

```
gunzip -k 8cell_rep2_trimmed2_WGBS_R1.fastq.gz 8cell_rep2_trimmed2_WGBS_R2.fastq.gz
Epiblast_rep2_trimmed2_WGBS_R1.fastq.gz Epiblast_rep2_trimmed2_WGBS_R2.fastq.gz
ICM_rep2_trimmed2_WGBS_R1.fastq.gz ICM_rep2_trimmed2_WGBS_R2.fastq.gz
```

```
gunzip -k 8cell_rep2_WGBS_R1.fastq.gz 8cell_rep2_WGBS_R2.fastq.gz
Epiblast_rep2_WGBS_R1.fastq.gz Epiblast_rep2_WGBS_R2.fastq.gz ICM_rep2_WGBS_R1.fastq.gz
ICM_rep2_WGBS_R2.fastq.gz
```

```
fastqc -o QC_Trimmed2
/home/students/sarmad1/Methylation/Trimmed2/8cell_rep2_trimmed2_WGBS_R1.fastq
fastqc -o QC_Trimmed2
/home/students/sarmad1/Methylation/Trimmed2/8cell_rep2_trimmed2_WGBS_R2.fastq
fastqc -o QC_Trimmed2
/home/students/sarmad1/Methylation/Trimmed2/Epiblast_rep2_trimmed2_WGBS_R1.fastq
fastqc -o QC_Trimmed2
/home/students/sarmad1/Methylation/Trimmed2/Epiblast_rep2_trimmed2_WGBS_R2.fastq
```

```
cp ICM_rep2_WGBS_R1.fastq.gz ICM_rep2_WGBS_R2.fastq.gz
/home/students/sarmad1/Methylation/Before_QC
```

```
/home/students/sarmad1/Methylation/Trimmed2/Epiblast_rep2_trimmed2_WGBS_R2.fastq
```

```
cp ICM_rep2_trimmed_WGBS_R1.fastq.gz ICM_rep2_trimmed_WGBS_R2.fastq.gz /home/students/
sarmad1/Methylation/Trimmed2
cp Epiblast_rep2_trimmed_WGBS_R1.fastq.gz Epiblast_rep2_trimmed_WGBS_R2.fastq.gz
/home/students/sarmad1/Methylation/Trimmed2
```

Rename from trimmed to trimmed2

```
gunzip -k ICM_rep2_WGBS_R1.fastq.gz ICM_rep2_WGBS_R2.fastq.gz
Epiblast_rep2_WGBS_R1.fastq.gz Epiblast_rep2_WGBS_R2.fastq.gz
```

```
fastqc -o /home/students/sarmad1/Methylation/After_QC2/
/home/students/sarmad1/Methylation/Before_QC/Epiblast_rep2_WGBS_R1.fastq
fastqc -o /home/students/sarmad1/Methylation/After_QC2/
/home/students/sarmad1/Methylation/Before_QC/Epiblast_rep2_WGBS_R2.fastq
fastqc -o /home/students/sarmad1/Methylation/After_QC2/
/home/students/sarmad1/Methylation/Before_QC/ICM_rep2_WGBS_R1.fastq
fastqc -o /home/students/sarmad1/Methylation/After_QC2/
/home/students/sarmad1/Methylation/Before_QC/ICM_rep2_WGBS_R2.fastq
```

```
bismark -p 4 --gzip /home/students/sarmad1/Methylation/Trimmed2/ -1
Epiblast_rep2_trimmed2_WGBS_R1.fastq.gz -2 Epiblast_rep2_trimmed2_WGBS_R2.fastq.gz
bismark -p 4 --gzip /home/students/sarmad1/Methylation/Trimmed2/ -1
ICM_rep2_trimmed2_WGBS_R1.fastq.gz -2 ICM_rep2_trimmed2_WGBS_R2.fastq.gz
bismark -p 4 --gzip /home/students/sarmad1/Methylation/Trimmed2/ -1
8cell_rep2_trimmed2_WGBS_R1.fastq.gz -2 8cell_rep2_trimmed2_WGBS_R2.fastq.gz
```

```
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/bismark -p 4 --gzip  
/home/students/sarmad1/Methylation/mm10/ -1 Epiblast_rep2_trimmed2_WGBS_R1.fastq.gz -2  
Epiblast_rep2_trimmed2_WGBS_R2.fastq.gz
```

```
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/bismark -p 4 --gzip  
/home/students/sarmad1/Methylation/mm10/ -1 ICM_rep2_trimmed2_WGBS_R1.fastq.gz -2  
ICM_rep2_trimmed2_WGBS_R2.fastq.gz
```

```
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/bismark -p 4 --gzip  
/home/students/sarmad1/Methylation/mm10/ -1 8cell_rep2_trimmed2_WGBS_R1.fastq.gz -2  
8cell_rep2_trimmed2_WGBS_R2.fastq.gz
```

```
gunzip -k ICM_rep2_trimmed2_WGBS_R1.fastq.gz ICM_rep2_trimmed2_WGBS_R2.fastq.gz  
Epiblast_rep2_trimmed2_WGBS_R1.fastq.gz Epiblast_rep2_trimmed2_WGBS_R2.fastq.gz
```

```
fastqc -o /home/students/sarmad1/Methylation/QC_Trimmed2/  
/home/students/sarmad1/Methylation/Trimmed2/ICM_rep2_trimmed2_WGBS_R1.fastq  
fastqc -o /home/students/sarmad1/Methylation/QC_Trimmed2/  
/home/students/sarmad1/Methylation/Trimmed2/ICM_rep2_trimmed2_WGBS_R2.fastq  
fastqc -o /home/students/sarmad1/Methylation/QC_Trimmed2/  
/home/students/sarmad1/Methylation/Trimmed2/Epiblast_rep2_trimmed2_WGBS_R1.fastq  
fastqc -o /home/students/sarmad1/Methylation/QC_Trimmed2/  
/home/students/sarmad1/Methylation/Trimmed2/Epiblast_rep2_trimmed2_WGBS_R2.fastq
```

```
bismark-0.22.3/bismark_genome_preparation /home/students/sarmad1/Methylation/mm10/
```

```
bismark-0.22.3/bismark_genome_preparation /home/students/sarmad1/Methylation/Trimmed2/
```

```
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/deduplicate_bismark --bam  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.bam  
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/deduplicate_bismark --bam  
Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.bam  
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/deduplicate_bismark --bam  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.bam
```

```
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/  
bismark_methylation_extractor --gzip --bedGraph  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam
```

```
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/  
bismark_methylation_extractor --gzip --bedGraph  
Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam
```

```
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/  
bismark_methylation_extractor --gzip --bedGraph  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam
```

```
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/bismark2report  
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/bismark2summary
```

```
sudo apt-get install libgs1-dev
```

```
http://smithlabresearch.org/software/methpipe/
```

Samtools View command

```
cd ~  
# optional. you may already have a src directory  
mkdir src  
cd ~/src  
git clone https://github.com/samtools/htslib  
git clone https://github.com/samtools/samtools  
cd samtools  
make  
cp samtools ~/bin
```

```
/home/students/sarmad1/methpipe-3.4.3/src/utils/to-mr -v -m bismark -  
o 8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam
```

```
/home/students/sarmad1/methpipe-3.4.3/src/utils/to-mr -v -m bismark -  
o Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr  
Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam
```

```
/home/students/sarmad1/methpipe-3.4.3/src/utils/to-mr -v -m bismark -  
o ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam
```

```
sort -k 1,1 -k 2,2n -k 3,3n -k 6,6 -o  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
```

```
sort -k 1,1 -k 2,2n -k 3,3n -k 6,6 -o  
Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr  
Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
```

```
sort -k 1,1 -k 2,2n -k 3,3n -k 6,6 -o  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
```

```
/home/students/sarmad1/methpipe-3.4.3/src/analysis/methcounts -cpg-  
only -c /home/students/sarmad1/Methylation/mm10/chr17.fa -o  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
```

```
/home/students/sarmad1/methpipe-3.4.3/src/analysis/methcounts -cpg-  
only -c /home/students/sarmad1/Methylation/mm10/chr17.fa -o  
Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.m  
eth Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
```

```
/home/students/sarmad1/methpipe-3.4.3/src/analysis/methcounts -cpg-  
only -c /home/students/sarmad1/Methylation/mm10/chr17.fa -o  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
```

```
grep CpG  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth  
> 8cell_rep2_trimmed2_WGBS_R1.symmetric_CpG.meth
```

```
grep CpG  
Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.m  
eth > Epiblast_rep2_trimmed2_WGBS_R1.symmetric_CpG.meth
```

```
grep CpG  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth >  
ICM_rep2_trimmed2_WGBS_R1.symmetric_CpG.meth
```

```
/home/students/sarmad1/methpipe-3.4.3/src/utils/symmetric-cpgs -v -o  
8cell_rep2_trimmed2_WGBS_R1.symmetric_CpG.meth  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth
```

```
/home/students/sarmad1/methpipe-3.4.3/src/utils/symmetric-cpgs -v -o  
Epiblast_rep2_trimmed2_WGBS_R1.symmetric_CpG.meth  
Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.m  
eth
```

```
/home/students/sarmad1/methpipe-3.4.3/src/utils/symmetric-cpgs -v -o  
ICM_rep2_trimmed2_WGBS_R1.symmetric_CpG.meth  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth
```

http://hgdownload.cse.ucsc.edu/admin/exe/linux.x86_64/

```
gunzip -k  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph.gz
```

```
gunzip -k  
Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph.g  
z
```

```
gunzip -k  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph.gz
```

Run from Local Terminal:

```
~/Softwares_bio/BLAT_Software/fetchChromSizes mm10 > mm10.chromSizes
```

```
~/Softwares_bio/BLAT_Software/bedGraphToBigWig  
8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph  
mm10.chromSizes 8cell_rep2_trimmed2_WGBS_R1.methylation.bigWig
```

```
~/Softwares_bio/BLAT_Software/bedGraphToBigWig  
Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph  
mm10.chromSizes Epiblast_rep2_trimmed2_WGBS_R1.methylation.bigWig
```

```
~/Softwares_bio/BLAT_Software/bedGraphToBigWig  
ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph  
mm10.chromSizes ICM_rep2_trimmed2_WGBS_R1.methylation.bigWig
```

```
cp 8cell_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam  
8cell_rep2_trimmed2_WGBS_R1_genomecov.bam
```

```
cp Epiblast_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam  
Epiblast_rep2_trimmed2_WGBS_R1_genomecov.bam
```

```
cp ICM_rep2_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam  
ICM_rep2_trimmed2_WGBS_R1_genomecov.bam
```

```
wget https://github.com/arq5x/bedtools2/releases/download/v2.29.1/bedtools-2.29.1.tar.gz  
tar -zxvf bedtools-2.29.1.tar.gz  
cd bedtools2  
make
```

```
~/sarmad1/src/samtools/samtools sort -n  
8cell_rep2_trimmed2_WGBS_R1_genomecov.bam -o  
8cell_rep2_trimmed2_WGBS_R1_genomecov.sorted
```

```
~/sarmad1/src/samtools/samtools sort -n  
Epiblast_rep2_trimmed2_WGBS_R1_genomecov.bam -o  
Epiblast_rep2_trimmed2_WGBS_R1_genomecov.sorted
```

```
~/sarmad1/src/samtools/samtools sort -n  
ICM_rep2_trimmed2_WGBS_R1_genomecov.bam -o  
ICM_rep2_trimmed2_WGBS_R1_genomecov.sorted
```

```
~/sarmad1/Methylation/bedtools/bedtools2/bin/genomeCoverageBed -bga -ibam  
8cell_rep2_trimmed2_WGBS_R1_genomecov.sorted >  
8cell_rep2_trimmed2_WGBS_R1_genomecov.sorted.bedGraph
```

```
~/sarmad1/Methylation/bedtools/bedtools2/bin/genomeCoverageBed -bga -ibam  
Epiblast_rep2_trimmed2_WGBS_R1_genomecov.sorted >  
Epiblast_rep2_trimmed2_WGBS_R1_genomecov.sorted.bedGraph
```

```
~/sarmad1/Methylation/bedtools/bedtools2/bin/genomeCoverageBed -bga -ibam  
ICM_rep2_trimmed2_WGBS_R1_genomecov.sorted >  
ICM_rep2_trimmed2_WGBS_R1_genomecov.sorted.bedGraph
```

Run from Local Terminal:

```
~/Softwares_bio/BLAT_Software/fetchChromSizes mm10 > mm10.chromSizes
```

```
~/Softwares_bio/BLAT_Software/bedGraphToBigWig  
8cell_rep2_trimmed2_WGBS_R1_genomecov.sorted.bedGraph  
mm10.chromSizes 8cell_rep2_trimmed2_WGBS_R1_genomecov.coverage.bigWig
```

```
~/Softwares_bio/BLAT_Software/bedGraphToBigWig  
Epiblast_rep2_trimmed2_WGBS_R1_genomecov.sorted.bedGraph  
mm10.chromSizes  
Epiblast_rep2_trimmed2_WGBS_R1_genomecov.coverage.bigWig
```

```
~/Softwares_bio/BLAT_Software/bedGraphToBigWig  
ICM_rep2_trimmed2_WGBS_R1_genomecov.sorted.bedGraph mm10.chromSizes  
ICM_rep2_trimmed2_WGBS_R1_genomecov.coverage.bigWig
```

```
scp  
~/Julia_Project_Files/8cell_rep2_trimmed2_WGBS_R1_genomecov.coverage.  
bigWig 8cell_rep2_trimmed2_WGBS_R1_genomecov.coverage.bigWig
```

```
scp  
~/Julia_Project_Files/Epiblast_rep2_trimmed2_WGBS_R1_genomecov.covera  
ge.bigWig Epiblast_rep2_trimmed2_WGBS_R1_genomecov.coverage.bigWig
```

```
scp  
~/Julia_Project_Files/ICM_rep2_trimmed2_WGBS_R1_genomecov.coverage.bi  
gWig ICM_rep2_trimmed2_WGBS_R1_genomecov.coverage.bigWig
```

```
scp  
~/Julia_Project_Files/ICM_rep2_trimmed2_WGBS_R1.methylation.bigWig  
8cell_rep2_trimmed2_WGBS_R1.methylation.bigWig <=
```

```
/home/students/sarmad1/methpipe-3.4.3/src/utils/merge-methcounts  
Epiblast_rep1_trimmed_WGBS_R1.symmetric_CpG.meth  
Epiblast_rep2_trimmed2_WGBS_R1.symmetric_CpG.meth -o Epiblast.meth
```

```
/home/students/sarmad1/methpipe-3.4.3/src/utils/merge-methcounts  
ICM_rep1_trimmed_WGBS_R1.symmetric_CpG.meth  
ICM_rep2_trimmed2_WGBS_R1.symmetric_CpG.meth -o ICM.meth
```

```
/home/students/sarmad1/methpipe-3.4.3/src/radmeth/methdiff -o  
Epiblast_ICM.methdiff Epiblast.meth ICM.meth
```

```
/home/students/sarmad1/methpipe-3.4.3/src/radmeth/methdiff -o  
ICM_Epiblast.methdiff ICM.meth Epiblast.meth
```

```
/home/students/sarmad1/methpipe-3.4.3/src/analysis/hmr -o Epiblast.hmr  
Epiblast.meth
```

```
/home/students/sarmad1/methpipe-3.4.3/src/analysis/hmr -o ICM.hmr ICM.meth
```

```
/home/students/sarmad1/methpipe-3.4.3/src/radmeth/dmr Epiblast_ICM.methdiff  
Epiblast.hmr ICM.hmr \Epiblast.meth ICM.meth
```

```
awk -F "[:\t]" '$5 >= 10 && $6 >= 5 {print $0}' Epiblast.meth \  
> Epiblast.filtered  
awk -F "[:\t]" '$5 >= 10 && $6 >= 5 {print $0}' ICM.meth \  
> ICM.filtered
```

```
awk -v OFS="\t" '{if($5>1000) print $1,$2,$3,$4,"1000"; else print  
$1,$2,$3,$4,int($5) }' ICM.meth > ICM.filtered.tobigbed
```

```
awk -v OFS="\t" '{if($5>1000) print $1,$2,$3,$4,"1000"; else print  
$1,$2,$3,$4,int($5) }' Epiblast.meth > Epiblast.filtered.tobigbed
```

```
bedToBigBed Epiblast.filtered.tobigbed mm10.chromSizes Epiblast.dmr.bb
```

```
bedToBigBed ICM.filtered.tobigbed mm10.chromSizes ICM.dmr.bb
```

```
/home/students/sarmad1/methpipe-3.4.3/src/utils/merge-methcounts -t  
Epiblast_rep1_trimmed_WGBS_R1.symmetric_CpG.meth  
Epiblast_rep2_trimmed2_WGBS_R1.symmetric_CpG.meth \  
ICM_rep1_trimmed_WGBS_R1.symmetric_CpG.meth  
ICM_rep2_trimmed2_WGBS_R1.symmetric_CpG.meth > proportion_table.txt
```

Make design_matrix

```
~/sarmad1/methpipe-3.4.3/src/utils/merge-methcounts -t  
proportion_table.txt > merged_design_matrix.txt
```

```
~/sarmad1/methpipe-3.4.3/src/radmeth/radmeth regression -factor case  
design_matrix.txt proportion_table.txt > cpgs.bed
```

```
~/sarmad1/methpipe-3.4.3/src/radmeth/radmeth adjust -bins 1:150:1  
cpgs.bed > cpgs.adjusted.bed
```

```
awk '$7 <= 0.01 "{ print $0; $}"' cpgs.adjusted.bed > dm_cpgs.bed
```

```
~/sarmad1/methpipe-3.4.3/src/radmeth/radmeth merge -p 0.01  
cpgs.adjusted.bed > dmrs.bed
```

```
grep chr11 your_file.gff > chr11.gff
```

```
sortBed -i myfile.gff | gff2bed > my_sorted_file.bed
```

```
~/sarmad1/Methylation/bedtools/bedtools2/obj/bin/sortBed -i  
mm10.chr17.gtf | ~/Softwares_bio/bedops/bin/gtf2bed > mm10.chr17.sorted.bed
```

END