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

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Background

Today we will analyze some RNASeq data from Himes et al. on the effects of a common steroid (dexamethasone) on the airway smooth muscle (ASM cells).

Are starting point is the "counts" data and "metadata" that contain the count values for each gene experiments (i.e cell lines with or without the drugs)

Data import

```
library(BiocManager)
```

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

Let's have a wee peak at these objects:

```
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
```

> Q1. How many genes are in this dataset?

```
nrow(counts)
```

```
[1] 38694
```

> Q. How many different experiments (columns in counts or rows in meta data) are there?

```
ncol(counts)
```

```
[1] 8
```

```
nrow(metadata)
```

```
[1] 8
```

```
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
```

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

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

```
[1] 4
```

Toy differential gene expression

To start our analysis, we need to calculate the mean counts of all of the genes in the "control" experiments

1. Extract all "control" columns from the `counts` object
2. Calculate the mean for all rows of these "control" columns
3. Do the same for "treated"
4. Do the same for "treated"
5. Compare these `control.mean` and `treated.mean` values

```r
control.inds <- metadata$dex == "control"
control.counts <- counts[ , control.inds]
```

```r
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 
```

```r
treated.inds <- metadata$dex == "treated"
treated.counts <- counts[ , treated.inds]
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
```

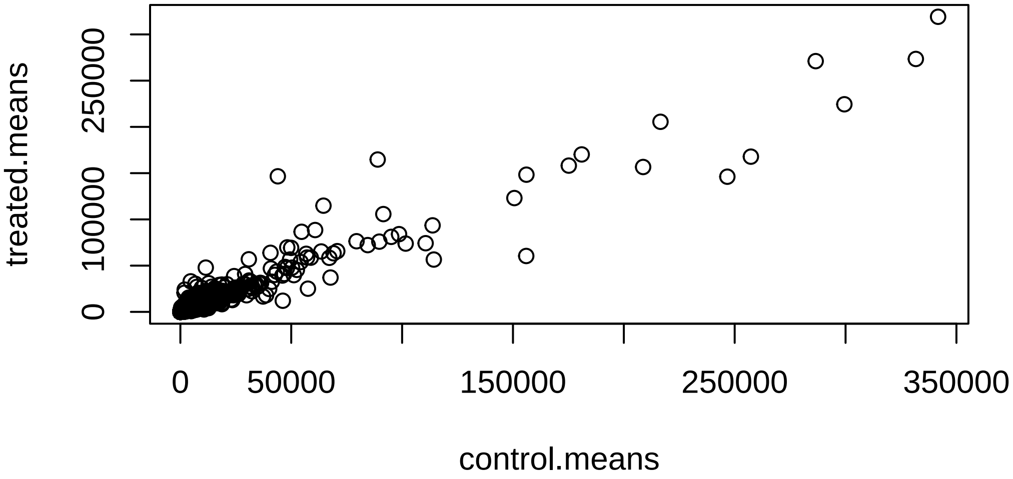
Store these together for ease of bookkeeping as `mean.counts`

```
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
```

Make a plot of control vs treated mean values for all genes

```
plot(meancounts)
```

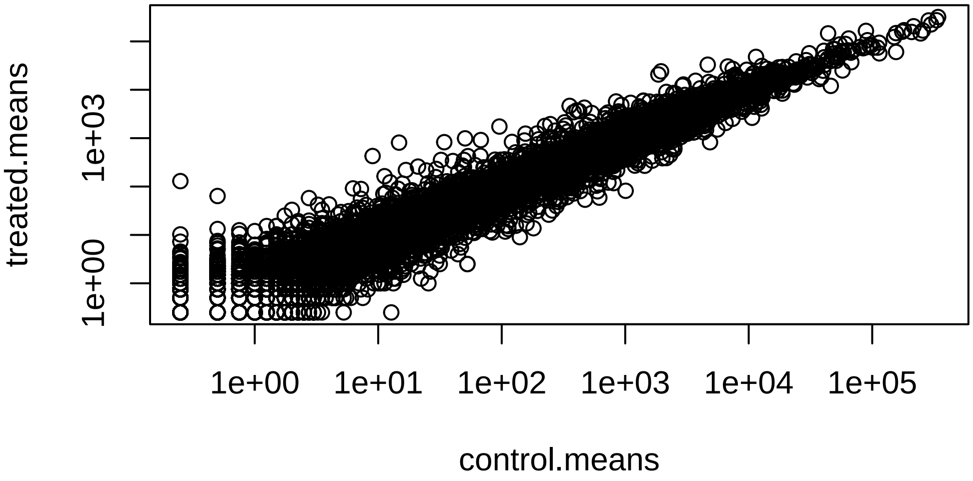


Make this a log log plot

```
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 often talk about metric like “log2 fold-change”

```
# treated/control
log2(10/10)
```

```
[1] 0
```

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

```
[1] -1
```

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

```
[1] 1
```

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

```
[1] 2
```

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

```
[1] -2
```

Let's calculate the log2 fold change for out treated over control mean counts.

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

```r
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
```

A common "rule of thumb" is a log2 fold change cutoff of +2 and -2 to call genes "Up regulated or"Down regulated".

Number of "up" genes

```r
sum(meancounts$log2fc >= +2, na.rm = T)
```

```
[1] 1910
```

Number of "down" genes at -2 threshold

```
sum(meancounts$log2fc <= -2, na.rm = T)
```

```
[1] 2330
```

These values may not be true because we need to consider that there is a range of values that may be considered as outliers

DESeq2 analysis

Let's do this analysis properly and keep our inner selves happy - i.e are the difference we see between drug and no drug significant given the replicate experiments

```
library(DESeq2)
```

For DESeq analysis we need 3 things

- Count values (`contData`)
- Metadata telling us about the columns in `countData` (`colData`)
- Design of the experiment (i.e what do you want to compare)

Our first function from DESeq2 will setup the input required for analysis by storing all these 3 things together

```
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
```

The main function in DESeq2 that runs the analysis is called `DESeq()`

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

```
estimating size factors
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

```r
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      -0.350703  0.168242 -2.084514 0.0371134
ENSG00000000005   0.000000             NA        NA        NA        NA
ENSG00000000419 520.134160       0.206107  0.101042  2.039828 0.0413675
ENSG00000000457 322.664844       0.024527  0.145134  0.168996 0.8658000
ENSG00000000460  87.682625      -0.147143  0.256995 -0.572550 0.5669497
ENSG00000000938   0.319167      -1.732289  3.493601 -0.495846 0.6200029
                     padj
                <numeric>
ENSG00000000003  0.163017
ENSG00000000005        NA
ENSG00000000419  0.175937
ENSG00000000457  0.961682
ENSG00000000460  0.815805
ENSG00000000938        NA
```

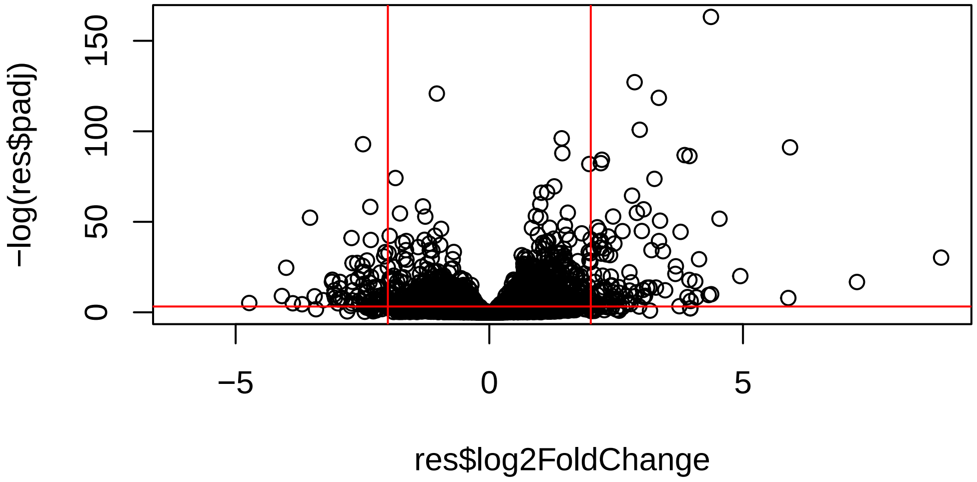
```r
36000 * 0.05
```

```
[1] 1800
```

Volcano Plots

This is a common summary result figures from these types of experiences and plot the log2 fold-change vs the adjusted p-value

```
plot(res$log2FoldChange, -log(res$padj))
abline(v=c(-2,2), col="red")
abline(h=-log(0.04), col= "red")
```



```
log(0.1)
```

```
[1] -2.302585
```

```
log(0.000001)
```

```
[1] -13.81551
```

Save our results

```r
write.csv(res, file="my_results.csv")
```