Class15

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Background

Our data for today come from Himes et al. RNASeq analysis of the drug dexamethasone, a synthetic glucocorticoid steroid with anti-inflammatory effects (Himes et al. 2014).

2. Import countData and colData

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

Let's have a look at these

```
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
```

```
nrow(counts)
```

```
## [1] 38694
```

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

```
## [1] 4
```

Q1. How many genes are in this dataset?

There are 38694 genes in this dataset.

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

There are 4 'control' cell lines.

3. Toy Differential Gene Expression

```
control <- metadata[metadata[,"dex"]=="control",]
control.counts <- counts[ ,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
```

```
library(dplyr)
```

```
##
## Attaching package: 'dplyr'
```

```
## The following objects are masked from 'package:stats':
##
##     filter, lag
```

```
## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

```
control <- metadata %>% filter(dex=="control")
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?

First I need to extract all the "control" columns. Then I'll take the row-wise mean to get the average count values for all genes in these 4 experiments.

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

```
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.inds <- metadata$dex == "treated"
treated.counts <- counts[ , treated.inds]
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
```

```
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
```

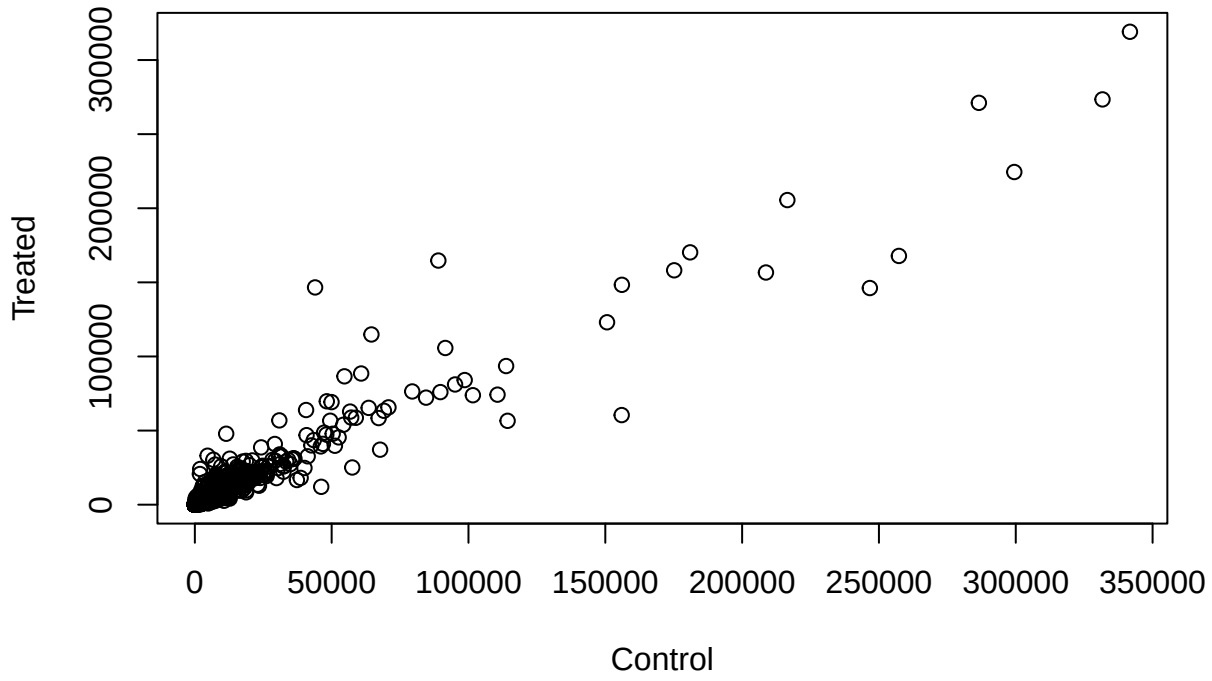
We will combine our meancount data for bookkeeping purposes.

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

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

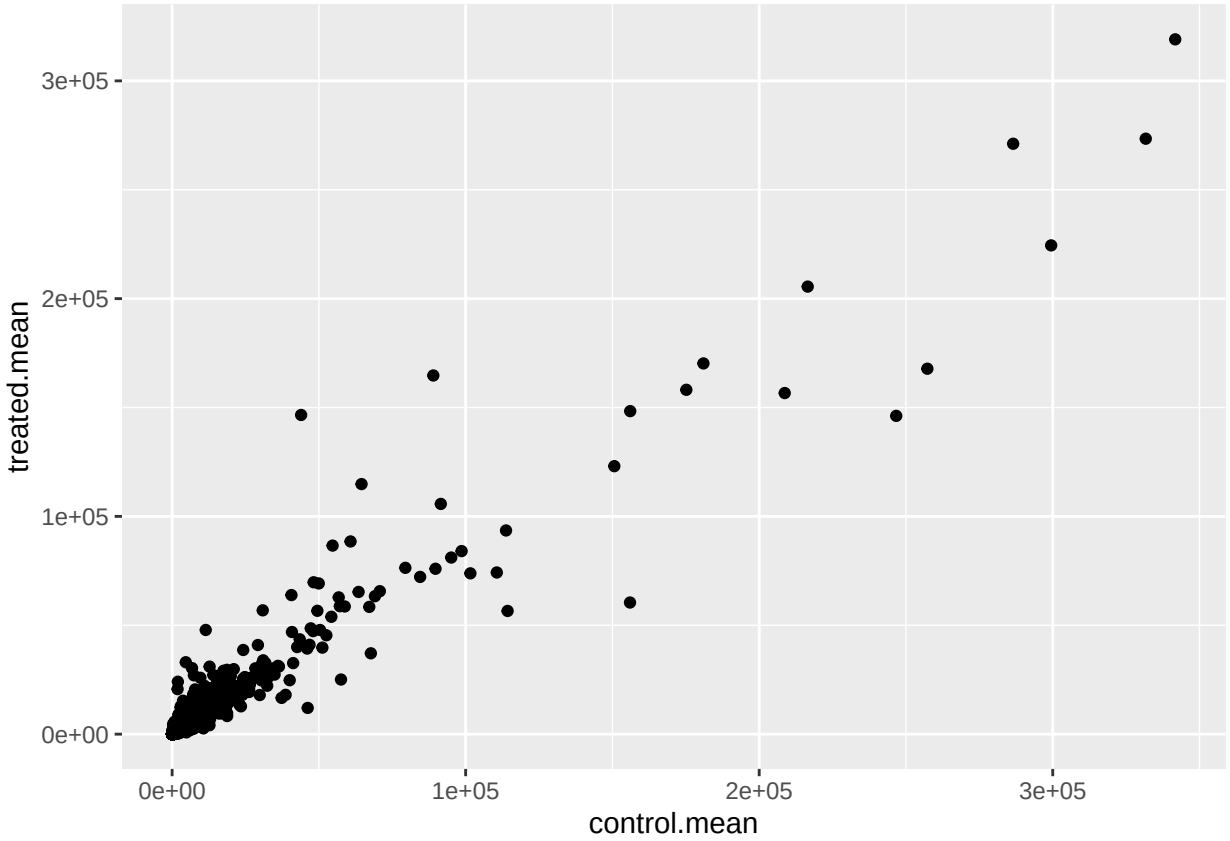
Q5 (a). Create a scatter plot showing the mean of the treated samples against the mean of the control samples. Your plot should look something like the following.

```
plot(meancounts[,1],meancounts[,2], xlab="Control", ylab="Treated")
```



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(x=control.mean, y=treated.mean) +
  geom_point()
```



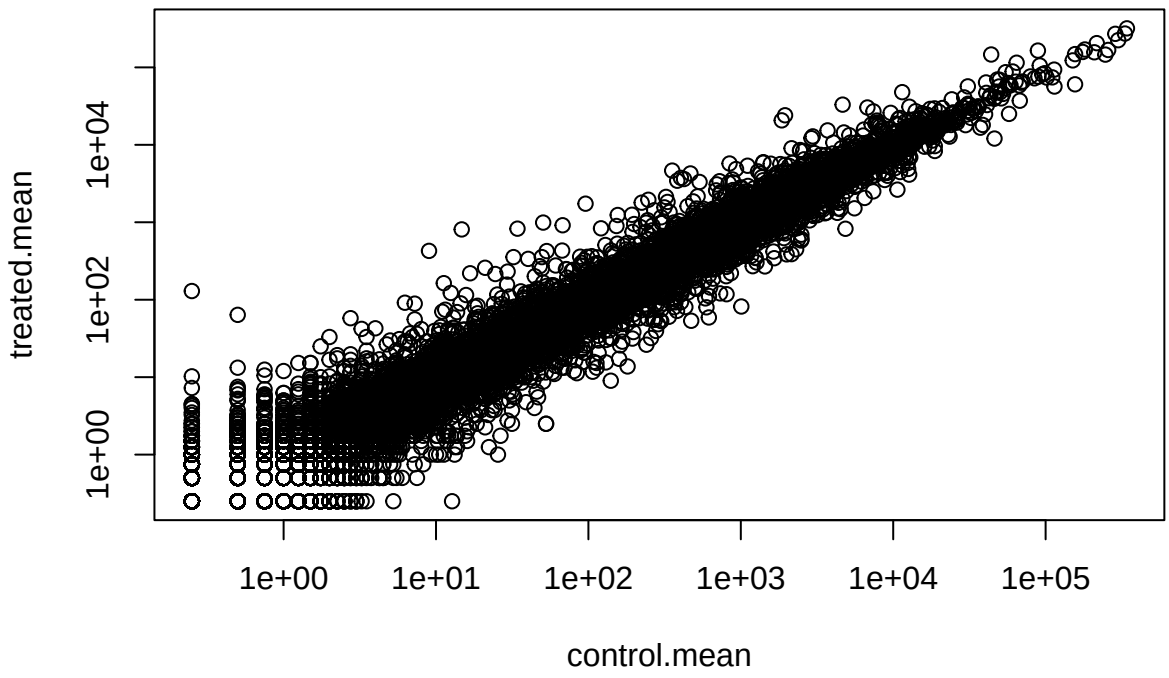
This indicates that we need a log transformation to see details of our data

> 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")
```

```
## 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
```



We often use log2 in this field because it has nice math properties that make interpretation easier.

We see 0 values for no change and + values for increases and minus values for decreases. This nice property leads us to work with **log2(fold-change)** all the time in the genomics and proteomics field.

```
log2(1)
```

```
## [1] 0
```

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

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

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

```
## [1] -1
```

Let's add the **log2(fold-change)** values to our `meancounts` dataframe.

```
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
```

I need to exclude the genes (i.e. rows) with zero counts because we can't say anything about these as we have no data for them. I can use the **which()** function with the `arr.ind=TRUE` argument to get the columns and rows where the TRUE values are (i.e. the zero counts in our case).

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

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

```
to.rm <- unique(zero.vals[, "row"])
head(sort(to.rm))
```

```
## [1]  2  6 65 70 73 81
```

Now remove these from our `meancounts` dataframe.

```
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
```

```
# How many do we have left?
nrow(mycounts)
```

```
## [1] 21817
```

> 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 will indicate which rows/columns are true. In this case, if it's true, it means that the rows, or genes, and columns, or samples, have zero counts. Since there are two columns, control.mean and treated.mean, that can have zero values, we need to use the unique() function in order to only count a row once even if it has zero values for both the samples.

How many genes are up regulated upon drug treatment? We will use a log2 threshold of +2 for this.

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

```
## [1] 250
```

How many genes are down regulated at the -2 fold change threshold?

```
sum(mycounts$log2fc < -2)
```

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

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

```
## [1] 250
```

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

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

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

250 genes are up regulated.

> Q9. Using the down.ind vector above can you determine how many down regulated genes we have at the greater than 2 fc level?

367 genes are down regulated.

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

No, not necessarily since we haven't analyzed the p-values. Analyzing the p-values will allow us to know if the results are statistically significant, but just know which genes are up regulated or down regulated based on a threshold won't tell us if the results are actually in fact statistically significant.

4. DESeq2 Analysis

```
library(DESeq2)
```

```
## Loading required package: S4Vectors
```

```
## Loading required package: stats4
```

```
## Loading required package: BiocGenerics
```

```
##
## Attaching package: 'BiocGenerics'

## The following objects are masked from 'package:dplyr':
##
##     combine, intersect, setdiff, union

## 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:dplyr':
##
##     first, rename

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

## Loading required package: IRanges

##
## Attaching package: 'IRanges'

## The following objects are masked from 'package:dplyr':
##
##     collapse, desc, slice

## Loading required package: GenomicRanges

## Loading required package: GenomeInfoDb

## Loading required package: SummarizedExperiment

## Loading required package: MatrixGenerics

## Loading required package: matrixStats
```

```
##
## Attaching package: 'matrixStats'

## The following object is masked from 'package:dplyr':
##
##     count

##
## 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")
```

```
##
##   Love, M.I., Huber, W., Anders, S. Moderated estimation of fold change
```

```
##   and dispersion for RNA-seq data with DESeq2 Genome Biology 15(12):550
##   (2014)
##
## A BibTeX entry for LaTeX users is
##
##   @Article{,
##     title = {Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2},
##     author = {Michael I. Love and Wolfgang Huber and Simon Anders},
##     year = {2014},
##     journal = {Genome Biology},
##     doi = {10.1186/s13059-014-0550-8},
##     volume = {15},
##     issue = {12},
##     pages = {550},
##   }
```

We need to first setup the input object for DESeq

```
dds <- DESeqDataSetFromMatrix(countData=counts,
                              colData=metadata,
                              design=~dex)
```

```
## converting counts to integer mode

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

```
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
```

Now we can run DESeq analysis

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

```
## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship
```

```
## final dispersion estimates

## fitting model and testing
```

To get at the results, we use the deseq `results()` function

```
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
```

```
summary(res)
```

```
##
## out of 25258 with nonzero total read count
## adjusted p-value < 0.1
## LFC > 0 (up)       : 1563, 6.2%
## LFC < 0 (down)     : 1188, 4.7%
## outliers [1]       : 142, 0.56%
## low counts [2]     : 9971, 39%
## (mean count < 10)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

Focus in on genes with a small p-value (i.e. show a significant change)

```
res05 <- results(dds, alpha=0.05)
summary(res05)
```

```
##
## out of 25258 with nonzero total read count
## adjusted p-value < 0.05
## LFC > 0 (up)       : 1236, 4.9%
```

```
## LFC < 0 (down)     : 933, 3.7%
## outliers [1]       : 142, 0.56%
## low counts [2]     : 9033, 36%
## (mean count < 6)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

5. Adding Annotation Data

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

```
## Warning: package 'AnnotationDbi' was built under R version 4.1.2
```

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

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

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

```
##
```

Let's have a look at what is in the `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"
```

We will use the `mapIDs` function to translate between identifiers from different databases.

```
res$symbol <- mapIds(org.Hs.eg.db,
                     keys=row.names(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    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.

We need ENTREZ ids for pathway analysis with KEGG

```
res$entrez <- mapIds(org.Hs.eg.db,
                     keys=row.names(res),
                     column="ENTREZID",
                     keytype="ENSEMBL",
                     multiVals="first")
```

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

```
res$uniprot <- mapIds(org.Hs.eg.db,
                     keys=row.names(res),
                     column="UNIPROT",
                     keytype="ENSEMBL",
                     multiVals="first")
```

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

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

```
ord <- order( res$padj )
#View(res[ord,])
head(res[ord,])
```

```
## 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>
## ENSG00000152583   954.771        4.36836 0.2371268   18.4220 8.74490e-76
## ENSG00000179094   743.253        2.86389 0.1755693   16.3120 8.10784e-60
## ENSG00000116584  2277.913       -1.03470 0.0650984  -15.8944 6.92855e-57
## ENSG00000189221  2383.754        3.34154 0.2124058   15.7319 9.14433e-56
## ENSG00000120129  3440.704        2.96521 0.2036951   14.5571 5.26424e-48
## ENSG00000148175 13493.920        1.42717 0.1003890   14.2164 7.25128e-46
##                       padj      symbol      entrez     uniprot
##                  <numeric> <character> <character> <character>
## ENSG00000152583 1.32441e-71     SPARCL1        8404  A0A024RDE1
## ENSG00000179094 6.13966e-56        PER1        5187      O15534
## ENSG00000116584 3.49776e-53     ARHGEF2        9181      Q92974
## ENSG00000189221 3.46227e-52        MAOA        4128      P21397
## ENSG00000120129 1.59454e-44       DUSP1        1843      B4DU40
## ENSG00000148175 1.83034e-42        STOM        2040      F8VSL7
##                             genename
##                          <character>
## ENSG00000152583           SPARC like 1
```

```
## ENSG00000179094 period circadian reg..
## ENSG00000116584 Rho/Rac guanine nucl..
## ENSG00000189221    monoamine oxidase A
## ENSG00000120129 dual specificity pho..
## ENSG00000148175               stomatin
```

```
write.csv(res[ord,], "deseq_results.csv")
```

Save our results

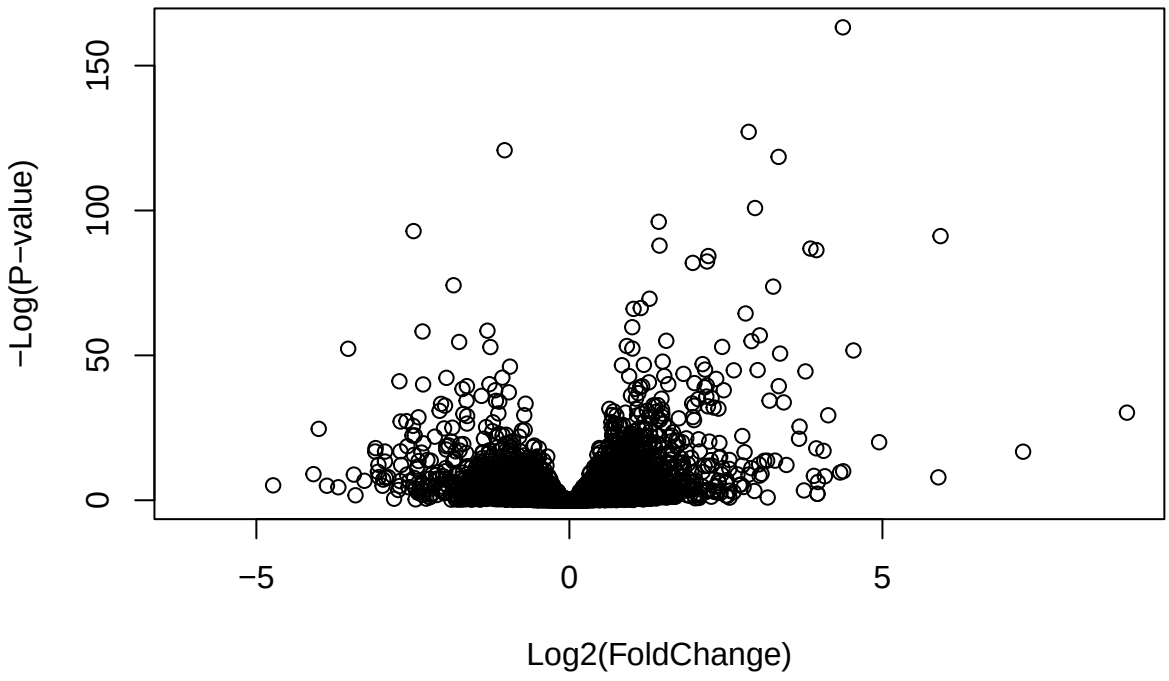
Write out whole results dataset (including genes that don't change significantly)

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

6. Data Visualization

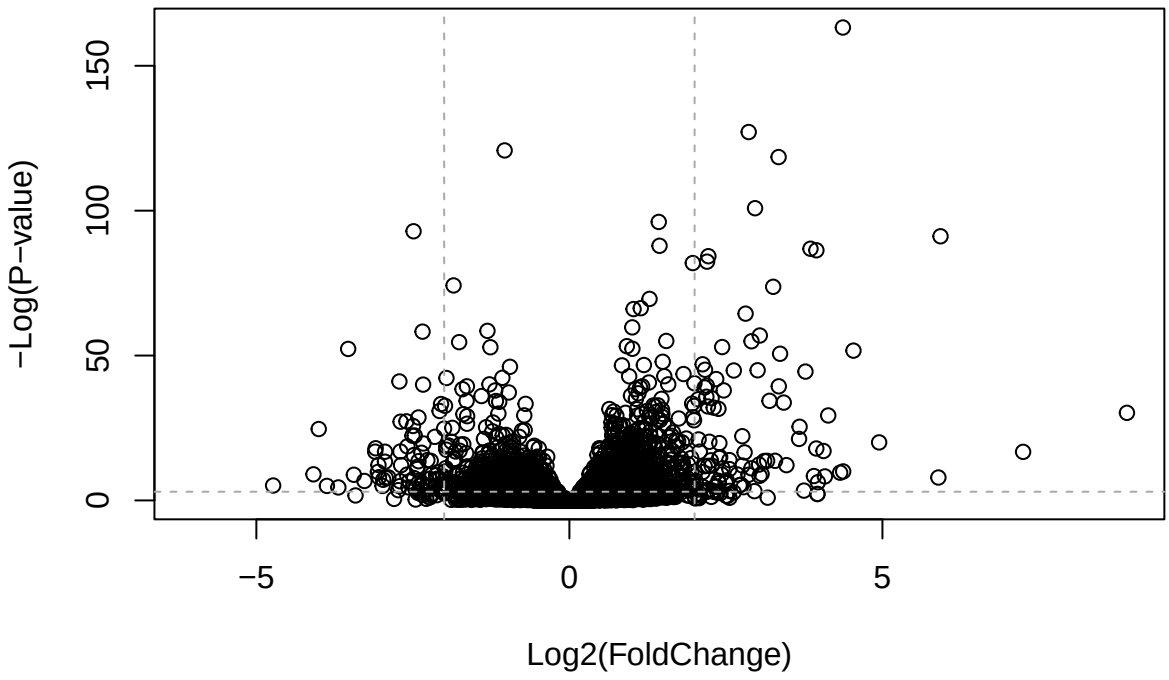
Let's make a commonly produced visualization from this data, namely a so-called volcano plot. These summary figures are frequently used to highlight the proportion of genes that are both significantly regulated and display a high fold change.

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



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

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



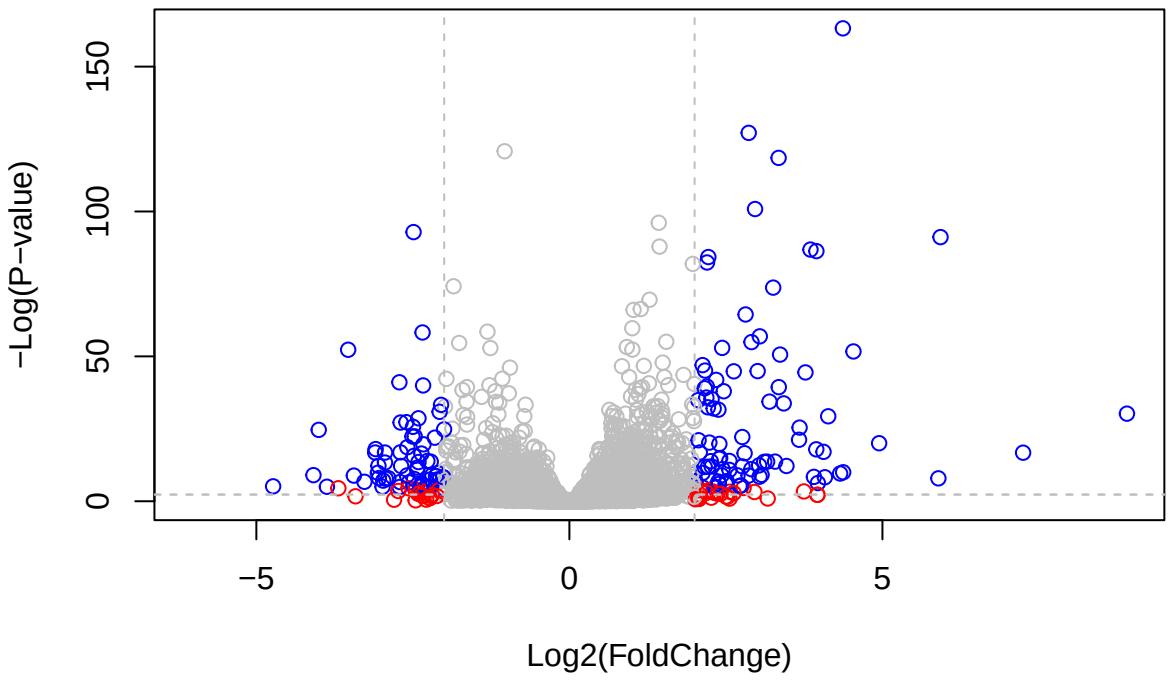
Let's add some color to this plot to draw attention to the genes (i.e. points) we care about - that is those with large fold-change and low p-values (i.e. high -log(p-values)).

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



Let's make another volcano plot with some gene labels. For this, we can use the **EnhancedVolcano** package

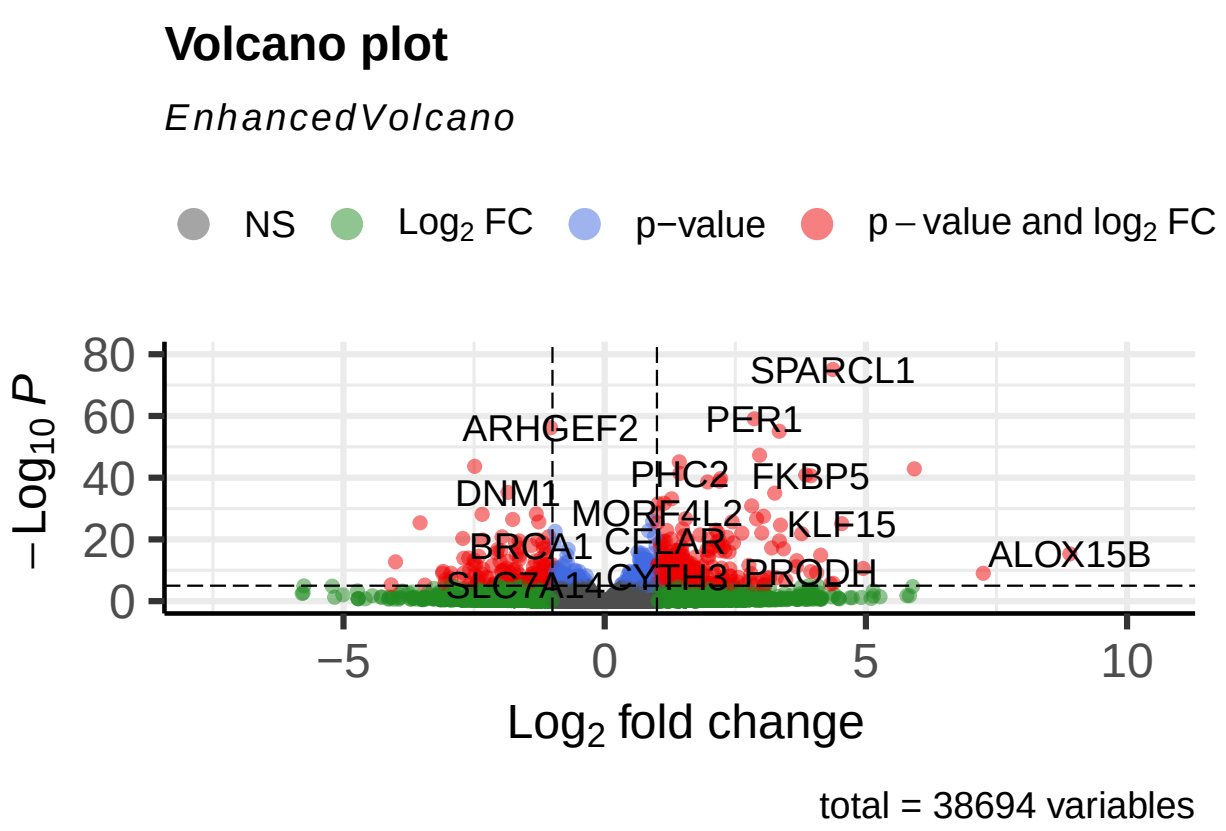
```
library(EnhancedVolcano)
```

```
## 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
```

```
x <- as.data.frame(res)

EnhancedVolcano(x,
    lab = x$symbol,
    x = 'log2FoldChange',
    y = 'pvalue')
```



7. Pathway Analysis

```
library(pathview)
```

```
## ##############################################################################
## Pathview is an open source software package distributed under GNU General
## Public License version 3 (GPLv3). Details of GPLv3 is available at
## http://www.gnu.org/licenses/gpl-3.0.html. Particullary, users are required to
## formally cite the original Pathview paper (not just mention it) in publications
## or products. For details, do citation("pathview") within R.
##
## The pathview downloads and uses KEGG data. Non-academic uses may require a KEGG
## license agreement (details at http://www.kegg.jp/kegg/legal.html).
## ##############################################################################
```

```
library(gage)
```

```
##
```

```
library(gageData)
```

```
data(kegg.sets.hs)

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

```
## $`hsa00232 Caffeine metabolism`
## [1] "10"   "1544" "1548" "1549" "1553" "7498" "9"
##
## $`hsa00983 Drug metabolism - other enzymes`
##  [1] "10"     "1066"   "10720"  "10941"  "151531" "1548"   "1549"   "1551"
##  [9] "1553"   "1576"   "1577"   "1806"   "1807"   "1890"   "221223" "2990"
## [17] "3251"   "3614"   "3615"   "3704"   "51733"  "54490"  "54575"  "54576"
## [25] "54577"  "54578"  "54579"  "54600"  "54657"  "54658"  "54659"  "54963"
## [33] "574537" "64816"  "7083"   "7084"   "7172"   "7363"   "7364"   "7365"
## [41] "7366"   "7367"   "7371"   "7372"   "7378"   "7498"   "79799"  "83549"
## [49] "8824"   "8833"   "9"      "978"
```

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

```
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
```

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

This separates out results by "greater" and "less" i.e. those that are up regulated and those that are down regulated

```
attributes(keggres)
```

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

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

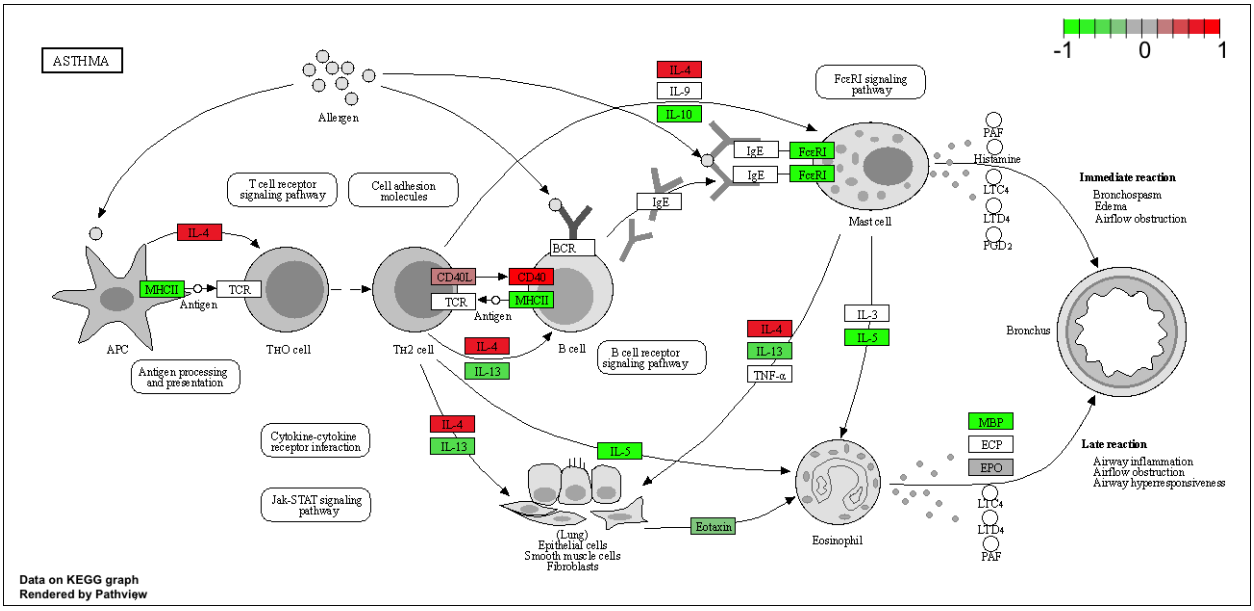
```
##                                      p.geomean stat.mean        p.val
## hsa05332 Graft-versus-host disease 0.0004250461 -3.473346 0.0004250461
## hsa04940 Type I diabetes mellitus  0.0017820293 -3.002352 0.0017820293
## hsa05310 Asthma                    0.0020045888 -3.009050 0.0020045888
##                                         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/LeynaN/BIMM143_Fall2021/bimm143_github/Class15
```

```
## 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/LeynaN/BIMM143_Fall2021/bimm143_github/Class15
```

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

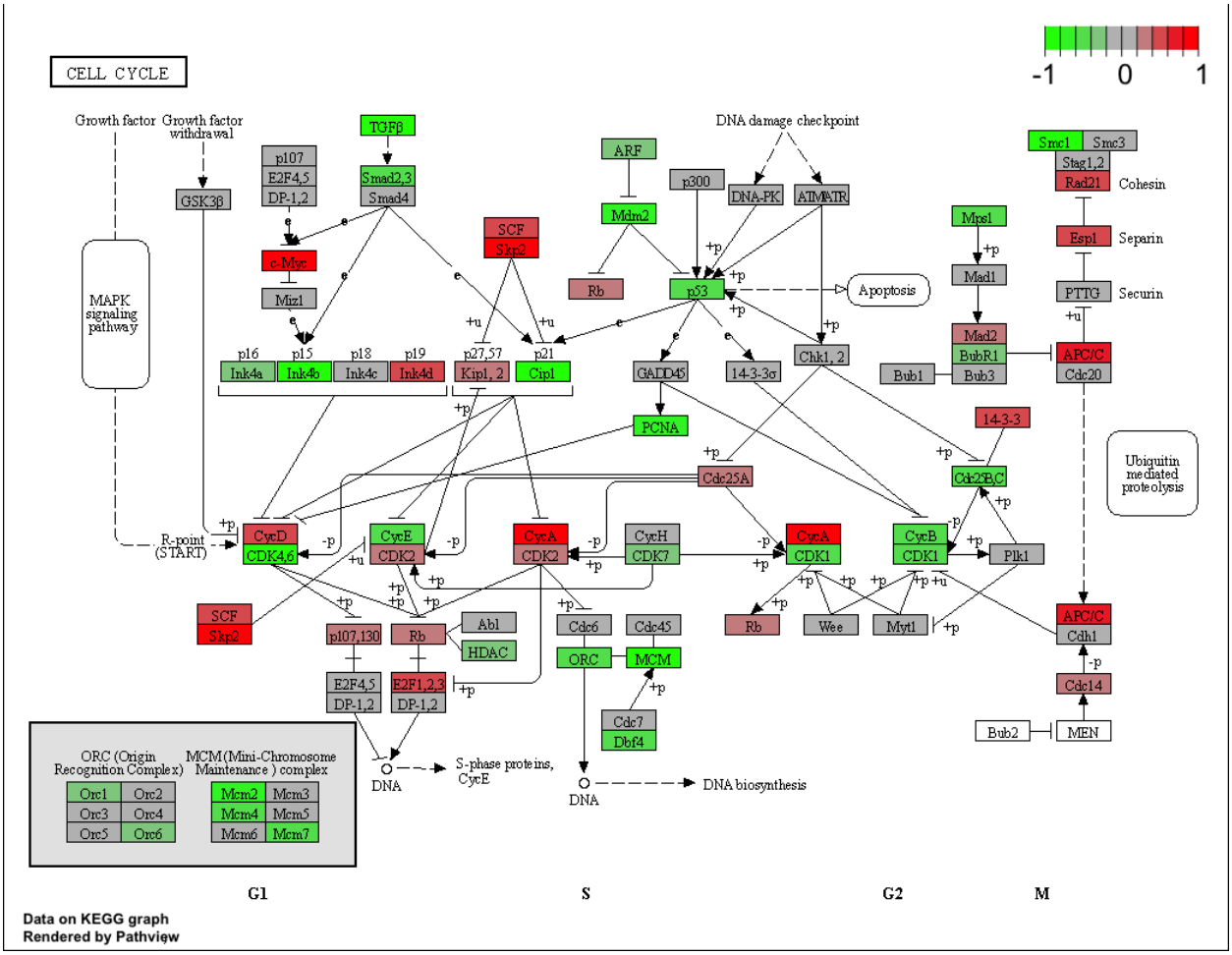
Q12. Can you do the same procedure as above to plot the pathview figures for the top 2 down-regulated pathways?

```
pathview(gene.data=foldchanges, pathway.id="hsa04110")
```

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

```
## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class15
```

```
## Info: Writing image file hsa04110.pathview.png
```

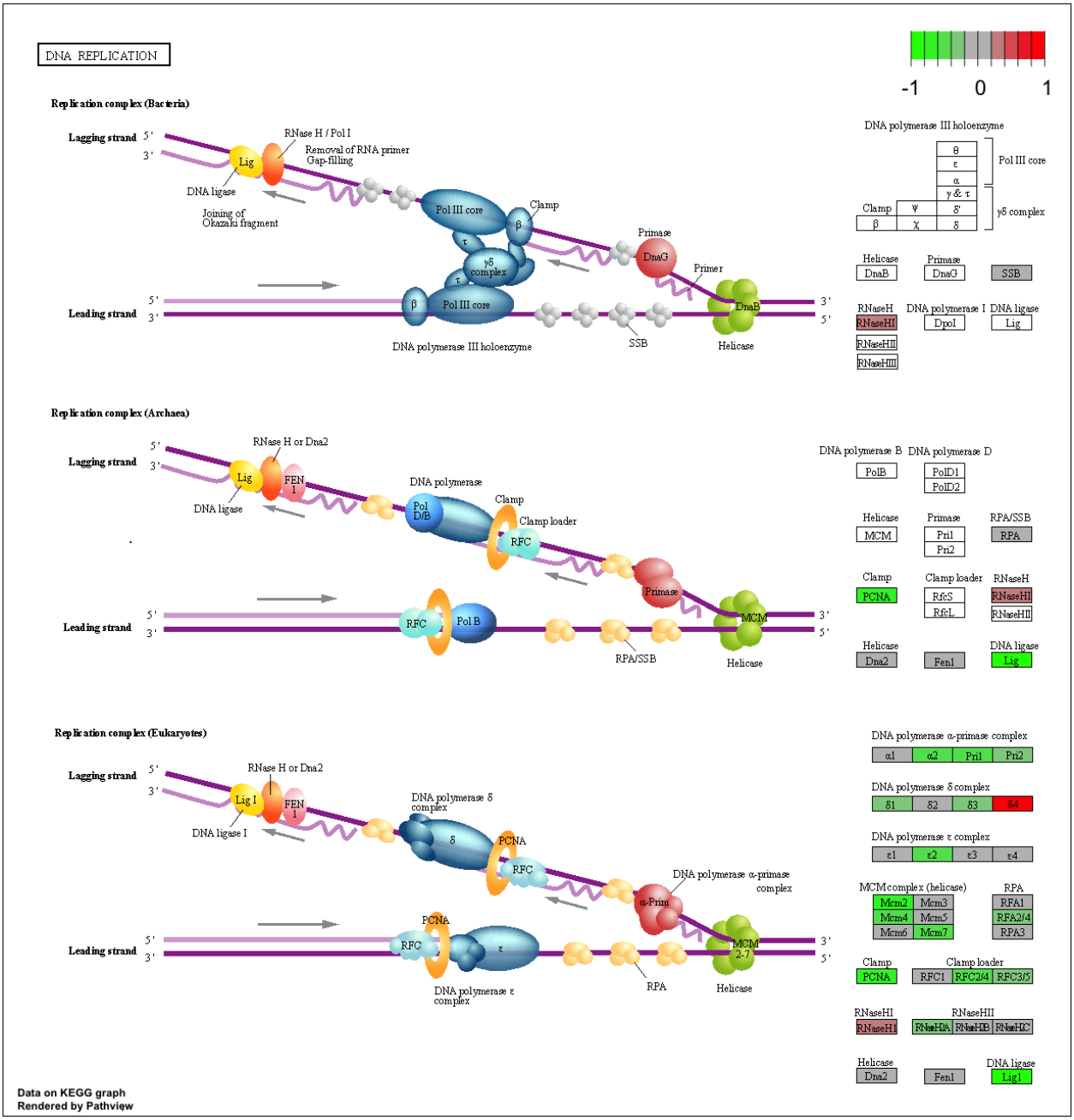



```
pathview(gene.data=foldchanges, pathway.id="hsa03030")
```

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

```
## Info: Working in directory /Users/LeynaN/BIMM143_Fall2021/bimm143_github/Class15
```

```
## Info: Writing image file hsa03030.pathview.png
```


DNA REPLICATION
-1
0
1
Replication complex (Bacteria)
Lagging strand
Leading strand
RNase H / Pol I
Removal of RNA primer
Gap-filling
Lig
DNA ligase
Joining of Okazaki fragment
Pol III core
Clamp
β
τ
γδ complex
Primase
DnaG
Primer
DnaB
SSB
Helicase
DNA polymerase III holoenzyme
DNA polymerase III holoenzyme
Pol III core
γδ complex
Clamp
Helicase
DnaB
Primase
DnaG
SSB
RNaseH
RNaseHI
RNaseHII
RNaseHIII
DNA polymerase I
DpoI
DNA ligase
Lig
Replication complex (Archaea)
RNase H or Dna2
FEN 1
DNA polymerase
Pol D/B
Clamp loader
RFC
Pol B
RPA/SSB
MCM
DNA polymerase B
PolB
DNA polymerase D
PolD1
PolD2
Helicase
MCM
Primase
Pri1
Pri2
RPA/SSB
RPA
Clamp
PCNA
Clamp loader
RfcS
RfcL
RNaseH
RNaseHI
RNaseHII
Helicase
Dna2
Fen1
DNA ligase
Lig
Replication complex (Eukaryotes)
RNase H or Dna2
Lig I
FEN 1
DNA ligase I
DNA polymerase δ complex
δ
PCNA
RFC
DNA polymerase α-primase complex
α-Prim
MCM 2-7
ε
DNA polymerase ε complex
RPA
Helicase
DNA polymerase α-primase complex
α1
α2
Pri1
Pri2
DNA polymerase δ complex
δ1
δ2
δ3
δ4
DNA polymerase ε complex
ε1
ε2
ε3
ε4
MCM complex (helicase)
Mcm2
Mcm3
Mcm4
Mcm5
Mcm6
Mcm7
RPA
RFA1
RFA2/4
RPA3
Clamp
PCNA
Clamp loader
RFC1
RFC2/4
RFC3/5
RNaseHI
RNaseH1
RNaseHII
RNaseHIIA
RNaseHIIB
RNaseHIIC
Helicase
Dna2
Fen1
DNA ligase
Lig1
Data on KEGG graph
Rendered by Pathview