Class 13: RNASeq Analysis with DESq2

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The data for this hands-on session comes from a published RNA-seq experiment where airway smooth muscle cells were treated with **dexamethasone** (dex), a synthetic glucocorticoid steroid with anti-inflammatory effects (Himes et al. 2014).

```
# Complete the missing code
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
```

> Q1. How many genes are in this dataset?

```
nrow(counts)
```

```
[1] 38694
```

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

```r
table(metadata$dex)
```

```

control treated 
      4       4 
```

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

```
[1] 4
```

Toy differential gene expression

Let's start by calculating the mean counts per gene in the "control" samples. We can then compare this value for each gene to the mean counts in the "treated" samples (i.e. columns).

- Step 1. Find which columns in the 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`

```r
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
```

```r
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
```

> Q3. How would you make the above code in either approach more robust? Is there a function that could help here? You can use a code that does not state the number of samples, like RowMeans

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

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)

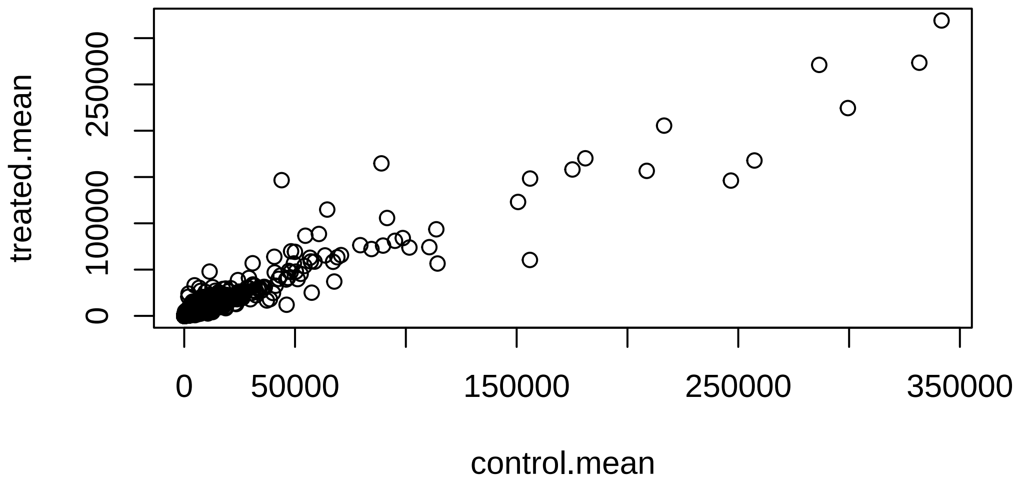
```
meancounts <- data.frame(control.mean, treated.mean)
```

```
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.

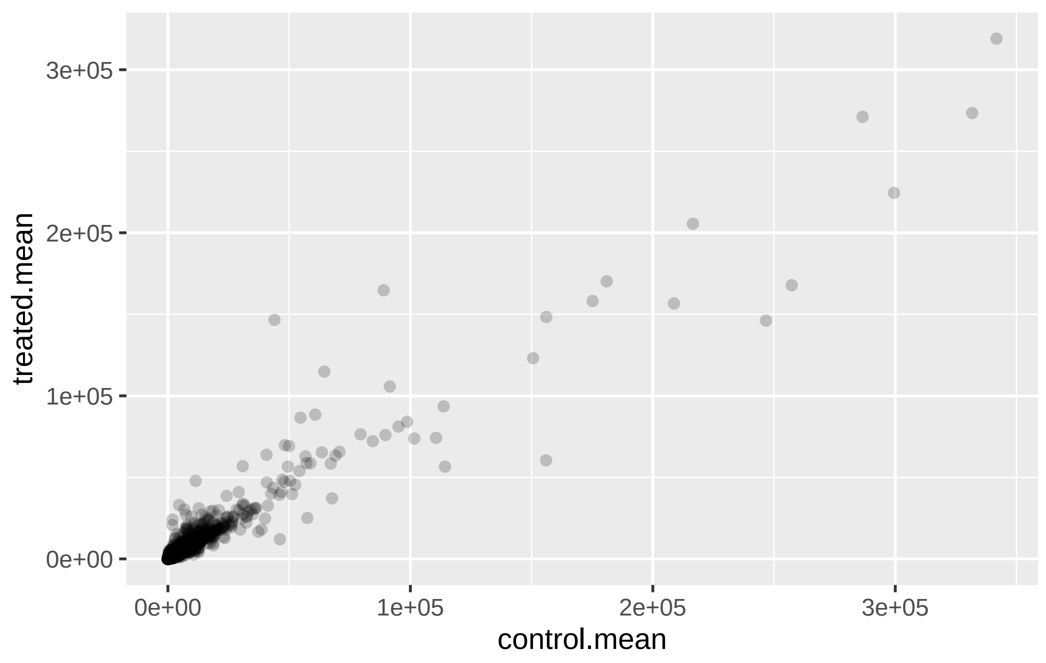
```
plot(meancounts)
```



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? a ggplot version:

```
library(ggplot2)

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

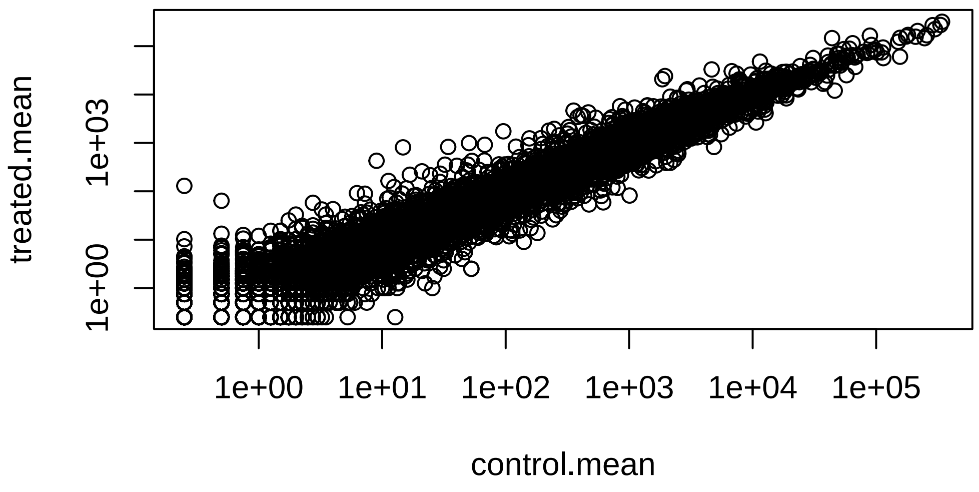


Q6. Try plotting both axes on a log scale. What is the argument to plot() that allows you to do this?

```r
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
```



Log transformation 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 butt we most often prefer log2 units.

```
#Treated/Control
log2(10/10)
```

```
[1] 0
```

What if there was a doubling

```
# Treated/Control
log2(20/10)
```

```
[1] 1
```

Half counts

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

```
[1] -1
```

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

```
[1] 2
```

```
log10(40/10)
```

```
[1] 0.60206
```

Let's add a log2 fold-change column 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
```

There are a couple of weird results. Namely, the NaN (not a number) and -INf 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. Again inspect your result (and the intermediate steps) to see if things make sense to you

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

The `!` mark flips TRUE values to FALSE and vice-versa…

```
x <- c(TRUE, FALSE, TRUE)
!x
```

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

```
x
```

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

```
which(x)
```

```
[1] 1 3
```

```
dim(mycounts)
```

```
[1] 21817     3
```

```
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
```

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.

> 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 arr. ind argument helps the which output the row and column positions and if you use it with TRUE, it will only give the true values and give the ones that have 0. If you use unique() it helps to not count the same row two times.

```
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?

```r
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?

```r
sum(down.ind)
```

```
[1] 367
```

> Q10. Do you trust these results? Why or why not? There could be huge variance and we do not have significance, we need to know if that difference could be significant.

But we forgot all about statistical significance of these differences…

We will use the DESeq2 package to do this analysis properly…

Using DESeq2

Like any package we must load it up with a `library()` call.

```r
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
```

Setup the input object required by DESeq

```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
```

Now we can run our DESeq analysis

```r
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.

```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
```

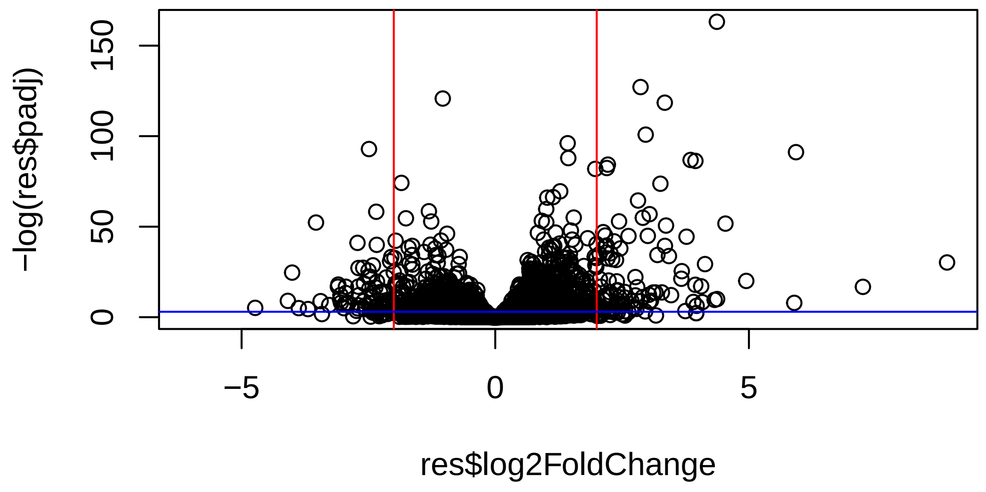
```
ENSG00000000419 520.134160      0.2061078  0.101059  2.039475 0.0414026
ENSG00000000457 322.664844      0.0245269  0.145145  0.168982 0.8658106
ENSG00000000460  87.682625     -0.1471420  0.257007 -0.572521 0.5669691
ENSG00000000938   0.319167     -1.7322890  3.493601 -0.495846 0.6200029
                     padj
                <numeric>
ENSG00000000003  0.163035
ENSG00000000005        NA
ENSG00000000419  0.176032
ENSG00000000457  0.961694
ENSG00000000460  0.815849
ENSG00000000938        NA
```

#A Summary results plot

Volcano plot.

This is a common type of summary figure that keeps both our inner biologist and inner stats nerd happy because it shows both P-values and log2 (fold-changes)

```
plot(res$log2FoldChange, -log(res$padj))
abline(v=2, col="red")
abline(v=-2, col="red")
abline( h=-log(0.05), col="blue")
```



```r
log(0.1)
```

```
[1] -2.302585
```

```r
log(0.00001)
```

```
[1] -11.51293
```

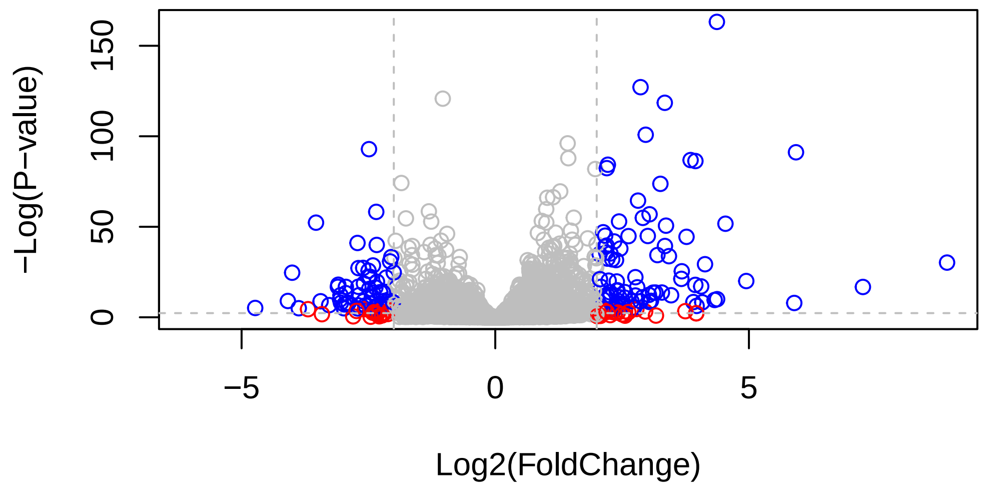
```r
# Setup our custom point color vector
mycols <- rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ]  <- "red"

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

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

# Cut-off lines
```

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



Save our results to date.....

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

```
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

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

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

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

```
org.Hs.eg.db
```

```
OrgDb object:
| DBSCHEMAVERSION: 2.1
| Db type: OrgDb
| Supporting package: AnnotationDbi
| DBSCHEMA: HUMAN_DB
| ORGANISM: Homo sapiens
| SPECIES: Human
| EGSOURCEDATE: 2023-Sep11
| EGSOURCENAME: Entrez Gene
| EGSOURCEURL: ftp://ftp.ncbi.nlm.nih.gov/gene/DATA
| CENTRALID: EG
| TAXID: 9606
| GOSOURCENAME: Gene Ontology
| GOSOURCEURL: http://current.geneontology.org/ontology/go-basic.obo
| GOSOURCEDATE: 2023-07-27
| GOEGSOURCEDATE: 2023-Sep11
| GOEGSOURCENAME: Entrez Gene
| GOEGSOURCEURL: ftp://ftp.ncbi.nlm.nih.gov/gene/DATA
| KEGGSOURCENAME: KEGG GENOME
| KEGGSOURCEURL: ftp://ftp.genome.jp/pub/kegg/genomes
| KEGGSOURCEDATE: 2011-Mar15
| GPSOURCENAME: UCSC Genome Bioinformatics (Homo sapiens)
| GPSOURCEURL:
| GPSOURCEDATE: 2023-Aug20
| ENSOURCEDATE: 2023-May10
| ENSOURCENAME: Ensembl
| ENSOURCEURL: ftp://ftp.ensembl.org/pub/current_fasta
| UPSOURCENAME: Uniprot
| UPSOURCEURL: http://www.UniProt.org/
| UPSOURCEDATE: Mon Sep 18 16:12:39 2023


Please see: help('select') for usage information
```

```
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"
```

Our current IDs are here: The main function we will use here is called `mapIds()`

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

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

These are in ENSEMBLE format. I want “SYMBOL” ids.

```
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
```

```
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       FIRRM       55732
ENSG00000000938        NA         FGR        2268
```

##Pathway Analysis

We will use the **gage** package along with **pathview** here to do genset enrichment (a.k.a pathway analysis) and figure generation respectively.

```
#1 message: false
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)
```

```r
library(gageData)
```

Lets have a peak at the first two pathways in KEGG

```r
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 of their importance.

It wants a vector of e.g. fold-changes.

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

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

```r
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.

```r
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 gneset we want to examine for overlap/enrichment…

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

Look at the results.

```r
attributes(keggres)
```

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

```r
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.

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

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

Info: Working in directory /Users/nicolenashed/Desktop/R Coding/class13

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

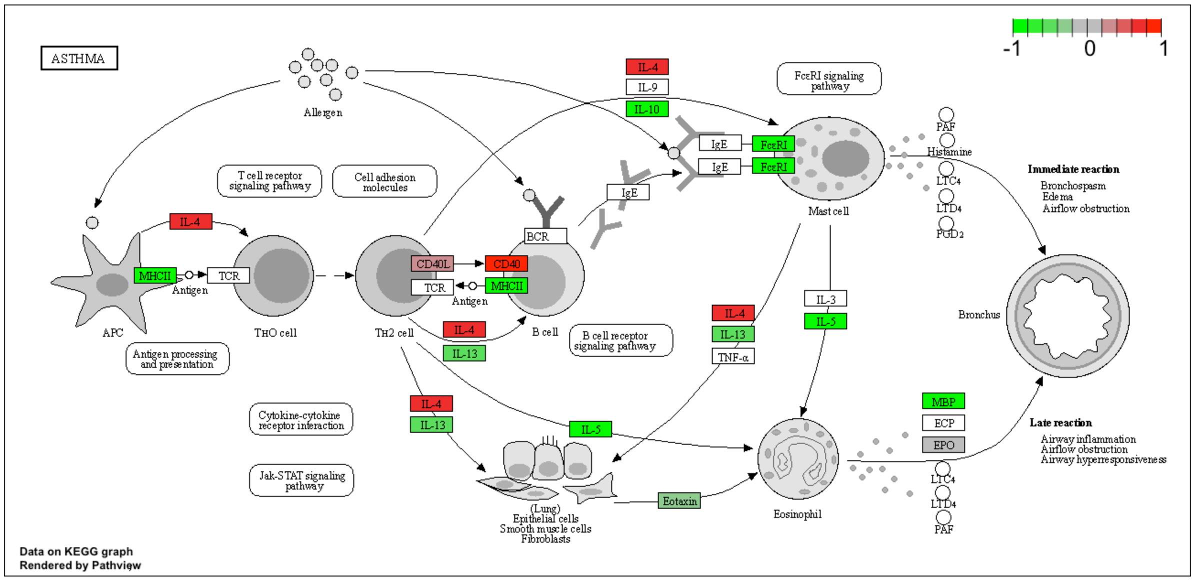


Figure 1: My Genes Involved in Asthma Pathway