Class 13: RNASeq pt.1

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The data for today's lab comes from a published RNA-seq experiment where airway smooth muscle cells were treated with dexamethasone, a synthetic glucocorticoid steroid with anti-inflammatory effects.

Import DATA

We need two things for this analysis: counts and metadata these are aclled "countData" and "colData" in the DESeq2 world.

```
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
```

The counts are prgamozed with a gene per row and experiment per column

```r
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?

```r
nrow(counts)
```

```
[1] 38694
```

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

4

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

```
[1] 4
```

Check on match of metadata and coldata

```r
colnames(counts)
```

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

```r
metadata$id
```

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

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

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

If you want to know that all the elements of a vector are TRUE we can use the `all()` function

```
all(c(T,T,T,F))
```

```
[1] FALSE
```

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

```
[1] TRUE
```

Examine Data

Analysis

I want to start by comparing "control" and "treated" columns. To this I will find the average for each gene (row) in all "control" columns. To this I will first find the average for each gene (row) in all "control" columns.

Let's extract all "control" columns first.

```
control.inds <- metadata$dex == "control"
```

```
control.counts <- counts[,control.inds]
```

Now find the mean count value per gene using the `apply()` function.

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

Now do the smae for the "treated" columns. i.e find `treated.mean` values

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

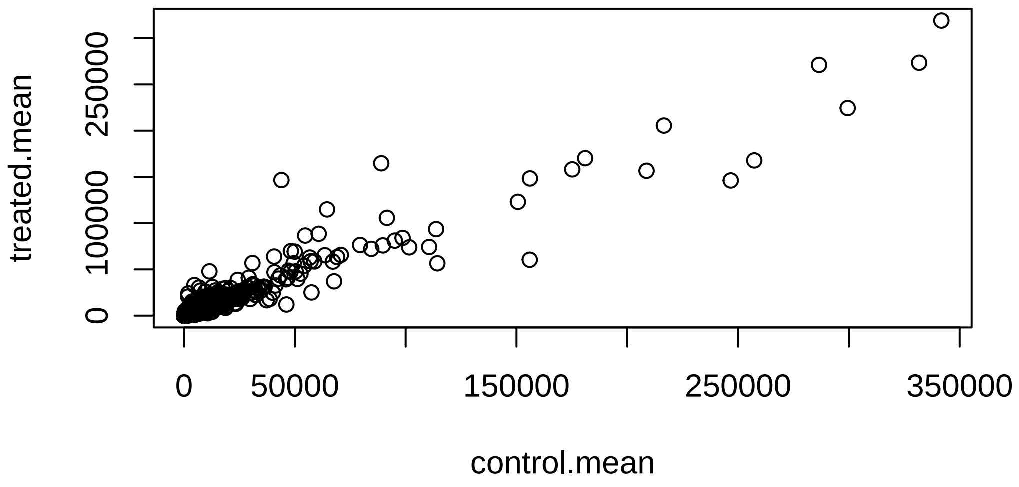
put these two mean vectors together for ease of book-keeping.

```
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
```

Let’s have a wee look with a quick plot

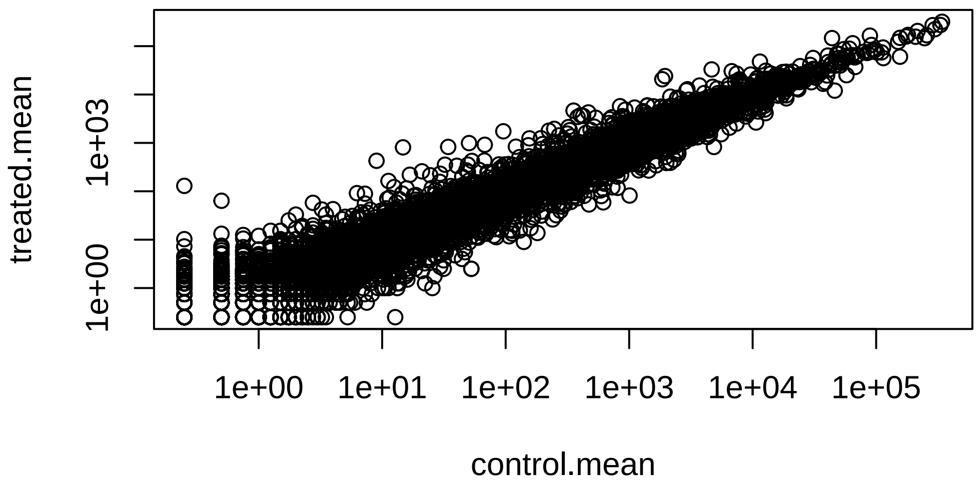
```
plot(meancounts)
```



```
plot(meancounts, log = "xy")
```

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

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



```
log(10, base=2)
```

```
[1] 3.321928
```

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

```
[1] 0
```

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

```
[1] 1
```

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

```
[1] -1
```

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

```
[1] 2
```

We most often work in log2 units because they have more intuitive interpertation

Here we calculate the log2 Fold-change of treated/control calues and add it to our wee data frame of results.

```r
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 some funky answers in there like NaN (Not a number) and -Inf(minus infinity) that all come because I have Zero count genes in my dataset.

It is common practice to filter these zero count genes out before we go too deep.

```r
to.keep.ind <- (rowSums(meancounts[,1:2] ==0)==0)

mycounts <- meancounts[to.keep.ind, ]
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
```

> Q how many genes do we have left after zero dount filtering?

```
nrow(mycounts)
```

```
[1] 21817
```

A common threshold for calling a gene "up" or "down" is log2 fold change of +2 or -2.

> Q. How many "up" regulted genes do we have?

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

```
[1] 314
```

DESeq analysis

We need to so this analysis properly with our inner stats person keep happy.

```
#l nessage: false
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
```

To use DESeq we need to get our input data in very particular format.

```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
```

Run DESeq anaylsis

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

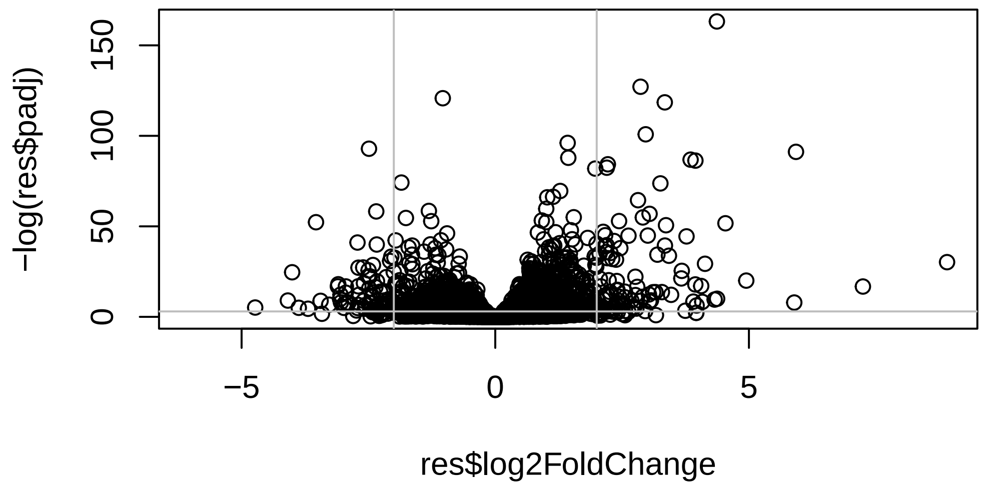
Get the results

```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
```

I want to make a figure showing an overview of all my results to date. A plot of **log2fold change** vs the **p-value** (adjusted p-value)

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



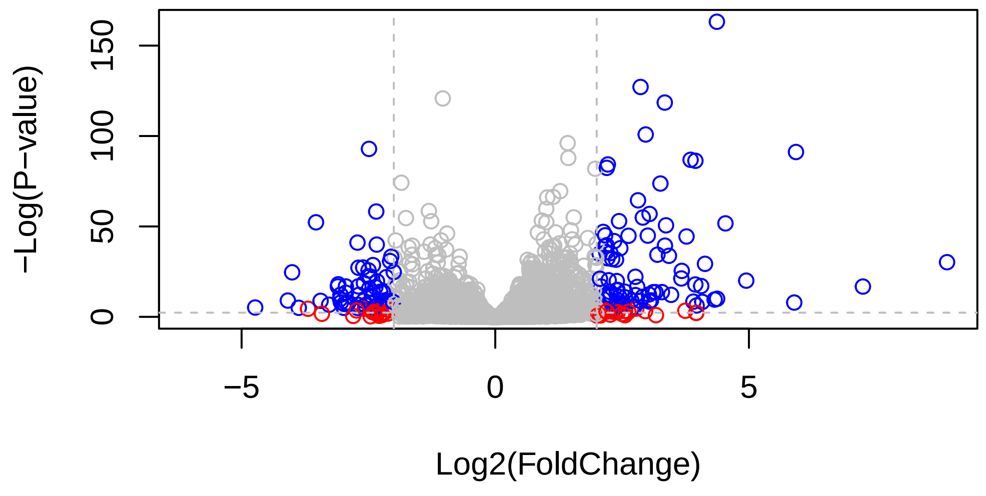
```r
log(0.5)
```

```
[1] -0.6931472
```

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

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


plot( res$log2FoldChange,  -log(res$padj),
 col=mycols, ylab="-Log(P-value)", xlab="Log2(FoldChange)" )
abline(v=c(-2,2), col="gray", lty=2)
abline(h=-log(0.1), col="gray", lty=2)
```



Add annotation data

We want to add gene symbols (i.e gene names) as well as other common identifiers from major databases for all our genes of interest

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

We can translate between the following IDs:

```
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"
```

```
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
```

My IDs are in the `rownames(res)`and they are found in the ENSEMBLE

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

We also want “GENENAME” and “ENTREZID”

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

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

```
res$entrez <- mapIds(org.Hs.eg.db,
                      keys=rownames(res),
                      keytype="ENSEMBL",
                      column="ENTREZID",
                      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       FIRRM FIGNL1 interacting r..       55732
ENSG00000000938        NA         FGR FGR proto-oncogene, ..        2268
```

Lets save our results to a new CSV file

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

Pathway Analysis

Here we will use the "gage" package to do some pathway analysis (aka geneset enrichment)

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

Have a wee peak at KEGG data

```
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"
```

To run gage we ned to provide it with a vector of fold change values (not our big full results table).

```
foldchanges <- res$log2FoldChange
#foldchange
```

Add the ENTREZ ids as names to this vector

```
c(chandra=10, alice=9, barry=7)
```

```
chandra   alice   barry 
     10       9       7 
```

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 run **gage** with this input and the kegg pathway

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

```
attributes(keggres)
```

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

```r
head(keggres$less)
```

```
                                                      p.geomean stat.mean
hsa05332 Graft-versus-host disease                 0.0004250461 -3.473346
hsa04940 Type I diabetes mellitus                  0.0017820293 -3.002352
hsa05310 Asthma                                    0.0020045888 -3.009050
hsa04672 Intestinal immune network for IgA production 0.0060434515 -2.560547
hsa05330 Allograft rejection                       0.0073678825 -2.501419
hsa04340 Hedgehog signaling pathway                0.0133239547 -2.248547
                                                          p.val      q.val
hsa05332 Graft-versus-host disease                 0.0004250461 0.09053483
hsa04940 Type I diabetes mellitus                  0.0017820293 0.14232581
hsa05310 Asthma                                    0.0020045888 0.14232581
hsa04672 Intestinal immune network for IgA production 0.0060434515 0.31387180
hsa05330 Allograft rejection                       0.0073678825 0.31387180
hsa04340 Hedgehog signaling pathway                0.0133239547 0.47300039
                                                   set.size         exp1
hsa05332 Graft-versus-host disease                       40 0.0004250461
hsa04940 Type I diabetes mellitus                        42 0.0017820293
hsa05310 Asthma                                          29 0.0020045888
hsa04672 Intestinal immune network for IgA production       47 0.0060434515
hsa05330 Allograft rejection                             36 0.0073678825
hsa04340 Hedgehog signaling pathway                      56 0.0133239547
```

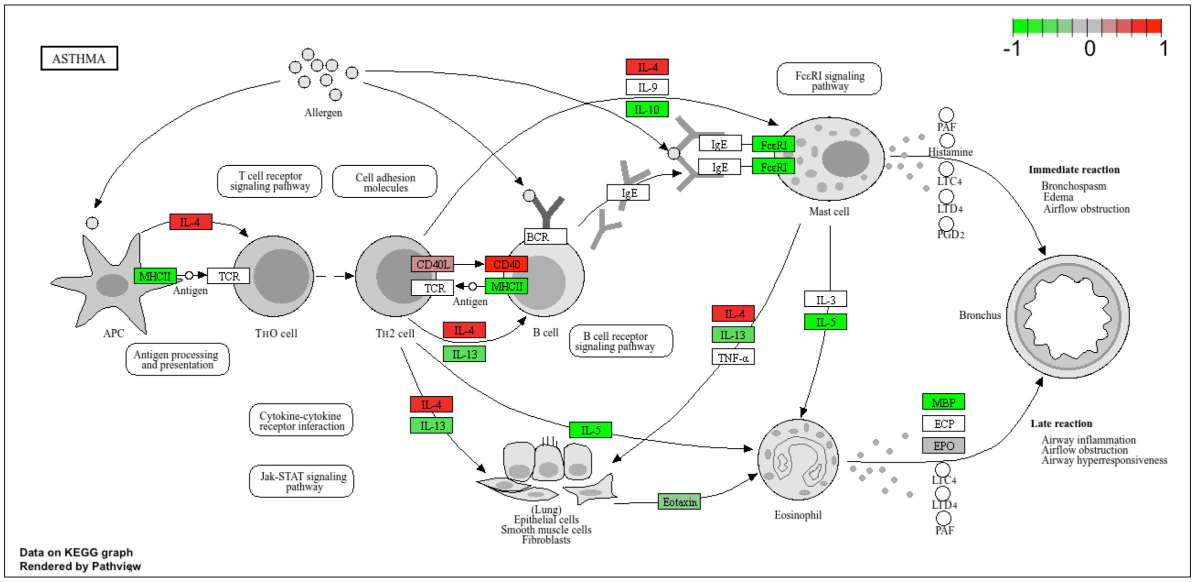
Let's have a look at the hsa05310 Asthma pathway with our genes highlighted using the `pathview()` function

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

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

Info: Working in directory /Users/xichen/Desktop/Bimm 143/Class 13

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


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