Package 'EventPointer'

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Type Package

Title An effective identification of alternative splicing events using junction arrays and RNA-Seq data

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Description EventPointer is an R package to identify alternative splicing events that involve either simple (case-control experiment) or complex experimental designs such as time course experiments and studies including paired-samples. The algorithm can be used to analyze data from either junction arrays (Affymetrix Arrays) or sequencing data (RNA-Seq).
The software returns a data.frame with the detected alternative splicing events: gene name, type of event (cassette, alternative 3',...,etc), genomic position, statistical significance and increment of the percent spliced in (Delta PSI) for all the events.
The algorithm can generate a series of files to visualize the detected alternative splicing events in IGV. This eases the interpretation of results and the design of primers for standard PCR validation.

Depends R (>= 3.4), SGSeq, Matrix, SummarizedExperiment

Imports GenomicFeatures, stringr, GenomeInfoDb, igraph, MASS, nnls, limma, matrixStats, RBGL, prodlim, graph, methods, utils, stats, doParallel, foreach, affxparser, GenomicRanges, S4Vectors, IRanges, qvalue, cobs, rhdf5, BSgenome, BSgenome.Hsapiens.UCSC.hg38, Biostrings

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VignetteBuilder knitr

Url https://github.com/jpromeror/EventPointer

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R topics documented:

`AllEvents_RNASeq` *Alternative splicing events detected by EventPointer*

Description

Alternative splicing events detected by EventPointer

Usage

```
data(AllEvents_RNASeq)
```

Format

A `list` object `AllEvents_RNASeq[[i]][[j]]` displays the jth splicing event for the ith gene.

Value

AllEvents_RNASeq object contains all the detected alternativesplicing events using EventPointer-methodology. The splicing events where detected using the BAM files from the dataset published in Seshagiri et al. 2012 andused in the SGSeq R package vignette.

AllEvents_RNASeq_MP *Alternative splicing multi-path events detected by EventPointer*

Description

Alternative splicing multi-path events detected by EventPointer

Usage

```
data(AllEvents_RNASeq_MP)
```

Format

A list object AllEvents_RNASeq[[i]][[j]] displays the jth splicing event for the ith gene.

Value

AllEvents_RNASeq_MP object contains all the detected alternative splicing events using Event-Pointer methodology for multi-path events. The splicing events where detected using the BAM files from the dataset published in Seshagiri et al. 2012 and used in the SGSeq R package vignette.

ArrayDatamultipath *Preprocessed arrays data with multi-path events*

Description

Preprocessed arrays data with multi-path events

Usage

```
data(ArrayDatamultipath)
```

Format

A data.frame with preprocessed arrays data. The preprocessing was done using aroma.affymetrix. See the package vignette for the preprocessing pipeline

Value

ArrayDatamultipath object contains preprocessed junction arrays data. The preprocessing was done using aroma.affymetrix R package, refer to EventPointer vignette for the pipeline used for the preprocessing. The data corresponds to 4 samples from the SUM149 Cell line hybridized to the HTA 2.0 Affymetrix array. The first two samples are control and the second ones are treated.

ArraysData *Preprocessed arrays data*

Description

Preprocessed arrays data

Usage

```
data(ArraysData)
```

Format

A `data.frame` with preprocessed arrays data. The preprocessing was done using `aroma.affymetrix`. See the package vignette for the preprocessing pipeline

Value

ArraysData object contains preprocessed junction arrays data. The preprocessing was done using `aroma.affymetrix` R package, refer to EventPointer vignette for the pipeline used for the preprocessing. The data corresponds to 4 samples from the SUM149 Cell line hybridized to the HTA 2.0 Affymetrix array. The first two samples are control and the second ones are treated.

CDFfromGTF *CDF file creation for EventPointer*

Description

Generates the CDF file to be used under the aroma.affymetrix framework

Usage

```
CDFfromGTF(input = "Ensembl", inputFile = NULL, PSR, Junc, PathCDF,
  microarray = NULL)
```

Arguments

`input`	Reference transcriptome used to build the CDF file. Must be one of: 'Ensembl', 'UCSC' , 'AffyGTF' or 'CustomGTF'.
`inputFile`	If input is 'AffyGTF' or 'CustomGTF', inputFile should point to the GTF file to be used.
`PSR`	Path to the Exon probes txt file
`Junc`	Path to the Junction probes txt file
`PathCDF`	Directory where the output will be saved
`microarray`	Microarray used to create the CDF file. Must be one of: HTA-2_0, ClariomD, RTA or MTA

Value

The function displays a progress bar to show the user the progress of the function. However, there is no value returned in R as the function creates three files that are used later by other EventPointer functions.1) EventsFound.txt : Tab separated file with all the information of all the alternative splcing events found. 2) .flat file : Used to build the corresponding CDF file. 3) .CDF file: Output required for the aroma.affymetrix preprocessing pipeline. Both the .flat and .CDF file take large ammounts of memory in the hard drive, it is recommended to have at least 1.5 GB of free space.

Examples

```
PathFiles<-system.file('extdata',package='EventPointer')
DONSON_GTF<-paste(PathFiles,'/DONSON.gtf',sep='')
PSRProbes<-paste(PathFiles,'/PSR_Probes.txt',sep='')
JunctionProbes<-paste(PathFiles,'/Junction_Probes.txt',sep='')
Directory<-tempdir()
microarray<-'HTA-2_0'

# Run the function

CDFfromGTF(input='AffyGTF',inputFile=DONSON_GTF,PSR=PSRProbes,Junc=JunctionProbes,
           PathCDF=Directory,microarray=microarray)
```

CDFfromGTF_Multipath *CDF file creation for EventPointer (MultiPath)*

Description

Generates the CDF file to be used under the aroma.affymetrix framework.

Usage

```
CDFfromGTF_Multipath(input = "Ensembl", inputFile = NULL, PSR, Junc,
  PathCDF, microarray = NULL, paths = 2)
```

Arguments

input	Reference transcriptome used to build the CDF file. Must be one of Ensembl, UCSC or GTF.
inputFile	If input is GTF, inputFile should point to the GTF file to be used.
PSR	Path to the Exon probes txt file
Junc	Path to the Junction probes txt file
PathCDF	Directory where the output will be saved
microarray	Microarray used to create the CDF file. Must be one of: HTA-2_0, ClariomD, RTA or MTA
paths	Maximum number of paths of the events to find.

Value

The function displays a progress bar to show the user the progress of the function. However, there is no value returned in R as the function creates three files that are used later by other EventPointer functions. 1) EventsFound.txt : Tab separated file with all the information of all the alternative splcing events found. 2) .flat file : Used to build the corresponding CDF file. 3) .CDF file: Output required for the aroma.affymetrix preprocessing pipeline. Both the .flat and .CDF file take large ammounts of memory in the hard drive, it is recommended to have at least 1.5 GB of free space.

Examples

```
PathFiles<-system.file('extdata',package='EventPointer')
DONSON_GTF<-paste(PathFiles,'/DONSON.gtf',sep='')
PSRProbes<-paste(PathFiles,'/PSR_Probes.txt',sep='')
JunctionProbes<-paste(PathFiles,'/Junction_Probes.txt',sep='')
Directory<-tempdir()
microarray<-'HTA-2_0'

# Run the function

CDFfromGTF_Multipath(input='AffyGTF',inputFile=DONSON_GTF,PSR=PSRProbes,Junc=JunctionProbes,
                     PathCDF=Directory,microarray=microarray,paths=3)
```

EventDetection *Detect splicing events using EventPointer methodology*

Description

Identification of all the alternative splicing events in the splicing graphs

Usage

```
EventDetection(Input, cores, Path)
```

Arguments

Input	Output of the PrepareBam_EP function
cores	Number of cores used for parallel processing
Path	Directory where to write the EventsFound_RNASeq.txt file

Value

list with all the events found for all the genes present in the experiment. It also generates a file called EventsFound_RNASeq.txt with the information of each event.

Examples

```
# Run EventDetection function
data(SG_RNASeq)
TxtPath<-tempdir()
AllEvents_RNASeq<-EventDetection(SG_RNASeq,cores=1,Path=TxtPath)
```

EventDetectionMultipath *Detect splicing multipath events using EventPointer methodology*

Description

Identification of all the multipath alternative splicing events in the splicing graphs

Usage

```
EventDetectionMultipath(Input, cores, Path, paths = 2)
```

Arguments

Input	Output of the PrepareBam_EP function
cores	Number of cores used for parallel processing
Path	Directory where to write the EventsFound_RNASeq.txt file
paths	Maximum number of paths of the events to find.

Value

list with all the events found for all the genes present in the experiment. It also generates a file called EventsFound_RNASeq.txt with the information each event.

Examples

```
# Run EventDetection function
 data(SG_RNASeq)
 TxtPath<-tempdir()
 AllEvents_RNASeq_MP<-EventDetectionMultipath(SG_RNASeq,cores=1,Path=TxtPath,paths=3)
```

EventPointer *EventPointer*

Description

Statistical analysis of alternative splcing events

Usage

```
EventPointer(Design, Contrast, ExFit, Eventstxt, Filter = TRUE,
  Qn = 0.25, Statistic = "LogFC", PSI = FALSE)
```

Arguments

Design	The design matrix for the experiment.
Contrast	The contrast matrix for the experiment.
ExFit	aroma.affymetrix pre-processed variable after using extractDataFrame(affy,addNames=TRUE)
Eventstxt	Path to the EventsFound.txt file generated by CDFfromGTF function.
Filter	Boolean variable to indicate if an expression filter is applied
Qn	Quantile used to filter the events (Bounded between 0-1, Q1 would be 0.25).
Statistic	Statistical test to identify differential splicing events, must be one of : LogFC, Dif_LogFC or DRS.
PSI	Boolean variable to indicate if Delta PSI should be calculated for every splicing event.

Value

Data.frame ordered by the splicing p.value . The object contains the different information for each splicing event such as Gene name, event type, genomic position, p.value, z.value and delta PSI.

Examples

```
  data(ArraysData)

  Dmatrix<-matrix(c(1,1,1,1,0,0,1,1),nrow=4,ncol=2,byrow=FALSE)
  Cmatrix<-t(t(c(0,1)))
 EventsFound<-paste(system.file('extdata',package='EventPointer'),'/EventsFound.txt',sep='')

Events<-EventPointer(Design=Dmatrix,
                     Contrast=Cmatrix,
                     ExFit=ArraysData,
                     Eventstxt=EventsFound,
                     Filter=TRUE,
                     Qn=0.25,
                     Statistic='LogFC',
                     PSI=TRUE)
```

EventPointer_IGV *EventPointer IGV Visualization*

Description

Generates of files to be loaded in IGV for visualization and interpretation of events

Usage

```
EventPointer_IGV(Events, input, inputFile = NULL, PSR, Junc, PathGTF,
  EventsFile, microarray = NULL)
```

Arguments

Events	Data.frame generated by EventPointer with the events to be included in the GTF file.
input	Reference transcriprome. Must be one of: 'Ensembl', 'UCSC' , 'AffyGTF' or 'CustomGTF'.
inputFile	If input is 'AffyGTF' or 'CustomGTF', inputFile should point to the GTF file to be used.
PSR	Path to the Exon probes txt file.
Junc	Path to the Junction probes txt file.
PathGTF	Directory where to write the GTF files.
EventsFile	Path to EventsFound.txt file generated with CDFfromGTF function.
microarray	Microarray used to create the CDF file. Must be one of: HTA-2_0, ClariomD, RTA or MTA

Value

The function displays a progress bar to show the user the progress of the function. Once the progress bar reaches 100 in PathGTF. The created files are: 1) paths.gtf : GTF file representing the alternative splicing events and 2) probes.gtf : GTF file representing the probes that measure each event and each path.

Examples

```
  PathFiles<-system.file('extdata',package='EventPointer')
  DONSON_GTF<-paste(PathFiles,'/DONSON.gtf',sep='')
  PSRProbes<-paste(PathFiles,'/PSR_Probes.txt',sep='')
  JunctionProbes<-paste(PathFiles,'/Junction_Probes.txt',sep='')
  Directory<-tempdir()

  data(ArraysData)

  Dmatrix<-matrix(c(1,1,1,1,0,0,1,1),nrow=4,ncol=2,byrow=FALSE)
  Cmatrix<-t(t(c(0,1)))
 EventsFound<-paste(system.file('extdata',package='EventPointer'),'/EventsFound.txt',sep='')

  Events<-EventPointer(Design=Dmatrix,
                       Contrast=Cmatrix,
                       ExFit=ArraysData,
                       Eventstxt=EventsFound,
                       Filter=TRUE,
                       Qn=0.25,
                       Statistic='LogFC',
                       PSI=TRUE)

EventPointer_IGV(Events=Events[1,,drop=FALSE],
                 input='AffyGTF',
                 inputFile=DONSON_GTF,
                 PSR=PSRProbes,
                 Junc=JunctionProbes,
                 PathGTF=Directory,
                EventsFile= EventsFound,
                microarray='HTA-2_0')
```

EventPointer_RNASeq *Statistical analysis of alternative splcing events for RNASeq data*

Description

Statistical analysis of all the alternative splicing events found in the given bam files.

Usage

```
EventPointer_RNASeq(Events, Design, Contrast, Statistic = "LogFC",
  PSI = FALSE)
```

Arguments

Events	Output from EventDetection function
Design	The design matrix for the experiment.
Contrast	The contrast matrix for the experiment.
Statistic	Statistical test to identify differential splicing events, must be one of : LogFC, Dif_LogFC and DRS.
PSI	Boolean variable to indicate if PSI should be calculated for every splicing event.

Value

Data.frame ordered by the splicing p.value . The object contains the different information for each splicing event such as Gene name, event type, genomic position, p.value, z.value and delta PSI.

Examples

```
 data(AllEvents_RNASeq)
 Dmatrix<-matrix(c(1,1,1,1,1,1,1,0,0,0,0,1,1,1,1),ncol=2,byrow=FALSE)
 Cmatrix<-t(t(c(0,1)))
Events <- EventPointer_RNASeq(AllEvents_RNASeq,Dmatrix,Cmatrix,Statistic='LogFC',PSI=TRUE)
```

EventPointer_RNASeq_IGV *EventPointer RNASeq IGV Visualization*

Description

Generates of files to be loaded in IGV for visualization and interpretation of events

Usage

```
EventPointer_RNASeq_IGV(Events, SG_RNASeq, EventsTxt, PathGTF)
```

Arguments

Events	Data.frame generated by EventPointer_RNASeq with the events to be included in the GTF file.
SG_RNASeq	Output from PrepareBam_EP function. Contains splicing graphs components.
EventsTxt	Path to EventsFound.txt file generated with EventDetection function
PathGTF	Directory where to write the GTF files.

Value

The function displays a progress bar to show the user the progress of the function. Once the progress bar reaches 100 file is written to the specified directory in PathGTF. The created file: 1) paths_RNASeq.gtf : GTF file representing the alternative splicing events.

Examples

```
data(AllEvents_RNASeq)
data(SG_RNASeq)

 # Run EventPointer

 Dmatrix<-matrix(c(1,1,1,1,1,1,1,1,0,0,0,0,1,1,1,1),ncol=2,byrow=FALSE)
 Cmatrix<-t(t(c(0,1)))
Events <- EventPointer_RNASeq(AllEvents_RNASeq,Dmatrix,Cmatrix,Statistic='LogFC',PSI=TRUE)

 # IGV Visualization

EventsTxt<-paste(system.file('extdata',package='EventPointer'),'/EventsFound_RNASeq.txt',sep='')
 PathGTF<-tempdir()
 EventPointer_RNASeq_IGV(Events,SG_RNASeq,EventsTxt,PathGTF)
```

EventPointer_RNASeq_TranRef

EventPointer_RNASeq_TranRef

Description

Statistical analysis of alternative splicing events with the output of GetPSI_FromTranRef

Usage

```
EventPointer_RNASeq_TranRef(Count_Matrix, Statistic = "LogFC", Design,
  Contrast)
```

Arguments

Count_Matrix	The list containing the expression data taken from the ouput of GetPSI_FromTranRef
Statistic	The type of statistic to apply. Default = 'LogFC' (can be 'logFC, 'Dif_LogFC','DRS')
Design	The design matrix of the experiment.
Contrast	The Contrast matrix of the experiment.

Value

a data.frame with the information of the names of the event, its p.values and the corresponding z.value. If there is more than one contrast, the function returns as many data.frames as number of contrast and all these data.frame are sotred in an unique list.

Examples

```
data(EventXtrans)
data(PSIss)
# Design and contrast matrix:

Design <- matrix(c(1,1,1,1,0,0,1,1),nrow=4)
Contrast <- matrix(c(0,1),nrow=2)

# Statistical analysis:


Fit <- EventPointer_RNASeq_TranRef(Count_Matrix =  PSIss$ExpEvs,
                                   Statistic = 'LogFC',Design = Design,
                                   Contrast = Contrast)
```

`EventsGTFfromTrancriptomeGTF`

Events .gtf from trancriptome .gtf

Description

Finds the alternative splicing events given a reference transcriptome.

Usage

```
EventsGTFfromTrancriptomeGTF(inputFile = NULL, Transcriptome = NULL,
  Pathtxt = NULL, PathGTF = NULL)
```

Arguments

`inputFile`	If input is GTF, inputFile should point to the GTF file to be used.
`Transcriptome`	the name of the transcriptome
`Pathtxt`	Directory to save the .txt of the events founded
`PathGTF`	Directory where the output will be saved

Value

a list containing five elements: three sparce matrices that relate which isoforms build up the paths (path1,path2 and pathRef) of each event. The fourth element contains the name of the reference annotation: only appear the name of the transcript. The final element is SG_List: a list with the information of the graph of each gene, this variable is necesary for Primers design step.

Examples

```
PathFiles<-system.file('extdata',package='EventPointer')
inputFile <- paste(PathFiles,'/gencode.v24.ann_2genes.gtf',sep='')
Transcriptome <- 'Gencode24_2genes'
Pathtxt <- tempdir()
PathGTF <- tempdir()

# Run the function

EventXtrans <- EventsGTFfromTrancriptomeGTF(inputFile = inputFile,
                                            Transcriptome = Transcriptome,
                                            Pathtxt=Pathtxt,PathGTF=PathGTF)
```

EventXtrans *relationship between isoforms and events*

Description

relationship between isoforms and events

Usage

```
data(EventXtrans)
```

Format

A `list` object `EventXtrans[[1]]` displays the isoform that build up the path1 of each event.

Value

EventXtrans object contains the relationship between the isoforms and the events. It is a list of 4 elements. the first three stored sparse matrices relating the isoforms with the events. The fourth element stores de names of the reference annotation used (isoforms names)

FindPrimers *FindPrimers*

Description

FindPrimers is the main function of the primers design option. The aim of this function is the design of PCR primers and TaqMan probes for detection and quantification of alternative splicing.

Depending on the assay we want to carry out the the algorithm will design the primers for a conventional PCR or the primers and TaqMan probes if we are performing a TaqMan assay.

In the case of a conventional PCR we will be able to detect the alternative splicing event. Besides, the algorithm gives as an output the length of the PCR bands that are going to appear. In the case of a TaqMan assay, we will not only detect but also quantify alternative splicing.

Usage

```
FindPrimers(SG, EventNum, Primer3Path, Dir, taqman = NA, nProbes = 1,
  nPrimerstwo = 3, ncommonForward = 3, ncommonReverse = 3,
  nExons = 5, nPrimers = 15, shortdistpenalty = 2000,
  maxLength = 1000, minsep = 100, wminsep = 200,
  valuethreePenalty = 1000, minexonlength = 25, wnpaths = 200,
  qualityfilter = 5000)
```

Arguments

SG	Information of the graph of the gene where the selected event belongs. This information is avaible in the output of EventsGTFfromTranscriptomeGTF function.
EventNum	The "EventNum" variable can be found in the returned .txt file from the EventsGTFfromTranscriptomeGTF function in the column "EventNumber" or in the output of EventPointer_RNASeq_TranRef, the number after the "_" character of the 'Event_ID'.
Primer3Path	Complete path where primer3_core.exe is placed.
Dir	Complete path where primer3web_v4_0_0_default_settings.txt file and primer3_config directory are stored.
taqman	1 if you want to get probes and primers for taqman. 0 if you want to get primers for conventional PCR.
nProbes	Number of probes for Taqman experiments. By default 1.
nPrimerstwo	Number of potential exon locations for primers using two primers (one forward and one reverse). By default 3.
ncommonForward	Number of potential exon locations for primers using one primer in forward and two in reverse. By default 3.
ncommonReverse	Number of potential exon locations for primers using two primer in forward and one in reverse. By default 3.
nExons	Number of combinations of ways to place primers in exons to interrogate an event after sorting. By default 5.
nPrimers	Once the exons are selected, number of primers combination sequences to search within the whole set of potential sequences. By default 5.
shortdistpenalty	Penalty for short exons following an exponential funciton(A * exp(-dist * shortdistpenalty)). By defautl 2000.
maxLength	Max length of exons that are between primers and for paths once we have calculated the sequence. By default 1000.
minsep	Distance from which it is penalized primers for being too close By default 100.
wminsep	Weigh of the penalization to primers for being too close By default 200.
valuethreePenalty	penalization for cases that need three primers instead of 2. By default 1000.
minexonlength	Minimum length that a exon has to have to be able to contain a primer. By default 25.
wnpaths	Penalty for each existing path By default 200.
qualityfilter	Results will show as maximum 3 combinations with a punctuation higher than qualityfilter By default 5000.

Value

The output of the function is a ‘data.frame‘ whose columns are:

For1Seq: Sequence of the first forward primer.

For2Seq: Sequence of the second forward primer in case it is needed.

Rev1Seq: Sequence of the first reverse primer.

Rev2Seq: Sequence of the second reverse primer in case it is needed.

For1Exon: Name of the exon of the first forward primer.

For2Exon: Name of the exon of the second forward primer in case it is needed.

Rev1Exon: Name of the exon of the first reverse primer.

Rev2Exon: Name of the exon of the second reverse primer in case it is needed.

FINALvalue: Final punctuation for that combination of exons and sequences. The lower it is this score, the better it is the combination.

DistPath1: Distances of the bands, in base pairs, that interrogate Path1 when we perform the conventional PCR experiment.

DistPath2: Distances of the bands, in base pairs, that interrogate Path2 ‘when we perform the conventional PCR experiment.

DistNoPath: Distances of the bands, in base pairs, that they do not interrogate any of the two paths when we perform the conventional PCR experiment.

SeqProbeRef: Sequence of the TaqMan probe placed in the Reference.

SeqProbeP1: Sequence of the TaqMan probe placed in the Path1.

SeqProbeP2: Sequence of the TaqMan probe placed in the Path2.

Examples

```
## Not run:

data("EventXtrans")
#From the output of EventsGTFfromTranscriptomeGTF we take the splicing graph information
SG_list <- EventXtrans$SG_List
#SG_list contains the information of the splicing graphs for each gene

#Let's supone we want to design primers for the event 1 of the gene ENSG00000254709.7

#We take the splicing graph information of the required gene
SG <- SG_list$ENSG00000254709.7

#We point the event number
EventNum <- 1

#Define rest of variables:
Primer3Path <- Sys.which("primer3_core")
Dir <- "C:\\PROGRA~2\\primer3\\"

MyPrimers <- FindPrimers(SG = SG,
                         EventNum = EventNum,
                         Primer3Path = Primer3Path,
                         Dir = Dir,
                         taqman = 1,
                         nProbes=1,
```

```
                        nPrimerstwo=4,
                        ncommonForward=4,
                        ncommonReverse=4,
                        nExons=10,
                        nPrimers =5,
                        maxLength = 1200)


## End(Not run)
```

`getbootstrapkallisto` *GetbootstrapKallisto*

Description

Function to load the values of the bootstrap returned by kallisto pipeline

Inputs:

Usage

```
getbootstrapkallisto(pathValues = NA, nb)
```

Arguments

`pathValues`	A vector with the complete directory to the folder of the output of kallisto
`nb`	number of bootstrap

Value

A list containing the quantification data and with the bootrap information.

Examples

```
PathFiles <- system.file('extdata',package='EventPointer')
PathFiles <- dir(paste0(PathFiles,'/output'),full.names = TRUE)

#load the data

mydatab <- getbootstrapkallisto(pathValues = PathFiles,nb = 20)
```

GetPSI_FromTranRef *GetPSI_FromTranRef*

Description

Get the values of PSI.A filer expresion is applied if the user select the option of filter.

Usage

```
GetPSI_FromTranRef(PathsxTranscript, Samples, Filter = TRUE, Qn = 0.25)
```

Arguments

PathsxTranscript	the outpu of EventGTFfromTrancriptomeGTF
Samples	the samples (in the rowname of the samples must be written only the name of the transcript)
Filter	Boolean variable to indicate if an expression filter is applied. Defaul T
Qn	Quantile used to filter the events (Bounded between 0-1, Q1 would be 0.25).

Value

The output of the function is a list containing two elements: a matrix with the values of PSI and a list containing as many matrices as number of events. In each matrix is stored the expression of the different paths of an event along the samples.

Examples

```
 data(EventXtrans)
 PathFiles <- system.file('extdata',package='EventPointer')
 filesnames <- dir(paste0(PathFiles,'/output'))
 PathFiles <- dir(paste0(PathFiles,'/output'),full.names = TRUE)
 dirtoload <- paste0(PathFiles,'/','abundance.tsv')
RNASeq <- read.delim(dirtoload[1],sep = '\t', colClasses = c(NA,'NULL','NULL','NULL',NA))
 for (n in 2:length(dirtoload)){
   RNASeq[,n+1] <- read.delim(dirtoload[n],sep = '\t',
                              colClasses = c('NULL','NULL','NULL','NULL',NA))
 }
 rownames(RNASeq)<-RNASeq[,1]
 RNASeq<-RNASeq[,-1]
 colnames(RNASeq) <- filesnames
 rownames(RNASeq) <- sapply(strsplit(rownames(RNASeq),'\\|'),function(X) return(X[1]))
 RNASeq<-as.matrix(RNASeq) #must be a matrix variable

 #Obtain values of PSI

PSIss <- GetPSI_FromTranRef(PathsxTranscript = EventXtrans,Samples = RNASeq,Filter = FALSE)

 PSI <- PSIss$PSI
 Expression <- PSIss$ExpEvs
```

MyPrimers *Data frame with primers design for conventional PCR*

Description

Data frame with primers design for conventional PCR

Usage

```
data(MyPrimers)
```

Format

A `data.frame` object displays the relative information for primers design for conventional PCR

Value

MyPrimers object contains a data.frame with the information of the design primers for conventioanl PCR.

MyPrimers_taqman *Data frame with primers design for taqman PCR*

Description

Data frame with primers design for taqman PCR

Usage

```
data(MyPrimers_taqman)
```

Format

A `data.frame` object displays the relative information for primers design for taqman PCR

Value

MyPrimers_taqman object contains a data.frame with the information of the design primers for taqman PCR.

PrepareBam_EP *Bam files preparation for EventPointer*

Description

Prepares the information contained in .bam files to be analyzed by EventPointer

Usage

```
PrepareBam_EP(Samples, SamplePath, Ref_Transc = "Ensembl",
  fileTransc = NULL, cores = 1, Alpha = 2)
```

Arguments

Samples	Name of the .bam files to be analyzed (Sample1.bam,Sample2.bam,...,etc).
SamplePath	Path where the bam files are stored.
Ref_Transc	Reference transcriptome used to name the genes found in bam files. Options are: Ensembl, UCSC or GTF.
fileTransc	Path to the GTF reference transcriptome ff Ref_Transc is GTF.
cores	Number of cores used for parallel processing.
Alpha	Internal SGSeq parameter to include or exclude regions

Value

SGFeaturesCounts object. It contains a GRanges object with the corresponding elements to build the different splicing graphs found and the counts related to each of the elements.

Examples

```
## Not run:
 # Obtain the samples and directory for .bam files

   BamInfo<-si
   Samples<-BamInfo[,2]
   PathToSamples <- system.file('extdata/bams', package = 'SGSeq')
   PathToGTF<-paste(system.file('extdata',package='EventPointer'),'/FBXO31.gtf',sep='')

  # Run PrepareBam function
   SG_RNASeq<-PrepareBam_EP(Samples=Samples,
                            SamplePath=PathToSamples,
                            Ref_Transc='GTF',
                            fileTransc=PathToGTF,
                            cores=1)

## End(Not run)
```

PSIss *relationship between isoforms and events*

Description

relationship between isoforms and events

Usage

```
data(PSIss)
```

Format

A object `PSIss[[1]]` displays the values of PSI and `PSIss[[2]]` the valeus of expression.

Value

PSIss object the values of PSI calculated by the funcion GetPSI_FromTranRef and also the values of expression.

PSI_Statistic *PSI_Statistic*

Description

Statistical analysis of the alternative splicing events. This function takes as input the values of PSI. Perform a statistical analysis based on permutation test

Usage

```
PSI_Statistic(PSI, Design, Contrast, nboot)
```

Arguments

`PSI`	A matrix with the values of the PSI.
`Design`	The design matrix for the experiment.
`Contrast`	The contrast matrix for the experiment.
`nboot`	The number of random analysis.

Value

The output of these functions is a list containing: two data.frame (deltaPSI and Pvalues) with the values of the deltaPSI and the p.values for each contrast, and a third element (LocalFDR) with the information of the local false discovery rate.

Examples

```
data(PSIss)
Design <- matrix(c(1,1,1,1,0,0,1,1),nrow=4)
Contrast <- matrix(c(0,1),nrow=1)

# Statistical analysis:

table <- PSI_Statistic(PSIss$PSI,Design = Design, Contrast = Contrast, nboot = 50)
```

SG_RNASeq *Splicing graph elements predicted from BAM files*

Description

Splicing graph elements predicted from BAM files

Usage

```
data(SG_RNASeq)
```

Format

A SGFeatureCounts objects with predicted splicing graph features and counts

Value

SG_RNASeq object displays the predicted features found in the BAM files from the dataset published in Seshagiri et al. 2012 and used in the SGSeq R package vignette.

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