# Lab 13

Lauren

The data for this hands-on session comes from a published RNA-seq experiment where airway smooth mucle cells were treated with **dexamthasone** (dex).

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

> 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
```

##Toy differential gene expression

```
control <- metadata[metadata[,"dex"]=="control",]
control.counts <- counts[ ,control$id]
control.mean <- rowSums( control.counts )/4
head(control.mean)
```

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

> Q3. How would you make the above code in either approach more robust? Is there a function that could help here?

Lets calculate the mean counts per gene in the "control" samples. We can then compare this value for each gene to the mean counts in the "treated" samples (columns).

-Step 1. Find which columns in the `counts` correspong to the "control" samples -Step 2. Calculate the mean value per gene in these columns -Step 3. Store my anser for later in `control.mean`

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

```
#apply(control.counts, 1, mean) OR

control.mean <- rowMeans(control.counts)
```

> Q4. Follow the same procedure for the treated samples (i.e. calculate the mean per gene across drug treated samples and assign to a labeled vector called treated.mean)

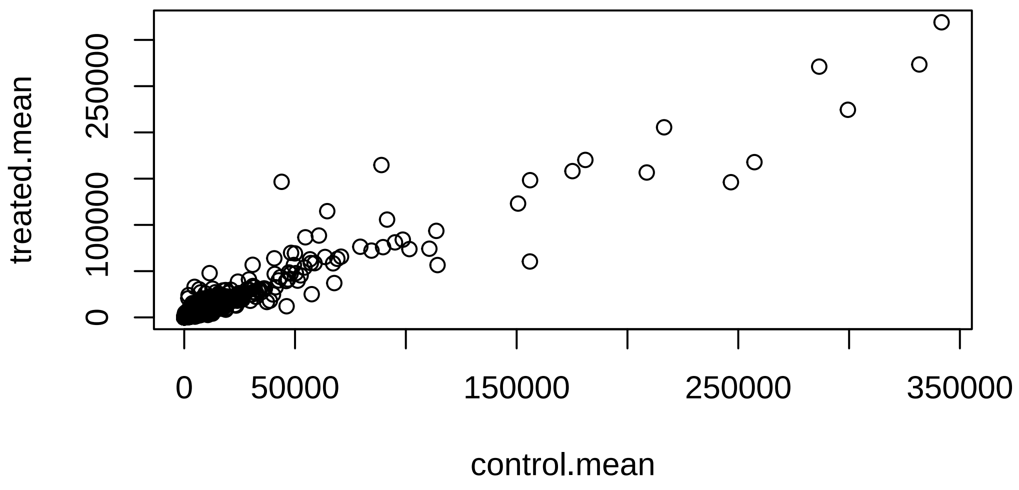
```
treated.inds <- metadata$dex == "treated"
treated.counts <- counts[,treated.inds]
treated.mean <- rowMeans(treated.counts)
```

To keep us tidy, lets put `control.mean` and `treated.mean` vectors together as 2 columns of new data

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

> Q5 (a). Create a scatter plot showing the mean of the treated samples against the mean of the control samples. Your plot should look something like the following.

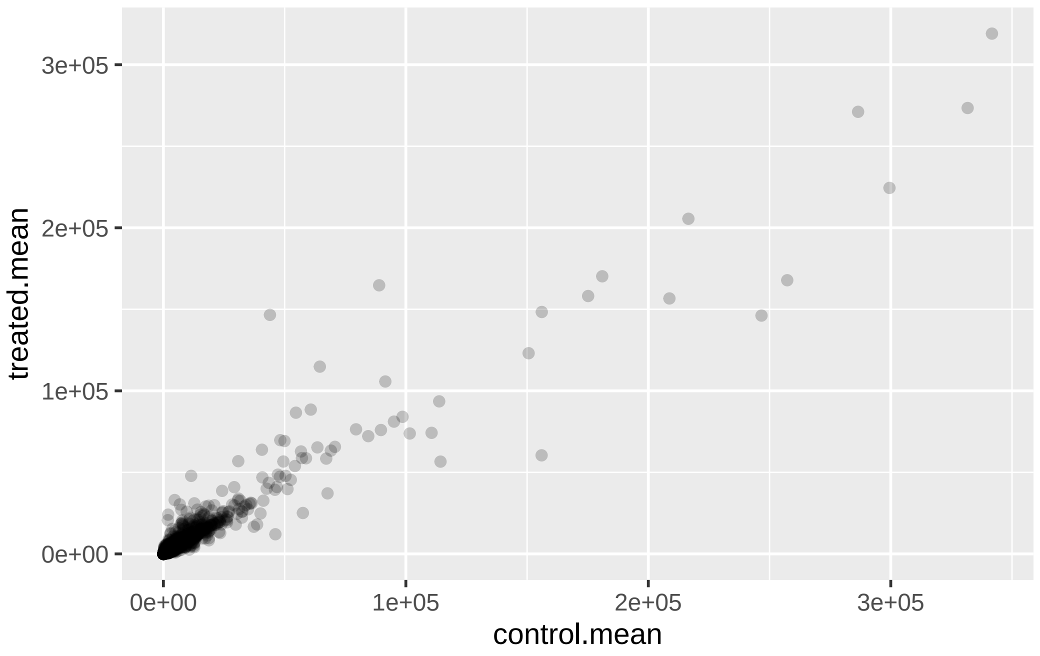
```
plot(meancounts)
```



Q5 (b).You could also use the ggplot2 package to make this figure producing the plot below. What geom_?() function would you use for this plot?

```
library(ggplot2)

ggplot(meancounts) +
  aes(control.mean, treated.mean) +
  geom_point(alpha=0.2)
```

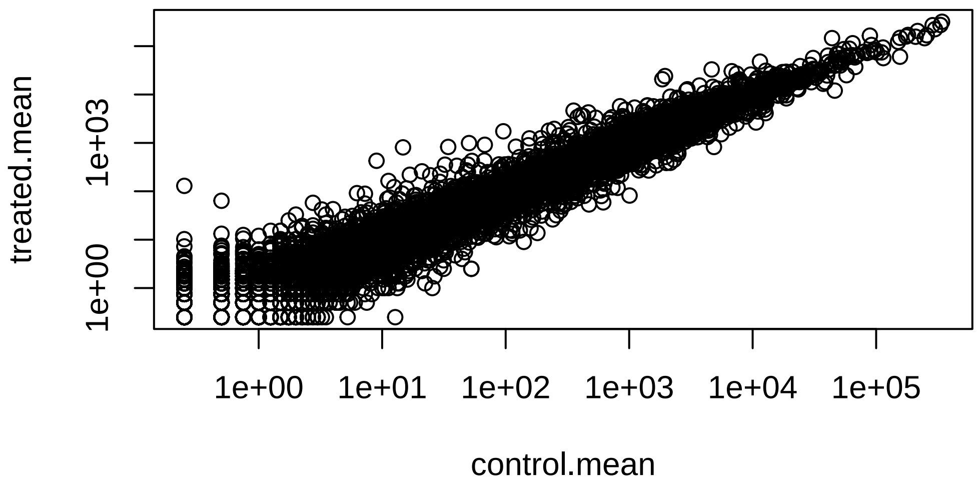


Q6. Try plotting both axes on a log scale. What is the argument to plot() that allows you to do this?

```
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
```



Log transformations are super useful when our data is skewed and measured over a wide range like this. We can use different log transformations like base10 or natural logs, but we most often prefer log2 units.

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

```
[1] 0
```

What if there was a doubling

```
#treated/control
log2(20/10)
```

```
[1] 1
```

What if there was a half count

```
#treated/control
log2(10/20)
```

```
[1] -1
```

Lets add a log2 fold change column to our little `meancounts` data.frame:

```
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 a couple weird results, so lets filter them.

```
to.rm.inds <- rowSums(meancounts[,1:2] == 0) > 0
mycounts <- meancounts[!to.rm.inds, ]
```

The `!` flips TRUE value to FALSE and vice-versa

```
dim(mycounts)
```

```
[1] 21817     3
```

```
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
```

> Q7. What is the purpose of the arr.ind argument in the which() function call above? Why would we then take the first column of the output and need to call the unique() function?

It gives the rows and columns where the outputs are TRUE (for both). The `unique()`finction makes sure that any rows aren't counted twice.

A common threshold used for calling something differentially expressed is a log2(FoldChange) of greater than 2 or less than -2. Let's filter the dataset both ways to see how many genes are up or down-regulated.

```
up.ind <- mycounts$log2fc > 2
down.ind <- mycounts$log2fc < (-2)
```

> Q8. Using the up.ind vector above can you determine how many up regulated genes we have at the greater than 2 fc level?

```
sum(up.ind)
```

```
[1] 250
```

> Q9. Using the down.ind vector above can you determine how many down regulated genes we have at the greater than 2 fc level?

```
sum(down.ind)
```

```
[1] 367
```

> Q10. Do you trust these results? Why or why not?

No, we aren't accounting for statistical significance with these results.

#Using DESeq2

like any package we must load it up using `library()`

```
library(DESeq2)
```

```
Loading required package: S4Vectors

Loading required package: stats4

Loading required package: BiocGenerics


Attaching package: 'BiocGenerics'
```

```
The following objects are masked from 'package:stats':

    IQR, mad, sd, var, xtabs


The following objects are masked from 'package:base':

    anyDuplicated, aperm, append, as.data.frame, basename, cbind,
    colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
    get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
    match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
    Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
    table, tapply, union, unique, unsplit, which.max, which.min



Attaching package: 'S4Vectors'


The following object is masked from 'package:utils':

    findMatches


The following objects are masked from 'package:base':

    expand.grid, I, unname


Loading required package: IRanges



Attaching package: 'IRanges'


The following object is masked from 'package:grDevices':

    windows


Loading required package: GenomicRanges


Loading required package: GenomeInfoDb


Loading required package: SummarizedExperiment
Loading required package: MatrixGenerics

Loading required package: matrixStats

Warning: package 'matrixStats' was built under R version 4.3.2


Attaching package: 'MatrixGenerics'

The following objects are masked from 'package:matrixStats':

    colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
    colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
    colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
    colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
    colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
    colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
    colWeightedMeans, colWeightedMedians, colWeightedSds,
    colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
    rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
    rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
    rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
    rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
    rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
    rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
    rowWeightedSds, rowWeightedVars

Loading required package: Biobase

Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.


Attaching package: 'Biobase'

The following object is masked from 'package:MatrixGenerics':

    rowMedians
```

```
The following objects are masked from 'package:matrixStats':

    anyMissing, rowMedians
```

```
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
```

Now we can run our DESeq analysis

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

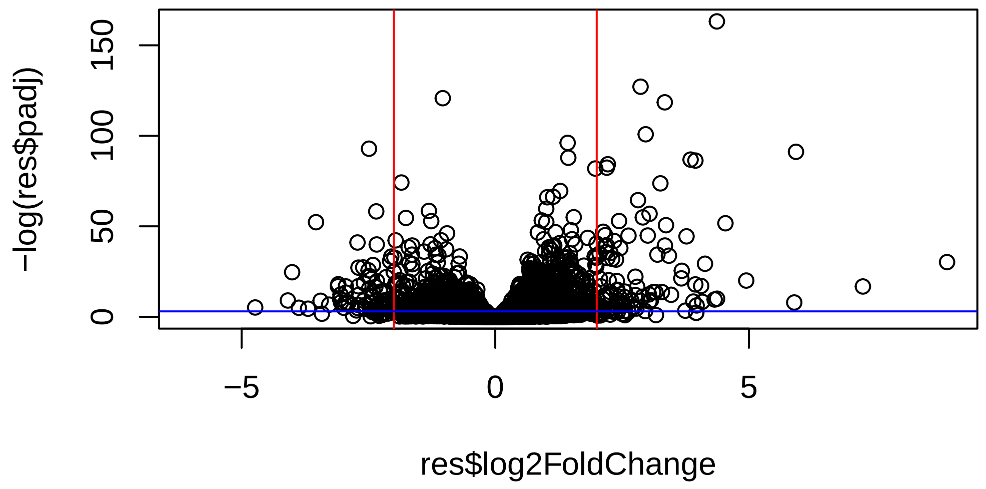
```
fitting model and testing
```

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

#Summary Results Plot

Volcano plot. This is a common type of summary figure that keeps both our inner biologist and inner stats nerd happy because it shows both P-value and Log2(Fold-Changes).

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



save our results to date:

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