**Analytical code of RRBS**

#Combine Fastq files of the same sample

$ cat O-germ1\_ATCATG\_L001\_R1\_001.fastq.gz O-germ1\_ATCATG\_L002\_R1\_001.fastq.gz > 1.fastq.gz

$ zcat 1.fastq.gz > 1.fastq

#Adaptor removal

$ cutadapt -a GATCGGAAGAGCACACG -O 10 -m 25 1.fastq -o 1c.fastq

#FastQC

$ fastqc -f fastq 1c.fastq --extract -nogroup -o 1\_qc

#Removal of “CCGG” located at the head of read

$ fastx\_trimmer -t 4 -i 1c.trimmed.fastq -Q 33 -o 1ct.trimmed.fastq

#Mapping

$ bismark --bowtie2 --path\_to\_bowtie /usr/local/bowtie2-2.0.5 /disk1/hiura/takashima/Mus\_musculus/UCSC/mm10/Sequence/Bismark/ 1ct.trimmed.fastq

$ mv 1ct.trimmed.fastq\_bismark\_bt2.sam 2\_mapping/ | mv 1ct.trimmed.fastq\_bismark\_bt2\_SE\_report.txt 2\_mapping/

#Calculation of cytosine methylation

$ bismark\_methylation\_extractor -o ./3\_report -s ./2\_mapping/1ct.trimmed.fastq\_bismark\_bt2.sam --comprehensive --merge\_non\_CpG --report

$ samtools view -bS ./2\_mapping/1ct.trimmed.fastq\_bismark\_bt2.sam > 1ct.trimmed.fastq\_bismark.bam

$ samtools sort 1ct.trimmed.fastq\_bismark.bam 1ct.trimmed.fastq\_bismark.sort

$ samtools index 1ct.trimmed.fastq\_bismark.sort.bam

$ perl /usr/local/src/bismark\_v0.7.12/bismark2bedGraph CpG\_context\_1ct.trimmed.fastq\_bismark.txt --cutoff 3 -o CpG\_context\_1ct\_trimmed.fastq\_bismark.bedgraph

#Conversion of bedgraph to bed files (RStudio in windows)

$ n1 <- read.csv("CpG\_context\_1ct\_trimmed.fastq\_bismark.bedgraph", header=F, sep="\t")

$ n2 <- read.csv("CpG\_context\_1ct\_trimmed.fastq\_bismark.bedgraph", header=F, sep="\t")

$ names(n1) <- c("V1", "V2", "V3", "methyl\_germ\_oil\_1")

$ names(n2) <- c("V1", "V2", "V3", "methyl\_germ\_oil\_2")

$ mix <- merge(n1, n2)

$ n1\_1 <- mix[,c(1:3, 4)]

$ n2\_1 <- mix[,c(1:3, 5)]

$ names(n1\_1) <- c("V1", "start", "end", "methyl\_germ\_oil\_1")

$ names(n2\_1) <- c("V1", "start", "end", "methyl\_germ\_oil\_2")

$ n1\_1$start <- formatC(n1\_1$start,format="d")

$ n1\_1$end <- formatC(n1\_1$end,format="d")

$ n2\_1$start <- formatC(n2\_1$start,format="d")

$ n2\_1$end <- formatC(n1\_1$end,format="d")

$ write.table(n1\_1, "C:/Users/hiura/Desktop/RRBS\_tando/tando\_RRBS\_bed/n1\_181011.bed", sep="\t", append=F, quote=F, row.names=F, col.names=F)

$ write.table(n2\_1, "C:/Users/hiura/Desktop/RRBS\_tando/tando\_RRBS\_bed/n1\_181011.bed", sep="\t", append=F, quote=F, row.names=F, col.names=F)

#Extraction of genome regions

$ dos2unix-6.0.4/dos2unix n1\_181011.bed

$ bedtools-2.17.0/bin/intersectBed -a n1\_181011.bed -b mm10\_pro.bed -wa -wb > pro\_n1\_181011.bed

$ bedtools-2.17.0/bin/intersectBed -a n1\_181011.bed -b mm10\_body.bed -wa -wb > body\_n1\_181011.bed

$ bedtools-2.17.0/bin/intersectBed -a n1\_181011.bed -b mm10\_CGI.bed -wa -wb > CGI\_n1\_181011.bed

$ bedtools-2.17.0/bin/intersectBed -a n1\_181011.bed -b mm10\_CGIshore.bed -wa -wb > CGIshore\_n1\_181011.bed

$ bedtools-2.17.0/bin/intersectBed -a n1\_181011.bed -b mm10\_ICR.bed -wa -wb > ICR\_n1\_181011.bed

$ bedtools-2.17.0/bin/intersectBed -a n1\_181011.bed -b mm10\_SINE.bed -wa -wb > SINE\_n1\_181011.bed

$ bedtools-2.17.0/bin/intersectBed -a n1\_181011.bed -b mm10\_LINE.bed -wa -wb > LINE\_n1\_181011.bed

$ bedtools-2.17.0/bin/intersectBed -a n1\_181011.bed -b mm10\_LTR.bed -wa -wb > LTR\_n1\_181011.bed

$ bedtools-2.17.0/bin/intersectBed -a n1\_181011.bed -b mm10\_repDNA.bed -wa -wb > repDNA\_n1\_181011.bed

#Calculation of cytosine methylation for each promoter

$ aaa <- read.csv("pro\_n1\_181011.bed", header=F, sep="\t")

$ aaa$ID <- paste(aaa$V5, aaa$V6, aaa$V7, aaa$V8)

$ aaa$V1 <- "chr"

$ num <- xtabs(~ID+V1, data=aaa)

$ mean <- xtabs(V4~ID+V1, data=aaa)

$ all <- cbind(num, mean)

$ all <- as.data.frame(all)

$ names(all) <- c("V1", "V2")

$ all$methyl <- all$V2/all$V1

$ write.table(as.matrix(all), "C:/Users/Hiura/Desktop/RRBS\_tando/tando\_RRBS\_bed/Pro\_mm10/pro\_n1\_181011.txt", append=F, quote=F, row.names=T, col.names=F)ol.names=F)