class 11: DESeq Lab - Transcriptomics and the analysis of RNA-Seq data

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1. Bioconductor and DESeq2 setup

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

```
## Loading required package: S4Vectors

## Loading required package: stats4

## Loading required package: BiocGenerics

##
## Attaching package: 'BiocGenerics'

## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, append, as.data.frame, basename, cbind, colnames,
##     dirname, do.call, duplicated, eval, evalq, Filter, Find, get, grep,
##     grepl, intersect, is.unsorted, lapply, Map, mapply, match, mget,
##     order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
##     rbind, Reduce, rownames, sapply, setdiff, sort, table, tapply,
##     union, unique, unsplit, which.max, which.min

##
## Attaching package: 'S4Vectors'

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

```
## Loading required package: IRanges

## Loading required package: GenomicRanges

## Loading required package: GenomeInfoDb

## Loading required package: SummarizedExperiment

## Loading required package: MatrixGenerics

## Loading required package: matrixStats

##
## Attaching package: 'MatrixGenerics'

## The following objects are masked from 'package:matrixStats':
##
##     colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
##     colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
##     colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
##     colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
##     colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
##     colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
##     colWeightedMeans, colWeightedMedians, colWeightedSds,
##     colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
##     rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
##     rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
##     rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
##     rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
##     rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
##     rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
##     rowWeightedSds, rowWeightedVars

## Loading required package: Biobase

## Welcome to Bioconductor
##
##     Vignettes contain introductory material; view with
##     'browseVignettes()'. To cite Bioconductor, see
##     'citation("Biobase")', and for packages 'citation("pkgname")'.

##
## Attaching package: 'Biobase'

## The following object is masked from 'package:MatrixGenerics':
##
##     rowMedians

## The following objects are masked from 'package:matrixStats':
##
##     anyMissing, rowMedians
```

2. Import countData and colData

```
# Use the read.csv() function to read these count data and metadata files.
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
```

Answer: There are 38,694 genes in this dataset.

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

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

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

Answer: We have 4 control cell lines in this dataset.

3. Toy differential gene expression

Lets perform some exploratory differential gene expression analysis.

First, need to extract all the control columns, then generate row-wise mean to get the average count values for all gene in these 4 experiments.

```
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
```

An alternative way to do this same thing using the dplyr package from the tidyverse is shown below. Which do you prefer and why?

```
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
```

```
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?

Answer: In both approaches, `control.mean` should be defined as `rowMeans(control.count)` to make the code more robust for using different datasets. Above example codes divided the mean by 4 because there are 4 control samples in the dataset (control.mean <- rowSums(control.counts)/4).

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

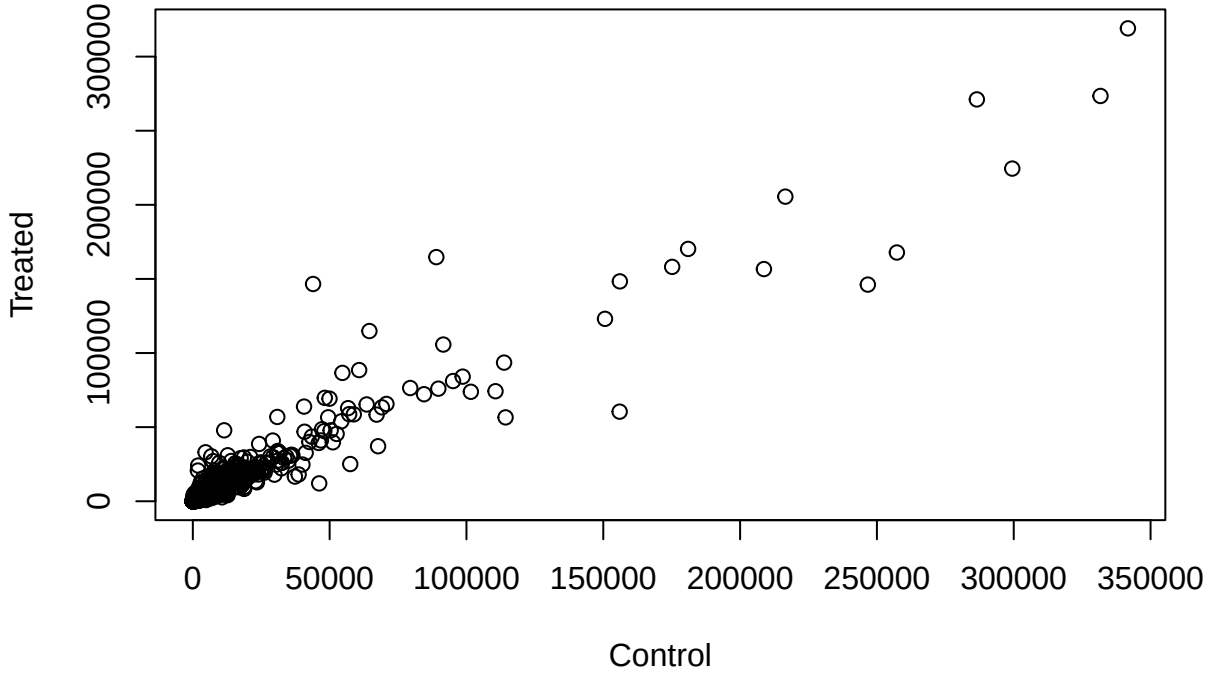
Answer: Store these results together in a new data fram called `meancounts`.

```
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.

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

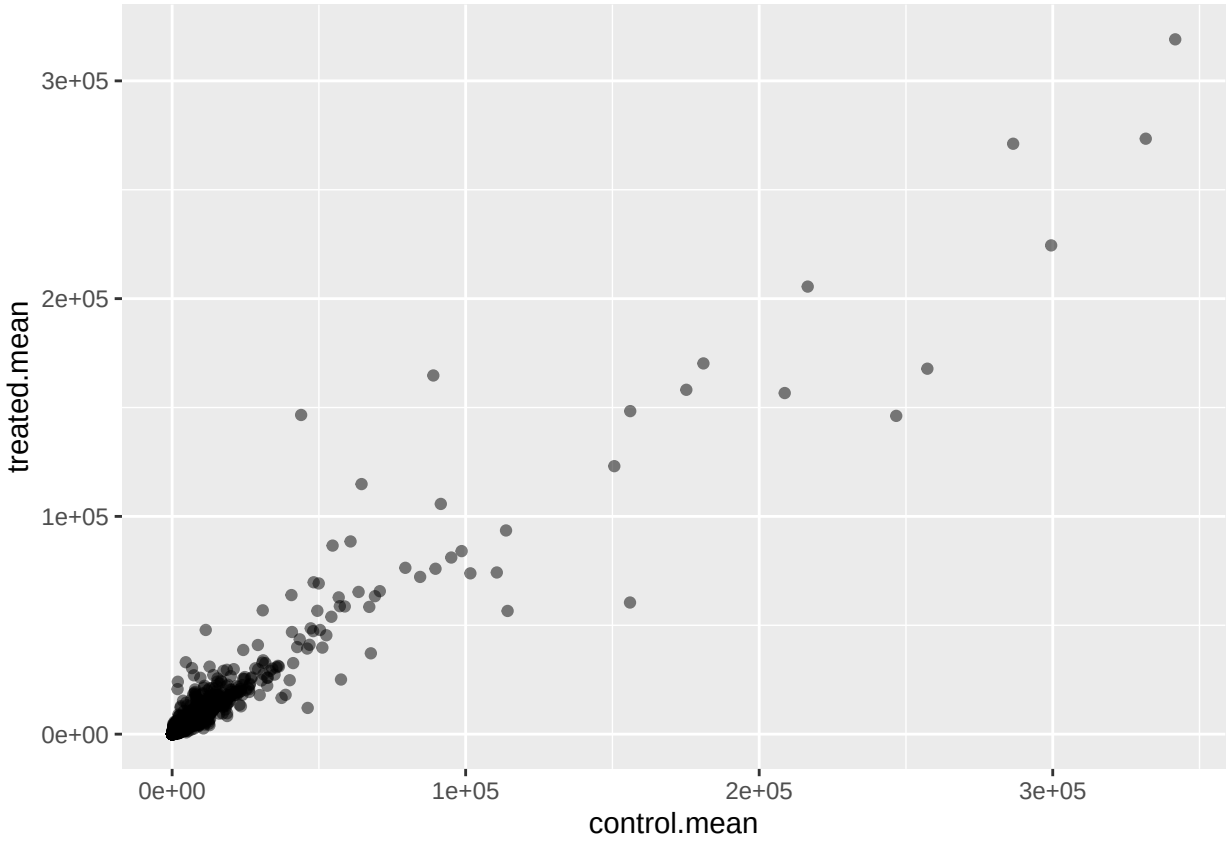


Answer:

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(meancounts[,1],meancounts[,2]) +
  labs(x="control.mean", y="treated.mean") +
  geom_point(alpha=0.5)
```



Answer: We can use `geom_points()` to produce the above plot.

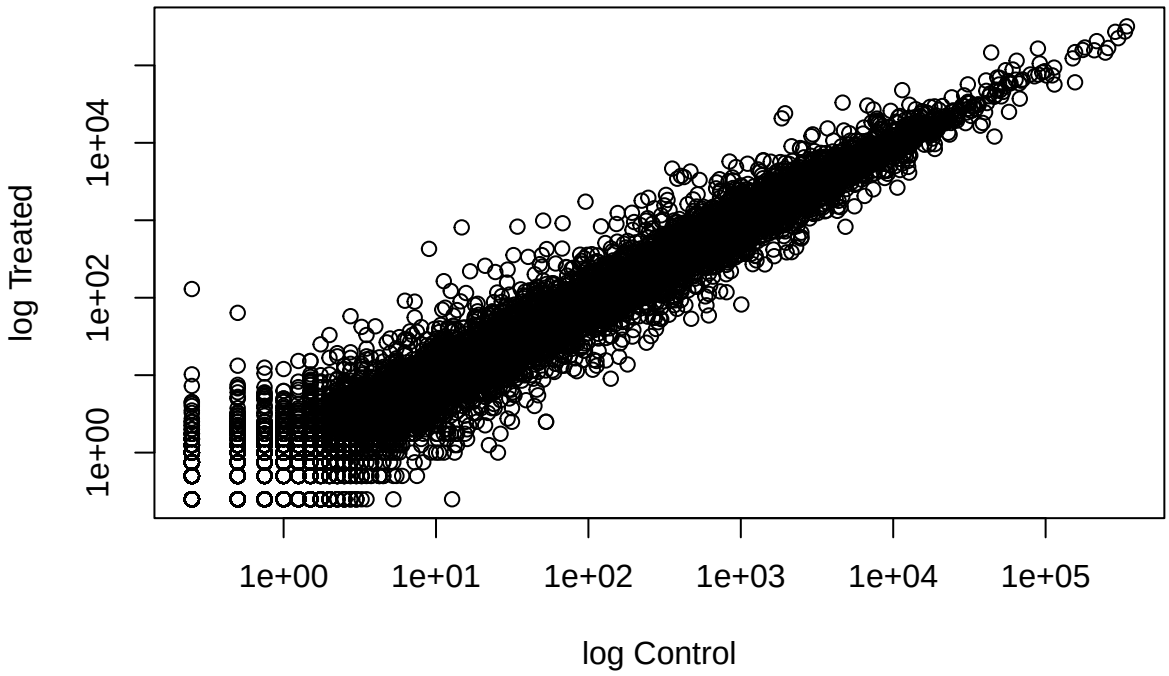
We will make a log-log plot to draw out this skewed data and see what is going on.

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

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



Answer: The `log` argument allows to plot both axes on a log scale.

We can find candidate differentially expressed genes by looking for genes with a large change between control and dex-treated samples. We usually look at the log2 of the fold change because it has a better mathematical property where: if there is no change, log2 value will be zero; if it doubled, log2 value will be 1; and if halved, log2 value will be -1.

So let's add a log2 fold change column to our results so far.

```
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
```

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.

```
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
```

How many genes are remaining?

```
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?

Answer: The purpose of the `arr.ind` argument in the `which()` function is to return the row and column indices where there are TRUE values. In this case, this will tell us which genes (rows) and samples (columns) have 0 count. Calling `unique()` function will ensure that we don't count any row twice if it has 0 entries in both samples (some rows and columns can have 0's at the same time and count twice).

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
```

Answer: We have 250 up-regulated genes at the 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
```

Answer: We have 367 down-regulated genes at the greater than 2 fc level.

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

Answer: No. Fold-change can be large without being statistically significant. We need to decide the statistical significance of the gene regulations.

4. DESeq2 analysis

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

Importing data

```
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
```

Running DESeq analysis

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

```
## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates
```

```
## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing
```

Getting results

```
res <- results(dds)
res
```

```
## log2 fold change (MLE): dex treated vs control
## Wald test p-value: dex treated vs control
## DataFrame with 38694 rows and 6 columns
##                   baseMean log2FoldChange     lfcSE      stat    pvalue
##                  <numeric>      <numeric> <numeric> <numeric> <numeric>
## ENSG00000000003  747.1942     -0.3507030  0.168246 -2.084470 0.0371175
## ENSG00000000005    0.0000             NA        NA        NA        NA
## ENSG00000000419  520.1342      0.2061078  0.101059  2.039475 0.0414026
## ENSG00000000457  322.6648      0.0245269  0.145145  0.168982 0.8658106
## ENSG00000000460   87.6826     -0.1471420  0.257007 -0.572521 0.5669691
## ...                    ...            ...       ...       ...       ...
## ENSG00000283115  0.000000             NA        NA        NA        NA
## ENSG00000283116  0.000000             NA        NA        NA        NA
## ENSG00000283119  0.000000             NA        NA        NA        NA
## ENSG00000283120  0.974916      -0.668258   1.69456 -0.394354  0.693319
## ENSG00000283123  0.000000             NA        NA        NA        NA
##                      padj
##                 <numeric>
## ENSG00000000003  0.163035
## ENSG00000000005        NA
## ENSG00000000419  0.176032
## ENSG00000000457  0.961694
## ENSG00000000460  0.815849
## ...                   ...
## ENSG00000283115        NA
## ENSG00000283116        NA
## ENSG00000283119        NA
## ENSG00000283120        NA
## ENSG00000283123        NA
```

We can get some basic summary tallies using the `summary()` function.

```
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
```

The results function contains a number of arguments to customize the results table. By default the argument alpha is set to 0.1. If the adjusted p value cutoff will be a value other than 0.1, alpha should be set to that value:

```
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

Our result table so far only contains the Ensemble gene IDs. However, alternative gene names and extra annotation are usually required for informative interpretation of our results. In this section we will add this necessary annotation data to our results.

We will use one of Bioconductor's main annotation packages to help with mapping between various ID schemes. Here we load the `AnnotationDbi` package and the annotation data package for humans `org.Hs.eg.db`.

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

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

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

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

```
res$symbol <- mapIds(org.Hs.eg.db,
                     keys=row.names(res), # Our genenames
                     keytype="ENSEMBL",        # The format of our genenames
                     column="SYMBOL",          # The new format we want to add
                     multiVals="first")
```

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

```
head(res)
```

```
## log2 fold change (MLE): dex treated vs control
## Wald test p-value: dex treated vs control
## DataFrame with 6 rows and 7 columns
##                   baseMean log2FoldChange     lfcSE      stat    pvalue
##                  <numeric>      <numeric> <numeric> <numeric> <numeric>
## ENSG00000000003 747.194195     -0.3507030  0.168246 -2.084470 0.0371175
## ENSG00000000005   0.000000             NA        NA        NA        NA
## ENSG00000000419 520.134160      0.2061078  0.101059  2.039475 0.0414026
## ENSG00000000457 322.664844      0.0245269  0.145145  0.168982 0.8658106
## ENSG00000000460  87.682625     -0.1471420  0.257007 -0.572521 0.5669691
## ENSG00000000938   0.319167     -1.7322890  3.493601 -0.495846 0.6200029
##                      padj      symbol
##                 <numeric> <character>
## ENSG00000000003  0.163035      TSPAN6
## ENSG00000000005        NA        TNMD
## ENSG00000000419  0.176032        DPM1
## ENSG00000000457  0.961694       SCYL3
## ENSG00000000460  0.815849    C1orf112
## ENSG00000000938        NA         FGR
```

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

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

Answer:

```
## '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, ..
```

We can arrange and view the results by the adjusted p-value.

```
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
```

Finally, let's write out the ordered significant results with annotations.

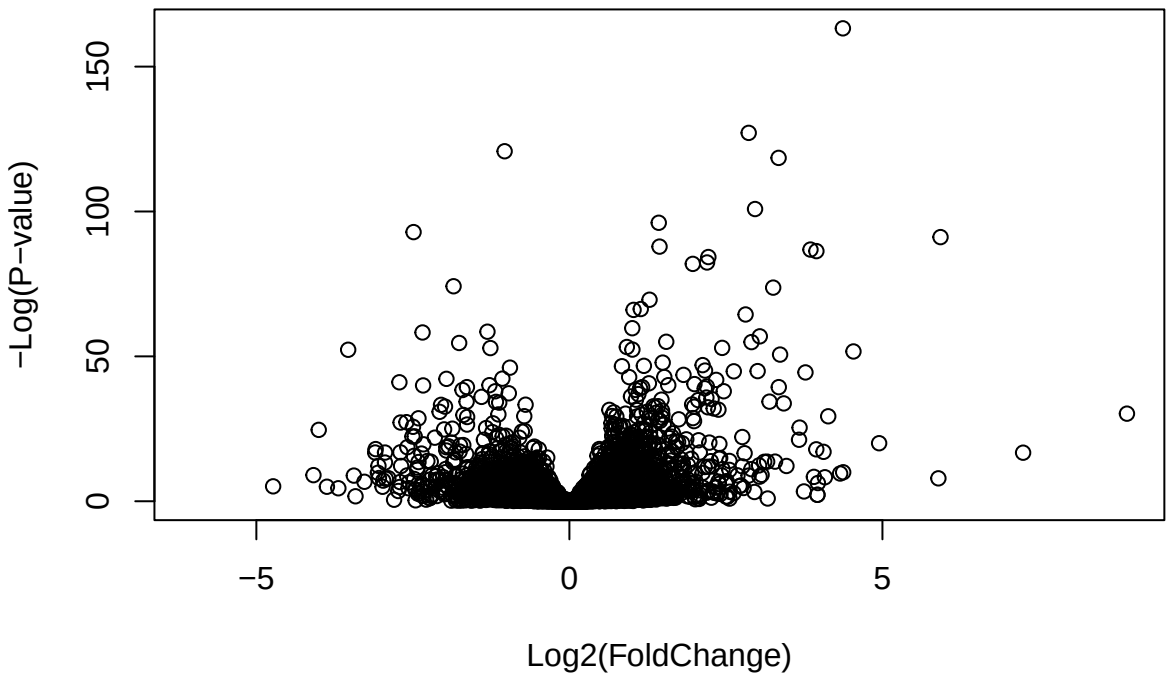
```
write.csv(res[ord,], "deseq_results.csv")
```

6. Data Visualization

Volcano plots

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.

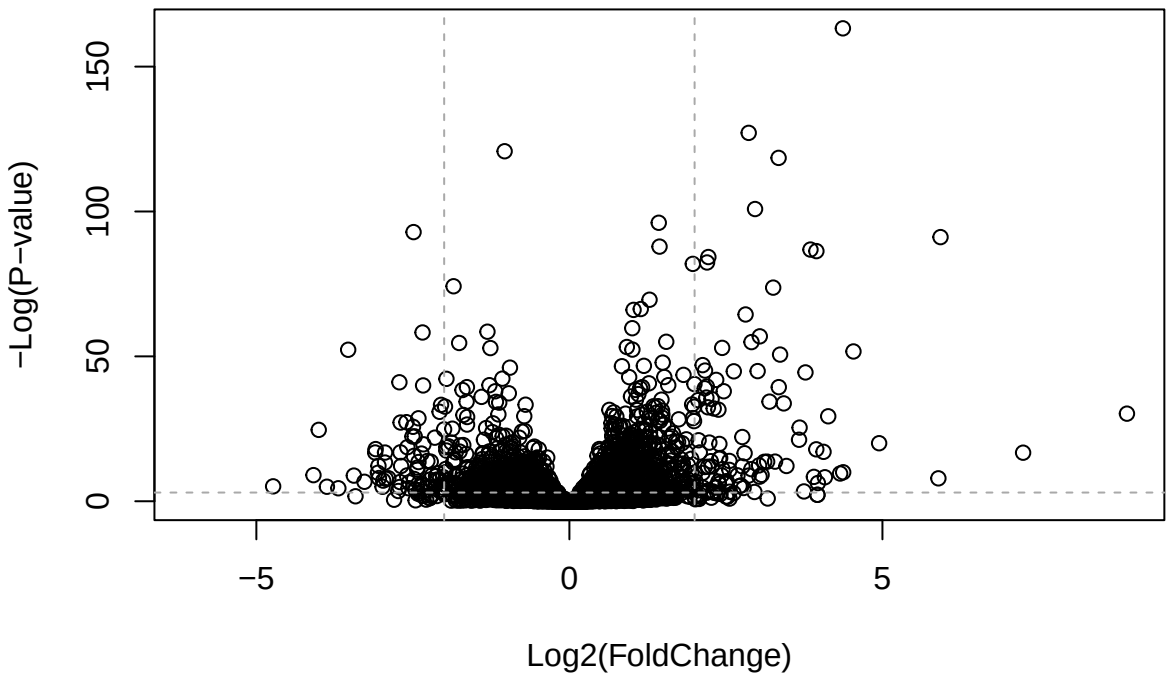
```
plot( res$log2FoldChange,  -log(res$padj),
      xlab="Log2(FoldChange)",
      ylab="-Log(P-value)")
```



To make this more useful we can add some guidelines (with the abline() function) and color (with a custom color vector) highlighting genes that have padj<0.05 and the absolute log2FoldChange>2.

```
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)
```



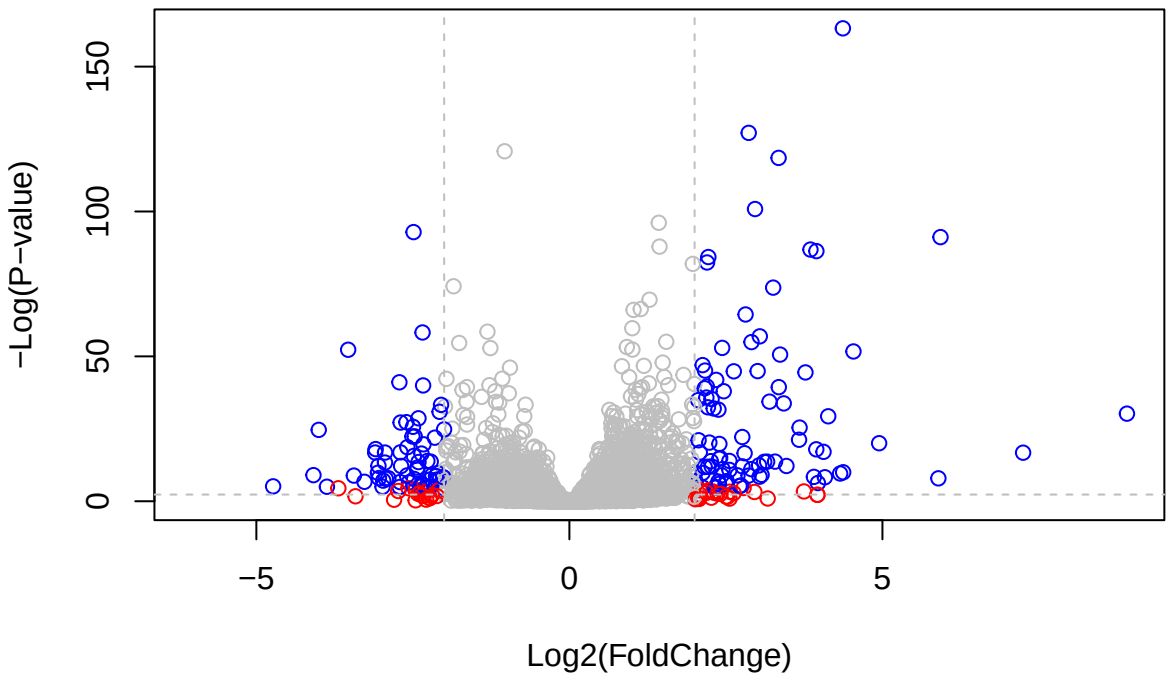
To color the points we will setup a custom color vector indicating transcripts with large fold change and significant differences between conditions:

```
# 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)
```



For even more customization you might find the EnhancedVolcano bioconductor package useful (Note. It uses ggplot under the hood):

First we will add the more understandable gene symbol names to our full results object res as we will use this to label the most interesting genes in our final plot.

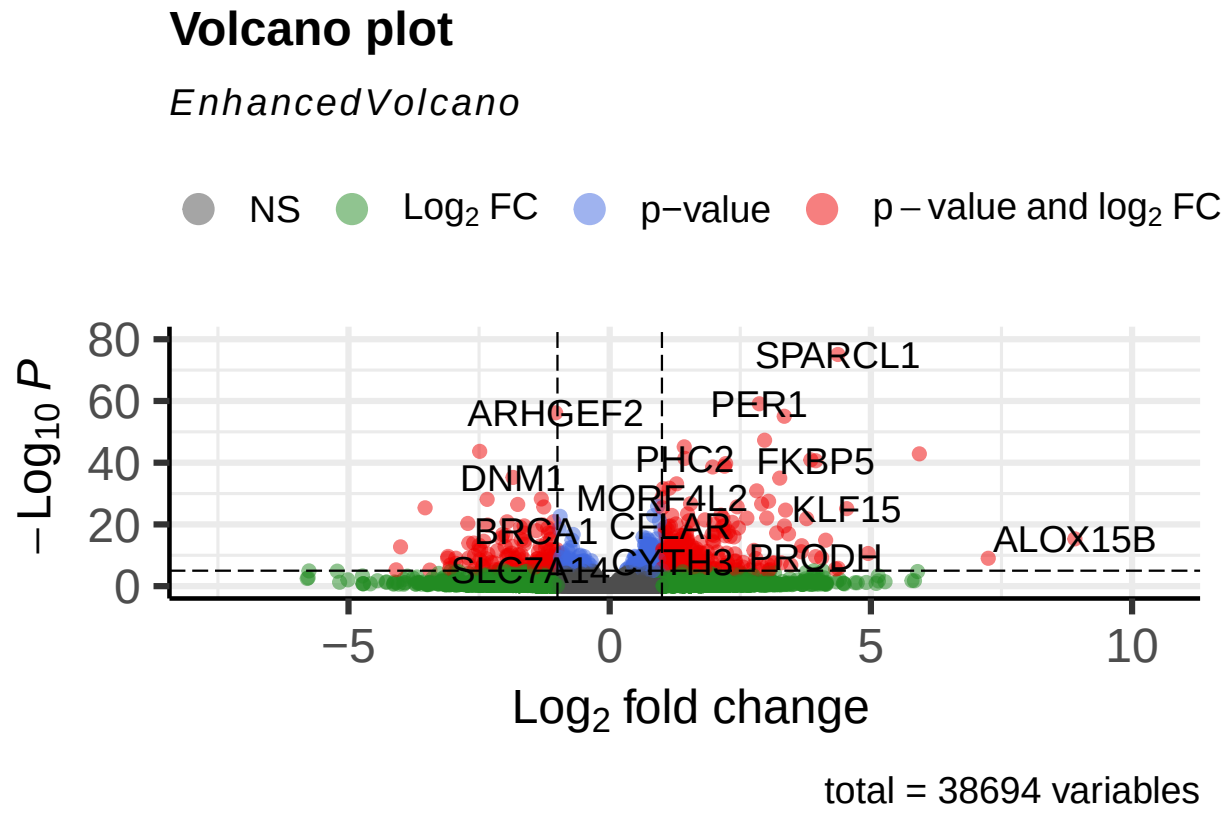
```
#BiocManager::install
library(EnhancedVolcano)
```

```
## 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

Pathway analysis with R and Bioconductor

GAGE package (which stands for Generally Applicable Gene set Enrichment): to do KEGG pathway enrichment analysis on our RNA-seq based differential expression results

The KEGG pathway database, unlike GO for example, provides functional annotation as well as information about gene products that interact with each other in a given pathway, how they interact (e.g., activation, inhibition, etc.), and where they interact (e.g., cytoplasm, nucleus, etc.). Hence KEGG has the potential to provide extra insight beyond annotation lists of simple molecular function, process etc. from GO terms.

```
#BiocManager::install( c("pathview", "gage", "gageData") )
library(pathview)
```

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

```
library(gage)
```

```
##
```

```
library(gageData)
```

```
data(kegg.sets.hs)

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

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

```
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
head(foldchanges)
```

```
##        7105       64102        8813       57147       55732        2268
## -0.35070302          NA  0.20610777  0.02452695 -0.14714205 -1.73228897
```

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

```
attributes(keggres)
```

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

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

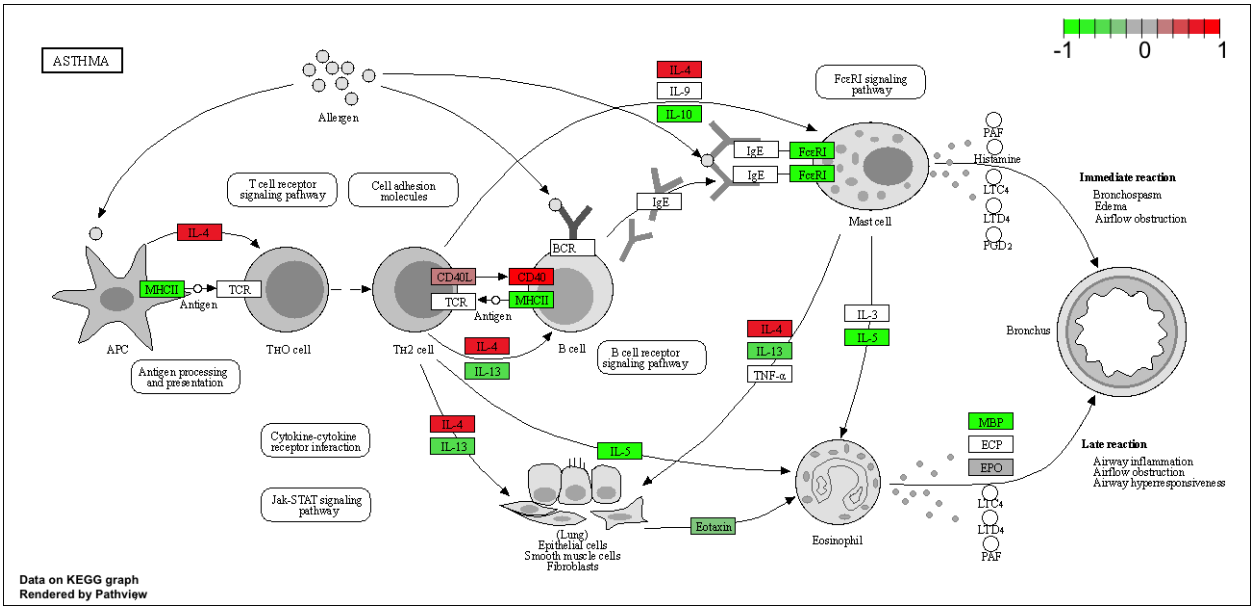
```
##                                      p.geomean stat.mean        p.val
## hsa05332 Graft-versus-host disease 0.0004250461 -3.473346 0.0004250461
## hsa04940 Type I diabetes mellitus  0.0017820293 -3.002352 0.0017820293
## hsa05310 Asthma                    0.0020045888 -3.009050 0.0020045888
##                                         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/seongtaesmacbook/Downloads/2022 WI/BIMM 143/class11
```

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



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

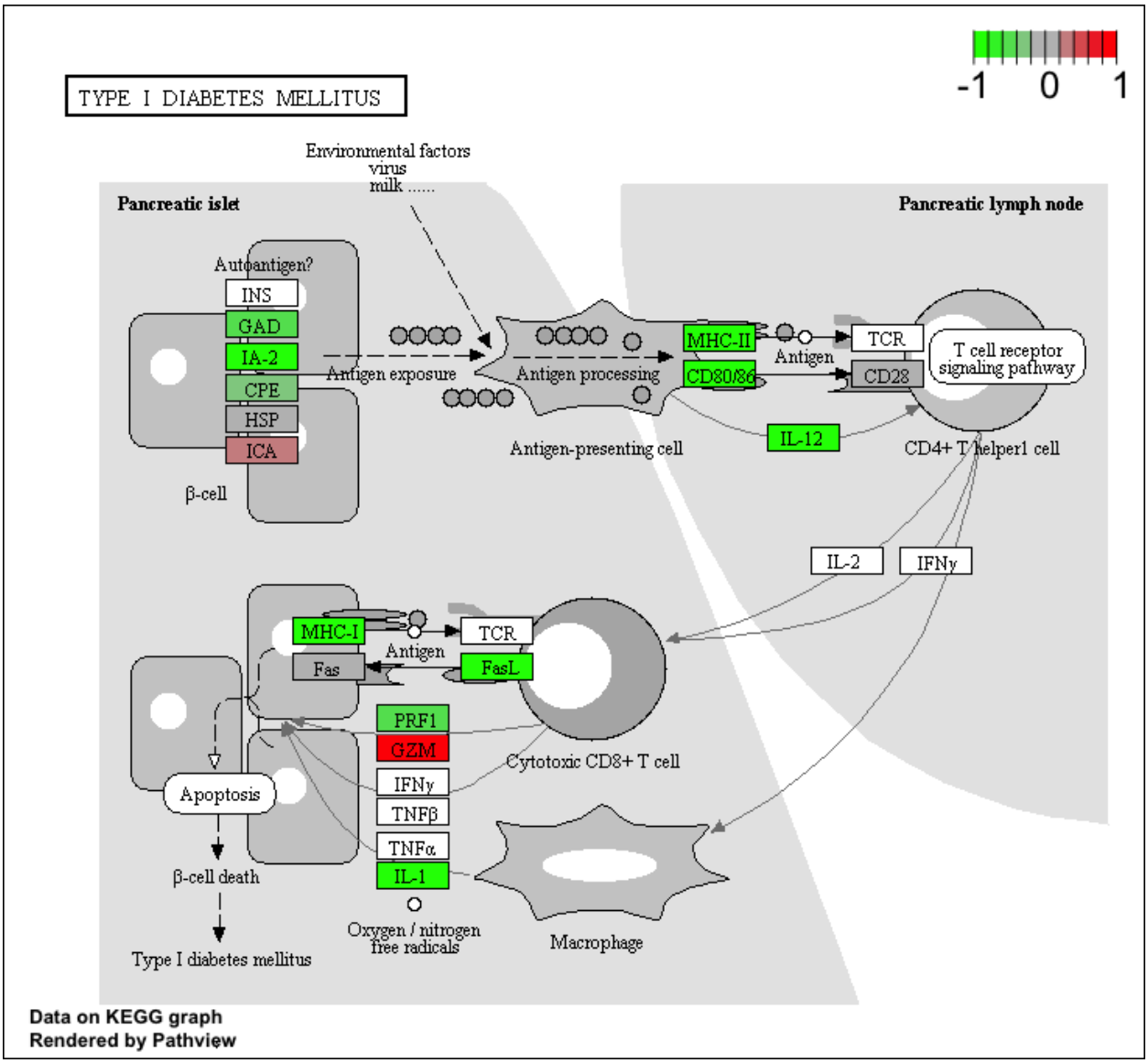
```
pathview(gene.data=foldchanges, pathway.id="hsa04940")
```

Answer:

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

```
## Info: Working in directory /Users/seongtaesmacbook/Downloads/2022 WI/BIMM 143/class11
```

```
## Info: Writing image file hsa04940.pathview.png
```

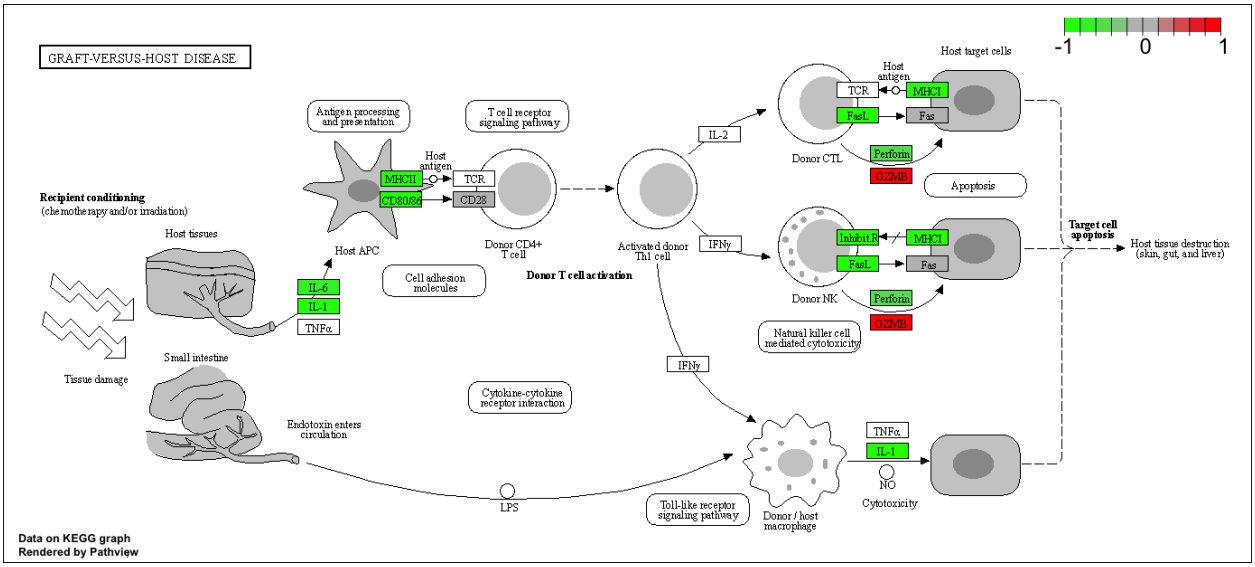



```
pathview(gene.data=foldchanges, pathway.id="hsa05332")
```

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

```
## Info: Working in directory /Users/seongtaesmacbook/Downloads/2022 WI/BIMM 143/class11
```

```
## Info: Writing image file hsa05332.pathview.png
```


GRAFT-VERSUS-HOST DISEASE
-1
0
1
Host target cells
Host antigen
TCR
MHCI
FasL
Fas
Donor CTL
Perforin
GZMB
Apoptosis
Antigen processing and presentation
T cell receptor signaling pathway
IL-2
Host antigen
MHCII
TCR
CD80/86
CD28
Recipient conditioning
(chemotherapy and/or irradiation)
Host tissues
Host APC
Donor CD4+ T cell
Activated donor Th1 cell
IFNγ
Inhibit.R
MHCI
FasL
Fas
Donor NK
Perforin
GZMB
Natural killer cell mediated cytotoxicity
Target cell apoptosis
Host tissue destruction (skin, gut, and liver)
IL-6
IL-1
TNFα
Cell adhesion molecules
Donor T cell activation
Tissue damage
Small intestine
IFNγ
Cytokine-cytokine receptor interaction
Endotoxin enters circulation
TNFα
IL-1
NO
Cytotoxicity
LPS
Toll-like receptor signaling pathway
Donor / host macrophage
Data on KEGG graph
Rendered by Pathview