Class 13: RNASeq pt.1

Yvonne Yu A16333006

The data utilized in today's lab comes from an old study by Himes et al. on a published RNA-seq experiment where airway smooth muscle cells were treated with dexamethasone, a synthetic glucocorticoid steroid with anti-inflammatory effects.

Import Data

Two things are needed for the analysis: counts and metadata, called "countData" and "colData", respectively, in the DESeq2 world.

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

Examine Data

```
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

The counts are organized with a gene per row and experiment per column.

```
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?

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

```
[1] 4
```

```
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"
```

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

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

To be able to determine if all of the elements of a vector are TRUE, then the `all()` function can be utilized.

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

```
[1] TRUE
```

Analysis

I want to start by comparing the "control" and "treated" columns. To do this, I will find the average counts for each gene (row) in all the "control" columns. Then the average of the "treated" columns will be identified. The two values would then be compared.

Extract the "control" columns first.

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

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

Determine the mean count value per gene for the control using the `apply()` function.

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

Extract the "treated" columns and determine the mean values for the treated columns. .

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

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

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

Combine the mean vectors into one dataframe.

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

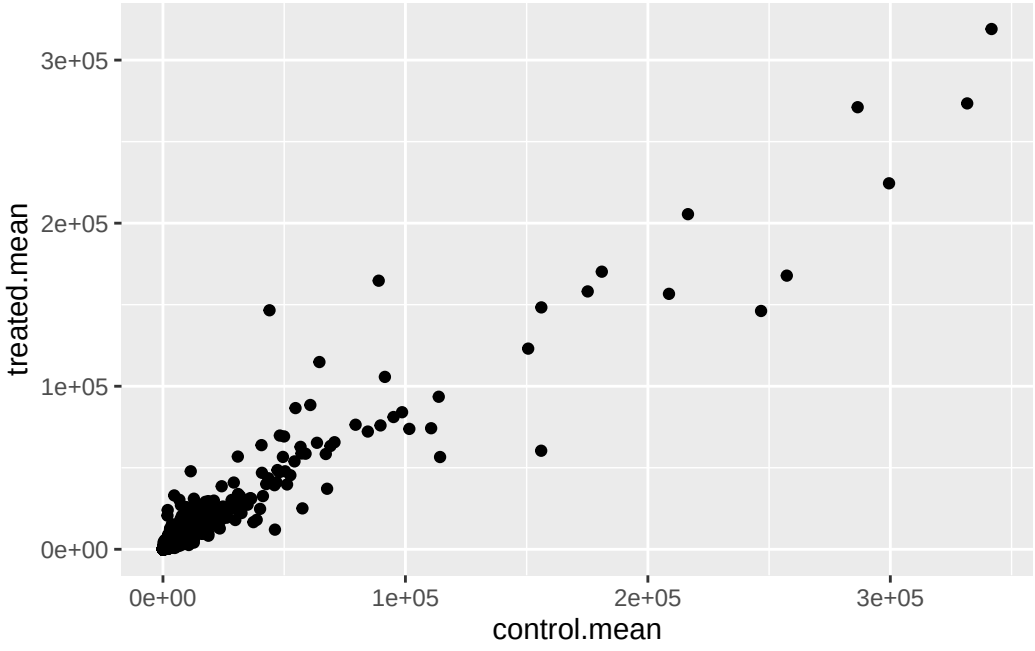
```
                control.mean treated.mean
ENSG00000000003       900.75       658.00
ENSG00000000005         0.00         0.00
ENSG00000000419       520.50       546.00
ENSG00000000457       339.75       316.50
ENSG00000000460        97.25        78.75
ENSG00000000938         0.75         0.00
```

Hypothetically, if there was no relation, the plotting of the two values would mean that there is a straight line down the diagonal.

Create a plot.

```
library(ggplot2)

ggplot(meancounts, aes(control.mean, treated.mean)) + geom_point()
```

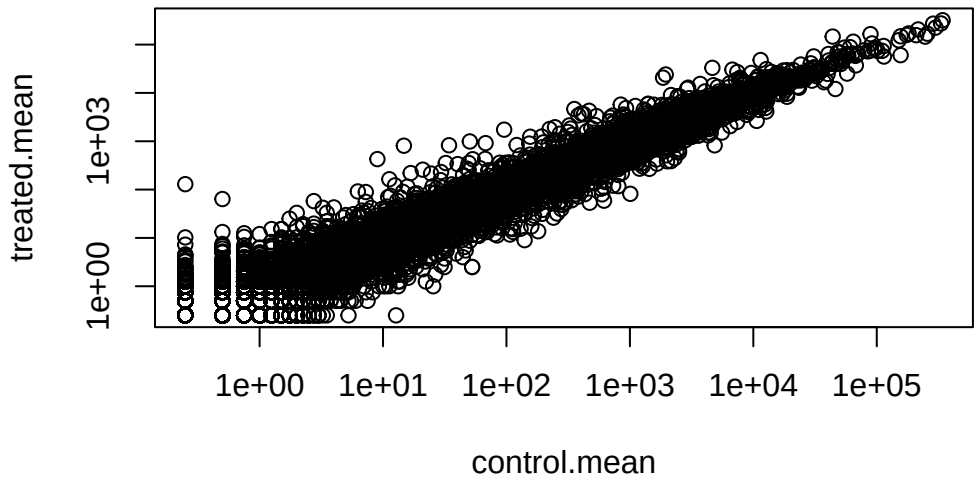


All the points are hidden at the origin, to be able to expose those points, use log to be able to identify more of the points without skewing the data.

```
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
```



Log2 units are typically used because of the more intuitive interpretation.

Log2 Fold change of treated/control values are calculated and added to the main dataframe 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
```

Because of the answers that were found inside the dataframe (like NaN = Not a Number, -Inf = negative infinity) that had resulted from zero counts genes in the dataset, it is common dataset to filter the 0 count genes out.

```
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

Need to do the analysis properly.

```
library(DESeq2)
```

To use DESeq, the input data is needed to be in a 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
```

Make a figure that gives an overview of all of the results. A plot of **log2 fold change** versus the **p-value** (adjusted p-value)

```
mycols <- rep("grey", nrow(res))
mycols[abs(res$log2FoldChange) >= 2 ] <- "red"
mycols[-log(res$padj) >= -log2(0.05) & abs(res$log2FoldChange) >=2] <- "blue"

plot(res$log2FoldChange, -log(res$padj),
     col = mycols, ylab = "Log2 of Adjusted P-value",
     xlab = "Log2 Fold Change")
abline(v = -2, col = "gray")
abline(v = 2, col = "gray")
abline(h = -log(0.05), col = "gray")
```