

Bismark analysis

#bismark genome preparation

bismark_genome_preparation /path/to/genome/

```
ibab@IBAB-MSc14-Comp003:~/data_analysis/bismark$ bismark_genome_preparation reference/
Writing bisulfite genomes out into a single MFA (multi FastA) file

Bisulfite Genome Indexer version v0.25.1 (last modified: 19 May 2022)

Step I - Prepare genome folders - completed

Total number of conversions performed:
C->T: 209834
G->A: 209332

Step II - Genome bisulfite conversions - completed

Bismark Genome Preparation - Step III: Launching the Bowtie 2 indexer
Please be aware that this process can - depending on genome size - take several hours!
Settings:
```

Alignment

bismark -bowtie2 reference/ R1.fastq.gz

```
Number of alignments to (merely theoretical) complementary strands being rejected in total: 0

Final Cytosine Methylation Report
=====
Total number of C's analysed: 875967

Total methylated C's in CpG context: 29030
Total methylated C's in CHG context: 851
Total methylated C's in CHH context: 2211
Total methylated C's in Unknown context: 3

Total unmethylated C's in CpG context: 17594
Total unmethylated C's in CHG context: 222073
Total unmethylated C's in CHH context: 604208
Total unmethylated C's in Unknown context: 532

C methylated in CpG context: 62.3%
C methylated in CHG context: 0.4%
C methylated in CHH context: 0.4%
C methylated in Unknown context (CN or CHN): 0.6%

Bismark completed in 0d 0h 0m 12s

=====
Bismark run complete
=====
```

bismark -bowtie2 reference/ R2.fastq.gz

```

Number of alignments to (merely theoretical) complementary strands being rejected in total: 0

Final Cytosine Methylation Report
=====
Total number of C's analysed: 485

Total methylated C's in CpG context: 10
Total methylated C's in CHG context: 8
Total methylated C's in CHH context: 282
Total methylated C's in Unknown context: 1

Total unmethylated C's in CpG context: 2
Total unmethylated C's in CHG context: 40
Total unmethylated C's in CHH context: 143
Total unmethylated C's in Unknown context: 0

C methylated in CpG context: 83.3%
C methylated in CHG context: 16.7%
C methylated in CHH context: 66.4%
C methylated in Unknown context (CN or CHN): 100.0%

Bismark completed in 0d 0h 0m 6s

=====
Bismark run complete

```

#methylation site extraction

bismark_methylation_extractor --gzip --bedGraph R1_bismark_bt2_pe.bam

```

=====
Methylation information will now be written into a bedGraph and coverage file
=====

Using the following files as Input:
/home/ibab/data_analysis/bismark/CpG_OT_R1_bismark_bt2.txt.gz /home/ibab/data_analysis/bisma
rk/CpG_OB_R1_bismark_bt2.txt.gz

Writing bedGraph to file: R1_bismark_bt2.bedGraph.gz
Also writing out a coverage file including counts methylated and unmethylated residues to file
: R1_bismark_bt2.bismark.cov.gz

Now writing methylation information for file >>CpG_OT_R1_bismark_bt2.txt.gz<< to individual fi
les for each chromosome
Finished writing out individual chromosome files for CpG_OT_R1_bismark_bt2.txt.gz
Now writing methylation information for file >>CpG_OB_R1_bismark_bt2.txt.gz<< to individual fi
les for each chromosome
Finished writing out individual chromosome files for CpG_OB_R1_bismark_bt2.txt.gz

Collecting temporary chromosome file information... Processing the following input file(s):
CpG_OT_R1_bismark_bt2.txt.gz.chrchrREF.methXtractor.temp

Sorting input file CpG_OT_R1_bismark_bt2.txt.gz.chrchrREF.methXtractor.temp by positions (usin
g -S of 2G)
Successfully deleted the temporary input file CpG_OT_R1_bismark_bt2.txt.gz.chrchrREF.methXtrac
tor.temp

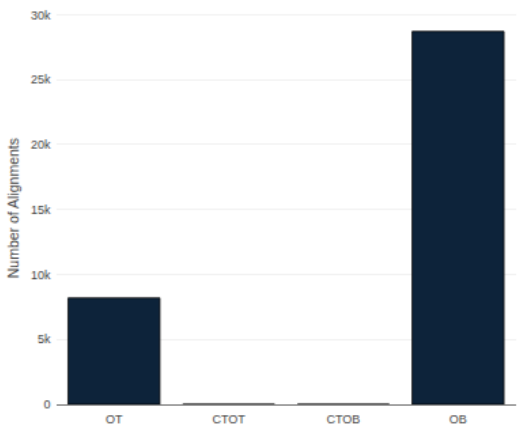
Finished BedGraph creation

```

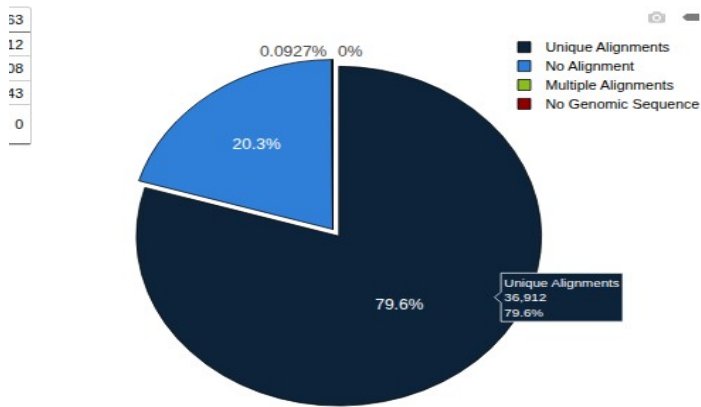
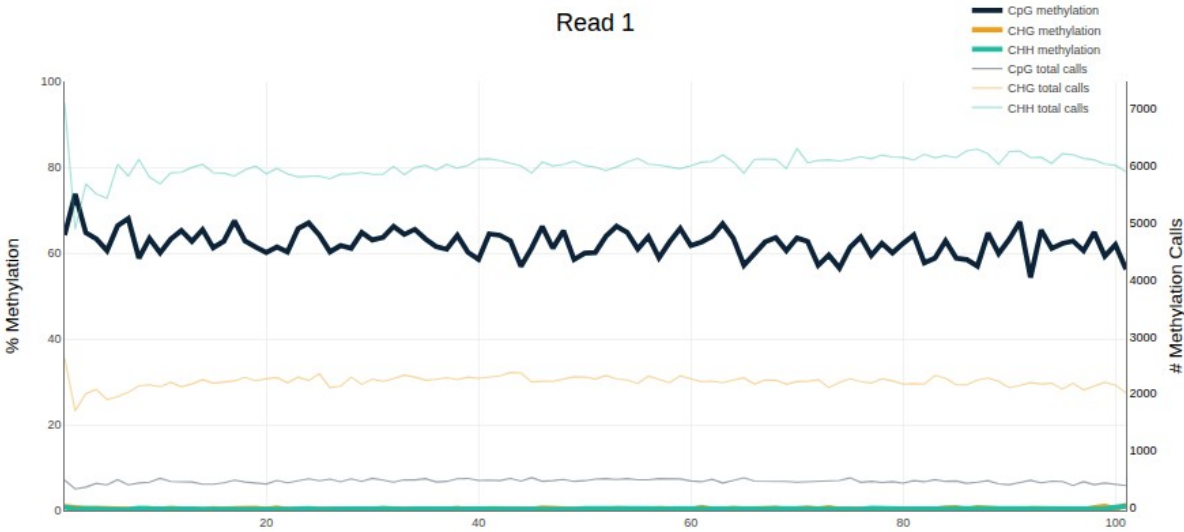
bismark_methylation_extractor --gzip --bedGraph R2_bismark_bt2_pe.bam

Alignment to Individual Bisulfite Strands

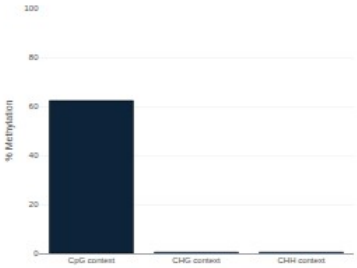
OT	8195	original top strand
CTOT	0	complementary to original top strand
CTOB	0	complementary to original bottom strand
OB	28717	original bottom strand



M-Bias Plot

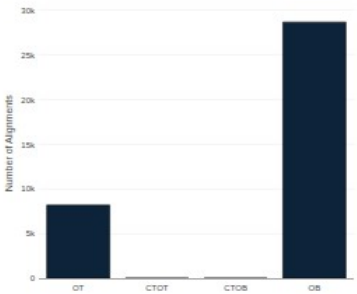


Total C's analysed	875967
Methylated C's in CpG context	29030
Methylated C's in CHG context	851
Methylated C's in CHH context	2211
Methylated C's in Unknown context	3
Unmethylated C's in CpG context	17594
Unmethylated C's in CHG context	222073
Unmethylated C's in CHH context	604208
Unmethylated C's in Unknown context	532
Percentage methylation (CpG context)	62.3%
Percentage methylation (CHG context)	0.4%
Percentage methylation (CHH context)	0.4%
Methylated C's in Unknown context	0.0%



Alignment to Individual Bisulfite Strands

OT	8195	original top strand
CTOT	0	complementary to original top strand
CTOB	0	complementary to original bottom strand
OB	28717	original bottom strand



Cytosine Methylation after Extraction

Total C's analysed	875967
Methylated C's in CpG context	29030
Methylated C's in CHG context	851
Methylated C's in CHH context	2211
Unmethylated C's in CpG context	17594
Unmethylated C's in CHG context	222073
Unmethylated C's in CHH context	604208
Percentage methylation (CpG context)	62.3%
Percentage methylation (CHG context)	0.4%
Percentage methylation (CHH context)	0.4%

