Lab4 732A51 Bioinformatics Group 9

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Question 1

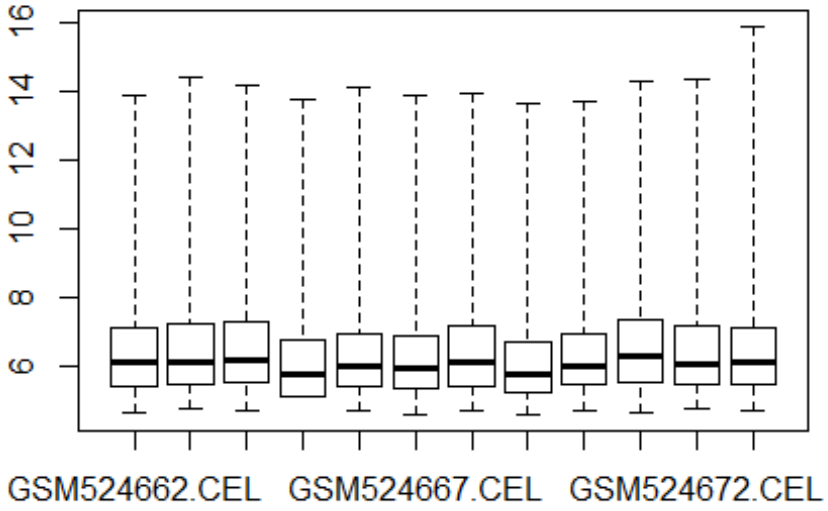
In overall, the code provided will analysis of gene expression data from HUVEC1 and Ocular Vascular Endothelial2 Cells.
The first step is to download the data with the code GSE20986 using `getGEOsuppFiles` function. Then untar, unzip it to data folder. A data frame called `phenodata` is created to hold the metadata of the data. It's also written to a file with the same name.

```
library(GEOquery)
#The data folder should be empty

x = getGEOSuppFiles("GSE20986")
x

##
size
## C:/Users/Duong Minh Duc/Documents/GitHub/Bioinformatics_Labs/Lab
4/GSE20986/GSE20986_RAW.tar 56360960
##
isdir
## C:/Users/Duong Minh Duc/Documents/GitHub/Bioinformatics_Labs/Lab
4/GSE20986/GSE20986_RAW.tar FALSE
##
mode
## C:/Users/Duong Minh Duc/Documents/GitHub/Bioinformatics_Labs/Lab
4/GSE20986/GSE20986_RAW.tar  666
##
mtime
## C:/Users/Duong Minh Duc/Documents/GitHub/Bioinformatics_Labs/Lab
4/GSE20986/GSE20986_RAW.tar 2018-12-13 18:00:21
##
ctime
## C:/Users/Duong Minh Duc/Documents/GitHub/Bioinformatics_Labs/Lab
4/GSE20986/GSE20986_RAW.tar 2018-12-13 17:59:41
##
atime
## C:/Users/Duong Minh Duc/Documents/GitHub/Bioinformatics_Labs/Lab
4/GSE20986/GSE20986_RAW.tar 2018-12-13 17:59:41
##
exe
```

```
## C:/Users/Duong Minh Duc/Documents/GitHub/Bioinformatics_Labs/Lab
4/GSE20986/GSE20986_RAW.tar  no

untar("GSE20986/GSE20986_RAW.tar", exdir = "data")
cels = list.files("data/", pattern = "[gz]")
sapply(paste("data", cels, sep = "/"), gunzip)

## data/GSM524662.CEL.gz data/GSM524663.CEL.gz data/GSM524664.CEL.gz
##              13555726              13555055              13555639
## data/GSM524665.CEL.gz data/GSM524666.CEL.gz data/GSM524667.CEL.gz
##              13560122              13555663              13557614
## data/GSM524668.CEL.gz data/GSM524669.CEL.gz data/GSM524670.CEL.gz
##              13556090              13560054              13555971
## data/GSM524671.CEL.gz data/GSM524672.CEL.gz data/GSM524673.CEL.gz
##              13554926              13555042              13555290

phenodata = matrix(rep(list.files("data"), 2), ncol =2)
class(phenodata)

## [1] "matrix"

phenodata <- as.data.frame(phenodata)
colnames(phenodata) <- c("Name", "FileName")
phenodata$Targets <- c("iris",
                       "retina",
                       "retina",
                       "iris",
                       "retina",
                       "iris",
                       "choroid",
                       "choroid",
                       "choroid",
                       "huvec",
                       "huvec",
                       "huvec")
#Write the list of downloaded content to a file
write.table(phenodata, "data/phenodata.txt", quote = F, sep = "\t", row.names
= F)
```

The, they use the `read.affy` function to read the data and stored it in an object called `celfiles`. The `boxplot` function will display the microarray distributions. The values in boxplots are the log base 2 intensities of both pm and mm probes.

```
library(simpleaffy)
#Using read.affy function to read..
celfiles <- read.affy(covdesc = "phenodata.txt", path = "data")
boxplot(celfiles)

##
```

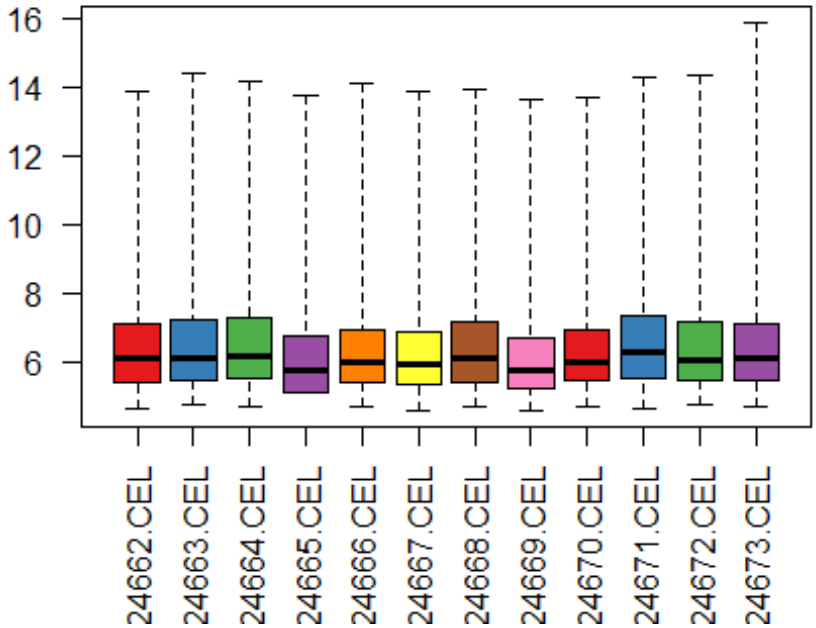


The second boxplot is still the same. But, it is couloured and the labels are made verticaled for easier reading.

```
library(RColorBrewer)
cols = brewer.pal(8, "Set1")
eset <- exprs(celfiles)
samples <- celfiles$Targets
colnames(eset)
```

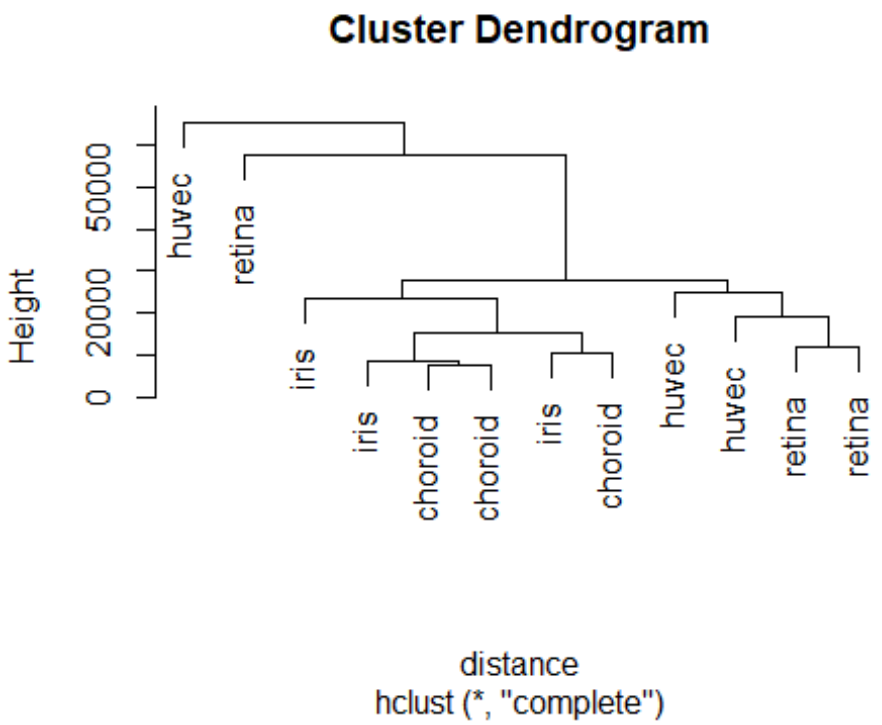
```
##  [1] "GSM524662.CEL" "GSM524663.CEL" "GSM524664.CEL" "GSM524665.CEL"
##  [5] "GSM524666.CEL" "GSM524667.CEL" "GSM524668.CEL" "GSM524669.CEL"
##  [9] "GSM524670.CEL" "GSM524671.CEL" "GSM524672.CEL" "GSM524673.CEL"
```

```
colnames(eset) <- samples
boxplot(celfiles, col = cols, las = 2) #las=2 make the axis labels horizontal
```



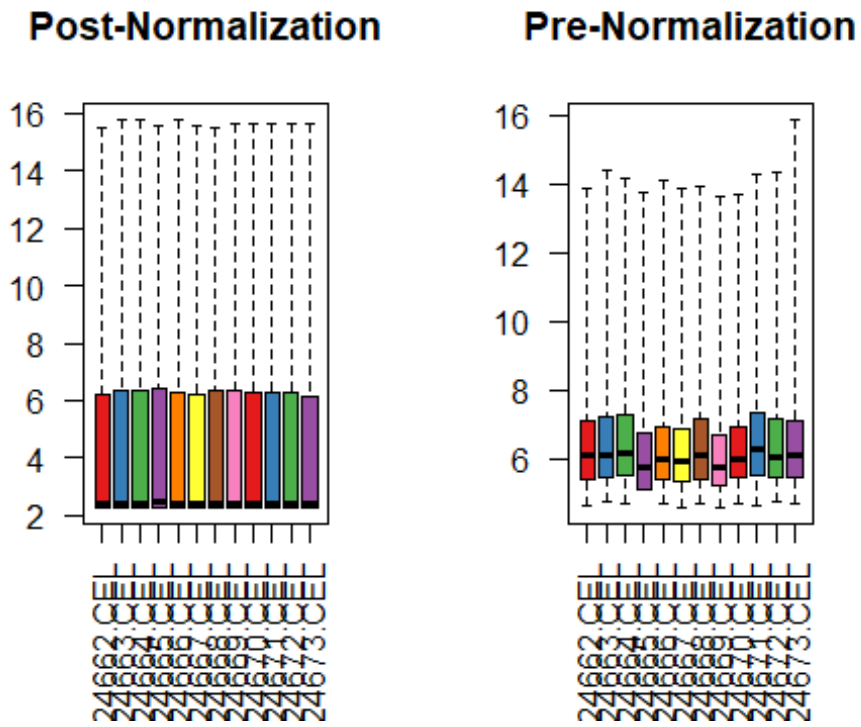
In the next step, they use `dist` function to calculate the distance of the data from 12 samples. Then, use `hclust` function to analysis hierarchical clusters and then plot it as a cluster dendrogram.

```
distance <- dist(t(eset), method = "maximum")
clusters <- hclust(distance)
plot(clusters)
```



The below block will convert `celfiles` objects (AffyBatch type) into an ExpressionSet though `gcrma` function. This function will use the robust multi-array average (RMA) expression measure with help of probe sequence. When converting, the data is being normalized. Two boxplots show the data before and after normalized is drawn to compare.

```
require(simpleaffy)
require(affyPLM)
celfiles.gcrma = gcrma(celfiles)

## Adjusting for optical effect.....Done.
## Computing affinities.Done.
## Adjusting for non-specific binding.....Done.
## Normalizing
## Calculating Expression

par(mfrow=c(1,2))
boxplot(celfiles.gcrma, col = cols, las = 2, main = "Post-Normalization")
boxplot(celfiles, col = cols, las = 2, main = "Pre-Normalization")
```



And then, they draw the cluster dndrogram of the normilizated data.

```
dev.off()

## null device
##           1

distance2 <- dist(t(exprs(celfiles.gcrma)), method = "maximum")
clusters2 <- hclust(distance2)
plot(clusters2)
```

In the next step, a matrix call `design` is created. It contains the name of the names of genes and which samples it belongs to. A contrast matrix is also created by the `makeContrasts` function. It includes three pairs of having versus the others.

```
library(limma)
phenodata

##             Name      FileName Targets
## 1  GSM524662.CEL GSM524662.CEL    iris
## 2  GSM524663.CEL GSM524663.CEL  retina
## 3  GSM524664.CEL GSM524664.CEL  retina
## 4  GSM524665.CEL GSM524665.CEL    iris
## 5  GSM524666.CEL GSM524666.CEL  retina
## 6  GSM524667.CEL GSM524667.CEL    iris
## 7  GSM524668.CEL GSM524668.CEL choroid
## 8  GSM524669.CEL GSM524669.CEL choroid
## 9  GSM524670.CEL GSM524670.CEL choroid
```

```
## 10 GSM524671.CEL GSM524671.CEL   huvec
## 11 GSM524672.CEL GSM524672.CEL   huvec
## 12 GSM524673.CEL GSM524673.CEL   huvec

samples <- as.factor(samples)
design <- model.matrix(~0+samples)
colnames(design)

## [1] "sampleschoroid" "sampleshuvec"   "samplesiris"    "samplesretina"

colnames(design) <- c("choroid", "huvec", "iris", "retina")
design

##    choroid huvec iris retina
## 1        0     0    1      0
## 2        0     0    0      1
## 3        0     0    0      1
## 4        0     0    1      0
## 5        0     0    0      1
## 6        0     0    1      0
## 7        1     0    0      0
## 8        1     0    0      0
## 9        1     0    0      0
## 10       0     1    0      0
## 11       0     1    0      0
## 12       0     1    0      0
## attr(,"assign")
## [1] 1 1 1 1
## attr(,"contrasts")
## attr(,"contrasts")$samples
## [1] "contr.treatment"

contrast.matrix = makeContrasts(
  huvec_choroid = huvec - choroid,
  huvec_retina = huvec - retina,
  huvec_iris = huvec - iris,
  levels = design)
```

In this step. They use the `design` matrix to fit the linear model `celfiles.gcrma` expressionSet created before by using the `LMFit` function. The result called `fit` is used in `contrasts.fit` function with the contrast matrix. They continue with extracting some t value, F value.. by the `eBayes` function.

```
fit = lmFit(celfiles.gcrma, design)
huvec_fit <- contrasts.fit(fit, contrast.matrix)
huvec_ebay <- eBayes(huvec_fit)
```

In the next step, the `topTable` function with number = 100000 will extract the top-ranked genes from the result before. `getSYMBOL` function is called to map that 100000 genes with the `hgu133plus2`. The final result is printed below.

```
library(hgu133plus2.db)
library(annotate)

probenames.list <- rownames(topTable(huvec_ebay, number = 100000))
getsymbols <- getSYMBOL(probenames.list, "hgu133plus2")
results <- topTable(huvec_ebay, number = 100000, coef = "huvec_choroid")
results <- cbind(results, getsymbols)
summary(results)
```

```
##      logFC             AveExpr             t                 P.Value
##  Min.   :-9.19111   Min.   : 2.279   Min.   :-39.77473   Min.   :0.0000
##  1st Qu.:-0.05967   1st Qu.: 2.281   1st Qu.: -0.70649   1st Qu.:0.1523
##  Median : 0.00000   Median : 2.480   Median :  0.00000   Median :0.5079
##  Mean   :-0.02353   Mean   : 4.375   Mean   :  0.07441   Mean   :0.5346
##  3rd Qu.: 0.03986   3rd Qu.: 6.241   3rd Qu.:  0.67455   3rd Qu.:1.0000
##  Max.   : 8.67086   Max.   :15.541   Max.   :296.84201   Max.   :1.0000
##
##    adj.P.Val            B             getsymbols
##  Min.   :0.0000   Min.   :-7.710   YME1L1  :   22
##  1st Qu.:0.6036   1st Qu.:-7.710   HFE     :   15
##  Median :1.0000   Median :-7.451   CFLAR   :   14
##  Mean   :0.7436   Mean   :-6.582   NRP2    :   14
##  3rd Qu.:1.0000   3rd Qu.:-6.498   ARHGEF12:   13
##  Max.   :1.0000   Max.   :21.290   (Other) :41857
##                                    NA's    :12740
```

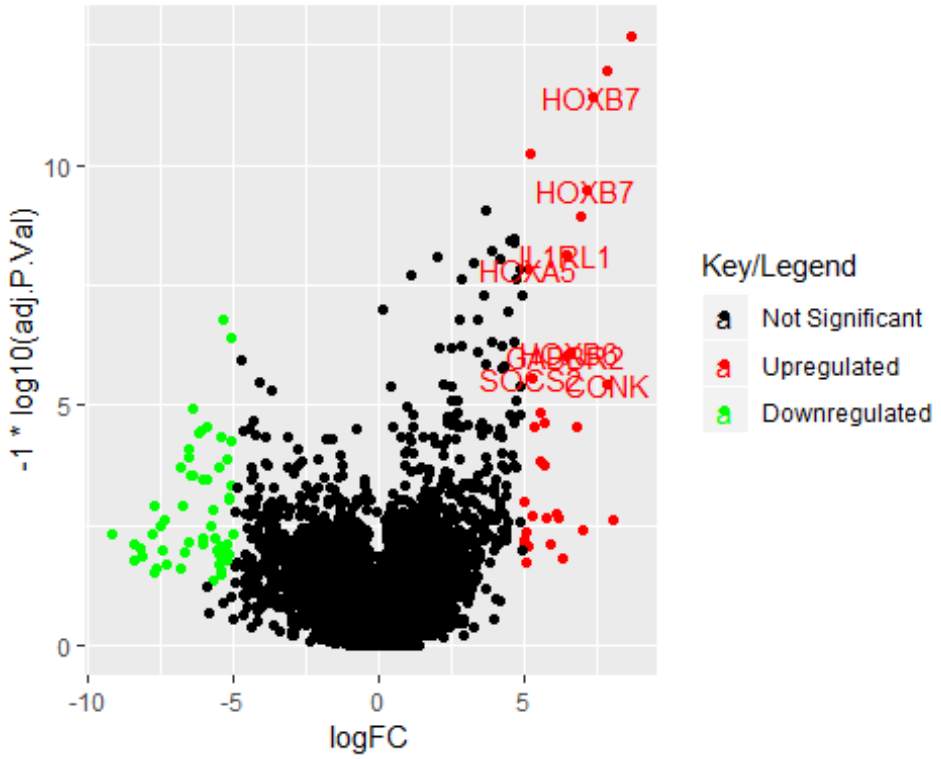
The results are grouped into three groups. Group 3 includes genes that adj.P.Val < 0.05 and logFC < -5. Group 2 contains gene that adj.P.Val < 0.05 and logFC > 5, and the rest is group 1. Number of gene in each groups is printed. Data in group 1 means Not Significant, group 2 means “Upregulated” and group 3 means “Downregulated”. A scatter plot is draw, in which x = logFC and y = -1*log10(adj.P.Val)

```
results$threshold <- "1"
a <- subset(results, adj.P.Val < 0.05 & logFC > 5)
results[rownames(a), "threshold"] <- "2"
b <- subset(results, adj.P.Val < 0.05 & logFC < -5)
results[rownames(b), "threshold"] <- "3"
table(results$threshold)
```

```
##
##     1     2     3
## 54587    33    55
```

```
library(ggplot2)
volcano <- ggplot(data = results,
                  aes(x = logFC, y = -1*log10(adj.P.Val),
                      colour = threshold,
                      label = getsymbols))

volcano <- volcano +
```

```
  geom_point() +
  scale_color_manual(values = c("black", "red", "green"),
                     labels = c("Not Significant", "Upregulated",
"Downregulated"),
                     name = "Key/Legend")

volcano +
  geom_text(data = subset(results, logFC > 5 & -1*log10(adj.P.Val) > 5),
aes(x = logFC, y = -1*log10(adj.P.Val), colour = threshold, label =
getsymbols)  )
```

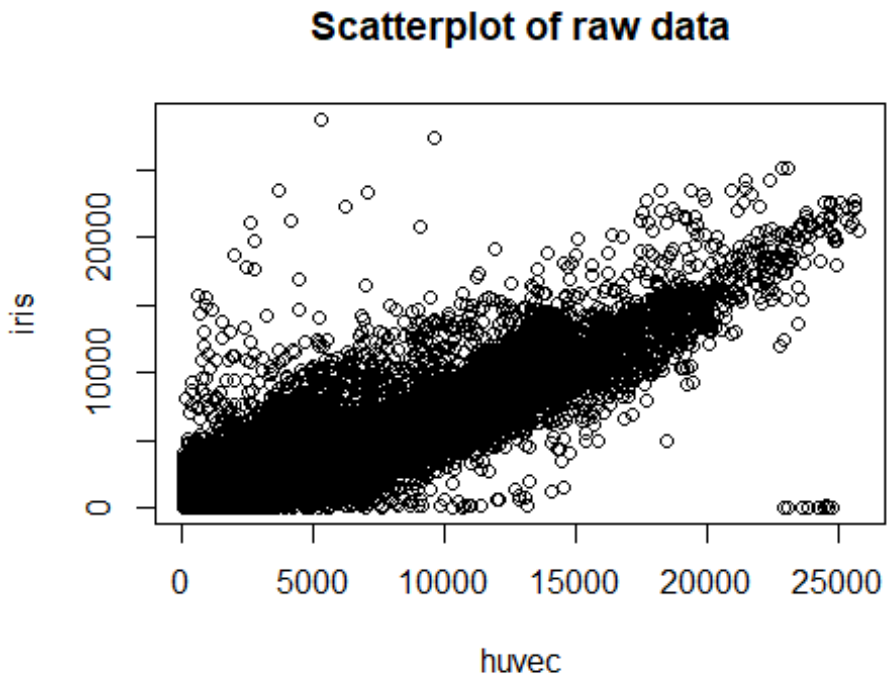


Question2

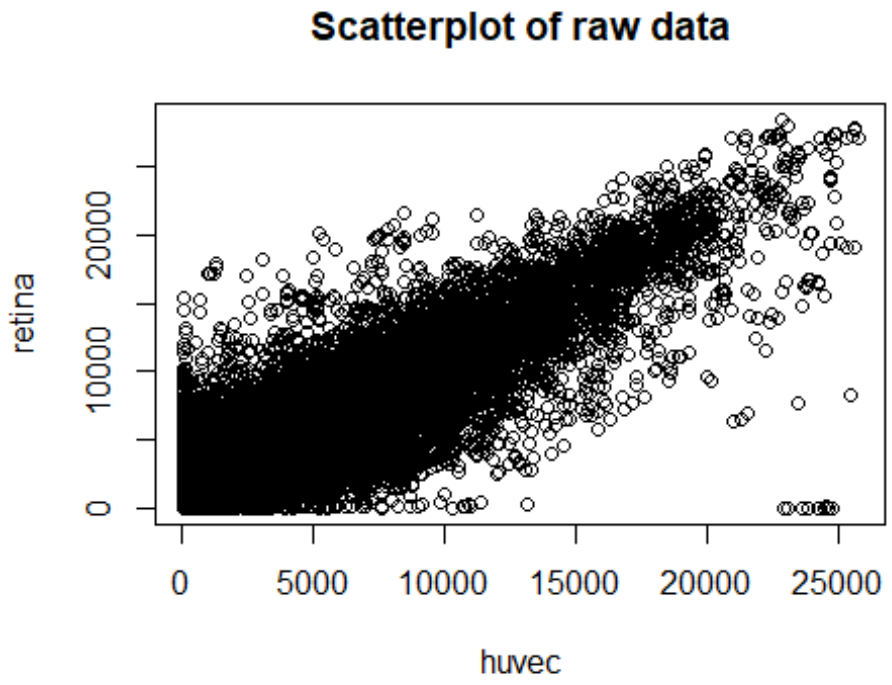
The three constrast are: + huvec - choroid,
+ huvec - retina,
+ huvec - iris

We will choose the first sample of each type to make analysis. Here is the plots of raw data.

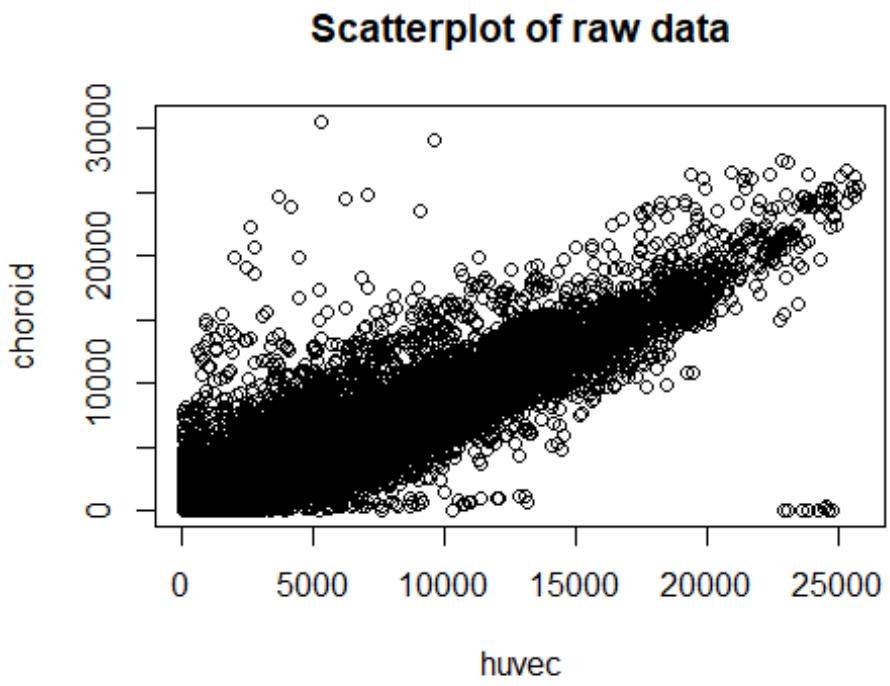
```
iris <- eset[,1]
retina <- eset[,2]
choroid <- eset[,7]
huvec <- eset[,10]

plot(x=huvec ,y=iris,xlab="huvec",ylab="iris", main="Scatterplot of raw
data")
```




```r
plot(x=huvec ,y=retina,xlab="huvec",ylab="retina", main="Scatterplot of raw data")
```

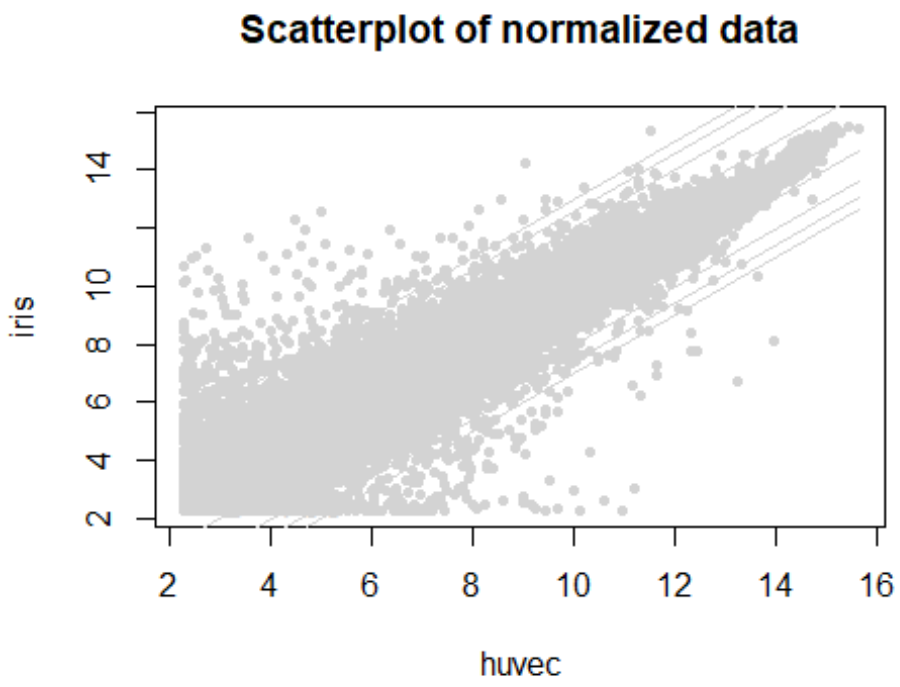


```
plot(x=huvec ,y=choroid,xlab="huvec",ylab="choroid", main="Scatterplot of raw
data")
```

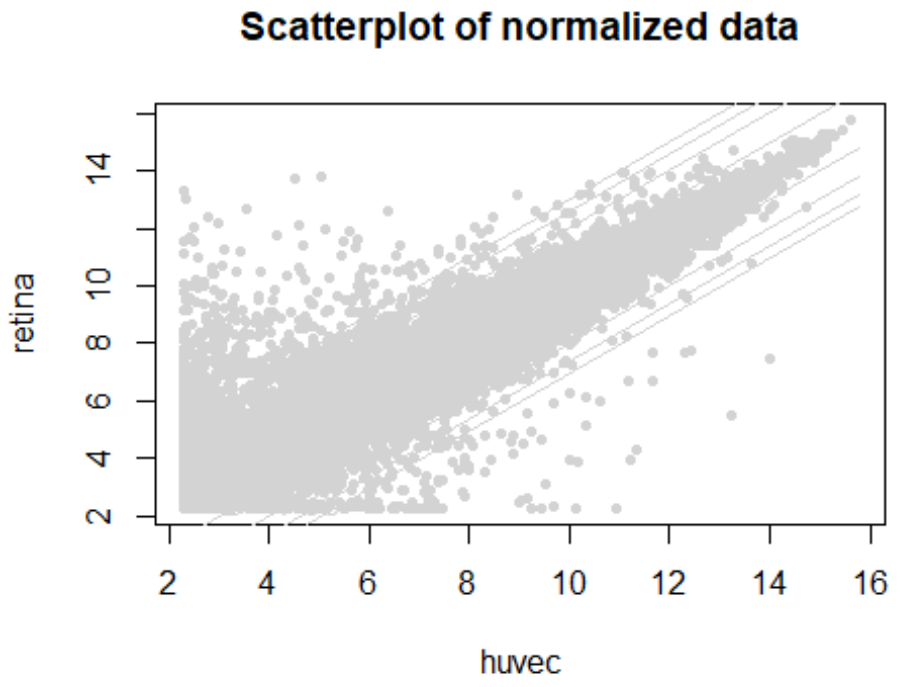


And here, for the normalized data:

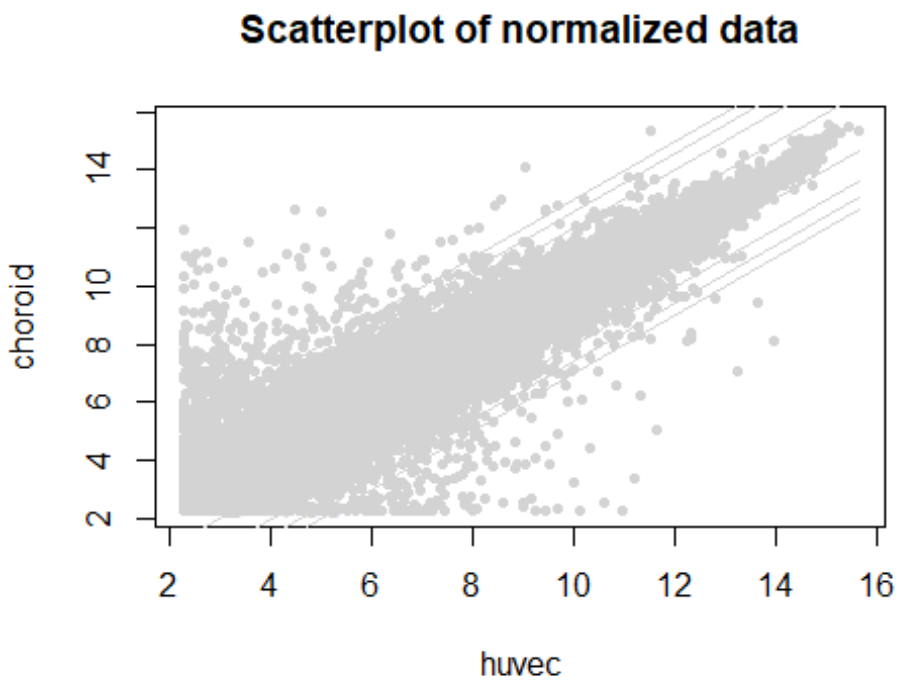
```
huvec_iris <- pairwise.comparison(celfiles.gcrma,"Targets",c("huvec","iris"))
huvec_retina <-
pairwise.comparison(celfiles.gcrma,"Targets",c("huvec","retina"))
huvec_choroid <-
pairwise.comparison(celfiles.gcrma,"Targets",c("huvec","choroid"))
plot(huvec_iris, main="Scatterplot of normalized data")
```




```r
plot(huvec_retina, main="Scatterplot of normalized data")
```

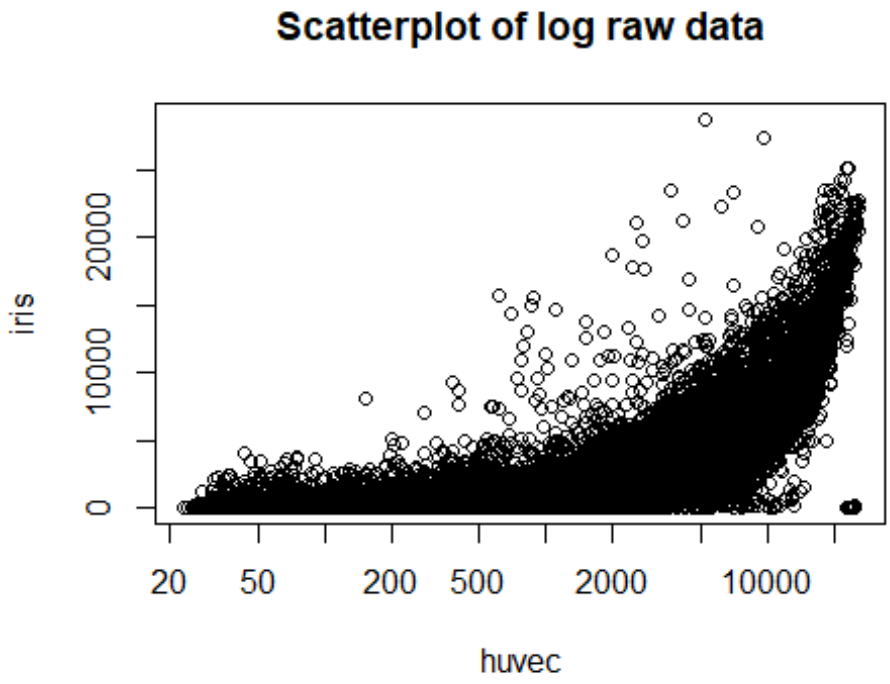



```r
plot(huvec_choroid, main="Scatterplot of normalized data")
```

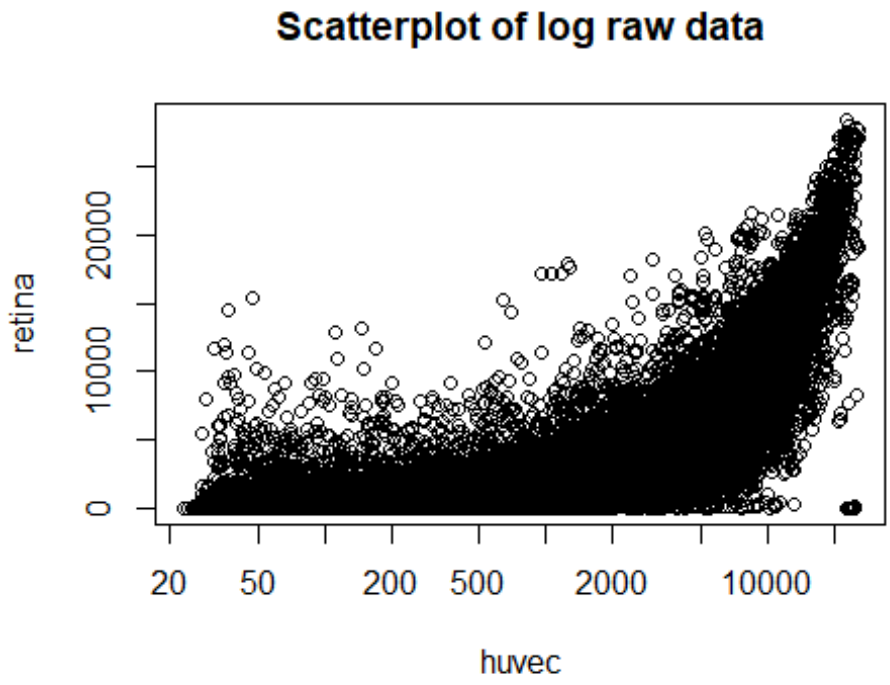


And here is log-scaled graph

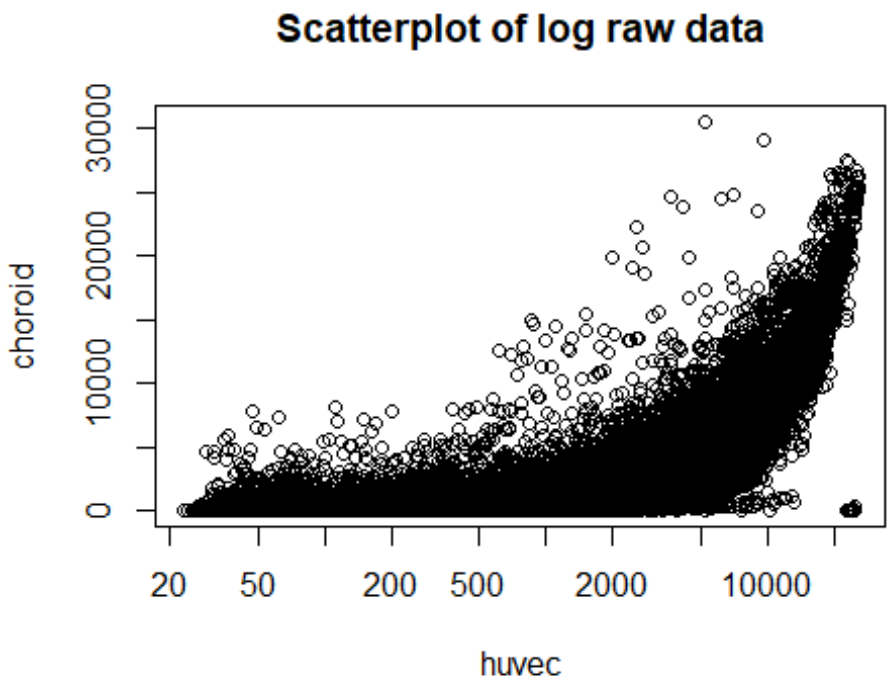
```
plot(x=huvec ,y=iris,xlab="huvec",ylab="iris", main="Scatterplot of log raw
data",log=c('x','y'))
```




```
plot(x=huvec ,y=retina,xlab="huvec",ylab="retina", main="Scatterplot of log
raw data",log=c('x','y'))
```

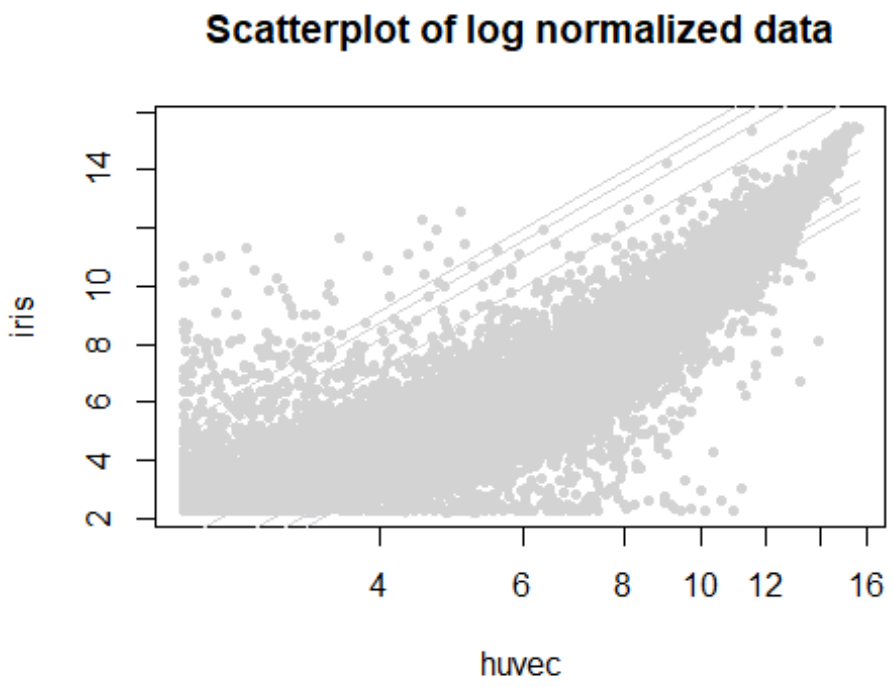


```
plot(x=huvec ,y=choroid,xlab="huvec",ylab="choroid", main="Scatterplot of log
raw data",log=c('x','y'))
```

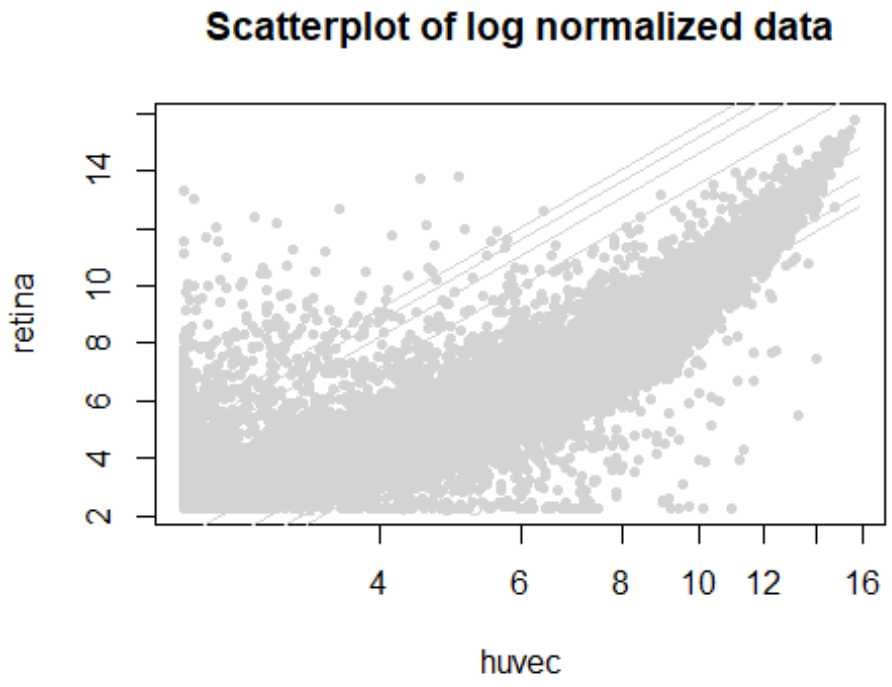


Here is log-scaled graph tirh normalized data

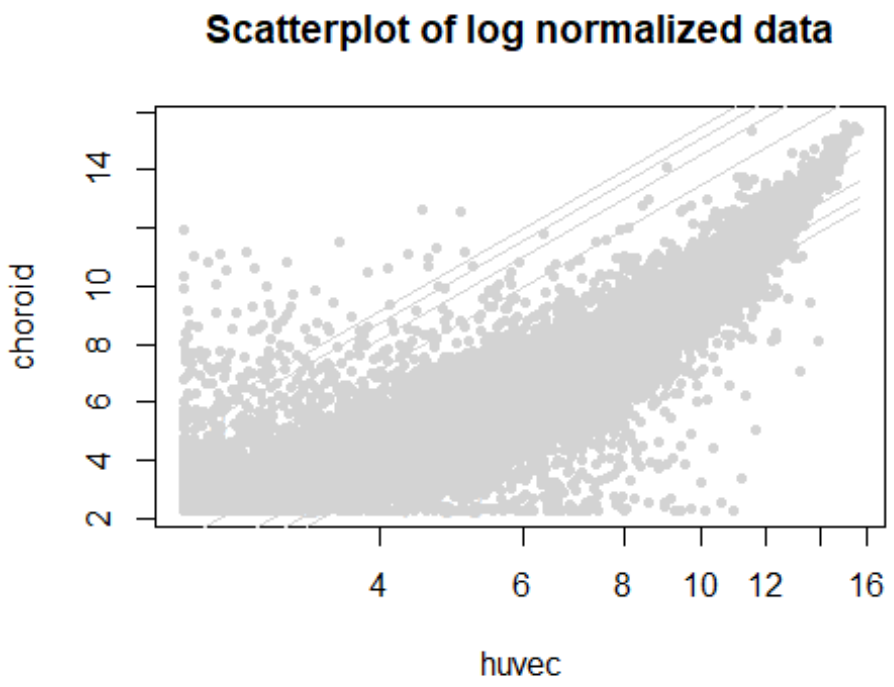
```
huvec_iris <- pairwise.comparison(celfiles.gcrma,"Targets",c("huvec","iris"))
huvec_retina <-
pairwise.comparison(celfiles.gcrma,"Targets",c("huvec","retina"))
huvec_choroid <-
pairwise.comparison(celfiles.gcrma,"Targets",c("huvec","choroid"))
plot(huvec_iris,log=c('x','y'), main="Scatterplot of log normalized data")
```




```r
plot(huvec_retina,log=c('x','y'), main="Scatterplot of log normalized data")
```

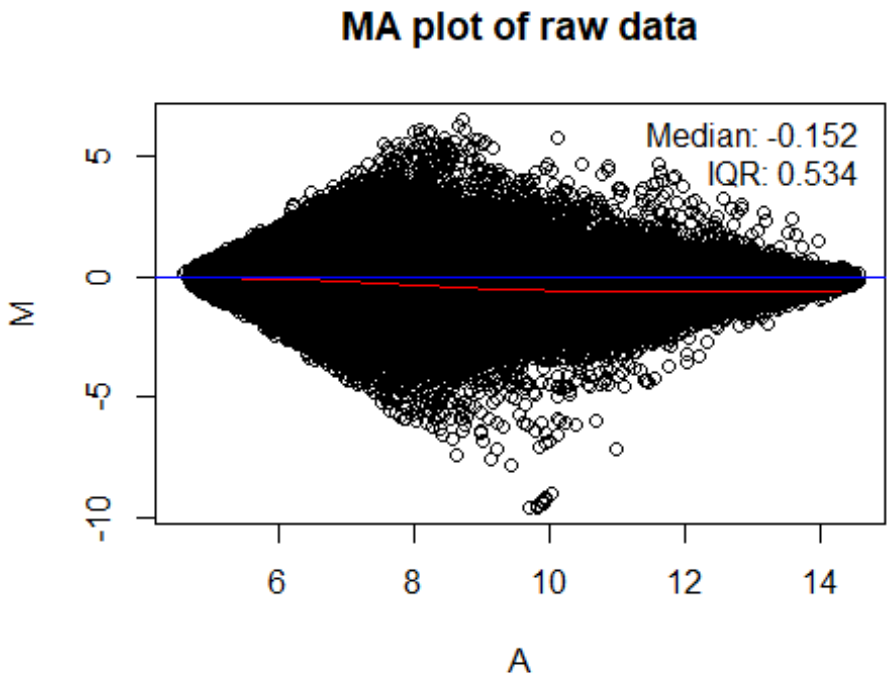



```r
plot(huvec_choroid,log=c('x','y'), main="Scatterplot of log normalized data")
```

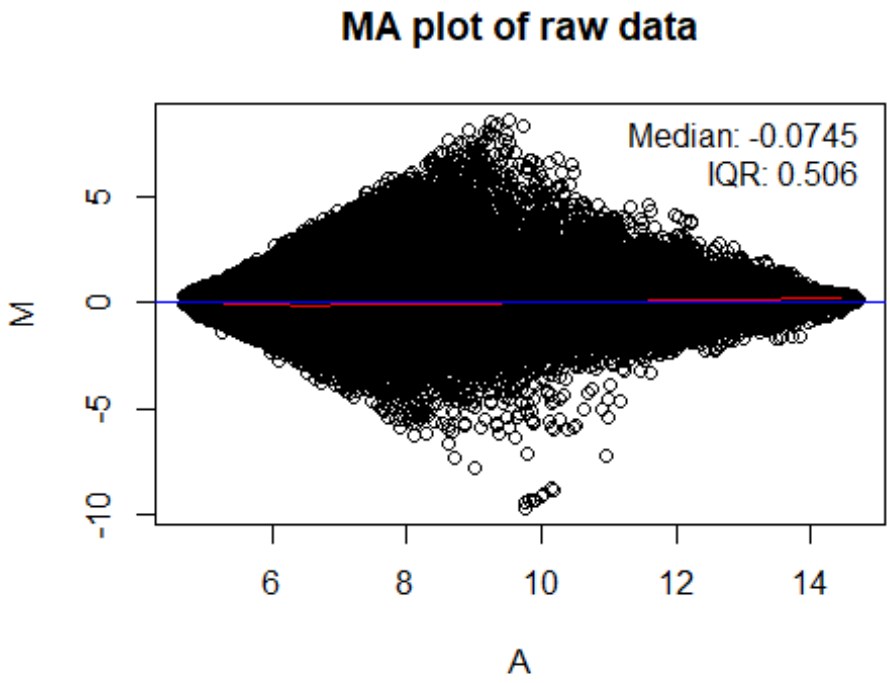


Here is MA plot

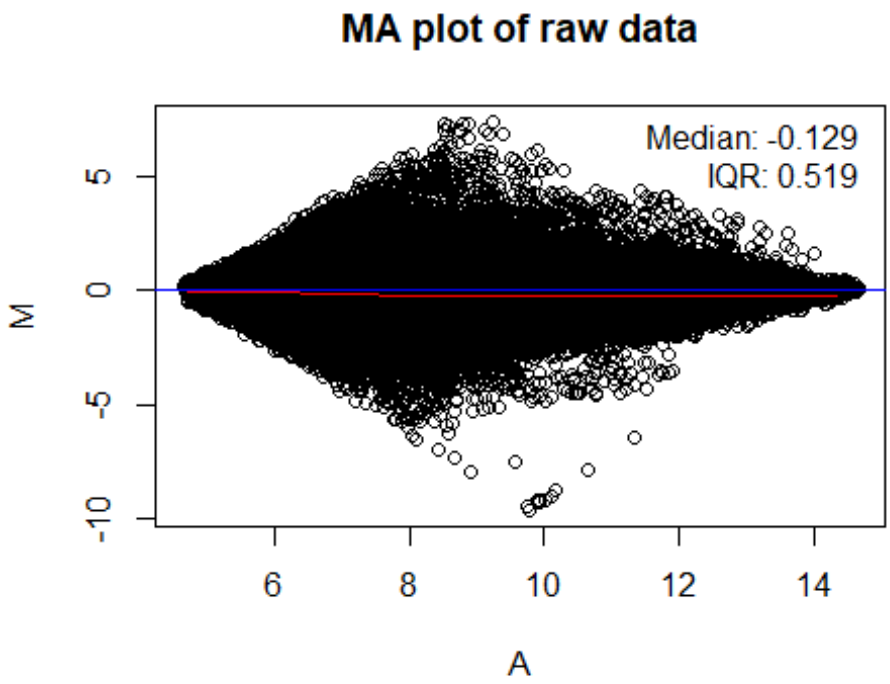
```
iris_huvec <- eset[,c(1,10)]
retina_huvec <- eset[,c(2,10)]
chronoid_huvec <- eset[,c(7,10)]
library(affy)
#inspired by wiki

ma.plot( rowMeans(log2(iris_huvec)), log2(iris_huvec[, 1])-log2(iris_huvec[,
2]), cex=1 , main="MA plot of raw data")
```




```
ma.plot( rowMeans(log2(retina_huvec)), log2(retina_huvec[, 1])-
log2(retina_huvec[, 2]), cex=1, main="MA plot of raw data" )
```

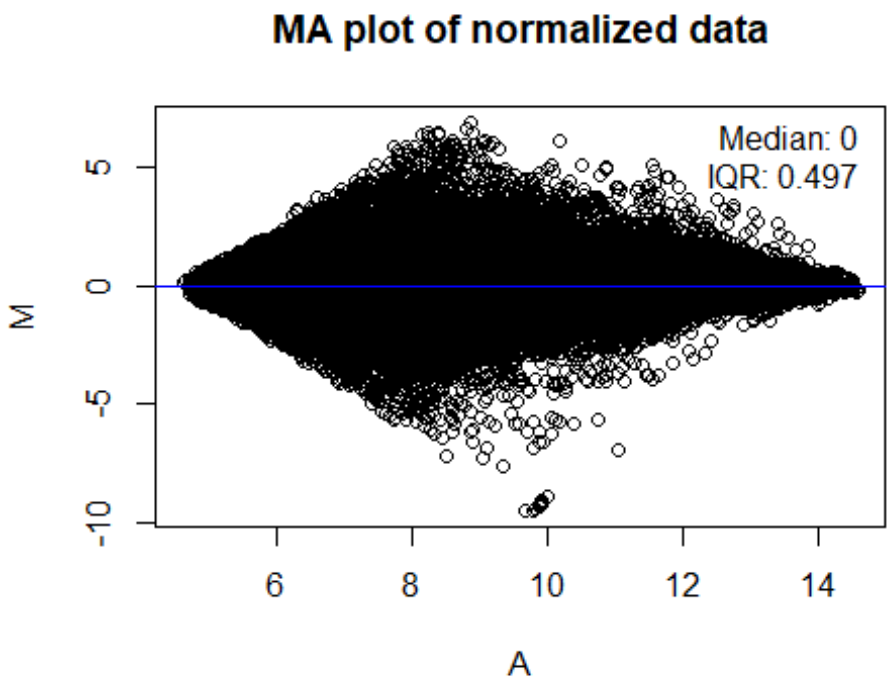


```
ma.plot( rowMeans(log2(chronoid_huvec)), log2(chronoid_huvec[, 1])-
log2(chronoid_huvec[, 2]), cex=1, main="MA plot of raw data" )
```

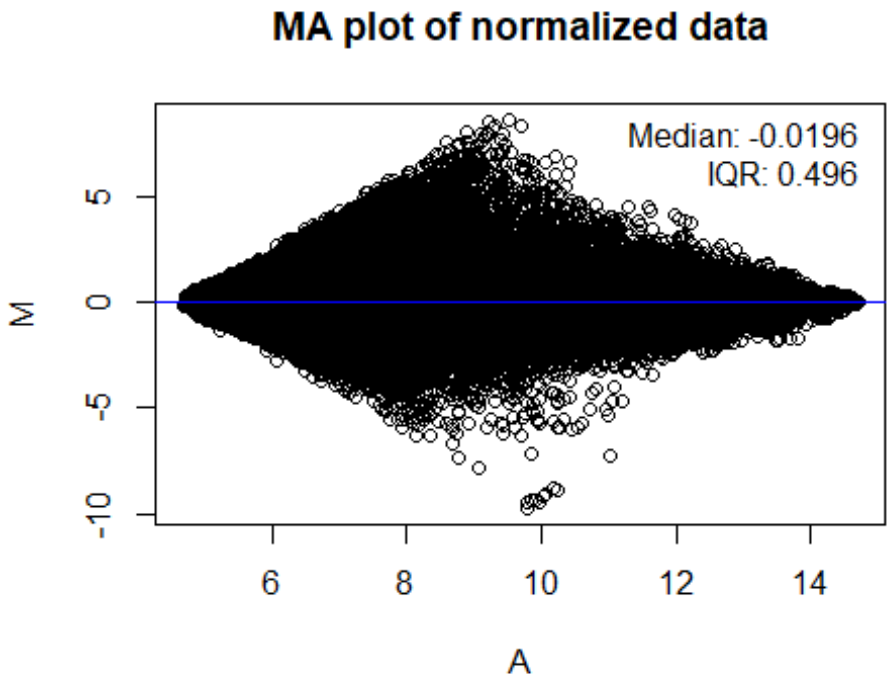


And MA plot with normalized data

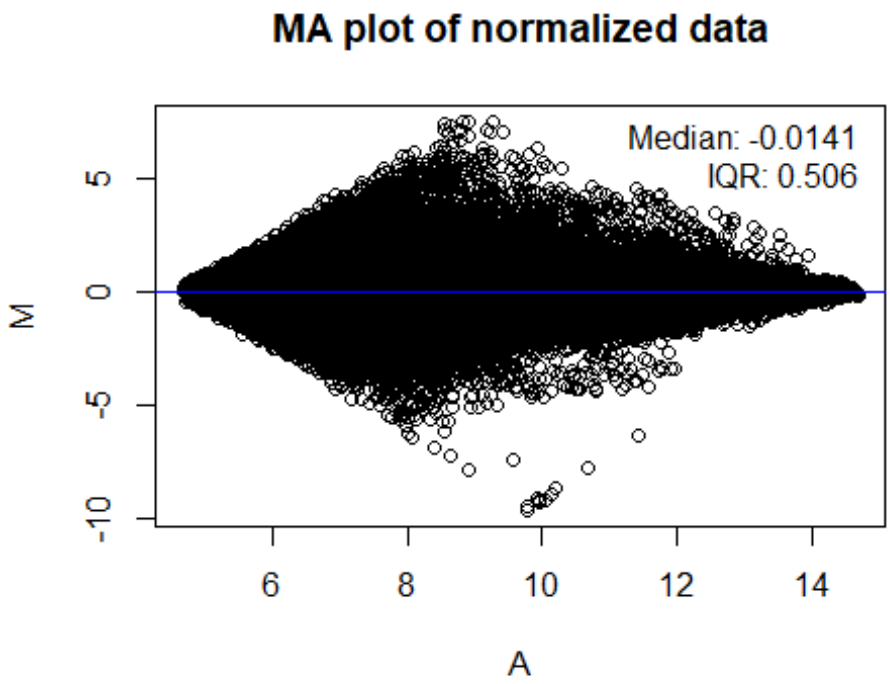
```
library(preprocessCore)

#do a quantile normalization
norm_iris_huvec <- normalize.quantiles(iris_huvec)
norm_retina_huvec <- normalize.quantiles(retina_huvec)
norm_chronoid_huvec <- normalize.quantiles(chronoid_huvec)



##normalized
ma.plot( rowMeans(log2(norm_iris_huvec)), log2(norm_iris_huvec[, 1])-
log2(norm_iris_huvec[, 2]), cex=1, main="MA plot of normalized data" )
```




```
ma.plot( rowMeans(log2(norm_retina_huvec)), log2(norm_retina_huvec[, 1])-
log2(norm_retina_huvec[, 2]), cex=1 , main="MA plot of normalized data")
```

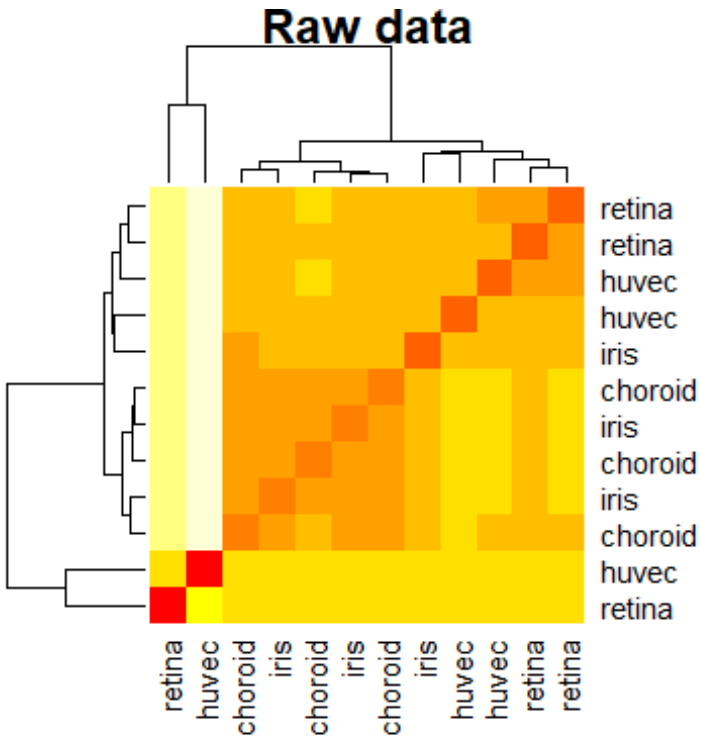


```
ma.plot( rowMeans(log2(norm_chronoid_huvec)), log2(norm_chronoid_huvec[, 1])-
log2(norm_chronoid_huvec[, 2]), cex=1 , main="MA plot of normalized data")
```

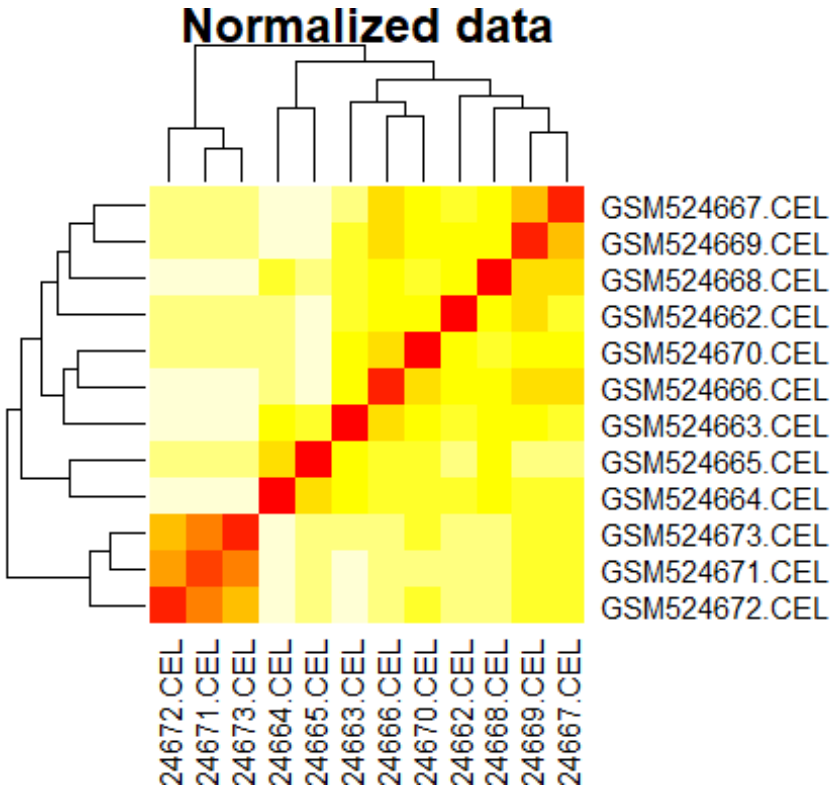


And here is the heat map:

```
par(mfrow=c(1,2))
heatmap(as.matrix(distance), main = "Raw data")
```




```r
heatmap(as.matrix(distance2), main = "Normalized data")
```



Question 3

Volcano plots:

```
results <- topTable(huvec_ebay, number = 100000, coef = "huvec_retina")
results <- cbind(results, getsymbols)
summary(results)
```

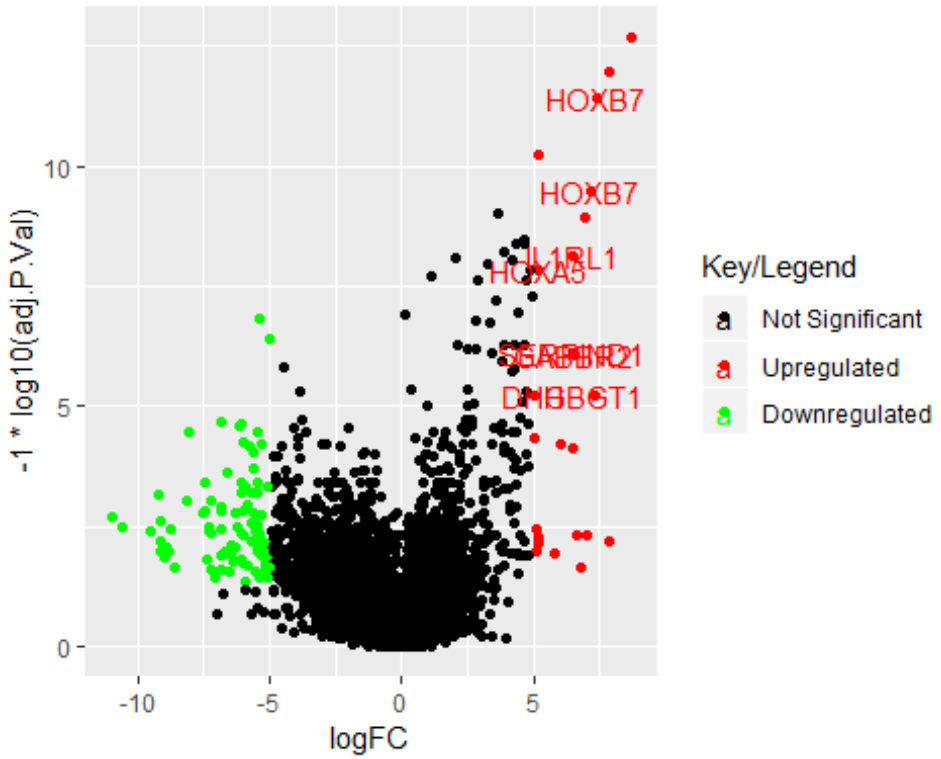
```
##      logFC              AveExpr             t                 P.Value
##  Min.   :-10.97621   Min.   : 2.279   Min.   :-40.23456   Min.   :0.0000
##  1st Qu.: -0.05050   1st Qu.: 2.281   1st Qu.: -0.60778   1st Qu.:0.1564
##  Median :  0.00000   Median : 2.480   Median :  0.00000   Median :0.5238
##  Mean   : -0.03205   Mean   : 4.375   Mean   :  0.08621   Mean   :0.5414
##  3rd Qu.:  0.04602   3rd Qu.: 6.241   3rd Qu.:  0.72396   3rd Qu.:1.0000
##  Max.   :  8.67086   Max.   :15.541   Max.   :296.84201   Max.   :1.0000
##
##    adj.P.Val            B             getsymbols
##  Min.   :0.0000   Min.   :-7.710   YME1L1  :   22
##  1st Qu.:0.6128   1st Qu.:-7.710   HFE     :   15
##  Median :1.0000   Median :-7.469   CFLAR   :   14
##  Mean   :0.7525   Mean   :-6.614   NRP2    :   14
##  3rd Qu.:1.0000   3rd Qu.:-6.522   ARHGEF12:   13
##  Max.   :1.0000   Max.   :21.290   (Other) :41857
##                                    NA's    :12740
```

```
results$threshold <- "1"
a <- subset(results, adj.P.Val < 0.05 & logFC > 5)
results[rownames(a), "threshold"] <- "2"
b <- subset(results, adj.P.Val < 0.05 & logFC < -5)
results[rownames(b), "threshold"] <- "3"
table(results$threshold)
```

```
##
##     1     2     3
## 54557    24    94
```

```
library(ggplot2)
volcano <- ggplot(data = results,
                  aes(x = logFC, y = -1*log10(adj.P.Val),
                      colour = threshold,
                      label = getsymbols))

volcano <- volcano +
  geom_point() +
  scale_color_manual(values = c("black", "red", "green"),
                     labels = c("Not Significant", "Upregulated",
"Downregulated"),
                     name = "Key/Legend")

volcano +
  geom_text(data = subset(results, logFC > 5 & -1*log10(adj.P.Val) > 5),
```

```
aes(x = logFC, y = -1*log10(adj.P.Val), colour = threshold, label =
getsymbols)  )
```

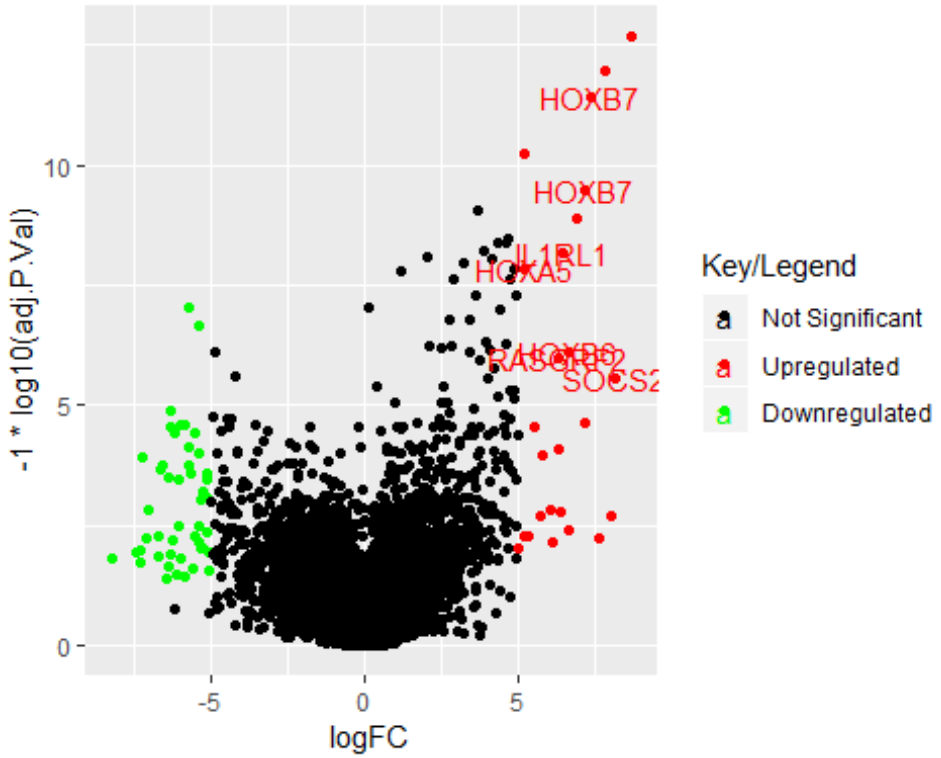



```
results <- topTable(huvec_ebay, number = 100000, coef = "huvec_iris")
results <- cbind(results, getsymbols)
summary(results)
```

```
##      logFC              AveExpr              t                 P.Value
##  Min.   :-8.26243   Min.   : 2.279   Min.   :-42.52934   Min.   :0.0000
##  1st Qu.:-0.08709   1st Qu.: 2.281   1st Qu.: -1.14547   1st Qu.:0.1252
##  Median : 0.00000   Median : 2.480   Median :  0.00000   Median :0.3678
##  Mean   :-0.02251   Mean   : 4.375   Mean   : -0.00841   Mean   :0.4888
##  3rd Qu.: 0.03905   3rd Qu.: 6.241   3rd Qu.:  0.66164   3rd Qu.:1.0000
##  Max.   : 8.67086   Max.   :15.541   Max.   :296.84201   Max.   :1.0000
##
##    adj.P.Val            B              getsymbols
##  Min.   :0.0000   Min.   :-7.710   YME1L1  :   22
##  1st Qu.:0.5008   1st Qu.:-7.710   HFE     :   15
##  Median :0.7355   Median :-7.230   CFLAR   :   14
##  Mean   :0.6798   Mean   :-6.440   NRP2    :   14
##  3rd Qu.:1.0000   3rd Qu.:-6.319   ARHGEF12:   13
##  Max.   :1.0000   Max.   :21.290   (Other) :41857
##                                    NA's    :12740
```

```
results$threshold <- "1"
a <- subset(results, adj.P.Val < 0.05 & logFC > 5)
results[rownames(a), "threshold"] <- "2"
```

```r
b <- subset(results, adj.P.Val < 0.05 & logFC < -5)
results[rownames(b), "threshold"] <- "3"
table(results$threshold)

##
##     1     2     3
## 54601    25    49

library(ggplot2)
volcano <- ggplot(data = results,
                  aes(x = logFC, y = -1*log10(adj.P.Val),
                      colour = threshold,
                      label = getsymbols))

volcano <- volcano +
  geom_point() +
  scale_color_manual(values = c("black", "red", "green"),
                     labels = c("Not Significant", "Upregulated",
"Downregulated"),
                     name = "Key/Legend")

volcano +
  geom_text(data = subset(results, logFC > 5 & -1*log10(adj.P.Val) > 5),
aes(x = logFC, y = -1*log10(adj.P.Val), colour = threshold, label =
getsymbols)  )
```



The most indicate signifcantly diferentially expressed genes in all vocano plots is “HOXB7” and “IL1RL1”

Question 4

HOXB7: This protein is a member of the Antp homeobox family. It encodes a protein with a homeobox DNA-binding domain. It is included in a cluster of homeobox B genes located on chromosome 17.

GO ID	Qualified GO term
GO:0000978	RNA polymerase II proximal promoter sequence-specific DNA binding
GO:0000981	RNA polymerase II transcription factor activity, sequence-specific
GO:0001077	transcriptional activator activity, RNA polymerase II proximal promoter sequence-specific DNA binding
GO:0003677	DNA binding
GO:0003700	DNA binding transcription factor activity

IL1RL1 A a member of the interleukin 1 receptor family. This receptor can be induced by proinflammatory stimuli, and may be involved in the function of helper T cells This gene is in a cluster in a region mapped to chromosome 2q12.

GO ID	Qualified GO term
GO:0002113	interleukin-33 binding
GO:0002114	interleukin-33 receptor activity
GO:0004896	cytokine receptor activity

GO:0004908 interleukin-1 receptor activity
GO:0005057 obsolete signal transducer activity, downstream of receptor