

# BINFF-402 — Genomics, Proteomics and Evolution

## Assignment 1

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## 1 Introduction

The Illumina Infinium 450K sequencer [1] was designed in order to sequence DNA methylation. It works by first applying bisulfite transformation to the DNA fragments to transform unmethylated Cytosines to Uracils, and leave methylated Cytosines unchanged, and then sequencing uses two different bead types: one to determine methylated loci and one to determine unmethylated loci [2].

The Infinium HumanMethylation450 BeadChip contains over 4.5e5 methylation sites probes, and two different chemical assays: Infinium I and Infinium II. In this assignment, only Infinium I is considered. Yet, Infinium II should also be considered in analyses, but separately [3].

In this document, methylation level is studied in the case of a DKO of genes DNMT1 and DNMT3b (respectively OMIM ids 126375 and 602900) [4] which are both methyltransferases. This double knockout induces a loss of hypermethylated CpG islands which is not present in case of single knockout of either of these genes [5].

The study has been performed with the R language. Note that all code has been written from scratch even though there is a wide range of available packages for bioinformatics in R. [6] See for instance the package IMA that is made on purpose for Infinium data analysis. [7]

All the R code used for this report can be found at <https://github.com/RobinPetit/BINF-F402>.

## 2 Analysis

### 2.1 $\beta$ -value distribution

The  $\beta$  distribution is shown in Figure 1. For each probe, the  $\beta$  value is computed to be  $\frac{M}{M+U+\alpha}$ , where  $M$  is the methylated score,  $U$  is the unmethylated score, and  $\alpha$  acts as a pseudo-count to avoid the case  $M = U = 0$  to lead to a division by zero. This  $\alpha$  value has been set to 100 [8].

It is clear that controls have a two-spikes  $\beta$  distribution: most probes have a methylation  $\beta$ -value around 0 or around 1. A high methylation  $\beta$ -value (close to 1) means that almost all of the sequenced cells had a methylated Cytosine at this position, whereas a low methylation  $\beta$ -value (close to 0) means that almost none of the sequenced cells had a methylated Cytosine at this position. Yet, as several different cells are sequenced at the same time, it is possible to have a Cytosine that is methylated in some cells, but not in others. This explains how  $\beta$ -value can take so many different values.

It is also clear that cases have a single-spike  $\beta$  distribution: most probes have a methylation  $\beta$ -value close to 0, but almost none has a  $\beta$ -value close to 1. Also, there is a higher density of intermediate  $\beta$ -values, i.e. between 0.2 and 0.8, which means that there is still methylation happening in the cases, but the methylation of DNA varies from one cell to another.

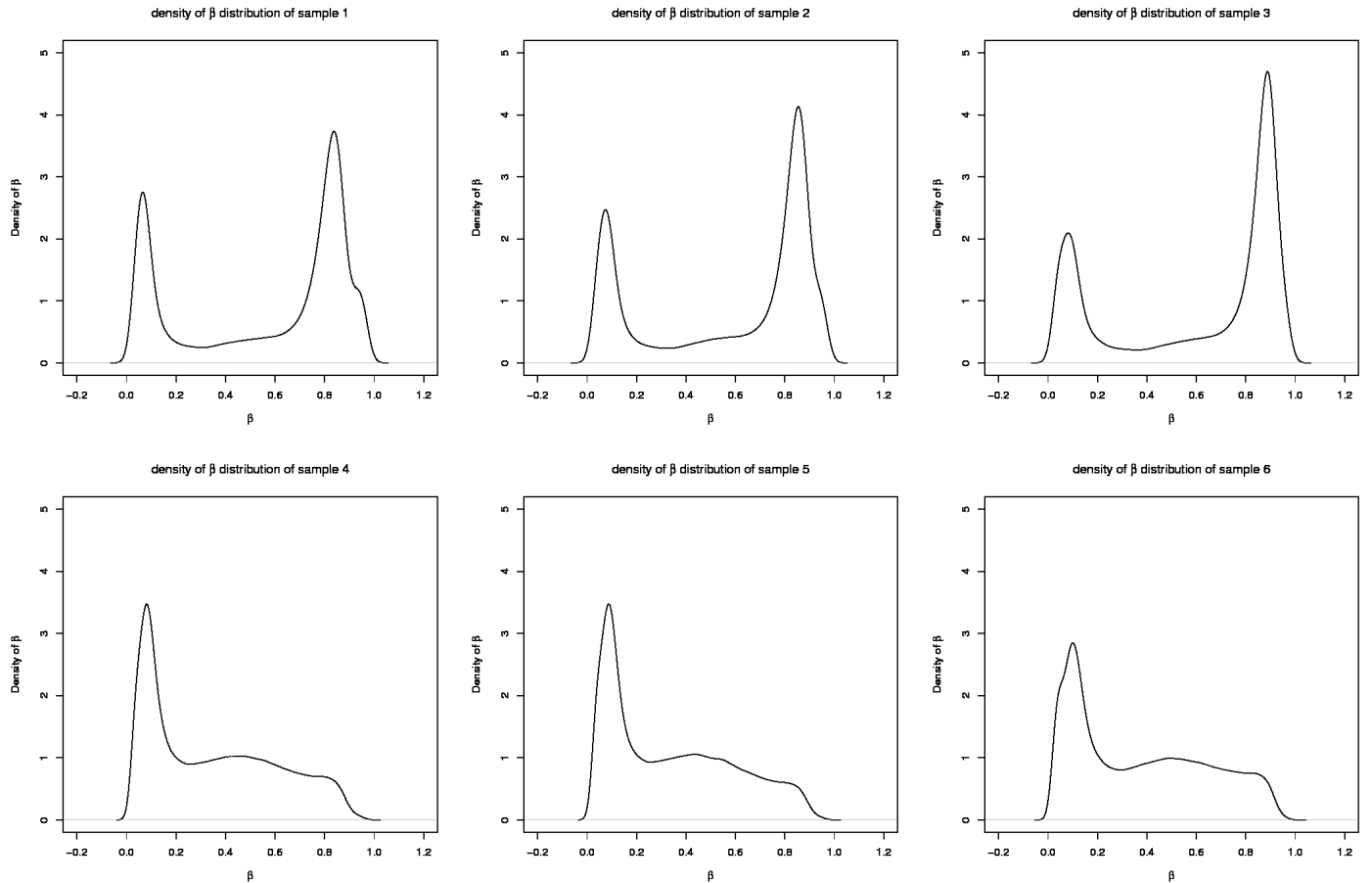


Figure 1: Distribution of the  $\beta$  value for each sample. Samples 1 to 3 are the controls, and samples 4 to 6 are the cases.

## 2.2 Global overview

An analysis of the methylation level of each chromosome is shown in Figure 2a. We can observe that the hypomethylation is present on each chromosome, with highest hypomethylation being on chromosomes 10, 16 and 20 and lowest hypomethylation is on chromosome Y, probably because the Y chromosome has a lower methylation level in control samples. Yet, chromosome X is also less hypomethylated than the other chromosomes, but sum of difference in methylation level for X and Y chromosomes reaches roughly the same value as the other chromosomes.

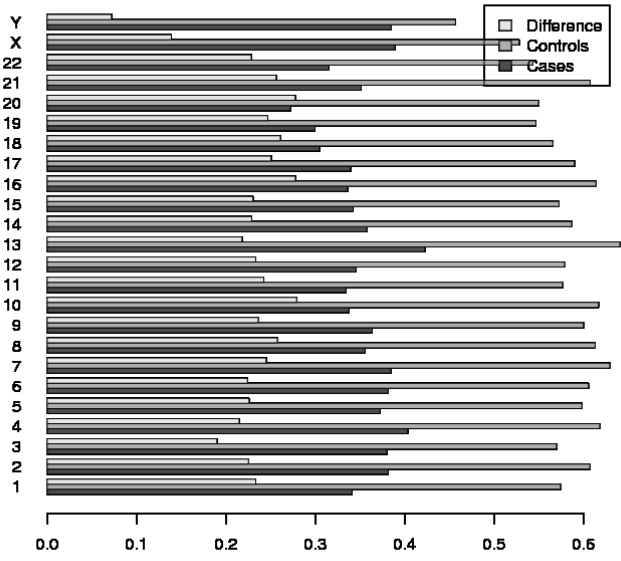
The distribution of the difference in methylation level per chromosome seems to be pretty uniform between 0.2 and 0.25.

Figure 2b shows the methylation level of chromosomes 19 to 21 as stated in the instructions.

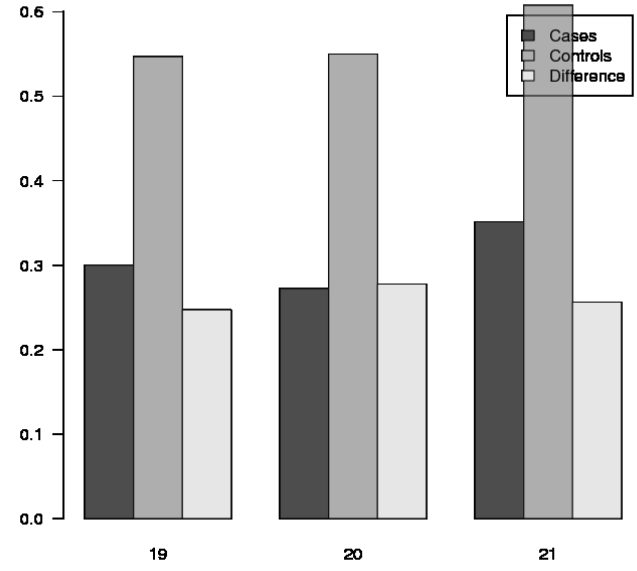
## 2.3 Number of probes per gene

The distribution of the number of probes per gene is shown in Figure 3: Figure 3a shows the distribution in which we observe that almost all of the genes have only a few probes related to them, so Figure 3 shows the same data in y log-scale.

Yet, as displayed on Figure 3b, the average number of probes per gene is around 32.36. This mean value is impacted by the very high values that are 2199, 2404, and 3897, but since only one such gene exist for each of these values, the impact is very low, due to the big amount of probes: 485577 (computed by either `nrow(infinium)` in the R code, or by `wc -l annotations.csv` minus 1 to remove the header).

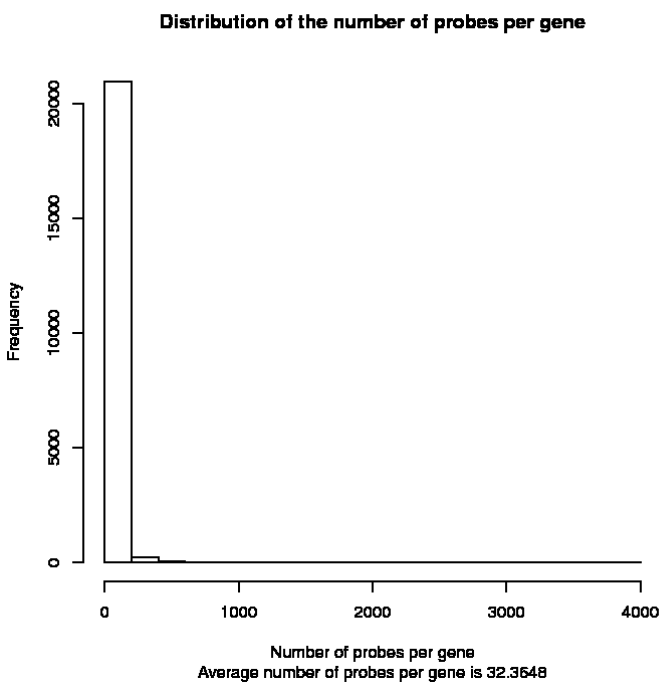


(a) Methylation level on each chromosome (1 to 22 plus X and Y).

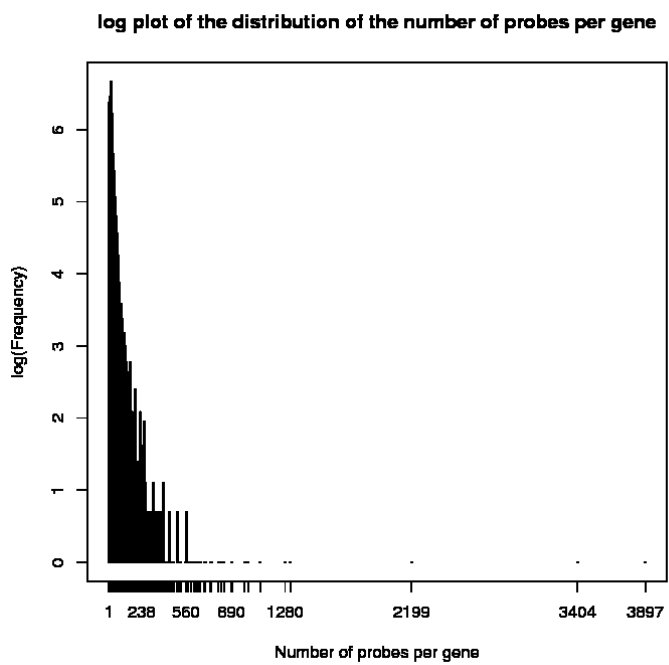


(b) Subplot of Figure 2a of chromosomes 19 to 21.

Figure 2: General analysis of the methylation level per chromosome for both controls and cases samples. Difference in methylation is defined as control minus case since cases are hypomethylated.



(a) Distribution of the number of probes per gene.



(b) Log-distribution of the number of probes per gene.

Figure 3: Distribution of the number of probes per gene in both linear and log scale.

### 3 R source code

First of all, libraries must be imported and constants must be defined. Only two libraries have been used files must be loaded into R:

```
1 INFINIUM_PATH <- 'Infinium450k_raw_data.txt';
2 ANNO_MINI_PATH <- 'HumanMethylation450_anno_mini.csv';
3 NB_CASES <- 3;
4 NB_CONTROLS <- NB_CASES;
5 CONTROLS <- 1:NB_CONTROLS;
6 CASES <- (1:NB_CASES) + NB_CONTROLS;
7 ALPHA_PSEUDO_COUNT <- 100;
8 ALL_CHROMOSOMES <- c(1:22, 'X', 'Y');
9 CHROMOSOMES_TO_TEST <- 19:21;
10
11 # Use first column as row names
12 infinium <- read.table(INFINIUM_PATH, header=T, dec=',', row.names=1);
13 annotations <- read.table(ANNO_MINI_PATH, header=T, sep=',', row.names=1);
```

In order to determine the  $\beta$  value of a sample, let's define a few functions:

```
1 compute.beta <- function(signalA, signalB) {
2   # Note the pseudo count $alpha = 100$ to avoid dividing by 0
3   return (signalB / (signalA + signalB + ALPHA_PSEUDO_COUNT));
4 }
5
6 # Get the column corresponding to the required sample
7 get.sample.signal <- function(table, sample.id, signalA=T) {
8   if(signalA) {
9     return (as.vector(table[[2*sample.id-1]]));
10  } else {
11    return (as.vector(table[[2*sample.id]]));
12  }
13 }
14
15 # Returns the $beta$ values of a given sample
16 get.beta <- function(infinium, sample.id) {
17   return (compute.beta(
18     get.sample.signal(infinium, sample.id, T),
19     get.sample.signal(infinium, sample.id, F)
20   ));
21 }
```

so that plotting becomes:

```
1 # Plot the beta distribution of each sample from 1 to 6
2 plot.beta.distribution.infinium <- function(infinium) {
3   for(sample.id in 1:(NB_CASES+NB_CONTROLS)) {
4     plot(
5       density(get.beta(infinium, sample.id)),
6       xlim=c(-.2, 1.2),
7       ylim=c(0, 5),
8       xlab=TeX('$\\beta$'),
9       ylab=TeX('Density of $\\beta$'),
10      main=TeX(paste('density of $\\beta$ distribution of sample', sample.id)),
11     );
12   }
13 }
14 plot.beta.distribution.infinium(infinium);
```

The methylation level per chromosome is performed as follows:

```
1 # Returns the subtable corresponding to the required chromosome
2 extract.chromosome <- function(annotations, chr) {
3   return (annotations[annotations$CHR == chr,]);
4 }
5
6 # Make the barplots of the average methylation level of each chromosome
7 plot.mean.methylation.level <- function(infinium, annotations) {
```

```

8 means <- matrix(rep(0, 3*length(ALL_CHROMOSOMES)), nrow=3, ncol=length(ALL_CHROMOSOMES));
9 idx <- 1;
10 for(chromosome in ALL_CHROMOSOMES) {
11   intersection <- intersect(rownames(infinium), rownames(extract.chromosome(annotations, chromosome)
12 ));
13   chromosome.infinium.subtable <- infinium[intersection,];
14   for(case.sample.id in CASES)
15     means[1,idx] <- means[1,idx] + mean(get.beta(chromosome.infinium.subtable, case.sample.id))/NB_
16 CASES;
17   for(control.sample.id in CONTROLS)
18     means[2,idx] <- means[2,idx] + mean(get.beta(chromosome.infinium.subtable, control.sample.id))/
19 NB_CONTROLS;
20   # Take difference control - case to see methylation difference per chromosome
21   means[3,] <- means[2,] - means[1,];
22   idx <- idx+1;
23 }
24
25 barplot(means, names.arg=ALL_CHROMOSOMES, horiz=T, beside=T, legend=c('Cases', 'Controls', '
26 Difference'), las=1);
27 barplot(means[,CHROMOSOMES_TO_TEST], names.arg=CHROMOSOMES_TO_TEST, beside=T, legend=c('Cases', '
28 Controls', 'Difference'), las=1);
29
30 }
31
32 plot.mean.methylation.level(infinium, annotations);

```

To find the number of probes per gene, only annotations are required since all ids are common to the infinium file and the annotations file:

```

1 # Plot the histograms of the distribution and log-distribution of the number of probes per gene
2 plot.number.of.genes.per.probe <- function(annotations) {
3   non.null.genes.associations <- as.vector(annotations[annotations$UCSC_RefGene_Name != '',]$UCSC_
4 RefGene_Name);
5   genes.counter <- table(unlist(sapply(non.null.genes.associations, function(s) {unique(strsplit(s, '
6 '))})));
7   hist(genes.counter, main='Distribution of the number of probes per gene',
8 sub=sprintf('Average number of probes per gene is %g', mean(genes.counter)),
9 xlab='Number of probes per gene',
10 ylab='Frequency');
11   plot(log(table(genes.counter)), main='log plot of the distribution of the number of probes per gene'
12 ,
13 xlab='Number of probes per gene',
14 ylab='log(Frequency)');
15 }

```

Note that `function(s) unique(strsplit(s, ' '))` allows to retrieve all the genes associated to a probe, but not taking the genes that come several times, thanks to the `unique` function. Making a table of the unlisted results (since `sapply` returns a list) makes R count the number of probes related to each gene on its own. Therefore, making a histogram of the table is sufficient to plot the distribution.

## References

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