Class 13

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##Background The data for this hands-on session comes from a published RNA-seq experiment where airway smooth muscle cells were treated with dexamethasone, a synthetic glucocorticoid steroid with anti-inflammatory effects (Himes et al. 2014). They found a number of differentially expressed genes but focus much of the discussion on a gene called CRISPLD2.

This gene encodes a secreted protein known to be involved in lung development, and SNPs in this gene in previous GWAS studies are associated with inhaled corticosteroid resistance and bronchodilator response in asthma patients.

##Bioconductor setup

```r
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, 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
```

##Import countData and colData

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

Now, take a look at the head of each.

```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
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
```

38,694 genes

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

```r
#View(metadata)
```

4 control cell lines

##Toy differential gene expression Lets perform some exploratory differential gene expression analysis.

We first need to find the sample ids for the labeled controls and then calculate the mean counts per gene across the samples.

```r
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
```

Q3. How would you make the above code in either approach more robust? Is there a function that could help here? rowMeans()

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)

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

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

Now lets combine our meancount data

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

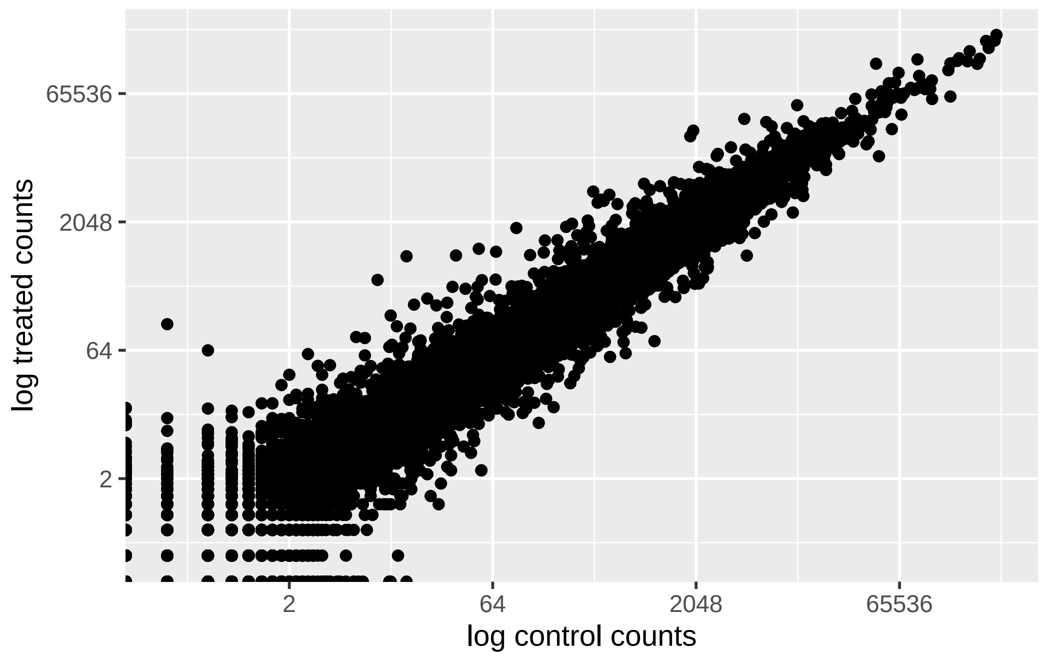
Q5 (a). Create a scatter plot showing the mean of the treated samples against the mean of the control samples.

```r
library(ggplot2)

ggplot(meancounts) +
  aes(x = meancounts[,1], y = meancounts [,2]) +
  geom_point() +
  labs(x = "log control counts", y = "log treated counts") +
  scale_x_continuous(trans="log2") +
  scale_y_continuous(trans="log2")
```

```
Warning in scale_x_continuous(trans = "log2"): log-2 transformation introduced
infinite values.

Warning in scale_y_continuous(trans = "log2"): log-2 transformation introduced
infinite values.
```



We often log2 data since if there is no change, the log2 value will be 0, if doubled it will be 1, and if halved it will be -1.

Here we calculate log2foldchange, add it to our meancounts data.frame and inspect the results either with the head() or the View() function for example.

```
meancounts$log2fc <- log2(meancounts[,"treated.mean"]/meancounts[,"control.mean"])
head(meancounts)
```

```
                control.mean treated.mean      log2fc
ENSG00000000003       900.75       658.00 -0.45303916
ENSG00000000005         0.00         0.00         NaN
ENSG00000000419       520.50       546.00  0.06900279
ENSG00000000457       339.75       316.50 -0.10226805
ENSG00000000460        97.25        78.75 -0.30441833
ENSG00000000938         0.75         0.00        -Inf
```

There are a couple of "weird" results. Namely, the NaN ("not a number") and -Inf (negative infinity) results. Let's filter our data to remove these genes.

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

to.rm <- unique(zero.vals[,1])
mycounts <- meancounts[-to.rm,]
head(mycounts)
```

```
                control.mean treated.mean      log2fc
ENSG00000000003       900.75       658.00 -0.45303916
ENSG00000000419       520.50       546.00  0.06900279
ENSG00000000457       339.75       316.50 -0.10226805
ENSG00000000460        97.25        78.75 -0.30441833
ENSG00000000971      5219.00      6687.50  0.35769358
ENSG00000001036      2327.00      1785.75 -0.38194109
```

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=TRUE argument will tell which() to return both the row and column indices where there are TRUE values. In this case this will tell us which genes and samples have 0 counts. Calling unique() ensures we don't count any row twice if it has zero entries in both samples.

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)
```

```
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

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

Q10. Do you trust these results? Why or why not? No I don't trust these, since from the dataset of a few thousand only a couple hundred are returned. There might hits that are not actually recorded in this data being represented.

##Setting up for DESeq

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

```
To cite package 'DESeq2' in publications use:

  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 will use the DESeqDataSetFromMatrix() function to build the required DESeqDataSet object and call it dds, short for our DESeqDataSet. If you get a warning about "some variables in design formula are characters, converting to factors" don't worry about it.

```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
```

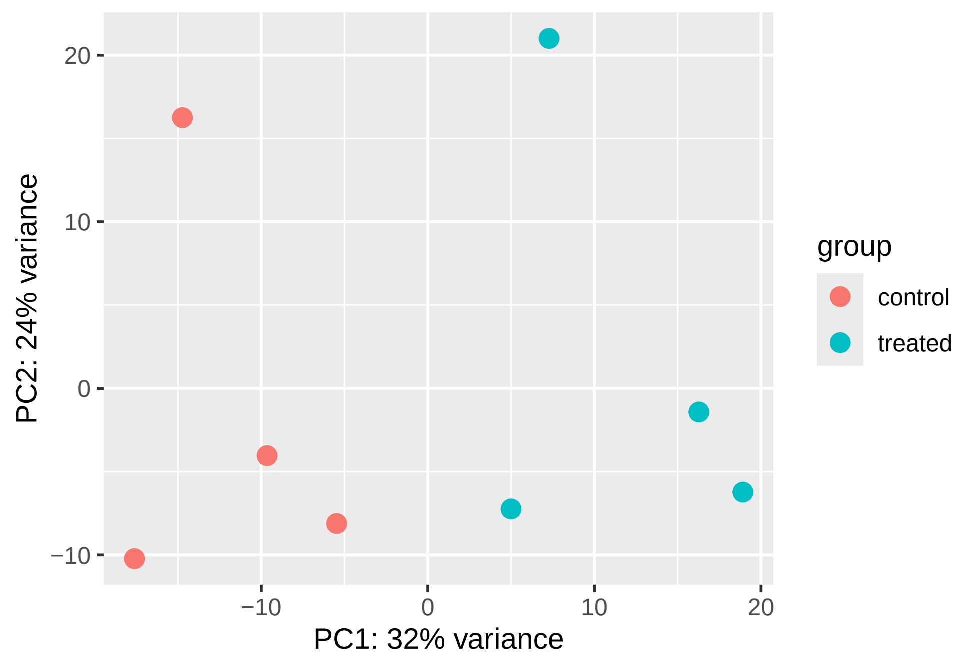
```r
dds
```

```
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
```

##Principal Component Analysis (PCA) Before running DESeq analysis we can look how the count data samples are related to one another via Principal Component Analysis (PCA). We must normalize the data via vst() transformation.

```r
vsd <- vst(dds, blind = FALSE)
plotPCA(vsd, intgroup = c("dex"))
```

```
using ntop=500 top features by variance
```

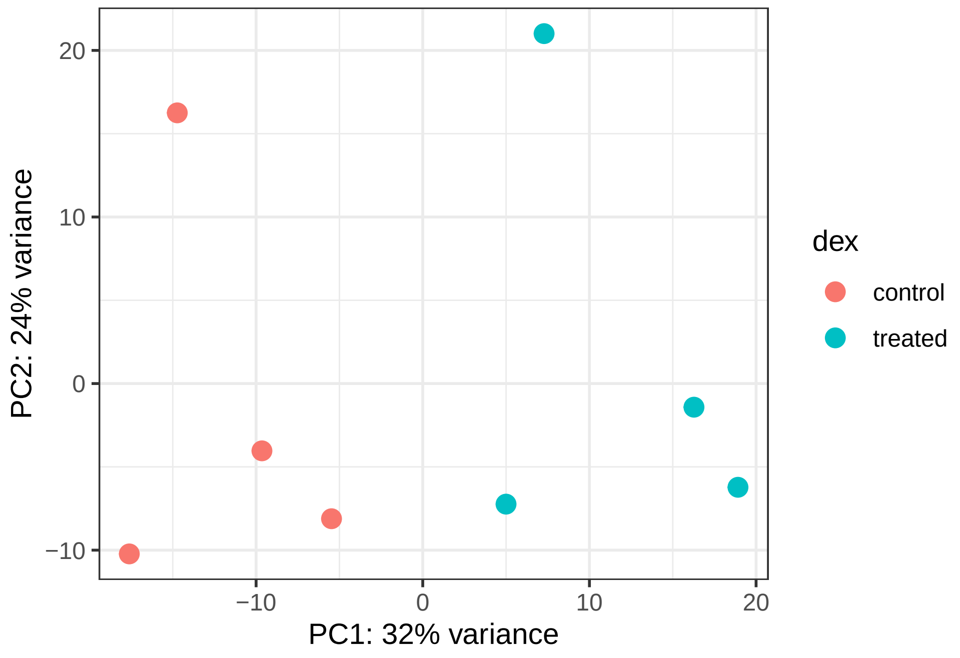


We can also build the PCA plot from scratch using the ggplot2 package. This is done by asking the plotPCA function to return the data used for plotting rather than building the plot.

```
pcaData <- plotPCA(vsd, intgroup=c("dex"), returnData=TRUE)
```

```
using ntop=500 top features by variance
```

```
percentVar <- round(100 * attr(pcaData, "percentVar"))
ggplot(pcaData) +
  aes(x = PC1, y = PC2, color = dex) +
  geom_point(size =3) +
  xlab(paste0("PC1: ", percentVar[1], "% variance")) +
  ylab(paste0("PC2: ", percentVar[2], "% variance")) +
  coord_fixed() +
  theme_bw()
```



##DESeq analysis Here, we're running the DESeq pipeline on the dds object, and reassigning the whole thing back to dds, which will now be a DESeqDataSet populated with all those values.

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

We can get results out of the object simply by calling the results() function on the DESeq-DataSet that has been run through the pipeline.

```
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
```

Convert the res object to a data.frame with the as.data.frame() function and then pass it to View() to bring it up in a data viewer.

```
res = as.data.frame(res)
```

```
summary(res)
```

```
    baseMean         log2FoldChange       lfcSE             stat
```

```
 Min.   :      0.0   Min.   :-6.030   Min.   :0.057   Min.   :-15.894
 1st Qu.:      0.0   1st Qu.:-0.425   1st Qu.:0.174   1st Qu.: -0.643
 Median :      1.1   Median :-0.009   Median :0.445   Median : -0.027
 Mean   :    570.2   Mean   :-0.011   Mean   :1.136   Mean   :  0.045
 3rd Qu.:    201.8   3rd Qu.: 0.306   3rd Qu.:1.848   3rd Qu.:  0.593
 Max.   :329280.4   Max.   : 8.906   Max.   :3.534   Max.   : 18.422
                     NA's   :13436    NA's   :13436   NA's   :13436
     pvalue            padj
 Min.   :0.000   Min.   :0.000
 1st Qu.:0.168   1st Qu.:0.203
 Median :0.533   Median :0.606
 Mean   :0.495   Mean   :0.539
 3rd Qu.:0.800   3rd Qu.:0.866
 Max.   :1.000   Max.   :1.000
 NA's   :13578   NA's   :23549
```

```r
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
```

##Add Annotation data

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.

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

We can use the mapIds() function to add individual columns to our results table. We provide the row names of our results table as a key, and specify that keytype=ENSEMBL. The column argument tells the mapIds() function which information we want, and the multiVals argument tells the function what to do if there are multiple possible values for a single input value.

```
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
```

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), # 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
```

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

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

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

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

```r
head(res)
```

```
                  baseMean log2FoldChange     lfcSE       stat     pvalue
ENSG00000000003 747.1941954    -0.35070302 0.1682457 -2.0844697 0.03711747
ENSG00000000005   0.0000000             NA        NA         NA         NA
ENSG00000000419 520.1341601     0.20610777 0.1010592  2.0394752 0.04140263
ENSG00000000457 322.6648439     0.02452695 0.1451451  0.1689823 0.86581056
ENSG00000000460  87.6826252    -0.14714205 0.2570073 -0.5725210 0.56696907
ENSG00000000938   0.3191666    -1.73228897 3.4936010 -0.4958463 0.62000288
                     padj symbol entrez    uniprot
ENSG00000000003 0.1630348 TSPAN6   7105 A0A024RCI0
ENSG00000000005        NA   TNMD  64102     Q9H2S6
ENSG00000000419 0.1760317   DPM1   8813     O60762
ENSG00000000457 0.9616942  SCYL3  57147     Q8IZE3
ENSG00000000460 0.8158486  FIRRM  55732 A0A024R922
ENSG00000000938        NA    FGR   2268     P09769
                                                                   genename
ENSG00000000003                                               tetraspanin 6
ENSG00000000005                                                 tenomodulin
ENSG00000000419 dolichyl-phosphate mannosyltransferase subunit 1, catalytic
ENSG00000000457                                    SCY1 like pseudokinase 3
ENSG00000000460   FIGNL1 interacting regulator of recombination and mitosis
ENSG00000000938              FGR proto-oncogene, Src family tyrosine kinase
```

Now view by adjusted p-value

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

```
                  baseMean log2FoldChange      lfcSE      stat       pvalue
ENSG00000152583   954.7709       4.368359 0.23712679  18.42204 8.744898e-76
ENSG00000179094   743.2527       2.863889 0.17556931  16.31201 8.107836e-60
ENSG00000116584  2277.9135      -1.034701 0.06509844 -15.89440 6.928546e-57
ENSG00000189221  2383.7537       3.341544 0.21240579  15.73189 9.144326e-56
ENSG00000120129  3440.7038       2.965211 0.20369513  14.55710 5.264243e-48
ENSG00000148175 13493.9204       1.427168 0.10038904  14.21638 7.251278e-46
                        padj  symbol entrez    uniprot
ENSG00000152583 1.324415e-71 SPARCL1   8404 A0A024RDE1
ENSG00000179094 6.139658e-56    PER1   5187     O15534
ENSG00000116584 3.497761e-53 ARHGEF2   9181     Q92974
```

```
ENSG00000189221 3.462270e-52    MAOA   4128     P21397
ENSG00000120129 1.594539e-44   DUSP1   1843     B4DU40
ENSG00000148175 1.830344e-42    STOM   2040     F8VSL7
                                                      genename
ENSG00000152583                                    SPARC like 1
ENSG00000179094                      period circadian regulator 1
ENSG00000116584 Rho/Rac guanine nucleotide exchange factor 2
ENSG00000189221                             monoamine oxidase A
ENSG00000120129                    dual specificity phosphatase 1
ENSG00000148175                                        stomatin
```

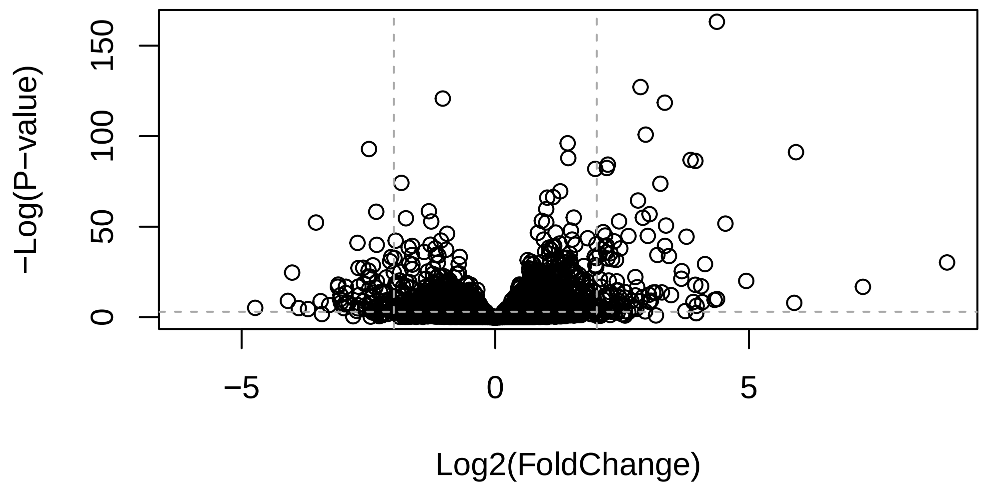
And write a csv file with the ordered results

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

##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),
 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)
```

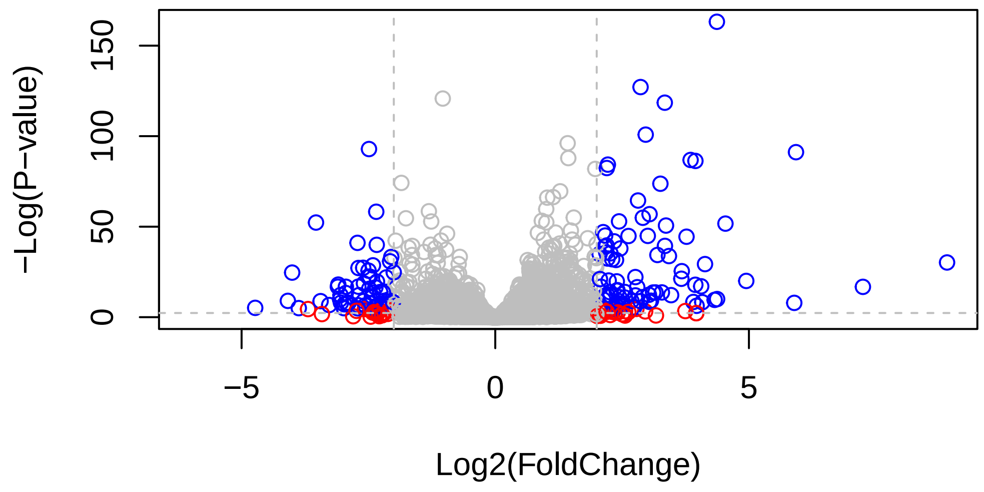


```
# Setup our custom point color vector
mycols <- rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ]  <- "red"

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)
```



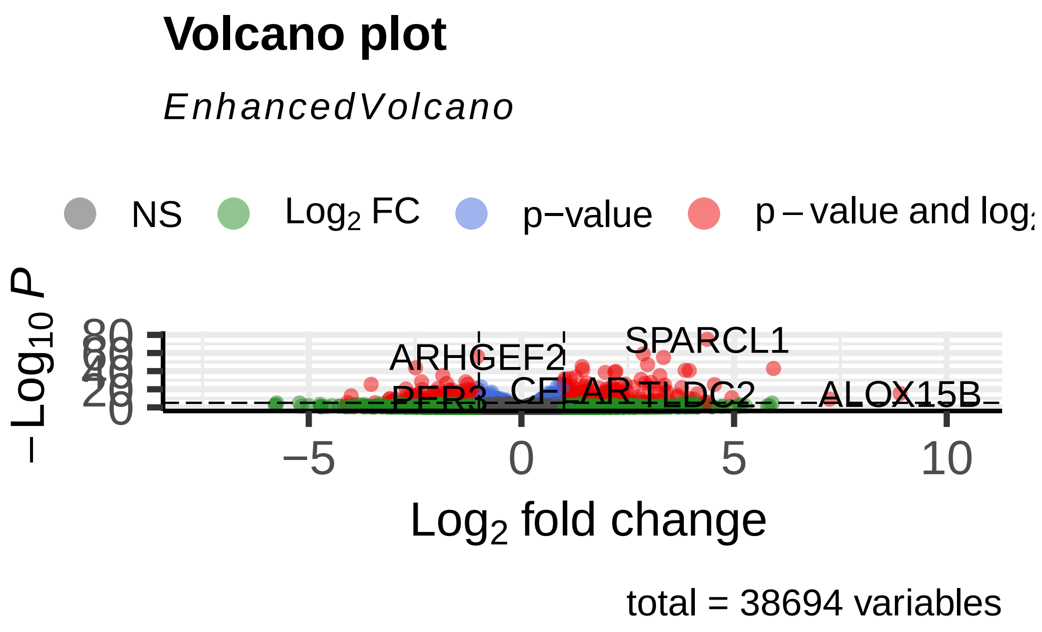
For an enhanced volcano plot, use the EnhanchedVolcano package from bioconductor.

```
library(EnhancedVolcano)
```

```
Loading required package: ggrepel
```

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

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



##Pathway Analysis Now that I have my annotations I can talk to different databases that use these IDs.

We will use the gage package to do geneset analysis (aka pathway analysis, geneset enrichment, overlap analysis). We will use KEGG first.

```
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)

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 is the main gage function that requires a named vector of fold changes where the name of the values are the entrez gene IDs

```r
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
```

Now, let's run the gage pathway analysis.

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

```r
attributes(keggres)
```

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

```
head(keggres$less, 3)
```

```
                                      p.geomean stat.mean        p.val
hsa05332 Graft-versus-host disease 0.0004250461 -3.473346 0.0004250461
hsa04940 Type I diabetes mellitus  0.0017820293 -3.002352 0.0017820293
hsa05310 Asthma                    0.0020045888 -3.009050 0.0020045888
                                        q.val set.size         exp1
hsa05332 Graft-versus-host disease 0.09053483       40 0.0004250461
hsa04940 Type I diabetes mellitus  0.14232581       42 0.0017820293
hsa05310 Asthma                    0.14232581       29 0.0020045888
```

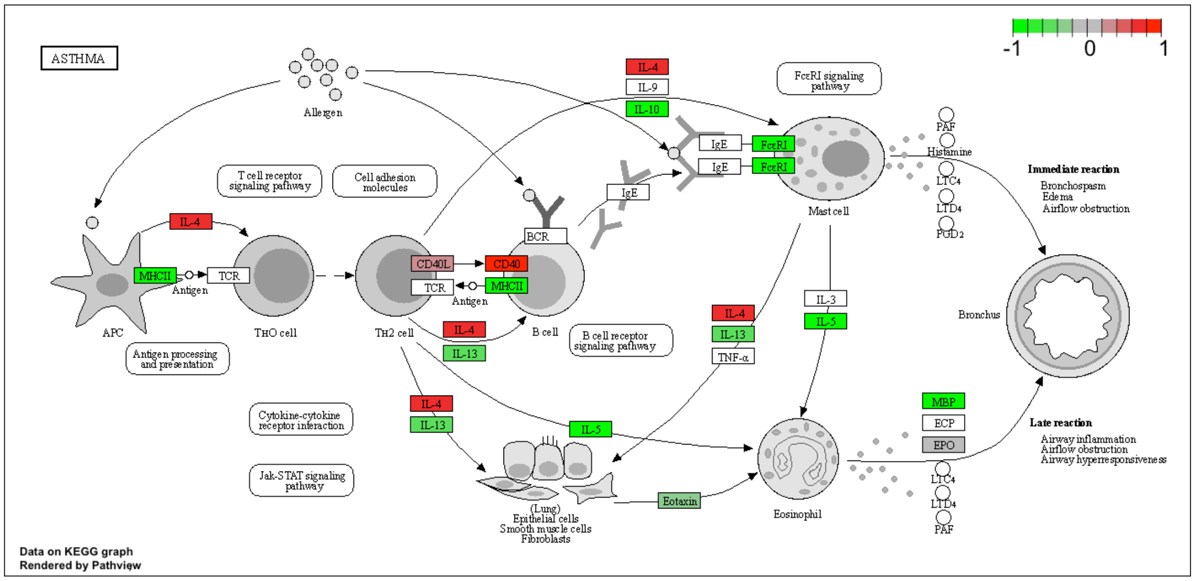
I can now use the returned pathway IDs from KEGG as input to the 'pathview' package to make pathway figures with our DEGs highlighted

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

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

```
Info: Working in directory /Users/kristiwong/Downloads/UCSD/UCSD Courses/BIOLOGY/BIMM143/BIMI
```

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



```r
# 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/kristiwong/Downloads/UCSD/UCSD Courses/BIOLOGY/BIMM143/BIM
```

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