Class 13 Transcriptomics A16246401

Scott MacLeod

Transcriptomics

In today's class we will expplore and analyze data from an RNASeq experiment where airway smooth muscles cells were treated with dexamethansone, a synthetic glucocorticoid steroid with anti-inflammatory effects (Himes et al. 2014).

Data Import

We have two input files, so-called "Count data" and "col data".

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

```
counts <- read.csv("airway_scaledcounts.csv",row.names=1)
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 the data set?

```
nrow(counts)
```

```
[1] 38694
```

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

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

```
[1] 4
```

4. Toy differential gene expression

Time to do some analysis.

We have 4 control and 4 treated samples/experiments/columns.

Make sure the metadata ID column matches the column in our count data.

```
colnames(counts)
```

```
[1] "SRR1039508" "SRR1039509" "SRR1039512" "SRR1039513" "SRR1039516"
[6] "SRR1039517" "SRR1039520" "SRR1039521"
```

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

```
[1] TRUE
```

To start I will calculate the `control.mean` and `treat.mean` values and compare them.

-Identify and extract the `control` only columns. -Determine the mean value for each gene (i.e row) -Do the same for `treated`.

First the `control`.

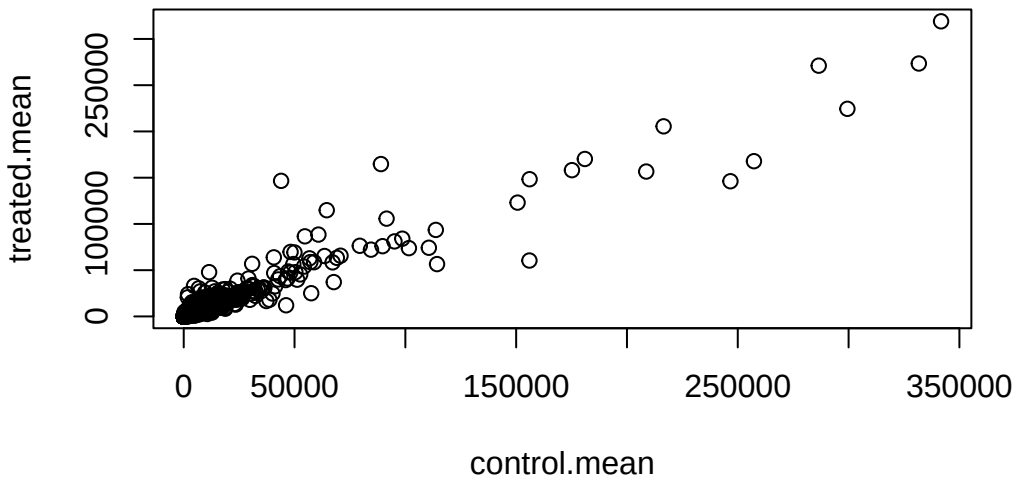
```
#Where does it tell me whicih columns are control?
control.inds <- metadata$dex == "control"
#Above shows how to extract which is control or treated. True is control
control.counts <- counts[,control.inds]
control.mean <- apply(control.counts, 1, mean)
```

Now let's get the mean for `treated`.

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

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

```
plot(meancounts)
```

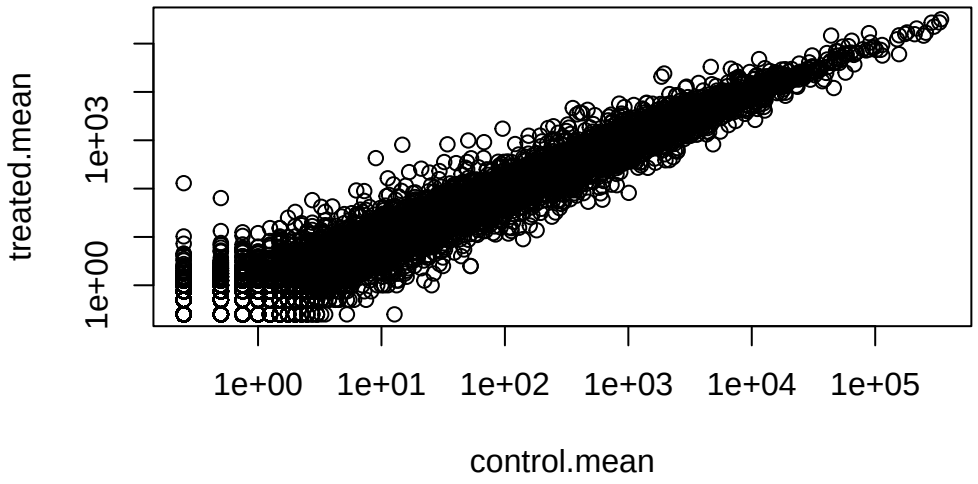


THis data is screaming at us to log transform as it is so heavily skewed and over such a wide rangee.

```
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
```



I want to compare the treated and then control values here and we will use fold change in log2 units to do this. Essentially: log2(treated/control)

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

Some log review: No difference

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

```
[1] 0
```

A doulbing in the treated:

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

```
[1] 1
```

```
log2(5/10)
```

```
[1] -1
```

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

```
[1] 2
```

```
log2(2.5/10)
```

```
[1] -2
```

A common rule of thumb cut-off for calling a gene "differentially expressed" is a log2 fold-change value of either $> +2$ or < -2 for "up regulated" and "down regulated" respectively.

```
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
```

We first need to remove zero count genes - as we can't say anything about these genes anyway and their division of log values are messing things up (divide by zero or log of 0) or the infiinity log problem.

```
zero.vals <- which(meancounts[,1:2]==0, arr.ind=TRUE)

to.rm <- unique(zero.vals[,1])
mycounts <- meancounts[-to.rm,]
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
```

This table is much better!

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

Q. How many genes do we have left that we can say something about (i.e. they don't have any zero counts)?

```
nrow(mycounts)
```

```
[1] 21817
```

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

```
up.ind <-sum(mycounts$log2fc > +2, na.rm =TRUE)
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?

```
down.ind <- sum(mycounts$log2fc < -2, na.rm =TRUE)
down.ind
```

```
[1] 367
```

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

We do trust these results becaue we have no done substanstial statistical analysis. The change is not significant! We are missing stats!

DESeq Analysis

Let's do this properly with the help of the DESeq2 package

```
library(DESeq2)
```

We have to use a specific data object for working DESeq.

```
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 our main analysis with the `DESeq()` fucntion.

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

To get the results out of our `dds` object we can use our DESeq function called `results()`:

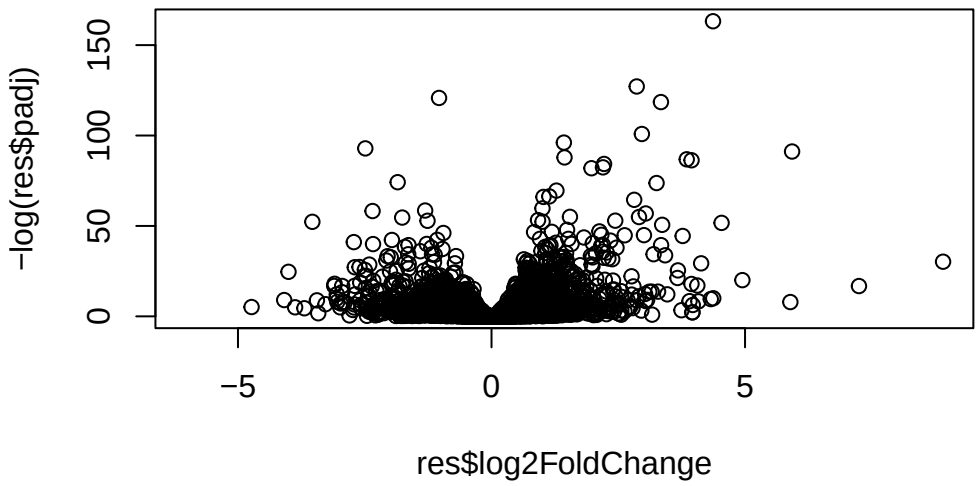
```
res <- results(dds)
head(res)
```

```
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
```

Volcano Plot

A very common and useful summary results figure from this type of analysis is called a volcano plot - a plot of log2FC vs P-value. We use the `padj`, the adjusted P-Value for multiple testing.

```
plot(res$log2FoldChange, -log(res$padj))
```



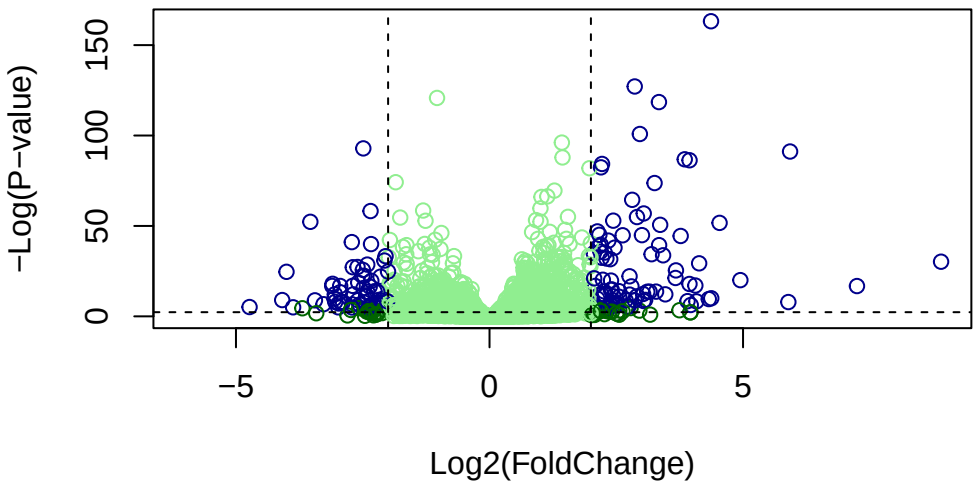
now let's add some color.

```
mycols <- rep("lightgreen", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ]  <- "darkgreen"

inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "darkblue"

plot( res$log2FoldChange,  -log(res$padj),
 col=mycols, ylab="-Log(P-value)", xlab="Log2(FoldChange)" )

abline(v=c(-2,2), col="black", lty=2)
abline(h=-log(0.1), col="black", lty=2)
```




```
log(0.000005)
```

```
[1] -12.20607
```

```
log(0.05)
```

```
[1] -2.995732
```

```
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"
```

```
res$symbol <- mapIds(org.Hs.eg.db,
                     keys=row.names(res), # Our genenames
                     keytype="ENSEMBL",        # The format of our genenames
                     column="SYMBOL",          # The new format we want to add
                     multiVals="first")
```

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

```
head(res)
```

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 7 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      symbol
                <numeric> <character>
ENSG00000000003  0.163035      TSPAN6
ENSG00000000005        NA        TNMD
ENSG00000000419  0.176032        DPM1
ENSG00000000457  0.961694       SCYL3
ENSG00000000460  0.815849    C1orf112
ENSG00000000938        NA         FGR
```

I also want entrez IDs

```
res$entrez <- mapIds(org.Hs.eg.db,
                     keys=row.names(res), # Our genenames
```

```
                    keytype="ENSEMBL",        # The format of our genenames
                    column="ENTREZID",          # The new format we want to add
                    multiVals="first")
```

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

```
head(res)
```

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 8 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      symbol      entrez
                <numeric> <character> <character>
ENSG00000000003  0.163035      TSPAN6        7105
ENSG00000000005        NA        TNMD       64102
ENSG00000000419  0.176032        DPM1        8813
ENSG00000000457  0.961694       SCYL3       57147
ENSG00000000460  0.815849    C1orf112       55732
ENSG00000000938        NA         FGR        2268
```

```
res$GENENAME <- mapIds(org.Hs.eg.db,
                    keys=row.names(res), # Our genenames
                    keytype="ENSEMBL",        # The format of our genenames
                    column="GENENAME",          # The new format we want to add
                    multiVals="first")
```

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

```
head(res)
```

```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 6 rows and 9 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      symbol      entrez              GENENAME
                <numeric> <character> <character>           <character>
ENSG00000000003  0.163035      TSPAN6        7105          tetraspanin 6
ENSG00000000005        NA        TNMD       64102            tenomodulin
ENSG00000000419  0.176032        DPM1        8813 dolichyl-phosphate m..
ENSG00000000457  0.961694       SCYL3       57147 SCY1 like pseudokina..
ENSG00000000460  0.815849    C1orf112       55732 chromosome 1 open re..
ENSG00000000938        NA         FGR        2268 FGR proto-oncogene, ..
```

##Pathway Analysis

Now that I have added the necessart annotation data I can talk to different databases that use these IDs.

We will use the **gage** package to do geneset analysis (a.k.a pathway analysis, geneset enrichment, overlap analysis)

```
library(pathview)
```

```
#############################################################################
Pathview is an open source software package distributed under GNU General
Public License version 3 (GPLv3). Details of GPLv3 is available at
http://www.gnu.org/licenses/gpl-3.0.html. Particullary, users are required to
formally cite the original Pathview paper (not just mention it) in publications
or products. For details, do citation("pathview") within R.

The pathview downloads and uses KEGG data. Non-academic uses may require a KEGG
license agreement (details at http://www.kegg.jp/kegg/legal.html).
#############################################################################
```

```
library(gage)
```

```
library(gageData)
```

We will use KEGG first

```
data(kegg.sets.hs)

# Examine the first 2 pathways in this kegg set for humans
head(kegg.sets.hs, 2)
```

```
$`hsa00232 Caffeine metabolism`
[1] "10"   "1544" "1548" "1549" "1553" "7498" "9"

$`hsa00983 Drug metabolism - other enzymes`
 [1] "10"     "1066"   "10720"  "10941"  "151531" "1548"   "1549"   "1551"
 [9] "1553"   "1576"   "1577"   "1806"   "1807"   "1890"   "221223" "2990"
[17] "3251"   "3614"   "3615"   "3704"   "51733"  "54490"  "54575"  "54576"
[25] "54577"  "54578"  "54579"  "54600"  "54657"  "54658"  "54659"  "54963"
[33] "574537" "64816"  "7083"   "7084"   "7172"   "7363"   "7364"   "7365"
[41] "7366"   "7367"   "7371"   "7372"   "7378"   "7498"   "79799"  "83549"
[49] "8824"   "8833"   "9"      "978"
```

The main `gage()` function requires a named vector of fold changes, where the names of the values are the Entrez gene IDs.

```
foldchange <- res$log2FoldChange
names(foldchange) <- res$entrez
head(foldchange)
```

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

Run the analysis

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

Lets look at what is in our results here

```
attributes(keggres)
```

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

```
head(keggres$less, 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
```

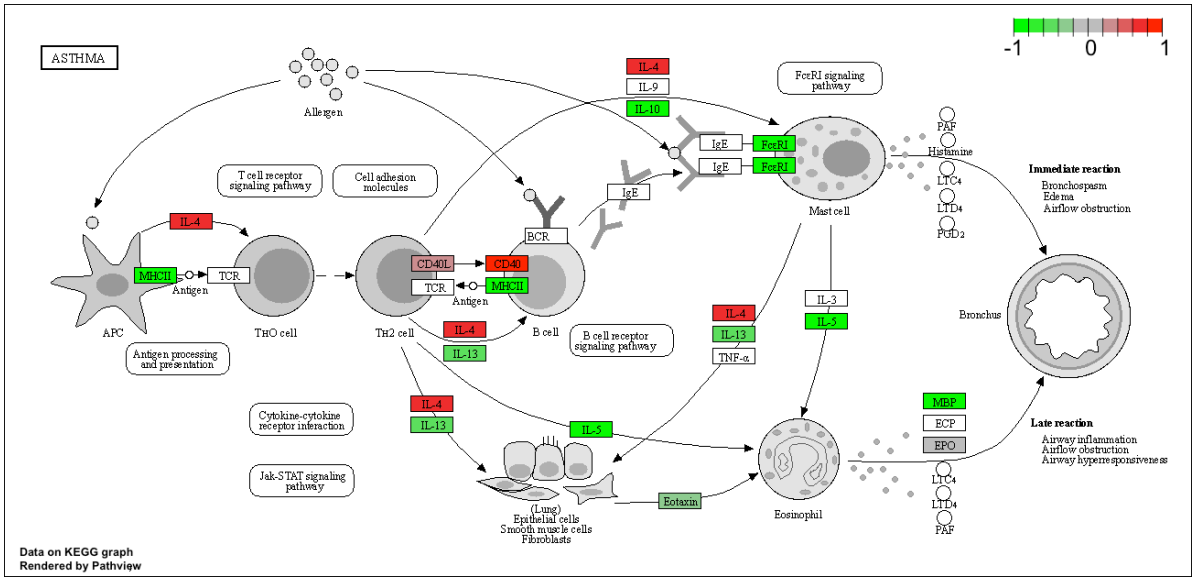
I can now use the returned pathway IDs from KEGG as input to the `pathview` package to make pathway figures with our DEGs.

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

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

```
Info: Working in directory /Users/scottmacleod/Desktop/BIMM 143/RStudio BIMM 143/class13
```

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


ASTHMA
-1
0
1
Allegen
IL-4
IL-9
IL-10
FcεRI signaling pathway
IgE
FcεRI
Mast cell
PAF
Histamine
LTC4
LTD4
PGD2
Immediate reaction
Bronchospasm
Edema
Airflow obstruction
T cell receptor signaling pathway
Cell adhesion molecules
BCR
MHCII
TCR
Antigen
APC
TH0 cell
TH2 cell
B cell
CD40L
CD40
IL-4
IL-13
B cell receptor signaling pathway
Antigen processing and presentation
IL-3
IL-5
IL-13
TNF-α
Bronchus
Cytokine-cytokine receptor interaction
Jak-STAT signaling pathway
(Lung)
Epithelial cells
Smooth muscle cells
Fibroblasts
Eotaxin
Eosinophil
MBP
ECP
EPO
Late reaction
Airway inflamnation
Airflow obstruction
Airway hyperresponsiveness
Data on KEGG graph
Rendered by Pathview