Class 13

Lena (A16420052)

```
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

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
```

Today we will examine RNA seq data from Himes et al.

Input countData and colData

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

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

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

```
[1] 4
```

```
# can use table() function as well
table(metadata$dex)
```

```
control treated
      4       4
```

Toy differential gene expression

Lets perform some exploratory differential gene expression analysis - step 1: find which columns in `counts` correspond to "control" samples -step 2: calculate the mean value per gene in these columns -step 3: store my answer for later in `control.mean`

This calculates the mean counts per gene across these samples:

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

Using dplyr instead

```
library(dplyr)
```

```
Attaching package: 'dplyr'

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

    combine

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

    count

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

    intersect, setdiff, union

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

    intersect

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

    collapse, desc, intersect, setdiff, slice, union

The following objects are masked from 'package:S4Vectors':

    first, intersect, rename, setdiff, setequal, union

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

    combine, intersect, setdiff, union
```

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

    filter, lag

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

    intersect, setdiff, setequal, union
```

```
#filter by $dex in metadata by "control"
control <- metadata %>% filter(dex=="control")
#select from id that are controls from count
control.counts <- counts %>% select(control$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?

We can use `rowMeans()` instead of `rowSums(control.counts)/4` to make the code more robust.

```
#barry's code
control.inds <- metadata$dex=="control"
metadata[control.inds, ]
```

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

using dplyr...

```
#treated <- metadata %>% filter(dex=="treated")
#treated.counts <- counts %>% select(control$id)
#treated.mean <- rowMeans(treated.counts)
#head(treated.mean)
```

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

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

To keep us tidy lets put `control.mean` and `treated.mean` vectors together as two columns of a new data frame

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

```
                control.mean treated.mean
ENSG00000000003       900.75       658.00
```

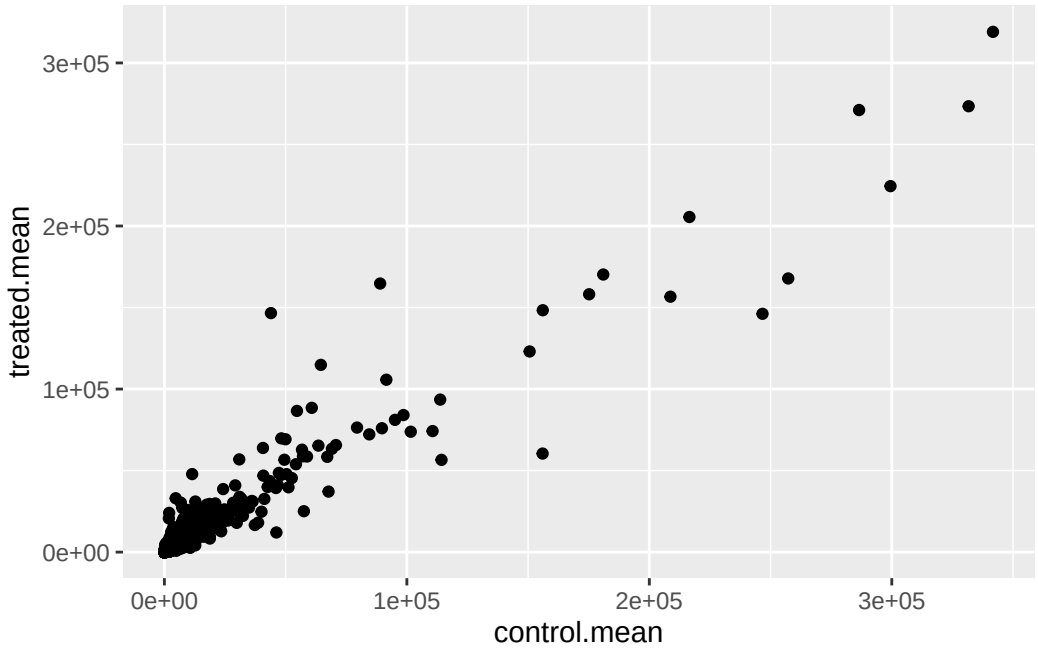
```
ENSG00000000005         0.00         0.00
ENSG00000000419       520.50       546.00
ENSG00000000457       339.75       316.50
ENSG00000000460        97.25        78.75
ENSG00000000938         0.75         0.00
```

```
colSums(meancounts)
```

```
control.mean treated.mean
    23005324     22196524
```

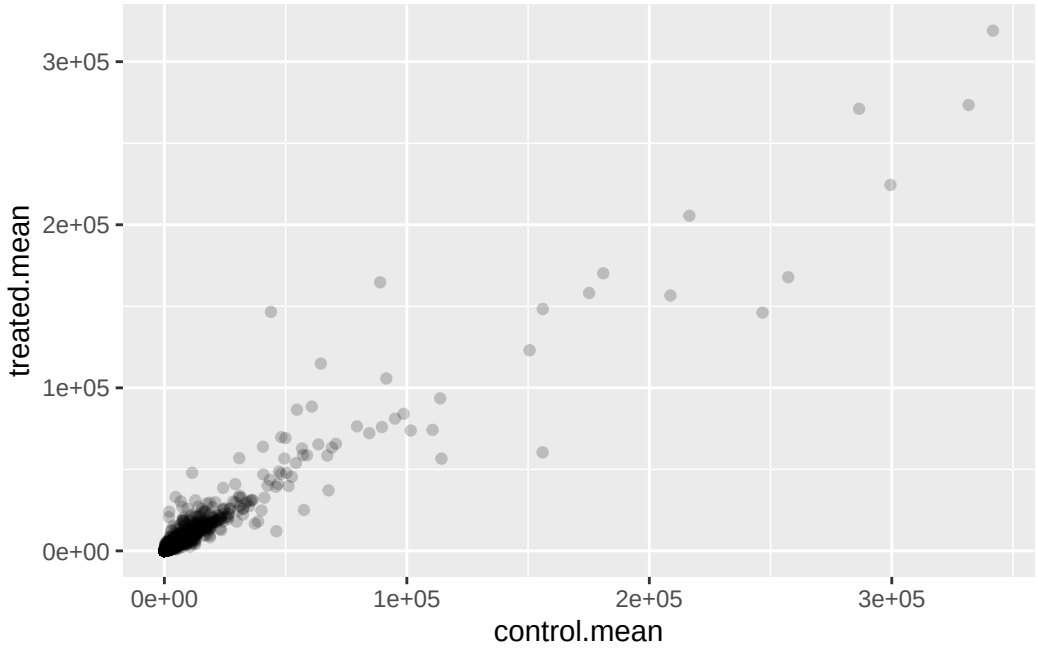
Q5 (a). Create a scatter plot showing the mean of the treated samples against the mean of the control samples. Your plot should look something like the following.

```
library(ggplot2)
ggplot(meancounts, aes(control.mean,treated.mean)) + geom_point()
```



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?

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

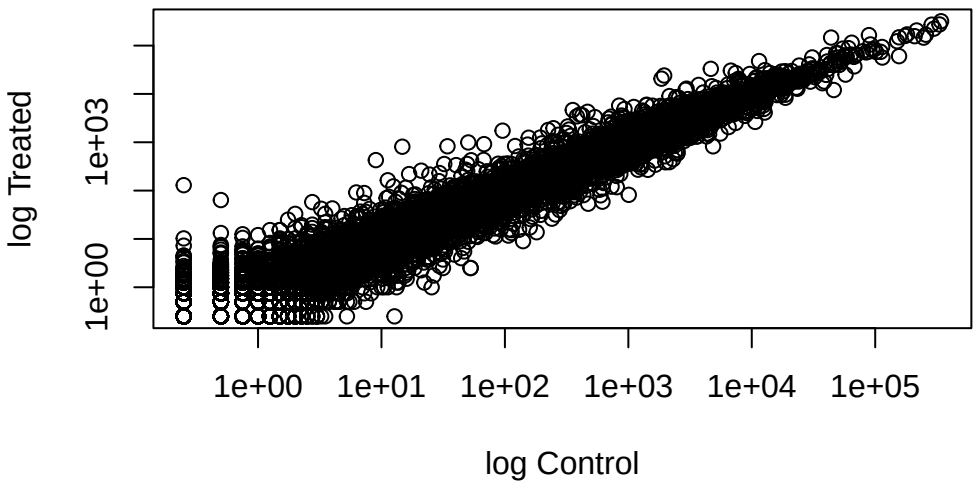


Q6. Try plotting both axes on a log scale. What is the argument to plot() that allows you to do this?

```
plot(meancounts, log="xy", xlab= "log Control", ylab= "log Treated")
```

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

```
[1] -1
```

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

```
[1] 1
```

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

```
[1] 2
```

Lets add a log2 fold-change colum to our little `mean.counts()` 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
```

```
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 of "weird" results. Namely, the NaN ("not a number") and -Inf (negative infinity) results.

The NaN is returned when you divide by zero and try to take the log. The -Inf is returned when you try to take the log of zero. It turns out that there are a lot of genes with zero expression. Let's filter our data to remove these genes.

```
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
```

```
#gets rid of any genes that are true (==1)
to.rm.inds <- rowSums(meancounts[, 1:2]==0)>0
head(meancounts[!to.rm.inds, ])
```

```
                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
```

The ! mark flips TRUE value to FALSE and vice versa...

```
random_var <- c(T, F, T)
which(random_var)
```

```
[1] 1 3
```

```
!random_var
```

```
[1] FALSE  TRUE FALSE
```

```
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
```

```
dim(mycounts)
```

```
[1] 21817     3
```

> 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?

The purpose of arr.ind=TRUE argument is to have the which() function return row and column indices where they equal TRUE. Calling the unique() function will help us make sure we do not count any rows twice if it has zero entries in both samples.

genes(row) samples(column)

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
```

There are 250 up regulated genes that are greater than 2 fc level. > 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
```

There are 367 down regulated genes that are greater than 2 fc level.

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

No I do not trust these results. Analysis has been based on large fold changes, but we are forgetting about statistical significance of these differences.

Setting up for DESeq

We will now use DEseq2 package to do this analysis properly

```
#call DEseq package
library(DESeq2)

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
```

Importing data

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

Get our results back from the `dds` object

```
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
```

Adding annotation data

We will use one of Bioconductor's main annotation packages to help with mapping between various ID schemes and add the necessary annotation data to our results.

```
library("AnnotationDbi")
```

```
Warning: package 'AnnotationDbi' was built under R version 4.3.2
```

```
Attaching package: 'AnnotationDbi'
```

```
The following object is masked from 'package:dplyr':

    select
```

```
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"
```

The main function we will use here is called `mapIds()`

Our current IDs are here:

```
#mapIds()
head(row.names(res))
```

```
[1] "ENSG00000000003" "ENSG00000000005" "ENSG00000000419" "ENSG00000000457"
[5] "ENSG00000000460" "ENSG00000000938"
```

```
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       FIRRM
ENSG00000000938        NA         FGR
```

Lets add GENENAME

```
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       FIRRM FIGNL1 interacting r..
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
```

A summary results plot

Volcano plot

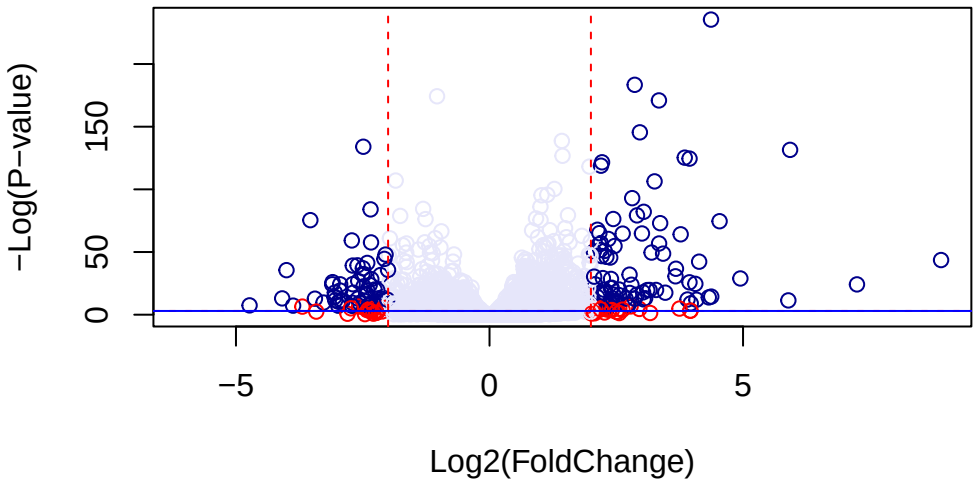
This is a common type of summary figure that keeps both of our inner biologist and inner stats nerd happy because it shows both P-values and Log2(Fold-Changes)

```
mycols <- rep("lavender", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ]  <- "red"
inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "darkblue"

plot(res$log2FoldChange, -log2(res$padj),
     col=mycols,
     xlab="Log2(FoldChange)",
      ylab="-Log(P-value)")

# Add some cut-off lines
abline(v=c(-2,2), col="red", lty=2)
abline(h=-log(0.05), col="red", lty=2)
```

```
abline(h=-log(0.05), col="blue")
```



Save our results to date...

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

Pathway Analysis

Install pathview package and gage packages`BiocManager::install( c("pathview", "gage", "gageData") )` to do geneset enrichement (aka pathway analysis) and figure generation respectively.

```
library(pathview)
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"
```

What we need for `gage()` is our genes in ENTREZ id format with a measure if their importance.

It wants a vector of eg. fold changes

```
foldchanges <-  res$log2FoldChange
head(foldchanges)
```

```
[1] -0.35070302          NA  0.20610777  0.02452695 -0.14714205 -1.73228897
```

```
x <- c(100, 80, 100)
names(x) <- c("desteny", "barry", "chris")
x
```

```
desteny   barry   chris
    100      80     100
```

Add ENTREZ ids as `names()` to my `foldchanges` vector.

```
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 `gage()` with this input vector and the genset we want to examine for overlap/enrichment...

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

Look at the results

```
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
```

We can view these pathways with our geneset genes highlighted using the `pathview()` function. E.g. for "Asthma" I will use the pathway.id hsa05310 as seen above

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

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

```
Info: Working in directory /Users/lenayang/Downloads/bimm 143/Class 13
```

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

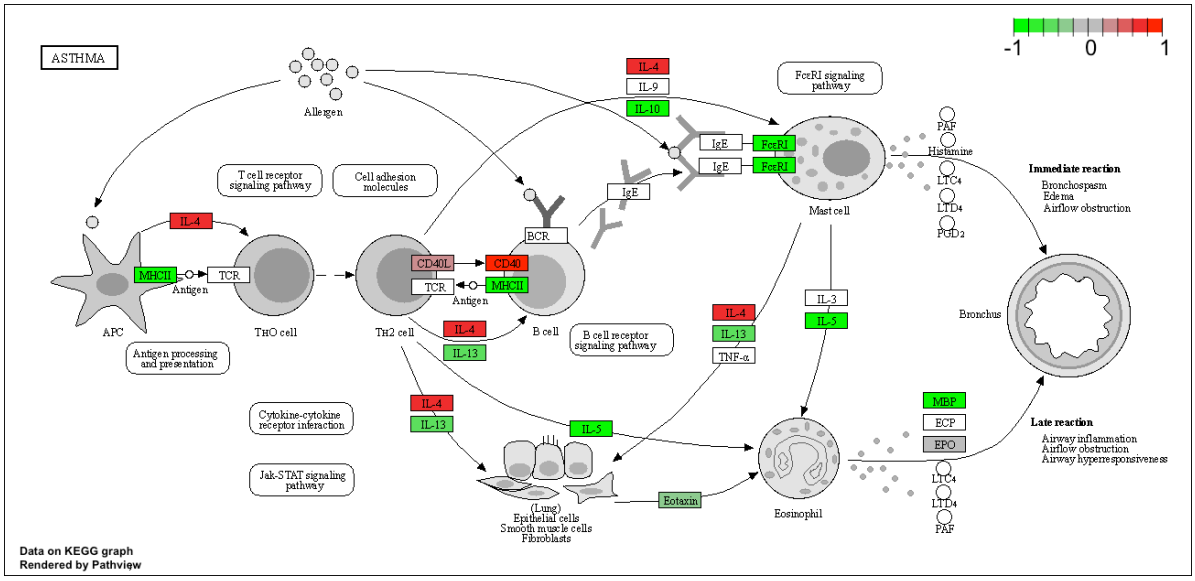


Figure 1: My genes involved in Asthma