gunzip -k 8cell_rep1_trimmed2_WGBS_R1.fastq.gz 8cell_rep1_trimmed2_WGBS_R2.fastq.gz
Epiblast_rep1_trimmed2_WGBS_R1.fastq.gz Epiblast_rep1_trimmed2_WGBS_R2.fastq.gz
ICM_rep1_trimmed2_WGBS_R1.fastq.gz ICM_rep1_trimmed2_WGBS_R2.fastq.gz

gunzip -k 8cell_rep1_WGBS_R1.fastq.gz 8cell_rep1_WGBS_R2.fastq.gz
Epiblast_rep1_WGBS_R1.fastq.gz Epiblast_rep1_WGBS_R2.fastq.gz ICM_rep1_WGBS_R1.fastq.gz
ICM_rep1_WGBS_R2.fastq.gz

fastqc -o QC_Reports
/home/students/Ali/Methylation/reads/8cell_rep1_trimmed2_WGBS_R1.fastq

fastqc -o QC_Reports
/home/students/Ali/Methylation/reads/8cell_rep1_trimmed2_WGBS_R2.fastq

fastqc -o QC_Reports
/home/students/Ali/Methylation/reads/Epiblast_rep1_trimmed2_WGBS_R1.fastq

fastqc -o QC_Reports
/home/students/Ali/Methylation/Trimmed2/Epiblast_rep1_trimmed2_WGBS_R2.fastq
cp ICM_rep1_WGBS_R1.fastq.gz ICM_rep1_WGBS_R2.fastq.gz

/home/students/Ali/Methylation/trimmedreads
/home/students/Ali/Methylation/trimmedreads/Epiblast_rep1_trimmed2_WGBS_R2.fastq
cp ICM_rep1_trimmed_WGBS_R1.fastq.gz ICM_rep1_trimmed_WGBS_R2.fastq.gz /home/students/
Ali/Methylation/Trimmed2
cp Epiblast_rep1_trimmed_WGBS_R1.fastq.gz Epiblast_rep1_trimmed_WGBS_R2.fastq.gz
/home/students/Ali/Methylation/Trimmed2

gunzip -k ICM_rep1_WGBS_R1.fastq.gz ICM_rep1_WGBS_R2.fastq.gz
Epiblast_rep1_WGBS_R1.fastq.gz Epiblast_rep1_WGBS_R2.fastq.gz

fastqc -o /home/students/Ali/Methylation/trimmedreads
/home/students/Ali/Methylation/Before_QC/Epiblast_rep1_WGBS_R1.fastq

fastqc -o /home/students/Ali/Methylation/ trimmedreads
/home/students/Ali/Methylation/Before_QC/Epiblast_rep1_WGBS_R2.fastq

fastqc -o /home/students/Ali/Methylation/ trimmedreads
/home/students/Ali/Methylation/Before_QC/ICM_rep1_WGBS_R1.fastq

fastqc -o /home/students/Ali/Methylation/ trimmedreads
/home/students/Ali/Methylation/Before_QC/ICM_rep1_WGBS_R2.fastq
bismark -p 4 --gzip /home/students/Ali/Methylation/Trimmedreads/ -1
Epiblast_rep1_trimmed2_WGBS_R1.fastq.gz -2 Epiblast_rep1_trimmed2_WGBS_R2.fastq.gz

bismark -p 4 --gzip /home/students/Ali/Methylation/Trimmedreads/ -1
ICM_rep1_trimmed2_WGBS_R1.fastq.gz -2 ICM_rep1_trimmed2_WGBS_R2.fastq.gz

bismark -p 4 --gzip /home/students/Ali/Methylation/Trimmed2/ -1
8cell_rep1_trimmed2_WGBS_R1.fastq.gz -2 8cell_rep1_trimmed2_WGBS_R2.fastq.gz
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/bismark -p 4 --gzip
/home/students/Ali/Methylation/mm10/ -1 Epiblast_rep1_trimmed2_WGBS_R1.fastq.gz -2

Epiblast_rep1_trimmed2_WGBS_R2.fastq.gz
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/bismark -p 4 --gzip
/home/students/Ali/Methylation/mm10/ -1 ICM_rep1_trimmed2_WGBS_R1.fastq.gz -2

ICM_rep1_trimmed2_WGBS_R2.fastq.gz
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/bismark -p 4 --gzip
/home/students/Ali/Methylation/mm10/ -1 8cell_rep1_trimmed2_WGBS_R1.fastq.gz -2

8cell_rep1_trimmed2_WGBS_R2.fastq.gz

gunzip -k ICM_rep1_trimmed2_WGBS_R1.fastq.gz ICM_rep1_trimmed2_WGBS_R2.fastq.gz
Epiblast_rep1_trimmed2_WGBS_R1.fastq.gz Epiblast_rep1_trimmed2_WGBS_R2.fastq.gz
fastqc -o /home/students/Ali/Methylation/QC_Trimmed2/
/home/students/Ali/Methylation/Trimmed2/ICM_rep1_trimmed2_WGBS_R1.fastq

fastqc -o /home/students/Ali/Methylation/QC_Trimmed2/
/home/students/Ali/Methylation/Trimmed2/ICM_rep1_trimmed2_WGBS_R2.fastq

fastqc -o /home/students/Ali/Methylation/QC_Trimmed2/
/home/students/Ali/Methylation/Trimmed2/Epiblast_rep1_trimmed2_WGBS_R1.fastq

fastqc -o /home/students/Ali/Methylation/QC_Trimmed2/
/home/students/Ali/Methylation/Trimmed2/Epiblast_rep1_trimmed2_WGBS_R2.fastq
bismark-0.22.3/bismark_genome_preparation /home/students/Ali/Methylation/mm10/
bismark-0.22.3/bismark_genome_preparation /home/students/Ali/Methylation/Trimmed2/

/home/students/PRACTICE_METHYLATION/bismark-0.22.3/deduplicate_bismark --bam
8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.bam

/home/students/PRACTICE_METHYLATION/bismark-0.22.3/deduplicate_bismark --bam
Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.bam

/home/students/PRACTICE_METHYLATION/bismark-0.22.3/deduplicate_bismark --bam
ICM_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.bam
/home/students/PRACTICE_METHYLATION/bismark-0.22.3/
bismark_methylation_extractor --gzip --bedGraph
8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam

/home/students/PRACTICE_METHYLATION/bismark-0.22.3/
bismark_methylation_extractor --gzip --bedGraph
Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam

/home/students/PRACTICE_METHYLATION/bismark-0.22.3/
bismark_methylation_extractor --gzip --bedGraph
ICM_rep1_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 libgsl-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/Ali/methpipe-3.4.3/src/utils/to-mr -v -m bismark -
o 8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam

/home/students/Ali/methpipe-3.4.3/src/utils/to-mr -v -m bismark -
o Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam

/home/students/Ali/methpipe-3.4.3/src/utils/to-mr -v -m bismark -
o ICM_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr

ICM_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam
sort -k 1,1 -k 2,2n -k 3,3n -k 6,6 -o
8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
sort -k 1,1 -k 2,2n -k 3,3n -k 6,6 -o
Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
sort -k 1,1 -k 2,2n -k 3,3n -k 6,6 -o
ICM_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
ICM_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
/home/students/Ali/methpipe-3.4.3/src/analysis/methcounts -cpgonly
-c /home/students/Ali/Methylation/mm10/chr17.fa -o
8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth
8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr

/home/students/Ali/methpipe-3.4.3/src/analysis/methcounts -cpgonly
-c /home/students/Ali/Methylation/mm10/chr17.fa -o
Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.m
eth Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr

/home/students/Ali/methpipe-3.4.3/src/analysis/methcounts -cpgonly
-c /home/students/Ali/Methylation/mm10/chr17.fa -o
ICM_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth
ICM_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.mr
grep CpG
8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth
> 8cell_rep1_trimmed2_WGBS_R1.symmetric_CpG.meth
grep CpG
Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.m
eth > Epiblast_rep1_trimmed2_WGBS_R1.symmetric_CpG.meth
grep CpG
ICM_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth >
ICM_rep1_trimmed2_WGBS_R1.symmetric_CpG.meth

/home/students/Ali/methpipe-3.4.3/src/utils/symmetric-cpgs -v -o
8cell_rep1_trimmed2_WGBS_R1.symmetric_CpG.meth
8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth

/home/students/Ali/methpipe-3.4.3/src/utils/symmetric-cpgs -v -o
Epiblast_rep1_trimmed2_WGBS_R1.symmetric_CpG.meth
Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.m
eth

/home/students/Ali/methpipe-3.4.3/src/utils/symmetric-cpgs -v -o
ICM_rep1_trimmed2_WGBS_R1.symmetric_CpG.meth
ICM_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.all_CpGs.meth
http://hgdownload.cse.ucsc.edu/admin/exe/linux.x86_64/
gunzip -k
8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph.gz
gunzip -k
Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph.g
z
gunzip -k
ICM_rep1_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_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph
mm10.chromSizes 8cell_rep1_trimmed2_WGBS_R1.methylation.bigWig

~/Softwares_bio/BLAT_Software/bedGraphToBigWig
Epiblast_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph

mm10.chromSizes Epiblast_rep1_trimmed2_WGBS_R1.methylation.bigWig

~/Softwares_bio/BLAT_Software/bedGraphToBigWig
ICM_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bedGraph
mm10.chromSizes ICM_rep1_trimmed2_WGBS_R1.methylation.bigWig
cp 8cell_rep1_trimmed2_WGBS_R1_bismark_bt2_pe.deduplicated.bam
8cell_rep1_trimmed2_WGBS_R1_genomecov.bam

cp **Epiblast_rep1_trimmed2_WGBS_R1**_bismark_bt2_pe.deduplicated.bam
Epiblast_rep1_trimmed2_WGBS_R1_genomecov.bam

cp **ICM_rep1_trimmed2_WGBS_R1**_bismark_bt2_pe.deduplicated.bam
ICM_rep1_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

~/Ali/src/samtools/samtools sort -n
8cell_rep1_trimmed2_WGBS_R1_genomecov.bam -o
8cell_rep1_trimmed2_WGBS_R1_genomecov.sorted

~/Ali/src/samtools/samtools sort -n
Epiblast_rep1_trimmed2_WGBS_R1_genomecov.bam -o
Epiblast_rep1_trimmed2_WGBS_R1_genomecov.sorted

~/Ali/src/samtools/samtools sort -n
ICM_rep1_trimmed2_WGBS_R1_genomecov.bam -o
ICM_rep1_trimmed2_WGBS_R1_genomecov.sorted

~/Ali/Methylation/bedtools/bedtools2/bin/genomeCoverageBed -bga -ibam
8cell_rep1_trimmed2_WGBS_R1_genomecov.sorted >
8cell_rep1_trimmed2_WGBS_R1_genomecov.sorted.bedGraph
~/Ali/Methylation/bedtools/bedtools2/bin/genomeCoverageBed -bga -ibam
Epiblast_rep1_trimmed2_WGBS_R1_genomecov.sorted >
Epiblast_rep1_trimmed2_WGBS_R1_genomecov.sorted.bedGraph
~/Ali/Methylation/bedtools/bedtools2/bin/genomeCoverageBed -bga -ibam
ICM_rep1_trimmed2_WGBS_R1_genomecov.sorted >
ICM_rep1_trimmed2_WGBS_R1_genomecov.sorted.bedGraph
Run from Local Terminal:
~/Softwares_bio/BLAT_Software/fetchChromSizes mm10 > mm10.chromSizes
~/Softwares_bio/BLAT_Software/bedGraphToBigWig
8cell_rep1_trimmed2_WGBS_R1_genomecov.sorted.bedGraph
mm10.chromSizes 8cell_rep1_trimmed2_WGBS_R1_genomecov.coverage.**bigWig**
~/Softwares_bio/BLAT_Software/bedGraphToBigWig
Epiblast_rep1_trimmed2_WGBS_R1_genomecov.sorted.bedGraph
mm10.chromSizes
Epiblast_rep1_trimmed2_WGBS_R1_genomecov.coverage.**bigWig**
~/Softwares_bio/BLAT_Software/bedGraphToBigWig
ICM_rep1_trimmed2_WGBS_R1_genomecov.sorted.bedGraph **mm10.chromSizes**
ICM_rep1_trimmed2_WGBS_R1_genomecov.coverage.**bigWig**
scp

~/Julia_Project_Files/8cell_rep1_trimmed2_WGBS_R1_genomecov.coverage.
bigWig 8cell_rep1_trimmed2_WGBS_R1_genomecov.coverage.bigWig
scp

~/Julia_Project_Files/**Epiblast_rep1_trimmed2_WGBS_R1**_genomecov.covera
ge.bigWig **Epiblast_rep1_trimmed2_WGBS_R1**_genomecov.coverage.bigWig
scp

~/Julia_Project_Files/**ICM_rep1_trimmed2_WGBS_R1**_genomecov.coverage.bi

gWig **ICM_rep1_trimmed2_WGBS_R1**_genomecov.coverage.bigWig
scp

~/Julia_Project_Files/**ICM_rep1_trimmed2_WGBS_R1.methylation**.bigWig
8cell_rep1_trimmed2_WGBS_R1.methylation.bigWig <=

/home/students/Ali/methpipe-3.4.3/src/utils/merge-methcounts
Epiblast_rep1_trimmed_WGBS_R1.symmetric_CpG.meth
Epiblast_rep1_trimmed2_WGBS_R1.symmetric_CpG.meth -o Epiblast.meth

/home/students/Ali/methpipe-3.4.3/src/utils/merge-methcounts
ICM_rep1_trimmed_WGBS_R1.symmetric_CpG.meth
ICM_rep1_trimmed2_WGBS_R1.symmetric_CpG.meth -o ICM.meth
/home/students/Ali/methpipe-3.4.3/src/radmeth/methdiff -o
Epiblast_ICM.methdiff Epiblast.meth ICM.meth
/home/students/Ali/methpipe-3.4.3/src/radmeth/methdiff -o
ICM_Epiblast.methdiff ICM.meth Epiblast.meth
/home/students/Ali/methpipe-3.4.3/src/analysis/hmr -o Epiblast.hmr
Epiblast.meth
/home/students/Ali/methpipe-3.4.3/src/analysis/hmr -o ICM.hmr ICM.meth
/home/students/Ali/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/Ali/methpipe-3.4.3/src/utils/merge-methcounts -t
Epiblast_rep1_trimmed_WGBS_R1.symmetric_CpG.meth
Epiblast_rep1_trimmed2_WGBS_R1.symmetric_CpG.meth \
ICM_rep1_trimmed_WGBS_R1.symmetric_CpG.meth
ICM_rep1_trimmed2_WGBS_R1.symmetric_CpG.meth > proportion_table.txt

Design_matrix
~/Ali/methpipe-3.4.3/src/utils/merge-methcounts -t
proportion_table.txt > merged_design_matrix.txt

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

~/Ali/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

~/Ali/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

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