Output files:

1. alignment result

Alignment result is in standard SAM format (see https://samtools.github.io/hts-specs/SAMv1.pdf for detailed information).

2. Calmeth result

The output files include an methylation output file (base sites) and a region methylation outfile (used for dmr) and a chromosome methylation output file.

Output file:

BS.mr.methratio.txt
BS.mr.methBins.txt
BS.mr_Region.CG.txt
BS.mr_Region.CHG.txt
BS.mr_Region.CHH.txt
BS.mr_loci.CG.txt
BS.mr_loci.CHG.txt
BS.mr_loci.CHH.txt

The methratio output file (BS.mr.methratio.txt) has a tab-separated format with 12 fields

1. Chromosome Chromosome.
2. Loci Coordinate.
3. Strand +/-
4. Context CG/CHG/CHH
5. C_count The number of C in this base pair.
6. CT_count The number of coverage in this base pair.
7. methRatio The methylation level.
8. eff_CT_count Adjust read coverage based on opposite strand.
9. rev_G_count The number of G in the reverse strand.
10. rev_GA_count The number of coverage in the reverse strand.
11. MethContext M/Mh/H/hU/U, M means the methylation level ≥ 80%, Mh means
12. 5context Five base pair across this Cytosine.

The chromosome methylation output file (BS.mr.methBins.txt) has a tab-separated format with 4 fields

1. Chr chromosome.
2. loci coordinate.
3. Methratio DNA methylation level of this region.
4. Context The DNA context calculated in this region.

The bins methylation level output file (BS.mr_Region.C*.txt), and this file can be used to do DMR detect.

1. Chromosome

2. Loci bins start coordinate
3. Strand +/-
4. Context CG/CHG/CHH
5. C_Count The number of methylated C reads in this region
6. CT_count The number of coverage C reads in this region.

The base pair methylation level output (BS.mr_loci.C*.txt) is same as region output. And it can be used to detect DMC.

3. Annotation output file

Output file (gene gtf as example):

BS.gene.GENE.cg.1.txt
BS.gene.GENE.chg.1.txt
BS.gene.GENE.chh.1.txt
BS.gene.Promoter.cg.1.txt
BS.gene.Promoter.chg.1.txt
BS.gene.Promoter.chh.1.txt
BS.gene.body.cg.1.txt
BS.gene.body.chg.1.txt
BS.gene.body.chh.1.txt
BS.gene.Methylevel.1.txt
BS.gene.AverMethylevel.1.txt
BS.gene.annoDensity.1.txt
BS.gene.TTS.cg.1.txt
BS.gene.TTS.chg.1.txt
BS.gene.TTS.chh.1.txt
BS.gene.TSS.cg.1.txt
BS.gene.TSS.chg.1.txt
BS.gene.TSS.chh.1.txt

The DNA methylation level on every gene (BS.gene.GENE.c*.1.txt).

Every line is one gene or other function element, and every gene has a tab-separated format with n fields.

1. Chrom chromosome.
2. Start gene or other function element start.
3. End gene or other function element end.
4. ID gene id.
5. Methy level on the 1 region of this gene
6. Methy level on the 2 region of this gene
7. …..
8. Methy level on the n region of this gene

Default: n = 317, this number can be changed by –s parameter.

The DNA methylation on the gene promoter or other function element upstream.

(BS.gene.Promoter.c*.1.txt)

1. Chr
2. Pos
3. Strand
4. Context
5. Meth_C
6. Cover
7. ID

The DNA methylation on the gene body or other function element body. (BS.gene.body.cg.1.txt)

The file format is same as promoter file above.

The average DNA methylation level of all the genes. (BS.gene.Methylevel.1.txt)

Every line is the different DNA methylation context (CG/CHG/CHH).

1. Context
2. Average methy level on the 1 region of the gene
3. Average methy level on the 2 region of the gene
4. …
5. Average methy level on the n region of the gene

Default: n = 117, this number can be changed by –s parameter.

The DNA methylation level on every gene. (BS.gene.AverMethylevel.1.txt)

Context	Regions	DNA methylation level
CG	UP	0.480916
CHG	UP	0.021739
CHH	UP	0.488764
CHG	UP	0.000000
CG	BODY	0.517162
CHG	BODY	0.015209
CHH	BODY	0.009640
CG	UP	0.936768
CHG	UP	0.018952
CHH	UP	0.013494

The gene density on the chromosome. (BS.gene.annoDensity.1.txt)

1. Chrom
2. Pos
3. Gene density
4. Strand +- means don’t separate +/- strand.

```
chr1    0       0.450020        +-
chr1    0       0.176230        +
chr1    0       -0.274020       -
chr1    1       0.857980        +-
chr1    1       0.188940        +
chr1    1       -0.696200       -
```

The DNA methylation level distribution of the gene Transcript Start Site (TSS) region. **(**BS.gene.TTS.c*.1.txt**)**

1. Gene ID
2. methy level on the 1 region of this gene
3. methy level on the 2 region of this gene
4. …
5. methy level on the n region of this gene

Default: n = 79, this number can be changed by –s parameter.

The DNA methylation level distribution of the gene Transcript Terminal Site (TTS) region. (BS.gene.TTS.c*.1.txt)

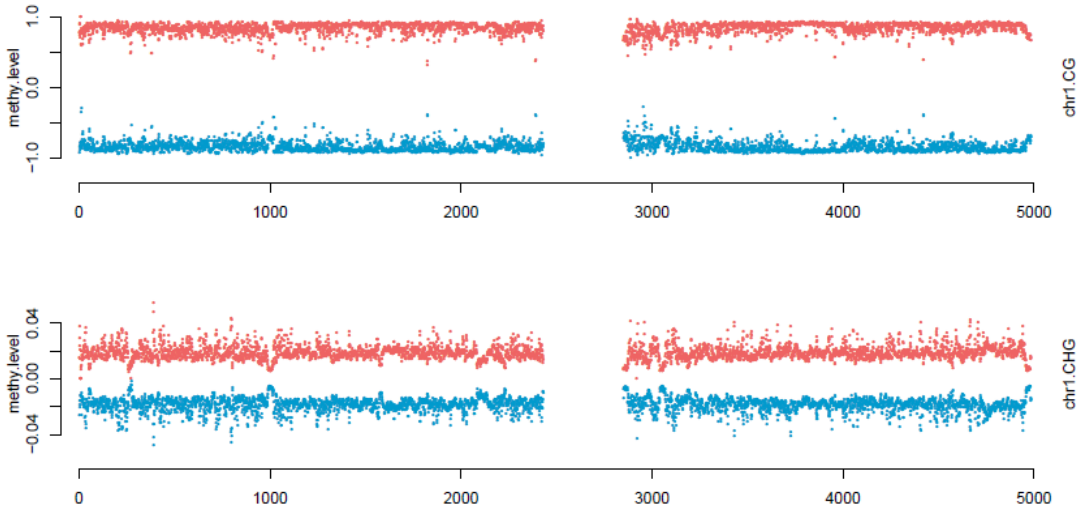
This file is same as TSS file.

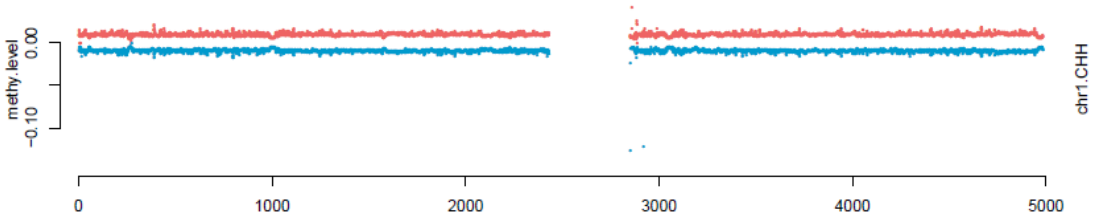
4. methyPlot output file

Output file:

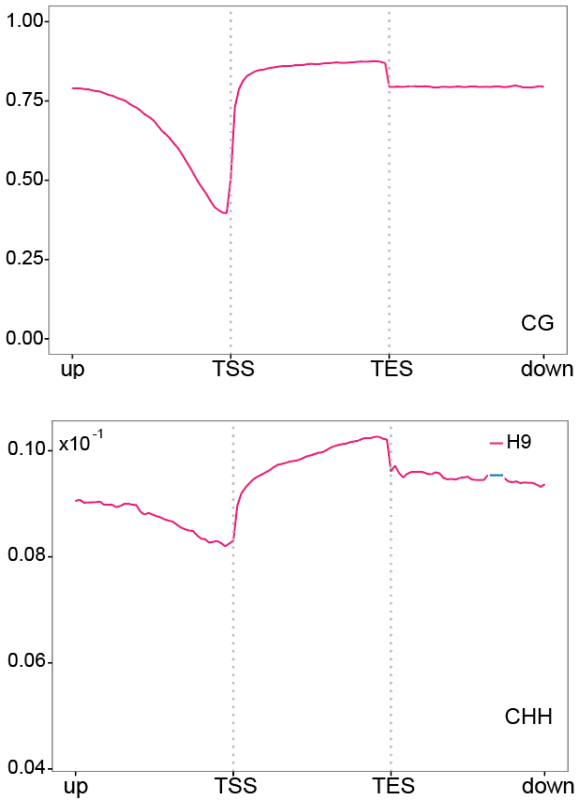
BS.chrosome.methy.distri.pdf
BS.methlevel.pdf
BS.elements.pdf
BS.density.pdf

The DNA methylation level distribution on genome (BS.chrosome.methy.distri.pdf). For example (H9 cell line chromosome 11):

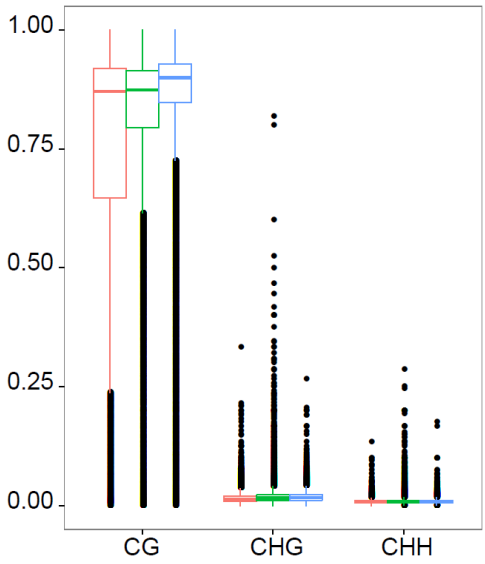




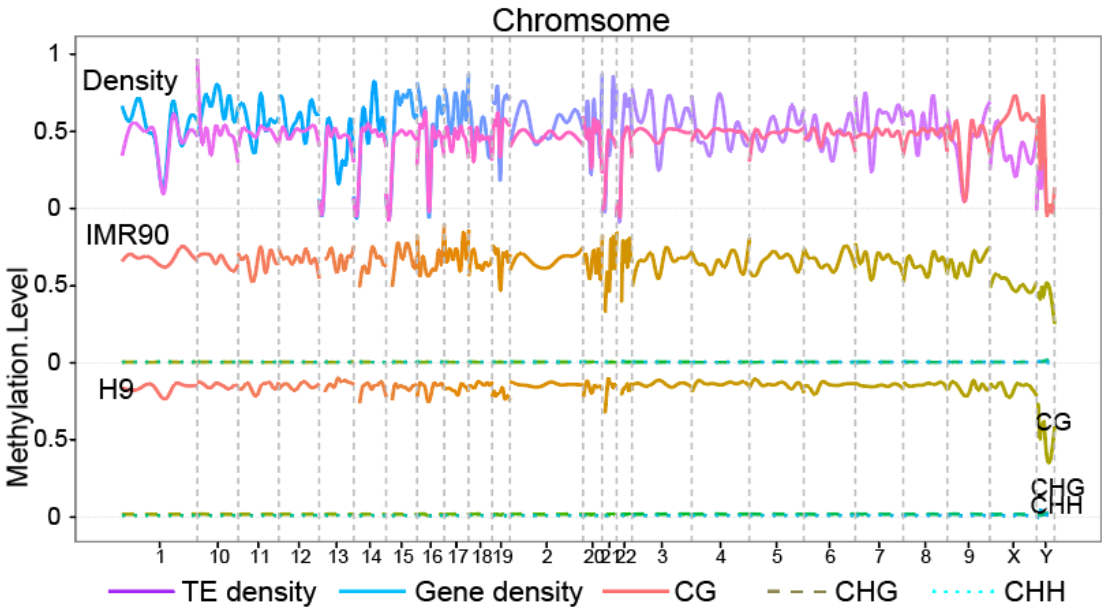
The DNA methylation distribution on the gene. (BS.methlevel.pdf)
For example (H9 cell line):



The DNA methylation level across gene. (BS.elements.pdf)
For example (H9 cell line):



The gene density and DNA methylation distribution on the genome. (BS.density.pdf)
For example(H9 and IMR90 cell line):



5. DNA methylation differential analysis output file.

DMC or DMR output file.

1. Chrom chromosome.
2. Position coordinate.
3. Starnd +/-
4. Context DNA methylation context.
5. Pvalue
6. adjust_pvalue
7. combine_pvalue
8. corrected_pvalue

9. cover_sample1 The number of coverage reads on this site in sample 2.
10. meth_sample1 The number of methylated reads on this site in sample 1.
11. cover_sample2 The number of coverage reads on this site in sample 2.
12. meth_sample2 The number of methylated reads on this site in sample 2.
13. meth.diff The methylation level minus
14. filter Pass/Failed

DMR output file detected by auto detect method.

1. Chrom
2. start
3. end
4. dmr score
5. meth.diff
6. aver_corrected_pvalue

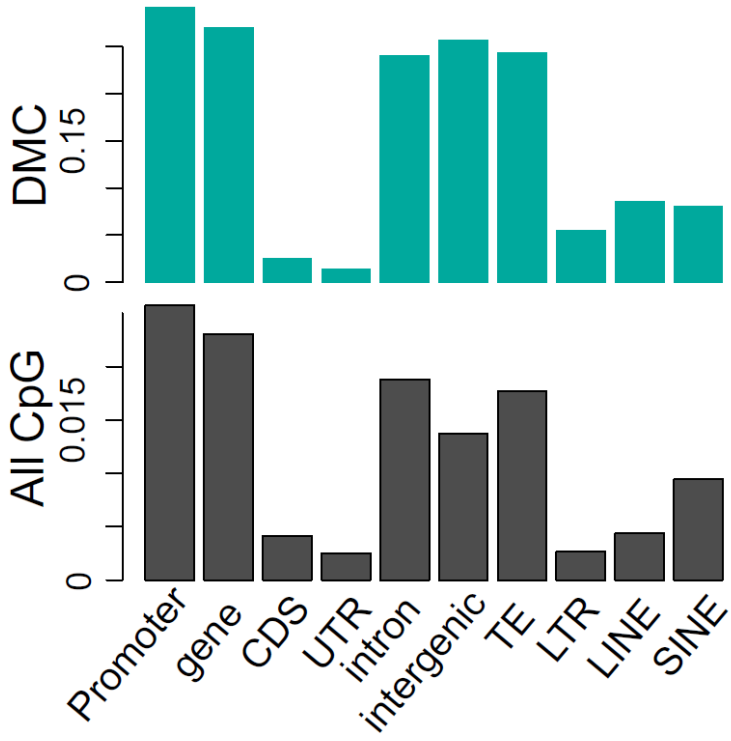
We can further filter the result by pvalue and methy.diff between two samples:

awk '$6<0.05 && sqrt($13*$13)>0.6 ' H9vsIMR90.gene.dm.txt > H9vsIMR90.gene.dm.filter.txt

6. DMC annotation

Output file:

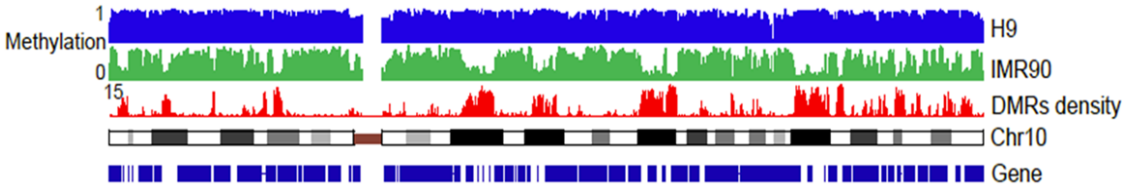
DMCanno.pdf



7. Others

We also provide bed file/bigwig file of the DNA methylation file and DMR output file, and we can upload this file to genome browser to seed the DMR distribution on the genome.

For example, DNA methylation disreibution on genome:



For example, (the DNA methylation different between H9 and IMR90, and upload this bed file to WashU genome browser.)