**1. Run FastQC and Trimmomatic (See Dataset S9).**

**2. Prepare the genome into bismark format.**

#!/bin/bash -l

#SBATCH --nodes=1

#SBATCH --ntasks=1

#SBATCH --cpus-per-task=16

#SBATCH --mem-per-cpu=16G

#SBATCH --time=36:00:00

#SBATCH --output=bismark1genprep\_Al4f.stdout

#SBATCH --mail-type=ALL

#SBATCH --job-name="bismark1genprep\_Al4f"

#SBATCH -p intel

module load bowtie

module load bowtie2

module load bismark

module load samtools

bismark\_genome\_preparation --bowtie2 ./GCF\_005508785.1\_pea\_aphid\_22Mar2018\_4r6ur\_genomic

**3. Bismark**

#!/bin/bash -l

#SBATCH --nodes=1

#SBATCH --ntasks=1

#SBATCH --cpus-per-task=16

#SBATCH --mem-per-cpu=16G

#SBATCH --time=36:00:00

#SBATCH --output=bismark1\_Al4ftest.stdout

#SBATCH --mail-type=ALL

#SBATCH --job-name="bismark1\_Al4ftest"

#SBATCH -p intel

module load bowtie

module load bowtie2

module load bismark

module load samtools

bismark --un --ambiguous --multicore 8 --bowtie2 --o ./bismarkResultsAl4f/ ./GCF\_005508785.1\_pea\_aphid\_22Mar2018\_4r6ur\_genomic/ -1 ./F1\_1\_R1\_paired.fastq -2 ./F1\_1\_R2\_paired.fastq

Repeat line for each sample

**4. Sort and index individual BAM files:**

**4A. To sort BAM files:**

#!/bin/bash -l

#SBATCH --nodes=1

#SBATCH --ntasks=1

#SBATCH --cpus-per-task=4

#SBATCH --mem-per-cpu=8G

#SBATCH --time=12:00:00

#SBATCH --output=samtoolsSort1.stdout

#SBATCH --mail-type=ALL

#SBATCH --job-name="samtoolsSort1"

#SBATCH -p intel

module load samtools

samtools sort ./bismarkResultsAl4f/F1\_1\_R1\_paired.fastq\_bismark\_bt2\_pe.bam -@ 4 -o ./bismarkResultsAl4f/F1\_1\_sorted.bam

samtools sort ./bismarkResultsAl4f/F1\_2\_R1\_paired.fastq\_bismark\_bt2\_pe.bam -@ 4 -o ./bismarkResultsAl4f/F1\_2\_sorted.bam

samtools sort ./bismarkResultsAl4f/F1\_3\_R1\_paired.fastq\_bismark\_bt2\_pe.bam -@ 4 -o ./bismarkResultsAl4f/F1\_3\_sorted.bam

samtools sort ./bismarkResultsAl4f/F1\_4\_R1\_paired.fastq\_bismakr\_bt2\_pe.bam -@ 4 -o ./bismarkResultsAl4f/F1\_4\_sorted.bam

samtools sort ./bismarkResultsAl4f/F1\_YA\_R1\_paired.fastq\_bismark\_bt2\_pe.bam -@ 4 -o ./bismarkResultsAl4f/F1\_YA\_sorted.bam

(Repeat one script for each subline Fava 1s, Fava 2s, Fava 3s)

**4B. To index sorted BAM files:**

#!/bin/bash -l

#SBATCH --nodes=1

#SBATCH --ntasks=1

#SBATCH --cpus-per-task=4

#SBATCH --mem-per-cpu=8G

#SBATCH --time=12:00:00

#SBATCH --output=samtoolsIndex\_AL4F.stdout

#SBATCH --mail-type=ALL

#SBATCH --job-name="samtoolsIndexAL4F"

#SBATCH -p intel

module load bowtie

module load bowtie2

module load samtools

samtools index -@ 4 ./bismarkResultsAl4f/F1\_1\_sorted.bam ./bismarkResultsAl4f/F1\_1\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F1\_2\_sorted.bam ./bismarkResultsAl4f/F1\_2\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F1\_3\_sorted.bam ./bismarkResultsAl4f/F1\_3\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F1\_4\_sorted.bam ./bismarkResultsAl4f/F1\_4\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F1\_YA\_sorted.bam ./bismarkResultsAl4f/F1\_YA\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F2\_1\_sorted.bam ./bismarkResultsAl4f/F2\_1\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F2\_2\_sorted.bam ./bismarkResultsAl4f/F2\_2\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F2\_3\_sorted.bam ./bismarkResultsAl4f/F2\_3\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F2\_4\_sorted.bam ./bismarkResultsAl4f/F2\_4\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F2\_YA\_sorted.bam ./bismarkResultsAl4f/F2\_YA\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F3\_1\_sorted.bam ./bismarkResultsAl4f/F3\_1\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F3\_2\_sorted.bam ./bismarkResultsAl4f/F3\_2\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F3\_3\_sorted.bam ./bismarkResultsAl4f/F3\_3\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F3\_4\_sorted.bam ./bismarkResultsAl4f/F3\_4\_sorted.bai

samtools index -@ 4 ./bismarkResultsAl4f/F3\_YA\_sorted.bam ./bismarkResultsAl4f/F3\_YA\_sorted.bai

**5A. Read multiple BAM files into methylKit**

myFileList=list("F1\_1\_sorted.bam","F1\_2\_sorted.bam","F1\_3\_sorted.bam","F1\_4\_sorted.bam","F1\_YA\_sorted.bam","F2\_1\_sorted.bam","F2\_2\_sorted.bam","F2\_3\_sorted.bam","F2\_4\_sorted.bam","F2\_YA\_sorted.bam","F3\_1\_sorted.bam","F3\_2\_sorted.bam","F3\_3\_sorted.bam","F3\_4\_sorted.bam","F3\_YA\_sorted.bam")

myNameList=list("F1\_1","F1\_2\_","F1\_3","F1\_4","F1\_YA","F2\_1","F2\_2","F2\_3","F2\_4","F2\_YA","F3\_1","F3\_2","F3\_3","F3\_4","F3\_YA")

myFiles=read.bismark(location=myFileList, sample.id=myNameList, assembly="Aphid", save.folder = "./", save.context = "CpG", read.context = "CpG", nolap = FALSE, mincov = 10, minqual = 20, phred64 = FALSE, treatment = c(0,1,2,3,4,0,1,2,3,4,0,1,2,3,4))

**5B. Convert the CpG output files: convertTxt.pl**

perl convertTxt.pl F1\_1\_CpG.txt>F1\_1\_CpG.converted.txt

perl convertTxt.pl F1\_2\_CpG.txt>F1\_2\_CpG.converted.txt

perl convertTxt.pl F1\_3\_CpG.txt>F1\_3\_CpG.converted.txt

perl convertTxt.pl F1\_4\_CpG.txt>F1\_4\_CpG.converted.txt

perl convertTxt.pl F1\_YA\_CpG.txt>F1\_YA\_CpG.converted.txt

perl convertTxt.pl F2\_YA\_CpG.txt>F2\_YA\_CpG.converted.txt

perl convertTxt.pl F2\_4\_CpG.txt>F2\_4\_CpG.converted.txt

perl convertTxt.pl F2\_3\_CpG.txt>F2\_3\_CpG.converted.txt

perl convertTxt.pl F2\_2\_CpG.txt>F2\_2\_CpG.converted.txt

perl convertTxt.pl F2\_1\_CpG.txt>F2\_1\_CpG.converted.txt

perl convertTxt.pl F3\_1\_CpG.txt>F3\_1\_CpG.converted.txt

perl convertTxt.pl F3\_2\_CpG.txt>F3\_2\_CpG.converted.txt

perl convertTxt.pl F3\_3\_CpG.txt>F3\_3\_CpG.converted.txt

perl convertTxt.pl F3\_4\_CpG.txt>F3\_4\_CpG.converted.txt

perl convertTxt.pl F3\_YA\_CpG.txt>F3\_YA\_CpG.converted.txt

**5C. Convert the annotation. GFF3->GenePred->Bed->modifiedBed.**

Download two programs, gff3ToGenePred and genePredToBed, from the following link (Mac versions): <http://hgdownload.cse.ucsc.edu/admin/exe/macOSX.x86_64/>

Add executable permissions to files:

chmod +x gff3ToGenePred

chmod +x genePRedToBed

Run those two files sequentially:

Usage: ./gff3ToGenePred [input file] [output file]

./gff3ToGenePred GCF\_005508785.1\_pea\_aphid\_22Mar2018\_4r6ur\_genomic.gff Al4f.GP

Usage: ./genePredToBed [input file] [output file]

./genePredToBed Al4f.GP Al4f.bed

Edit BED format file: convertBED.pl

Usage: perl convertBED.pl [input file] > [output file]

ex) perl convertBED.pl Al4f.bed >Al4f.modified.bed

**5D. Load converted files**

> library(methylKit)

> setwd("/Users/Dan/Desktop/Data/Bcyte\_Dissection\_Project/WGBSseq\_Cluster/Al4f\_Assembly/methylKit/")

> newFileList=list("F1\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F3\_YA\_CpG.converted.txt")

> myNameList=list("F1\_1","F1\_2","F1\_3","F1\_4","F1\_YA","F2\_1","F2\_2","F2\_3","F2\_4","F2\_YA","F3\_1","F3\_2","F3\_3","F3\_4","F3\_YA")

> myCpG<-methRead(newFileList, sample.id = myNameList, assembly = "Al4f", context = "CpG", treatment = c(0,1,2,3,4,0,1,2,3,4,0,1,2,3,4))

**5E. Differentially methylated sites: 1 vs 2:**

> myCpG1vs2<-methRead(list("F1\_1\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F3\_2\_CpG.converted.txt"), sample.id = list("F1\_1","F2\_1","F3\_1","F1\_2","F2\_2","F3\_2"), assembly = "Al4f",context = "CpG", treatment = c(0,0,0,1,1,1))

> methCpG1vs2<-unite(myCpG1vs2, destrand=FALSE)

> myDiffCpG1vs2<-calculateDiffMeth(methCpG1vs2)

**5F. Add thresholds (>25% difference, q-value < 0.05)**

myDiffCpG1vs2\_25p<-getMethylDiff(myDiffCpG1vs2, difference=25, qvalue=0.05)

**5G. Save DMS data to excel.**

write.csv(myDiffCpG1vs2\_25p, "myDiffCpG1vs2\_25p.csv")

**5H. Gene context determination:**

> gene.obj <- genomation::readTranscriptFeatures("Al4f.modified.bed")

> genomation::annotateWithGeneParts(as(myDiffCpG1vs2\_25p,"GRanges"),gene.obj)

**6. Create PCA Plot**

library(methylKit)

setwd("/Users/Dan/Desktop/Data/Bcyte\_Dissection\_Project/WGBSseq\_Cluster/Al4f\_Assembly/methylKit/")

newFileList=list("F1\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F3\_YA\_CpG.converted.txt")

myNameList=list("F1\_1","F1\_2","F1\_3","F1\_4","F1\_YA","F2\_1","F2\_2","F2\_3","F2\_4","F2\_YA","F3\_1","F3\_2","F3\_3","F3\_4","F3\_YA")

myCpG<-methRead(newFileList, sample.id = myNameList, assembly = "Al4f", context = "CpG", treatment = c(0,1,2,3,4,0,1,2,3,4,0,1,2,3,4))

methCpG<-unite(myCpG,destrand=FALSE)

.

PCASamples(methCpG)

**7. Repeat 5 w/ other samples: Differentially methylated sites:**

**7A. 2 vs 3:**

> myCpG2vs3<-methRead(list("F1\_2\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F3\_3\_CpG.converted.txt"), sample.id = list("F1\_2","F2\_2","F3\_2","F1\_3","F2\_3","F3\_3"), assembly = "Al4f",context = "CpG", treatment = c(1,1,1,2,2,2))

> methCpG2vs3<-unite(myCpG2vs3, destrand=FALSE)

> myDiffCpG2vs3<-calculateDiffMeth(methCpG2vs3)

> myDiffCpG2vs3\_25p<-getMethylDiff(myDiffCpG2vs3, difference=25, qvalue=0.05)

> write.csv(myDiffCpG2vs3\_25p, "myDiffCpG2vs3\_25p.csv")

> gene.obj <- genomation::readTranscriptFeatures("Al4f.modified.bed")

> genomation::annotateWithGeneParts(as(myDiffCpG2vs3\_25p,"GRanges"),gene.obj)

**7B. Differentially methylated sites: 3 vs 4:**

> library(methylKit)

> setwd("/Users/Dan/Desktop/Data/Bcyte\_Dissection\_Project/WGBSseq\_Cluster/Al4f\_Assembly/methylKit/")

> newFileList=list("F1\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F3\_YA\_CpG.converted.txt")

> myNameList=list("F1\_1","F1\_2","F1\_3","F1\_4","F1\_YA","F2\_1","F2\_2","F2\_3","F2\_4","F2\_YA","F3\_1","F3\_2","F3\_3","F3\_4","F3\_YA")

> myCpG<-methRead(newFileList, sample.id = myNameList, assembly = "Al4f", context = "CpG", treatment = c(0,1,2,3,4,0,1,2,3,4,0,1,2,3,4))

> myCpG3vs4<-methRead(list("F1\_3\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F3\_4\_CpG.converted.txt"), sample.id = list("F1\_3","F2\_3","F3\_3","F1\_4","F2\_4","F3\_4"), assembly = "Al4f",context = "CpG", treatment = c(2,2,2,3,3,3))

> methCpG3vs4<-unite(myCpG3vs4, destrand=FALSE)

> myDiffCpG3vs4<-calculateDiffMeth(methCpG3vs4)

> myDiffCpG3vs4\_25p<-getMethylDiff(myDiffCpG3vs4, difference=25, qvalue=0.05)

There are 2243 CpG sites that’s differentially methylated.

> write.csv(myDiffCpG3vs4\_25p, "myDiffCpG3vs4\_25p.csv")

> gene.obj <- genomation::readTranscriptFeatures("Al4f.modified.bed")

> genomation::annotateWithGeneParts(as(myDiffCpG3vs4\_25p,"GRanges"),gene.obj)

 **7C. Differentially methylated sites: 4 vs YA:**

> library(methylKit)

> setwd("/Users/Dan/Desktop/Data/Bcyte\_Dissection\_Project/WGBSseq\_Cluster/Al4f\_Assembly/methylKit/")

> newFileList=list("F1\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F3\_YA\_CpG.converted.txt")

> myNameList=list("F1\_1","F1\_2","F1\_3","F1\_4","F1\_YA","F2\_1","F2\_2","F2\_3","F2\_4","F2\_YA","F3\_1","F3\_2","F3\_3","F3\_4","F3\_YA")

> myCpG<-methRead(newFileList, sample.id = myNameList, assembly = "Al4f", context = "CpG", treatment = c(0,1,2,3,4,0,1,2,3,4,0,1,2,3,4))

> myCpG4vsYA<-methRead(list("F1\_4\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_YA\_CpG.converted.txt"), sample.id = list("F1\_4","F2\_4","F3\_4","F1\_YA","F2\_YA","F3\_YA"), assembly = "Al4f",context = "CpG", treatment = c(3,3,3,4,4,4))

> methCpG4vsYA<-unite(myCpG4vsYA, destrand=FALSE)

> myDiffCpG4vsYA<-calculateDiffMeth(methCpG4vsYA)

(So after calculating differentially methylated sites, there are 12055124 CpG sites.)

> myDiffCpG4vsYA\_25p<-getMethylDiff(myDiffCpG4vsYA, difference=25, qvalue=0.05)

(There are 1173 CpG sites that’s differentially methylated.)

> write.csv(myDiffCpG4vsYA\_25p, "myDiffCpG4vsYA\_25p.csv")

> gene.obj <- genomation::readTranscriptFeatures("Al4f.modified.bed")

> genomation::annotateWithGeneParts(as(myDiffCpG4vsYA\_25p,"GRanges"),gene.obj)

**7D. LSS 1 vs REST**

library(methylKit)

setwd("/Users/Dan/Desktop/Data/Bcyte\_Dissection\_Project/WGBSseq\_Cluster/Al4f\_Assembly/methylKit/")

newFileList=list("F1\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F3\_YA\_CpG.converted.txt")

myNameList=list("F1\_1","F1\_2","F1\_3","F1\_4","F1\_YA","F2\_1","F2\_2","F2\_3","F2\_4","F2\_YA","F3\_1","F3\_2","F3\_3","F3\_4","F3\_YA")

myCpG<-methRead(newFileList, sample.id = myNameList, assembly = "Al4f", context = "CpG", treatment = c(0,1,1,1,1,0,1,1,1,1,0,1,1,1,1))

methCpG<-unite(myCpG,destrand=FALSE)

myCpG1vsREST<-methRead(list("F1\_1\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_YA\_CpG.converted.txt"), sample.id = list("F1\_1","F2\_1","F3\_1","F1\_2","F2\_2","F3\_2","F1\_3","F2\_3","F3\_3","F1\_4","F2\_4","F3\_4","F1\_YA","F2\_YA","F3\_YA"), assembly = "Al4f",context = "CpG", treatment = c(0,0,0,1,1,1,1,1,1,1,1,1,1,1,1))

methCpG1vsREST<-unite(myCpG1vsREST, destrand=FALSE)

.

myDiffCpG1vsREST<-calculateDiffMeth(methCpG1vsREST)

myDiffCpG1vsREST\_25p<-getMethylDiff(myDiffCpG1vsREST, difference=25, qvalue=0.05)

write.csv(myDiffCpG1vsREST\_25p, "myDiffCpG1vsREST\_25p.csv")

> gene.obj <- genomation::readTranscriptFeatures("Al4f.modified.bed")

> genomation::annotateWithGeneParts(as(myDiffCpG1vsREST\_25p,"GRanges"),gene.obj)

**7E. LSS 2 vs REST**

library(methylKit)

setwd("/Users/Dan/Desktop/Data/Bcyte\_Dissection\_Project/WGBSseq\_Cluster/Al4f\_Assembly/methylKit/")

newFileList=list("F1\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F3\_YA\_CpG.converted.txt")

myNameList=list("F1\_1","F1\_2","F1\_3","F1\_4","F1\_YA","F2\_1","F2\_2","F2\_3","F2\_4","F2\_YA","F3\_1","F3\_2","F3\_3","F3\_4","F3\_YA")

myCpG<-methRead(newFileList, sample.id = myNameList, assembly = "Al4f", context = "CpG", treatment = c(1,0,1,1,1,1,0,1,1,1,1,0,1,1,1))

methCpG<-unite(myCpG,destrand=FALSE)

myCpG2vsREST<-methRead(list("F1\_2\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F1\_1\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_YA\_CpG.converted.txt"), sample.id = list("F1\_2","F2\_2","F3\_2","F1\_1","F2\_1","F3\_1","F1\_3","F2\_3","F3\_3","F1\_4","F2\_4","F3\_4","F1\_YA","F2\_YA","F3\_YA"), assembly = "Al4f",context = "CpG", treatment = c(0,0,0,1,1,1,1,1,1,1,1,1,1,1,1))

methCpG2vsREST<-unite(myCpG2vsREST, destrand=FALSE)

myDiffCpG2vsREST<-calculateDiffMeth(methCpG2vsREST)

myDiffCpG2vsREST\_25p<-getMethylDiff(myDiffCpG2vsREST, difference=25, qvalue=0.05)

write.csv(myDiffCpG2vsREST\_25p, "myDiffCpG2vsREST\_25p.csv")

> gene.obj <- genomation::readTranscriptFeatures("Al4f.modified.bed")

> genomation::annotateWithGeneParts(as(myDiffCpG2vsREST\_25p,"GRanges"),gene.obj)

**7F. LSS 3 vs REST**

library(methylKit)

setwd("/Users/Dan/Desktop/Data/Bcyte\_Dissection\_Project/WGBSseq\_Cluster/Al4f\_Assembly/methylKit/")

newFileList=list("F1\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F3\_YA\_CpG.converted.txt")

myNameList=list("F1\_1","F1\_2","F1\_3","F1\_4","F1\_YA","F2\_1","F2\_2","F2\_3","F2\_4","F2\_YA","F3\_1","F3\_2","F3\_3","F3\_4","F3\_YA")

myCpG<-methRead(newFileList, sample.id = myNameList, assembly = "Al4f", context = "CpG", treatment = c(1,1,0,1,1,1,1,0,1,1,1,1,0,1,1))

methCpG<-unite(myCpG,destrand=FALSE)

myCpG3vsREST<-methRead(list("F1\_3\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F1\_1\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_YA\_CpG.converted.txt"), sample.id = list("F1\_3","F2\_3","F3\_3","F1\_1","F2\_1","F3\_1","F1\_2","F2\_2","F3\_2","F1\_4","F2\_4","F3\_4","F1\_YA","F2\_YA","F3\_YA"), assembly = "Al4f",context = "CpG", treatment = c(0,0,0,1,1,1,1,1,1,1,1,1,1,1,1))

methCpG3vsREST<-unite(myCpG3vsREST, destrand=FALSE)

myDiffCpG3vsREST<-calculateDiffMeth(methCpG3vsREST)

myDiffCpG3vsREST\_25p<-getMethylDiff(myDiffCpG3vsREST, difference=25, qvalue=0.05)

write.csv(myDiffCpG3vsREST\_25p, "myDiffCpG3vsREST\_25p.csv")

> gene.obj <- genomation::readTranscriptFeatures("Al4f.modified.bed")

> genomation::annotateWithGeneParts(as(myDiffCpG3vsREST\_25p,"GRanges"),gene.obj)

**7G. LSS 4 vs REST**

library(methylKit)

setwd("/Users/Dan/Desktop/Data/Bcyte\_Dissection\_Project/WGBSseq\_Cluster/Al4f\_Assembly/methylKit/")

newFileList=list("F1\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F3\_YA\_CpG.converted.txt")

myNameList=list("F1\_1","F1\_2","F1\_3","F1\_4","F1\_YA","F2\_1","F2\_2","F2\_3","F2\_4","F2\_YA","F3\_1","F3\_2","F3\_3","F3\_4","F3\_YA")

myCpG<-methRead(newFileList, sample.id = myNameList, assembly = "Al4f", context = "CpG", treatment = c(1,1,1,0,1,1,1,1,0,1,1,1,1,0,1))

methCpG<-unite(myCpG,destrand=FALSE)

myCpG4vsREST<-methRead(list("F1\_4\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F1\_1\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_YA\_CpG.converted.txt"), sample.id = list("F1\_4","F2\_4","F3\_4","F1\_1","F2\_1","F3\_1","F1\_2","F2\_2","F3\_2","F1\_3","F2\_3","F3\_3","F1\_YA","F2\_YA","F3\_YA"), assembly = "Al4f",context = "CpG", treatment = c(0,0,0,1,1,1,1,1,1,1,1,1,1,1,1))

methCpG4vsREST<-unite(myCpG4vsREST, destrand=FALSE)

myDiffCpG4vsREST<-calculateDiffMeth(methCpG4vsREST)

myDiffCpG4vsREST\_25p<-getMethylDiff(myDiffCpG4vsREST, difference=25, qvalue=0.05)

write.csv(myDiffCpG4vsREST\_25p, "myDiffCpG4vsREST\_25p.csv")

> gene.obj <- genomation::readTranscriptFeatures("Al4f.modified.bed")

> genomation::annotateWithGeneParts(as(myDiffCpG4vsREST\_25p,"GRanges"),gene.obj)

**7H. LSS YA vs REST**

library(methylKit)

setwd("/Users/Dan/Desktop/Data/Bcyte\_Dissection\_Project/WGBSseq\_Cluster/Al4f\_Assembly/methylKit/")

newFileList=list("F1\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F1\_YA\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F3\_4\_CpG.converted.txt","F3\_YA\_CpG.converted.txt")

myNameList=list("F1\_1","F1\_2","F1\_3","F1\_4","F1\_YA","F2\_1","F2\_2","F2\_3","F2\_4","F2\_YA","F3\_1","F3\_2","F3\_3","F3\_4","F3\_YA")

myCpG<-methRead(newFileList, sample.id = myNameList, assembly = "Al4f", context = "CpG", treatment = c(1,1,1,1,0,1,1,1,1,0,1,1,1,1,0))

methCpG<-unite(myCpG,destrand=FALSE)

myCpGYAvsREST<-methRead(list("F1\_YA\_CpG.converted.txt","F2\_YA\_CpG.converted.txt","F3\_YA\_CpG.converted.txt","F1\_1\_CpG.converted.txt","F2\_1\_CpG.converted.txt","F3\_1\_CpG.converted.txt","F1\_2\_CpG.converted.txt","F2\_2\_CpG.converted.txt","F3\_2\_CpG.converted.txt","F1\_3\_CpG.converted.txt","F2\_3\_CpG.converted.txt","F3\_3\_CpG.converted.txt","F1\_4\_CpG.converted.txt","F2\_4\_CpG.converted.txt","F3\_4\_CpG.converted.txt"), sample.id = list("F1\_YA","F2\_YA","F3\_YA","F1\_1","F2\_1","F3\_1","F1\_2","F2\_2","F3\_2","F1\_3","F2\_3","F3\_3","F1\_4","F2\_4","F3\_4"), assembly = "Al4f",context = "CpG", treatment = c(0,0,0,1,1,1,1,1,1,1,1,1,1,1,1))

methCpGYAvsREST<-unite(myCpGYAvsREST, destrand=FALSE)

.

myDiffCpGYAvsREST<-calculateDiffMeth(methCpGYAvsREST)

myDiffCpGYAvsREST\_25p<-getMethylDiff(myDiffCpGYAvsREST, difference=25, qvalue=0.05)

write.csv(myDiffCpGYAvsREST\_25p, "myDiffCpGYAvsREST\_25p.csv")

> gene.obj <- genomation::readTranscriptFeatures("Al4f.modified.bed")

> genomation::annotateWithGeneParts(as(myDiffCpGYAvsREST\_25p,"GRanges"),gene.obj)