Class 13: RNASeq with DESeq2

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Today we wil analyze some RNASeq data from Himes et al. on the effects of dexamrthasone (dex), a synthetic glucocorticoid steroid on air smooth muscle cells (ASM).

Data import

```
## Complete the missing code
counts <- read.csv("airway_scaledcounts.csv", row.names=1)
metadata <-  read.csv("airway_metadata.csv", row.names=1)
```

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)
```

```
               dex celltype     geo_id
SRR1039508 control   N61311 GSM1275862
SRR1039509 treated   N61311 GSM1275863
SRR1039512 control  N052611 GSM1275866
SRR1039513 treated  N052611 GSM1275867
SRR1039516 control  N080611 GSM1275870
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
```

Toy differential expression analysis

Calculate the mean per gene count values for all "control" samples (i.e. columns in `counts`) and do the same for "treated" and then compare them.

1. Find all "control" values/columns in `counts`

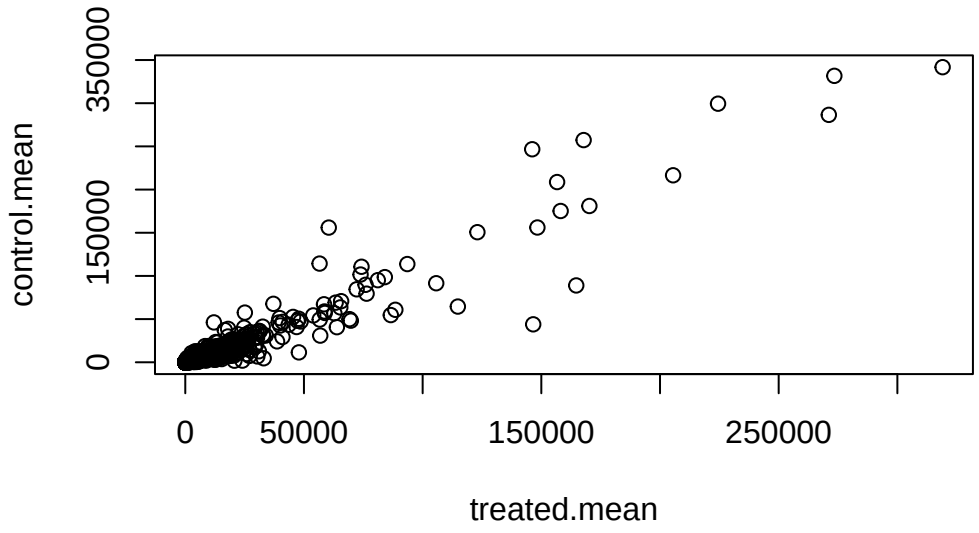
```
control.inds <- metadata$dex == "control"
control.counts <- counts[,control.inds]
```

2. Find the mean per gene across all control columns.

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

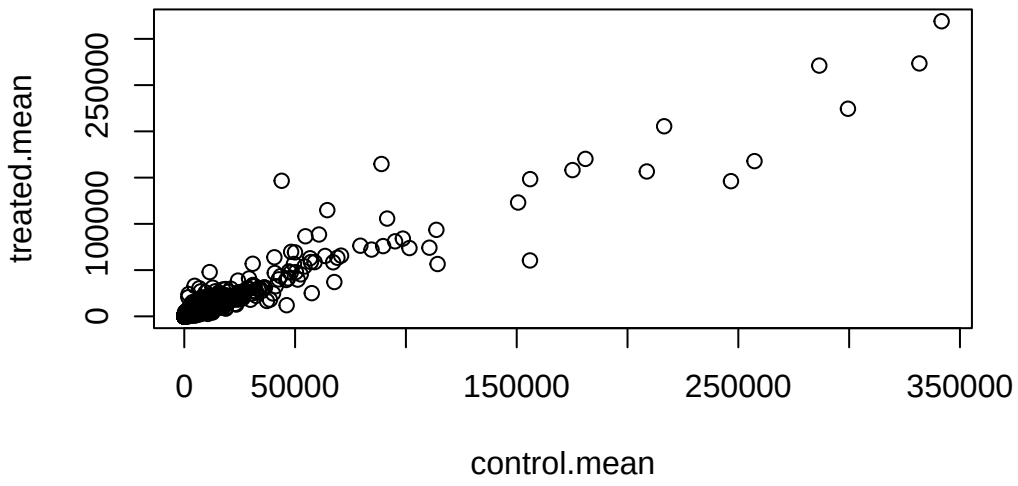
3. Do the same steps to find the `treated.mean` values

```
treated.inds <- metadata$dex == "treated"
treated.counts <- counts[,treated.inds]
treated.mean <- apply(treated.counts, 1, mean)
plot(treated.mean, control.mean)
```




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

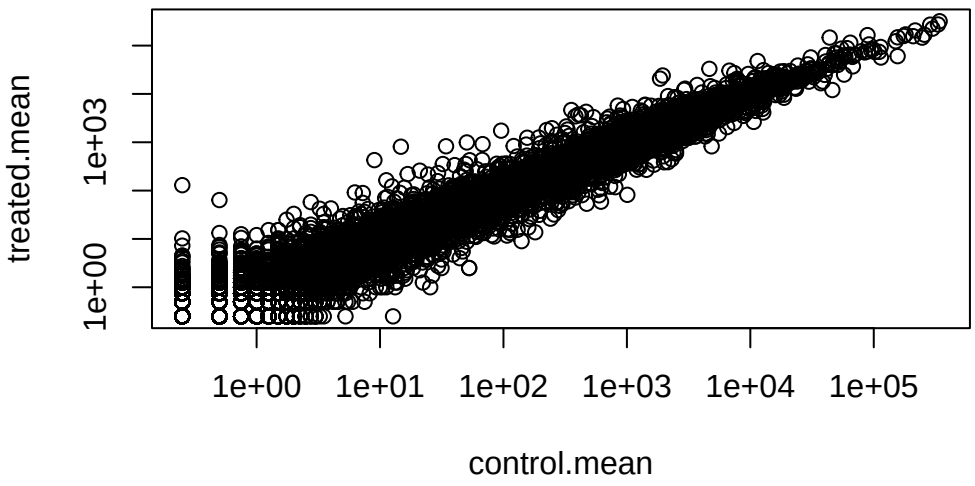
```
plot(meancounts)
```




```
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 most frequently use log2 transformations for this type of data.

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

```
[1] 0
```

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

```
[1] 1
```

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

```
[1] -1
```

These log2 values make the interpretation of "fold-change" a little easier and a rule-of-thumb in the filed is a log2 fold-change of 2+ or -2 is where we start to pay attention.

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

```
[1] 2
```

Let's calculate the log2 (fold-change) and add it to our `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
```

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

> Q. How many genes do I have left after this zero count filtering?

```
nrow(mycounts)
```

```
[1] 21817
```

> Q. How many genes are "up" regulated upon drug treatment at a threshold of +2 log2-fold-change?

1. I need to extract the log2fc values
2. I need to find those that are above +2
3. Count them

```
sum(mycounts$log2fc > 2)
```

```
[1] 250
```

> Q. How many genes are "down" regulated upon drug treatment at a threshold of -2 log2-fc??

```
sum(mycounts$log2fc < -2)
```

```
[1] 367
```

Wow hold on we are missing the stats here. Is the difference in the mean counts significant???

Let's do this analysis the right way with stats and use the **DESeq2** package

DESeq analysis

```
library(DESeq2)
```

The first function that we will use will setup the data in the way (format) DESeq wants it.

```
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 package is called `DESeq()` and we can run it on our `dds` object

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

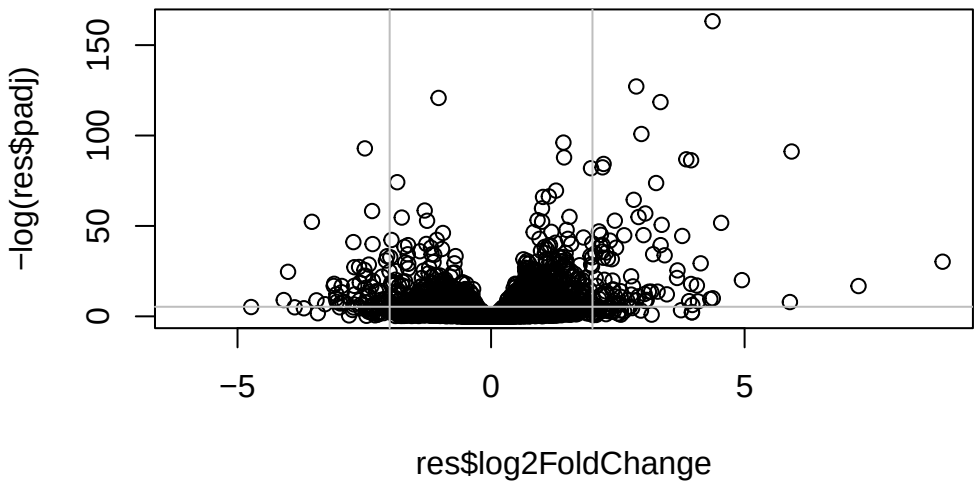
I will get the results from dds with the `results()` function:

```
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 common overall results figure from this analysis. This is designed to keep our iner biologist and inner stats nerd happy - it plots fold-change vs P-value

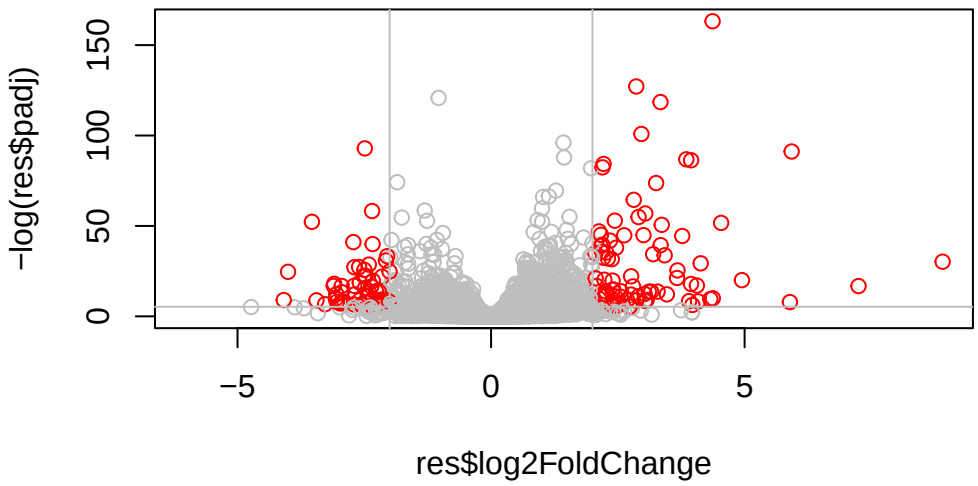
```
plot(res$log2FoldChange, -log(res$padj))
abline(v=c(-2,2), col="gray")
abline(h=-log(0.005), col="gray")
```



Add some color to this plot:

```
mycols <- rep("gray", nrow(res))
mycols[res$log2FoldChange > 2] <- "red"
mycols[res$log2FoldChange < -2] <- "red"
mycols[res$padj > 0.005] <- "gray"

plot(res$log2FoldChange, -log(res$padj), col=mycols)
abline(v=c(-2,2), col="gray")
abline(h=-log(0.005), col="gray")
```



I want to save my results to date out to disc

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

We will pick this up next day and add **annotation** (i.e. what are these genes of interest) and do **pathway analysis** (what biology) are they known to be involved with.

I need to translate our gene identifiers "ENSG0000..." into gene names that the rest of the world can understand.

To fo this "annotation" I will use the "AnnotationDbi" package. I can install this with `BiocManager::install()`

```
library(AnnotationDbi)
library(org.Hs.eg.db)
```

```
columns(org.Hs.eg.db)
```

```
 [1] "ACCNUM"       "ALIAS"        "ENSEMBL"      "ENSEMBLPROT"  "ENSEMBLTRANS"
 [6] "ENTREZID"     "ENZYME"       "EVIDENCE"     "EVIDENCEALL"  "GENENAME"
```

```
[11] "GENETYPE"     "GO"           "GOALL"        "IPI"          "MAP"
[16] "OMIM"         "ONTOLOGY"     "ONTOLOGYALL"  "PATH"         "PFAM"
[21] "PMID"         "PROSITE"      "REFSEQ"       "SYMBOL"       "UCSCKG"
[26] "UNIPROT"
```

I will use `mapIds()` function to "map" my identifiers to those from different databases. I will go between "ENSEMBL" and "SYMBOL" (and then after "GENENAME").

```
res$symbol <- mapIds(org.Hs.eg.db,
       keys = rownames(res),
       keytype = "ENSEMBL",
       column = "SYMBOL")
```

```
'select()' returned 1:many mapping between keys and columns
```

```
#head(res)
```

Add "GENENAME"

```
res$genename <- mapIds(org.Hs.eg.db,
       keys = row.names(res),
       keytype = "ENSEMBL",
       column = "GENENAME")
```

```
'select()' returned 1:many mapping between keys and columns
```

Add "ENTREZID"

```
res$entrez <- mapIds(org.Hs.eg.db,
       keys = rownames(res),
       keytype = "ENSEMBL",
       column = "ENTREZID")
```

```
'select()' returned 1:many mapping between keys and columns
```

Save our annotated results object.

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

Pathway Analysis

Now that we have our results with added annotation we can do some pathway mapping.

Let's use the *gage* package to look for KEGG pathways in our results (genes of interest). I will also use the *pathview* package

```
library(pathview)
library(gage)
library(gageData)

data(kegg.sets.hs)
```

What **gage** wants as input is not my big table/data.frame of results. It just wants a "vector of importance". For RNASeq data like we have this is our log2FC values...

```
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
head(foldchanges)
```

```
       7105       64102        8813       57147       55732        2268
-0.35070302          NA  0.20610777  0.02452695 -0.14714205 -1.73228897
```

Now let's run the gage pathway analysis.

```
# Get the results
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

What is in this `keggres` object?

```
attributes(keggres)
```

```
$names
[1] "greater" "less"    "stats"
```

```
head(keggres$les, 3)
```

```
                                     p.geomean stat.mean        p.val
hsa05332 Graft-versus-host disease 0.0004250461 -3.473346 0.0004250461
hsa04940 Type I diabetes mellitus  0.0017820293 -3.002352 0.0017820293
hsa05310 Asthma                    0.0020045888 -3.009050 0.0020045888
                                        q.val set.size         exp1
hsa05332 Graft-versus-host disease 0.09053483       40 0.0004250461
hsa04940 Type I diabetes mellitus  0.14232581       42 0.0017820293
hsa05310 Asthma                    0.14232581       29 0.0020045888
```

Let's use the pathview package to look at one of these highlighted KEGG pathways with our genes highighted. "hsa05310 Asthma"

```
pathview(gene.data=foldchanges, pathway.id="hsa05310")
```

```
'select()' returned 1:1 mapping between keys and columns
```

```
Info: Working in directory /Users/noellim/Desktop/BIMM143/class13
```

```
Info: Writing image file hsa05310.pathview.png
```

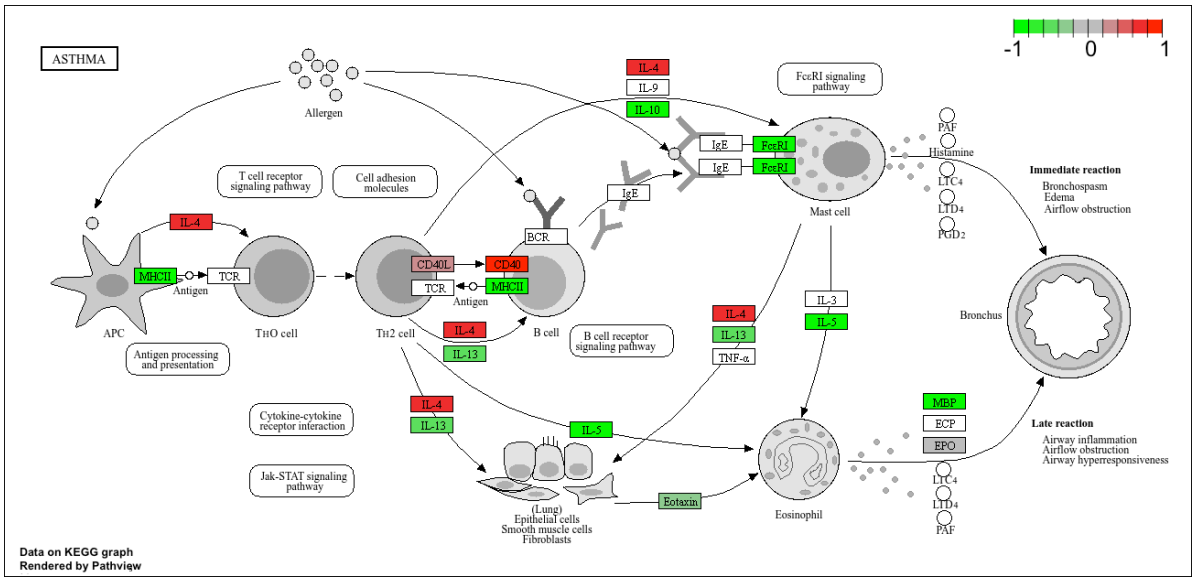


Figure 1: Asthma pathway with my DEGs