Lab12

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##Background

RNA-seq: after all the alignments and whatnot the result is a **count matrix**. Columns are the experiments (+/- drug, KO, etc.)

countData -> input for DESeq2 colData-> tells you about the design of the experiment. Rows match columns from count data. Metadata folder.

Today we will analyse some RNASeq data from Himes et al. on the effects of a commons teroid (dex) on airway smooth muscle cells (ASMs). For this analysis, we need two main inputs.

1. countData: table of **counts** per gene (in rows) across experiments (in columns)
2. colData: **metadata** about the design of the experiments. Rows here match columns in `countdata`

loading libraries

```r
library(BiocManager)
library(DESeq2)
library(ggplot2)
library(dplyr)
```

##Data Import

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

Lets have a wee peak

```
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
```

ENSG –> gene code. Each one corresponds SRR–> experiment details, explained in metadata file. geo_id

> Q1. How many "genes" are in this dataset

```
nrow(counts)
```

```
[1] 38694
```

There are 38694 genes in this dataset

> Q2. How many experiments (i.e. columns in counts, or rows in metadata)

```
ncol(counts)
```

```
[1] 8
```

```
nrow(metadata)
```

```
[1] 8
```

8 experiments

> Q3. How many "control" experiments are there

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

```
[1] 4
```

4 control conditions

Toy analysis example

Step 1: Extract the "control" columns from `counts` **Step 2:** Calculate the mean value for each gene in these "control" columns

Steps 3-4: Do the same for the "treated" columns **Step 5:** Compare

Step 1

```
control.inds<-metadata$dex=="control" #use this to access the control the columns
control.counts<-counts[ , control.inds] #making a control count matrix
```

Step 2

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

Steps 3, 4

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

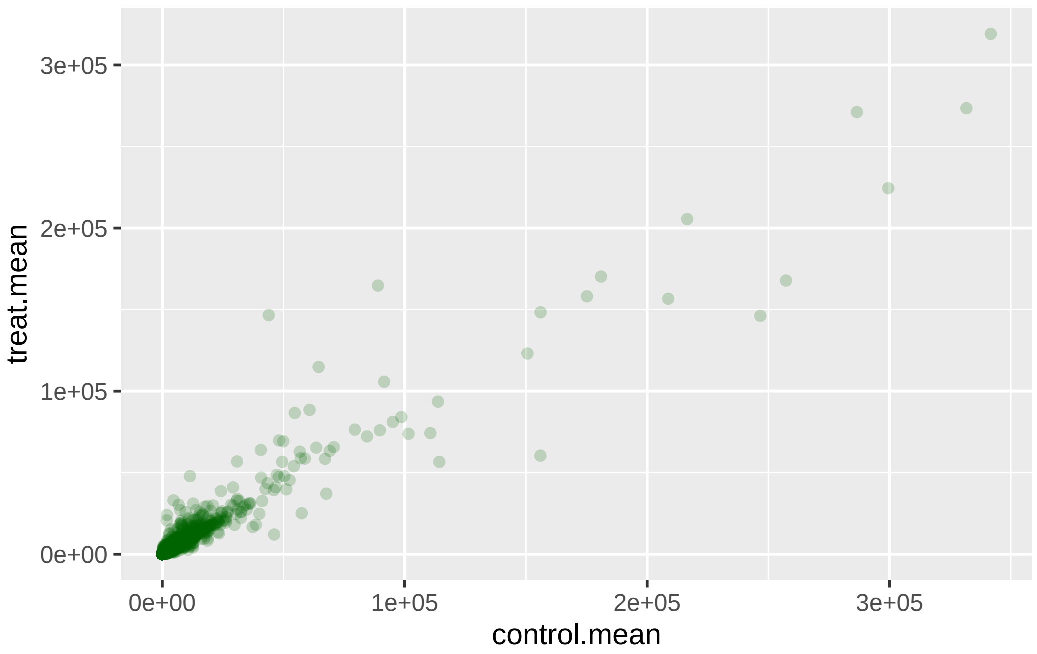
For ease of book-keeping, we can store these together in one df - `meancounts`

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

```
                control.mean treat.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
```

Step 5: Compare

```r
ggplot(meancounts,aes(control.mean,treat.mean))+
  geom_point(alpha=0.2,col="darkgreen")
```



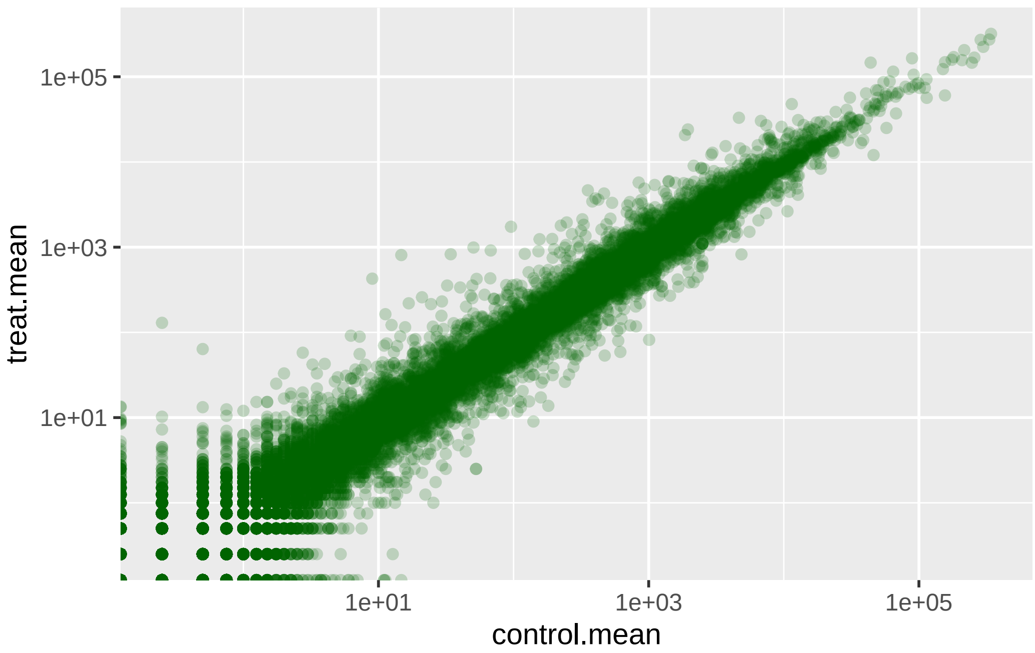
This does not look like 38000 data points

Use log scale to account for the heavy skew.

```
ggplot(meancounts,aes(control.mean,treat.mean))+
  geom_point(alpha=0.2,col="darkgreen")+
  scale_x_log10()+
  scale_y_log10()
```

```
Warning in scale_x_log10(): log-10 transformation introduced infinite values.

Warning in scale_y_log10(): log-10 transformation introduced infinite values.
```



Points along the diagonal likely have no change - this is a heavy portion of the data. To account for this, we calculate "fold change" as a way to see differences. Generally for fold change we use log2 - Why?

```
#treated/control
10/10
```

```
[1] 1
```

```
log2(10/10) #log2 says that there is no change here.
```

```
[1] 0
```

```r
log2(20/10) #expression increased with treatment
```

```
[1] 1
```

```r
log2(10/20) #expression decreased with treatment
```

```
[1] -1
```

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

```
                control.mean treat.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
```

NaN, -Inf - not a number, -infinity. 0/0 is not a number, so we can either add pseudocounts or just filter it out.

A common rule-of-thumb for calling something upregulated is a log2FC > +2, downregulated log2FC < -2

filter out zero

```r
#filter out zero count genes

#nonzero.inds<-rowSums(counts!=0)
#mycounts<-meancounts[nonzero.inds,]
#nrow(mycounts)

#OR
zero.inds<-which(meancounts[,1:2] == 0,arr.ind=T)[,1]
mygenes<-meancounts[-zero.inds,]
head(mygenes)
```

```
                control.mean treat.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 are upregulated

```r
sum(mygenes$log2fc>=2)
```

```
[1] 314
```

> Q. how many genes are downregulated

```r
sum(mygenes$log2fc <= -2)
```

```
[1] 485
```

This is good, however this has no statistical significance attached to it. We need to calculate that - can be done with DESeq

##DESeq analysis

```r
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 the DESeq package to run analysis is called `DESeq()`

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

```
estimating size factors
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

results

```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     -0.3507030  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
```

baseMean- mean value across all columns log2FC - pval - padj - 5% of 36000 things is still a large number. So we adjust the pvalues to account for this - batch correction.

```r
36000*0.05
```

```
[1] 1800
```

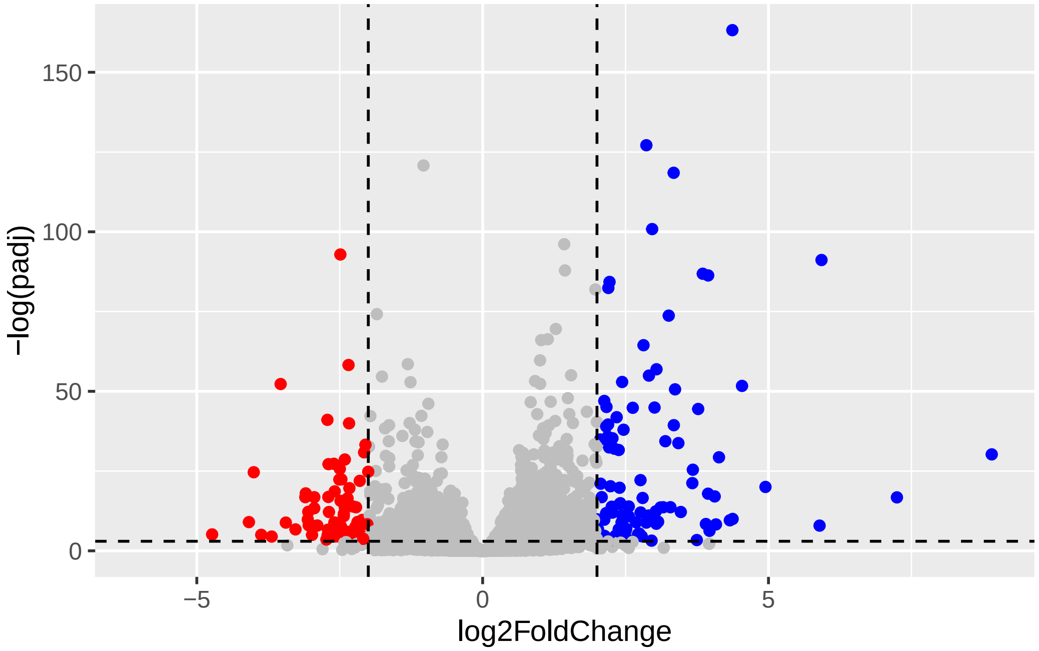
Volcano plot

This is a plot of log2FC vs. adjusted pval (padj)

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

ggplot(res, aes(log2FoldChange,-log(padj)))+
  geom_point(col=mycols)+
  geom_vline(xintercept=c(-2,2), color = "black", linetype = "dashed")+
  geom_hline(yintercept = -log(0.05),color = "black", linetype = "dashed")
```

```
Warning: Removed 23549 rows containing missing values or values outside the scale range
(`geom_point()`).
```



```
#we take -log2FC
```

Save our results

```
write.csv(res,file="myresults.csv")
```