# Class 12: Transcriptomics and the analysis of RNA-Seq data

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Here we will use the DESeq2 package for RNASeq analysis. The data for today's class comes from a study of airway smooth muscle cells were treated with dexamethasone, a synthetic glucocorticoid steroid with anti-inflammatory effects (Himes et al. 2014).

### Import their data

WE need two things for this analysis:

-countData(Counts for every transcript/gene in each experiment) -colData (metadata that describes the experimental setup)

```
countData <- read.csv("airway_scaledcounts.csv",row.names = 1 )
head(countData)
```

| | 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 |

```
metadata <- read.csv("airway_metadata.csv")
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
7 SRR1039520 control  N061011 GSM1275874
8 SRR1039521 treated  N061011 GSM1275875
```

Q1. How many genes are in this dataset?

```
nrow(countData)
```

```
[1] 38694
```

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

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

```
control treated
      4       4
```

Another way

```
sum(metadata$dex == "control")
```

```
[1] 4
```

-Step 1. Calculate the mean of the control samples (i.e. columns in countData) Calculate the mean of the treated samples

(a) We need to find which columns are "control" samples.

- look in the metadata (a.k.a. colData), $dex column

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

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

(b) Extract all the control columns from `countData` and call it `control.counts`

```
control.counts <- countData[ , control.inds]
head(control.counts)
```

```
                SRR1039508 SRR1039512 SRR1039516 SRR1039520
ENSG00000000003        723        904       1170        806
ENSG00000000005          0          0          0          0
ENSG00000000419        467        616        582        417
ENSG00000000457        347        364        318        330
ENSG00000000460         96         73        118        102
ENSG00000000938          0          1          2          0
```

(c) Calculate the eman value across the rows of `control.counts` i.e. calculate the mean count values for each gene in the control samples.

```
control.means <- rowMeans(control.counts)
head(control.means)
```

```
ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
         900.75            0.00          520.50          339.75           97.25
ENSG00000000938
           0.75
```

-Step 2. Calculate the mean of the treated samples...

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

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

```
treated.counts <- countData[ , treated.inds]
head(treated.counts)
```

```
                SRR1039509 SRR1039513 SRR1039517 SRR1039521
ENSG00000000003        486        445       1097        604
ENSG00000000005          0          0          0          0
ENSG00000000419        523        371        781        509
ENSG00000000457        258        237        447        324
ENSG00000000460         81         66         94         74
ENSG00000000938          0          0          0          0
```

```
treated.means <- rowMeans(treated.counts)
head(treated.means)
```

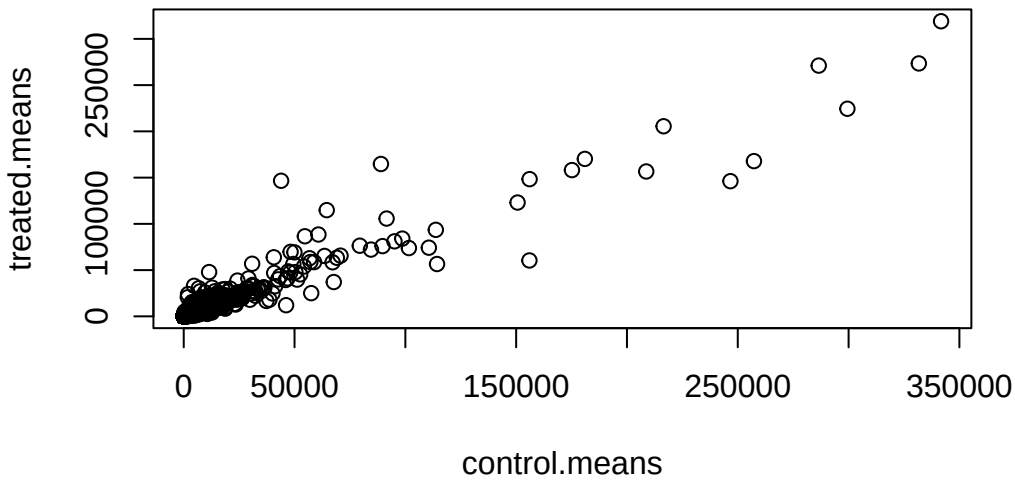
```
ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
         658.00            0.00          546.00          316.50           78.75
ENSG00000000938
           0.00
```

We now have control and treated mean count values. For ease of book-keeping I will combine these vectors into a new data.frame called `meancounts`.

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

```
                control.means treated.means
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
```

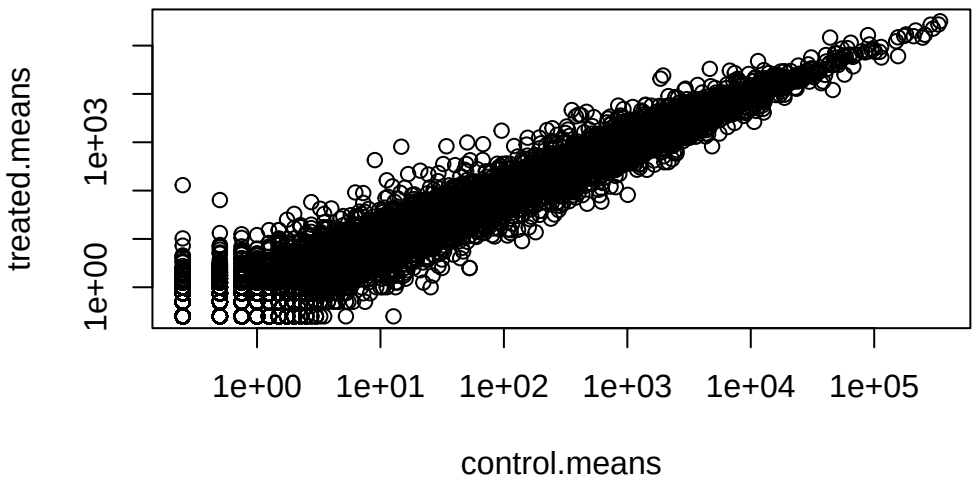
```
plot(meancounts)
```




```
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 use log transofrms fror skewed data such as this and because we really care most about realtive changes in magnitude.

We mos often use log2 as our transform as the math is easier to interpret than log10 or others.

If we have no change -i.e. same values in control and treated we will have a log2 value of zero.

```
log2(20/20)
```

```
[1] 0
```

If ia have dpuble the amount i.e. 20 compated to the 10 for example. I will have a log2 fold-change of +1

```
log2(20/10)
```

```
[1] 1
```

If I have half the amount I will have a log2 fold-change of -1

```
log2(10/20)
```

```
[1] -1
```

```
log2(40/10)
```

```
[1] 2
```

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

```
                control.means treated.means      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
```

Q. How many genes are up regulated at the common treshold of +2 log2FC values?

```
sum(meancounts$log2fc >= 2, na.rm = TRUE)
```

```
[1] 1910
```

Hold on what about the stats! Yes, these are big changes bit are these changes significant?!

To do this properly, we will turn to the DESeq2 package.

## DESeq2 analysis:

```
library(DESeq2)
```

To use DESeq we need our input contData and colData in a specific format that DESeq wants:

```
dds <- DESeqDataSetFromMatrix(countData = countData,
                              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
```

To run analysis, I can now use the main DESeq2 function called `DESeq()` with `dds` as input.

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

To get the results out of. this `dds` object we can use the `results()` function from the package.

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

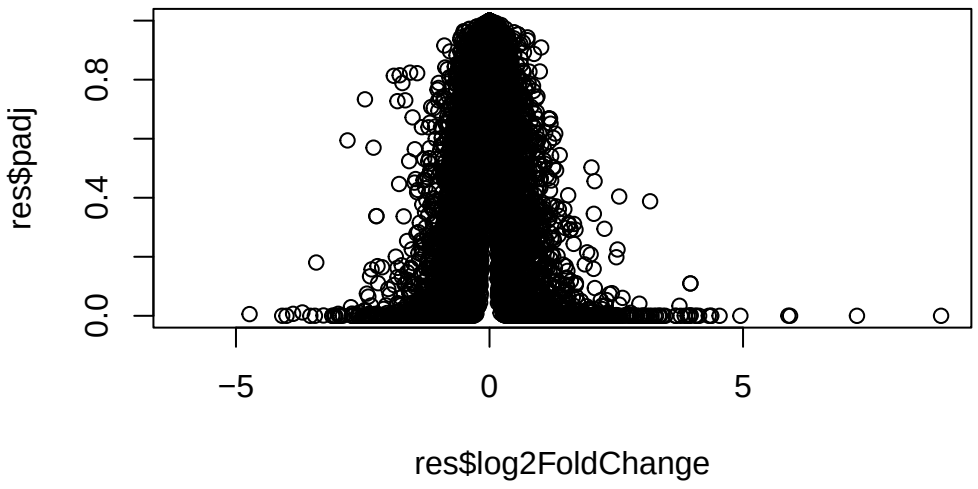
```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 6 columns
                  baseMean log2FoldChange     lfcSE      stat    pvalue
                 <numeric>      <numeric> <numeric> <numeric> <numeric>
ENSG00000000003 747.194195     -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
```

### Volcano plot:

Let's make a final (for today) plot of log2 fold-change vs the adjusted P-value.

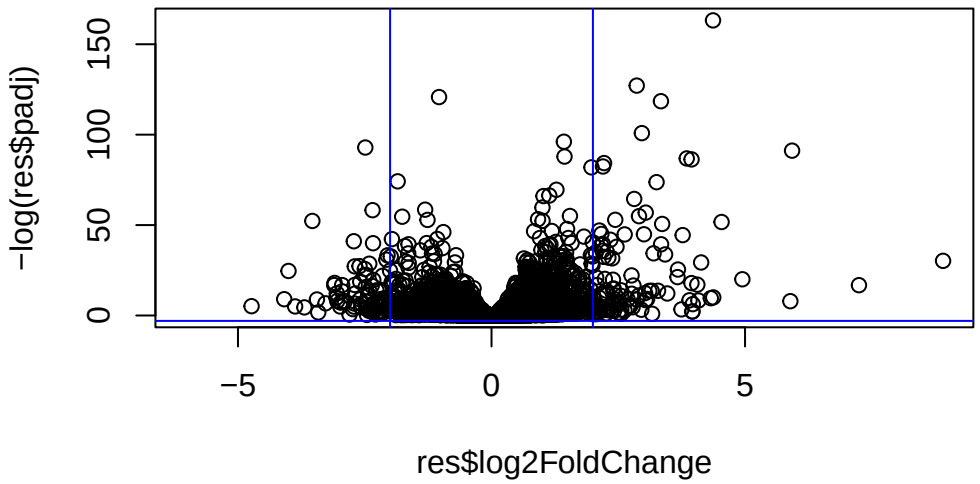
```
plot(res$log2FoldChange, res$padj)
```




It is the low P-values that we care about and these are lost in the skewed plot above. Let's

take the log of the $padj values for our plot.

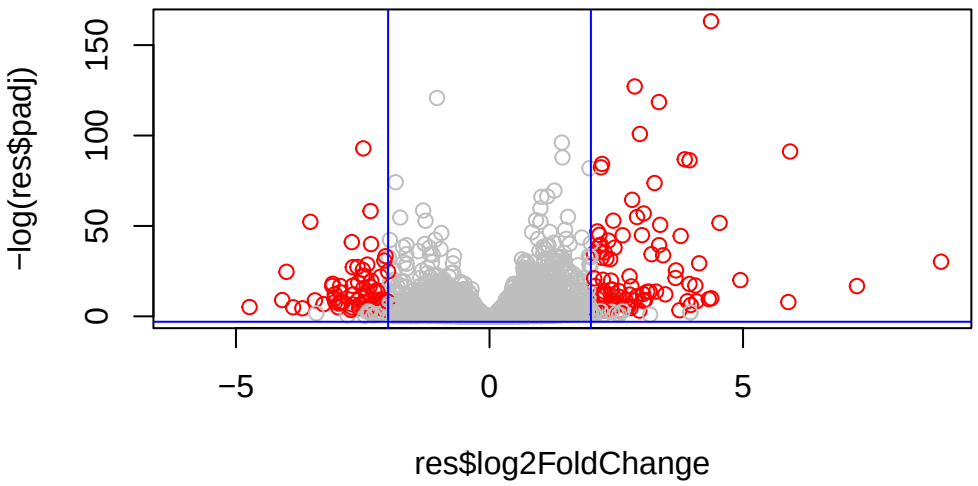
```
plot(res$log2FoldChange, -log(res$padj))
abline(v=c(+2,-2), col="blue")
abline(h=log(0.05), col="blue")
```




Finally we can make a color vector to use in the plot to better highlight the genes we care about.

```
mycols <- rep("gray", nrow(res))
mycols[abs(res$log2FoldChange) >= 2] <- "red"
mycols[res$padj >0.05 ] <- "gray"

plot(res$log2FoldChange, -log(res$padj), col=mycols)
abline(v=c(+2,-2), col="blue")
abline(h=log(0.05), col="blue")
```


−log(res$padj)
0
50
100
150
−5
0
5
res$log2FoldChange