

Package ‘AllelicImbalance’

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Type Package

Title Investigates allele specific expression

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Description Provides a framework for allelic specific expression investigation using RNA-seq data.

License GPL-3

URL <https://github.com/pappewaio/AllelicImbalance>

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R topics documented:

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AllelicImbalance-package
*A package meant to provide all basic functions for high-throughput
allele specific expression analysis*

Description

Package AllelicImbalance has functions for importing, filtering and plotting high-throughput data to make an allele specific expression analysis. A major aim of this package is to provide functions to collect as much information as possible from regions of choice, and to be able to explore the allelic expression of that region in detail.

Details

Package: AllelicImbalance
Type: Package
Version: 1.2.0
Date: 2014-08-24
License: GPL-3

Overview - standard procedure

Start out creating a GRange object defining the region of interest. This can also be done using getAreaFromGeneNames providing gene names as arguments. Then use BamImpGAList to import reads from that region and find potential SNPs using scanForHeterozygotes. Then retrieve the allele counts of heterozygote sites by the function getAlleleCount. With this data create an ASEset. At this point all pre-requisites for a 'basic' allele specific expression analysis is available. Two ways to go on could be to apply [chisq.test](#) or [barplot](#) on this ASEset object.

Author(s)

Author: Jesper Robert Gadin Author: Lasse Folkersen
Maintainer: Jesper Robert Gadin <j.r.gadin@gmail.com>

References

Reference to published application note (work in progress)

See Also

- `code?ASEset`

annotation-wrappers *AnnotationDb wrappers*

Description

These functions acts as wrappers to retrieve information from annotation database objects (annotationDb objects) or (transcriptDb objects)

Usage

```

getGenesFromAnnotation(OrgDb, GR, leftFlank = 0, rightFlank = 0,
  getUCSC = FALSE, verbose = FALSE)

getGenesVector(OrgDb, GR, leftFlank = 0, rightFlank = 0, verbose = FALSE)

getExonsFromAnnotation(TxDb, GR, leftFlank = 0, rightFlank = 0,
  verbose = FALSE)

getExonsVector(TxDb, GR, leftFlank = 0, rightFlank = 0, verbose = FALSE)

getTranscriptsFromAnnotation(TxDb, GR, leftFlank = 0, rightFlank = 0,
  verbose = FALSE)

getTranscriptsVector(TxDb, GR, leftFlank = 0, rightFlank = 0,
  verbose = FALSE)

getCDSFromAnnotation(TxDb, GR, leftFlank = 0, rightFlank = 0,
  verbose = FALSE)

getCDSVector(TxDb, GR, leftFlank = 0, rightFlank = 0, verbose = FALSE)

getAnnotationDataFrame(GR, strand = "+", annotationType = NULL,
  OrgDb = NULL, TxDb = NULL, verbose = FALSE)

```

Arguments

OrgDb	An OrgDb object
GR	A GenomicRanges object with sample area
leftFlank	An integer specifying number of additional nucleotides around the SNPs for the leftFlank
rightFlank	An integer specifying number of additional nucleotides around the SNPs for the rightFlank

getUCSC	A logical indicating if UCSC transcript IDs should also be retrieved
verbose	A logical making the functions more talkative
TxDb	A transcriptDb object
strand	Two options, '+' or '-'
annotationType	select one or more from 'gene', 'exon', 'transcript', 'cds'.

Details

These functions retrieve regional annotation from OrgDb or TxDb objects, when given GRanges objects.

Value

GRanges object with ranges over the genes in the region.

The getGenesVector function will return a character vector where each element are gene symbols separated by comma

GRanges object with ranges over the exons in the region.

The getTranscriptsFromAnnotation function will return a GRanges object with ranges over the transcripts in the region.

The getCDSFromAnnotation function will return a GRanges object with ranges over the CDSFs in the region.

The getExonsVector function will return a character vector where each element are exons separated by comma

The getTranscriptsVector function will return a character vector where each element are transcripts separated by comma

The getCDSVector function will return a character vector where each element are CDSs separated by comma

The getAnnotationDataFrame function will return a data.frame with annotations. This function is used internally by i.e. the barplot-function

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
data(ASEset)
require(org.Hs.eg.db)
require(TxDb.Hsapiens.UCSC.hg19.knownGene)
OrgDb <- org.Hs.eg.db
TxDb <- TxDb.Hsapiens.UCSC.hg19.knownGene

#use for example BcfFiles as the source for SNPs of interest
GR <- rowRanges(ASEset)
#get annotation
g <- getGenesFromAnnotation(OrgDb,GR)
```

```
e <- getExonsFromAnnotation(TxDB,GR)
t <- getTranscriptsFromAnnotation(TxDB,GR)
c <- getCDSFromAnnotation(TxDB,GR)
```

annotationBarplot *add annotation to AllelicImbalance barplot*

Description

adds a customizable annotation functionality for AllelicImbalance barplots.

Usage

```
annotationBarplot(strand, snp, lowerLeftCorner, annDfPlus, annDfMinus,
  cex = 0.7, ypos = 0, interspace = 1)
```

Arguments

strand	strand, "+", "-", "*" or "both"
snp	integer for the described snp
lowerLeftCorner	position of the plot to add legend to (default c(0,0))
annDfPlus	annotation dataframe plus strand
annDfMinus	annotation dataframe minus strand
cex	size of annotation text
ypos	relative y-axis position for the annotation text
interspace	space between each annotation block

Details

the function is preferably called from within the AllelicImbalance barplot method.

Author(s)

Jesper R. Gadin

Examples

```
#code placeholders
#< create a barplot without annotation >
#< add annotation >
```

ASEset-barplot

*barplot ASEset objects***Description**

Generates barplots for ASEset objects. Two levels of plotting detail are provided: a detailed barplot of read counts by allele useful for fewer samples and SNPs, and a less detailed barplot of the fraction of imbalance, useful for more samples and SNPs.

Usage

```
barplot(height, ...)
```

```
## S4 method for signature 'ASEset'
barplot(height, type = "count", sampleColour.top = NULL,
  sampleColour.bot = NULL, legend = TRUE, pValue = TRUE, strand = "*",
  testValue = NULL, testValue2 = NULL, OrgDb = NULL, TxDb = NULL,
  annotationType = c("gene", "exon", "transcript"), main = NULL,
  ylim = NULL, yaxis = TRUE, xaxis = FALSE, ylab = TRUE,
  ylab.text = NULL, xlab.text = "samples", xlab = TRUE,
  legend.colnames = "", las.ylab = 1, las.xlab = 2, cex.main = 1,
  cex.pValue = 0.7, cex.ylab = 0.7, cex.xlab = 0.7, cex.legend = 0.6,
  add = FALSE, lowerLeftCorner = c(0, 0), size = c(1, 1),
  addHorizontalLine = 0.5, add.frame = TRUE,
  filter.pValue.fraction = 0.99, legend.fill.size = 1,
  legend.interspace = 1, verbose = FALSE,
  top.fraction.criteria = "maxcount", cex.annotation = 0.7,
  ypos.annotation = 0, annotation.interspace = 1, ...)
```

Arguments

height	An ASEset object
...	for simpler generics when extending function
type	'count' or 'fraction'
sampleColour.top	User specified colours for top fraction
sampleColour.bot	User specified colours for bottom fraction
legend	Display legend
pValue	Display p-value
strand	four options, '+', '-', 'both' or '*'
testValue	if set, a matrix or vector with user p-values
testValue2	if set, a matrix or vector with user p-values
OrgDb	an OrgDb object which provides annotation

TxDB	a TxDb object which provides annotation
annotationType	select one or more from 'gene','exon','transcript','cds'.
main	text to use as main label
ylim	set plot y-axis limit
yaxis	whether the y-axis is to be displayed or not
xaxis	whether the x-axis is to be displayed or not
ylab	showing labels for the tic marks
ylab.text	ylab text
xlab.text	xlab text
xlab	showing labels for the tic marks
legend.colnames	gives colnames to the legend matrix
las.ylab	orientation of ylab text
las.xlab	orientation of xlab text
cex.main	set main label size (max 2)
cex.pValue	set pValue label size
cex.ylab	set ylab label size
cex.xlab	set xlab label size
cex.legend	set legend label size
add	boolean indicates if a new device should be started
lowerLeftCorner	integer that is only useful when add=TRUE
size	Used internally by locationplot. Rescales each small barplot window
addHorizontalLine	adds a horizontal line that marks the default fraction of 0.5 - 0.5
add.frame	boolean to give the new plot a frame or not
filter.pValue.fraction	numeric between 0 and 1 that filter away pValues where the main allele has this frequency.
legend.fill.size	size of the fill/boxes in the legend (default:NULL)
legend.interspace	set legend space between fills and text
verbose	Makes function more talkative
top.fraction.criteria	'maxcount', 'ref' or 'phase'
cex.annotation	size of annotation text
ypos.annotation	relative ypos for annotation text
annotation.interspace	space between annotation text

Details

`filter.pValue.fraction` is intended to remove p-value annotation with very large difference in frequency, which could just be a sequencing mistake. This is to avoid p-values like $1e-235$ or similar.

`sampleColourUser` specified colours, either given as named colours ('red', 'blue', etc) or as hexadecimal code. Can be either length 1 for all samples, or else of a length corresponding to the number of samples for individual colouring.

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [ASEset](#) class which the `barplot` function can be called up on.

Examples

```
data(ASEset)
barplot(ASEset[1])
```

ASEset-class

ASEset objects

Description

Object that holds allele counts, genomic positions and map-bias for a set of SNPs

Usage

```
alleleCounts(x, strand = "*", return.class = "list")

## S4 method for signature 'ASEset'
alleleCounts(x, strand = "*", return.class = "list")

alleleCounts(x, strand = "*") <- value

## S4 replacement method for signature 'ASEset'
alleleCounts(x, strand = "*") <- value

mapBias(x, ...)

## S4 method for signature 'ASEset'
mapBias(x, return.class = "list")

fraction(x, ...)
```

```
## S4 method for signature 'ASEset'
fraction(x, strand = "*",
        top.fraction.criteria = "maxcount", verbose = FALSE)

arank(x, return.type = "names", return.class = "list", strand = "*", ...)

frequency(x, ...)

genotype(x, ...)

## S4 method for signature 'ASEset'
genotype(x, return.class = "matrix")

genotype(x) <- value

## S4 replacement method for signature 'ASEset'
genotype(x) <- value

countsPerSnp(x, ...)

## S4 method for signature 'ASEset'
countsPerSnp(x, return.class = "matrix",
            return.type = "mean", strand = "*")

countsPerSample(x, ...)

## S4 method for signature 'ASEset'
countsPerSample(x, return.class = "matrix",
                return.type = "mean", strand = "*")

phase(x, ...)

## S4 method for signature 'ASEset'
phase(x, return.class = "matrix")

phase(x) <- value

## S4 replacement method for signature 'ASEset'
phase(x) <- value

ref(x)

## S4 method for signature 'ASEset'
ref(x)

ref(x) <- value

## S4 replacement method for signature 'ASEset,ANY'
```

```

ref(x) <- value

alt(x)

## S4 method for signature 'ASEset'
alt(x)

alt(x) <- value

## S4 replacement method for signature 'ASEset,ANY'
alt(x) <- value

```

Arguments

x	ASEset object
strand	which strand of '+', '-' or '*'
return.class	return 'list' or 'array'
value	replacement variable
...	additional arguments
top.fraction.criteria	'maxcount', 'ref' or 'phase'
verbose	makes function more talkative
return.type	return 'names', rank or 'counts'

Details

An ASEset object differs from a regular SummarizedExperiment object in that the assays contains an array instead of matrix. This array has ranges on the rows, sampleNames on the columns and variants in the third dimension.

It is possible to use the commands `barplot` and `locationplot` on an ASEset object see more details in [barplot](#) and [locationplot](#).

Three different alleleCount options are available. The simplest one is the `*` option, and is for experiments where the strand information is not known e.g. non-stranded data. The unknown strand could also be for strand specific data when the aligner could not find any strand associated with the read, but this should normally not happen, and if it does probably having an extremely low mapping quality. Then there are an option too add plus and minus stranded data. When using this, it is essential to make sure that the RNA-seq experiment under analysis has in fact been created so that correct strand information was obtained. The most functions will by default have their strand argument set to `'*'`.

The phase information is stored by the convention of 'maternal chromosome/paternal chromosome', with 0 as reference allele and 1 as alternative allele. 'l' when the phase is known and '/' when the phase is unknown. Internally the information will be stored as a three dimensional array, dim 1 for SNPs, dim 2 for Samples and dim 3 which is fixed and stores maternal chromosome, paternal chromosome and phased (1 equals TRUE).

Value

An object of class ASEset containing location information and allele counts for a number of SNPs measured in a number of samples on various strand, as well as mapBias information. All data is stored in a manner similar to the [SummarizedExperiment](#) class.

Table

table(...)

Arguments:

... An ASEset object that contains the variants of interest

The generics for table does not easily allow more than one argument so in respect to the different strand options, table will return a SimpleList with length 3, one element for each strand.

Frequency

frequency(x, return.class = "list", strand = "*", threshold.count.sample = 15)

Arguments:

x An ASEset object that contains the variants of interest

x threshold.count.samplesif sample has fewer counts the function return NA.

Constructor

ASEsetFromCountList(rowRanges, countListNonStranded = NULL, countListPlus = NULL, countListMinus = NULL, countListUnknown = NULL, colData = NULL, mapBiasExpMean = array(), verbose=FALSE, ...)

Arguments:

rowRanges A GenomicRanges object that contains the variants of interest

countListNonStranded A list where each entry is a matrix with allele counts as columns and sample counts as rows

countListPlus A list where each entry is a matrix with allele counts as columns and sample counts as rows

countListMinus A list where each entry is a matrix with allele counts as columns and sample counts as rows

countListUnknown A list where each entry is a matrix with allele counts as columns and sample counts as rows

colData A DataFrame object containing sample specific data

mapBiasExpMean A 3D array describing mapping bias. The SNPs are in the 1st dimension, samples in the 2nd dimension and variants in the 3rd dimension.

verbose Makes function more talkative

... arguments passed on to SummarizedExperiment constructor

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [SummarizedExperiment](#) for ranges operations.

Examples

```
#make example countList
set.seed(42)
countListPlus <- list()
snps <- c('snp1', 'snp2', 'snp3', 'snp4', 'snp5')
for(snp in snps){
  count<-matrix(rep(0,16),ncol=4,dimnames=list(
c('sample1', 'sample2', 'sample3', 'sample4'),
c('A', 'T', 'G', 'C')))
  #insert random counts in two of the alleles
  for(allele in sample(c('A', 'T', 'G', 'C'),2)){
count[,allele]<-as.integer(rnorm(4,mean=50,sd=10))
  }
  countListPlus[[snp]] <- count
}

#make example rowRanges
rowRanges <- GRanges(
  seqnames = Rle(c('chr1', 'chr2', 'chr1', 'chr3', 'chr1')),
  ranges = IRanges(1:5, width = 1, names = head(letters,5)),
  snp = paste('snp',1:5,sep='')
)

#make example colData
colData <- DataFrame(Treatment=c('ChIP', 'Input', 'Input', 'ChIP'),
  row.names=c('ind1', 'ind2', 'ind3', 'ind4'))

#make ASEset
a <- ASEsetFromCountList(rowRanges, countListPlus=countListPlus,
colData=colData)

#example phase matrix (simple form)
p1 <- matrix(sample(c(1,0),replace=TRUE, size=nrow(a)*ncol(a)),nrow=nrow(a), ncol(a))
p2 <- matrix(sample(c(1,0),replace=TRUE, size=nrow(a)*ncol(a)),nrow=nrow(a), ncol(a))
p <- matrix(paste(p1,sample(c("|", "|", "/"), size=nrow(a)*ncol(a), replace=TRUE), p2, sep=""),
nrow=nrow(a), ncol(a))

phase(a) <- p
```

Description

Generates gbarplots for ASEset objects. Two levels of plotting detail are provided: a detailed gbarplot of read counts by allele useful for fewer samples and SNPs, and a less detailed gbarplot of the fraction of imbalance, useful for more samples and SNPs.

Usage

```
gbarplot(x, type = "count", strand = "*", verbose = FALSE, ...)
```

Arguments

x	An ASEset object
type	'count' or 'fraction'
strand	four options, '+', '-', 'both' or '*'
verbose	Makes function more talkative
...	for simpler generics when extending function

Details

This function serves the same purpose as the normal barplot, but with trellis graphics using lattice, to be able to integrate well with Gviz track functionality.

Author(s)

Jesper R. Gadin

See Also

- The [ASEset](#) class which the gbarplot function can be called up on.
- The [barplot](#) non trellis barplot.

Examples

```
data(ASEset)
gbarplot(ASEset[1])
```

ASEset-glocationplot *glocationplot ASEset objects*

Description

plotting ASE effects over a specific genomic region using Gviz functionality

Usage

```
glocationplot(x, type = "fraction", strand = "*", BamGAL = NULL,
  GenomeAxisTrack = FALSE, trackNameDeAn = paste("deTrack", type),
  TxDb = NULL, sizes = NULL, add = FALSE, verbose = FALSE, ...)
```

Arguments

x	an ASEset object.
type	'fraction' or 'count'
strand	'+', '-', '*' or 'both'. This argument determines which strand is plotted. See <code>getAlleleCounts</code> for more information of choice of strand.
BamGAL	GAlignmentsList covering the same genomic region as the ASEset
GenomeAxisTrack	include an genomic axis track
trackNameDeAn	trackname for deAnnotation track
TxDb	a TxDb object which provides annotation
sizes	vector with the sum 1. Describes the size of the tracks
add	add to existing plot
verbose	if set to TRUE it makes function more talkative
...	arguments passed on to <code>barplot</code> function

Details

The `glocationplot` methods visualises the distribution of ASE over a larger region on one chromosome. It takes an ASEset object as well as additional information on plot type (see `gbarplot`), strand type (see `getAlleleCounts`), Annotation tracks are created from the `Gviz` package. It is obviously important to make sure that the genome build used is set correctly, e.g. 'hg19'.

`sizes` has to be of the same length as the number of tracks used.

Author(s)

Jesper R. Gadin

See Also

- The `ASEset` class which the `glocationplot` function can be called up on.

Examples

```
data(ASEset)
genome(ASEset) <- 'hg19'

glocationplot(ASEset, strand='+')

#for ASEsets with fewer SNPs the 'count' type plot is useful
glocationplot(ASEset, type='count', strand='+')
```

ASEset-gviztrack *ASEset-gviztrack ASEset objects*

Description

plotting ASE effects over a specific genomic region

Usage

```
ASEDAnnotationTrack(x, GR = rowRanges(x), type = "fraction", strand = "*",
  trackName = paste("deTrack", type), verbose = TRUE, ...)
```

```
## S4 method for signature 'ASEset'
ASEDAnnotationTrack(x, GR = rowRanges(x),
  type = "fraction", strand = "*", trackName = paste("deTrack", type),
  verbose = TRUE, ...)
```

```
CoverageDataTrack(x, GR = rowRanges(x), BamList = NULL, strand = NULL,
  start = NULL, end = NULL, trackNameVec = NULL, meanCoverage = FALSE,
  verbose = TRUE, ...)
```

Arguments

x	an ASEset object.
GR	genomic range of plotting
type	'fraction' or 'count'
strand	'+', '-'. This argument determines which strand is plotted.
trackName	name of track (ASEDAnnotationTrack)
verbose	Setting verbose=TRUE gives details of procedure during function run
...	arguments passed on to barplot function
BamList	GAlignmentsList object of reads from the same genomic region as the ASEset
start	start position of reads to be plotted
end	end position of reads to be plotted
trackNameVec	names of tracks (CoverageDataTrack)
meanCoverage	mean of coverage over samples (CoverageGataTrack)

Details

For information of how to use these tracks in more ways, visit the Gviz package manual.

Author(s)

Jesper R. Gadin

See Also

- The [ASEset](#) class which the functions can be called up on.

Examples

```

data(ASEset)
x <- ASEset[,1:2]
r <- reads[1:2]
genome(x) <- 'hg19'
seqlevels(r) <- seqlevels(x)

GR <- GRanges(seqnames=seqlevels(x),
ranges=IRanges(start=min(start(x)),end=max(end(x))),
strand='+', genome=genome(x))

deTrack <- ASEAnnotationTrack(x, GR=GR, type='fraction',strand='+')
covTracks <- CoverageDataTrack(x,BamList=r,strand='+')

lst <- c(deTrack,covTracks)

sizes <- c(0.5,rep(0.5/length(covTracks),length(covTracks)))
#temporarily do not run this function
#plotTracks(lst, from=min(start(x)), to=max(end(x)),
#sizes=sizes, col.line = NULL, showId = FALSE, main='mainText',
#cex.main=1, title.width=1, type='histogram')

```

ASEset-locationplot *locationplot ASEset objects*

Description

plotting ASE effects over a specific genomic region

Usage

```

locationplot(x, ...)

## S4 method for signature 'ASEset'
locationplot(x, type = "fraction", strand = "*",
yaxis = TRUE, xaxis = FALSE, xlab = FALSE, ylab = TRUE,
xlab.text = "", ylab.text = "", legend.colnames = "", size = c(0.8,
1), main = NULL, pValue = FALSE, cex.main = 0.7, cex.ylab = 0.6,
cex.legend = 0.5, OrgDb = NULL, TxDb = NULL, verbose = TRUE,
top.fraction.criteria = "maxcount", ...)

```

Arguments

x	an ASEset object.
...	arguments passed on to barplot function
type	'fraction' or 'count'
strand	'+', '-', '*' or 'both'. This argument determines which strand is plotted. See <code>getAlleleCounts</code> for more information on strand.
yaxis	whether the y-axis is to be displayed or not
xaxis	whether the x-axis is to be displayed or not
xlab	showing labels for the tic marks
ylab	showing labels for the tic marks
xlab.text	xlab text
ylab.text	ylab text
legend.colnames	gives colnames to the legend matrix
size	will give extra space in the margins of the inner plots
main	text to use as main label
pValue	Display p-value
cex.main	set main label size
cex.ylab	set ylab label size
cex.legend	set legend label size
OrgDb	an OrgDb object from which to plot a gene map. If given together with argument TxDb this will only be used to extract genesymbols.
TxDb	a TxDb object from which to plot an exon map.
verbose	Setting verbose=TRUE gives details of procedure during function run
top.fraction.criteria	'maxcount', 'ref' or 'phase'

Details

The locationplot methods visualises how fractions are distributed over a larger region of genes on one chromosome. It takes an ASEset object as well as additional information on plot type (see [barplot](#)), strand type (see [getAlleleCounts](#)), colouring, as well as annotation. The annotation is taken either from the bioconductor OrgDb-sets, the TxDb sets or both. It is obviously important to make sure that the genome build used is the same as used in aligning the RNA-seq data.

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [ASEset](#) class which the locationplot function can be called up on.

Examples

```

data(ASEset)
locationplot(ASEset)

#SNPs are plotted in the order in which they are found.
#This can be sorted according to location as follows:
locationplot(ASEset[order(start(rowRanges(ASEset))),])

#for ASEsets with fewer SNPs the 'count' type plot is
# useful for detailed visualization.
locationplot(ASEset,type='count',strand='*')

```

```

ASEset-scanForHeterozygotes
      scanForHeterozygotes

```

Description

Identifies the positions of SNPs found in BamGR reads.

Usage

```

scanForHeterozygotes(BamList, ...)

## S4 method for signature 'GAlignmentsList'
scanForHeterozygotes(BamList,
  minimumReadsAtPos = 20, maximumMajorAlleleFrequency = 0.9,
  minimumMinorAlleleFrequency = 0.1, minimumBiAllelicFrequency = 0.9,
  verbose = TRUE, ...)

```

Arguments

BamList	A GAlignmentsList object
...	argument to pass on
minimumReadsAtPos	minimum number of reads required to call a SNP at a given position
maximumMajorAlleleFrequency	maximum frequency allowed for the most common allele. Setting this parameter lower will minimise the SNP calls resulting from technical read errors, at the cost of missing loci with potential strong ASE
minimumMinorAlleleFrequency	minimum frequency allowed for the second most common allele. Setting this parameter higher will minimise the SNP calls resulting from technical read errors, at the cost of missing loci with potential strong ASE

minimumBiAllelicFrequency	minimum frequency allowed for the first and second most common allele. Setting a Lower value for this parameter will minimise the identification of loci with three or more alleles in one sample. This is useful if sequencing errors are suspected to be common.
verbose	logical indicating if process information should be displayed

Details

This function scans all reads stored in a `GAlignmentsList` for possible heterozygote positions. The user can balance the sensitivity of the search by modifying the `minimumReadsAtPos`, `maximumMajorAlleleFrequency` and `minimumBiAllelicFrequency` arguments.

Value

`scanForHeterozygotes` returns a `GRanges` object with the SNPs for the `BamList` object that was used as input.

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [getAlleleCounts](#) which is a function that count the number of reads overlapping a site.

Examples

```
data(reads)
s <- scanForHeterozygotes(reads, verbose=FALSE)
```

ASEset.sim

ASEset.sim object

Description

ASEset with simulated data with SNPs within the first 200bp of chromosome 17, which is required to have example data for the `refAllele` function.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
##load eample data (Not Run)
#data(ASEset.sim)
```

ASEsetFromBam	<i>ASEset from bam file</i>
---------------	-----------------------------

Description

count alleles and create an ASEset direct from bam file instead of reading into R first.

Usage

```
ASEsetFromBam(gr, ...)  
  
## S4 method for signature 'GRanges'  
ASEsetFromBam(gr, pathToDir, PE = TRUE,  
  flagsMinusStrand = c(83, 163), flagsPlusStrand = c(99, 147),  
  strandUnknown = FALSE, ...)
```

Arguments

gr	GenomicRanges of SNPs to create ASEset for
...	passed on to ASEsetFromBam function
pathToDir	Directory of bam files with index in same directory
PE	if paired end or not (default: TRUE)
flagsMinusStrand	flags that mark reads coming from minus strand
flagsPlusStrand	flags that mark reads coming from plus strand
strandUnknown	default: FALSE

Details

counts the alleles in a bam file based on GRanges positions.

Author(s)

Jesper R. Gadin

Examples

```
data(GRvariants)  
gr <- GRvariants  
  
##no execution at the moment  
#pathToDir <- system.file('inst/extdata/ERP000101_subset', package='AllelicImbalance')  
#a <- ASEsetFromBam(gr, pathToDir)
```

barplot-lattice-support

lattice barplot inner functions for ASEset objects

Description

Generates lattice barplots for ASEset objects. Two levels of plotting detail are provided: a detailed barplot of read counts by allele useful for fewer samples and SNPs, and a less detailed barplot of the fraction of imbalance, useful for more samples and SNPs.

Usage

```
barplotLatticeFraction(identifier, ...)
```

```
barplotLatticeCounts(identifier, ...)
```

Arguments

`identifier`, the single snp name to plot
... used to pass on variables

Details

`filter.pValue.fraction` is intended to remove p-value annotation with very large difference in frequency, which could just be a sequencing mistake. This is to avoid p-values like $1e-235$ or similar.

`sampleColourUser` specified colours, either given as named colours ('red', 'blue', etc) or as hexadecimal code. Can be either length 1 for all samples, or else of a length corresponding to the number of samples for individual colouring.

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [ASEset](#) class which the barplot function can be called up on.

Examples

```
a <- ASEset
name <- rownames(a)[1]

barplotLatticeFraction(identifier=name, x=a, astrand="+")
barplotLatticeCounts(identifier=name, x=a, astrand="+")
```

binom.test	<i>binomial test</i>
------------	----------------------

Description

Performs a binomial test on an ASEset object.

Usage

```
## S4 method for signature 'ASEset'  
binom.test(x, n = "*")
```

Arguments

x	ASEset object
n	strand option

Details

the test can only be applied to one strand at the time.

Value

binom.test returns a matrix

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [chisq.test](#) which is another test that can be applied on an [ASEset](#) object.

Examples

```
#load example data  
data(ASEset)  
  
#make a binomial test  
binom.test(ASEset, '*')
```

boxplot	<i>boxplots</i>
---------	-----------------

Description

uses base graphics box plot

Usage

```
## S4 method for signature 'ReferenceBias'
boxplot(x, strand = "*", labels.axis = TRUE, ...)
```

Arguments

x	ReferenceBias object
strand	'+', '-' or '*'
labels.axis	logical
...	arguments to forward to internal boxplots function

Details

The boxplot will show the density over frequencies for each sample

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data

data("ReferenceBias")
boxplot(ReferenceBias)
```

chisq.test	<i>chi-square test</i>
------------	------------------------

Description

Performs a chisq.test on an ASEset object.

Usage

```
## S4 method for signature 'ASEset'
chisq.test(x, y = "*")
```


Arguments

x	ASEset object
y	strand option

Details

The test is performed on one strand in an ASEset object.

Value

chisq.test returns a matrix with the chisq.test P-value for each SNP and sample

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [binom.test](#) which is another test that can be applied on an [ASEset](#) object.

Examples

```
#load example data
data(ASEset)

#make a chi-square test on default non-stranded strand
chisq.test(ASEset)
```

countAllelesFromBam *alleleCounts from bam file*

Description

count alleles before creating ASEset.

Usage

```
countAllelesFromBam(gr, ...)

## S4 method for signature 'GRanges'
countAllelesFromBam(gr, pathToDir, flag = NULL,
  scanBamFlag = NULL, return.class = "array", verbose = TRUE)
```

Arguments

gr	GRanges that contains SNPs of interest
...	arguments to pass on
pathToDir	path to directory of bam files
flag	specify one flag to use as filter, default is no filtering. allowed flags are 99, 147, 83 and 163
scanBamFlag	set a custom flag to use as filter
return.class	type of class for the returned object
verbose	makes function more talkative

Details

counts the alleles in a bam file based on GRanges positions.

Author(s)

Jesper R. Gadin

Examples

```
data(GRvariants)
gr <- GRvariants

##not run at the moment
#pathToDir <- system.file('inst/extdata/ERP000101_subset', package='AllelicImbalance')
#ar <- countAllelesFromBam(gr, pathToDir)
```

coverageMatrixListFromGAL

coverage matrix of GAlignmentsList

Description

Get coverage per nucleotide for reads covering a region

Usage

```
coverageMatrixListFromGAL(BamList, ...)

## S4 method for signature 'GAlignmentsList'
coverageMatrixListFromGAL(BamList, strand = "*",
  ignore.empty.bam.row = TRUE)
```

Arguments

BamList	GAlignmentsList containing reads over the region to calculate coverage
...	arguments to pass on
strand	strand has to be '+' or '-'
ignore.empty.bam.row	argument not in use atm

Details

a convenience function to get the coverage from a list of reads stored in GAlignmentsList, and returns by default a list with one matrix, and information about the genomic start and stop positions.

Author(s)

Jesper R. Gadin

Examples

```
r <- reads
seqlevels(r) <- '17'
covMatList <- coverageMatrixListFromGAL(BamList=r, strand='+')
```

defaultMapBias	<i>Generate default mapbias from genotype</i>
----------------	---

Description

Create mapbias array from genotype matrix requires genotype information

Usage

```
defaultMapBias(x, ...)

## S4 method for signature 'ASEset'
defaultMapBias(x)
```

Arguments

x	ASEset object
...	internal arguments

Details

Default mapbias will be 0.5 for bi-allelic snps and 1 for homozygots. For genotypes with NA, 0.5 will be placed on all four alleles. Therefore tri-allelic can not be used atm. Genotype information has to be placed in the genotype(x) assay.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data
data(ASEset.sim)

fasta <- system.file('extdata/hg19.chr17.subset.fa', package='AllelicImbalance')
refAllele(ASEset.sim,fasta=fasta)
a <- refAllele(ASEset.sim,fasta=fasta)
```

defaultPhase	<i>defaultPhase</i>
--------------	---------------------

Description

used to populate the phase slot in an ASEset object

Usage

```
defaultPhase(i, ...)

## S4 method for signature 'numeric'
defaultPhase(i, j, ...)
```

Arguments

i	number of rows
...	arguments to forward to internal functions
j	number of columns

Details

will set everything to 0

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
i <- 5
j <- 10
defaultPhase(i,j)
```

detectAI	<i>detectAI</i>
----------	-----------------

Description

detection of AllelicImbalance

Usage

```
detectAI(x, ...)
```

```
## S4 method for signature 'ASEset'
detectAI(x, return.class = "DetectedAI", strand = "*",
  threshold.frequency = 0, threshold.count.sample = 1,
  threshold.delta.frequency = 0, threshold.pvalue = 0.05,
  inferGenotype = FALSE, random.ref = FALSE, function.test = "binom.test",
  verbose = TRUE, gc = FALSE)
```

Arguments

x	ASEset
...	internal arguments
return.class	class to return (atm only class 'logical')
strand	strand to infer from
threshold.frequency	least fraction to classify (see details)
threshold.count.sample	least amount of counts to try to infer allele
threshold.delta.frequency	minimum of frequency difference from 0.5 (or mapbias adjusted value)
threshold.pvalue	pvalue over this number will be filtered out
inferGenotype	infer genotypes based on count data in ASEset object
random.ref	set the reference as random if you dont know. Affects interpretation of results.
function.test	At the moment the only available option is 'binomial.test'
verbose	makes function more talkative
gc	use garbage collection when possible to save space

Details

threshold.frequency is the least fraction needed to classify as bi tri or quad allelic SNPs. If 'all' then all of bi tri and quad allelic SNPs will use the same threshold. Everything under the treshold will be regarded as noise. 'all' will return a matrix with snps as rows and uni bi tri and quad will be

columns. For this function Anything that will return TRUE for tri-allelic will also return TRUE for uni and bi-allelic for the same SNP an Sample.

return.type 'ref' return only AI when reference allele is more expressed. 'alt' return only AI when alternative allele is more expressed or 'all' for both 'ref' and 'alt' alleles. Reference allele is the one present in the reference genome on the forward strand.

threshold.delta.frequency and function.test will use the value in mapBias(x) as expected value.

function.test will use the two most expressed alleles for testing. Make therefore sure there are no tri-allelic SNPs or somatic mutations among the SNPs in the ASEset.

inferGenotype(), set TRUE it should be used with as much samples as possible. If you split up the samples and run detectAI() on each sample separately, please make sure you have inferred the genotypes in before hand, alternatively used the genotypes detected by another variantCaller or chip-genotypes. Use ONLY biallelic genotypes.

Author(s)

Jesper R. Gadin

Examples

```
#load example data
data(ASEset)
a <- ASEset

dai <- detectAI(a)
```

DetectedAI-class	<i>DetectedAI class</i>
------------------	-------------------------

Description

Object that holds results from AI detection.

Usage

```
referenceFrequency(x, ...)

## S4 method for signature 'DetectedAI'
referenceFrequency(x, return.class = "array")

thresholdFrequency(x, ...)

## S4 method for signature 'DetectedAI'
thresholdFrequency(x, return.class = "array")

thresholdCountSample(x, ...)

## S4 method for signature 'DetectedAI'
```

```
thresholdCountSample(x, return.class = "array")

thresholdDeltaFrequency(x, ...)

## S4 method for signature 'DetectedAI'
thresholdDeltaFrequency(x, return.class = "array")

thresholdPvalue(x, ...)

## S4 method for signature 'DetectedAI'
thresholdPvalue(x, return.class = "array")
```

Arguments

x	ASEset object or list of ASEsets
...	pass arguments to internal functions
return.class	type of class returned eg. "list or ""array".

Details

The DetectedAI-class contains

Value

An object of class DetectedAI containing logics for thresholds of interest.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
data(ASEset)
a <- ASEset
dai <- detectAI(a)

#summary(gba)
#write.tables(dai)
```

fractionPlotDf

Plot Dataframe

Description

Summarizes information to ease creating plots

Usage

```
fractionPlotDf(x, snp, strand = "*", top.fraction.criteria = "maxcount",
  ...)

## S4 method for signature 'ASEset'
fractionPlotDf(x, snp, strand = "*",
  top.fraction.criteria = "maxcount", ...)
```

Arguments

x	ASEset
snp	rownames identifier for ASEset or row number
strand	'+', '-' or '*'
top.fraction.criteria	'maxcount', 'ref' or 'phase'
...	arguments to forward to internal functions

Details

Main purpose is to reduce the amount of overall code and ease maintenance.

top.fraction.criteria can take three options, maxcount, ref and phase. The top allele will be every second row in the data frame, with start from row 2. The maxcount argument will put the allele with most reads on top of the bivariate fraction. Similarly the ref argument will put always the reference allele on top. The phase arguments puts the maternal phase always on top. The top.fraction.criteria for the ref or phase arguments requires that both ref and alt is set in mcols(ASEset).

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#test on example ASEset
data(ASEset)
a <- ASEset
df <- fractionPlotDf(a, 1, strand="+")
```

 gba

global analysis wrapper

Description

A wrapper to make a global analysis based on paths for BAM, VCF and GFF files

Usage

```
gba(pathBam, ...)  
  
## S4 method for signature 'character'  
gba(pathBam, pathVcf, pathGFF = NULL, verbose)
```

Arguments

pathBam	path to bam file
...	arguments to pass on
pathVcf	path to vcf file
pathGFF	path to gff file
verbose	makes function more talkative

Author(s)

Jesper R. Gadin

Examples

```
#empty as function doesn't exist
```

genofilters	<i>genotype filter methods</i>
-------------	--------------------------------

Description

useful genotype filters

Arguments

x	ASEset object
---	---------------

Details

These filters are called upon ASEset objects

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data  
data(ASEset)  
a <- ASEset  
genotype(a) <- inferGenotypes(a)  
hets <- hetFilt(a)
```

getAlleleCounts	<i>snp count data</i>
-----------------	-----------------------

Description

Given the positions of known SNPs, this function returns allele counts from a BamGRL object

Usage

```
getAlleleCounts(BamList, ...)
```

```
## S4 method for signature 'GAlignmentsList'
getAlleleCounts(BamList, GRvariants, strand = "*",
  return.class = "list", verbose = TRUE, ...)
```

Arguments

BamList	A GAlignmentsList object or GRangesList object containing data imported from a bam file
...	parameters to pass on
GRvariants	A GRanges object that contains positions of SNPs to retrieve
strand	A length 1 character with value '+', '-', or '*'. This argument determines if getAlleleCounts will retrieve counts from all reads, or only from reads marked as '+', '-' or '*' (unknown), respectively.
return.class	'list' or 'array'
verbose	Setting verbose=TRUE makes function more talkative

Details

This function is used to retrieve the allele counts from specified positions in a set of RNA-seq reads. The BamList argument will typically have been created using the impBamGAL function on bam-files. The GRvariants is either a GRanges with user-specified locations or else it is generated through scanning the same bam-files as in BamList for heterozygote locations (e.g. using scanForHeterozygotes). The GRvariants will currently only accept locations having width=1, corresponding to bi-allelic SNPs. In the strand argument, specifying '*' is the same as retrieving the sum count of '+' and '-' reads (and unknown strand reads in case these are found in the bam file). '*' is the default behaviour and can be used when the RNA-seq experiments strand information is not available.

Value

getAlleleCounts returns a list of several data.frame objects, each storing the count data for one SNP.

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [scanForHeterozygotes](#) which is a function to find possible heterozygote sites in a `GAlignmentsList` object

Examples

```
#load example data
data(reads)
data(GRvariants)

#get counts at the three positions specified in GRvariants
alleleCount <- getAlleleCounts(BamList=reads,GRvariants,
strand='*')

#if the reads had contained stranded data, these two calls would
#have given the correct input objects for getAlleleCounts
alleleCountPlus <- getAlleleCounts(BamList=reads,GRvariants,
strand='+')
alleleCountMinus <- getAlleleCounts(BamList=reads,GRvariants,
strand='-')
```

getAreaFromGeneNames *Get Gene Area*

Description

Given a character vector with genesymbols and an `OrgDb` object, this function returns a `GRanges` giving the coordinates of the genes.

Usage

```
getAreaFromGeneNames(genesymbols, ...)

## S4 method for signature 'character'
getAreaFromGeneNames(genesymbols, OrgDb, leftFlank = 0,
  rightFlank = 0, na.rm = FALSE, verbose = TRUE)
```

Arguments

<code>genesymbols</code>	A character vector that contains genesymbols of genes from which we wish to retrieve the coordinates
<code>...</code>	arguments to pass on
<code>OrgDb</code>	An <code>OrgDb</code> object containing gene annotation
<code>leftFlank</code>	A integer specifying number of additional nucleotides before the genes
<code>rightFlank</code>	A integer specifying number of additional nucleotides after the genes
<code>na.rm</code>	A boolean removing genes that returned NA from the annotation
<code>verbose</code>	Setting <code>verbose=TRUE</code> makes function more talkative

Details

This function is a convenience function that can be used to determine which genomic coordinates to specify to e.g. `impBamGAL` when retrieving reads.

The function cannot handle genes that do not exist in the annotation. To remove these please set the `na.rm=TRUE`.

Value

`getAreaFromGeneNames` returns a `GRanges` object with genomic coordinates around the specified genes

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data
data(ASEset)

#get counts at the three positions specified in GRvariants
library(org.Hs.eg.db )
searchArea<-getAreaFromGeneNames(c('PAX8','TLR7'), org.Hs.eg.db)
```

```
getDefaultMapBiasExpMean
      Map Bias
```

Description

an allele frequency array

Usage

```
getDefaultMapBiasExpMean(alleleCountList, ...)

getDefaultMapBiasExpMean3D(alleleCountList, ...)

## S4 method for signature 'list'
getDefaultMapBiasExpMean(alleleCountList)

## S4 method for signature 'ANY'
getDefaultMapBiasExpMean3D(alleleCountList)
```

Arguments

alleleCountList
A GRangesList object containing read information
... parameters to pass on

Details

This function will assume there is no bias that comes from the mapping of reads, and therefore create a matrix with expected frequency of 0.5 for each allele.

Value

getDefaultMapBiasExpMean returns a matrix with a default expected mean of 0.5 for every element.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data
data(ASEset)
#access SnpAflist
alleleCountList <- alleleCounts(ASEset)
#get default map bias exp mean
matExpMean <- getDefaultMapBiasExpMean(alleleCountList)
```

getSnpIdFromLocation *Get rsIDs from locations of SNP*

Description

Given a GRanges object of SNPs and a SNPlocs annotation, this function attempts to replace the names of the GRanges object entries with rs-IDs.

Usage

```
getSnpIdFromLocation(GR, ...)
```

S4 method for signature 'GRanges'

```
getSnpIdFromLocation(GR, SNPloc, return.vector = FALSE,
  verbose = TRUE)
```

Arguments

GR	A GRanges that contains positions of SNPs to look up
...	arguments to pass on
SNPloc	A SNPlocs object containing information on SNP locations (e.g. SNPlocs.Hsapiens.dbSNP.xxxxxxxx)
return.vector	Setting return.vector=TRUE returns vector with rsIds
verbose	Setting verbose=TRUE makes function more talkative

Details

This function is used to try to identify the rs-IDs of SNPs in a GRanges object.

Value

getSnpIdFromLocation returns the same GRanges object it was given with, but with updated with rs.id information.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data
data(ASEset)

#get counts at the three positions specified in GRvariants
if(require(SNPlocs.Hsapiens.dbSNP.20120608)){
  updatedGRanges<-getSnpIdFromLocation(rowRanges(ASEset), SNPlocs.Hsapiens.dbSNP.20120608)
  rowRanges(ASEset)<-updatedGRanges
}
```

GlobalAnalysis-class *GlobalAnalysis class*

Description

Object that holds results from a global AI analysis including reference bias estimations and AI detection.

Arguments

x	ASEset object or list of ASEsets
TxDB	A transcriptDb object
...	pass arguments to internal functions

Details

The GlobalAnalysis-class contains summaries and "pre-configured and pre-calculated lattice plots" needed to create an AI-report

Value

An object of class GlobalAnalysis containing all data to make report.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
data(ASEset)
#a <- ASEset
#gba <- gba(a)

#report(gba)
#write.tables(gba)
#graphs(gba)
#as.list(gba)
```

GRvariants

GRvariants object

Description

this data is a GRanges object that contains the ranges for three example SNPs.

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [reads](#) which is another example object

Examples

```
#load example data
data(GRvariants)
```

`histplot`*histogram plots*

Description

uses base graphics hist plot

Usage

```
## S4 method for signature 'ReferenceBias'  
hist(x, strand = "*", ...)  
  
## S4 method for signature 'ASEset'  
hist(x, strand = "*", type = "mean", log = 1, ...)
```

Arguments

<code>x</code>	ReferenceBias object or ASEset object
<code>strand</code>	'+', '-' or '*'
<code>...</code>	arguments to forward to interal boxplots function
<code>type</code>	'mean' (only one option atm)
<code>log</code>	an integer to log each value (integer 10 for log10)

Details

The histogram will show the density over frequencies for each sample

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
##load example data  
  
#data(ASEset)  
#a <- ASEset  
#hist(a)
```

implodeList.old	<i>implode list of arguments into environment</i>
-----------------	---

Description

apply on list of variables to be put in the local environment

Usage

```
implodeList.old(x)
```

Arguments

x list of variables

Details

help the propagation of e.g. graphical paramters

Author(s)

Jesper R. Gadin

Examples

```
lst <- list(hungry='yes', thirsty='no')
implodeList.old(lst)
#the check ls()
ls()
```

import-bam	<i>Import Bam</i>
------------	-------------------

Description

Imports a specified genomic region from a bam file using a GRanges object as search area.

Usage

```
impBamGAL(UserDir, ...)

## S4 method for signature 'character'
impBamGAL(UserDir, searchArea, files = NULL,
          XStag = FALSE, verbose = TRUE, ...)
```

Arguments

UserDir	The relative or full path of folder containing bam files.
...	arguments to pass on
searchArea	A <code>GenomicRanges</code> object that contains the regions of interest
files	use character vector to specify one or more files to import. The default imports all bam files from the directory.
XStag	Setting <code>XStag=TRUE</code> stores the strand specific information in the <code>mcols</code> slot 'XS'
verbose	makes the function more talkative.

Details

If the sequence data is strand-specific you may want to set `XStag=TRUE`. The strand specific information has then to be stored in the meta columns with column name 'XS'. If the aligner did not set the XS-tag and the data is strand-specific it is still possible to infer the strand from the bit flags after importing the reads to R. Depending on the strand-specific protocol different combinations of the flags will have to be used. For illumina fr-secondstrand, 83 and 163 are minus strand reads and 99 and 147 are plus strand reads.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#Declare searchArea
searchArea <- GRanges(seqnames=c('17'), ranges=IRanges(79478301,79478361))

#Relative or full path
pathToFiles <- system.file('extdata/ERP000101_subset', package='AllelicImbalance')

#all files in directory
reads <- impBamGAL(pathToFiles,searchArea,verbose=FALSE)
#specified files in directory
reads <- impBamGAL(pathToFiles,searchArea,
files=c("ERR009160.bam", "ERR009167.bam"),verbose=FALSE)
```

import-bam-2

Import Bam-2

Description

Imports bla bal bal a specified genomic region from a bam file using a `GenomicRanges` object as search area.

Usage

```
impBamGRL.old(UserDir, searchArea, verbose = TRUE)
```

Arguments

UserDir	The relative or full path of folder containing bam files.
searchArea	A GenomicRanges object that contains the regions of interest
verbose	Setting verbose=TRUE gives details of procedure during function run.

Details

These functions are right on tahea wrappers to import bam files into R and store them into either GRanges, GAlignments or GappedAlignmentpairs objects.

It is recommended to use the impBamGAL() which takes information of gaps into account. It is also possible to use the other variants as well, but then pre-filtering becomes important keys to understand because gapped, intron-spanning reads will cause problems. This is because the GRanges objects can not handle if gaps are present and will then give a wrong result when calculating the allele (SNP) count table.

Value

impBamGRL returns a GRangesList object containing the RNA-seq reads in the region defined by the searchArea argument. impBamGAL returns a list with GAlignments objects containing the RNA-seq reads in the region defined by the searchArea argument. funImpBamGAPL returns a list with GappedAlignmentPairs object containing the RNA-seq reads in the region defined by the searchArea argument.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#Declare searchArea
searchArea <- GRanges(seqnames=c('17'), ranges=IRanges(79478301,79478361))

#Relative or full path
pathToFiles <- system.file('extdata/ERP000101_subset', package='AllelicImbalance')
```

import-bcf

Import Bcf Selection

Description

Imports a selection of a bcf file or files specified by a GenomicRanges object as search area.

Usage

```
impBcfGRL(UserDir, ...)  
  
## S4 method for signature 'character'  
impBcfGRL(UserDir, searchArea = NULL, verbose = TRUE,  
  ...)  
  
impBcfGR(UserDir, ...)  
  
## S4 method for signature 'character'  
impBcfGR(UserDir, searchArea = NULL, verbose = TRUE,  
  ...)
```

Arguments

UserDir	The relative or full path of folder containing bam files.
...	parameters to pass on
searchArea	A GenomicRanges object that contains the regions of interest
verbose	Setting verbose=TRUE gives details of the procedure during function run.

Details

A wrapper to import bcf files into R in the form of GenomicRanges objects.

Value

BcfImpGRL returns a GRangesList object. BcfImpGR returns one GRanges object of all unique entries from one or more bcf files.

Note

Make sure there is a complementary index file *.bcf.bci for each bcf file in UserDir. If there is not, then the functions impBcfGRL and impBcfGR will try to create them.

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The `impBamGRL` for importing bam files
- The [getAlleleCounts](#) for how to get allele(SNP) counts
- The [scanForHeterozygotes](#) for how to find possible heterozygote positions

Examples

```
#Declare searchArea
searchArea <- GRanges(seqnames=c('17'), ranges=IRanges(79478301,79478361))

#Relative or full path
pathToFiles <- system.file('extdata/ERP000101_subset', package='AllelicImbalance')

#import
reads <- impBcfGRL(pathToFiles, searchArea, verbose=FALSE)
```

inferAlleles *inference of SNPs of ASEset*

Description

inference of SNPs

Usage

```
inferAlleles(x, strand = "*", return.type = "bi", threshold.frequency = 0,
             threshold.count.sample = 1, inferOver = "eachSample", allow.NA = FALSE)
```

Arguments

x	ASEset
strand	strand to infer from
return.type	'uni' 'bi' 'tri' 'quad' 'all'
threshold.frequency	least fraction to classify (see details)
threshold.count.sample	least amount of counts to try to infer allele
inferOver	'eachSample' or 'allSamples'
allow.NA	treat NA as zero when TRUE

Details

threshold.frequency is the least fraction needed to classify as bi tri or quad allelic SNPs. If 'all' then all of bi tri and quad allelic SNPs will use the same threshold. Everything under the threshold will be regarded as noise. 'all' will return a matrix with snps as rows and uni bi tri and quad will be columns. For this function Anything that will return TRUE for tri-allelic will also return TRUE for uni and bi-allelic for the same SNP an Sample.

Author(s)

Jesper R. Gadin

Examples

```
data(ASEset)
i <- inferAlleles(ASEset)
```

inferAltAllele	<i>inferAltAllele</i>
----------------	-----------------------

Description

inference of the alternate allele based on count data

Arguments

x	matrix see examples
return.class	class of returned object
...	arguments to forward to internal functions

Details

The inference essentially ranks all alleles and the most expressed allele not declared as reference will be inferred as the alternative allele. At the moment only inference of bi-allelic alternative alleles are available.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load data
data(ASEset)

inferAltAllele(ASEset)
```

inferGenotypes	<i>inference of genotypes from ASEset count data</i>
----------------	--

Description

inference of genotypes

Usage

```
inferGenotypes(x, strand = "*", return.class = "matrix",
  return.allele.allowed = "bi", threshold.frequency = 0,
  threshold.count.sample = 1)
```

Arguments

x ASEset
strand strand to infer from
return.class 'matrix' or 'vector'
return.allele.allowed
vector with 'bi' 'tri' or 'quad'. 'uni' Always gets returned
threshold.frequency
least fraction to classify (see details)
threshold.count.sample
least amount of counts to try to infer allele

Details

Often necessary information to link AI to SNPs outside coding region

Author(s)

Jesper R. Gadin

Examples

```
data(ASEset)
g <- inferGenotypes(ASEset)
```

initialize-ASEset *Initialize ASEset*

Description

Functions to construct ASEset objects

Usage

```
ASEsetFromCountList(rowRanges, countListUnknown = NULL,
  countListPlus = NULL, countListMinus = NULL, colData = NULL,
  mapBiasExpMean = NULL, phase = NULL, verbose = FALSE, ...)

ASEsetFromArrays(rowRanges, countsUnknown = NULL, countsPlus = NULL,
  countsMinus = NULL, colData = NULL, mapBiasExpMean = NULL,
  phase = NULL, verbose = FALSE, ...)
```

Arguments

rowRanges	A GenomicRanges object that contains the variants of interest
countListUnknown	A list where each entry is a matrix with allele counts as columns and sample counts as rows
countListPlus	A list where each entry is a matrix with allele counts as columns and sample counts as rows
countListMinus	A list where each entry is a matrix with allele counts as columns and sample counts as rows
colData	A DataFrame object containing sample specific data
mapBiasExpMean	A 3D array where the SNPs are in the 1st dimension, samples in the 2nd dimension and variants in the 3rd dimension.
phase	A matrix or an array containing phase information.
verbose	Makes function more talkative
...	arguments passed on to SummarizedExperiment constructor
countsUnknown	An array containing the countinformation
countsPlus	An array containing the countinformation
countsMinus	An array containing the countinformation

Details

The resulting ASEset object is based on the SummarizedExperiment, and will therefore inherit the same accessors and ranges operations.

If both countListPlus and countListMinus are given they will be used to calculate countListUnknown, which is the sum of the plus and minus strands.

countListPlus, countListMinus and countListUnknown are i.e. the outputs from the [getAlleleCounts](#) function.

Value

ASEsetFromCountList returns an ASEset object.

Note

ASEsetFromCountList requires the same input data as an SummarizedExperiment, but with minimum one assay for the allele counts.

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [SummarizedExperiment](#) for ranges operations.

Examples

```

#make example alleleCountListPlus
set.seed(42)
countListPlus <- list()
snps <- c('snp1', 'snp2', 'snp3', 'snp4', 'snp5')
for(snp in snps){
  count<-matrix(rep(0,16),ncol=4,dimnames=list(
    c('sample1', 'sample2', 'sample3', 'sample4'),
    c('A', 'T', 'G', 'C')))
  #insert random counts in two of the alleles
  for(allele in sample(c('A', 'T', 'G', 'C'),2)){
    count[,allele]<-as.integer(rnorm(4,mean=50,sd=10))
  }
  countListPlus[[snp]] <- count
}

#make example alleleCountListMinus
countListMinus <- list()
snps <- c('snp1', 'snp2', 'snp3', 'snp4', 'snp5')
for(snp in snps){
  count<-matrix(rep(0,16),ncol=4,dimnames=list(
    c('sample1', 'sample2', 'sample3', 'sample4'),
    c('A', 'T', 'G', 'C')))
  #insert random counts in two of the alleles
  for(allele in sample(c('A', 'T', 'G', 'C'),2)){
    count[,allele]<-as.integer(rnorm(4,mean=50,sd=10))
  }
  countListMinus[[snp]] <- count
}

#make example rowRanges
rowRanges <- GRanges(
  seqnames = Rle(c('chr1', 'chr2', 'chr1', 'chr3', 'chr1')),
  ranges = IRanges(1:5, width = 1, names = head(letters,5)),
  snp = paste('snp',1:5,sep='')
)

#make example colData
colData <- DataFrame(Treatment=c('ChIP', 'Input', 'Input', 'ChIP'),
  row.names=c('ind1', 'ind2', 'ind3', 'ind4'))

#make ASEset
a <- ASEsetFromCountList(rowRanges, countListPlus=countListPlus,
  countListMinus=countListMinus, colData=colData)

```

initialize-DetectedAI *Initialize DetectedAI*

Description

Functions to construct DetectedAI objects

Usage

```
DetectedAIFromArray(x = "ASEset", strand = "*",
  reference.frequency = NULL, threshold.frequency = NULL,
  threshold.count.sample = NULL, threshold.delta.frequency = NULL,
  threshold.pvalue = NULL, threshold.frequency.names = NULL,
  threshold.count.sample.names = NULL,
  threshold.delta.frequency.names = NULL, threshold.pvalue.names = NULL,
  ...)
```

Arguments

x	ASEset
strand	set strand to detectAI over "+","-","*"
reference.frequency	frequencies of reference alleles based allele counts
threshold.frequency	logical array for frequency thresholds
threshold.count.sample	logical array for per sample allele count thresholds
threshold.delta.frequency	logical array for delta frequency thresholds.
threshold.pvalue	logical array for pvalue thresholds (max 1, min 0)
threshold.frequency.names	character vector
threshold.count.sample.names	character vector
threshold.delta.frequency.names	character vector
threshold.pvalue.names	character vector
...	internal arguments

Details

produces a class container for reference bias calculations

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
data(ASEset)
a <- ASEset
dai <- detectAI(a)
```

initialize-GlobalAnalysis
Initialize GlobalAnalysis

Description

Functions to construct GlobalAnalysis objects

Usage

```
GAnalysis(x = "ASEset", ...)
```

Arguments

x	ASEset
...	internal arguments

Details

produces a class container for a global analysis

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
data(ASEset)
a <- ASEset
# gba <- gba(a)
```

initialize-ReferenceBias
Initialize ReferenceBias

Description

Functions to construct ReferenceBias objects

Usage

```
RBias(x = "ASEset", ...)
```

Arguments

x	ASEset
...	internal arguments

Details

produces a class container for reference bias calculations

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
data(ASEset)
a <- ASEset
refbiasObject <- RBias(a)
```

legendBarplot	<i>add legend to AllelicImbalance barplot</i>
---------------	---

Description

adds a very customizable legend function for AllelicImbalance barplots.

Usage

```
legendBarplot(lowerLeftCorner, size, rownames, colnames, boxsize = 1,
  boxspace = 1, fgCol, bgCol, ylegendPos = 1, xlegendPos = 0.96,
  cex = 1)
```

Arguments

lowerLeftCorner	
size	position of the plot to add legend to (default c(0,0))
rownames	scale the plot, default is 1
colnames	rownames in legend
boxsize	colnames in legend
boxspace	size of each box fill
fgCol	space inbetween the box fill
bgCol	color for allele1
ylegendPos	color for allele2
xlegendPos	placement of the legend within the plot for y
cex	placement of the legend within the plot for x
	size of legend text

Details

the function is preferably called from within the AllelicImbalance barplot method.

Author(s)

Jesper R. Gadin

Examples

```
#code placeholders
#< create a barplot with legend >
#< add legend >
```

makeMaskedFasta	<i>makes masked fasta reference</i>
-----------------	-------------------------------------

Description

Replaces all selected positions in a fasta file with the character N

Usage

```
makeMaskedFasta(fastaIn, ...)

## S4 method for signature 'character'
makeMaskedFasta(fastaIn, fastaOut, posToReplace,
  splitOnSeqlevels = TRUE, verbose = TRUE)
```

Arguments

fastaIn	character string of the path for the fasta file to be used
...	arguments to pass on
fastaOut	character string of the path for the masked fasta file (no extension)
posToReplace	GRanges object with the genomic ranges to replace
splitOnSeqlevels	write on file for each seqlevel to save memory
verbose	makes function more talkative

Author(s)

Jesper R. Gadin

Examples

```
data(ASEset.sim)
gr <- rowRanges(ASEset.sim)
fastaIn <- system.file('extdata/hg19.chr17.subset.fa', package='AllelicImbalance')
makeMaskedFasta(fastaIn=fastaIn, fastaOut="fastaOut", posToReplace=gr)
```

mapBiasRef	<i>mapBias for reference allele</i>
------------	-------------------------------------

Description

Create a matrix of bias for the reference allele

Usage

```
mapBiasRef(x, ...)  
  
## S4 method for signature 'ASEset'  
mapBiasRef(x)
```

Arguments

x	ASEset object
...	internal arguments

Details

select the expected frequency for the reference allele

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data  
data(ASEset)  
a <- ASEset  
  
mat <- mapBiasRef(a)
```

phaseArray2Matrix	<i>phaseArray2Matrix</i>
-------------------	--------------------------

Description

used to convert the phase from the visually friendly matrix to array.

Usage

```
phaseArray2Matrix(x, ...)

## S4 method for signature 'array'
phaseArray2Matrix(x, ...)
```

Arguments

```
x          array see examples
...        arguments to forward to internal functions
```

Details

A more effective way of store the phase data in the ASEset object

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load data
data(ASEset)
a <- ASEset

#example phase matrix
p1 <- matrix(sample(c(1,0),replace=TRUE, size=nrow(a)*ncol(a)),nrow=nrow(a), ncol(a))
p2 <- matrix(sample(c(1,0),replace=TRUE, size=nrow(a)*ncol(a)),nrow=nrow(a), ncol(a))
p <- matrix(paste(p1,sample(c("|",",","/"), size=nrow(a)*ncol(a), replace=TRUE), p2, sep=""),
nrow=nrow(a), ncol(a))

ar <- phaseMatrix2Array(p)

#Convert back
mat <- phaseArray2Matrix(ar)
```

phaseMatrix2Array *phaseMatrix2Array*

Description

used to convert the phase from the visually friendly matrix to array.

Usage

```
phaseMatrix2Array(x, ...)

## S4 method for signature 'matrix'
phaseMatrix2Array(x, dimnames = NULL, ...)
```

Arguments

x matrix see examples
 ... arguments to forward to internal functions
 dimnames list with dimnames

Details

A more effective way of store the phase data in the ASEset object

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load data
data(ASEset)
a <- ASEset

#example phase matrix
p1 <- matrix(sample(c(1,0),replace=TRUE, size=nrow(a)*ncol(a)),nrow=nrow(a), ncol(a))
p2 <- matrix(sample(c(1,0),replace=TRUE, size=nrow(a)*ncol(a)),nrow=nrow(a), ncol(a))
p <- matrix(paste(p1,sample(c("|","|","/"), size=nrow(a)*ncol(a), replace=TRUE), p2, sep=""),
nrow=nrow(a), ncol(a))

ar <- phaseMatrix2Array(p)
```

randomRef

Random ref allele from genotype

Description

Create a vector of random reference alleles

Usage

```
randomRef(x, ...)
```

S4 method for signature 'ASEset'

```
randomRef(x, inferGenotypes = FALSE)
```

Arguments

x ASEset object
 ... internal arguments
 inferGenotypes infer genotypes from count matrix

Details

Randomly shuffles which of the two alleles for each genotype that is indicated as reference allele.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data
data(ASEset.sim)
a <- ASEset.sim

mcols(a)[["ref"]] <- randomRef(a, inferGenotypes=TRUE)
```

reads	<i>reads object</i>
-------	---------------------

Description

This data set corresponds to the BAM-file data import illustrated in the vignette. The data set consists of a chromosome 17 region from 20 RNA-seq experiments of HapMap samples.

Author(s)

Jesper R. Gadin, Lasse Folkersen

References

Montgomery SB et al. Transcriptome genetics using second generation sequencing in a Caucasian population. Nature. 2010 Apr 1;464(7289):773-7.

See Also

- The [GRvariants](#) which is another example object

Examples

```
##load eample data (Not Run)
#data(reads)
```

refAllele *Reference allele*

Description

Extract the allele based on SNP location from the reference fasta file

Usage

```
refAllele(x, fasta)
```

Arguments

x	ASEset object
fasta	path to fasta file, index should be located in the same folder

Details

The alleles will be placed in the rowRanges() meta column 'ref'

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data
data(ASEset.sim)

fasta <- system.file('extdata/hg19.chr17.subset.fa', package='AllelicImbalance')
a <- refAllele(ASEset.sim, fasta=fasta)
```

ReferenceBias-class *ReferenceBias class*

Description

Object that holds Reference bias information

Usage

```

refbiasByAnnotation(x, ...)

## S4 method for signature 'ReferenceBias'
refbiasByAnnotation(x, TxDb)

## S4 method for signature 'ReferenceBias'
frequency(x, return.class = "matrix",
  strand = "*")

hetPerSample(x, ...)

## S4 method for signature 'ReferenceBias'
hetPerSample(x, return.class = "vector",
  strand = "*")

hetPerSnp(x, ...)

## S4 method for signature 'ReferenceBias'
hetPerSnp(x, return.class = "vector",
  strand = "*")

```

Arguments

x	ReferenceBias object
...	pass arguments to internal functions
TxDb	A transcriptDb object
return.class	class to return
strand	'+', '-' or '*'

Details

Reference bias-class contain annotated fractions of the reference allele

Shortly the ReferenceBias-class most prominent purpose is quickly to be able to dispatch on methods like, 'plot', 'summary' and similar.

Value

An object of class ReferenceBias storing reference fractions.

Constructor

```

refBias(x = ASEset)
  Arguments:
  x an ASEset object

```

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
data(ASEset)
a <- ASEset
rb <- RBias(a)
```

ReferenceBias-show *genotype filter methods*

Description

useful genotype filters

Usage

```
## S4 method for signature 'ReferenceBias'
show(object)
```

Arguments

object ReferenceBias object

Details

These filters are called upon ASEset objects

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data
cat("there is no example data yet for this method")
```

refFraction	<i>reference fraction</i>
-------------	---------------------------

Description

The fractions for all heterozygote reference alleles

Usage

```
refFraction(x, ...)
```

Arguments

x	ASEset object
...	arguments to forward to internal functions

Details

Necessary to measure the effect of mapbias over heterozygous SNPs

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
#load example data
data(ASEset)
a <- ASEset
rf <- refFraction(a, strand="*")
```

regionSummary	<i>regionSummary</i>
---------------	----------------------

Description

Gives a summary of AI-consistency for a transcript

Usage

```
regionSummary(x, ...)

## S4 method for signature 'ASEset'
regionSummary(x, region, strand = "*", ...)
```

Arguments

x	ASEset object
...	arguments to forward to internal functions
region	GenomicRanges object to summarize over
strand	can be "+", "-" or "*"

Details

From a given set of e.g. transcripts exon ranges the function will return a summary for the sum of all exons. Phase information is required.

Author(s)

Jesper R. Gadin, Lasse Folkersen

Examples

```
data(ASEset)
a <- ASEset
# Add phase
set.seed(1)
p1 <- matrix(sample(c(1,0),replace=TRUE, size=nrow(a)*ncol(a)),nrow=nrow(a), ncol(a))
p2 <- matrix(sample(c(1,0),replace=TRUE, size=nrow(a)*ncol(a)),nrow=nrow(a), ncol(a))
p <- matrix(paste(p1,sample(c("|","|","/"), size=nrow(a)*ncol(a), replace=TRUE), p2, sep=""),
nrow=nrow(a), ncol(a))

phase(a) <- p

#add alternative allele information
mcols(a)[["alt"]] <- inferAltAllele(a)

# in this example every snp is on its own exon
txGR <- granges(a)
t <- regionSummary(a, txGR)
```

scanForHeterozygotes.old

scanForHeterozygotes-old

Description

Identifies the positions of SNPs found in BamGR reads.

Usage

```
scanForHeterozygotes.old(BamList, minimumReadsAtPos = 20,
  maximumMajorAlleleFrequency = 0.9, minimumBiAllelicFrequency = 0.9,
  maxReads = 15000, verbose = TRUE)
```

Arguments

BamList	A GAlignmentsList object
minimumReadsAtPos	minimum number of reads required to call a SNP at a given position
maximumMajorAlleleFrequency	maximum frequency allowed for the most common allele. Setting this parameter lower will minimise the SNP calls resulting from technical read errors, at the cost of missing loci with potential strong ASE
minimumBiAllelicFrequency	minimum frequency allowed for the first and second most common allele. Setting a Lower value for this parameter will minimise the identification of loci with three or more alleles in one sample. This is useful if sequencing errors are suspected to be common.
maxReads	max number of reads of one list-element allowed
verbose	logical indicating if process information should be displayed

Details

This function scans all reads stored in a GAlignmentsList for possible heterozygote positions. The user can balance the sensitivity of the search by modifying the minimumReadsAtPos, maximumMajorAlleleFrequency and minimumBiAllelicFrequency arguments.

Value

scanForHeterozygotes.old returns a GRanges object with the SNPs for the BamList object that was used as input.

Author(s)

Jesper R. Gadin, Lasse Folkersen

See Also

- The [getAlleleCounts](#) which is a function that count the number of reads overlapping a site.

Examples

```
data(reads)
s <- scanForHeterozygotes.old(reads, verbose=FALSE)
```

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