Lab13

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```
counts <- read.csv("airway_scaledcounts.csv", row.names=1)
metadata <- read.csv("airway_metadata.csv")
```

```
head(counts)
```

```
                SRR1039508 SRR1039509 SRR1039512 SRR1039513 SRR1039516
ENSG00000000003        723        486        904        445       1170
ENSG00000000005          0          0          0          0          0
ENSG00000000419        467        523        616        371        582
ENSG00000000457        347        258        364        237        318
ENSG00000000460         96         81         73         66        118
ENSG00000000938          0          0          1          0          2
                SRR1039517 SRR1039520 SRR1039521
ENSG00000000003       1097        806        604
ENSG00000000005          0          0          0
ENSG00000000419        781        417        509
ENSG00000000457        447        330        324
ENSG00000000460         94        102         74
ENSG00000000938          0          0          0
```

```
head(metadata)
```

```
          id     dex celltype     geo_id
1 SRR1039508 control   N61311 GSM1275862
2 SRR1039509 treated   N61311 GSM1275863
3 SRR1039512 control  N052611 GSM1275866
4 SRR1039513 treated  N052611 GSM1275867
5 SRR1039516 control  N080611 GSM1275870
6 SRR1039517 treated  N080611 GSM1275871
```

Q1. How many genes are in this dataset?

```
nrow(counts)
```

```
[1] 38694
```

Q2. How many 'control' cell lines do we have?

```
table(metadata$dex)
```

```
control treated
      4       4
```

###check on match of metadata and coldata

```
colnames(counts)
```

```
[1] "SRR1039508" "SRR1039509" "SRR1039512" "SRR1039513" "SRR1039516"
[6] "SRR1039517" "SRR1039520" "SRR1039521"
```

```
metadata$id
```

```
[1] "SRR1039508" "SRR1039509" "SRR1039512" "SRR1039513" "SRR1039516"
[6] "SRR1039517" "SRR1039520" "SRR1039521"
```

```
metadata$id==colnames(counts)
```

```
[1] TRUE TRUE TRUE TRUE TRUE TRUE TRUE TRUE
```

if you want to know that all elements of a vector are TRUE we can use the 'all()' function

```
all(metadata$id==colnames(counts))
```

```
[1] TRUE
```

##Analysis

I want to start by comparing "control" and "treated" columns. to this will find find the average for each gene(row) in all "control" columns. then I will find the average in the treated columns. then I will compare them.

let's extract all "control" columns first

```
control.inds <- metadata$dex=="control"
```

```
control.counts <- counts[,control.inds]
```

Now find the mean count value per gene using the 'apply()' function.

```
control.mean <- apply(control.counts, 1,mean)
```

Now do the same for the treated columns. i.e. find `treated.mean values.

```
treated.inds <- metadata$dex=="treated"
```

```
treated.counts <- counts[,treated.inds]
```

```
treated.mean <- apply(treated.counts, 1, mean)
```

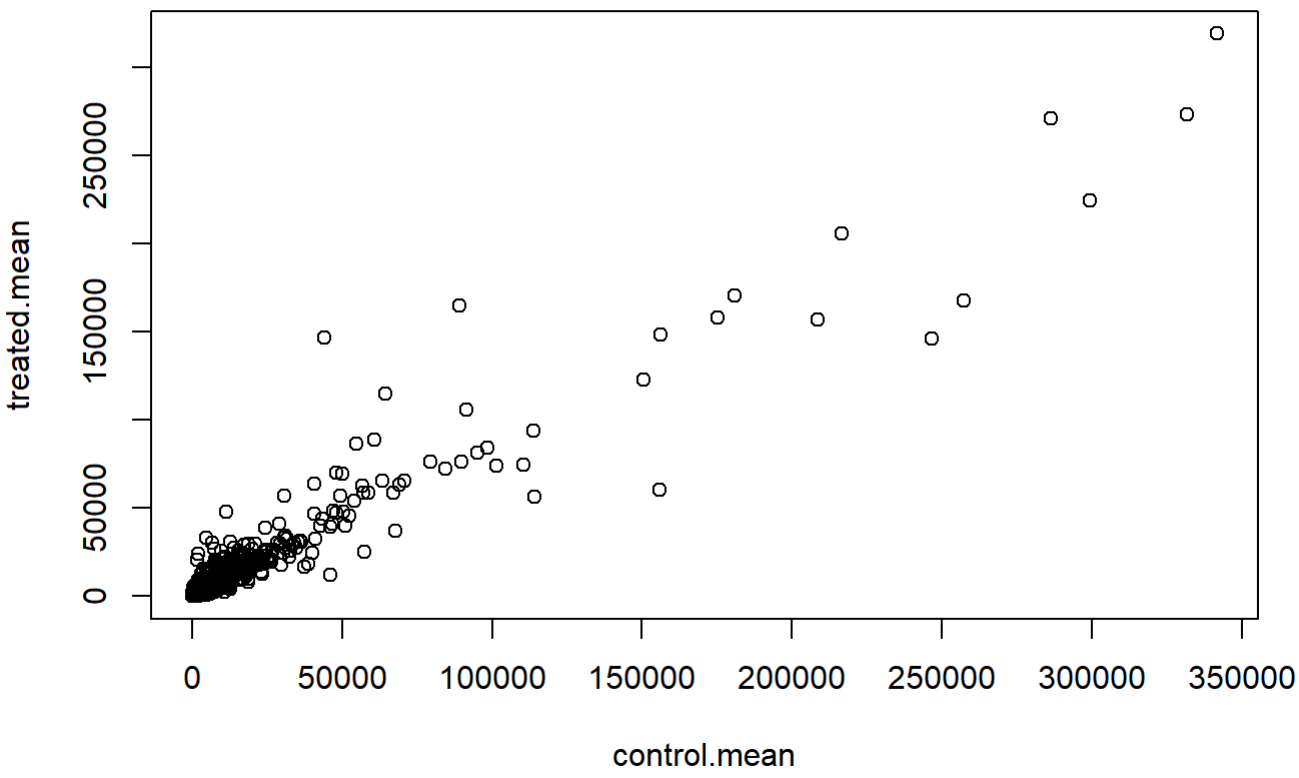
PUT THESE TWO MEAN VECTORS TOGETHER FOR EASE OF BOOK-KEEPING

```
meancounts <- data.frame(control.mean, treated.mean)
head(meancounts)
```

```
                control.mean treated.mean
ENSG00000000003       900.75       658.00
ENSG00000000005         0.00         0.00
ENSG00000000419       520.50       546.00
ENSG00000000457       339.75       316.50
ENSG00000000460        97.25        78.75
ENSG00000000938         0.75         0.00
```

Let's have a quick plot.

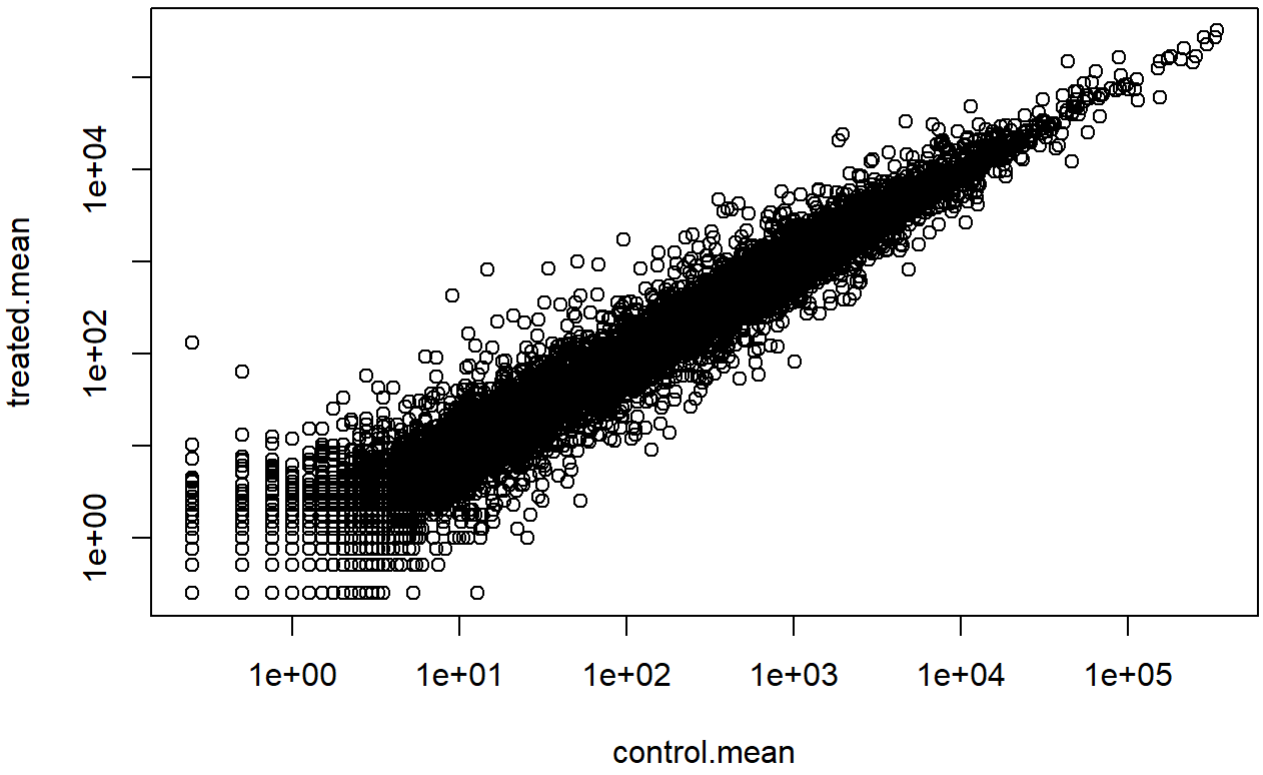
```
plot(meancounts)
```



```
plot(meancounts, log="xy")
```

```
Warning in xy.coords(x, y, xlabel, ylabel, log): 15032 x values <= 0 omitted
from logarithmic plot

Warning in xy.coords(x, y, xlabel, ylabel, log): 15281 y values <= 0 omitted
from logarithmic plot
```



we most often work in log2 units because they have a more simple interpretation

here we calculate the log2 fold-change of treated/control values and add it to our data frame of results

```
meancounts$log2fc <- log2(meancounts$treated.mean/meancounts$control.mean)
head(meancounts)
```

```
                control.mean treated.mean      log2fc
ENSG00000000003       900.75       658.00 -0.45303916
ENSG00000000005         0.00         0.00         NaN
ENSG00000000419       520.50       546.00  0.06900279
ENSG00000000457       339.75       316.50 -0.10226805
ENSG00000000460        97.25        78.75 -0.30441833
ENSG00000000938         0.75         0.00        -Inf
```

there are some funky answers in there like NaN (not a number) and -infinity that all come because I have zero count gene in my dataset

it is common practice to filter these zero count genes out before we go too deep.

```
to.keep.inds <- (rowSums(meancounts[,1:2]==0)==0)
mycounts <- meancounts[to.keep.inds,]
head(mycounts)
```

```
                control.mean treated.mean      log2fc
ENSG00000000003       900.75       658.00 -0.45303916
ENSG00000000419       520.50       546.00  0.06900279
ENSG00000000457       339.75       316.50 -0.10226805
ENSG00000000460        97.25        78.75 -0.30441833
ENSG00000000971      5219.00      6687.50  0.35769358
ENSG00000001036      2327.00      1785.75 -0.38194109
```

Q. How many genes do we have left after zero count filtering?

```
nrow(mycounts)
```

```
[1] 21817
```

A common threshold for calling a gene up or down is a log2 fold change of +2 or -2.

Q. How many “up” regulated genes do we have?

```
sum(mycounts$log2fc >=+2)
```

```
[1] 314
```

##DESeq analysis

we need to do this analysis properly with our inner stats person kept happy.

```
library(DESeq2)
```

To use DESeq we need to get our input data in very particular format.

```
dds <- DESeqDataSetFromMatrix(countData = counts, colData = metadata, design = ~dex)
```

```
converting counts to integer mode

Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
design formula are characters, converting to factors
```

Run DESeq analysis

```
dds <- DESeq(dds)
```

```
estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

Get the results

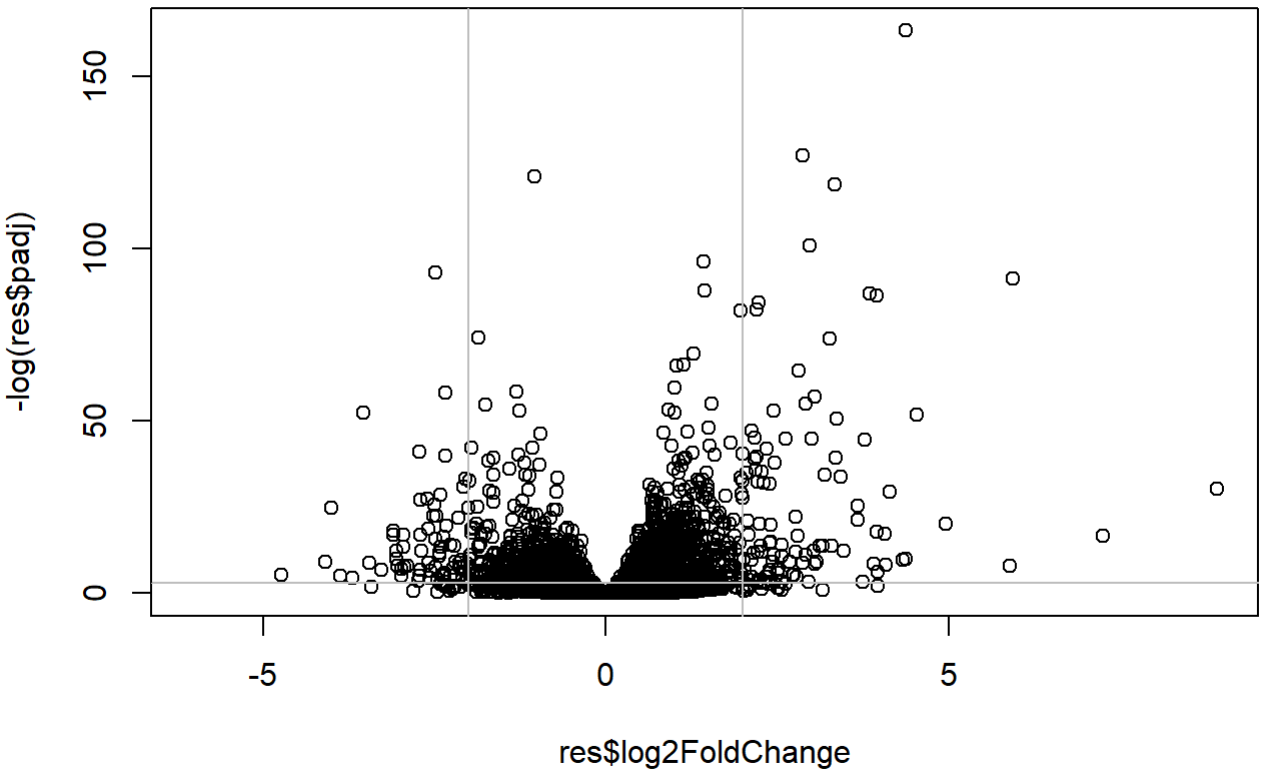
```
res <- results(dds)
head(res)
```

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 6 columns
                  baseMean log2FoldChange     lfcSE      stat    pvalue
                 <numeric>      <numeric> <numeric> <numeric> <numeric>
ENSG00000000003 747.194195     -0.3507030  0.168246 -2.084470 0.0371175
ENSG00000000005   0.000000             NA        NA        NA        NA
ENSG00000000419 520.134160      0.2061078  0.101059  2.039475 0.0414026
ENSG00000000457 322.664844      0.0245269  0.145145  0.168982 0.8658106
ENSG00000000460  87.682625     -0.1471420  0.257007 -0.572521 0.5669691
ENSG00000000938   0.319167     -1.7322890  3.493601 -0.495846 0.6200029
                     padj
                <numeric>
ENSG00000000003  0.163035
ENSG00000000005        NA
ENSG00000000419  0.176032
ENSG00000000457  0.961694
ENSG00000000460  0.815849
ENSG00000000938        NA
```

I want to make a figure showing an overview of all my results to date.

A plot of **log2 fold** change vs the **p value**(adjusted p-value)

```
plot(res$log2FoldChange, -log(res$padj))
abline(v=-2, col="gray")
abline(v=2, col="gray")
abline(h=-log(0.05), col="gray")
```

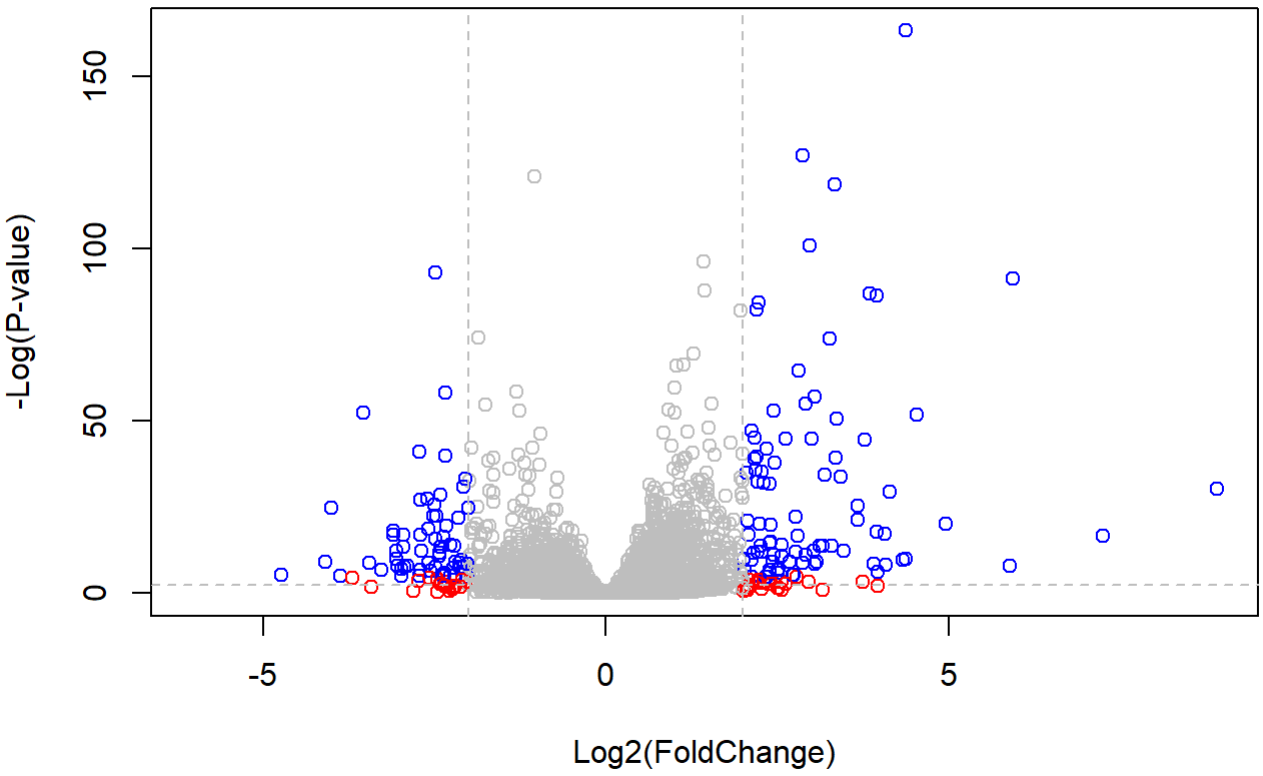



```
mycols <- rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ]  <- "red"

inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

# Volcano plot with custom colors
plot( res$log2FoldChange,  -log(res$padj),
 col=mycols, ylab="-Log(P-value)", xlab="Log2(FoldChange)" )

# Cut-off lines
abline(v=c(-2,2), col="gray", lty=2)
abline(h=-log(0.1), col="gray", lty=2)
```


-Log(P-value)
0
50
100
150
-5
0
5
Log2(FoldChange)