Microarray Data Analysis

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Load Packages

```
suppressPackageStartupMessages({
    library(here)
    library(oligo)
})
```

Load the dataset

```
dataset <- read.celfiles(list.files(here("data/microarray"), full.names = TRUE))
```

```
## Platform design info loaded.
```

```
## Reading in : /home/jialin/Courses/code6150/data/microarray/GSM651310.CEL.gz
## Reading in : /home/jialin/Courses/code6150/data/microarray/GSM651315.CEL.gz
## Reading in : /home/jialin/Courses/code6150/data/microarray/GSM651320.CEL.gz
## Reading in : /home/jialin/Courses/code6150/data/microarray/GSM651325.CEL.gz
```

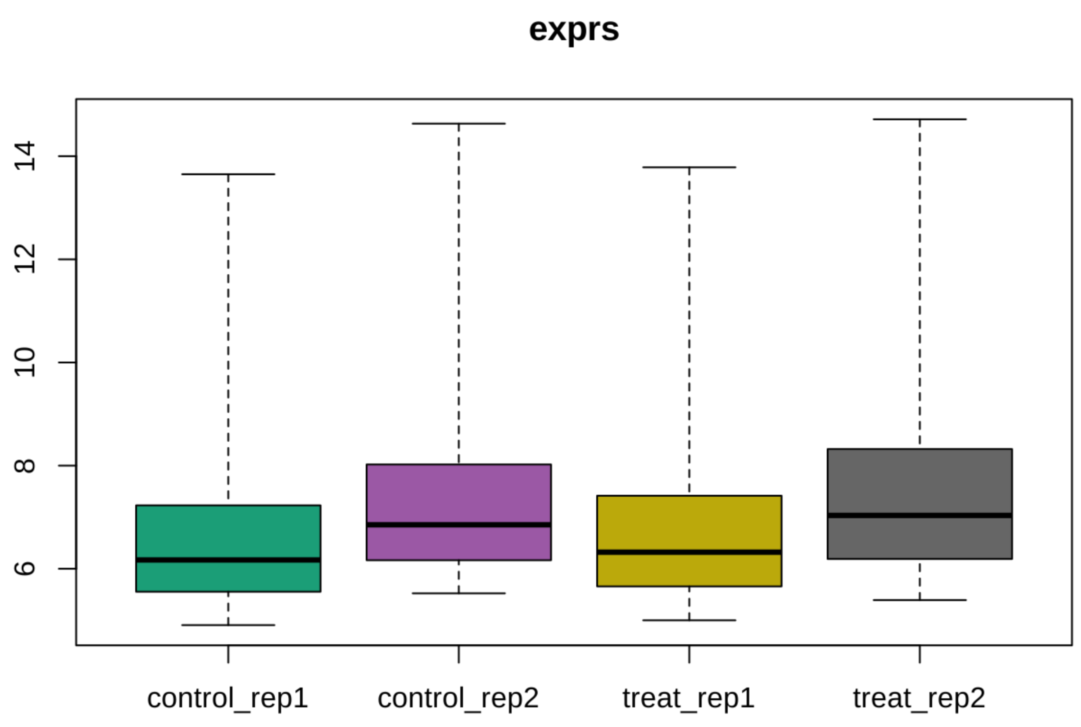
```
dataset <- dataset[, c(paste0(c("GSM651310", "GSM651320", "GSM651315", "GSM651325"),
".CEL.gz"))]
sampleNames(dataset) <- c("control_rep1", "control_rep2", "treat_rep1", "treat_rep2")
pData(dataset)$group <- c("control", "control", "treat", "treat")
dataset
```

```
## ExpressionFeatureSet (storageMode: lockedEnvironment)
## assayData: 409600 features, 4 samples
##   element names: exprs
## protocolData
##   rowNames: control_rep1 control_rep2 treat_rep1 treat_rep2
##   varLabels: exprs dates
##   varMetadata: labelDescription channel
## phenoData
##   rowNames: control_rep1 control_rep2 treat_rep1 treat_rep2
##   varLabels: index group
##   varMetadata: labelDescription channel
## featureData: none
## experimentData: use 'experimentData(object)'
## Annotation: pd.hg.u95av2
```

Quality control

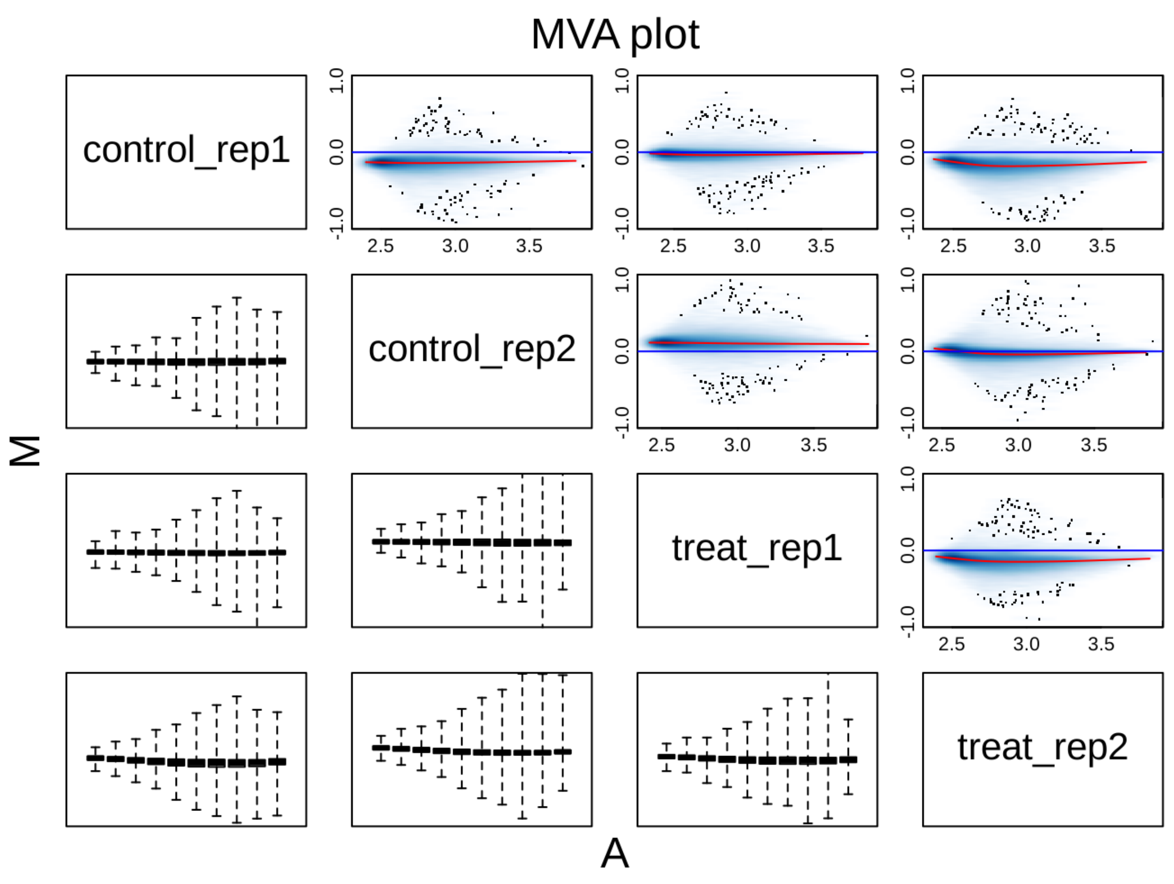
Boxplot

```
boxplot(dataset, target = "core")
```



MA plot

```
oligo::MAplot(dataset, pairs = TRUE, ylim = c(-1, 1))
```



Quality report of the raw data

The following will generate a quality report of the raw microarray data in `docs/microarray_qualitymetrics` directory.

```
library(arrayQualityMetrics)
arrayQualityMetrics(expressionset = dataset,
    outdir = "docs/microarray_qualitymetrics",
    force = TRUE, do.logtransform = TRUE,
    intgroup = c("group"))
```

RMA

The RMA method proceeds with background subtraction, normalization and summarization using a deconvolution method for background correction, quantile normalization and the RMA (robust multichip average) algorithm for summarization.

```
edata <- oligo::rma(dataset)
```

```
## Background correcting
## Normalizing
## Calculating Expression
```

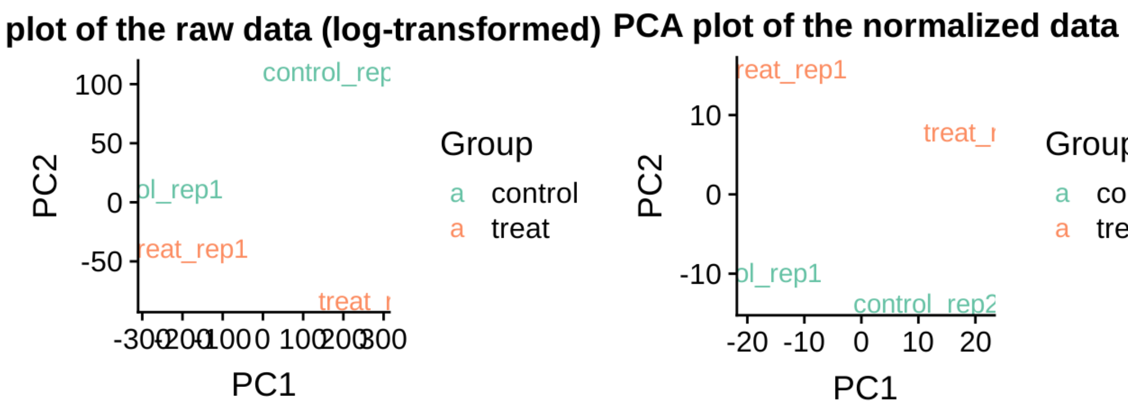
```
edata
```

```
## ExpressionSet (storageMode: lockedEnvironment)
## assayData: 12625 features, 4 samples
##   element names: exprs
## protocolData
##   rowNames: control_rep1 control_rep2 treat_rep1 treat_rep2
##   varLabels: exprs dates
##   varMetadata: labelDescription channel
## phenoData
##   rowNames: control_rep1 control_rep2 treat_rep1 treat_rep2
##   varLabels: index group
##   varMetadata: labelDescription channel
## featureData: none
## experimentData: use 'experimentData(object)'
## Annotation: pd.hg.u95av2
```

Access the quality after normalization

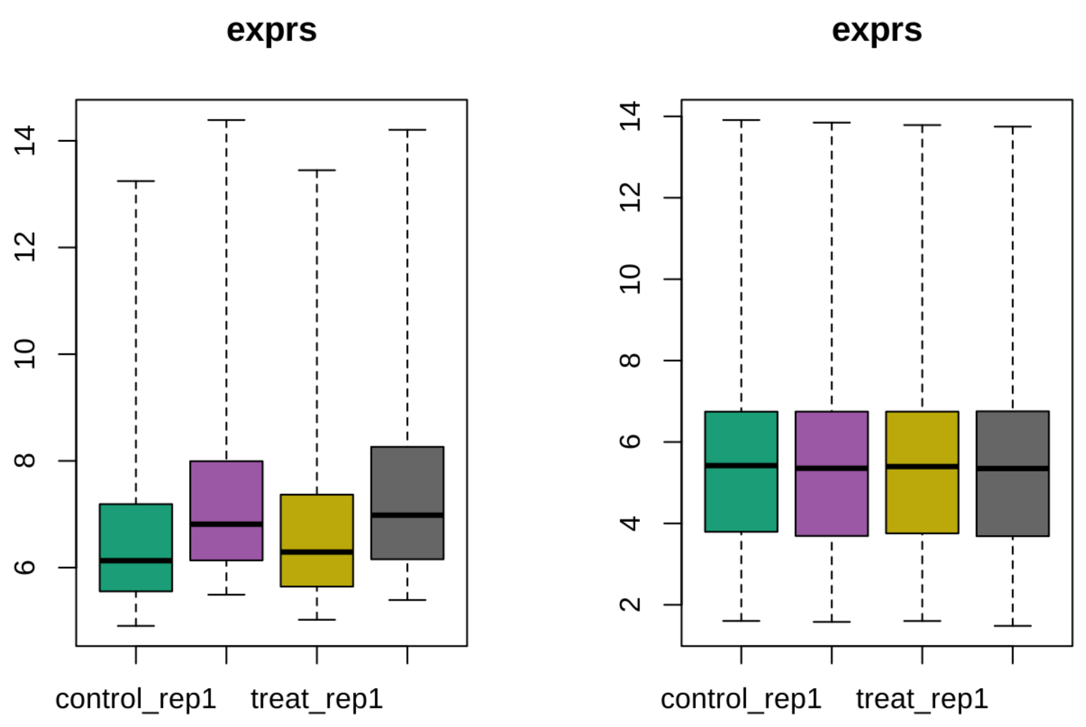
PCA plot before and after normalization

```
library(ggplot2)
library(cowplot)
plot_grid(
    local({
        PCA_raw <- prcomp(t(log2(exprs(dataset))), scale = FALSE)

        dataGG <- data.frame(PC1 = PCA_raw$x[,1], PC2 = PCA_raw$x[,2],
                         Group = pData(dataset)$group)

        qplot(PC1, PC2, data = dataGG, color = Group,
              main = "PCA plot of the raw data (log-transformed)", asp = 1.0, geom =
"text",
        label = sampleNames(dataset)) + scale_colour_brewer(palette = "Set2")
    }),
    local({
        PCA <- prcomp(t(exprs(edata)), scale = FALSE)
        dataGG <- data.frame(PC1 = PCA$x[,1], PC2 = PCA$x[,2],
                  Group = pData(dataset)$group)
        qplot(PC1, PC2, data = dataGG, color =  Group,
            main = "PCA plot of the normalized data", asp = 1.0, geom = "text",
            label = sampleNames(edata)) +
            scale_colour_brewer(palette = "Set2")
    })
)
```



Boxplot before and after normalization

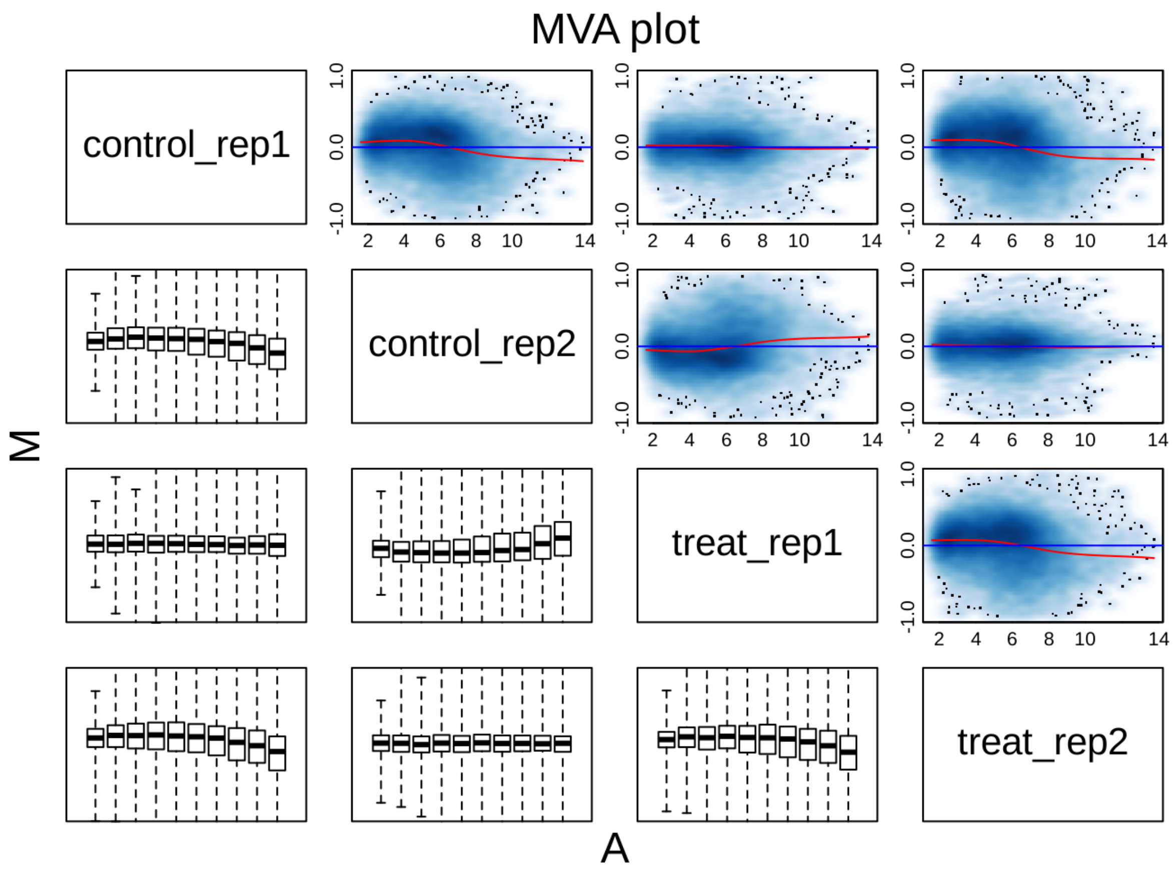
```
par(mfrow = c(1,2))
boxplot(dataset)
boxplot(edata)
```



```
par(mfrow = c(1,1))
```

MA plot after normalization

```
oligo::MAplot(edata, pairs = TRUE, ylim = c(-1, 1))
```



Heatmap with sample-to-sample distance after normalization

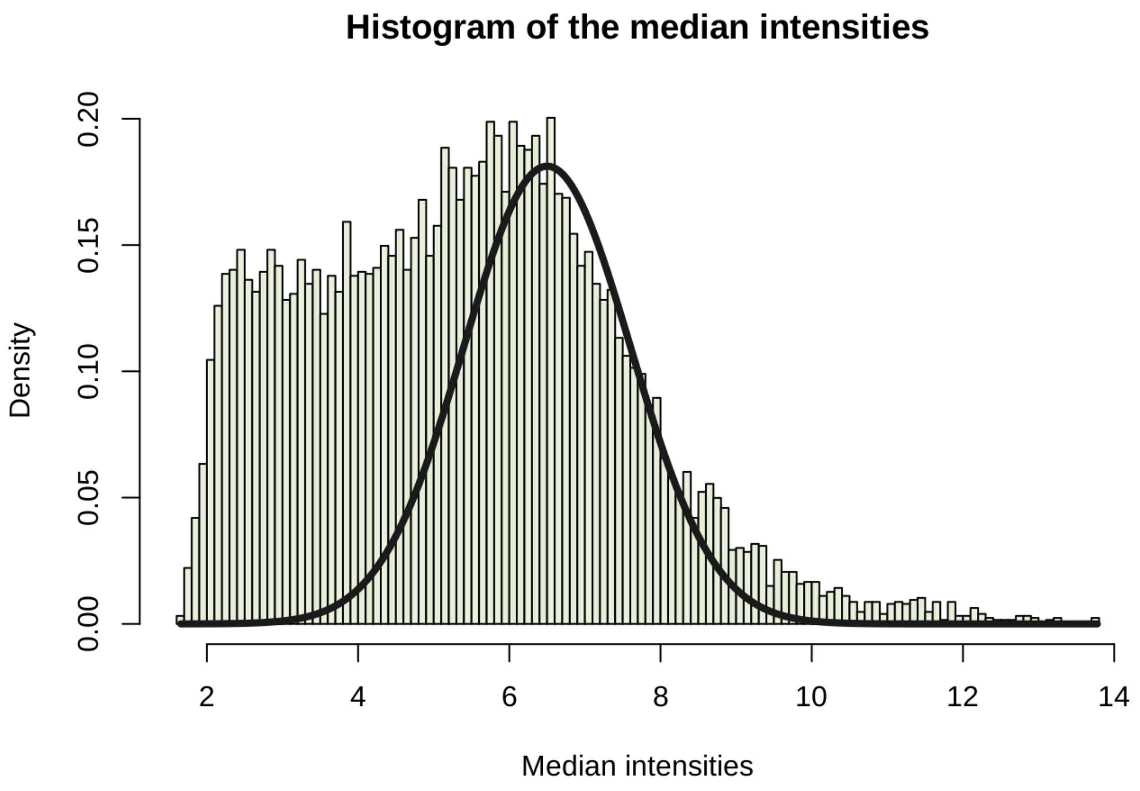
It can not provide too much information for us since the number of samples is limited.

```
library(RColorBrewer)
library(pheatmap)
dists <- as.matrix(dist(t(exprs(edata)), method = "manhattan"))
diag(dists) <- NA
hmcol <- colorRampPalette(rev(brewer.pal(9, "PuOr")))(255)
pheatmap(dists, col = rev(hmcol), clustering_distance_rows = "manhattan",
                    clustering_distance_cols = "manhattan")
```

Filter based on intensity

Microarray data commonly show a large number of probes in the background intensity range. They also do not change much across arrays. Hence they combine a low variance with a low intensity. We want to filter these results as they may contribute to false positive results in the differential expression analysis.

```
edata_medians <- rowMedians(exprs(edata))
hist_res <- hist(edata_medians, 100, col="#e7efd8", freq = FALSE,
                 main = "Histogram of the median intensities",
                 xlab = "Median intensities")
emp_mu <- hist_res$breaks[which.max(hist_res$density)]
emp_sd <- mad(edata_medians)/2
prop_cental <- 0.50
lines(sort(edata_medians),
      prop_cental*dnorm(sort(edata_medians), mean = emp_mu, sd = emp_sd),
    col = "grey10", lwd = 4)
```



```
cut_val <- 0.05 / prop_cental
thresh_median <- qnorm(0.05 / prop_cental, emp_mu, emp_sd)
samples_cutoff <- 2
idx_thresh_median <- apply(exprs(edata), 1, function(x){
                   sum(x > thresh_median) >= samples_cutoff})
table(idx_thresh_median)
```

```
## idx_thresh_median
## FALSE  TRUE
##  5510  7115
```

```
edata <- subset(edata, idx_thresh_median)
```

Identification of differentially expressed genes

Create a simple design matrix.

```
library(limma)
f <- factor(c("control", "control", "treat", "treat"))
design <- model.matrix(~ 0 + f)
colnames(design)
```

```
## [1] "fcontrol" "ftreat"
```

```
colnames(design) <- c("control", "treat")
design
```

```
##   control treat
## 1       1     0
## 2       1     0
## 3       0     1
## 4       0     1
## attr(,"assign")
## [1] 1 1
## attr(,"contrasts")
## attr(,"contrasts")$f
## [1] "contr.treatment"
```

We can fit the linear model, define appropriate contrast to test the hypothesis on treatment effect and compute the moderated t–statistics by calling the `eBayes` function.

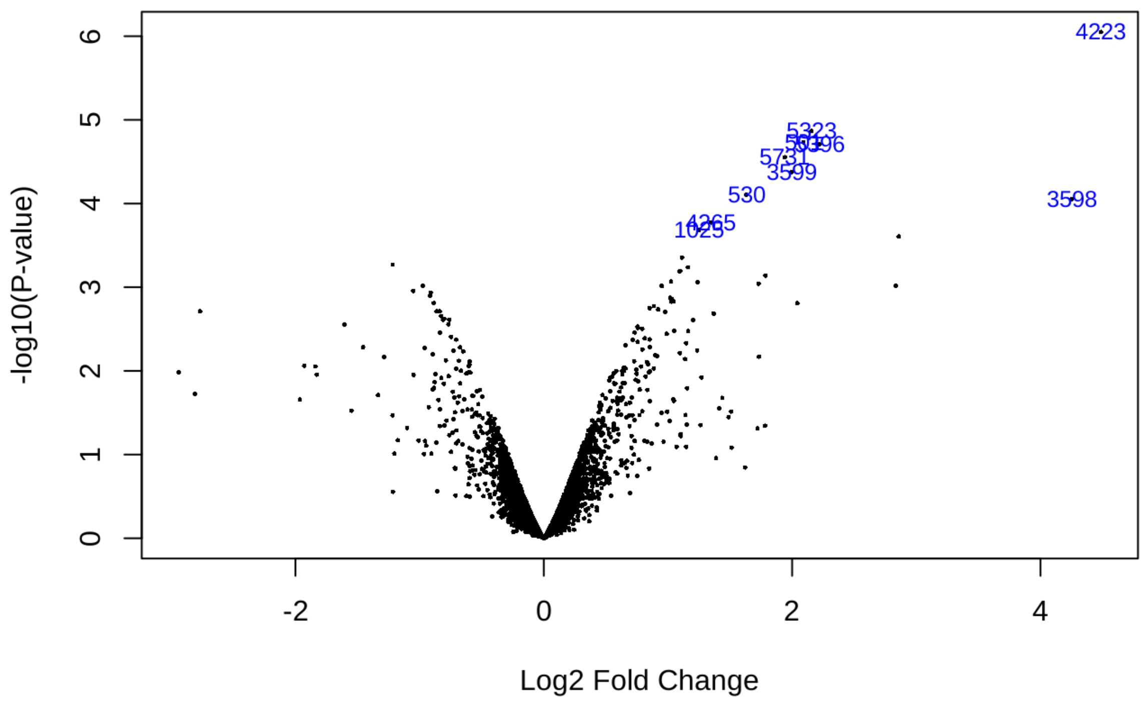
```
data.fit <- lmFit(exprs(edata), design)
head(data.fit$coefficients)
```

```
##             control    treat
## 100_g_at  7.668756 7.715222
## 1000_at   7.500988 7.509880
## 1003_s_at 6.080299 5.955531
## 1004_at   5.637423 5.510949
## 1005_at   7.805104 9.312773
## 1006_at   7.453394 6.403360
```

```
contrast.matrix <- makeContrasts(treat-control,levels=design)
data.fit.con <- contrasts.fit(data.fit,contrast.matrix)
data.fit.eb <- eBayes(data.fit.con)
```

Volcano plot to show the distribution of fold change and p value.

```
volcanoplot(data.fit.eb,highlight=10)
```



Then we sort the results by their absolute t-statistics.

```
top <- topTable(data.fit.eb, number = Inf)
head(top)
```

```
##              logFC  AveExpr        t      P.Value  adj.P.Val        B
## 37701_at 4.487768 7.288447 23.90482 8.923836e-07 0.00634931 3.279131
## 39528_at 2.155203 7.214587 14.46714 1.363637e-05 0.03491603 2.484241
## 1776_at  2.090149 7.318168 13.66205 1.855341e-05 0.03491603 2.354451
## 39681_at 2.219663 5.720843 13.51932 1.962953e-05 0.03491603 2.329679
## 40202_at 1.941558 6.657501 12.65317 2.798882e-05 0.03982809 2.166516
## 36630_at 1.995351 7.579214 11.72145 4.209550e-05 0.04991825 1.962962
```

Check how many results can we get if we use a p value cutoff by 0.001 or an adjusted p value cutoff by 0.05.

```
table(top$adj.P.Val < 0.05)
```

```
##
## FALSE  TRUE
##  7109     6
```

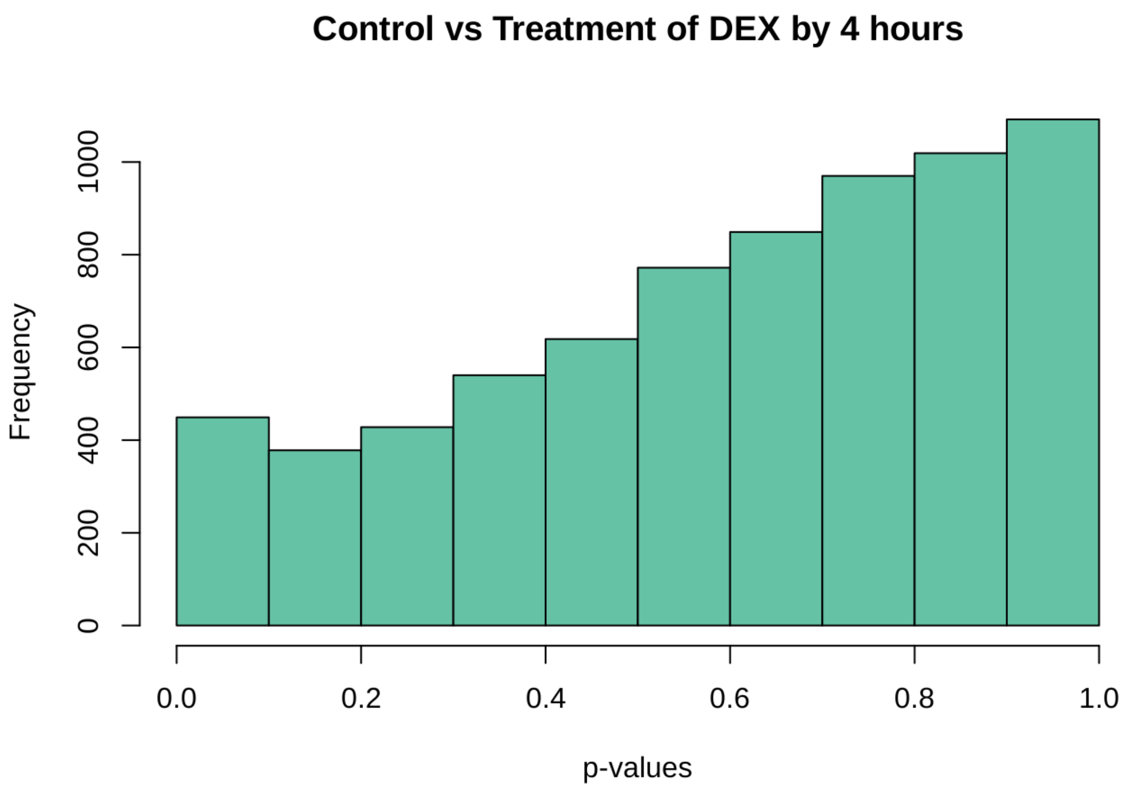
```
table(top$P.Value < 0.001)
```

```
##
## FALSE  TRUE
##  7092    23
```

We notice that the number of differential expressed genes are limited, which indicates the the effect of 4 hour DEX treatment may not be significant in altering the gene expression.

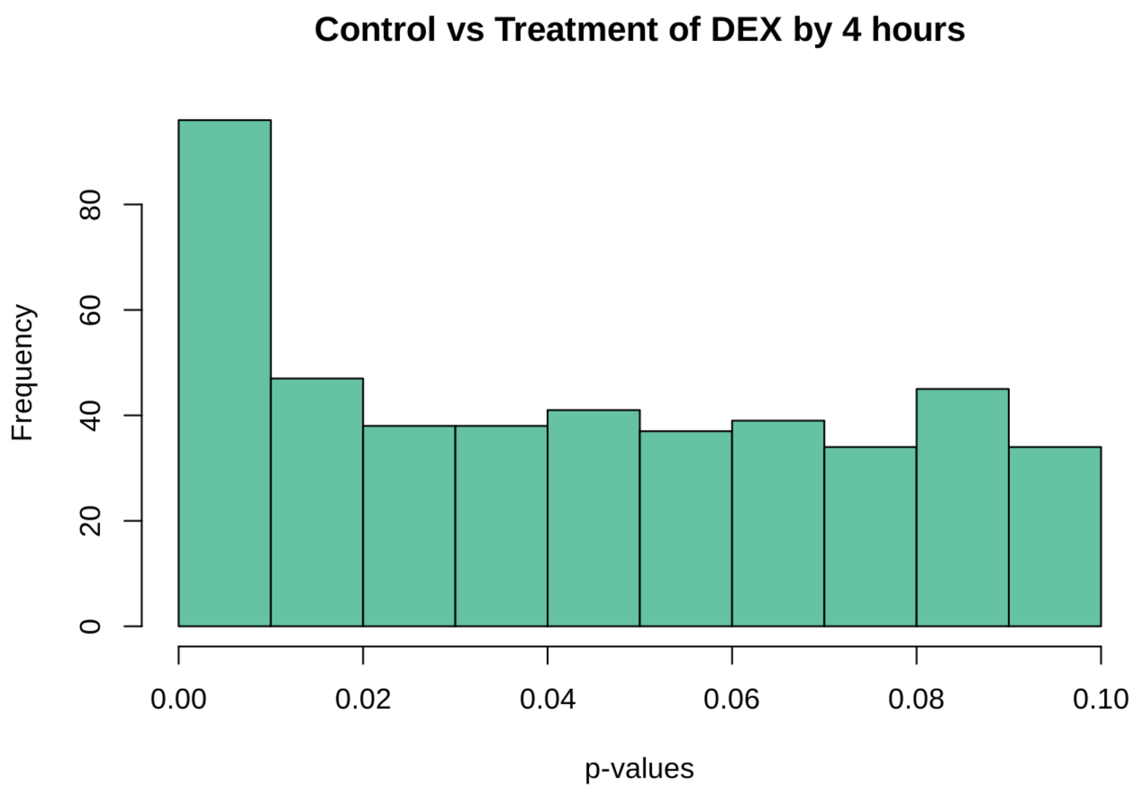
Then plot a histogram of p value distribution.

```
hist(top$P.Value, col = brewer.pal(3, name = "Set2")[1],
      main = "Control vs Treatment of DEX by 4 hours", xlab = "p-values")
```



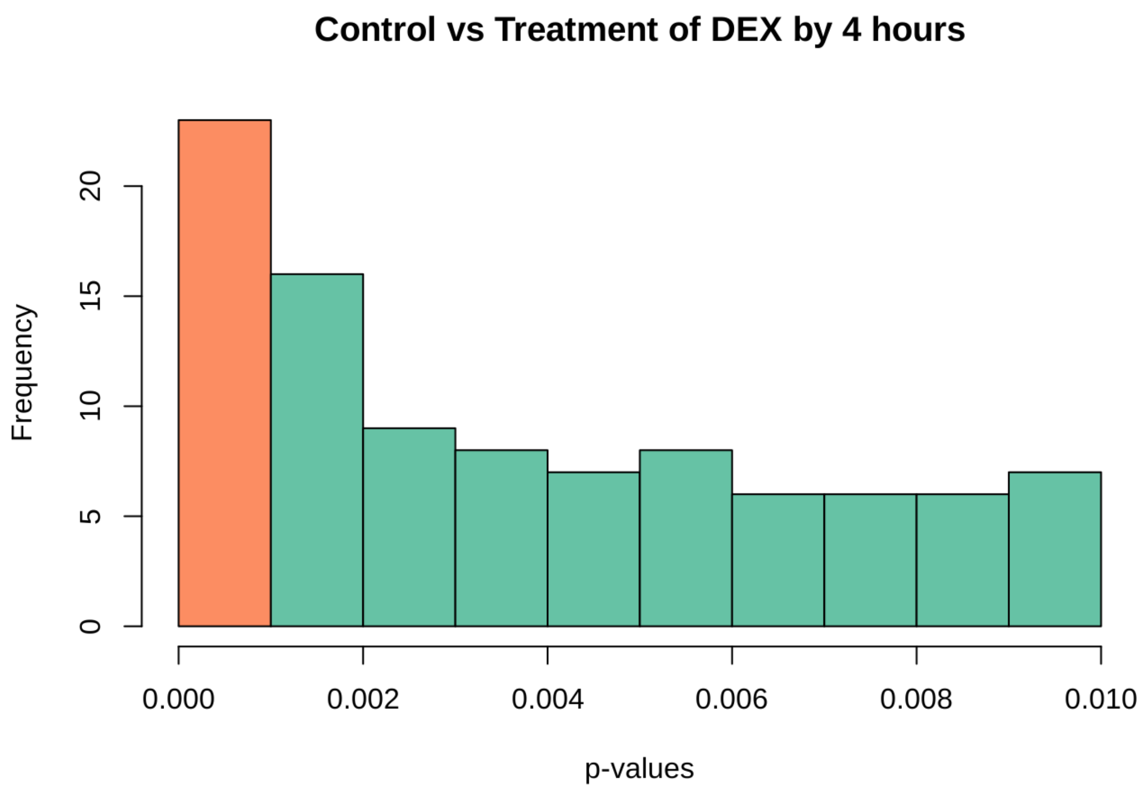
We zoom in the plot to see p value distribution between 0 and 0.1.

```
hist(top$P.Value[top$P.Value < 0.1], col = brewer.pal(3, name = "Set2")[1],
      main = "Control vs Treatment of DEX by 4 hours", xlab = "p-values")
```



Zoom in again and the orange bin indicates the differentially expressed genes if we use a p value cutoff by 0.001.

```
local({
    breaks <- 0:10/1000
    color1 <- brewer.pal(3, name = "Set2")[1]
    color2 <- brewer.pal(3, name = "Set2")[2]
    color <- rep.int(color1, length(breaks) - 1)
    color[1] <- color2
    data <- top$P.Value[top$P.Value < 0.01]
    hist(data, col = color,
         main = "Control vs Treatment of DEX by 4 hours", xlab = "p-values", breaks =
breaks)
})
```



Annotating genes

We need to annotate the gene names by the probe IDs.

```
suppressPackageStartupMessages({
    library(hgu95av2.db)
})
get_symbol <- function(probeid) {
    ans <- mapIds(hgu95av2.db::hgu95av2.db,
                  keys = probeid, keytype = "PROBEID", column = "SYMBOL", multiVals =
"first")
    unname(ans)
}
get_genename <- function(probeid) {
    ans <- mapIds(hgu95av2.db::hgu95av2.db,
                  keys = probeid, keytype = "PROBEID", column = "GENENAME", multiVals
= "first")
    unname(ans)
}
top$symbol <- get_symbol(rownames(top))
```

```
## 'select()' returned 1:many mapping between keys and columns
```

```
top$gene_name <- get_genename(rownames(top))
```

```
## 'select()' returned 1:many mapping between keys and columns
```

Generate a table of differentially expressed genes

We use a p value cutoff by 0.001.

```
top <- top[, c("symbol", "gene_name", "logFC", "P.Value", "adj.P.Val")]
top <- cbind(data.frame(probeID = rownames(top), stringsAsFactors = FALSE), top)
rownames(top) <- NULL
top <- top[top$P.Value < 0.001,]
knitr::kable(top)
```

probeID	symbol	gene_name	logFC	P.Value	adj.P.Val
37701_at	RGS2	regulator of G protein signaling 2	4.4877683	0.0000009	0.0063493
39528_at	RRAD	RRAD, Ras related glycolysis inhibitor and calcium channel regulator	2.1552028	0.0000136	0.0349160
1776_at	RRAD	RRAD, Ras related glycolysis inhibitor and calcium channel regulator	2.0901488	0.0000186	0.0349160
39681_at	ZBTB16	zinc finger and BTB domain containing 16	2.2196632	0.0000196	0.0349160
40202_at	KLF9	Kruppel like factor 9	1.9415580	0.0000280	0.0398281
36630_at	TSC22D3	TSC22 domain family member 3	1.9953514	0.0000421	0.0499183
1814_at	TGFBR2	transforming growth factor beta receptor 2	1.6309718	0.0000782	0.0785749
36629_at	TSC22D3	TSC22 domain family member 3	4.2511045	0.0000883	0.0785749
37760_at	BAIAP2	BAI1 associated protein 2	1.3443442	0.0001677	0.1325401
31850_at	GCLC	glutamate-cysteine ligase catalytic subunit	1.2492825	0.0002082	0.1481454
34721_at	FKBP5	FK506 binding protein 5	2.8581334	0.0002476	0.1601256
38013_at	MTUS1	microtubule associated scaffold protein 1	1.1129654	0.0004426	0.2624371
36100_at	VEGFA	vascular endothelial growth factor A	-1.2173336	0.0005370	0.2872803
33113_at	CITED2	Cbp/p300 interacting transactivator with Glu/Asp rich carboxy-terminal domain 2	1.1614203	0.0005779	0.2872803
37225_at	KANK1	KN motif and ankyrin repeat domains 1	1.0982152	0.0006381	0.2872803
40855_at	SAMD4A	sterile alpha motif domain containing 4A	1.0942596	0.0006460	0.2872803
37430_at	ALOX15B	arachidonate 15-lipoxygenase, type B	1.7852935	0.0007265	0.2993091
634_at	PRSS8	serine protease 8	1.0246834	0.0008545	0.2993091
1052_s_at	CEBPD	CCAAT enhancer binding protein delta	1.2372588	0.0008687	0.2993091
33272_at	SAA1	serum amyloid A1	1.7293550	0.0009106	0.2993091
40522_at	GLUL	glutamate-ammonia ligase	2.8331430	0.0009608	0.2993091
33900_at	FSTL3	follistatin like 3	0.9502991	0.0009643	0.2993091
33131_at	SOX4	SRY-box 4	-0.9740144	0.0009675	0.2993091