GSE143893

ukke

6 december 2021

```r
library(affy)
library(arrayQualityMetrics)
library(ArrayExpress)
library(limma)
library(siggenes)
```

Methylation Data

We selected the dataset with accession number GSE143893 as a methylation dataset. This data contains Whole Genome Bisulfite Sequencing of CD4+ T cells from mice developmentally exposed to vehicle or TCDD prior to and during influenza infection. We loaded only samples from mice that were not treated with TCDD.

General info

Methylation profiling by high throughput sequencing:GSE143893 We selected the dataset with accession number GSE143893 as a methylation dataset. This data contains Whole Genome Bisulfite Sequencing of CD4+ T cells from mice developmentally exposed to vehicle or TCDD prior to and during influenza infection. We loaded only samples from mice that were not treated with TCDD.

Intensity values

loading in the data

```r
library(methylKit)
```

```
## Warning: package 'methylKit' was built under R version 4.0.3
```

```
##
## Attaching package: 'methylKit'
```

```
## The following object is masked from 'package:AnnotationDbi':
##
##     select
```

```r
file.list=list( "C:/Users/tobia/Documents/AHAT/GSM4276332_Vehicle-Naive1_CpG.txt",
                "C:/Users/tobia/Documents/AHAT/GSM4276334_Vehicle-Naive2_CpG.txt",
                "C:/Users/tobia/Documents/AHAT/GSM4276336_Vehicle-Naive3_CpG.txt",
                "C:/Users/tobia/Documents/AHAT/GSM4276338_Vehicle-Infected1_CpG.txt",
                "C:/Users/tobia/Documents/AHAT/GSM4276340_Vehicle-Infected2_CpG.txt",
                "C:/Users/tobia/Documents/AHAT/GSM4276342_Vehicle-Infected3_CpG.txt")
# read the files to a methylRawList object: myobj immediately filter so that sites supported by less th
myobj=methRead(file.list,
           sample.id=list("vehicle_naive1","vehicle_naive2", "vehicle_naive3","vehicle_infected1","vehic
           assembly="GRCm38.p5",
           treatment=c(0,0,0,1,1,1),
           context="CpG",
           mincov = 10
           )
```

```
## Received list of locations.
```

```
## Reading file.
## Reading file.
## Reading file.
## Reading file.
## Reading file.
## Reading file.
```

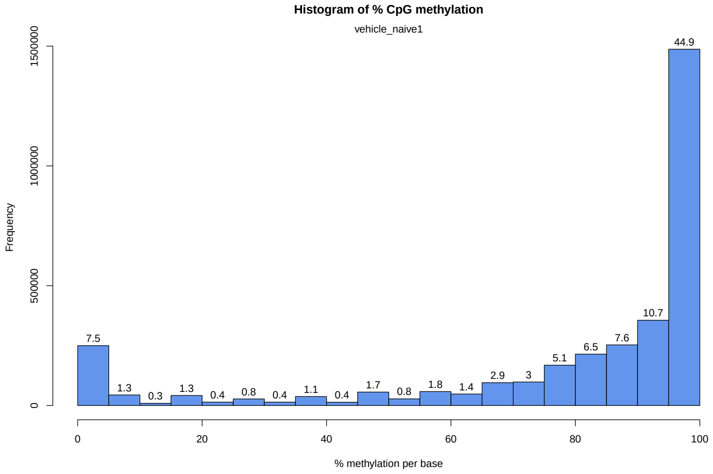
```
head(myobj)
```

```
## [[1]]
## methylRaw object with 3313657 rows
## --------------
##    chr   start     end strand coverage numCs numTs
## 1 chr1 3003380 3003380      -       10     9     1
## 2 chr1 3009138 3009138      -       10     9     1
## 3 chr1 3011266 3011266      -       12    11     1
## 4 chr1 3012097 3012097      -       11    11     0
## 5 chr1 3014974 3014974      +       12    12     0
## 6 chr1 3017888 3017888      -       13     9     4
## --------------
## sample.id: vehicle_naive1
## assembly: GRCm38.p5
## context: CpG
## resolution: base
##
##
## [[2]]
## methylRaw object with 2388205 rows
## --------------
##    chr   start     end strand coverage numCs numTs
## 1 chr1 3003583 3003583      -       10    10     0
## 2 chr1 3005999 3005999      -       11     8     3
## 3 chr1 3007581 3007581      -       13    11     2
## 4 chr1 3012097 3012097      -       11    11     0
## 5 chr1 3014612 3014612      -       12    12     0
## 6 chr1 3017888 3017888      -       12     6     6
## --------------
## sample.id: vehicle_naive2
## assembly: GRCm38.p5
## context: CpG
## resolution: base
##
##
## [[3]]
## methylRaw object with 3445871 rows
## --------------
##    chr   start     end strand coverage numCs numTs
## 1 chr1 3007581 3007581      -       13    11     2
## 2 chr1 3009138 3009138      -       11    10     1
## 3 chr1 3012097 3012097      -       12    12     0
## 4 chr1 3020815 3020815      -       10     8     2
## 5 chr1 3020843 3020843      -       10     6     4
## 6 chr1 3020878 3020878      -       13    13     0
## --------------
## sample.id: vehicle_naive3
## assembly: GRCm38.p5
## context: CpG
## resolution: base
##
##
## [[4]]
## methylRaw object with 3853966 rows
## --------------
##    chr   start     end strand coverage numCs numTs
## 1 chr1 3003380 3003380      -       13    13     0
## 2 chr1 3003899 3003899      -       10    10     0
## 3 chr1 3007581 3007581      -       11     8     3
## 4 chr1 3011266 3011266      -       12    10     2
## 5 chr1 3012840 3012840      +       14     9     5
## 6 chr1 3014602 3014602      -       10    10     0
## --------------
## sample.id: vehicle_infected1
## assembly: GRCm38.p5
## context: CpG
## resolution: base
##
##
## [[5]]
## methylRaw object with 3939620 rows
## --------------
##    chr   start     end strand coverage numCs numTs
## 1 chr1 3003227 3003227      -       10    10     0
## 2 chr1 3003340 3003340      -       10    10     0
## 3 chr1 3014602 3014602      -       11    11     0
## 4 chr1 3014612 3014612      -       18    17     1
## 5 chr1 3014742 3014742      -       10     8     2
## 6 chr1 3020795 3020795      -       11    10     1
## --------------
## sample.id: vehicle_infected2
## assembly: GRCm38.p5
## context: CpG
## resolution: base
##
##
## [[6]]
## methylRaw object with 4769378 rows
## --------------
##    chr   start     end strand coverage numCs numTs
## 1 chr1 3011266 3011266      -       13    12     1
## 2 chr1 3011314 3011314      -       12    10     2
## 3 chr1 3012097 3012097      -       10    10     0
## 4 chr1 3014533 3014533      -       12    12     0
## 5 chr1 3014556 3014556      -       16    16     0
## 6 chr1 3014612 3014612      -       16    15     1
## --------------
## sample.id: vehicle_infected3
## assembly: GRCm38.p5
## context: CpG
## resolution: base
```

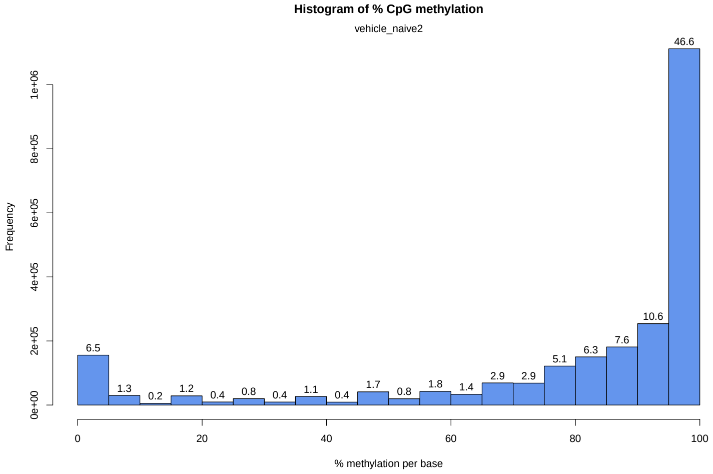
viewing the data

View the methylation rates per sample with the plot function that is provided in the package.

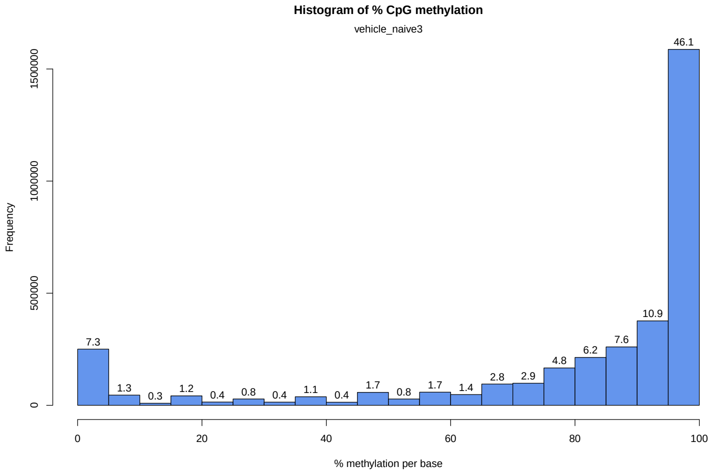
```
naive_1 <- getMethylationStats(myobj[[1]],plot=TRUE,both.strands=FALSE)
```



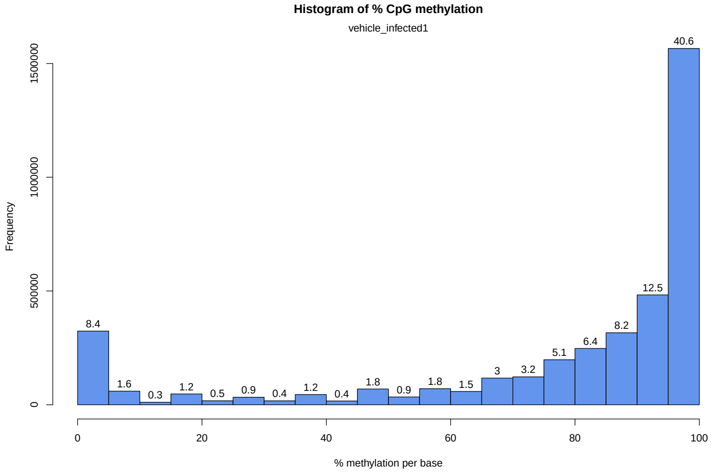
```
naive_2 <- getMethylationStats(myobj[[2]],plot=TRUE,both.strands=FALSE)
```



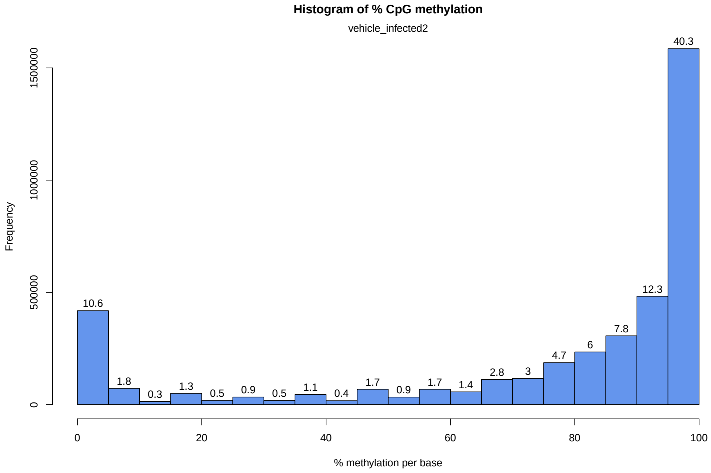
```
naive_3 <- getMethylationStats(myobj[[3]],plot=TRUE,both.strands=FALSE)
```



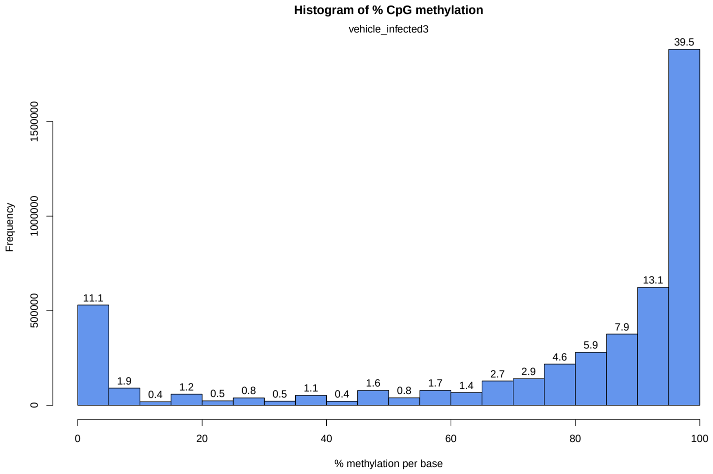
```
infected_1 <- getMethylationStats(myobj[[4]],plot=TRUE,both.strands=FALSE)
```



```
infected_2 <- getMethylationStats(myobj[[5]],plot=TRUE,both.strands=FALSE)
```

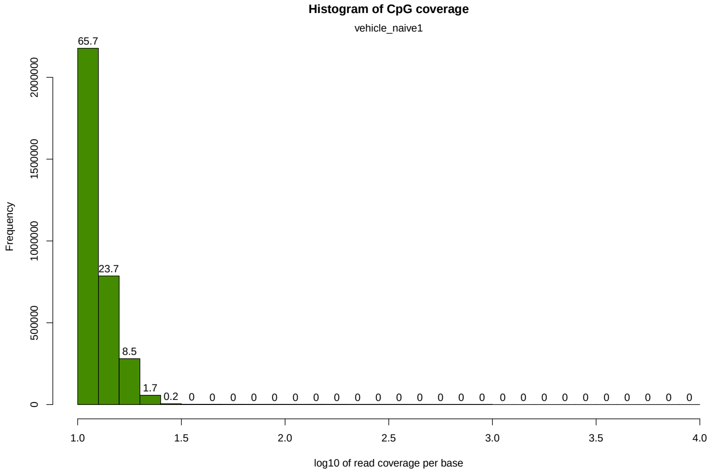


```
infected_3 <- getMethylationStats(myobj[[6]],plot=TRUE,both.strands=FALSE)
```

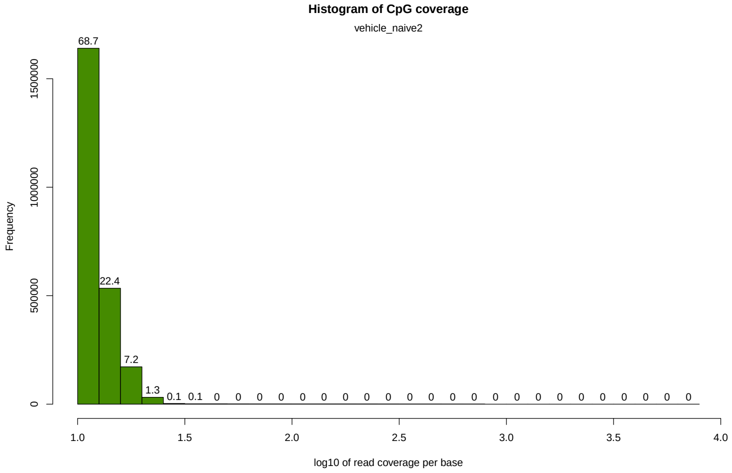


View the coverage rates per sample with the plot function that is provided in the package.

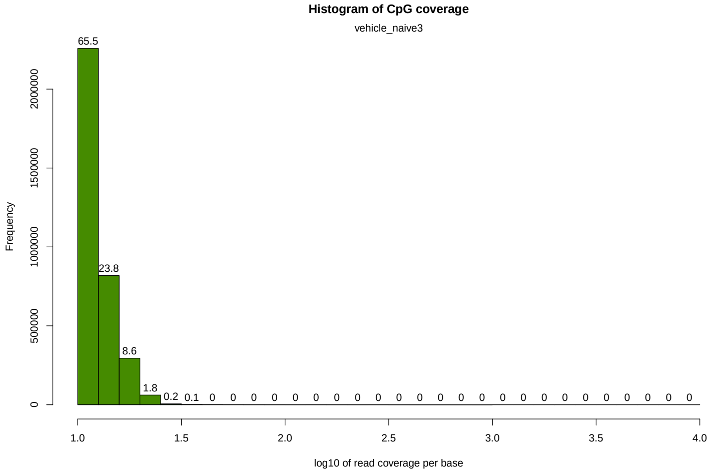
```
naive_1 <- getCoverageStats(myobj[[1]],plot=TRUE,both.strands=FALSE)
```



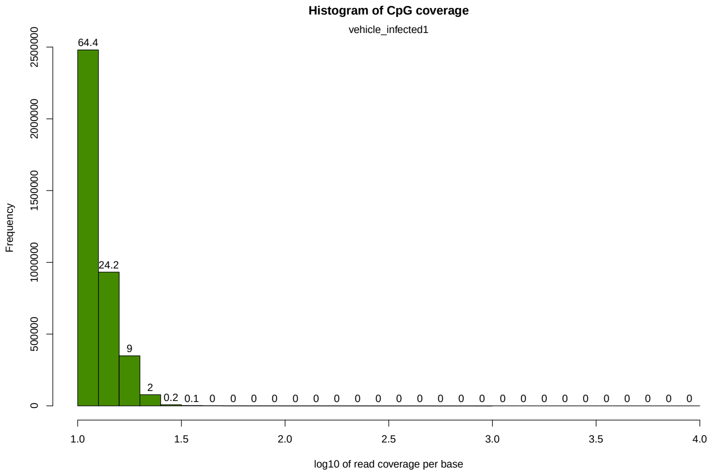
```
naive_2 <- getCoverageStats(myobj[[2]],plot=TRUE,both.strands=FALSE)
```



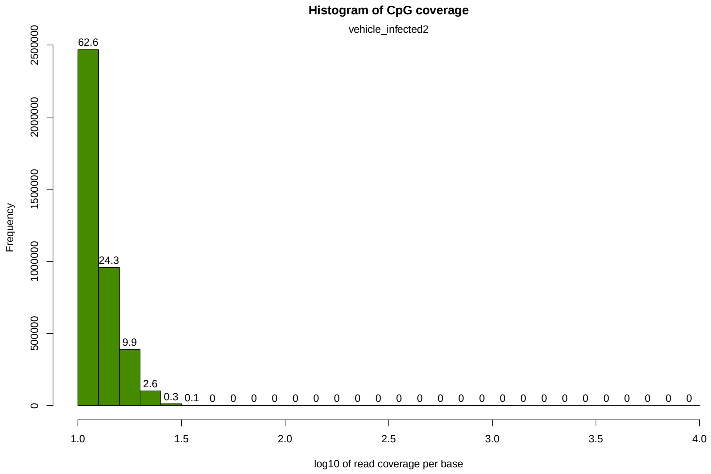
```
naive_3 <- getCoverageStats(myobj[[3]],plot=TRUE,both.strands=FALSE)
```



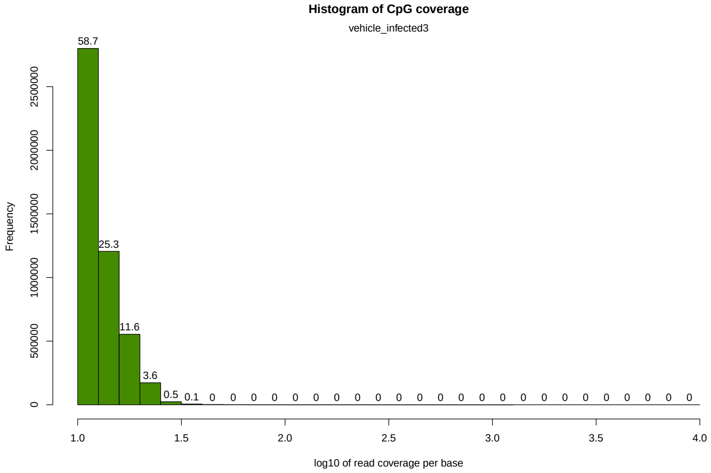
```
infected_1 <- getCoverageStats(myobj[[4]],plot=TRUE,both.strands=FALSE)
```



```
infected_2 <- getCoverageStats(myobj[[5]],plot=TRUE,both.strands=FALSE)
```



```
infected_3 <- getCoverageStats(myobj[[6]],plot=TRUE,both.strands=FALSE)
```



filtering the data is not necessary because no signs of PCR bias are observed (features with very high coverage). But is strongly recommended by methylkit so. . . .

```
# filter out extreme coverage values (top 0.1%)
filtered <- filterByCoverage(myobj, hi.count = 99.9)

# normalize coverage to to avoid bias introduced by systematically more sequenced samples
normalized <- normalizeCoverage(filtered)
```

merging samples

```
# destrand parameter can be set to TRUE as we're working with base-pair resolution CpG methylation data
meth=unite(normalized, destrand=TRUE)
```

```
## destranding...
```

```
## uniting...
```

```
head(meth)
```

```
##           chr start   end strand coverage1 numCs1 numTs1 coverage2 numCs2 numTs2
## 1 GL456210.1   773   773      +        34     32      2        26     24      2
## 2 GL456210.1   779   779      +        36     36      0        28     24      4
## 3 GL456210.1  7360  7360      +        16     13      3        15     13      2
## 4 GL456210.1 17333 17333      +        20     10     10        11      9      2
## 5 GL456210.1 26873 26873      +        12     12      0        14     14      0
## 6 GL456210.1 33611 33611      +        34     24     10        29     21      8
##   coverage3 numCs3 numTs3 coverage4 numCs4 numTs4 coverage5 numCs5 numTs5
## 1        12     12      0        22     22      0        14     14      0
## 2        13     13      0        24     24      0        16     15      1
## 3        19     13      6        29     17     12        16     13      3
## 4        15      9      6        11      7      4        13      8      5
## 5        11     10      1        18     18      0        11     10      1
## 6        21     13      8        30     21      9        28     17     11
##   coverage6 numCs6 numTs6
## 1        14     14      0
## 2        13     11      2
## 3          19      14       5
## 4          27      19       8
## 5          10      10       0
## 6          31      24       7
```

get the sample correlation

```
getCorrelation(meth,plot=F)
```

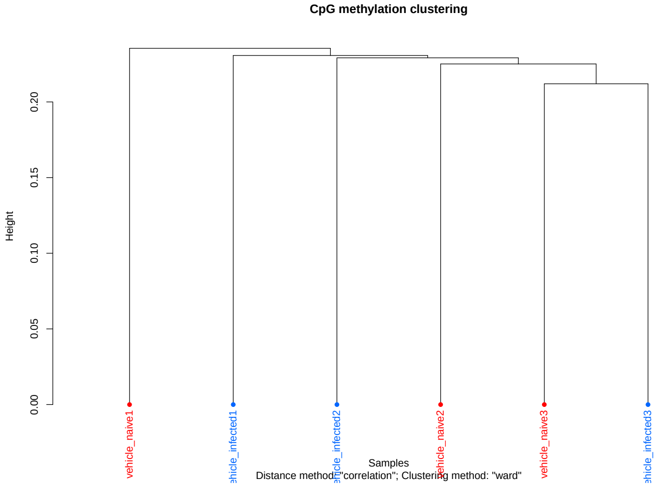
```
##                    vehicle_naive1 vehicle_naive2 vehicle_naive3
## vehicle_naive1          1.0000000      0.8918948      0.8959197
## vehicle_naive2          0.8918948      1.0000000      0.8957106
## vehicle_naive3          0.8959197      0.8957106      1.0000000
## vehicle_infected1       0.8900042      0.8923888      0.8952116
## vehicle_infected2       0.8916590      0.8929707      0.8954318
## vehicle_infected3       0.8965660      0.8973933      0.9013212
##                    vehicle_infected1 vehicle_infected2 vehicle_infected3
## vehicle_naive1             0.8900042         0.8916590         0.8965660
## vehicle_naive2             0.8923888         0.8929707         0.8973933
## vehicle_naive3             0.8952116         0.8954318         0.9013212
## vehicle_infected1          1.0000000         0.8950831         0.8993060
## vehicle_infected2          0.8950831         1.0000000         0.9004814
## vehicle_infected3          0.8993060         0.9004814         1.0000000
```

All samples have a very high correlation rate.

Cluster the samples samples

```
clusterSamples(meth, dist="correlation", method="ward")
```

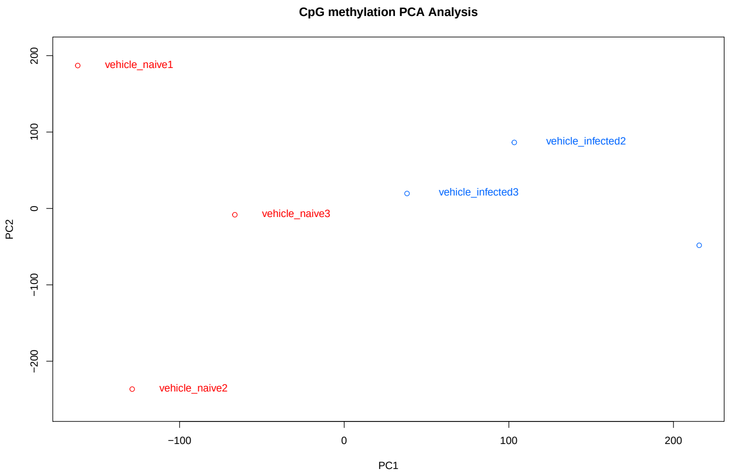
```
## The "ward" method has been renamed to "ward.D"; note new "ward.D2"
```



```
##
## Call:
## hclust(d = d, method = HCLUST.METHODS[hclust.method])
##
## Cluster method   : ward.D
## Distance         : pearson
## Number of objects: 6
```

PCA

```r
PCASamples(meth)
```



This PCA plot is not similar to the other pca-plots we made => make same figure with our figure style.

```r
# preprocessing steps used by methylkit (code obtained from open source scripts on their github)
mat = getData(meth)
meth.mat = mat[, meth@numCs.index]/
  (mat[,meth@numCs.index] + mat[,meth@numTs.index] )
names(meth.mat)=meth@sample.ids

# remove rows (bases) that are to simillar to avoid error from scale. parameter
sds=rowSds(as.matrix(meth.mat))
cutoff=quantile(sds,0.5)
meth.mat=meth.mat[sds>cutoff,]

# transpose the data before PcA as this function requires the variables to b columms
pca <- prcomp(t(meth.mat), center = T, scale. = T)


summary(pca)
```

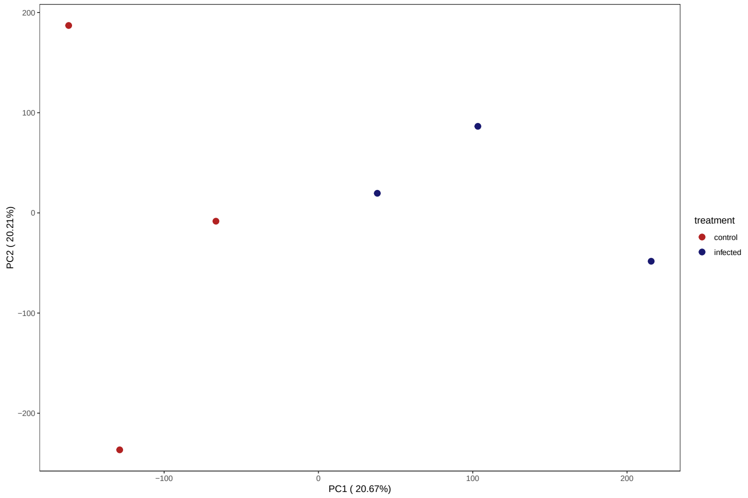
```
## Importance of components:
##                              PC1      PC2      PC3      PC4      PC5       PC6
## Standard deviation     145.4873 142.2571 141.5512 138.7873 135.8371 3.092e-12
## Proportion of Variance   0.2135   0.2041   0.2021   0.1943   0.1861 0.000e+00
## Cumulative Proportion    0.2135   0.4176   0.6197   0.8139   1.0000 1.000e+00
```

```r
# save as dataframe and add treatment variable
pca_out <- as.data.frame(pca$x)
pca_out$treatment <- c("control", "control", "control","infected","infected" ,"infected")
pca_out$sample <- c("rep1", "rep2", "rep3", "rep1", "rep2", "rep3")

# get lablels
percentage <- round(pca$sdev / sum(pca$sdev) * 100, 2)
percentage <- paste( colnames(pca_out), "(", paste( as.character(percentage), "%", ")", sep="") )

ggplot(data = pca_out)+
  geom_point(aes(x = PC1, y = PC2, colour = treatment, label=sample), size=3)+
  #geom_text(aes(x = PC1, y = PC2, colour = treatment, label=sample),hjust=0.5, vjust=1.15)+
  theme_bw()+
  xlab(percentage[1])+
  ylab(percentage[2])+
  labs(colour = "treatment")+
  theme(plot.title = element_text(hjust = 0.5))+
  scale_colour_manual(values = c("firebrick", "midnightblue"))+
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank())
```

```
## Warning: Ignoring unknown aesthetics: label
```



```
ggsave("PCA_WGBS.png", dpi=750, height = 5, width = 8)
```

get differentially methylated bases

```
myDiff=calculateDiffMeth(meth)
```

```
## two groups detected:
##  will calculate methylation difference as the difference of
## treatment (group: 1) - control (group: 0)
```

```
myDiff25p=getMethylDiff(myDiff,difference=50,qvalue=0.05)
diffMethPerChr(myDiff,plot=FALSE,qvalue.cutoff=0.05, meth.cutoff=25)
```

```
## Warning in eval(quote(list(...)), env): NAs introduced by coercion
```

```
## $diffMeth.per.chr
##            chr number.of.hypermethylated percentage.of.hypermethylated
## 2         chr1                         3                   0.023485204
## 13        chr2                         2                   0.014286735
## 14        chr3                         5                   0.056605910
## 15        chr4                         0                   0.000000000
## 16        chr5                         1                   0.007189589
## 17        chr6                         1                   0.010610080
## 18        chr7                         2                   0.016335865
## 19        chr8                         3                   0.024135157
## 20        chr9                         1                   0.009399380
## 3        chr10                         2                   0.019892580
## 4        chr11                         0                   0.000000000
## 5        chr12                         0                   0.000000000
## 6        chr13                         2                   0.023482447
## 7       chr14                         2                   0.027348557
## 8       chr15                         1                   0.012134450
## 9       chr16                         1                   0.016095284
## 10      chr17                         3                   0.032119914
## 11      chr18                         0                   0.000000000
## 12      chr19                         1                   0.016911889
## 1  JH584304.1                         1                   0.123762376
## 21       chrX                         4                   0.078988942
##    number.of.hypomethylated percentage.of.hypomethylated
## 2                         2                  0.015656803
## 13                        3                  0.021430102
## 14                        5                  0.056605910
## 15                        9                  0.061859922
## 16                        1                  0.007189589
## 17                        4                  0.042440318
## 18                        4                  0.032671731
## 19                        5                  0.040225261
## 20                        1                  0.009399380
## 3                         2                  0.019892580
## 4                         3                  0.024246343
## 5                         3                  0.035215401
## 6                         4                  0.046964894
## 7                         6                  0.082045672
## 8                         1                  0.012134450
## 9                         4                  0.064381136
## 10                        2                  0.021413276
## 11                        5                  0.082209799
## 12                        5                  0.084559445
## 1                         0                  0.000000000
## 21                        1                  0.019747235
##
## $diffMeth.all
##   number.of.hypermethylated percentage.of.hypermethylated
## 1                        35                    0.01763997
##   number.of.hypomethylated percentage.of.hypomethylated
## 1                       70                   0.03527995
```

```
myDiff25p
```

```
##                chr    start      end strand       pvalue       qvalue meth.diff
## 823    JH584304.1    34304    34304      + 8.199773e-25 1.489274e-19  58.17783
## 2181         chr1 23116177 23116177      + 1.509287e-06 1.305345e-02 -50.82067
## 25320       chr11 12515663 12515663      + 3.913577e-06 2.328643e-02 -50.73359
## 54015       chr14 14346963 14346963      + 3.793240e-07 5.299560e-03 -51.04396
## 57803       chr14 63756321 63756321      + 2.402432e-07 3.636157e-03  52.82230
## 73359       chr16 67339031 67339031      + 1.342956e-05 3.752504e-02 -50.11583
## 85272       chr18 12517640 12517640      + 2.020320e-07 3.636157e-03 -53.55898
## 113429       chr3 57941935 57941935      + 5.898043e-17 5.356126e-12  81.43590
## 163682       chr7 79299862 79299862      + 1.501372e-07 3.636157e-03  51.22549
```

```
Diffmeth <-calculateDiffMeth(meth, overdispersion="MN",test="Chisq")
```

```
## two groups detected:
##  will calculate methylation difference as the difference of
## treatment (group: 1) - control (group: 0)
```

```r
Diff25p=getMethylDiff(Diffmeth,difference=50,qvalue=0.05)
diffMethPerChr(Diffmeth,plot=FALSE,qvalue.cutoff=0.05, meth.cutoff=25)
```

```
## $diffMeth.per.chr
##    chr number.of.hypermethylated percentage.of.hypermethylated
## 1 chr3                         2                    0.02264236
## 2 chr4                         0                    0.00000000
##   number.of.hypomethylated percentage.of.hypomethylated
## 1                        0                  0.000000000
## 2                        1                  0.006873325
##
## $diffMeth.all
##   number.of.hypermethylated percentage.of.hypermethylated
## 1                         2                   0.001007998
##   number.of.hypomethylated percentage.of.hypomethylated
## 1                        1                 0.0005039992
```

There appear to be a very low number of differentially methylated positions between cells from mice infected with influenza and those that are not infected with influenza.

annotating differentially methylated bps

```r
library(genomation)
```

```
## Warning: package 'genomation' was built under R version 4.0.3

## Loading required package: grid

##
## Attaching package: 'genomation'

## The following objects are masked from 'package:methylKit':
##
##     getFeatsWithTargetsStats, getFlanks, getMembers,
##     getTargetAnnotationStats, plotTargetAnnotation
```

```r
# load the hgr18 bed file.
gene.obj=readTranscriptFeatures("C:/Users/tobia/Documents/AHAT/mm10_RefSeq.bed.gz")
```

```
## Reading the table...

## Calculating intron coordinates...

## Calculating exon coordinates...

## Calculating TSS coordinates...

## Calculating promoter coordinates...

## Outputting the final GRangesList...
```

```r
# annotate to promotors, exons, introns.
diffAnn=annotateWithGeneParts(as(myDiff25p,"GRanges"),gene.obj)
```

```
## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the oth
##   - in 'x': JH584304.1
##   - in 'y': chr2, chr4, chr5, chr6, chr8, chr9, chrM, chrX, chrY, chr10, chr12, chr13, chr15, chr17,
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).
## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the othe
##   - in 'x': JH584304.1
##   - in 'y': chr2, chr4, chr5, chr6, chr8, chr9, chrM, chrX, chrY, chr10, chr12, chr13, chr15, chr17,
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).

## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the othe
##   - in 'x': JH584304.1
##   - in 'y': chr2, chr4, chr5, chr6, chr8, chr9, chrM, chrX, chrY, chr10, chr12, chr13, chr15, chr17,
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).

## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the othe
##   - in 'x': chr2, chr4, chr5, chr6, chr8, chr9, chrM, chrX, chrY, chr10, chr12, chr13, chr15, chr17,
##   - in 'y': JH584304.1
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).

## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the othe
##   - in 'x': chr2, chr4, chr5, chr6, chr8, chr9, chrM, chrX, chrY, chr10, chr12, chr13, chr15, chr17,
##   - in 'y': JH584304.1
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).

## Warning in .Seqinfo.mergexy(x, y): Each of the 2 combined objects has sequence levels not in the othe
##   - in 'x': chr2, chr4, chr5, chr6, chr8, chr9, chrM, chrX, chrY, chr10, chr12, chr13, chr15, chr17,
##   - in 'y': JH584304.1
##   Make sure to always combine/compare objects based on the same reference
##   genome (use suppressWarnings() to suppress this warning).
```

```r
# get associated transcription start sites
getAssociationWithTSS(diffAnn)
```

```
##      target.row dist.to.feature   feature.name feature.strand
## 2654          2           -7812    XR_373275.2              +
## 446           3          -50704 XM_006514485.3              -
## 1756          4            -134 XM_006517940.1              +
## 3580          5          -13886    XR_383493.2              -
## 2992          6          106991    XR_876148.2              -
## 30            7           13434 XM_006525690.2              +
## 2433          8           -7173    XR_867305.2              +
## 5296          9             914    XR_882244.1              +
```

```r
# save features as vector
features <- c("no feature linked", getAssociationWithTSS(diffAnn)[[3]])
features
```

```
## [1] "no feature linked" "XR_373275.2"       "XM_006514485.3"
## [4] "XM_006517940.1"    "XR_383493.2"       "XR_876148.2"
## [7] "XM_006525690.2"    "XR_867305.2"       "XR_882244.1"
```

```r
dist_features <- c(NA, getAssociationWithTSS(diffAnn)[[2]])

summary <- as.data.frame(myDiff25p)
# add feature information to myDiff25p
summary$feature <- features
summary$dist.to.feature <- dist_features
```

```
summary
```

```
##                 chr    start      end strand       pvalue       qvalue meth.diff
## 823    JH584304.1    34304    34304      + 8.199773e-25 1.489274e-19  58.17783
## 2181         chr1 23116177 23116177      + 1.509287e-06 1.305345e-02 -50.82067
## 25320       chr11 12515663 12515663      + 3.913577e-06 2.328643e-02 -50.73359
## 54015       chr14 14346963 14346963      + 3.793240e-07 5.299560e-03 -51.04396
## 57803       chr14 63756321 63756321      + 2.402432e-07 3.636157e-03  52.82230
## 73359       chr16 67339031 67339031      + 1.342956e-05 3.752504e-02 -50.11583
## 85272       chr18 12517640 12517640      + 2.020320e-07 3.636157e-03 -53.55898
## 113429       chr3 57941935 57941935      + 5.898043e-17 5.356126e-12  81.43590
## 163682       chr7 79299862 79299862      + 1.501372e-07 3.636157e-03  51.22549
##                   feature dist.to.feature
## 823    no feature linked              NA
## 2181         XR_373275.2           -7812
## 25320     XM_006514485.3          -50704
## 54015     XM_006517940.1            -134
## 57803        XR_383493.2          -13886
## 73359        XR_876148.2          106991
## 85272     XM_006525690.2           13434
## 113429       XR_867305.2           -7173
## 163682       XR_882244.1             914
```

```
diffAnn@members
```

```
##       prom exon intron
##  [1,]    0    0      0
##  [2,]    0    0      0
##  [3,]    0    0      0
##  [4,]    1    1      1
##  [5,]    0    0      1
##  [6,]    0    0      1
##  [7,]    0    0      1
##  [8,]    0    0      0
##  [9,]    1    1      1
```

```
promoters=regionCounts(normalized,gene.obj$promoters)

head(promoters[[1]])
```

```
##    chr   start     end strand coverage numCs numTs
## 1 chr1 3360551 3362551      +       11    10     1
## 2 chr1 3670498 3672498      -      285    16   270
## 3 chr1 3671278 3673278      -      160    15   146
## 4 chr1 4232728 4234728      -       25    17     8
## 5 chr1 4242365 4244365      -       11     8     3
## 6 chr1 4359314 4361314      -       26    21     5
```

```
getTargetAnnotationStats(diffAnn,percentage=TRUE,precedence=TRUE)
```

```
##   promoter       exon     intron intergenic
##      22.22       0.00      33.33      44.44
```

same for more stringent

```
# annotate to promotors, exons, introns.
diffAnn_str=annotateWithGeneParts(as(Diff25p,"GRanges"),gene.obj)
```

```r
# get associated transcription start sites
getAssociationWithTSS(diffAnn_str)
```

```
##      target.row dist.to.feature feature.name feature.strand
## 2433          1           -7173  XR_867305.2              +
```

```r
# save features as vector
features <- c(getAssociationWithTSS(diffAnn_str)[[3]])

dist_features <- c(getAssociationWithTSS(diffAnn_str)[[2]])

summary <- as.data.frame(Diff25p)
# add feature information to myDiff25p
summary$feature <- features
summary$dist.to.feature <- dist_features
summary
```

```
##         chr    start      end strand       pvalue       qvalue meth.diff
## 113429 chr3 57941935 57941935      + 5.898043e-17 1.117464e-11   81.4359
##            feature dist.to.feature
## 113429 XR_867305.2           -7173
```

```r
diffAnn_str@members
```

```
##      prom exon intron
## [1,]    0    0      0
```

```r
getTargetAnnotationStats(diffAnn_str,percentage=TRUE,precedence=TRUE)
```

```
##   promoter       exon     intron intergenic
##          0          0          0        100
```