META2 toolkit manual

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About META2

META2 (DNA METhylation Annotator & Analyzer), aims to detect differential status of cross-cell DNA methylation, annotate the detected loci and perform downstream analysis. It contains multiple functions to run a series of operations on DNA methylation analysis and cross cell interrogation. Currently only RRBS (Reduced Representation Bisulfite Sequencing) can be directly run through the whole analysis procedure, but the package functions are be used to analyze the other datasets with the permitted input formats.

META2 contains several statistical analysis and integrative visualization functions. Part of those functions are listed as below,

1. **fun_findDMC**: the function aims to identify the differentially methylated CpG loci (DMC), together filter out the statistically significant DMCs;
2. **fun_plotMethLoci**: the function is designed to plot the differentially methylated loci (based on differential methylated level between control vs treatment). The function can plot the dot or line style for the methylated loci across the whole genome (ch1 ~ chr22, chrX and chrY);
3. **fun_combineDMC**: the function aims to combine the isolated DMCs into a complete DMC cluster with a predefined length, then those clusters can further become differential methylated region (DMR) candidates;
4. **fun_findDMR**: the function is to filter differentially methylated loci and combine those isolated loci into region with its length specified, and finally output the identified differential methylated region (DMR) candidates. This function is an integration of fun_findDMC and fun_combineDMC;
5. **fun_intplotDMR**: the function is to generate the integrative diagram for the identified differential methylated region (DMR) candidate, which contains reference sequence information within the interested DMR.

The RRBS datasets used as a study case in this manual include: (1) T47DDS_r1.bed.gz: T47D cell with DMSO treatment, replicate 1; (2) T47DDS_r2.bed.gz: T47D cell with DMSO treatment, replicate 2; (3) T47DE2_r1.bed.gz: T47D cell with E2 treatment, replicate 1; (4) T47DE2_r2.bed.gz: T47D cell with E2 treatment, replicate 2.

References:

1. Tang, B., DMAK: a curated pan-cancer DNA methylation annotation knowledgebase. T & F Bioengineered, 2016.
2. Tang, B., Zhu W., and Wu C., Cross-cell DNA methylation annotation and analysis for pan-cancer study. B. Journal of Pharmacology, 2016.

1. Basic Preprocess (Pairwise Sample Raw Data Analysis)

We adopt the cell line, T-47D (DMSO and E2 treatment with 2 replicates), as the study case for the following demonstration. And the raw data samples are zipped as ".bed.gz" format, each denoted as below:

(1) T47DDS_r1.bed.gz (2) T47DDS_r2.bed.gz (3) T47DE2_r1.bed.gz (4) T47DE2_r2.bed.gz

We firstly load and convert the sample list (indicating the four samples) into a methylRaw object file.

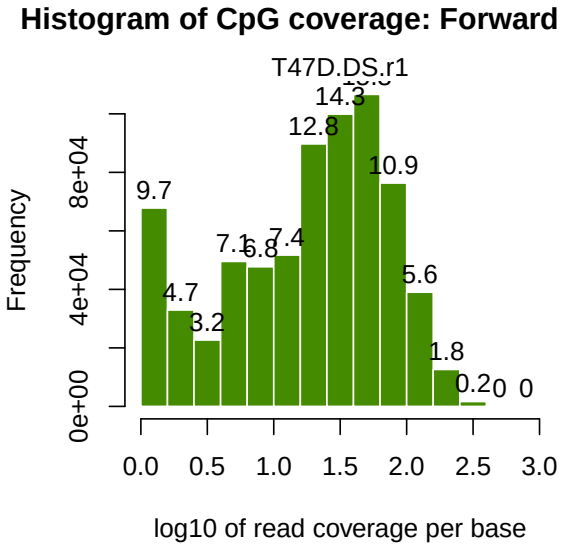
```
file<-list("T47DDS_r1.bed.gz","T47DDS_r2.bed.gz",
           "T47DE2_r1.bed.gz","T47DE2_r2.bed.gz")
obj<-read(file,
          sample.id=list("T47D.DS.r1","T47D.DS.r2","T47D.E2.r1","T47D.E2.r2"),
          treatment=c(0,0,1,1),
          assembly="hg19",header=F,context="CpG",resolution="base",
          pipeline=list(fraction=F,chr.col=1,start.col=2,end.col=3,
                        coverage.col=5,strand.col=6,freqC.col=11))
#load("obj.rda")
obj
```

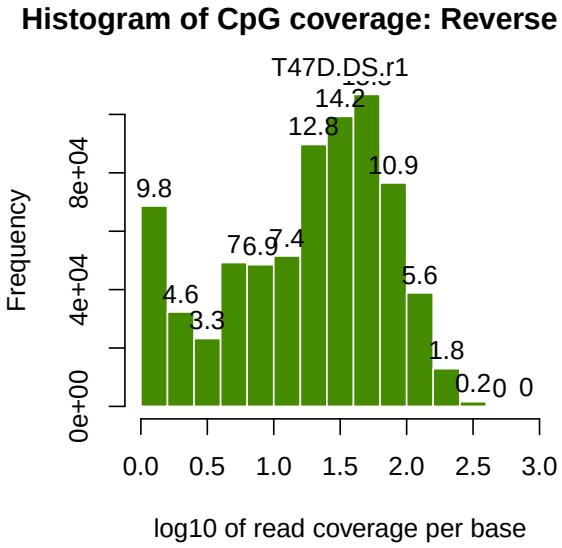
```
## methylRawList object with 4 methylRaw objects
##
## methylRaw object with 1398323 rows
## --------------
##    chr     start       end strand coverage numCs numTs
## 1 chr1 100002989 100002990      +        4     0     4
## 2 chr1 100003153 100003154      -       81     2    79
## 3 chr1   1000170   1000171      +       15     5    10
## 4 chr1   1000190   1000191      +       15     1    14
## 5 chr1   1000191   1000192      -       18     0    18
## 6 chr1   1000198   1000199      +       15     1    14
## --------------
## sample.id: T47D.DS.r1
## assembly: hg19
## context: CpG
## resolution: base
##
## methylRaw object with 1419227 rows
## --------------
##    chr     start       end strand coverage numCs numTs
## 1 chr1 100002989 100002990      +        1     1     0
## 2 chr1 100003153 100003154      -       52     4    48
## 3 chr1   1000170   1000171      +       20     7    13
## 4 chr1   1000190   1000191      +       20     0    20
## 5 chr1   1000191   1000192      -       13     0    13
## 6 chr1   1000198   1000199      +       20     2    18
## --------------
## sample.id: T47D.DS.r2
## assembly: hg19
## context: CpG
## resolution: base
##
## methylRaw object with 1277382 rows
## --------------
##    chr     start       end strand coverage numCs numTs
## 1 chr1 100003153 100003154      -        1     0     1
## 2 chr1   1000170   1000171      +       18     3    15
## 3 chr1   1000190   1000191      +       18     0    18
## 4 chr1   1000191   1000192      -       14     0    14
## 5 chr1   1000198   1000199      +       18     2    16
## 6 chr1   1000199   1000200      -       14     0    14
```

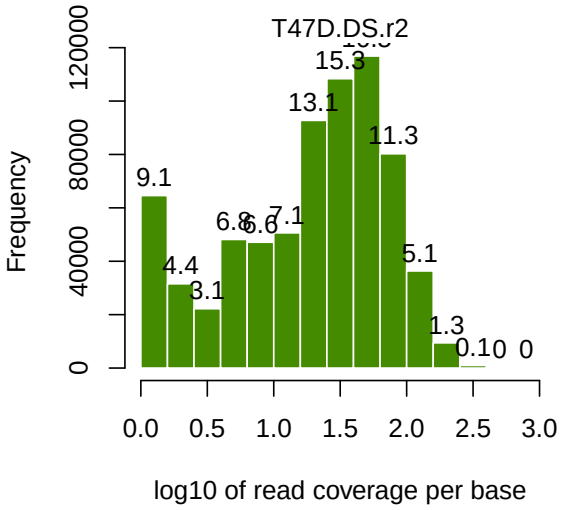
```
## --------------
## sample.id: T47D.E2.r1
## assembly: hg19
## context: CpG
## resolution: base
##
## methylRaw object with 1254565 rows
## --------------
##    chr     start       end strand coverage numCs numTs
## 1 chr1 100003153 100003154      -        1     0     1
## 2 chr1   1000170   1000171      +       33     9    24
## 3 chr1   1000190   1000191      +       33     3    30
## 4 chr1   1000191   1000192      -       12     0    12
## 5 chr1   1000198   1000199      +       33     5    28
## 6 chr1   1000199   1000200      -       12     1    11
## --------------
## sample.id: T47D.E2.r2
## assembly: hg19
## context: CpG
## resolution: base
##
## treament: 0 0 1 1
```

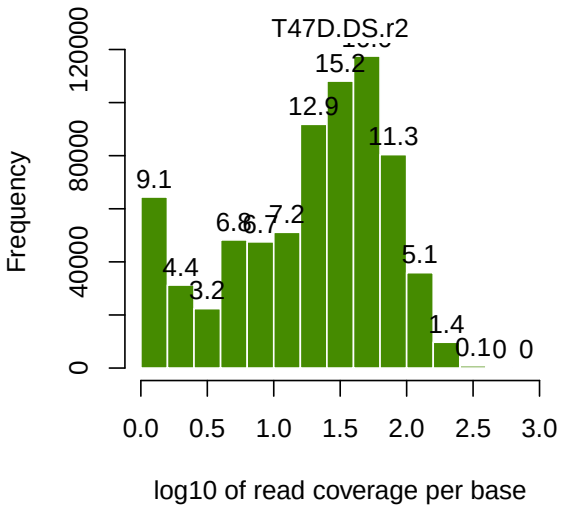
2. Pairwise Sample Methylation Level Distribution and Correlation Analysis

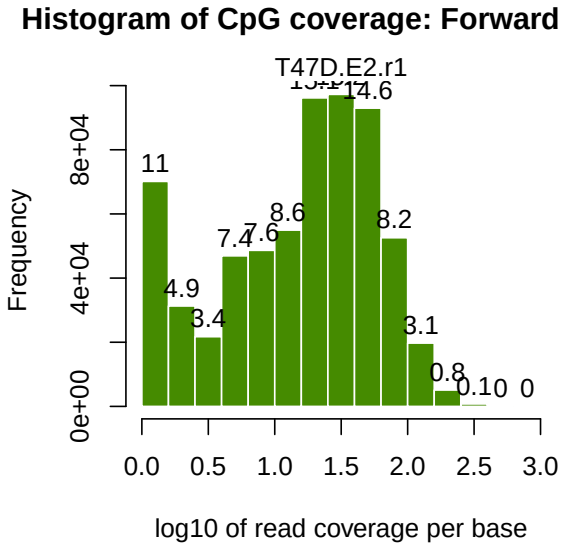
Below are the histogram of CpG methylation level (%) for the four pairwise samples. The histogram plots depict the both strands of the input sample data.

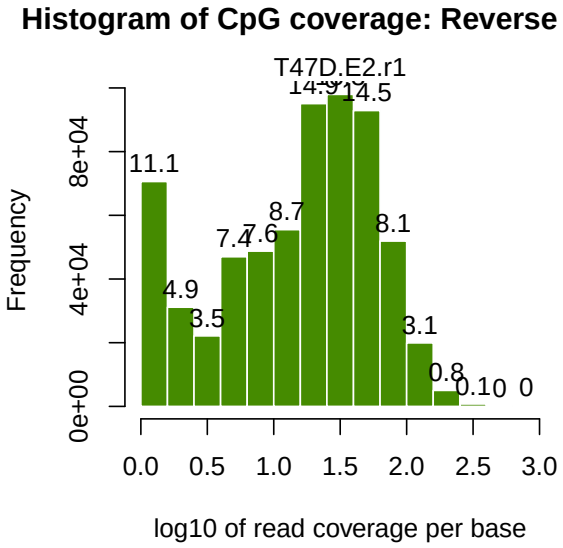

Histogram of CpG coverage: Forward stra
T47D.DS.r1
Frequency
0e+00
4e+04
8e+04
9.7
4.7
3.2
7.1
6.8
7.4
12.8
14.3
10.9
5.6
1.8
0.2
0
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
log10 of read coverage per base

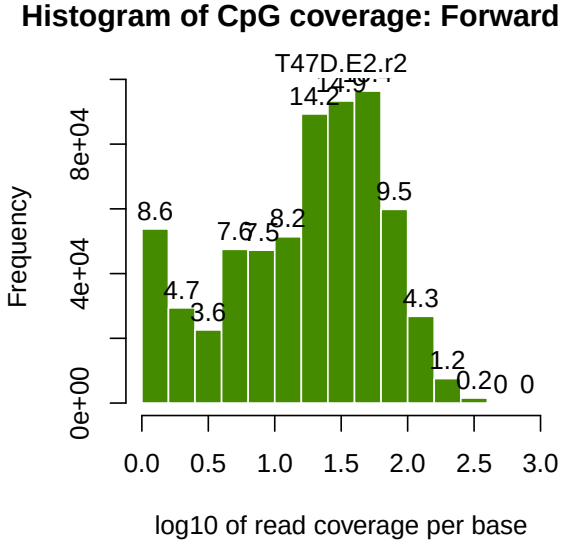

Histogram of CpG coverage: Reverse stra
T47D.DS.r1
Frequency
0e+00
4e+04
8e+04
9.8
4.6
3.3
7
6.9
7.4
12.8
14.2
10.9
5.6
1.8
0.2
0
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
log10 of read coverage per base

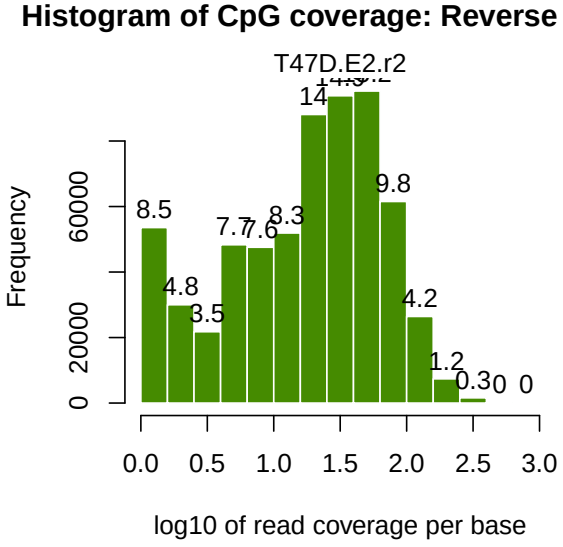

Histogram of CpG coverage: Forward stra
T47D.DS.r2
Frequency
0
40000
80000
120000
9.1
4.4
3.1
6.8
6.6
7.1
13.1
15.3
11.3
5.1
1.3
0.10
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
log10 of read coverage per base


Histogram of CpG coverage: Reverse stra
T47D.DS.r2
Frequency
0
40000
80000
120000
9.1
4.4
3.2
6.8
6.7
7.2
12.9
15.2
11.3
5.1
1.4
0.10
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
log10 of read coverage per base





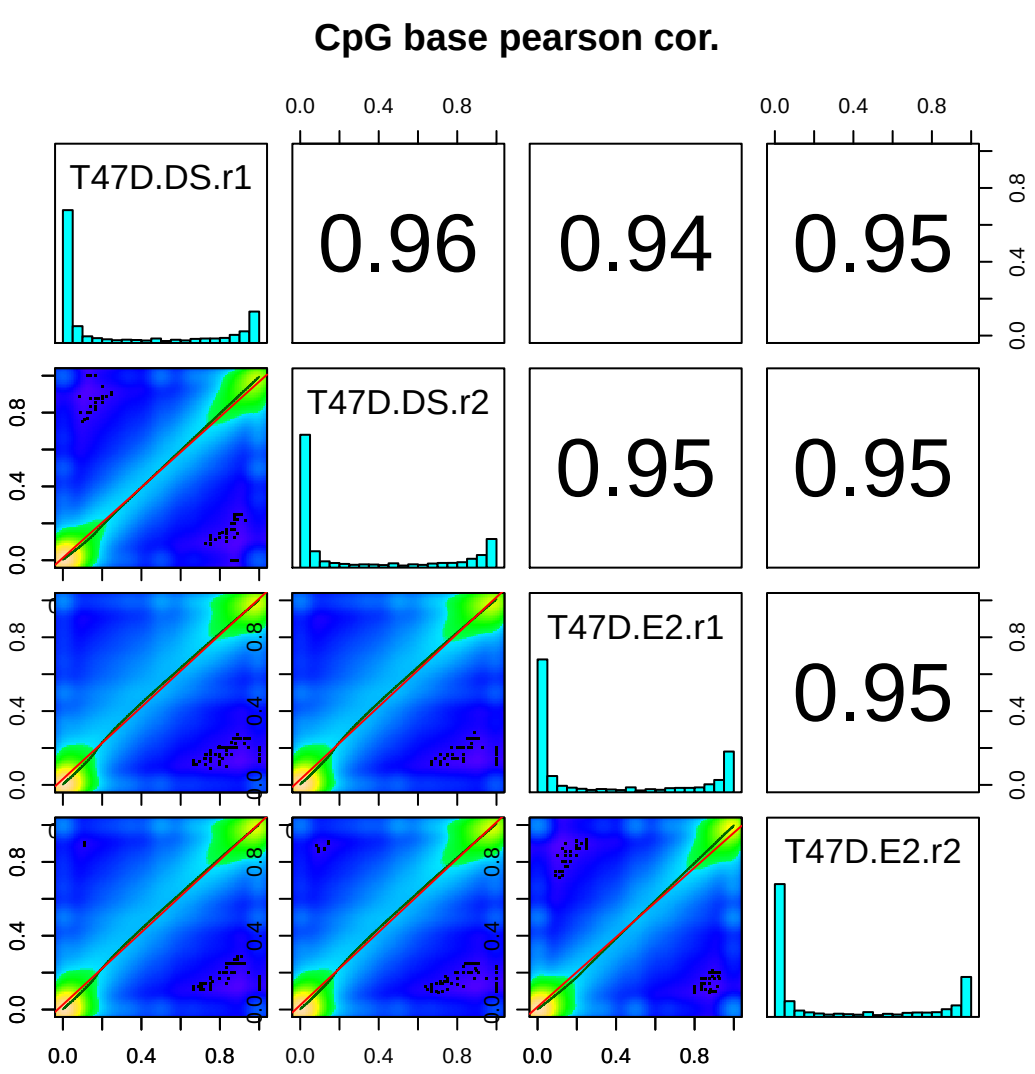




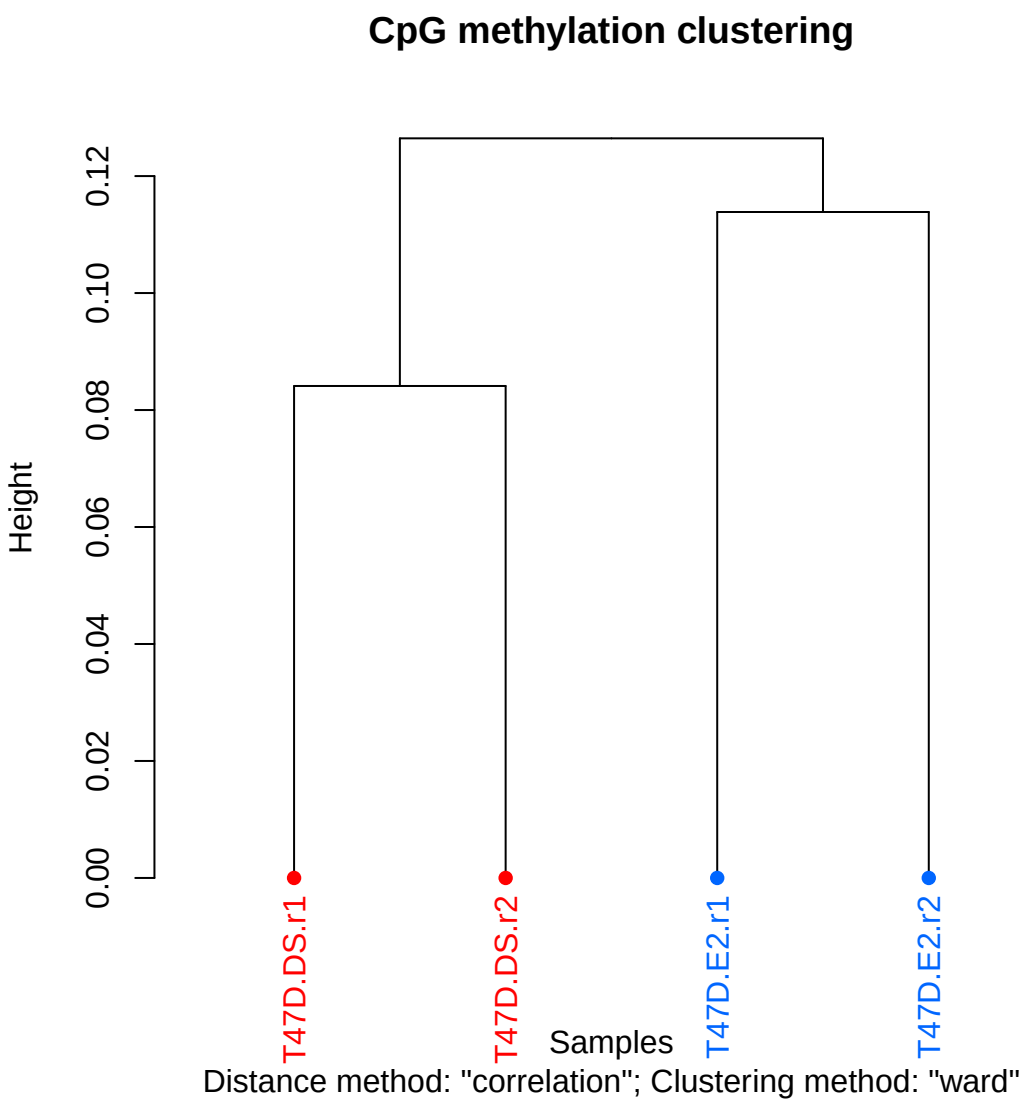
2.1 Pairwise sample correlation analysis:

```
## methylBase object with 6 rows
## --------------
##    chr start   end strand coverage1 numCs1 numTs1 coverage2 numCs2 numTs2
## 1 chr1 10785 10786      +        10     10      0        11     11      0
## 2 chr1 10788 10789      +        10      9      1        11     11      0
## 3 chr1 10794 10795      +        10      9      1        11     11      0
## 4 chr1 10810 10811      +        10     10      0        11     11      0
## 5 chr1 10812 10813      +        10     10      0        11     11      0
## 6 chr1 10815 10816      +        10     10      0        11     11      0
##   coverage3 numCs3 numTs3 coverage4 numCs4 numTs4
## 1        39     39      0        76     75      1
## 2        39     39      0        76     76      0
```

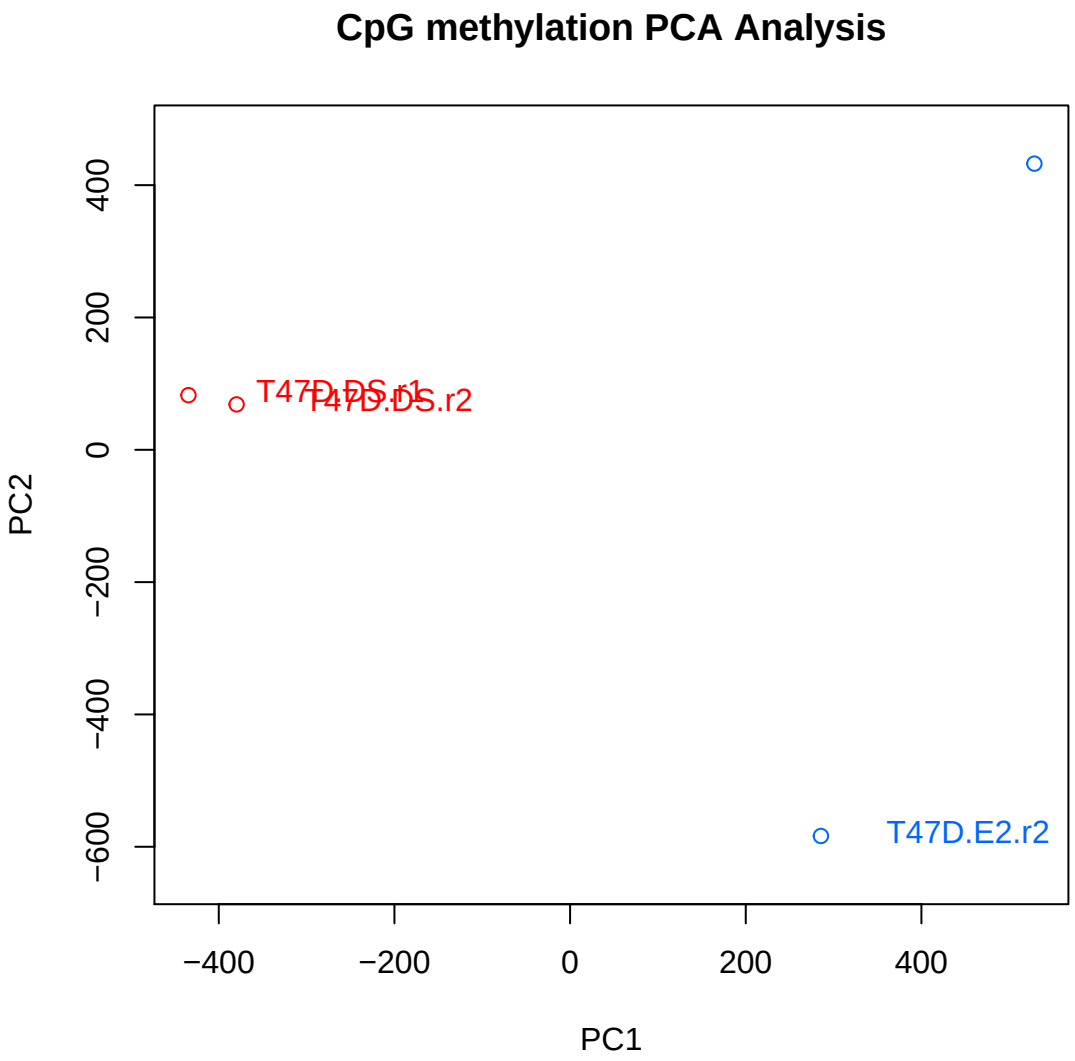
```
## 3        39     39      0        76     76      0
## 4        39     39      0        76     74      2
## 5        39     39      0        76     74      2
## 6        39     36      3        76     75      1
## --------------
## sample.ids: T47D.DS.r1 T47D.DS.r2 T47D.E2.r1 T47D.E2.r2
## destranded FALSE
## assembly: hg19
## context: CpG
## treament: 0 0 1 1
## resolution: base

##            T47D.DS.r1 T47D.DS.r2 T47D.E2.r1 T47D.E2.r2
## T47D.DS.r1  1.0000000  0.9598207  0.9401965  0.9464834
## T47D.DS.r2  0.9598207  1.0000000  0.9450299  0.9507292
## T47D.E2.r1  0.9401965  0.9450299  1.0000000  0.9466383
## T47D.E2.r2  0.9464834  0.9507292  0.9466383  1.0000000
```



Then we perform the clustering of the samples based on correlation.




```
##
## Call:
## hclust(d = d, method = HCLUST.METHODS[hclust.method])
##
## Cluster method   : ward.D
## Distance         : pearson
## Number of objects: 4
```



3. Differential Methylation Annotation and Analysis

The calculated differential methylation candiates, listed as below,

```
myDiff <- calculateDiffMeth(meth)
```

```
## Warning: glm.fit: fitted probabilities numerically 0 or 1 occurred
```

```
## Warning: glm.fit: fitted probabilities numerically 0 or 1 occurred
```

```
#save(file="myDiff.rda",myDiff)
#load("myDiff.rda")
myDiff   # 1135337 rows
```

```
## methylDiff object with 1135337 rows
## --------------
##    chr start   end strand     pvalue    qvalue  meth.diff
## 1 chr1 10785 10786      + 0.56168482 0.9449035 -0.8695652
## 2 chr1 10788 10789      + 0.05195282 0.5644036  4.7619048
## 3 chr1 10794 10795      + 0.05195282 0.5644036  4.7619048
## 4 chr1 10810 10811      + 0.41085680 0.8442256 -1.7391304
## 5 chr1 10812 10813      + 0.41085680 0.8442256 -1.7391304
## 6 chr1 10815 10816      + 0.24289096 0.7604375 -3.4782609
## --------------
## sample.ids: T47D.DS.r1 T47D.DS.r2 T47D.E2.r1 T47D.E2.r2
## destranded FALSE
## assembly: hg19
## context: CpG
## treament: 0 0 1 1
## resolution: base
```

```
# get Significant hyper-methylated bases
myDiff25p.hyper=get.methylDiff(myDiff,difference=25,qvalue=0.01,type="hyper")
head(myDiff25p.hyper,3)
```

```
## methylDiff object with 3 rows
## --------------
##       chr   start     end strand       pvalue       qvalue meth.diff
## 8    chr1  137985  137986      + 7.251977e-13 4.324026e-09  27.14186
## 2370 chr1 1093875 1093876      + 2.652323e-13 1.820171e-09  58.57403
## 3478 chr1 1227508 1227509      - 4.622883e-05 9.543191e-03  29.19325
## --------------
## sample.ids: T47D.DS.r1 T47D.DS.r2 T47D.E2.r1 T47D.E2.r2
## destranded FALSE
## assembly: hg19
## context: CpG
## treament: 0 0 1 1
## resolution: base
```

```
# get hypo-methylated bases
myDiff25p.hypo=get.methylDiff(myDiff,difference=25,qvalue=0.01,type="hypo")
head(myDiff25p.hypo,3)
```

```
## methylDiff object with 3 rows
## --------------
##          chr    start      end strand       pvalue       qvalue meth.diff
## 52919   chr1 58716087 58716088      + 3.700485e-05 8.086863e-03 -41.77215
## 112860 chr10 39023615 39023616      - 3.973488e-13 2.596202e-09 -27.76575
## 120918 chr10 73767217 73767218      + 3.924071e-05 8.436917e-03 -35.22267
## --------------
## sample.ids: T47D.DS.r1 T47D.DS.r2 T47D.E2.r1 T47D.E2.r2
## destranded FALSE
## assembly: hg19
## context: CpG
## treament: 0 0 1 1
## resolution: base
```

```r
# get all differentially methylated bases
myDiff25p=get.methylDiff(myDiff,difference=25,qvalue=0.01)
dim(myDiff25p)
```

```
## [1] 3651    7
```

```r
dim(myDiff25p.hyper)[1]+dim(myDiff25p.hypo)[1]
```

```
## [1] 3651
```

```r
head(myDiff25p)
```

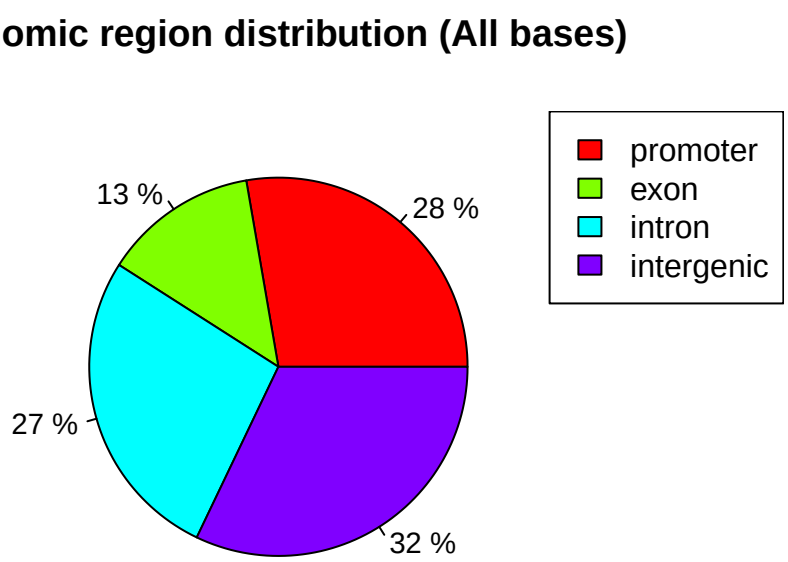
```
## methylDiff object with 6 rows
## --------------
##       chr   start     end strand       pvalue       qvalue meth.diff
## 8    chr1  137985  137986      + 7.251977e-13 4.324026e-09  27.14186
## 2370 chr1 1093875 1093876      + 2.652323e-13 1.820171e-09  58.57403
## 3478 chr1 1227508 1227509      - 4.622883e-05 9.543191e-03  29.19325
## 4139 chr1 1286881 1286882      + 6.049905e-08 5.681008e-05  40.09259
## 4218 chr1 1293042 1293043      + 1.494626e-05 4.181681e-03  26.85755
## 6028 chr1 1852664 1852665      - 4.131871e-05 8.766236e-03  54.44444
## --------------
## sample.ids: T47D.DS.r1 T47D.DS.r2 T47D.E2.r1 T47D.E2.r2
## destranded FALSE
## assembly: hg19
## context: CpG
## treament: 0 0 1 1
## resolution: base

## GRangesList object of length 4:
## $exons
## GRanges object with 462321 ranges and 2 metadata columns:
##             seqnames               ranges strand |     score         name
##                <Rle>            <IRanges>  <Rle> | <integer>  <character>
##        [1]     chr1 [66999825, 67000051]      + |         1    NM_032291
##        [2]     chr1 [67091530, 67091593]      + |         2    NM_032291
##        [3]     chr1 [67098753, 67098777]      + |         3    NM_032291
##        [4]     chr1 [67101627, 67101698]      + |         4    NM_032291
##        [5]     chr1 [67105460, 67105516]      + |         5    NM_032291
##        ...      ...                 ...    ... .       ...          ...
##   [462317]    chr22 [51214200, 51214279]      - |         5 NM_001003789
##   [462318]    chr22 [51215098, 51215177]      - |         4 NM_001003789
##   [462319]    chr22 [51216380, 51216409]      - |         3 NM_001003789
##   [462320]    chr22 [51220616, 51220779]      - |         2 NM_001003789
##   [462321]    chr22 [51221929, 51222087]      - |         1 NM_001003789
##
## ...
## <3 more elements>
## -------
## seqinfo: 24 sequences from an unspecified genome; no seqlengths
```

```
# Annotate differentially methylated base with Promoter/Exon/Intron by annotation information
myDiff25p_Anno=annotate.WithGenicParts(myDiff25p, gene.obj)
myDiff25p_Anno
```

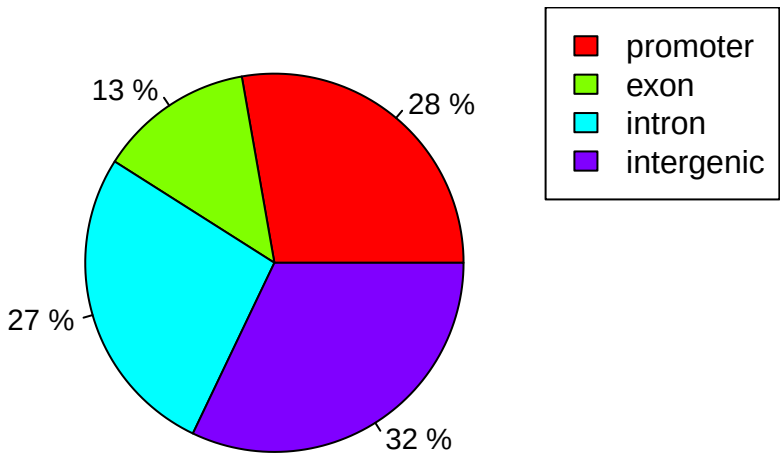
```
## summary of target set annotation with genic parts
## 3651 rows in target set
## --------------
## --------------
## percentage of target features overlapping with annotation :
##   promoter       exon     intron intergenic
##   27.71843   24.89729   39.41386   32.10079
##
##
## percentage of target features overlapping with annotation (with promoter>exon>intron precedence) :
##   promoter       exon     intron intergenic
##   27.71843   13.22925   26.95152   32.10079
##
##
## percentage of annotation boundaries with feature overlap :
##  promoter      exon    intron
## 1.7324043 0.2104598 0.5030322
##
##
## summary of distances to the nearest TSS :
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##       0     736    7587   31520   32060 1221000
```

```
# Similarly, we can read the CpG Island Annotation and annotate differentially
# methylated bases/regions with them.
diffCpGann=annotate.WithFeature.Flank(myDiff25p,
                                      cpg.obj$CpGi,
                                      cpg.obj$shores,
                                      feature.name="CpG Islands",
                                      flank.name="CpG Shores")
diffCpGann.hyper=annotate.WithFeature.Flank(myDiff25p.hyper,
                                            cpg.obj$CpGi, cpg.obj$shores,
                                            feature.name="CpG Islands",
                                            flank.name="CpG Shores")
diffCpGann.hypo=annotate.WithFeature.Flank(myDiff25p.hypo,
                                           cpg.obj$CpGi,
                                           cpg.obj$shores,
                                           feature.name="CpG Islands",
                                           flank.name="CpG Shores")
```

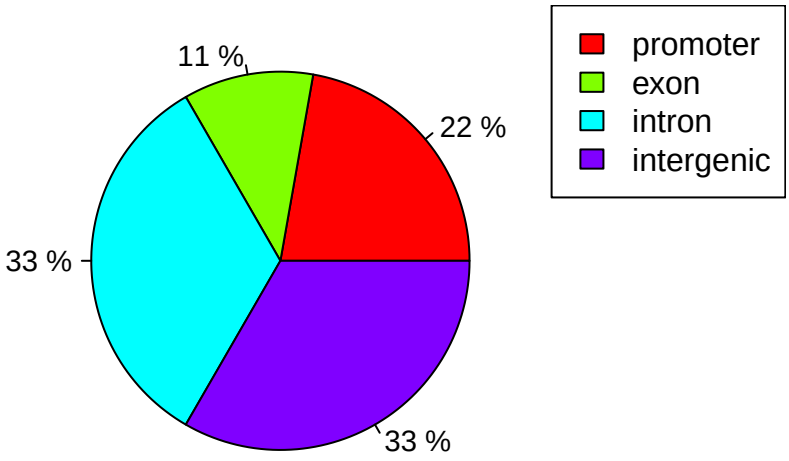
Firstly, we can plot the general genomic region distribution with all CpG bases.



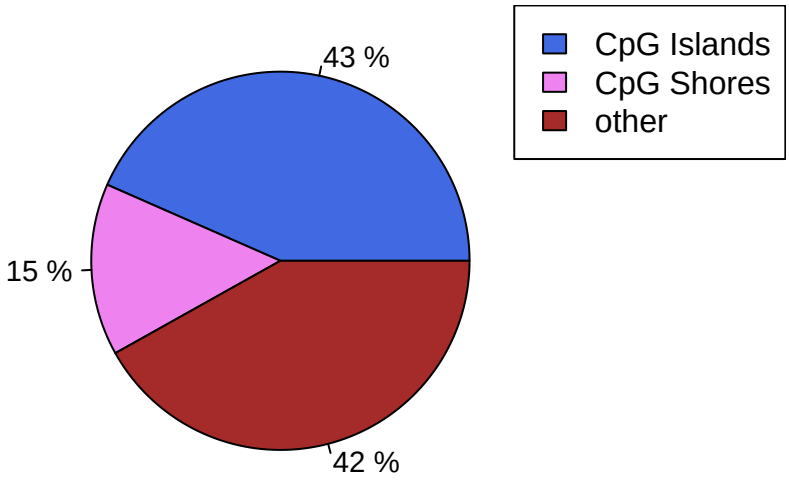
Secondly, we investigate the genomic region distribution with those hypermethylated CpG bases.



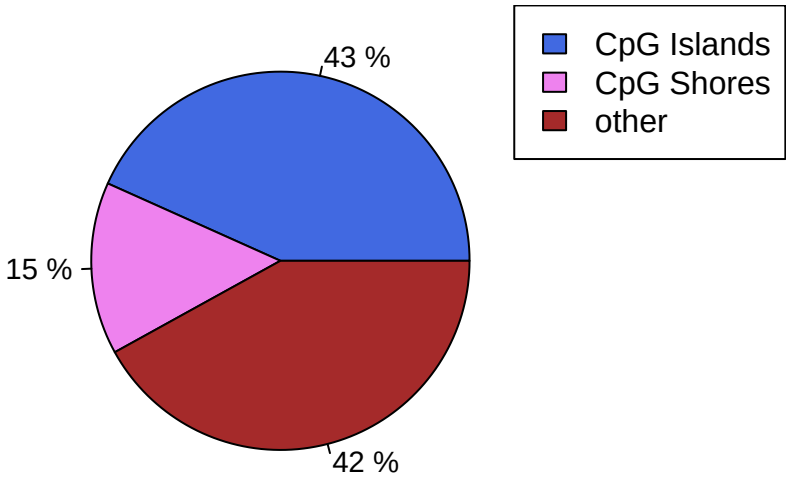
Then, we can also investigate the genomic region distribution with all hypomethylated CpG bases.



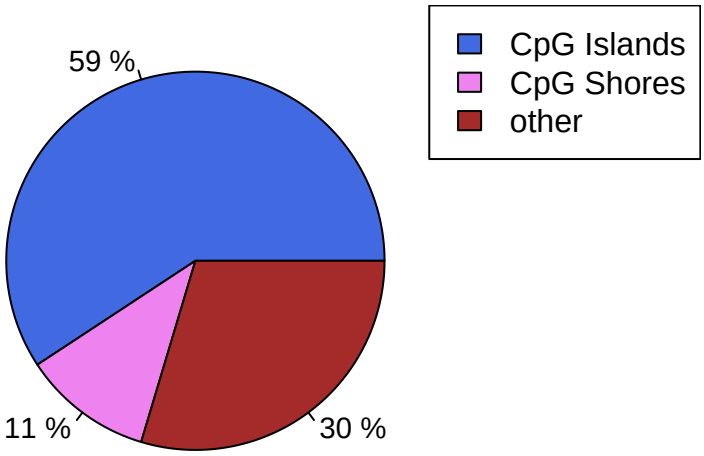
Similarly if we zoom into the specified CpG regions, e.g. CpG island, CpG shore, and other regions. And we can also discuss the CpG region distribution with all CpG bases.



And for the CpG region distribution with all hypermethylated CpG bases.



And for the CpG region distribution with all hypomethylated CpG bases.




```
##    chr start   end strand     pvalue    qvalue       diff
## 1 chr1 10785 10786      + 0.56168482 0.9449035 -0.8695652
## 2 chr1 10788 10789      + 0.05195282 0.5644036  4.7619048
## 3 chr1 10794 10795      + 0.05195282 0.5644036  4.7619048
## 4 chr1 10810 10811      + 0.41085680 0.8442256 -1.7391304
## 5 chr1 10812 10813      + 0.41085680 0.8442256 -1.7391304
```

```
##    chr   start     end strand       pvalue       qvalue     diff
## 1 chr1  137985  137986      + 7.251977e-13 4.324026e-09 27.14186
## 2 chr1 1093875 1093876      + 2.652323e-13 1.820171e-09 58.57403
## 3 chr1 1227508 1227509      - 4.622883e-05 9.543191e-03 29.19325
## 4 chr1 1286881 1286882      + 6.049905e-08 5.681008e-05 40.09259
## 5 chr1 1293042 1293043      + 1.494626e-05 4.181681e-03 26.85755
```

4. Differentially Methylated Region (DMR) Analysis

First, the raw methylated loci should take the format as below,

```
#  chr   start     end strand       pvalue       qvalue     diff
# chr1  137985  137986      + 7.251977e-13 4.324026e-09 27.14186
# chr1 1093875 1093876      + 2.652323e-13 1.820171e-09 58.57403
# chr1 1227508 1227509      - 4.622883e-05 9.543191e-03 29.19325
```

Let's check the first 5 rows (basic errors may be found at this step),

```
head(data)
```

```
##    chr start   end strand     pvalue    qvalue       diff
## 1 chr1 10785 10786      + 0.56168482 0.9449035 -0.8695652
## 2 chr1 10788 10789      + 0.05195282 0.5644036  4.7619048
## 3 chr1 10794 10795      + 0.05195282 0.5644036  4.7619048
## 4 chr1 10810 10811      + 0.41085680 0.8442256 -1.7391304
## 5 chr1 10812 10813      + 0.41085680 0.8442256 -1.7391304
## 6 chr1 10815 10816      + 0.24289096 0.7604375 -3.4782609
```

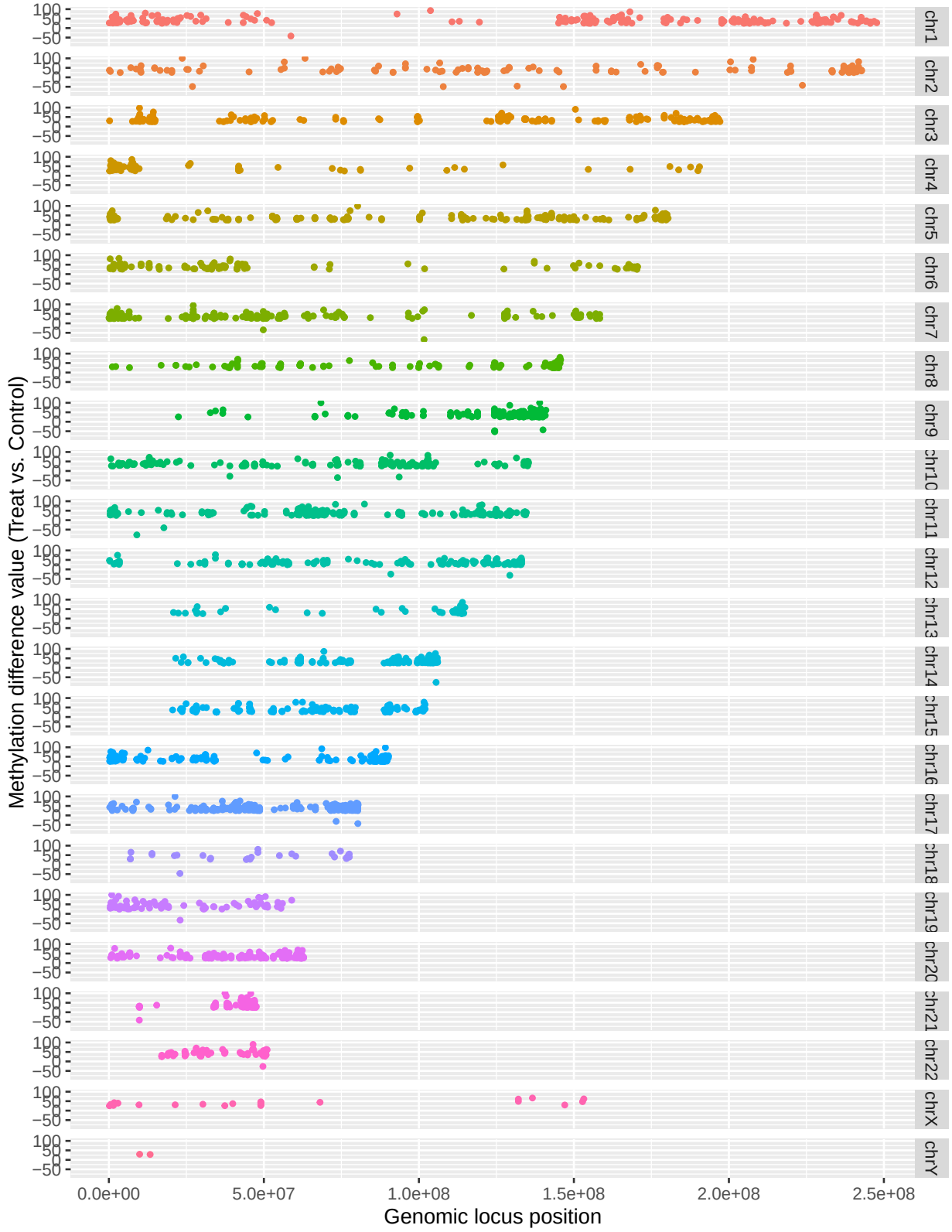
Then similarly, we may check the statistically significant methylated loci (Diff $> 25\%$, $p < 0.01$) as below,

```
head(dat)
```

```
##    chr   start     end strand       pvalue       qvalue     diff
## 1 chr1  137985  137986      + 7.251977e-13 4.324026e-09 27.14186
## 2 chr1 1093875 1093876      + 2.652323e-13 1.820171e-09 58.57403
## 3 chr1 1227508 1227509      - 4.622883e-05 9.543191e-03 29.19325
## 4 chr1 1286881 1286882      + 6.049905e-08 5.681008e-05 40.09259
## 5 chr1 1293042 1293043      + 1.494626e-05 4.181681e-03 26.85755
## 6 chr1 1852664 1852665      - 4.131871e-05 8.766236e-03 54.44444
```

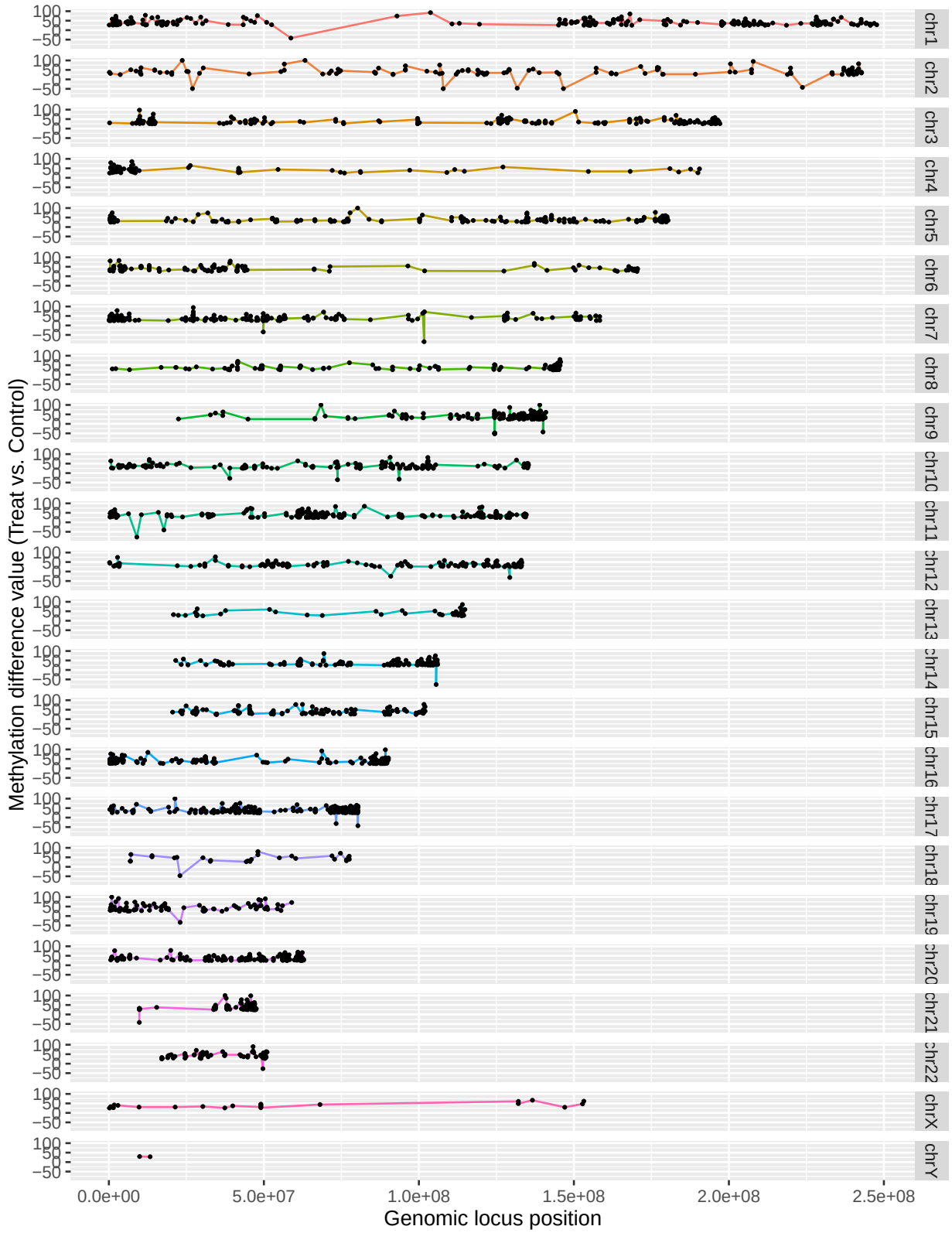
Then we may plot the statistically significant methylated loci (based on differential methylated level between control vs treatment samples) across the whole genome (ch1 ~ chr22, chrX and chrY).

```
# library(META2)
# fun_plotMethLoci(df, plotLine=F)
```



Similarly, we may be interested in the line curve convering all differential methylated regions.

```
# library(META2)
# fun_plotMethLoci(df, plotLine=T)
```



Then we filter out all those regions with length <= 1000 bp across chr1 to chr22.

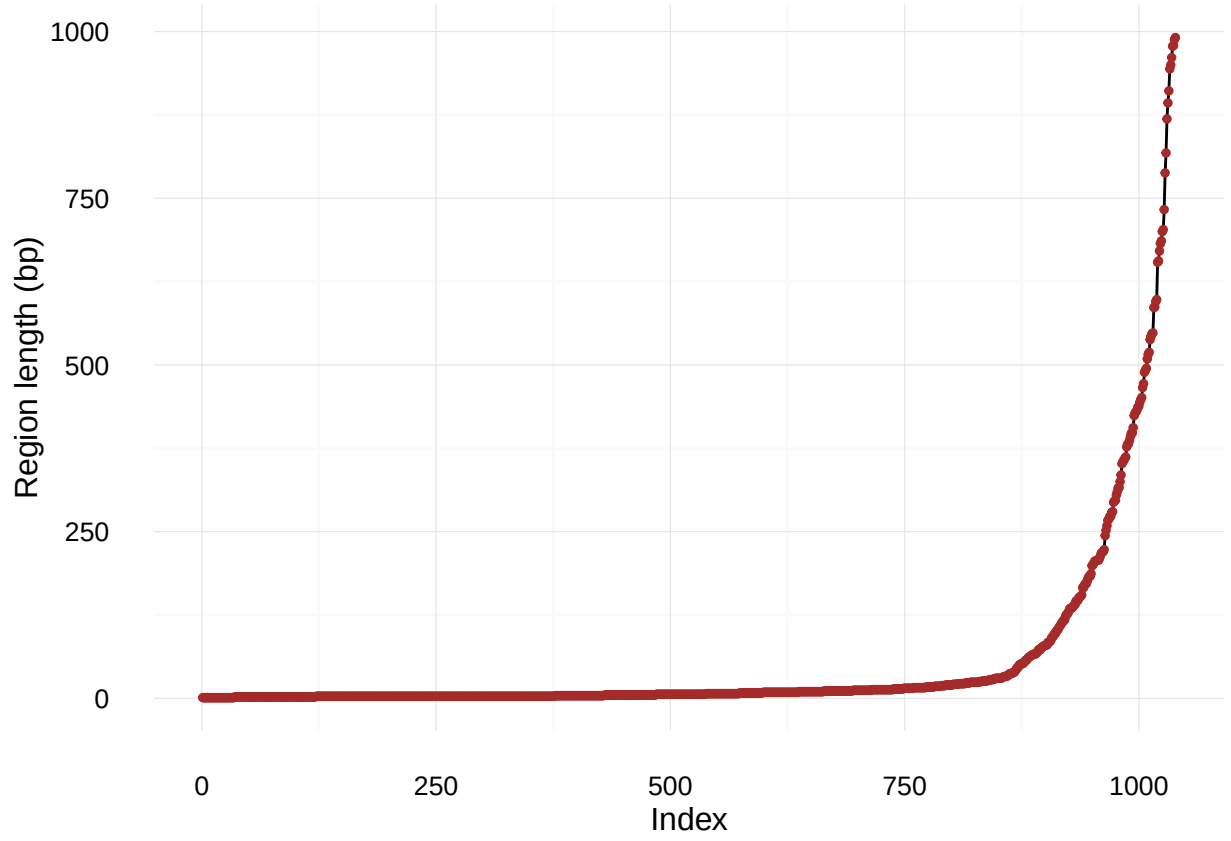
```
# library(META2)
# fun_findDMR <- function(data=df, sp=1000)
```

We can check those separate region's data format as below

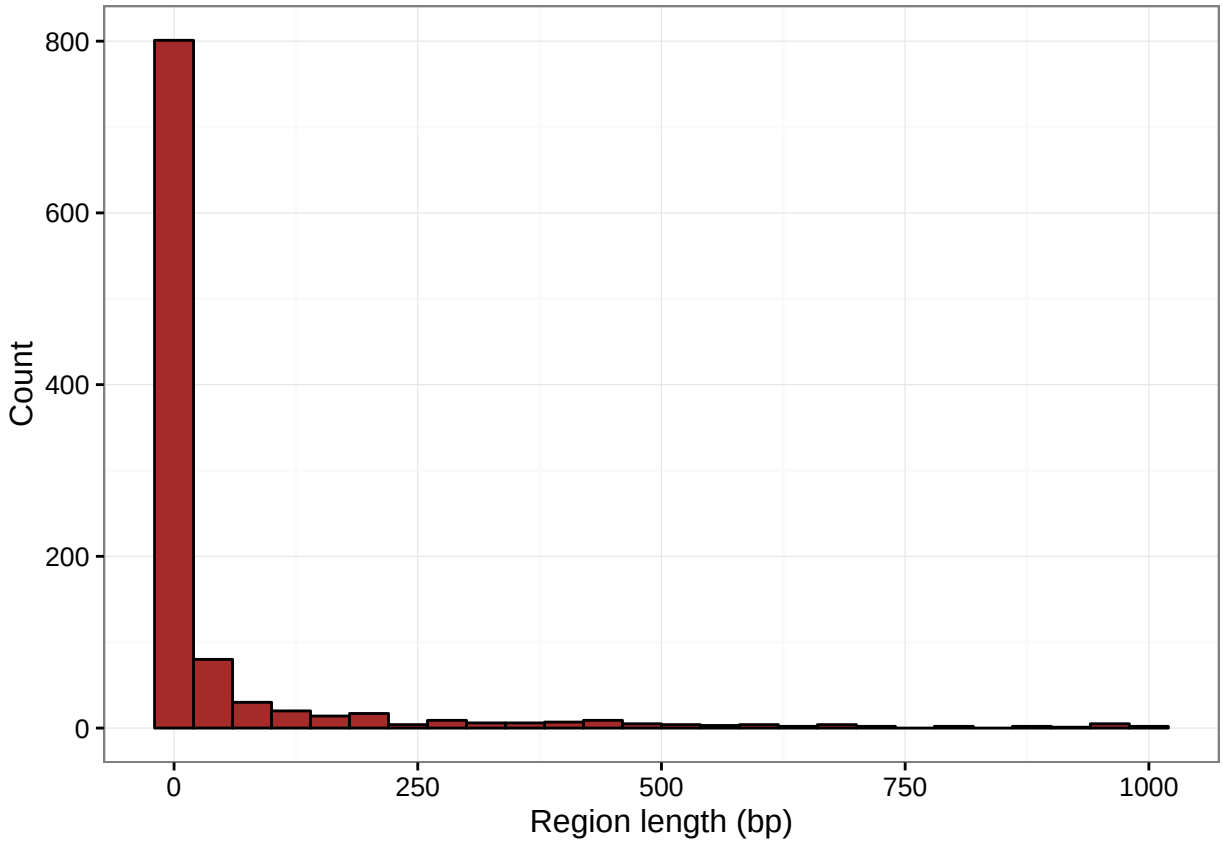
```
head(LOC)
```

```
##     V1        V2        V3
## 1 chr1   2276955   2277021
## 2 chr1 149222907 149222910
## 3 chr1 153233634 153234123
## 4 chr1 153234123 153234132
## 5 chr1 153651998 153652001
## 6 chr1 154733269 154733272
```

And their statistical properties as below, first the sorted region length diagram,



Then, the histogram for the region length distribution,



Then we further combine those regions <= 1000 bp across chr1 to chr22.

And we may check the combined region format as below,

```
##    chr     start       end
## 1 chr1   2276955   2277021
## 2 chr1 149222907 149222910
## 3 chr1 153233634 153234132
## 5 chr1 153651998 153652001
## 6 chr1 154733269 154733272
## 7 chr1 156831164 156831167
```

5. Integrative Visulization of DMR Analysis

Finally we annotate the regions with reference sequences from UCSC (hg 19) and transcript information convered by the region. Note some regions have no transcripts convered within the length, i.e. those transcripts are not influenced directly by DNA methylation. For such case, the readers are suggested to use the gene annotation instead.

And for illustration, we may choose one annotated region and display its integrated propery as below,

The selected region takes three main columns, and it is listed as below,

```
loc
```

```
##     chr     start       end
## 18 chr1 197743880 197744626
```

Then we can detect the seletced region contains the below methylated loci list,

```
head(Reg_loc)
```

```
##        chr     start       end strand       pvalue       qvalue      diff
## 78858 chr1 197743880 197743881      + 4.315018e-07 0.0002743774  29.60078
## 78859 chr1 197743881 197743882      - 4.374712e-01 0.8610859041 -11.97917
## 78860 chr1 197743891 197743892      + 6.834133e-06 0.0023194520  24.71276
## 78861 chr1 197743943 197743944      - 1.141918e-03 0.0862283348  30.35714
## 78862 chr1 197743957 197743958      - 8.960121e-03 0.2781009918  23.21429
## 78863 chr1 197743960 197743961      - 4.093930e-04 0.0442596060  32.14286
```

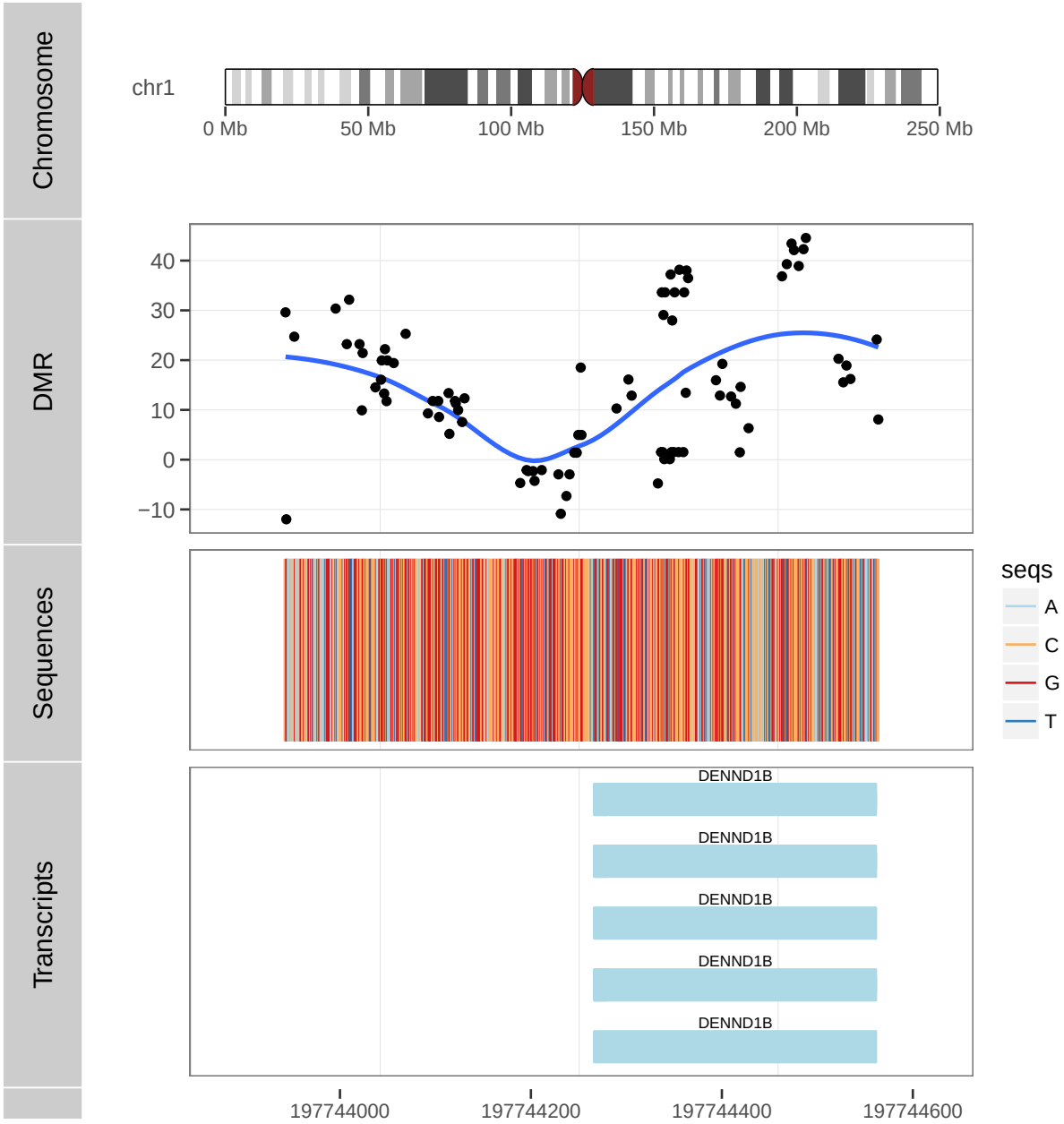
The integrative illustration for the annotated region selected for demonstration.

```
# library(META2)
# fun_intplotDMR(loc, Reg_loc)
```

```
## Warning: replacing previous import 'ggplot2::Position' by
## 'BiocGenerics::Position' when loading 'ggbio'
```

```
## Warning: The plyr::rename operation has created duplicates for the
## following name(s): (`colour`)
```

```
## Warning: The plyr::rename operation has created duplicates for the
## following name(s): (`colour`)
```



6. Session Information

```
## R version 3.3.1 (2016-06-21)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 10586)
##
## locale:
## [1] LC_COLLATE=Chinese (Simplified)_China.936
## [2] LC_CTYPE=Chinese (Simplified)_China.936
## [3] LC_MONETARY=Chinese (Simplified)_China.936
## [4] LC_NUMERIC=C
## [5] LC_TIME=Chinese (Simplified)_China.936
```

```
##
## attached base packages:
## [1] stats4    parallel  stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] Homo.sapiens_1.3.1
##  [2] TxDb.Hsapiens.UCSC.hg19.knownGene_3.2.2
##  [3] org.Hs.eg.db_3.3.0
##  [4] GO.db_3.3.0
##  [5] OrganismDbi_1.14.1
##  [6] GenomicFeatures_1.24.4
##  [7] AnnotationDbi_1.34.4
##  [8] Biobase_2.32.0
##  [9] BSgenome.Hsapiens.UCSC.hg19_1.4.0
## [10] BSgenome_1.40.1
## [11] rtracklayer_1.32.1
## [12] Biostrings_2.40.2
## [13] XVector_0.12.0
## [14] GenomicRanges_1.24.2
## [15] GenomeInfoDb_1.8.3
## [16] IRanges_2.6.1
## [17] S4Vectors_0.10.2
## [18] ggbio_1.20.1
## [19] Repitools_1.18.2
## [20] BiocGenerics_0.18.0
## [21] ggplot2_2.1.0
## [22] methylKit_0.9.4
##
## loaded via a namespace (and not attached):
##   [1] colorspace_1.2-6             biovizBase_1.20.0
##   [3] DNAcopy_1.46.0               base64enc_0.1-3
##   [5] dichromat_2.0-0              listenv_0.6.0
##   [7] affyio_1.42.0                interactiveDisplayBase_1.10.3
##   [9] codetools_0.2-14             splines_3.3.0
##  [11] R.methodsS3_1.7.1            knitr_1.14
##  [13] Formula_1.2-1                Rsamtools_1.24.0
##  [15] annotate_1.50.0              cluster_2.0.4
##  [17] vsn_3.40.0                   R.oo_1.20.0
##  [19] graph_1.50.0                 shiny_0.14
##  [21] httr_1.2.1                   Matrix_1.2-7.1
##  [23] limma_3.28.20                formatR_1.4
##  [25] acepack_1.3-3.3              htmltools_0.3.5
##  [27] tools_3.3.0                  gtable_0.2.0
##  [29] affy_1.50.0                  reshape2_1.4.1
##  [31] Rcpp_0.12.7                  gdata_2.17.0
##  [33] preprocessCore_1.34.0        R.devices_2.14.0
##  [35] stringr_1.1.0                globals_0.7.0
##  [37] mime_0.5                     ensembldb_1.4.7
##  [39] gtools_3.5.0                 XML_3.98-1.4
##  [41] future_1.0.1                 AnnotationHub_2.4.2
##  [43] edgeR_3.14.0                 zlibbioc_1.18.0
##  [45] MASS_7.3-45                  scales_0.4.0
##  [47] VariantAnnotation_1.18.5     BiocInstaller_1.22.3
```

```
##  [49] RBGL_1.48.1                 SummarizedExperiment_1.2.3
##  [51] RColorBrewer_1.1-2          yaml_2.1.13
##  [53] gridExtra_2.2.1             aroma.affymetrix_3.0.0
##  [55] biomaRt_2.28.0              rpart_4.1-10
##  [57] reshape_0.8.5               latticeExtra_0.6-28
##  [59] stringi_1.1.1               RSQLite_1.0.0
##  [61] genefilter_1.54.2           Ringo_1.36.0
##  [63] PSCBS_0.61.0                caTools_1.17.1
##  [65] BiocParallel_1.6.3          truncnorm_1.0-7
##  [67] chron_2.3-47                matrixStats_0.50.2
##  [69] bitops_1.0-6                Rsolnp_1.16
##  [71] evaluate_0.9                lattice_0.20-34
##  [73] R.huge_0.9.0                GenomicAlignments_1.8.4
##  [75] labeling_0.3                GGally_1.2.0
##  [77] plyr_1.8.4                  magrittr_1.5
##  [79] R6_2.1.3                    gplots_3.0.1
##  [81] Hmisc_3.17-4                DBI_0.5-1
##  [83] foreign_0.8-67              gsmoothr_0.1.7
##  [85] R.filesets_2.10.0           survival_2.39-5
##  [87] RCurl_1.95-4.8              nnet_7.3-12
##  [89] R.rsp_0.30.0                KernSmooth_2.23-15
##  [91] rmarkdown_1.0               grid_3.3.0
##  [93] data.table_1.9.6            digest_0.6.10
##  [95] aroma.core_3.0.0            xtable_1.8-2
##  [97] R.cache_0.12.0              httpuv_1.3.3
##  [99] R.utils_2.4.0               aroma.apd_0.6.0
## [101] munsell_0.4.3
```