

# Package ‘REDseq’

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**Type** Package

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

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**Depends** R (>= 3.5.0), BiocGenerics, BSgenome.Celegans.UCSC.ce2, multtest, Biostrings, BSgenome, ChIPpeakAnno

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

**biocViews** Sequencing, SequenceMatching, Preprocessing

**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)

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REDseq-package	<i>REDseq</i>
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## 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)

Lihua Julie Zhu

Maintainer: Lihua Julie Zhu <julie.zhu@umassmed.edu>

## 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.

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5. Robinson, M.D., D.J. McCarthy, and G.K. Smyth, edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics*, 2010. 26(1): p. 139-40.
6. Zhu, L.J., et al., ChIPpeakAnno: a Bioconductor package to annotate ChIP-seq and ChIP-chip data. *BMC Bioinformatics*, 2010. 11: p. 237.
7. Pages, H., BSgenome package. <http://bioconductor.org/packages/2.8/bioc/vignettes/BSgenome/inst/doc/GenomeSearching.pdf>
8. Zhu, L.J., et al., REDseq: A Bioconductor package for Analyzing High Throughput Sequencing Data from Restriction Enzyme Digestion. (In preparation)

### 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	<i>Assign mapped sequence tags to corresponding restriction enzyme (RE) cut sites</i>
------------------	---

---

### 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 sites 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	<i>Build a genome wide cut site map for a Restriction Enzyme (RE)</i>
------------	---

---

**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, TRUE 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 conditions $\geq$ 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	<i>an example REmap dataset</i>
-------------	---------------------------------

---

### 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	<i>an example sequencing dataset from a restoration enzyme digestion (RED) experiment</i>
----------------	---

---

### 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))
```

---

searchPattern	<i>search pattern(s) in a genome</i>
---------------	--------------------------------------

---

**Description**

internal function for searching pattern(s) in a genome used by buildREmap leveraging BSgenome package

**Usage**

```
searchPattern(dict0, BSgenomeName, outfile = "")
```

**Arguments**

dict0	DNAStringSet object, see examples
BSgenomeName	BSgenome object, please refer to available.genomes in BSgenome package for details
outfile	The file path to write the search results as a bed file

**Value**

results are saved in the file specified by outfile

**Author(s)**

Lihua Julie Zhu

**References**

<http://bioconductor.org/packages/2.8/bioc/vignettes/BSgenome/inst/doc/GenomeSearching.pdf>

**See Also**

buildREmap

**Examples**

```
library(REDseq)
patternFile = system.file("extdata", "examplePattern.fa", package="REDseq")
example.dict0 <- readDNAStringSet(patternFile, "fasta")
library(BSgenome.Celegans.UCSC.ce2)
REDseq:::searchPattern(dict0 = example.dict0, BSgenomeName=Celegans, outfile=tempfile())
```

---

summarizeByRE	<i>Output count/weight summary by restriction enzyme cut site ID (REid)</i>
---------------	---

---

### 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	<i>Output count/weight summary by sequences</i>
----------------	---

---

**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")
```

---

writeHits	<i>write the hits of pattern search to a bed file</i>
-----------	---

---

**Description**

write the hits of pattern search to a bed file, internal function used by searchPattern

**Usage**

```
writeHits(seqname, matches, strand, file = "", append = FALSE)
```

**Arguments**

seqname	Chromosome name
matches	XStringViews object storing matched chromosome locations
strand	strand of the match
file	file path where the hits is written to
append	TRUE if append to existing file, false if start a new file

**Value**

results are saved in the file specified by outfile

**Author(s)**

Lihua Julie Zhu

**References**

<http://bioconductor.org/packages/2.8/bioc/vignettes/BSgenome/inst/doc/GenomeSearching.pdf>

**See Also**

searchPattern, buildREmap

**Examples**

```
library(REDseq)
x <- DNASTring("AAGCGCGATATG")
m <- matchPattern("GCGC", x)
REDseq::writeHits(seqname="chr1", m, strand="+", file="exampleWriteHits.bed", append=FALSE)
```

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