Class11 Lab

Morgan Farrell

2/23/2022

#RNAseq analysis from Asthma study Himes et. al.

```
#install.packages("BiocManager")
#BiocManager::install("DESeq2")
#library(BiocManager)
#library(DESeq2)
```

#Import data set, countData and colData

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

Look at the 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
```

```
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
```

There are 38694 genes

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

```
metadata$dex
```

```
## [1] "control" "treated" "control" "treated" "control" "treated" "control"
## [8] "treated"
```

There are 4 control cell lines

Make sure the control id from the count and metadata match

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

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

```
all(c(T, F, T)) #how the all function works
```

```
## [1] FALSE
```

Test the 'all()' function on the conditional argument we wrote

```
if(all(metadata$id == colnames(counts))){
  cat("yep")
}
```

```
## yep
```

Compare control vs treated cell lines

```
control <- metadata[metadata$dex =="control",] #subset control data from the metadata set
head(control)
```

```
##           id     dex celltype     geo_id
## 1 SRR1039508 control   N61311 GSM1275862
## 3 SRR1039512 control  N052611 GSM1275866
## 5 SRR1039516 control  N080611 GSM1275870
## 7 SRR1039520 control  N061011 GSM1275874
```

```
control.counts <- counts[ ,control$id] #extract control ids from the count data
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
```

Q3. How would you make the above code in either approach more robust? Because we are sub-setting the metadata and the count data by row labels it works for larger datasets too. If we were only working with column locations that may change with different datasets. Also we put the subsetted information into a vector to store all the data we pulled out, opposed to just printing out the entire dataset.

Get treated experiments too, using a similar method to control

```
treated <- metadata[metadata$dex =="treated",]
treated.counts <- counts[ ,treated$id]
head(treated.counts)
```

```
##                 SRR1039509 SRR1039513 SRR1039517 SRR1039521
## ENSG00000000003        486        445       1097        604
## ENSG00000000005          0          0          0          0
## ENSG00000000419        523        371        781        509
## ENSG00000000457        258        237        447        324
## ENSG00000000460         81         66         94         74
## ENSG00000000938          0          0          0          0
```

Find the means of treated and control experiments

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

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

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.mean <- rowMeans( treated.counts )
head(treated.mean)
```

```
## ENSG00000000003 ENSG00000000005 ENSG00000000419 ENSG00000000457 ENSG00000000460
##          658.00            0.00          546.00          316.50           78.75
## ENSG00000000938
##            0.00
```

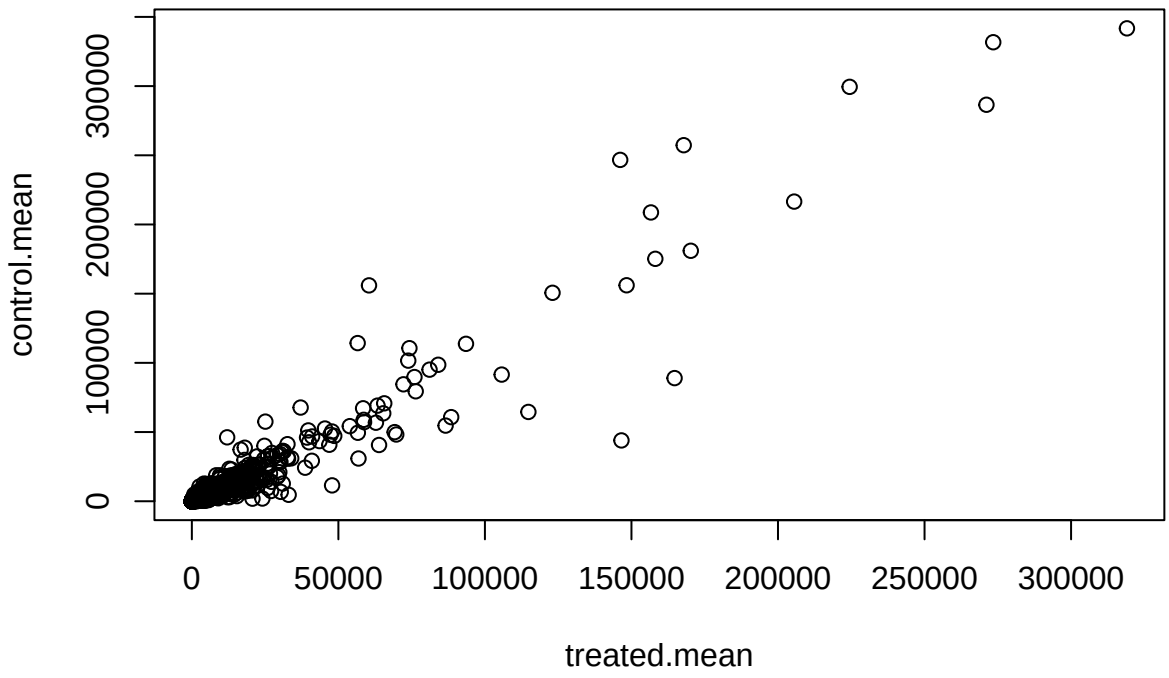
Combine the two datasetts for plotting

```
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
```

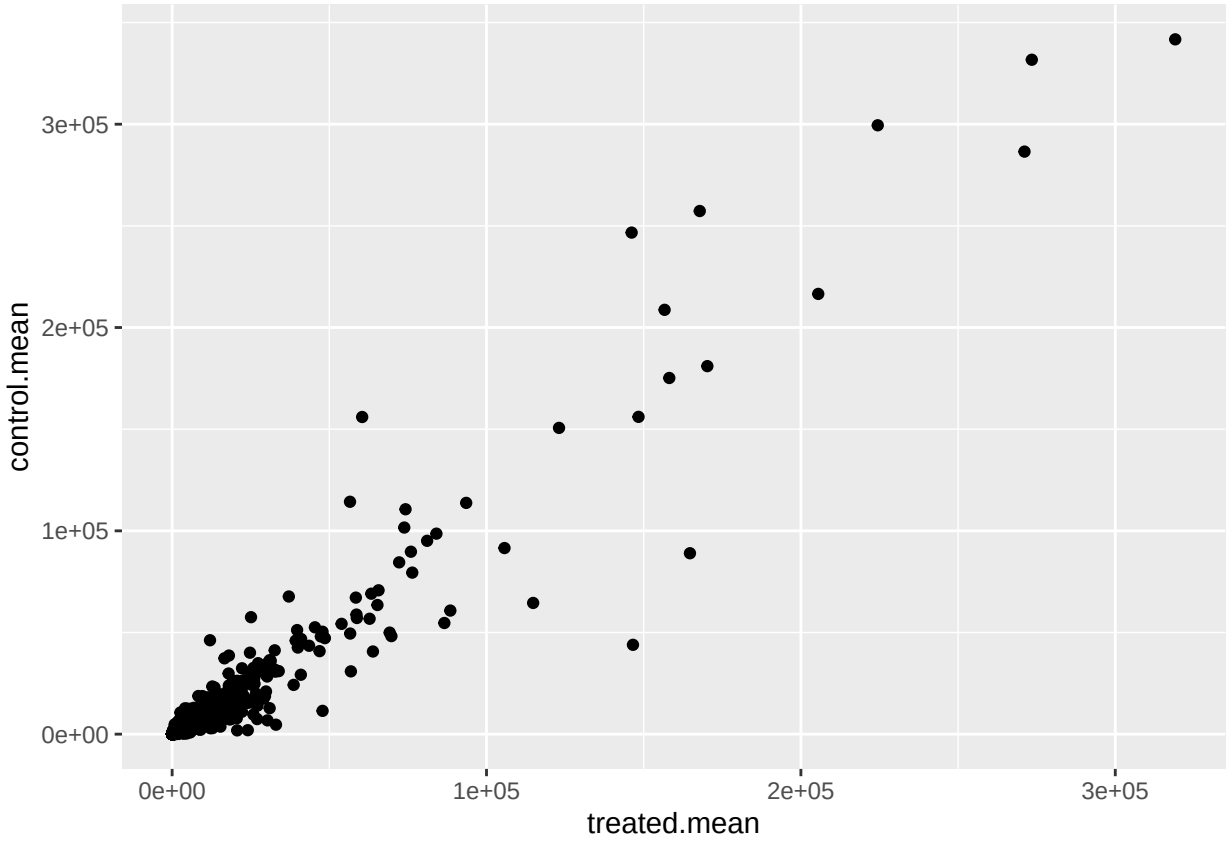
Q5 (a). Create a scatter plot showing the mean of the treated samples against the mean of the control samples. Your plot should look something like the following.

```
plot(treated.mean, control.mean)
```



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(treated.mean, control.mean)) +
  geom_point()
```

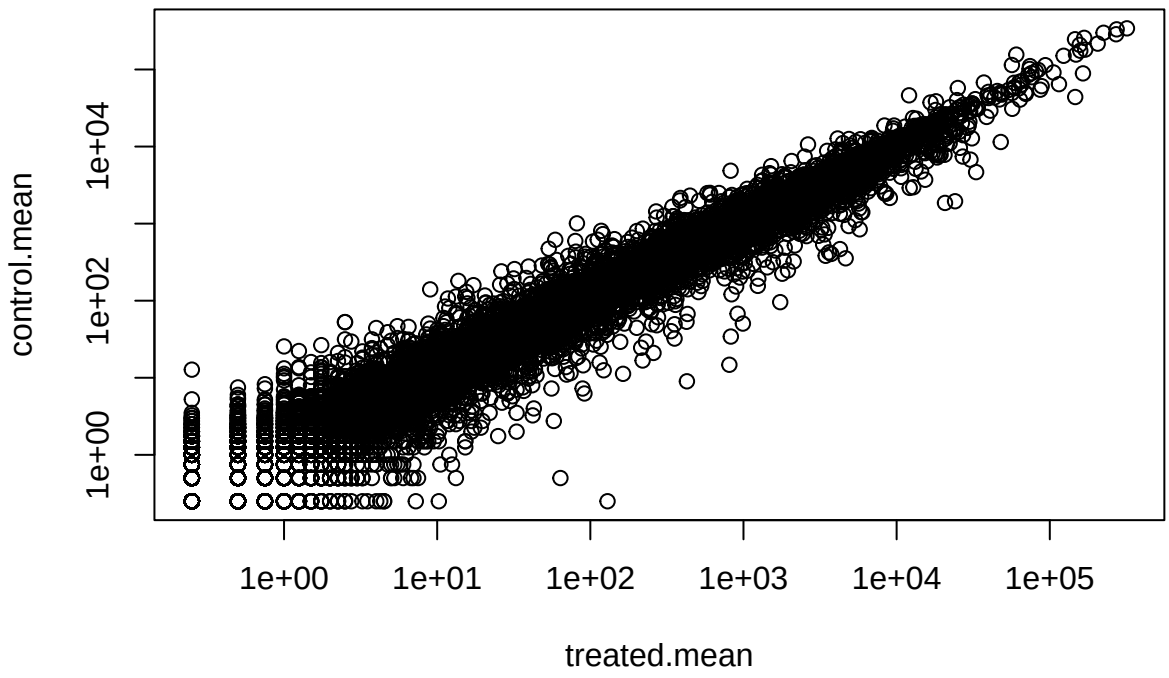


Q6. Try plotting both axes on a log scale. What is the argument to plot() that allows you to do this?

```
plot(treated.mean, control.mean, log="xy")
```

```
## Warning in xy.coords(x, y, xlabel, ylabel, log): 15281 x values <= 0 omitted
## from logarithmic plot
```

```
## Warning in xy.coords(x, y, xlabel, ylabel, log): 15032 y values <= 0 omitted
## from logarithmic plot
```



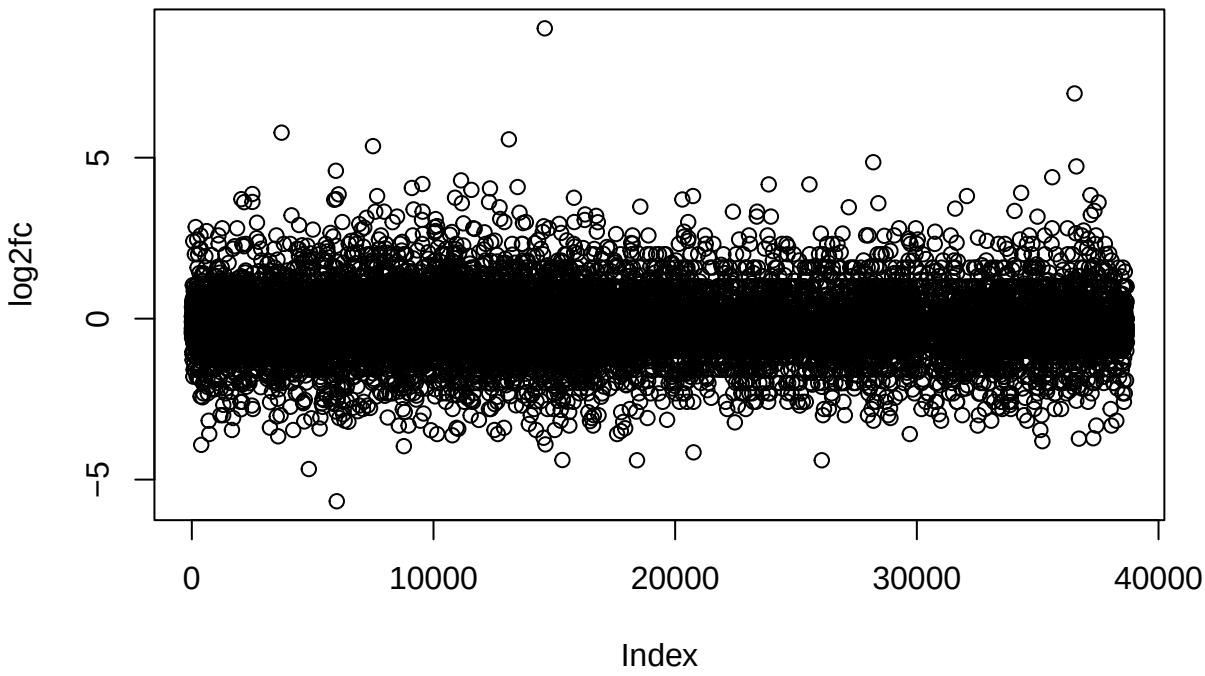
We often use log 2 transformation, because it indicates fold change

```
log2(80/20)
```

```
## [1] 2
```

log2 fold change on data

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



Put all our data in a data.frame

```
meancounts <- data.frame(control.mean, treated.mean, log2fc)
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
```

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?

arr.ind is logical; should array indices be returned when x is an array? So it sorts the matching info into an array with the row and column locations

Get rid of 0s and infinities

```
zero.vals <- which(meancounts[,1:2]== "0", arr.ind = TRUE) #use which function to find the 0s and retur
head(zero.vals)
```

```
##                  row col
## ENSG00000000005    2   1
## ENSG00000004848   65   1
## ENSG00000004948   70   1
## ENSG00000005001   73   1
## ENSG00000006059  121   1
## ENSG00000006071  123   1
```

Find the unique rows with these 0s

```
to.rm <- unique(which(meancounts[,1:2]== "0", arr.ind = TRUE)[,"row"]) #genes to exclude sorted by the
mycounts <- meancounts[-to.rm,] #remove the vector with the 0s
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
```

Genes left after removing the ones with 0s

```
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 <- mycounts$log2fc > 2
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?

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

```
## [1] 367
```

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

Not entirely. These differences in the mean may not be statistically significant based on many factors. The control could be skewed by low sample size for example. We should probably do more summary statistics for both groups before determining significance.

Use DESeq2 for these summary statistics on this dataset

```
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, 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 objects are masked from 'package:base':
##
##     expand.grid, I, unname

## Loading required package: IRanges
```

```
## Loading required package: GenomicRanges

## Loading required package: GenomeInfoDb

## Loading required package: SummarizedExperiment

## Loading required package: MatrixGenerics

## Loading required package: matrixStats

##
## 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
```

```
#citation("DESeq2")
```

Create a DESeq data matrix with our data

```
dds <- DESeqDataSetFromMatrix(countData=counts,
                              colData=metadata,
                              design=~dex) #how are we comparing the data look at the dex column for th
```

```
## converting counts to integer mode

## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
## design formula are characters, converting to factors
```

```
dds
```

```
## class: DESeqDataSet
## dim: 38694 8
## metadata(1): version
## assays(1): counts
## rownames(38694): ENSG00000000003 ENSG00000000005 ... ENSG00000283120
##   ENSG00000283123
## rowData names(0):
## colnames(8): SRR1039508 SRR1039509 ... SRR1039520 SRR1039521
## colData names(4): id dex celltype geo_id
```

```
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)
res
```

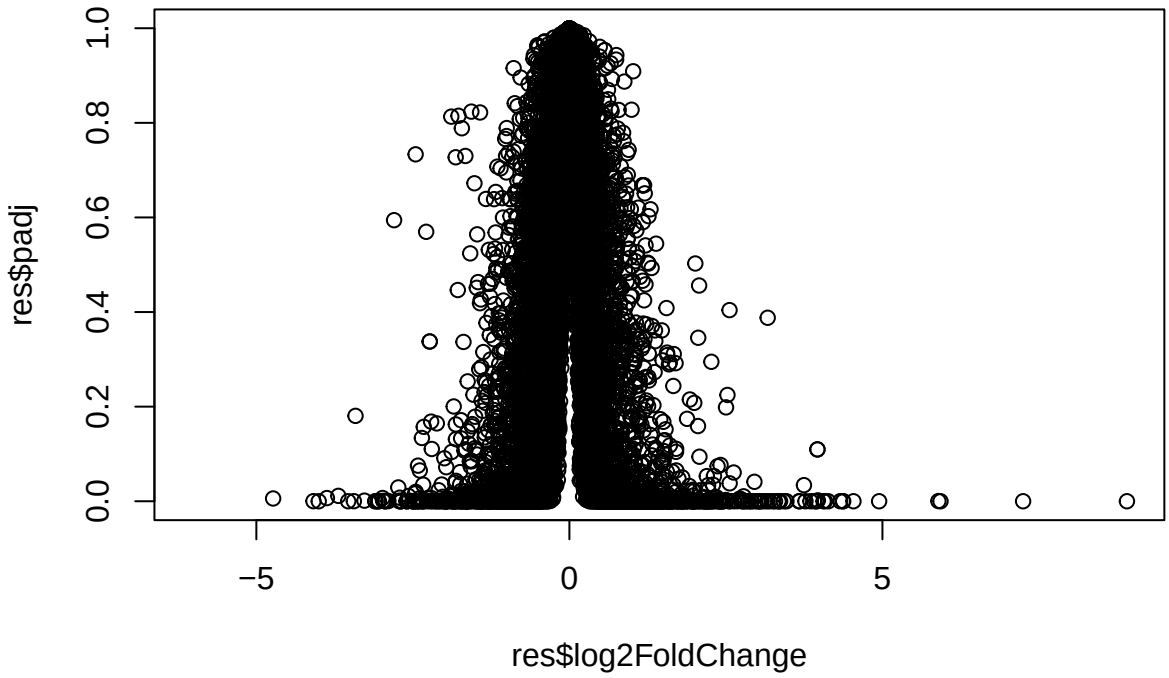
```
## log2 fold change (MLE): dex treated vs control
## Wald test p-value: dex treated vs control
## DataFrame with 38694 rows and 6 columns
##                  baseMean log2FoldChange     lfcSE      stat    pvalue
##                 <numeric>      <numeric> <numeric> <numeric> <numeric>
## ENSG00000000003  747.1942     -0.3507030  0.168246 -2.084470 0.0371175
## ENSG00000000005    0.0000             NA        NA        NA        NA
```

```
## ENSG00000000419  520.1342      0.2061078  0.101059  2.039475 0.0414026
## ENSG00000000457  322.6648      0.0245269  0.145145  0.168982 0.8658106
## ENSG00000000460   87.6826     -0.1471420  0.257007 -0.572521 0.5669691
## ...                  ...             ...       ...       ...       ...
## ENSG00000283115  0.000000             NA        NA        NA        NA
## ENSG00000283116  0.000000             NA        NA        NA        NA
## ENSG00000283119  0.000000             NA        NA        NA        NA
## ENSG00000283120  0.974916      -0.668258   1.69456 -0.394354  0.693319
## ENSG00000283123  0.000000             NA        NA        NA        NA
##                      padj
##                 <numeric>
## ENSG00000000003  0.163035
## ENSG00000000005        NA
## ENSG00000000419  0.176032
## ENSG00000000457  0.961694
## ENSG00000000460  0.815849
## ...                   ...
## ENSG00000283115        NA
## ENSG00000283116        NA
## ENSG00000283119        NA
## ENSG00000283120        NA
## ENSG00000283123        NA
```

Main results figure as a volcano plot

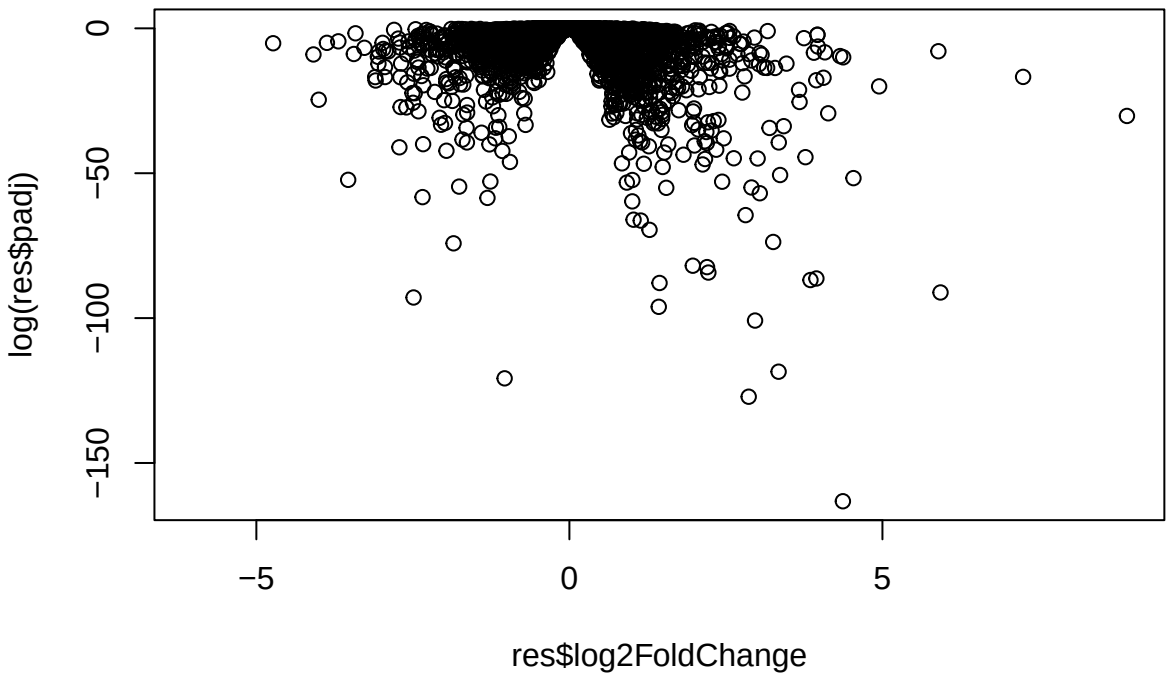
Log2 fold change vs p- value

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



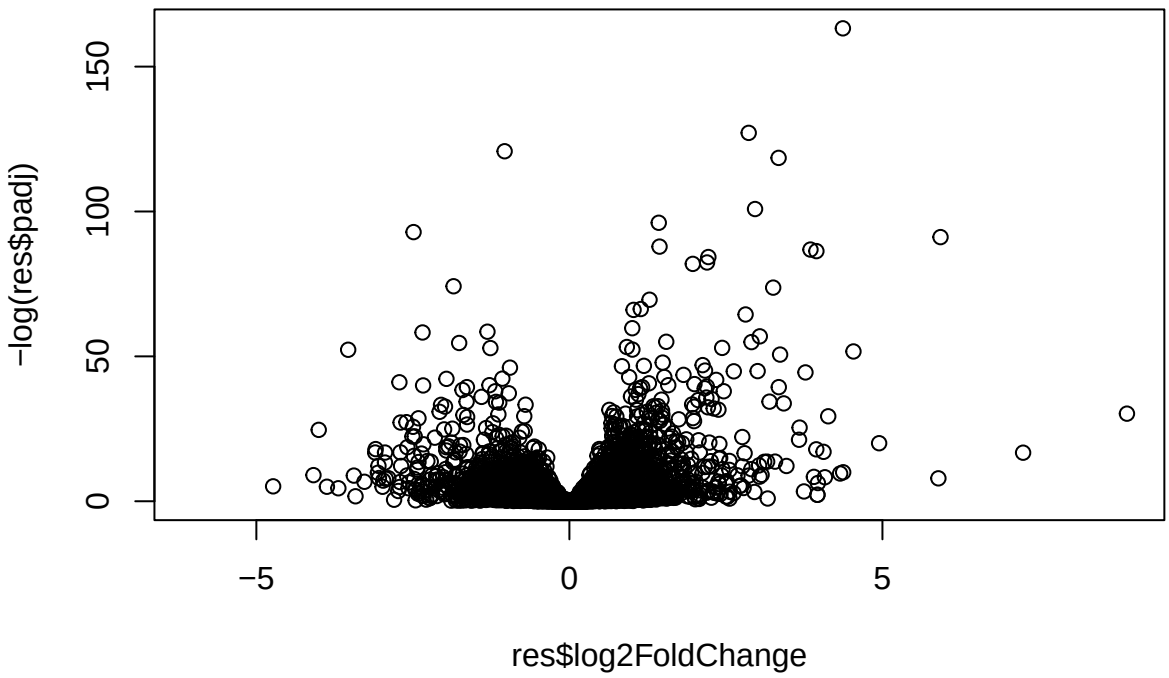
Log transformation of this plot

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



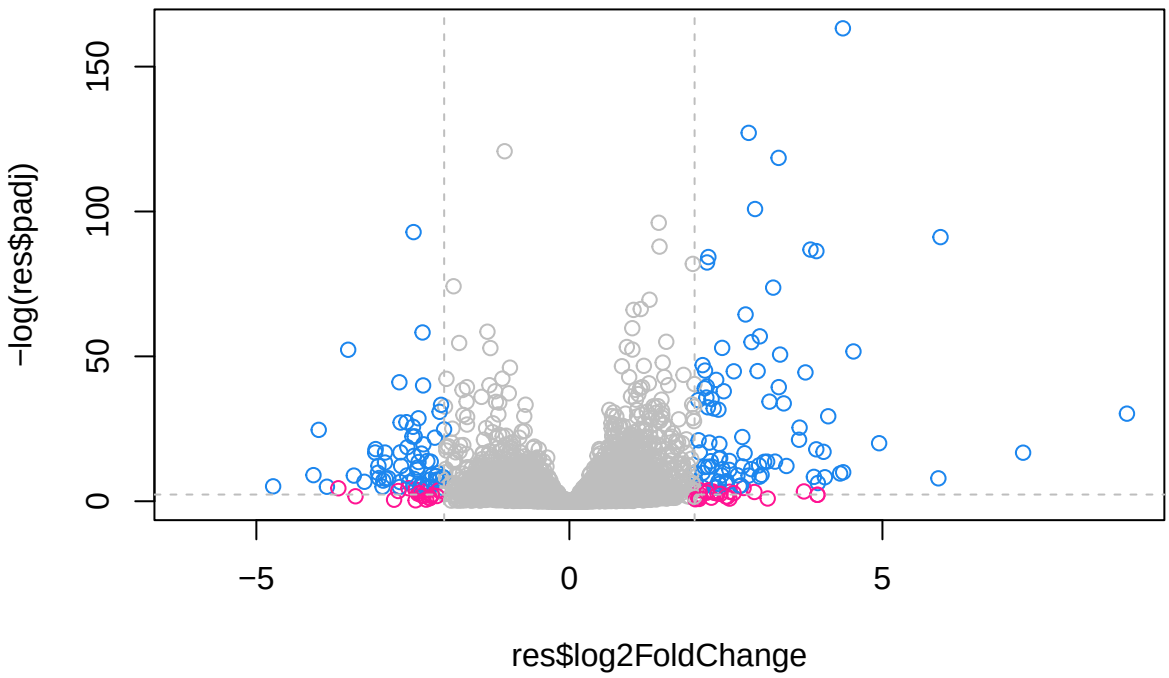
Flip the plot by putting a - in front of our log padj

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




```
mycols <- rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ]  <- "deeppink"
inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "dodgerblue2"
plot(res$log2FoldChange, -log(res$padj), col= mycols)

# Cut-off lines
abline(v=c(-2,2), col="gray", lty=2)
abline(h=-log(0.1), col="gray", lty=2)
```



Add annotation data

Install all the proper packages

```
#BiocManager::install("EnhancedVolcano")
library(EnhancedVolcano)
```

```
## Loading required package: ggrepel
```

```
## Registered S3 methods overwritten by 'ggalt':
##   method                  from
##   grid.draw.absoluteGrob  ggplot2
##   grobHeight.absoluteGrob ggplot2
##   grobWidth.absoluteGrob  ggplot2
##   grobX.absoluteGrob      ggplot2
##   grobY.absoluteGrob      ggplot2
```

```
library(AnnotationDbi)
#BiocManager::install("org.Hs.eg.db")
library(org.Hs.eg.db)
```

```
##
```

Now use 'mapIDs()" function from the AnnotationDbi package to find

```
#First what is available
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
```

Q11. Run the mapIds() function two more times to add the Entrez ID and UniProt accession and GENENAME as new columns called res$entrez$, res$uniprot and res$genename.

Add in all of these new columns based on the mapID function

```
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 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               genename
##                 <numeric> <character>            <character>
## ENSG00000000003  0.163035      TSPAN6          tetraspanin 6
## ENSG00000000005        NA        TNMD            tenomodulin
## ENSG00000000419  0.176032        DPM1 dolichyl-phosphate m..
## ENSG00000000457  0.961694       SCYL3 SCY1 like pseudokina..
## ENSG00000000460  0.815849    C1orf112 chromosome 1 open re..
## ENSG00000000938        NA         FGR FGR proto-oncogene, ..
```

```
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 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               genename      entrez
##                 <numeric> <character>            <character> <character>
## ENSG00000000003  0.163035      TSPAN6          tetraspanin 6        7105
## ENSG00000000005        NA        TNMD            tenomodulin       64102
## ENSG00000000419  0.176032        DPM1 dolichyl-phosphate m..        8813
## ENSG00000000457  0.961694       SCYL3 SCY1 like pseudokina..       57147
## ENSG00000000460  0.815849    C1orf112 chromosome 1 open re..       55732
## ENSG00000000938        NA         FGR FGR proto-oncogene, ..        2268
```

```
res$uniprot <- mapIds(org.Hs.eg.db,
                      keys=row.names(res),      # Our genenames
                      keytype="ENSEMBL",        # The format of our genenames
                      column="UNIPROT",          # 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 10 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               genename      entrez
##                 <numeric> <character>            <character> <character>
## ENSG00000000003  0.163035      TSPAN6          tetraspanin 6        7105
## ENSG00000000005        NA        TNMD            tenomodulin       64102
## ENSG00000000419  0.176032        DPM1 dolichyl-phosphate m..        8813
## ENSG00000000457  0.961694       SCYL3 SCY1 like pseudokina..       57147
## ENSG00000000460  0.815849    C1orf112 chromosome 1 open re..       55732
## ENSG00000000938        NA         FGR FGR proto-oncogene, ..        2268
##                    uniprot
##                <character>
## ENSG00000000003  A0A024RCI0
## ENSG00000000005      Q9H2S6
## ENSG00000000419      O60762
## ENSG00000000457      Q8IZE3
## ENSG00000000460  A0A024R922
## ENSG00000000938      P09769
```

```
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)
```

```
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"
```

```
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 we can run gene set enrichment (aka pathway analysis) We can find out what keggres is using the 'attributes()' function

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

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

First look at the downregulated list (less)

```
# Look at the first three down (less) pathways
head(keggres$less, 3)
```

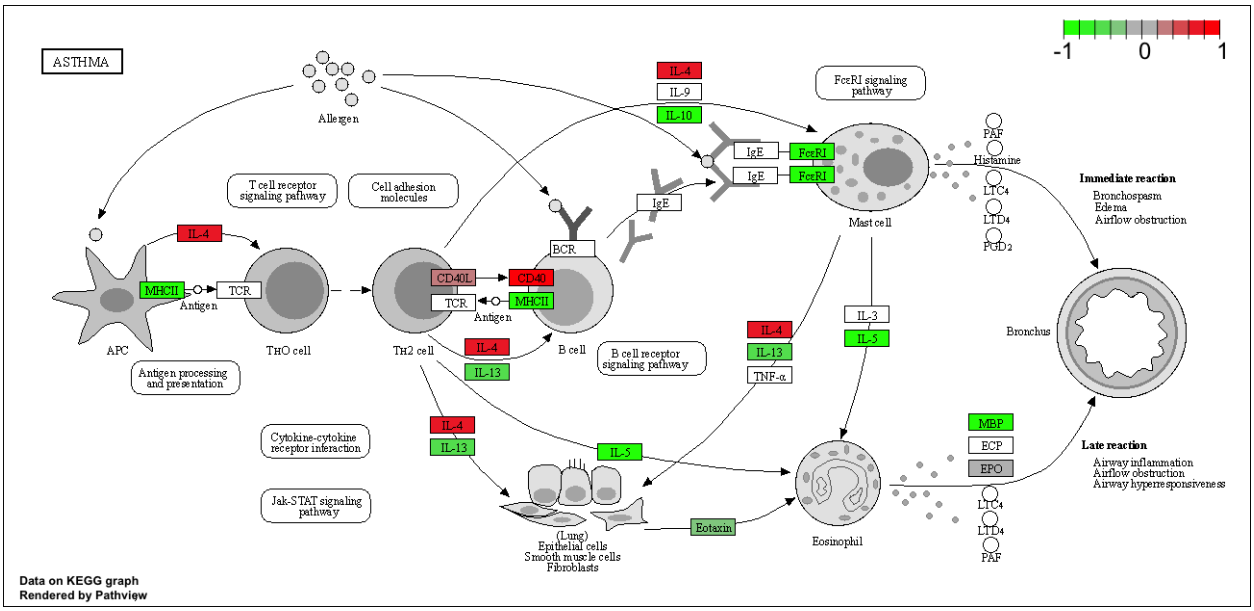
```
##                                      p.geomean stat.mean        p.val
## hsa05332 Graft-versus-host disease 0.0004250461 -3.473346 0.0004250461
## hsa04940 Type I diabetes mellitus  0.0017820293 -3.002352 0.0017820293
## hsa05310 Asthma                    0.0020045888 -3.009050 0.0020045888
##                                         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
```

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

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

```
## Info: Working in directory /Users/morganfarrell/Documents/PhD Research/UCSD Courses/BGGN213- Bioinfo
```

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




```
# A different PDF based output of the same data
pathview(gene.data=foldchanges, pathway.id="hsa05310", kegg.native=FALSE)
```

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

```
## Info: Working in directory /Users/morganfarrell/Documents/PhD Research/UCSD Courses/BGGN213- Bioinfo
```

```
## Info: Writing image file hsa05310.pathview.pdf
```

Save the results to a CSV

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