The REDseq user's guide

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Contents

1 Introduction

Restriction Enzyme digestion (RED) followed by high throughput sequencing (REDseq) enables genome wide differentiation of highly accessible regions and inaccessible regions. Comparing the profiles of restriction enzyme (RE) digestion among different cell types, developmental stages, disease stages, or different tissues facilitates deciphering of complex regulation network of cell differentiation, developmental control, and disease etiology and progression. We have developed a Bioconductor package called *REDSeq* to address the fundamental upstream analysis tasks of REDseq dataset. We have implemented functions for building genomic map of restriction enzyme sites (`buildREmap`), assigning sequencing tags to

RE sites (`assignSeq2REsite`), visualizing genome-wide distribution of differentially cut regions (`distanceHistSeq2RE`) and the distance distribution of sequence tags to corresponding RE sites (`distanceHistSeq2RE`), generating count table for identifying statistically significant RE sites (`summarizeByRE`). We have leveraged *BSgenome* on implementing function `buildREmap` for building genome-wide RE maps. The input data for `assignSeq2REsite` are represented as RangedData, for efficiently associating sequences with RE sites. It 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. The user-defined offset guards against imperfect sticky end repair and primer addition process. 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. For experiment with at least two conditions with biological replicates, count summary generated from `summarizeByRE` can be easily used for identifying differentially cut RE sites using either *DESeq* or *edgeR*. Differentially cut RE sites can be annotated to the nearest gene using *ChIPpeakAnno*. In addition, for early stage experiments without replicates, `compareREDseq` outputs differentially cut RE sites between two experimental conditions using Fisher's Exact Test. For experiment with one experimental condition, `binom.test.REDseq` outputs differentially cut RE sites in the genome. Multiplicity adjustment functions from *multtest* package were integrated in both functions.

2 Examples of using REDseq

2.1 Task 1: Build a RE map for a genome

Given a fasta/fastq file containing the restriction enzyme recognition site and a BSgenome object, the function `buildREmap` builds a genome-wide RE map.

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

>>> Finding all hits in sequences chrI ...
>>> DONE searching
>>> Finding all hits in sequences chrII ...
>>> DONE searching
>>> Finding all hits in sequences chrIII ...
>>> DONE searching
>>> Finding all hits in sequences chrIV ...
>>> DONE searching
>>> Finding all hits in sequences chrV ...
>>> DONE searching
>>> Finding all hits in sequences chrX ...
>>> DONE searching
>>> Finding all hits in sequences chrM ...
>>> DONE searching
```

2.2 Task 2: Assign mapped sequence tags to RE site

Given a mapped sequence tags as a RangedData and REmap as a RangedData, `assignSeq2REsite` function assigns mapped sequence tags to RE site depending on the strategy users select. There are five strategies implemented, i.e., unique, average, estimate, best and random. For details, type help(`assignSeq2REsite`) in a R session.

```
>        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")


Thu Apr  4 01:10:41 2013 Validating input ...
Thu Apr  4 01:10:41 2013 Prepare map data ...
Thu Apr  4 01:10:41 2013 Align to chromosome  2  ...
Thu Apr  4 01:10:41 2013 Finished 1st round of aligning! Start the 2nd round of aligning ...
Thu Apr  4 01:10:41 2013 Align to chromosome  2  ...
Thu Apr  4 01:10:42 2013 Start filtering ...


>        r.best= assignSeq2REsite(example.REDseq, example.map,
+        cut.offset = 1, seq.length = 36, allowed.offset = 5,
+        min.FragmentLength = 60, max.FragmentLength = 300, partitionMultipleRE = "best")


Thu Apr  4 01:10:42 2013 Validating input ...
Thu Apr  4 01:10:42 2013 Prepare map data ...
Thu Apr  4 01:10:42 2013 Align to chromosome  2  ...
Thu Apr  4 01:10:42 2013 Finished 1st round of aligning! Start the 2nd round of aligning ...
Thu Apr  4 01:10:42 2013 Align to chromosome  2  ...
Thu Apr  4 01:10:42 2013 Start filtering ...
Thu Apr  4 01:10:42 2013 Partitioning reads over RE sites within  300 ...
Thu Apr  4 01:10:42 2013 get count for each RE ...


>        r.random = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
+        seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
+        max.FragmentLength = 300, partitionMultipleRE = "random")


Thu Apr  4 01:10:42 2013 Validating input ...
Thu Apr  4 01:10:42 2013 Prepare map data ...
Thu Apr  4 01:10:43 2013 Align to chromosome  2  ...
Thu Apr  4 01:10:43 2013 Finished 1st round of aligning! Start the 2nd round of aligning ...
Thu Apr  4 01:10:43 2013 Align to chromosome  2  ...
Thu Apr  4 01:10:43 2013 Start filtering ...
Thu Apr  4 01:10:43 2013 Partitioning reads over RE sites within  300 ...


>        r.average = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
+        seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
+         max.FragmentLength = 300, partitionMultipleRE = "average")


Thu Apr  4 01:10:43 2013 Validating input ...
Thu Apr  4 01:10:43 2013 Prepare map data ...
Thu Apr  4 01:10:43 2013 Align to chromosome  2  ...
Thu Apr  4 01:10:43 2013 Finished 1st round of aligning! Start the 2nd round of aligning ...
Thu Apr  4 01:10:43 2013 Align to chromosome  2  ...
Thu Apr  4 01:10:43 2013 Start filtering ...
Thu Apr  4 01:10:43 2013 Partitioning reads over RE sites within  300 ...


>        r.estimate = assignSeq2REsite(example.REDseq, example.map, cut.offset = 1,
+        seq.length = 36, allowed.offset = 5, min.FragmentLength = 60,
+        max.FragmentLength = 300, partitionMultipleRE = "estimate")
```

```
Thu Apr  4 01:10:43 2013 Validating input ...
Thu Apr  4 01:10:43 2013 Prepare map data ...
Thu Apr  4 01:10:43 2013 Align to chromosome  2  ...
Thu Apr  4 01:10:43 2013 Finished 1st round of aligning! Start the 2nd round of aligning ...
Thu Apr  4 01:10:43 2013 Align to chromosome  2  ...
Thu Apr  4 01:10:44 2013 Start filtering ...
Thu Apr  4 01:10:44 2013 Partitioning reads over RE sites within  300 ...
Thu Apr  4 01:10:44 2013 get count for each RE ...


>         head(r.estimate$passed.filter)


    SEQid             REid Chr strand SEQstart  SEQend REstart    REend Distance
1 00000036 Sau96I.chr10.29   2     -1  3012058 3012093 3012090 3012094      -32
2 00000037 Sau96I.chr10.29   2      1  3012091 3012126 3012090 3012094        1
3 00000038 Sau96I.chr10.29   2      1  3012096 3012131 3012090 3012094        6
4 00000039 Sau96I.chr10.30   2     -1  3012266 3012301 3012299 3012303      -33
5 00000040 Sau96I.chr10.30   2      1  3012300 3012335 3012299 3012303        1
6 00000052 Sau96I.chr10.40   2     -1  3017881 3017916 3017916 3017920      -35
  Weight
1      1
2      1
3      1
4      1
5      1
6      1
```

2.3   Task 3: Visualize the distribution of cut frequency in selected genomic regions and the distance distribution of sequence tags to corresponding RE sites

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

```
> plotCutDistribution(example.assignedREDseq,example.map, chr="2",
+ xlim =c(3012000, 3020000))
```
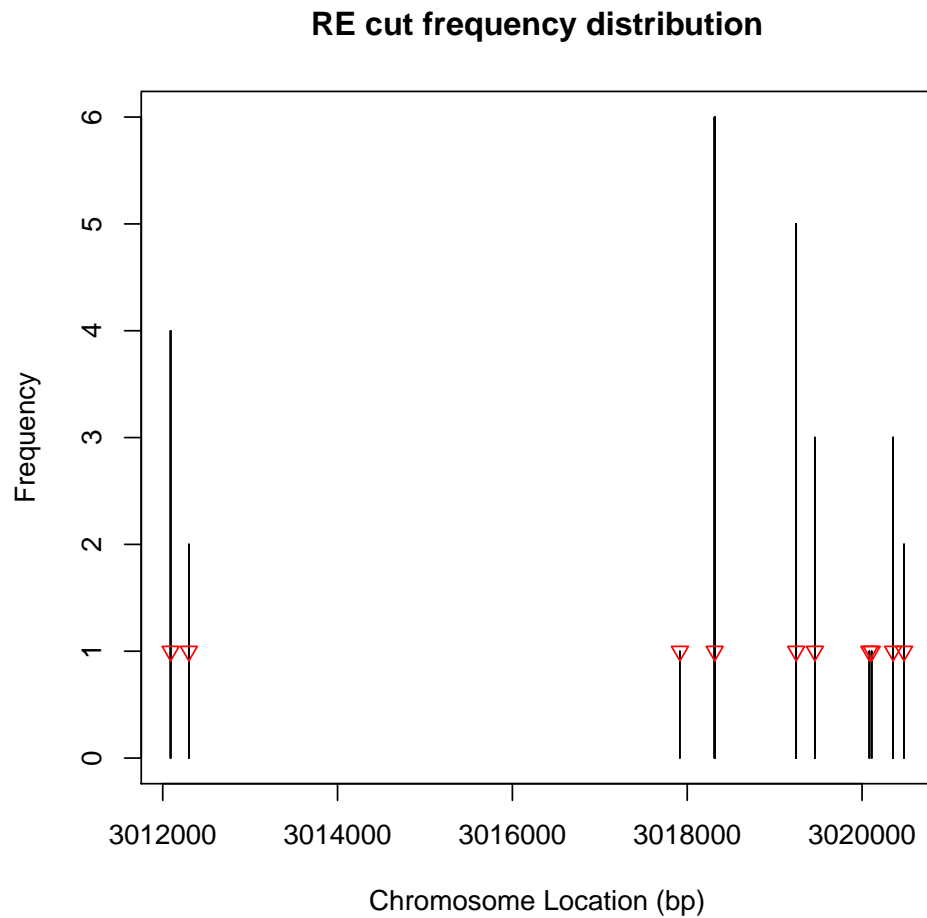
RE cut frequency distribution

Figure 1: Plot to show the distribution of cut frequency in the selected genomic-regions with the function `plotCutDistribution`. The red triangle is the expected cut frequency for each RE site.

```
> distanceHistSeq2RE(example.assignedREDseq,ylim=c(0,25))
```
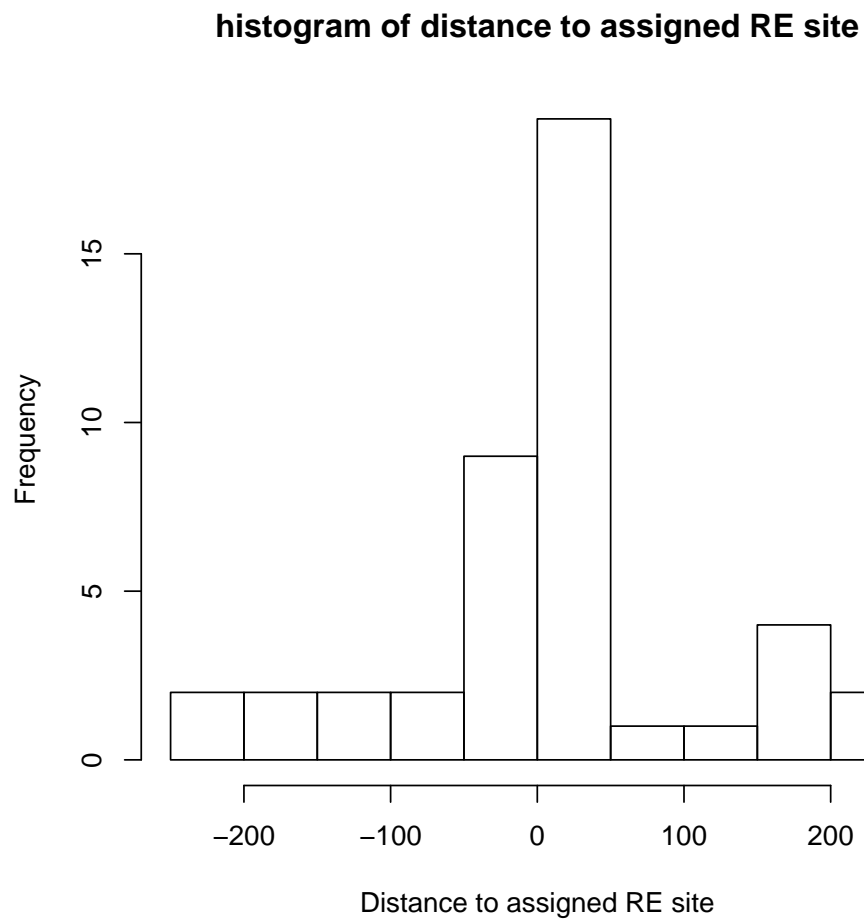
histogram of distance to assigned RE site

Figure 2: Plot to show the distribution of distance of sequence tags to associated RE sites with the function `distanceHistSeq2RE`.

2.4 Task 4: Generating count table for identifying statistically significant RE sites

Once you have obtained the assigned RE sites, you can use the function `summarizeByRE` to obtain a count table for identifying statistically significant RE sites using *DEseq* or *edgeR*.

```
> REsummary  =summarizeByRE(example.assignedREDseq,by="Weight")
```

2.5 Task 5: Identifying differential cut RE sites for experiment with one experiment condition

```
> binom.test.REDseq(REsummary)
```

	p.value	total.weight.count	REid	cut.frequency
1	2.804822e-47	9	Sau96I.chr10.42	0.28125
2	9.061718e-31	6	Sau96I.chr10.43	0.18750
3	3.595919e-20	4	Sau96I.chr10.29	0.12500
4	4.959892e-15	3	Sau96I.chr10.50	0.09375
5	4.959892e-15	3	Sau96I.chr10.45	0.09375
6	4.959901e-10	2	Sau96I.chr10.30	0.06250
7	4.959901e-10	2	Sau96I.chr10.51	0.06250
8	3.199950e-05	1	Sau96I.chr10.40	0.03125
9	3.199950e-05	1	Sau96I.chr10.49	0.03125
10	3.199950e-05	1	Sau96I.chr10.47	0.03125

	BH.adjusted.p.value
1	2.804822e-46
2	4.530859e-30
3	1.198640e-19
4	9.919784e-15
5	9.919784e-15
6	7.085573e-10
7	7.085573e-10
8	3.199950e-05
9	3.199950e-05
10	3.199950e-05

2.6 Task 6: Identifying differential cut RE sites for early stage experiment without replicates

```
> 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)
```

	p.value	control.count	treated.count	REid	control.total	treated.total
1	6.233642e-16	0	40	RE4	111	100
2	1.159388e-10	100	50	RE3	111	100
3	1.035503e-01	1	5	RE2	111	100
4	2.943364e-01	10	5	RE1	111	100

	odds.ratio	BH.adjusted.p.value
1	Inf	2.493457e-15
2	0.1112945	2.318777e-10
3	5.7478720	1.380671e-01
4	0.5331227	2.943364e-01

3 References

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4. bibitemAnders10 Anders, S. and W. Huber, Differential expression analysis for sequence count data. Genome Biol, 2010. 11(10): p. R106.

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.

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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)

4 Session Info

```
> sessionInfo()

R version 3.0.0 (2013-04-03)
Platform: x86_64-unknown-linux-gnu (64-bit)

locale:
 [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
 [3] LC_TIME=en_US.UTF-8        LC_COLLATE=C
 [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
 [7] LC_PAPER=C                 LC_NAME=C
 [9] LC_ADDRESS=C               LC_TELEPHONE=C
[11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C

attached base packages:
[1] grid      parallel  stats     graphics  grDevices utils     datasets
[8] methods   base
```

```
other attached packages:
 [1] REDseq_1.6.0                       ChIPpeakAnno_2.8.0
 [3] GenomicFeatures_1.12.0             limma_3.16.0
 [5] org.Hs.eg.db_2.9.0                 GO.db_2.9.0
 [7] RSQLite_0.11.2                     DBI_0.2-5
 [9] AnnotationDbi_1.22.0               BSgenome.Ecoli.NCBI.20080805_1.3.17
[11] biomaRt_2.16.0                     VennDiagram_1.5.1
[13] multtest_2.16.0                    Biobase_2.20.0
[15] BSgenome.Celegans.UCSC.ce2_1.3.19  BSgenome_1.28.0
[17] Biostrings_2.28.0                  GenomicRanges_1.12.0
[19] IRanges_1.18.0                     BiocGenerics_0.6.0

loaded via a namespace (and not attached):
 [1] MASS_7.3-26      RCurl_1.95-4.1     Rsamtools_1.12.0  XML_3.96-1.1
 [5] bitops_1.0-5     rtracklayer_1.20.0 splines_3.0.0     stats4_3.0.0
 [9] survival_2.37-4  tools_3.0.0        zlibbioc_1.6.0
```