   begin=seq(100, by=100, length.out=5 ),
   end=seq(110, by=100, length.out=5 ),
   strand=rep("+",5))
 )

testExObj<- InterestResult(
  resultFiles= paste(paste("testFile",1:3, sep="_"),"bam", sep="."),
  counts= matrix(1:30, ncol=3, nrow=10, byrow=TRUE,
   dimnames= list(c(), paste("s", 1:3, sep="_"))),
  scaledRetention= matrix(1:30, ncol=3, nrow=10, byrow=TRUE,
   dimnames= list(c(), paste("s", 1:3, sep="_"))),
  scaleLength= FALSE,
  scaleFragment= FALSE,
  sampleAnnotation= data.frame(
   files=paste(paste("testFile",1:3, sep="_"),"bam", sep="."),
   names=paste("s", 1:3, sep="_"),
   row.names=paste("s", 1:3, sep="_")),
  rowData=data.frame(id= paste("e", 1:10, sep="_"),
   chr= rep("chr1", 10),
   begin= c(seq(90, by=100, length.out=5),
    seq(111, by=100, length.out=5)),
   end= c(seq(99, by=100, length.out=5),
    seq(120, by=100, length.out=5 )),
   strand=rep("+",10))
 )


(testIntExObj<- interestResultIntEx(intObj=testIntObj, exObj=testExObj,
 mean.na.rm=TRUE, postExName="ex_junc" ) )
```

`intexIndex` *Extract index of intron or exon rows*

Description

Extract row numbers where introns (or exons dependant on user's request) are located in an object of type `SummarizedExperiment`.

Usage

```
intexIndex(x, intExCol="int_ex", what="intron")
```

Arguments

`x`	Object of type `SummarizedExperiment`.
`intExCol`	Column name (or number) that represents whether each row is "intron" or "exon" in `rowData` of `x`.
`what`	A character string that defines whether the index for the introns or exons should be returned. Accepts either `"exon"` or `"intron"` (default) as values.

Value

A numeric vector which includes the index of the introns/exons.

Author(s)

Ali Oghabian

See Also

`u12NbIndex`

Examples

```
# Show the few first index of rows that represent the introns
head(intexIndex(mdsChr22Obj, what="intron"))
```

`lfc` *Log fold change*

Description

Log fold change estimation and normalized log fold change using `edgeR` package.

Usage

```
lfc(x, fcType="edgeR", sampleAnnoCol=c(), sampleAnnotation=c(),
 silent=TRUE, group=c(), rejection.region="doubletail",
 pseudoCnt=1, log2=TRUE, ...)
```

Arguments

x	Object of type SummarizedExperiment.
fcType	Available as "scaledRetention" or "edgeR" (as default) corresponding to either log fold change of scaled retention values or degeR normalized log fold change values.
sampleAnnoCol	Which columnn of colData of x to consider for the analysis.
sampleAnnotation	A vector of size 2 which cotains values from colData of SummarizedExperiment object; e.g. if getAnnotation(x)[,sampleAnnoCol]= c("test", "test", "ctrl","ctrl", ...) , and the goal is to compare "test" and "ctrl" samples, sampleAnnotation should either be c("test", "ctrl") or c("ctrl", "test").
silent	Whether run exactTestInterest silently, without warnings.
group	Vector to manually define the sample groups (or annotations). It is ignored if sampleAnnoCol is defined.
rejection.region	The rejection.region parameter in exactTest, considered only if fcType is "edgeR".
pseudoCnt	Pseudo count for log transformation (default=1).
log2	Logical value either TRUE (default) or FALSE indicating whether the fold-changes should be log 2 transformed.
...	Other parameter settings from the exactTestInterest function.

Value

Vector including fold change values.

Author(s)

Ali Oghabian

See Also

exactTestInterest, u12DensityPlotIntron

Examples

```
lfcFpkm<- lfc(mdsChr22Obj, fcType="scaledRetention",
 sampleAnnoCol="test_ctrl",
 sampleAnnotation=c("ctrl", "test"),
 silent=TRUE, group=c(), pseudoFpkm=1, log2=TRUE)


lfcEdgeRFpkm<- lfc(mdsChr22Obj, fcType="edgeR",
 sampleAnnoCol="test_ctrl",
 sampleAnnotation=c("ctrl", "test"),
 silent=TRUE, group=c(), pseudoFpkm=1, log2=TRUE)
```

mdsChr22ExObj *Object of SummarizedExperiment type for exon-exon junction of MDS data*

Description

The Results of `interest()` analysis in exon-exon junction mode, for the genes that feature U12-type introns and are located on Chr22 in MDS data.

Usage

```
data(mdsChr22ExObj)
```

Format

An Object of class `SummarizedExperiment` that contains intron retention results generated by `interest()` function on MDS data consisting of bone-marrows samples of 8 MDS patients with ZRSR2 mutations, 4 patients without the mutation and 4 healthy individuals.

`@colData` A "DataFrame" (from "S4Vectors" package) that its rownames can be set as the sample identification names and the other columns are various annotations for the samples. Its column names are characters that describe the annotations.

`@assays` List of size 2 that includes two numeric matrices: `counts` that includes raw read counts of the sequencing reads mapped to introns and exons, and (2) `scaledRetention`, i.e. the normalized read counts.

`@NAMES` A NULL value.

`@elementMetadata` A "DataFrame" (from "S4Vectors" package) that include intron and exon annotations.

`@metadata` A list of size 2 that includes parameter settings for the `interest()` and `interest.sequential()` runs.

Value

Object of class `SummarizedExperiment`.

Source

Madan, V., et.al., Aberrant splicing of U12-type introns is the hallmark of ZRSR2 mutant myelodysplastic syndrome. Nat Communication 2015 Jan 14;6:6042. doi: 10.1038/ncomms7042.

mdsChr22IntSpObj *Object of SummarizedExperiment type for intron spanning reads of MDS data*

Description

The Results of `interest()` analysis in intron-spanning mode, for the genes that feature U12-type introns and are located on Chr22 in MDS data.

Usage

```
data(mdsChr22ExObj)
```

Format

An Object of class `SummarizedExperiment` that contains intron retention results generated by `interest()` function on MDS data consisting of bone-marrows samples of 8 MDS patients with ZRSR2 mutations, 4 patients without the mutation and 4 healthy individuals.

`@colData` A "DataFrame" (from "S4Vectors" package) that its rownames can be set as the sample identification names and the other columns are various annotations for the samples. Its column names are characters that describe the annotations.

`@assays` List of size 2 that includes two numeric matrices: `counts` that includes raw read counts of the sequencing reads mapped to introns and exons, and (2) `scaledRetention`, i.e. the normalized read counts.

`@NAMES` A NULL value.

`@elementMetadata` A "DataFrame" (from "S4Vectors" package) that include intron and exon annotations.

`@metadata` A list of size 2 that includes parameter settings for the `interest()` and `interest.sequential()` runs.

Value

Object of class `SummarizedExperiment`.

Source

Madan, V., et.al., Aberrant splicing of U12-type introns is the hallmark of ZRSR2 mutant myelodysplastic syndrome. Nat Communication 2015 Jan 14;6:6042. doi: 10.1038/ncomms7042.

mdsChr22Obj *Object of SummarizedExperiment type for intron retention MDS data*

Description

The Results of `interest()` analysis in Intron-retention mode, for the genes that feature U12-type introns and are located on Chr22 in MDS data.

Usage

```
data(mdsChr22Obj)
```

Format

An Object of class `SummarizedExperiment` that contains intron retention results generated by `interest()` function on MDS data consisting of bone-marrows samples of 8 MDS patients with ZRSR2 mutations, 4 patients without the mutation and 4 healthy individuals.

`@colData` A "DataFrame" (from "S4Vectors" package) that its rownames can be set as the sample identification names and the other columns are various annotations for the samples. Its column names are characters that describe the annotations.

`@assays` List of size 2 that includes two numeric matrices: `counts` that includes raw read counts of the sequencing reads mapped to introns and exons, and (2) `scaledRetention`, i.e. the normalized read counts.

`@NAMES` A NULL value.

`@elementMetadata` A "DataFrame" (from "S4Vectors" package) that include intron and exon annotations.

`@metadata` A list of size 2 that includes parameter settings for the `interest()` and `interest.sequential()` runs.

Value

Object of class `SummarizedExperiment`.

Source

Madan, V., et.al., Aberrant splicing of U12-type introns is the hallmark of ZRSR2 mutant myelodysplastic syndrome. Nat Communication 2015 Jan 14;6:6042. doi: 10.1038/ncomms7042.

mergeInterestResult *merge two SummarizedExperiment objects into one*

Description

Build a new object bu merging data of two SummarizedExperiment objects.

Usage

```
mergeInterestResult(x, y)
```

Arguments

x	Object of type SummarizedExperiment.
y	Object of type SummarizedExperiment.

Value

An object of calss SummarizedExperiment.

Author(s)

Ali Oghabian

See Also

interest, InterestResult.

Examples

```
geneId<- paste("gene", c(rep(1,5), rep(2,5), rep(3,5), rep(4,5)),
 sep="_")
readCnt1<- sample(1:100, 20)
readCnt2<- sample(1:100, 20)
readCnt3<- sample(1:100, 20)
readCnt4<- sample(1:100, 20)
fpkm1<- readCnt1/(tapply(readCnt1, geneId, sum))[geneId]
fpkm2<- readCnt2/(tapply(readCnt2, geneId, sum))[geneId]
fpkm3<- readCnt3/(tapply(readCnt3, geneId, sum))[geneId]
fpkm4<- readCnt4/(tapply(readCnt4, geneId, sum))[geneId]

# Creating object using test data
interestDat<- data.frame(
  int_ex=rep(c(rep(c("exon","intron"),2),"exon"),4),
  int_ex_num= rep(c(1,1,2,2,3),4),
  gene_id= geneId,
  sam1_readCnt=readCnt1,
  sam2_readCnt=readCnt2,
```

```
  sam3_readCnt=readCnt3,
  sam4_readCnt=readCnt4,
  sam1_fpkm=fpkm1,
  sam2_fpkm=fpkm2,
  sam3_fpkm=fpkm3,
  sam4_fpkm=fpkm4
)
readFreqColIndex<- grep("_readCnt$",colnames(interestDat))
scaledRetentionColIndex<- grep("_fpkm$",colnames(interestDat))

scalRetTmp<- as.matrix(interestDat[ ,scaledRetentionColIndex])
colnames(scalRetTmp)<-gsub("_fpkm$","", colnames(scalRetTmp))

frqTmp<- as.matrix(interestDat[ ,readFreqColIndex])
colnames(frqTmp)<-gsub("_readCnt$","", colnames(frqTmp))

#Object including data for Males
interestResObjM<-InterestResult(
 resultFiles=paste("file",1:2, sep="_"),
 rowData= interestDat[, -c(readFreqColIndex,
  scaledRetentionColIndex)],
 counts= frqTmp[,1:2],
 scaledRetention= scalRetTmp[,1:2],
 scaleLength=TRUE,
 scaleFragment=FALSE,
 sampleAnnotation=data.frame(
  sampleName=paste("sam",1:2, sep=""),
  gender=c("M","M"),
  health=c("healthy","unhealthy"),
  row.names=paste("sam", 1:2, sep="")
 )
)

#Object including data for Females
interestResObjF<-InterestResult(
 resultFiles=paste("file",3:4, sep="_"),
 rowData= interestDat[, -c(readFreqColIndex,
  scaledRetentionColIndex)],
 counts= frqTmp[,3:4],
 scaledRetention= scalRetTmp[,3:4],
 scaleLength=TRUE,
 scaleFragment=FALSE,
 sampleAnnotation=data.frame(
  sampleName=paste("sam",3:4, sep=""),
  gender=c("F","F"),
  health=c("healthy","unhealthy"),
  row.names=paste("sam", 3:4, sep="")
 )
)

#Build new object
newObj<- mergeInterestResult(interestResObjM, interestResObjF)
```

```
#View newObj
print(newObj)
```

---

plot-method *plot - method*

Description

plot method for SummarizedExperiment objects.

Usage

```
## S4 method for signature 'SummarizedExperiment,ANY'
plot(x, summary="none",
 subsetRows=NULL, what="scaled", intronExon="intron",
 logScaleBase=NULL, logPseudoCnt=1, plotLoess=TRUE,
 loessCol="red", loessLwd=1, loessLty=1, cexText=1,
 marPlot=c(2,2,2,2), mgpPlot=c(1, 1, 0), cexAxis=1,
 writeCor=TRUE, corCex=1, corMethod="pearson", corCol="grey63",
 upperCorXY=c("topleft", NULL), lowerCorXY=c("topleft", NULL),
 na.rm=TRUE, cex=1, sampleAnnoCol=c(), lowerPlot=FALSE,
 upperPlot=TRUE, ...)
```

Arguments

x	Object of type SummarizedExperiment generated by either interest(), interest.sequential() or readInterestResults().
summary	Whether to plot the mean or median of the values over the sample with the same annotations, or plot the values for each individual sample separately. The available options are "mean", "median", or "none".
subsetRows	Vector either constructed of TRUE/FALSE values or constructed of numeric values that could be used to choose rows of x i.e. the SummarizedExperiment object.
what	Whether plot "scaled" (default) or read counts ("counts").
intronExon	Whether plot intron retention, i.e. "intron" (default) or exon-junction "exon".
logScaleBase	Base of the log transform of the values, if defined. By default the value is NULL meaning that the values would not be log transformed.
logPseudoCnt	Pseudocount for the log transformation (default=1).
plotLoess	Whether fit and plot LOESS curve line (default="red").
loessCol	loess line colour (default="red").
loessLwd	loess line width (default=1).
loessLty	loess line type (default=1).
cexText	Size of the text for sample names or annotations (default=1).

marPlot	Plot margins (default=c(2,2,2,2)). See ?par for more information.
mgpPlot	Plotting mgp parameter (default=c(1, 1, 0)). See ?par for more information.
cexAxis	Size of the text for the axis (default=1).
writeCor	Write correlation values (default=TRUE).
corCex	Text size of correlation values (default=1).
corMethod	Method used for correlation calculation. For more information see cor from stats package of R.
corCol	Color of the text of correlation (default="grey").
upperCorXY	The coordinates of the correlation text in the upper panel plots (default= c("topleft", NULL)).
lowerCorXY	The coordinates of the correlation text in the lower panel plots (default= c("topleft", NULL)).
na.rm	whether remove the rows with missing values (default=TRUE).
cex	size of the plot text and symbols (default=1).
sampleAnnoCol	Which columnn of colData of object SummarizedExperiment to consider for plotting.
lowerPlot	Whether plot the lower panel (default=FALSE).
upperPlot	Whether plot the upper panel (default=TRUE).
...	Other arguments to pass to the plot() function.

Value

Returns NULL.

Author(s)

Ali Oghabian

See Also

Class: SummarizedExperiment-class Method: counts-method boxplot-method

Examples

```
geneId<- paste("gene", c(rep(1,5), rep(2,5), rep(3,5), rep(4,5)),
 sep="_")
readCnt1<- sample(1:100, 20)
readCnt2<- sample(1:100, 20)
readCnt3<- sample(1:100, 20)
readCnt4<- sample(1:100, 20)
fpkm1<- readCnt1/(tapply(readCnt1, geneId, sum))[geneId]
fpkm2<- readCnt2/(tapply(readCnt2, geneId, sum))[geneId]
fpkm3<- readCnt3/(tapply(readCnt3, geneId, sum))[geneId]
fpkm4<- readCnt4/(tapply(readCnt4, geneId, sum))[geneId]
```

```
# Creating object using test data
interestDat<- data.frame(
  int_ex=rep(c(rep(c("exon","intron"),2),"exon"),4),
  int_ex_num= rep(c(1,1,2,2,3),4),
  gene_id= geneId,
  sam1_readCnt=readCnt1,
  sam2_readCnt=readCnt2,
  sam3_readCnt=readCnt3,
  sam4_readCnt=readCnt4,
  sam1_fpkm=fpkm1,
  sam2_fpkm=fpkm2,
  sam3_fpkm=fpkm3,
  sam4_fpkm=fpkm4
)
readFreqColIndex<- grep("_readCnt$",colnames(interestDat))
scaledRetentionColIndex<- grep("_fpkm$",colnames(interestDat))

scalRetTmp<- as.matrix(interestDat[ ,scaledRetentionColIndex])
colnames(scalRetTmp)<-gsub("_fpkm$","", colnames(scalRetTmp))

frqTmp<- as.matrix(interestDat[ ,readFreqColIndex])
colnames(frqTmp)<-gsub("_readCnt$","", colnames(frqTmp))


InterestResultObj<- InterestResult(
 resultFiles=paste("file",1:4, sep="_"),
 rowData= interestDat[ , -c(readFreqColIndex,
  scaledRetentionColIndex)],
 counts= frqTmp,
 scaledRetention= scalRetTmp,
 scaleLength=TRUE,
 scaleFragment=FALSE,
 sampleAnnotation=data.frame(
  sampleName=paste("sam",1:4, sep=""),
  gender=c("M","M","F","F"), row.names=paste("sam", 1:4, sep="")
 )
)

InterestResultObj2<- addAnnotation(x=InterestResultObj,
 sampleAnnotationType="health",
 sampleAnnotation=c("healthy","unhealthy","healthy","unhealthy")
)

#Plotting
plot(InterestResultObj)
plot(InterestResultObj, sampleAnnoCol="gender", summary="mean")
plot(InterestResultObj2, sampleAnnoCol=3, summary="mean")
plot(InterestResultObj2, summary="none")
```

---

psi *Psi values estimation*

Description

Calculating the relative inclusion level of intron or Psi values base on two count matrices from a single or two separate objects. The values for each intron is in the range of [0,1], where 0 means complete splicing or no retention of the intron and 1 represnet complete 100

Usage

```
psi (x, y, intCol, exCol, pseudoCnt=0)
```

Arguments

x	Object of type SummarizedExperiment.
y	Optional; i.e. an object of type SummarizedExperiment.
intCol	Column numbers or column names in counts matrix of x which include the number of reads mapped to the introns.
exCol	Column numbers or column names in counts matrix of x (or if defined y) which include the number of reads spanning the introns (or mapping exons flanking the introns).
pseudoCnt	Pseudo counts to sum to the denominator of the devision to avoid devision to zero.

Value

data.frame with column size equal to the size of intCol parameter, and row size equal to the number of rows in x. It contains the psi values (i,e.values between 0 and 1 showing the fraction of spliced in transcripts).

Author(s)

Ali Oghabian

See Also

interestResultIntEx

Examples

```
mdsChr22IntObj<- mdsChr22Obj[which(rowData(mdsChr22Obj)$int_ex=="intron"), ]

#Build object including intron-retention and exon-junction results
mdsChr22RefIntExObj<- interestResultIntEx(intObj=mdsChr22Obj,
 exObj=mdsChr22ExObj, mean.na.rm=TRUE, postExName="ex_junc",
 intExCol="int_ex" )
# Calculate Psi
psiRes<- psi(mdsChr22RefIntExObj,
 intCol=which(colData(mdsChr22RefIntExObj)$intronExon=="intron"),
 exCol=which(colData(mdsChr22RefIntExObj)$intronExon=="exon"))
# show Psi results
head(psiRes)
```

pwmU12db *PWM of U12 and U2-type introns splice sites*

Description

PWM of U12 and U2-type introns splice sites and it is based on the U12DB database.

Usage

```
data("pwmU12db")
```

Format

A list that contains Position Weight Matrices (PWM) of donor site, branch point and acceptor site of U12-type introns and the PWMs of donor site and acceptor site of U2-type introns. It is based on the U12DB database.

`pwmDonU12` A position weigh matrix for the donor site of the U12-type introns, with 4 rows and 46 columns. The rows of the matrix represent "A", "C", "G", and "T" nucleotides and the columns represent the postions in the genome. Each position in the matrix include a weight (i.e. number between 0 and 1) which indicates how common the corresponding base (represented by the row of the matrix) is observed in the corerespoding position (represented by the colum of the matrix).

`pwmBpU12` A position weigh matrix for the branch point of the U12-type introns, with 4 rows and 9 columns.

`pwmAccU12` A position weigh matrix for the acceptor site of the U12-type introns, with 4 rows and 46 columns.

`pwmDonU2` A position weigh matrix for the donor site of the U2-type introns, with 4 rows and 25 columns.

`pwmAccU2` A position weigh matrix for the acceptor site of the U12-type introns, with 4 rows and 46 columns.

Value

List of 5 numeric matrices representing the PWMs of donor site of U12-type introns, branch point site of U12-type introns, acceptor site of U12-type introns, donor site of U2-type introns, and acceptor site of U2-type introns.

Source

Alioto, T.S. U12DB: a database of orthologous U12-type spliceosomal introns. Nucleic Acids Research 2006, doi: 10.1093/nar/gkl796

qlfInterest *quasi-likelihood F-test*

Description

Compute quasi-likelihood F-test using edgeR package. For more information see glmQLFit and glmQLFTest functions in edgeR package.

Usage

```
qlfInterest(x, design=c(), silent=TRUE, disp="common",
 coef=c(), contrast=NULL,
 poisson.bound=TRUE, ...)
```

Arguments

x	Object of type SummarizedExperiment.
design	Design matrix.
silent	Whether run silently, i.e. without printing the top differential expression tags. The default is TRUE.
disp	The method of estimating the dispersion in the data. Available options are: "common", "trended", "tagwiseInitCommon" and "tagwiseInitTrended". It is also possible to assign a number.
coef	Integer or character vector indicating which coefficients of the linear model are to be tested equal to zero. See glmQLFTest for more information.
contrast	Numeric vector or matrix specifying contrasts of the linear model coefficients to be tested equal to zero. See glmQLFTest for more information.
poisson.bound	Logical value, if TRUE (i.e. default) the pvalue would be higher than when obtained fom likelihood ratio test while Negative Binomial dispersion is zero.
...	Other parameter settings for the glmQLFTest function in the edgeR package.

Value

All values produced by glmQLFTest plus the following :

dispersionType	The name of the type of dispersion used.
dispersion	The estimated dispersion values.

Author(s)

Ali Oghabian

See Also

exactTestInterest, glmInterest, treatInterest

Examples

```
#Test retention differentiation across the 3 types of sampels
group <- getAnnotation(mdsChr22Obj)[,"type"]
qlfRes<- qlfInterest(x=mdsChr22Obj,
 design=model.matrix(~group), silent=TRUE,
 disp="tagwiseInitTrended", coef=2:3, contrast=NULL)

qlfRes
```

`readInterestResults` *Read interest/interest.sequential results text files*

Description

Reads one or multiple text file results generated by the `interest` or `interest.sequential` functions and builds an object of `SummarizedExperiment-class` class.

Usage

```
readInterestResults(resultFiles, sampleNames,
 sampleAnnotation, commonColumns, freqCol, scaledRetentionCol,
 scaleLength, scaleFragment, reScale=FALSE, geneIdCol,
 repeatsTableToFilter=c())
```

Arguments

`resultFiles`	Vector of character strings which includes the path to the tab-separated files resulted by the `interest` function.
`sampleNames`	Vector of character strings which includes the name of the samples. It should be the same size as the `resultFiles` parameter.
`sampleAnnotation`	Data frame with the same row number as the size of `resultFiles` and `sampleNames` parameter. The column names represent the annotation names and values in each column represent the annotations of the samples.
`commonColumns`	Columns in the result file which include intron/exon annotations and are common across all files defined in `resultFiles`.
`freqCol`	Column in the result file which include the read counts for introns/exons.
`scaledRetentionCol`	Column in the result file which include the scaled retention values for introns/exons.
`scaleLength`	Logical value, indicating whether the intron/exon retention levels are scaled to the length of the introns/exons. If `reScale` is `TRUE` the scaled retention levels would be rescalculated when reading the data.
`scaleFragment`	Logical value, indicating whether the intron/exon retention levels are scaled to the fragments mapped to the genes. If `reScale` is `TRUE` the scaled retention levels would be rescalculated when reading the data.

reScale — Logical value, indicating whether the scaled retention levels would be rescalculated when reading the data. By default it does not calculate and trusts the user to set the `scaleLength` and `scaleFragment` parameters correctly, i.e. as it was set in the `interest()` or `interest.sequential()` analysis.

geneIdCol — The number or name of the column in `resultFiles` which represents the gene/transcript names. It would be used for summing up the number of mapped fragments to the genes when scaling the retention levels. It is only used if `reScale` and `scaleFragment` arguments are set `TRUE`.

repeatsTableToFilter — A data.frame table with similar stucture to the `reference`. It includes `chr`, `begin`, and `end` columns. If defined, all reads mapped to the described regions would be ingnored and the Intron/exon lengths would be corrected to exclude the to exclude the regions with repetitive DNA sequences. See `getRepeatTable`. It is only used if `reScale` and `scaleLength` arguments are set `TRUE`.

Value

An object of calss `SummarizedExperiment-class`.

Author(s)

Ali Oghabian

See Also

`interest`, `InterestResult`.

Examples

```
geneId<- paste("gene", c(rep(1,7), rep(2,7), rep(3,7), rep(4,7)),
 sep="_")
readCnt1<- sample(1:100, 28)
readCnt2<- sample(1:100, 28)
readCnt3<- sample(1:100, 28)
readCnt4<- sample(1:100, 28)
fpkm1<- readCnt1/(tapply(readCnt1, geneId, sum))[geneId]
fpkm2<- readCnt2/(tapply(readCnt2, geneId, sum))[geneId]
fpkm3<- readCnt3/(tapply(readCnt3, geneId, sum))[geneId]
fpkm4<- readCnt4/(tapply(readCnt4, geneId, sum))[geneId]

#Create tmp director
tmpDir=file.path(tempdir(),"InterestResult")
dir.create(tmpDir)

# Build text files similar to files resulted by interest
dfTmp=data.frame(
  int_ex=rep(c(rep(c("exon","intron"),3),"exon"),4),
  int_ex_num= rep(c(1,1,2,2,3,3,4),4),
  int_type=rep(c(NA,"U2",NA,"U12",NA,"U2",NA),4),
  strand=rep("*",28),
```

```
  gene_id= geneId,
  sam1_readCnt=readCnt1,
  sam2_readCnt=readCnt2,
  sam3_readCnt=readCnt3,
  sam4_readCnt=readCnt4,
  sam1_fpkm=fpkm1,
  sam2_fpkm=fpkm2,
  sam3_fpkm=fpkm3,
  sam4_fpkm=fpkm4
)

writeDf<-function(df, file){
 write.table(df, file, col.names=TRUE,
  row.names=FALSE, quote=FALSE, sep='\t')
}

writeDf(dfTmp[, c(1:5,6,10)], paste(tmpDir, "df1.tsv", sep="/"))
writeDf(dfTmp[, c(1:5,7,11)], paste(tmpDir, "df2.tsv", sep="/"))
writeDf(dfTmp[, c(1:5,8,12)], paste(tmpDir, "df3.tsv", sep="/"))
writeDf(dfTmp[, c(1:5,9,13)], paste(tmpDir, "df4.tsv", sep="/"))

# Build object from generated text file results
testObj<-readInterestResults(
 resultFiles=paste(tmpDir,
  c("df1.tsv", "df2.tsv", "df3.tsv", "df4.tsv"), sep="/"),
 sampleNames=c("sam1","sam2","sam3","sam4"),
 sampleAnnotation= data.frame( gender=c("M","M","F","F"),
  health=c("healthy","unhealthy","healthy","unhealthy")),
 commonColumns=1:5, freqCol=6, scaledRetentionCol=7,
 scaleLength=FALSE, scaleFragment=TRUE, reScale=FALSE)

#View object
testObj
```

`referencePrepare` *Creates reference file*

Description

Creates reference file for IntEREst functions, e.g. `interest()`. The function uses functions of `biomaRt` library.

Usage

```
referencePrepare( outFileTranscriptsAnnotation="",
 annotateGeneIds=TRUE,
 u12IntronsChr=c(), u12IntronsBeg=c(), u12IntronsEnd=c(),
 u12IntronsRef, collapseExons=TRUE, sourceBuild="UCSC",
```

```
ucscGenome="hg19", ucscTableName="knownGene",
ucscUrl="http://genome-euro.ucsc.edu/cgi-bin/",
biomart="ENSEMBL_MART_ENSEMBL",
biomartDataset="hsapiens_gene_ensembl",
biomartTranscriptIds=NULL, biomartExtraFilters=NULL,
biomartIdPrefix="ensembl_", biomartHost="www.ensembl.org",
biomartPort=80, circSeqs="", taxonomyId=NA,
filePath="", fileFormat=c("auto", "gff3", "gtf"), fileDatSrc=NA,
fileOrganism=NA, fileChrInf=NULL,
fileDbXrefTag=c(), addCollapsedTranscripts=TRUE,
ignore.strand=FALSE )
```

Arguments

`outFileTranscriptsAnnotation`
: If defined outputs transcripts annotations.

`annotateGeneIds`
: Wether annotate and add the gene ids information.

`collapseExons`
: Whether collapse (i.e. reduce) the exonic regions. TRUE by default.

`sourceBuild`
: The source to use to build the reference data, `"UCSC"`, `"biomaRt"`, and `"file"` (for GFF3 or GTF files) are supported.

`ucscGenome`
: The genome to use. `"hg19"` is the default. See `genome` parameter of `makeTxDbFromUCSC` function of `txdbmaker` library for more information.

`ucscTableName`
: The UCSC table name to use. See `tablename` parameter of `makeTxDbFromUCSC` function of `txdbmaker` library for more information.

`ucscUrl`
: The UCSC URL address. See `url` parameter of `makeTxDbFromUCSC` function of `txdbmaker` library for more information.

`u12IntronsChr`
: A vector of character strings that includes chromsomal locations of the U12 type introns. If defined together with `u12IntronsBeg` and `u12IntronsBeg`, they would be used to annotate the U12-type introns.

`u12IntronsBeg`
: A vector of numbers that defines the begin (or start) coordinates of the u12-type introns.

`u12IntronsEnd`
: A vector of numbers that defines the end coordinates of the u12-type introns.

`u12IntronsRef`
: A GRanges object that includes the coordinates of the U12 type introns. If defined, it would be used to annotate the U12-type introns.

`biomart`
: BioMart database name. See `biomart` parameter of `makeTxDbFromBiomart` function of `txdbmaker` library for more information.

`biomartDataset`
: BioMart dataset name; default is "hsapiens_gene_ensembl". See `dataset` parameter of `makeTxDbFromBiomart` function of `txdbmaker` library for more information.

`biomartTranscriptIds`
: optional parameter to only retrieve transcript annotation results for a defined set of transcript ids. See `transcript_ids` parameter of `makeTxDbFromBiomart` function of `txdbmaker` library for more information.

biomartExtraFilters
: A list of names; i.e. additional filters to use in the BioMart query. See filters parameter of makeTxDbFromBiomart function of txdbmaker library for more information.

biomartIdPrefix
: A list of names; i.e. additional filters to use in the BioMart query. See id_prefix parameter of makeTxDbFromBiomart function of txdbmaker library for more information.

biomartHost
: Host to connect to; the default is "www.ensembl.org". For older versions of the GRCH you can provide the archive websites, e.g. for GRCH37 you can use "grch37.ensembl.org".

biomartPort
: The port to use in the HTTP communication with the host. Default is 80.

circSeqs
: A character vector that includes chromosomes that should be marked as circular. See circ_seqs parameter of makeTxDbFromBiomart and makeTxDbFromUCSC functions of txdbmaker library for more information.

taxonomyId
: This parameter can be used to provide taxonomy Ids. It is set to NA by default. You can check the taxonomy Ids with the available.species() function in GenomeInfoDb package. For more information see taxonomyId parameter of makeTxDbFromBiomart and makeTxDbFromUCSC functions of txdbmaker library.

filePath
: Character string i.e. the path to file. Used if sourceBuild is "file".

fileFormat
: The format of the input file. "auto", "gff3" and "gtf" is supported.

fileDatSrc
: Character string describing the source of the data file. Used if sourceBuild is "file".

fileOrganism
: The genus and species name of the organism. Used if sourceBuild is "file".

fileChrInf
: Dataframe that includes information about the chromosome. The first column represents the chromosome name and the second column is the length of the chromosome. Used if sourceBuild is "file".

fileDbXrefTag
: A vector of chracater strings which if defined it would be used as feature names. Used if sourceBuild is "file".

addCollapsedTranscripts
: Whether add a column that includes the collapsed transcripts information. Used if collapseExons is TRUE.

ignore.strand
: Whether consider the strands in the reference. If set TURE the strands would be ingnored.

Value

Data frame that includes the coordinates and annotations of the introns and exons of the transcripts, i.e. the reference.

Author(s)

Ali Oghabian

Examples

```
# Build test gff3 data
tmpGen<- u12[u12[,"ens_trans_id"]=="ENST00000413811",]
tmpEx<-tmpGen[tmpGen[,"int_ex"]=="exon",]
exonDat<- cbind(tmpEx[,3], ".",
 tmpEx[,c(7,4,5)], ".", tmpEx[,6], ".",paste("ID=exon",
 tmpEx[,11], "; Parent=ENST00000413811", sep="") )
trDat<- c(tmpEx[1,3], ".", "mRNA", as.numeric(min(tmpEx[,4])),
 as.numeric(max(tmpEx[,5])), ".", tmpEx[1,6], ".",
 "ID=ENST00000413811")

outDir<- file.path(tempdir(),"tmpFolder")
dir.create(outDir)
outDir<- normalizePath(outDir)

gff3File=paste(outDir, "gffFile.gff", sep="/")

cat("##gff-version 3\n",file=gff3File, append=FALSE)
cat(paste(paste(trDat, collapse="\t"),"\n", sep=""),
 file=gff3File, append=TRUE)

write.table(exonDat, gff3File,
 row.names=FALSE, col.names=FALSE,
 sep='\t', quote=FALSE, append=TRUE)

# Selecting U12 introns info from 'u12' data
u12Int<-u12[u12$int_ex=="intron"&u12$int_type=="U12",]

# Test the function
refseqRef<- referencePrepare (sourceBuild="file",
 filePath=gff3File, u12IntronsChr=u12Int[,"chr"],
 u12IntronsBeg=u12Int[,"begin"],
 u12IntronsEnd=u12Int[,"end"], collapseExons=TRUE,
 fileFormat="gff3", annotateGeneIds=FALSE)
```

`subInterestResult` *Extract subset of object*

Description

Build a new object using subset of data in an `SummarizedExperiment` object.

Usage

```
subInterestResult(x, selectRow, selectCol,
 sampleAnnoCol, sampleAnnotation=c())
```

Arguments

x	Object of type SummarizedExperiment.
selectRow	Numeric or TRUE/FALSE Vector indicating what rows to extract.
selectCol	A vector with Numeric values, character strings (sample names) or TRUE/FALSE Vector indicating what columns to extract.
sampleAnnoCol	Which columnn of colData of object x to consider for subset data extraction.
sampleAnnotation	Vector including the annotations to consider for subset data extraction. They should be present in the sampleAnnoCol column of the colData of x.

Value

An object of calss SummarizedExperiment.

Author(s)

Ali Oghabian

See Also

interest, InterestResult.

Examples

```
geneId<- paste("gene", c(rep(1,7), rep(2,7), rep(3,7), rep(4,7)),
 sep="_")
readCnt1<- sample(1:100, 28)
readCnt2<- sample(1:100, 28)
readCnt3<- sample(1:100, 28)
readCnt4<- sample(1:100, 28)
fpkm1<- readCnt1/(tapply(readCnt1, geneId, sum))[geneId]
fpkm2<- readCnt2/(tapply(readCnt2, geneId, sum))[geneId]
fpkm3<- readCnt3/(tapply(readCnt3, geneId, sum))[geneId]
fpkm4<- readCnt4/(tapply(readCnt4, geneId, sum))[geneId]

# Creating object using test data
interestDat<-data.frame(
  int_ex=rep(c(rep(c("exon","intron"),3),"exon"),4),
  int_ex_num= rep(c(1,1,2,2,3,3,4),4),
  int_type=rep(c(NA,"U2",NA,"U12",NA,"U2",NA),4),
  strand=rep("*",28),
  gene_id= geneId,
  sam1_readCnt=readCnt1,
  sam2_readCnt=readCnt2,
  sam3_readCnt=readCnt3,
  sam4_readCnt=readCnt4,
  sam1_fpkm=fpkm1,
  sam2_fpkm=fpkm2,
  sam3_fpkm=fpkm3,
  sam4_fpkm=fpkm4
```

```
)
readFreqColIndex<- grep("_readCnt$",colnames(interestDat))
scaledRetentionColIndex<- grep("_fpkm$",colnames(interestDat))
samNames<-paste("sam", 1:4, sep="")
frqTmp<-as.matrix(interestDat[, readFreqColIndex])
sclTmp<-as.matrix(interestDat[, scaledRetentionColIndex])
colnames(frqTmp)<- samNames
colnames(sclTmp)<- samNames
interestResObj<- InterestResult(
 resultFiles=paste("file",1:4, sep="_"),
 rowData= interestDat[, -c(readFreqColIndex,
  scaledRetentionColIndex)],
 counts= frqTmp,
 scaledRetention= sclTmp ,
 scaleLength=TRUE,
 scaleFragment=FALSE,
 sampleAnnotation=data.frame(
  sampleName=paste("sam",1:4, sep=""),
  gender=c("M","M","F","F"),
  health=c("healthy","unhealthy","healthy","unhealthy"),
  row.names=samNames
 )
)

#Build new object
newObj<- subInterestResult(interestResObj, selectRow=1:20)

#View newObj
print(newObj)
```

`treatInterest` *Differential retention test relative to a threshold*

Description

Compute a genewise statistical test relative to a fold-change threshold using edgeR package. For more information see `glmTreat` function in edgeR package.

Usage

```
treatInterest(x, design=c(), silent=TRUE, disp="common",
 coef=c(), contrast=NULL, lfc=0, ...)
```

Arguments

x	Object of class `SummarizedExperiment`.
design	Design matrix.

silent	Whether run silently, i.e. without printing the top differential expression tags. Default is TRUE.
disp	The method of estimating the dispersion in the data. Available options are: "common", "trended", "tagwiseInitCommon" and "tagwiseInitTrended". It is also possible to assign a number.
coef	Integer or character vector indicating which coefficients of the linear model are to be tested equal to zero. See glmTreat for more information.
contrast	Numeric vector or matrix specifying contrasts of the linear model coefficients to be tested equal to zero. See glmTreat for more information.
lfc	Numeric scalar i.e. the log fold change threshold.
...	Other parameter settings for the glmFit function in the edgeR package.

Value

All values produced by glmTreat plus the following :

dispersionType	The name of the type of dispersion used.
dispersion	The estimated dispersion values.

Author(s)

Ali Oghabian

See Also

exactTestInterest, qlfInterest, glmInterest

Examples

```
group <- getAnnotation(mdsChr22Obj)[,"type"]

#Test retention differentiation across the 3 types of sampels
# The log fold change threshold is 0
treatRes<- treatInterest(x=mdsChr22Obj,
 design=model.matrix(~group), silent=TRUE,
 disp="tagwiseInitTrended", coef=2:3, contrast=NULL, lfc=0)
treatRes
```

---

u12 *U12 data*

Description

Intron/exon annotations of genes featuring U12 introns. It is based on HG19/GRCh37 (converted from hg17/NCBI35). Moreover the u12 genes are based on the U12DB database.

Usage

```
data("u12")
```

Format

A data frame with 22713 observations on the following 17 variables.

`id` a numeric vector

`int_ex_id` a character vector

`chr` a character vector

`begin` a numeric vector

`end` a numeric vector

`strand` a numeric vector

`int_ex` a character vector

`trans_type` a character vector

`ens_gene_id` a character vector

`ens_trans_id` a character vector

`int_ex_num` a numeric vector

`gene_name` a character vector

`trans_name` a character vector

`overlap_no` a numeric vector

`int_type` a character vector

`int_subtype` a character vector

Value

Data frame that includes the coordinates and annotations of the introns and exons of the transcripts, i.e. the reference.

Source

Alioto, T.S. U12DB: a database of orthologous U12-type spliceosomal introns. Nucleic Acids Research 2006, doi: 10.1093/nar/gkl796

u12Boxplot *U12 boxplot*

Description

A boxplot method for U12 and U2-type introns of SummarizedExperiment objects.

Usage

```
u12Boxplot(x, sampleAnnoCol=NA, intExCol="int_ex",
 intTypeCol="int_type", intronExon, col="white",
 boxplotNames=c(), lasNames=3, outline=FALSE, addGrid=FALSE, ...)
```

Arguments

x	Object of type SummarizedExperiment.
sampleAnnoCol	Which columnn of colData in x to consider for plotting.
intExCol	Column name (or number) that represents whether each row of x assays is "intron" or "exon".
intTypeCol	Column name (or number) that represents what type of intron each row of x assays represents.
intronExon	Whether plot intron retention (set intronExon="intron") or exon-exon junction (set intronExon="exon") levels.
col	Vector showing box colours. It is either of size 1 or the same size as the number of groups to be plotted.
boxplotNames	Names to write under boxes. If not defined, as names, it pastes U12/U2 (intron annotation) to the sample group annotations separated by a space " ".
lasNames	Orientation of the box names.
outline	If outline is TRUE the outlier points are drawn otherwise if FALSE (default) they are not.
addGrid	Whether add a grid under the boxplots (FALSE by default).
...	Other arguments to pass to the boxplot() function.

Value

A SummarizedExperiment object.

Author(s)

Ali Oghabian

See Also

u12BoxplotNb

Examples

```
u12Boxplot(mdsChr22Obj, sampleAnnoCol="type",
    intExCol="int_ex",  intTypeCol="intron_type", intronExon="intron",
    col=rep(c("orange", "yellow"),3) ,  lasNames=3,
    outline=FALSE, ylab="FPKM", cex.axis=0.8)
```

`u12BoxplotNb` *boxplot U12 introns retention levels (or flanking exons junction levels) and (up/down)stream U2 introns (or exons junction levels)*

Description

boxplot U12 introns and (Up/Down)stream U2 introns in `SummarizedExperiment` objects.

Usage

```
u12BoxplotNb(x, sampleAnnoCol=2, intExCol="int_ex",
 intTypeCol="int_type", intronExon, strandCol="strand", geneIdCol,
 col=c(), names=c(), lasNames=1, outline=FALSE, plotLegend=TRUE,
 cexLegend=1, xLegend="topright", yLegend=NULL, bgLegend="transparent",
 legend=c(), addGrid=FALSE, ...)
```

Arguments

`x`	Object of type `SummarizedExperiment`.
`sampleAnnoCol`	Which columnn of `colData` of `x` to consider for plotting.
`intExCol`	Column name (or number) that represents whether each row of `x` assays is "intron" or "exon".
`intTypeCol`	Column name (or number) that represents what type of intron each row of `x` assays represents.
`intronExon`	Whether plot intron retention (set `intronExon="intron"`) or exon-exon junction (set `intronExon="exon"`) levels.
`strandCol`	Column name (or number) that represents the strand of each row of assays in `x`. The values in the column are either "+", "-" or "*".
`geneIdCol`	Column name (or number) that represents the gene ID of each row of assays in `x`.
`col`	Vector containing box colours. It is either of size 1 or the same size as the number of boxes resulted based on the grouping of the samples defined by `sampleAnnoCol`.
`names`	Names to write under group of boxes.
`lasNames`	Orientation of the box names.

outline	If outline is TRUE the outlier points are drawn otherwise if FALSE (default) they are not.
plotLegend	Whether show legend (TRUE by default).
cexLegend	Size of the text in legend .
xLegend, yLegend	Position of legend in the plot. For more info see x and y parameters in legend.
bgLegend	Bakcground colour of the legend box. It is "transparent" by default.
legend	The replacement texts to be used in legend.
addGrid	Whether add a grid under the boxplots (FALSE by default).
...	Other arguments to pass to the boxplot() function.

Value

Returns NULL

Author(s)

Ali Oghabian

See Also

u12Boxplot

Examples

```
u12BoxplotNb(mdsChr22Obj, sampleAnnoCol="type", lasNames=1,
    intExCol="int_ex", intTypeCol="intron_type", intronExon="intron",
    boxplotNames=c(), outline=FALSE, plotLegend=TRUE,
    geneIdCol="collapsed_transcripts_id", xLegend="topleft",
    col=c("pink", "lightblue", "lightyellow"), ylim=c(0,600000),
    ylab="FPKM", cex.axis=0.8)
```

---

u12DensityPlot — *Density plot of fld changes of intron retention and exon-exon junction levels*

Description

Density plot of fold change of the retention levels of U12- vs U2- type intron, or exon-exon junction levels of the flanking exons. For the density plot of the foldchange of intron retention levels the u12DensityPlotIntron() function or u12DensityPlot() function with intronExon= "intron" can be used. For density plot of the foldchange of exon-exon junction levels use u12DensityPlot() function with intronExon= "exon".

Usage

```
u12DensityPlot(x,
 type=c("U12", "U2Up", "U2Dn", "U2UpDn", "U2Rand"),
 fcType="edgeR", sampleAnnotation=c(),  sampleAnnoCol=c(),
 group=c(), intExCol="int_ex", intTypeCol="int_type", intronExon,
 strandCol="strand", geneIdCol="collapsed_transcripts",
 naUnstrand=FALSE, col=1, lty=1, lwd=1, plotLegend=TRUE,
 cexLegend=1, xLegend="topright", yLegend=NULL, legend=c(),
 randomSeed=NULL, xlab="", ...)

u12DensityPlotIntron(x,
 type= c("U12", "U2Up", "U2Dn", "U2UpDn", "U2Rand"),
 fcType= "edgeR", sampleAnnotation=c(),  sampleAnnoCol=c(),
 group=c(), intExCol="int_ex", intTypeCol="int_type",
 strandCol= "strand", geneIdCol= "collapsed_transcripts",
 naUnstrand=FALSE, col=1, lty=1, lwd=1, plotLegend=TRUE,
 cexLegend=1, xLegend="topright", yLegend=NULL, legend=c(),
 randomSeed=NULL, xlab="", ...)
```

Arguments

x	Object of type SummarizedExperiment.
type	A vector that includes the type of introns to plot. Available options are U12 introns "U12", U2 introns at downstream of U12 introns "U2Dn", U2 introns at upstream of U12 introns "U2Up", U2 introns at upstream or downstream of U12 introns suitable for when the coorduinates in object x are unstranded (their strand is "*") "U2UpDn", random U2 introns from object x "U2Rand". Settings "U2Up", "U2Dn" and "U2UpDn" are useful only if the refernce is linearly ordered. References with exons only resulted by referencePrepare and unionRefTr are NOT necessarily linearly ordered.
fcType	Available as "fpkm" or "edgeR" (as default) corresponding to either log fold change of fpkm values or degeR normalized log fold change values.
sampleAnnoCol	Which columnn of colData of x to consider for plotting.
sampleAnnotation	A vector of size 2 which cotains values from colData of SummarizedExperiment object; e.g. if getAnnotation(x)[,sampleAnnoCol]= c("test", "test", "ctrl","ctrl", ...) , and the goal is to compare "test" and "ctrl" samples, sampleAnnotation should either be c("test", "ctrl") or c("ctrl", "test").
group	Vector to manually define the sample groups (or annotations). It is ignored if sampleAnnoCol is defined.
intExCol	Column name (or number) that represents whether each row of x assays is "intron" or "exon".
intTypeCol	Column name (or number) that represents what type of intron each row of x assays represents.
intronExon	Whether plot intron retention (set intronExon="intron") or exon-exon junction (set intronExon="exon") levels.

strandCol	Column name (or number) that represents the strand of each row of assays in x. The values in the column are either "+", "-" or "*".
geneIdCol	Column name (or number) that represents the gene ID of each row of assays in x.
naUnstrand	Replace unstranded results, i.e. introns or exon with "*" strand, with NA (to be excluded).
col	A vector with the size of 1 or the same size as the type parameter which includes the colour/colours of the plotted density lines (default=1).
lty	A vector with the size of 1 or the same size as the type parameter which includes the type of the plotted density lines (default=1).
lwd	A vector with the size of 1 or the same size as the type parameter which includes the width of the plotted density lines (default=1).
plotLegend	Whether show legend (TRUE by default).
cexLegend	Size of the text in legend .
xLegend, yLegend	Position of legend in the plot. For more info see x and y parameters in legend.
legend	The replacement texts to be used in legend.
randomSeed	Seed value for random number generator.
xlab	The lable of the X axis of the plot; by default it is "".
...	Other parameter settings from the plot function.

Value

Returns NULL.

Author(s)

Ali Oghabian

See Also

exactTestInterest, lfc

Examples

```
u12DensityPlotIntron(mdsChr22Obj,
    type= c("U12", "U2Up", "U2Dn", "U2UpDn", "U2Rand"),
    fcType= "edgeR", sampleAnnoCol="test_ctrl",
    sampleAnnotation=c("ctrl","test"), intExCol="int_ex",
    intTypeCol="intron_type", strandCol= "strand",
    geneIdCol= "collapsed_transcripts_id", naUnstrand=FALSE, col=c(2,3,4,5,6),
    lty=c(1,2,3,4,5), lwd=1, plotLegend=TRUE, cexLegend=0.7,
    xLegend="topright", yLegend=NULL, legend=c(), randomSeed=10,
    ylim=c(0,0.6), xlab=expression("log"[2]*" fold change FPKM"))
```

u12Index *Extract index of U12 introns rows*

Description

Extract row numbers of U12 introns in an object of class SummarizedExperiment.

Usage

```
u12Index(x, intExCol="int_ex", intTypeCol="int_type", intronExon="intron")
```

Arguments

x	Object of type SummarizedExperiment.
intExCol	Column name (or number) that represents whether each row of x assays is "intron" or "exon".
intTypeCol	Column name (or number) that represents what type of intron each row of x assays represents.
intronExon	Whether extract U12 type introns (set intronExon="intron") or exon-exon junction (set intronExon="exon") flanking U12 introns.

Value

A numeric vector which includes the index of U12 introns.

Author(s)

Ali Oghabian

See Also

u12NbIndex

Examples

```
head(u12Index(mdsChr22Obj, intTypeCol="intron_type"))
```

u12NbIndex *Extract index of U2 introns (up/down)stream of U12 introns rows*

Description

Extract row numbers of U2-type introns (up/down)stream of U12-type introns (in the `@interestDf` attribute of an object of class `SummarizedExperiment`).

Usage

```
u12NbIndex(x, intExCol="int_ex", intTypeCol="int_type",
 strandCol="strand", geneIdCol="collapsed_transcripts",
 naUnstrand=FALSE)
```

Arguments

`x`	Object of type `SummarizedExperiment`.
`intExCol`	Column name (or number) that represents whether each row of `x` assays is "intron" or "exon".
`intTypeCol`	Column name (or number) that represents what type of intron each row of `x` assays represents.
`strandCol`	Column name (or number) that represents the strand of each row of assays in `x`. The values in the column are either "+", "-" or "*".
`geneIdCol`	Column name (or number) that represents the gene ID of each row of assays in `x`.
`naUnstrand`	Replace unstranded results, i.e. introns or exon with "*" strand, with NA. If set as FALSE (default) "*" strand would be same as "+" strand.

Value

`upIntron`	A numeric vector which includes the index of U2-type intron upstream the U12-type introns.
`downIntron`	A numeric vector which includes the index of U2-type intron downstream the U12-type introns.
`upExon`	A numeric vector which includes the index of exon upstream the U12-type introns.
`downExon`	A numeric vector which includes the index of exon downstream the U12-type introns.

Author(s)

Ali Oghabian

See Also

`u12Index`

Examples

```
head(u12NbIndex(mdsChr22Obj, intExCol="int_ex",
 intTypeCol="intron_type", strandCol="strand",
 geneIdCol="collapsed_transcripts_id", naUnstrand=FALSE))
# Return NA if no strand information available
head(u12NbIndex(mdsChr22Obj, intExCol="int_ex",
 intTypeCol="intron_type", strandCol="strand",
 geneIdCol="collapsed_transcripts_id", naUnstrand=TRUE))
```

---

unionRefTr *Union introns/exons of transcripts*

Description

Performs union on the overlapping introns/exons so that the final merged transcripts would feature from each exon or intron, one copy.

Usage

```
unionRefTr( referenceChr, referenceBegin, referenceEnd, referenceTr,
 referenceIntronExon, intronExon="exon", silent=FALSE)
```

Arguments

`referenceChr`	Chromosome names of the references (e.g. introns).
`referenceBegin`	A vector that corresponds to the begin coordinates of the reference.
`referenceEnd`	A vector that corresponds to the end coordinates of the reference.
`referenceTr`	A character vector that includes transcription IDs.
`referenceIntronExon`	A vector with the same size as the `referenceChr`, `referenceBegin` and `referenceEnd` which contains `'intron'` and `'exon'` describing what (either intron or exon) each element of the 3 vectors represents.
`intronExon`	Should be assigned either `'intron'` or `'exon'` or `c('intron','exon')` based on whether match the PWM to the intronic, exonic, or intronic and exonic regions of the reference. By default it seeks matches in intronic regions (intronExon='intron').
`silent`	Whether run silently.

Value

Data frame containing merged transcripts structure. The merged transcripts feature from each intron or exon, one copy ONLY.

Author(s)

Ali Oghabian

See Also

annotateU12.

Examples

```
unU12Ex<-unionRefTr( referenceChr=u12[1:94,"chr"],
 referenceBegin=u12[1:94,"begin"], referenceEnd=u12[1:94,"end"],
 referenceTr=u12[1:94,"trans_name"],
 referenceIntronExon=u12[1:94,"int_ex"], intronExon="exon", silent=TRUE)

unU12Int<-unionRefTr( referenceChr=u12[1:94,"chr"],
 referenceBegin=u12[1:94,"begin"], referenceEnd=u12[1:94,"end"],
 referenceTr=u12[1:94,"trans_name"],
 referenceIntronExon=u12[1:94,"int_ex"], intronExon="intron", silent=TRUE)

unU12IntEx<-unionRefTr( referenceChr=u12[1:94,"chr"],
 referenceBegin=u12[1:94,"begin"], referenceEnd=u12[1:94,"end"],
 referenceTr=u12[1:94,"trans_name"],
 referenceIntronExon=u12[1:94,"int_ex"], intronExon=c("intron","exon"),
 silent=TRUE)
```

---

updateRowDataCol *Updating contents of* rowData *of* SummarizedExperiment *objects*

Description

Updates the values in a single column of the rowData of SummarizedExperiment objects.

Usage

```
updateRowDataCol(x,  updateCol, value)
```

Arguments

x	Object of type SummarizedExperiment.
updateCol	Name or the number of the column in the rowData of x to be updated with the new values. if the updateCol does not match to any column names it will be added as a new column.
value	The new Replacing values.

Value

Returns an object of type SummarizedExperiment.

Author(s)

Ali Oghabian

See Also

annotateU12

Examples

```
test<- mdsChr22Obj
# See the the frequency of each intron type annotation
table(rowData(test)$intron_type)

#Change U2 to u2
newIntType<- as.character(rowData(test)$intron_type)
newIntType[newIntType=="U2" &
 !is.na(newIntType=="U2")]<- "u2"
#Updating values
test<- updateRowDataCol(test, updateCol="intron_type",
 value=newIntType)
#See the frequency of the updated intron type annotations
table(rowData(test)$intron_type)

#Adding a new column
test<- updateRowDataCol(test, updateCol="new_column",
 value=rep(NA, nrow(rowData(test))) )
head(rowData(test))
```

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