Package: REDseq (via r-universe)

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Type Package

Title Analysis of high-throughput sequencing data processed by restriction enzyme digestion

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Imports AnnotationDbi, graphics, IRanges (>= 1.13.5), stats, utils

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Description The package includes functions to build restriction enzyme cut site (RECS) map, distribute mapped sequences on the map with five different approaches, find enriched/depleted RECSs for a sample, and identify differentially enriched/depleted RECSs between samples.

License GPL (>=2)

Config/pak/sysreqs make libbz2-dev libicu-dev liblzma-dev libpng-dev libxml2-dev libssl-dev xz-utils zlib1g-dev

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`REDseq-package` *REDseq*

Description

REDSeq is a Bioconductor package for building genomic map of restriction enzyme sites REmap, assigning sequencing tags to RE sites using five different strategies, visualizing genome-wide distribution of differentially cut regions with the REmap as reference and the distance distribution of sequence tags to corresponding RE sites, generating count table for identifying statistically significant RE sites using edgeR or DEseq.

Details

Package:	REDseq
Type:	Package
Version:	1.0
Date:	2011-05-10
License:	GPL
LazyLoad:	yes

~~ An overview of how to use the package, including the most important functions ~~

Author(s)

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References

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See Also

buildREmap, assignSeq2REsit, plotCutDistribution, distanceHistSeq2RE, summarizeByRE, summarizeBySeq, compareREseq, binom.test.REDseq

Examples

```
 if(interactive()){
 library(ChIPpeakAnno)
 REpatternFilePath = system.file("extdata", "examplePattern.fa", package="REDseq")
 library(BSgenome.Celegans.UCSC.ce2)
 buildREmap( REpatternFilePath, BSgenomeName=Celegans, outfile=tempfile())
 library(REDseq)
 data(example.REDseq)
 data(example.map)
 r.unique = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
max.FragmentLength = 300, partitionMultipleRE = "unique")
 r.average = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
 seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
max.FragmentLength = 300, partitionMultipleRE = "average")
 r.random = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
 seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
max.FragmentLength = 300, partitionMultipleRE = "random")
 r.best = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
 seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
max.FragmentLength = 300, partitionMultipleRE = "best")
 r.estimate = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
 seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
max.FragmentLength = 300, partitionMultipleRE = "estimate")
 r.estimate$passed.filter
 r.estimate$notpassed.filter
 data(example.assignedREDseq)
 plotCutDistribution(example.assignedREDseq,example.map,
chr="2", xlim =c(3012000, 3020000))
 distanceHistSeq2RE(example.assignedREDseq,ylim=c(0,20))
 summarizeByRE(example.assignedREDseq,by="Weight",sampleName="example")
 REsummary  =summarizeByRE(example.assignedREDseq,by="Weight")
 binom.test.REDseq(REsummary)
}
```

assignSeq2REsite *Assign mapped sequence tags to corresponding restriction enzyme (RE) cut sites*

Description

Given the sequence tags aligned to a genome as a GRanges, and a map built using the buildREmap function, assignSeq2REsite first identifies RE sites that have mapped sequence tags around the cut position taking consideration of user-defined offset, sequence length and strand in the aligned sequences. These RE sites are used as seeds for assigning the remaining tags depending on which of five strategies the users select for partitioning sequences associated with multiple RE sites, i.e., unique, average,estimate, best and random. Please note that the default setting is for single-end sequencing data. For paired-end sequencing data, please create inputS.RD and inputE.RD from input.RD first with start(input.RD) and end(input.RD), where inputS.RD contains the start of the input.RD and inputE.RD contains the end of the input.RD. Then call assignSeq2REsite twice with inputS.RD and inputE.RD respectively. Please set min.FragmentLength = 0, max.FragmentLength = 1, seq.length = 1 with both calls.

Usage

```
assignSeq2REsite(input.RD, REmap.RD, cut.offset = 1, seq.length = 36,
allowed.offset = 5, min.FragmentLength = 60, max.FragmentLength = 300,
partitionMultipleRE = c("unique", "average", "estimate","best", "random"))
```

Arguments

input.RD	GRanges as mapped sequences: see example below
REmap.RD	GRanges as restriction enzyme (RE) cut site map: see example below
cut.offset	The cut offset from the start of the RE recognition sequence: index is 0 based, i.e.,1 means the RE cuts at position 2.
seq.length	Sequence length: 36 means that the sequence tags are 36-base long.
allowed.offset	Offset allowed to count for imperfect sticky end repair and primer addition.
min.FragmentLength	Minimum fragment length of the sequences size-selected for sequencing
max.FragmentLength	Maximum fragment length of the sequences size-selected for sequencing
partitionMultipleRE	The strategy for partitioning sequences associated with multiple RE sites. For strategy unique, only sequence tags that are associated with a unique RE site within the distance between min.FragmentLength and max.FragmentLength are kept for downstream analysis. For strategy average, sequence tags are partitioned equally among associated RE sites. For strategy estimate, sequence tags are partitioned among associated RE sites with a weight function, which is determined using the count distribution of the RE seed sites described in the description section above. For strategy best, sequence tags are assigned to the most

probable RE sties with the same weight function as that in strategy estimate. For strategy random, the sequence tags are randomly assigned to one of the multiple associated RE sites.

Value

`passed.filter` Sequences assigned to RE(s), see the example r.unique$passed.filter

`notpassed.filter`
Sequences not assigned to any RE, see example r.unique$notpassed.filter

`mREwithDetail` Detailed assignment information for sequences associated with multiple RE sites. Only available when partitionMultipleRE is set to average or estimate, see r.estimate$mREwithDetail in the examples

Author(s)

Lihua Julie Zhu

References

1. Roberts, R.J., Restriction endonucleases. CRC Crit Rev Biochem, 1976. 4(2): p. 123-64.
2.Kessler, C. and V. Manta, Specificity of restriction endonucleases and DNA modification methyltransferases a review (Edition 3). Gene, 1990. 92(1-2): p. 1-248.
3. Pingoud, A., J. Alves, and R. Geiger, Restriction enzymes. Methods Mol Biol, 1993. 16: p. 107-200.

See Also

buildREMap, example.REDseq, example.map, example.assignedREDseq

Examples

```
 library(REDseq)
 data(example.REDseq)
 data(example.map)
 r.unique = assignSeq2REsite(example.REDseq, example.map,
cut.offset = 1, seq.length = 36, allowed.offset = 5,
min.FragmentLength = 60, max.FragmentLength = 300,
partitionMultipleRE = "unique")
 r.average = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
max.FragmentLength = 300, partitionMultipleRE = "average")
 r.random = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
 max.FragmentLength = 300, partitionMultipleRE = "random")
 r.best = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
 max.FragmentLength = 300, partitionMultipleRE = "best")
 r.estimate = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
 max.FragmentLength = 300, partitionMultipleRE = "estimate")
```

```
r.estimate$passed.filter
r.estimate$notpassed.filter
```

`binom.test.REDseq` *Binomial test for REDseq dataset*

Description

For any early stage experiment with one experimental condition and one biological replicate, binom.test.REDseq computes p-value for each RE site in the genome.

Usage

```
binom.test.REDseq(REsummary, col.count = 2, multiAdj = TRUE,
multiAdjMethod = "BH", prior.p = 0.000001)
```

Arguments

`REsummary`	A matrix returned from summarizeByRE with a RE id column, a count/weight column. See examples
`col.count`	The column where the total count/weight is
`multiAdj`	Whether apply multiple hypothesis testing adjustment, TURE or FALSE
`multiAdjMethod`	Multiple testing procedures, for details, see mt.rawp2adjp in multtest package
`prior.p`	It is the probability of assigning a mapped sequence tag to a given RE site. Assuming each RE site gets cut equally, then the prior.p = 1/number of total RE sites in the genome.

Value

`p.value`	p-value of the test
`*.count`	weight/count from the input REsummary
`REid`	the id of the restriction enzyme from the input REsummary
`cut.frequency`	cut frequency
`*.adjusted.p.value`	applicable if multiAdj=TRUE, adjusted p.value using * method specified in multiAdjMethod

Author(s)

Lihua Julie Zhu

See Also

compareREDseq

Examples

```
library(REDseq)
REsummary = cbind(c("RE1", "RE2", "RE3"), c(10,1,100))
colnames(REsummary) = c("REid", "control")
binom.test.REDseq(REsummary)
```

---

buildREmap *Build a genome wide cut site map for a Restriction Enzyme (RE)*

Description

Build a genome-wide cut map for a Restriction Enzyme (RE)

Usage

```
buildREmap(REpatternFilePath, format = "fasta", BSgenomeName, outfile)
```

Arguments

REpatternFilePath	File path storing the recognition pattern of a RE
format	format of the pattern file, either "fasta" (the default) or "fastq
BSgenomeName	BSgenome object, please refer to available.genomes in BSgenome package for details
outfile	temporary output file for writing the matched chromosome location to

Value

Output REmap as a GRanges

Author(s)

Lihua Julie Zhu

Examples

```
library(REDseq)
REpatternFilePath = system.file("extdata", "examplePattern.fa", package="REDseq")
library(BSgenome.Celegans.UCSC.ce2)
buildREmap( REpatternFilePath, BSgenomeName=Celegans, outfile=tempfile())
```

compareREDseq *Compare two RED Sequencing Dataset*

Description

For early stage experiment without replicates, compareREDseq outputs differentially cut RE sites between two experimental conditions using Fisher's Exact Test.

Usage

```
compareREDseq(REsummary, col.count1 = 2, col.count2 = 3, multiAdj = TRUE,
 multiAdjMethod = "BH", maxP = 1, minCount = 1)
```

Arguments

REsummary	A matrix with a RE id column, 2 count/weight column, see examples
col.count1	The column where the total count/weight for the 1st experimental condition is
col.count2	The column where the total count/weight for the 2nd experimental condition is
multiAdj	Whether apply multiple hypothesis testing adjustment, TURE or FALSE
multiAdjMethod	Multiple testing procedures, for details, see mt.rawp2adjp in multtest package
maxP	The maximum p-value to be considered to be significant
minCount	For a RE site to be included, the tag count from at least one of the experimental condictions >= minimumCount

Value

p.value	the p-value of the test
*.count	weight/count from the input column col.count1 and col.count2
*.total	total weight/count from input column col.count1 and col.count2
REid	the id of the restriction enzyme from the input
odds.ratio	an estimate of the odds ratio for 2nd experimental condition vs. 1st experimental condition
*.adjusted.p.value	applicable if multiAdj=TRUE, adjusted p.value using the method * specified in multiAdjMethod

Author(s)

Lihua Julie Zhu

See Also

binom.test.REDseq

Examples

```
library(REDseq)
x= cbind(c("RE1", "RE2", "RE3", "RE4"), c(10,1,100, 0),c(5,5,50, 40))
colnames(x) = c("REid", "control", "treated")
compareREDseq(x)
```

`distanceHistSeq2RE` *Plot the distance distribution from sequence to the associated RE sites*

Description

Give an overview of the distance distribution from all assigned sequences to the associated RE sites. If average or estimate is used for assigning sequences to RE sites, the count for histogram drawing will be adjusted with the weight assigned.

Usage

```
distanceHistSeq2RE(assignedSeqs, longestDist = 1000,
title = "histogram of distance to assigned RE site",
xlab = "Distance to assigned RE site", ylab = "Frequency", ylim="")
```

Arguments

`assignedSeqs`	result returned from assignSeq2REsite
`longestDist`	longest distance to keep in the plot
`title`	an overall title for the plot
`xlab`	a title for the x axis
`ylab`	a title for the y axis
`ylim`	range of y to be plotted

Author(s)

Lihua Julie Zhu

See Also

assignSeq2REsite, distanceHistSeq2RE

Examples

```
library(REDseq)
data(example.assignedREDseq)
distanceHistSeq2RE(example.assignedREDseq,ylim=c(0,20))
```

`example.assignedREDseq` *an example assigned REDseq dataset*

Description

an example assigned REDseq dataset generated from assignSeq2REsite

Usage

```
data(example.assignedREDseq)
```

Format

The format is: List of 3
$ passed.filter :'data.frame': Sequences that passed the filters:
..$ SEQid :Sequence ID
..$ REid : Restriction Enzyme Site ID
..$ Chr : Chromosome
..$ strand : Strand
..$ SEQstart: Sequence Start
..$ SEQend : Sequence End
..$ REstart : Restriction Enzyme Site Start
..$ REend : Restriction Enzyme Site End
..$ Distance: Distance from SEQstart to REstart
..$ Weight : Weighted count for this REid and this SEQid
$ notpassed.filter:'data.frame' : Sequences that did not pass the filters
..$ SEQid :Sequence ID
..$ REid : Restriction Enzyme Site ID
..$ Chr : Chromosome
..$ strand : Strand
..$ SEQstart: Sequence Start
..$ SEQend : Sequence End
..$ REstart : Restriction Enzyme Site Start
..$ REend : Restriction Enzyme Site End
..$ Distance: Distance from SEQstart to REstart
..$ Weight : Weighted count for this REid and this SEQid
$ mREwithDetail :'data.frame': Detailed information about the sequences that are associated with multiple REid - for debugging:
..$ SEQid :Sequence ID
..$ REid : Restriction Enzyme Site ID
..$ Chr : Chromosome
..$ strand : Strand
..$ SEQstart: Sequence Start
..$ SEQend : Sequence End
..$ REstart : Restriction Enzyme Site Start
..$ REend : Restriction Enzyme Site End

..$ Distance: Distance from SEQstart to REstart
..$ Weight : Weighted count for this REid and this SEQid
..$ count : count of seed for this REid and SEQid
..$ total.count: total number of seeds that are associated with this SEQid

Examples

```
library(REDseq)
data(example.assignedREDseq)
## maybe str(example.assignedREDseq) ; plot(example.assignedREDseq) ...
```

`example.map` *an example REmap dataset*

Description

an example REmap dataset as GRanges generated from buildREmap

Usage

```
data(example.map)
```

Format

The format is: Formal class 'GRanges' [package "GenomicRanges"]

Examples

```
library(REDseq)
data(example.map)
## maybe str(example.map) ; plot(example.map) ...
```

`example.REDseq` *an example sequencing dataset from a restoration enzyme digestion (RED) experiment*

Description

an example RED sequencing dataset as a GRanges

Usage

```
data(example.REDseq)
```

Format

The format is: Formal class 'GRanges' [package "GenomicRanges"]

Examples

```
library(REDseq)
data(example.REDseq)
## maybe str(example.REDseq) ; plot(example.REDseq) ...
```

`plotCutDistribution` *plot cut frequencies of RE sites along a given chromosome*

Description

plot cut frequencies of RE sites along a chromosome, which gives a bird-eye view of genome-wide frequent-cut regions and RE inaccessible regions.

Usage

```
plotCutDistribution(assignedSeqs,REmap, chr="chr1",xlim,
title="RE cut frequency distribution",
xlab="Chromosome Location (bp)",ylab="Frequency",
round=TRUE, n.sequence)
```

Arguments

`assignedSeqs`	result returned from assignSeq2REsite
`REmap`	REmap used in assignSeq2REsite and generated from buildREmap
`chr`	chromosome to be plotted
`xlim`	range of x to be plotted
`title`	an overall title for the plot
`xlab`	a title for the x axis
`ylab`	a title for the y axis
`round`	TRUE: the sum of the weight is rounded up if the fraction part is greater than 0.5. FALSE: as it is.
`n.sequence`	total uniquely mapped sequences in the dataset for estimating the expected count for each RE site. If omitted, the expected count for each RE site will be set as 1 as default.

Author(s)

Lihua Julie Zhu

See Also

assignSeq2REsite, distanceHistSeq2RE

Examples

```
 library(REDseq)
 data(example.assignedREDseq)
 data(example.map)
 plotCutDistribution(example.assignedREDseq,example.map,
chr="2", xlim =c(3012000, 3020000))
```

---

summarizeByRE *Output count/weight summary by restriction enzyme cut site ID (REid)*

Description

Output count/weight summary by REid with each row representing each REid

Usage

```
summarizeByRE(assignedSeqs, by=c("Weight", "REid"),sampleName="",round=TRUE)
```

Arguments

assignedSeqs	output from assignSeq2REsite
by	Weight if sum up the weight for each REid, REid if sum the occurrence of each REid.
sampleName	The name of the sample used as the count column name.
round	TRUE: the sum of the weight is rounded up if the fraction part is greater than 0.5. FALSE: as it is.

Value

a matrix with REid as the first column and total count/weight as the second column, that can be used for the downstream analysis with DEseq or edgeR.

Author(s)

Lihua Julie Zhu

See Also

summarizeBySeq, assignSeq2REsite

Examples

```
 library(REDseq)
 data(example.assignedREDseq)
 summarizeByRE(example.assignedREDseq,by="REid",sampleName="example")
 summarizeByRE(example.assignedREDseq,by="Weight",sampleName="example")
```

summarizeBySeq *Output count/weight summary by sequences*

Description

Output count/weight summary by sequences with each row representing each sequences

Usage

```
summarizeBySeq(assignedSeqs, by =c("Weight", "SEQid"))
```

Arguments

assignedSeqs	output from assignSeq2REsite
by	Weight if sum up the weight for each sequence, SEQid if sum the occurrence of each sequence

Value

a matrix with SEQid as the first column and total count/weight as the second column

Author(s)

Lihua Julie Zhu

See Also

summarizeByRE, assignSeq2REsite

Examples

```
library(REDseq)
data(example.assignedREDseq)
summarizeBySeq(example.assignedREDseq, by="Weight")
summarizeBySeq(example.assignedREDseq,by="SEQid")
```

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