Class 12: Transcriptomics and the Analysis of RNA-Seq Data

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Important countData and colData

```
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
```

There are 38694 genes in this dataset.

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

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

```
control treated
      4       4
```

There are 4 'control' cell lines.

Let's check to see if the id names in metadata match the order of the columns in the countData.

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

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

We can use the `all()` function to check that all its inputs are true.

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

```
[1] TRUE
```

Let's extract only the control columns.

```
control.inds <- metadata$dex == "control"
control.ids <- metadata$id[control.inds]
control.counts <- counts[, control.ids]
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
```

I want a single summary counts value for each gene in the control experiments. I will start by taking the average.

```
# you can also use apply(control.counts, 1, mean)
control.mean <- rowMeans(control.counts)
```

We need to do the same thing to get the `treated.mean`.

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

Toy differential gene expression

This bit of code will first find the sample `id` for those labeled control. It will then calculate the mean counts per gene across the samples.

```
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 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
```

I like using the first method better, because I don't like typing the %>% in dplyr.

Q3. How would you make the above code in either approach more robust?

You can use the function `apply()` to include more functions.

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`)

See above code for `treated.mean`.

We will combine our meancount data for bookkeeping purposes.

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

```
                control.mean treated.mean
ENSG00000000003       900.75       658.00
ENSG00000000005         0.00         0.00
ENSG00000000419       520.50       546.00
ENSG00000000457       339.75       316.50
ENSG00000000460        97.25        78.75
ENSG00000000938         0.75         0.00
```

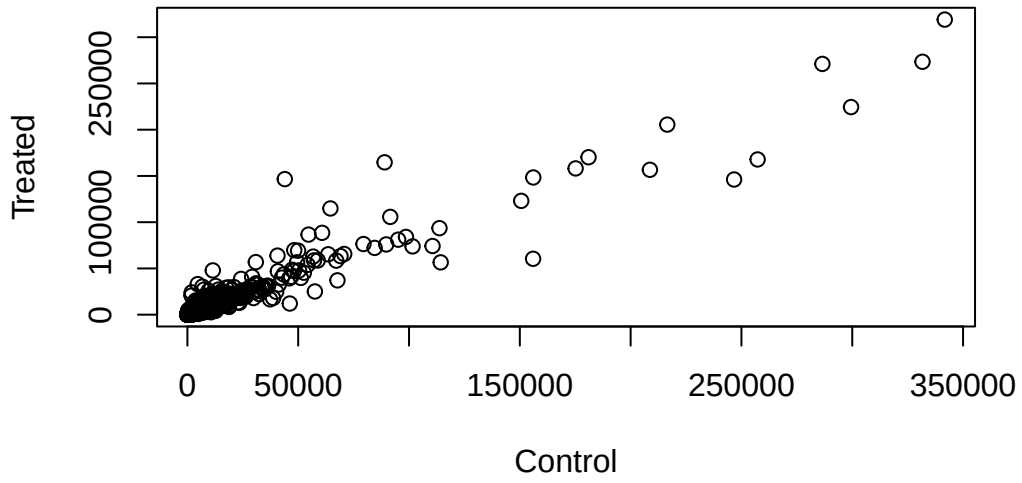
`colSums()` the data to show the sum of the mean counts across all genes for each group.

```
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.

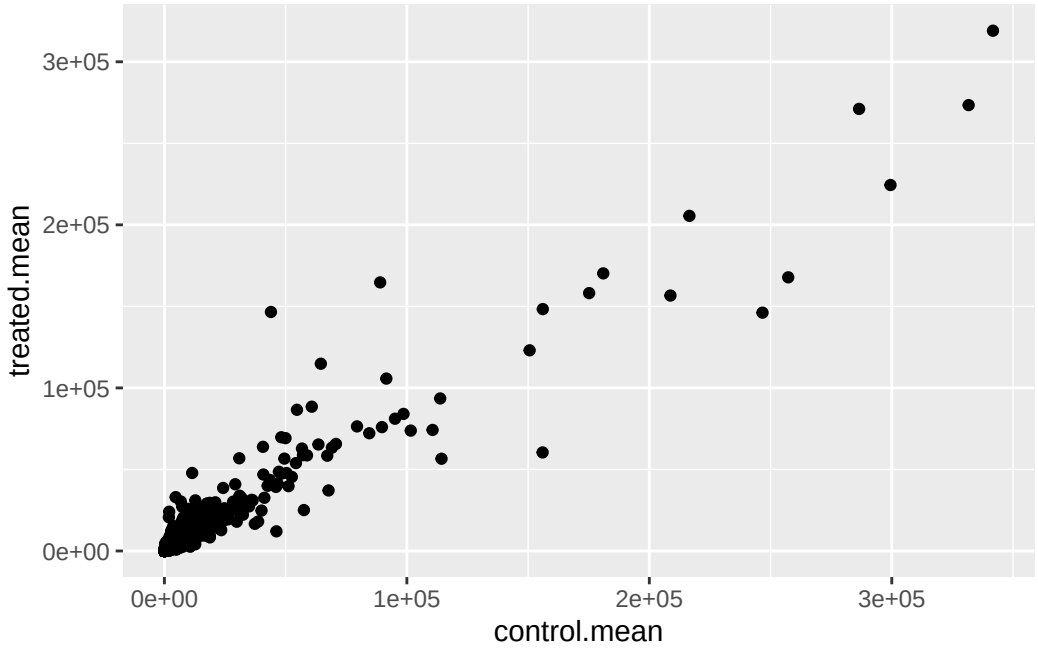
```
plot(meancounts$control.mean, meancounts$treated.mean, xlab = "Control", ylab = "Treated")
```



Q5 (b). You could also use the **ggplot2** pacakge to make this figure producing the plot below. What **geom_?()** function would you use for this plot?

You would use **geom_point()**.

```
library(ggplot2)
ggplot(meancounts, aes(control.mean, treated.mean)) + geom_point()
```



There are too many points around the origin, even though there are 60,000 points. Use a log

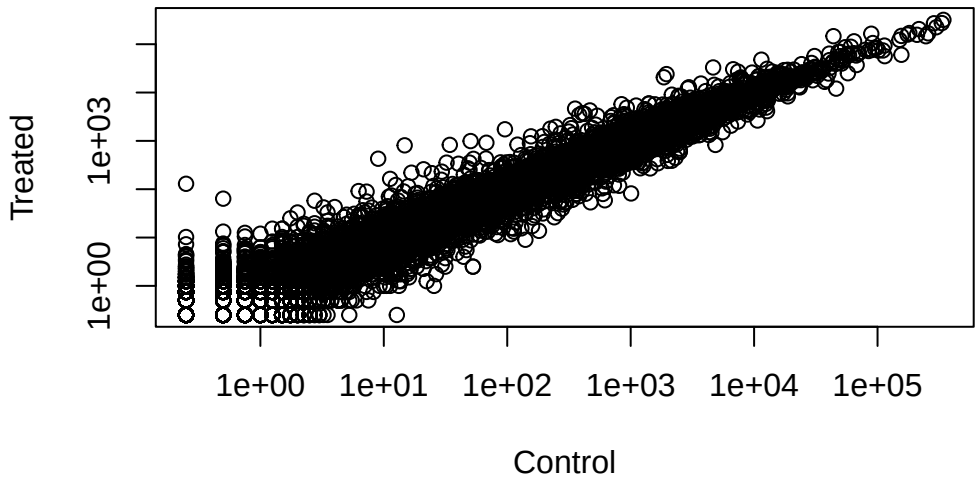
function to visualize more points.

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

```
plot(meancounts$control.mean, meancounts$treated.mean, xlab = "Control", ylab = "Treated",
```

```
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
```



The most useful and most straightforward to understand is log2 transform.

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

```
[1] 0
```

0 means no change.

Doubling

```
log2(40/20)
```

```
[1] 1
```

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

```
[1] -1
```

```
log2(80/20)
```

```
[1] 2
```

We can calculate the log2fold change, add it to our `meancounts` data.frame and inspect the results either with the **head()** or the **View()** function.

```
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
```

Let's filter our data to remove the genes with "NaN" (not a number) and -Inf (negative infinity) results.

```
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` argument returns the array indices if x is an array. In this case, it returns the zero values. The **unique()** function is used to eliminate or delete the duplicate values or rows present. In this case, it deletes the zero values if it has zero entries in both samples.

A common threshold used for calling something differentially expressed is a log2(FoldChange) og 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 upregulated genes we have at the greater than 2 fc level?

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

```
[1] 250
```

There are 250 genes that are 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(mycounts$log2fc < -2)
```

```
[1] 367
```

There are 367 downregulated genes that are greater than 2 fc level.

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

Not really, because we haven't really seen if these results are statistically significant, based on p-values.

DESeq2 analysis

```
library(DESeq2)
```

```
Warning: package 'DESeq2' was built under R version 4.2.2
```

```
Warning: package 'S4Vectors' was built under R version 4.2.2
```

```
Warning: package 'BiocGenerics' was built under R version 4.2.1
```

```
Warning: package 'IRanges' was built under R version 4.2.1
```

```
Warning: package 'GenomicRanges' was built under R version 4.2.2
```

```
Warning: package 'GenomeInfoDb' was built under R version 4.2.2
```

```
Warning: package 'SummarizedExperiment' was built under R version 4.2.1
```

```
Warning: package 'MatrixGenerics' was built under R version 4.2.1
```

```
Warning: package 'Biobase' was built under R version 4.2.1
```

```
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},
  }
```

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
```

DESeq analysis

You have to run **DESeq()** before looking at results.

```
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
```

```
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
```

Adjusting the p-value to 0.05. Its default value is 0.1.

```
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
```

Adding annotation data

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

```
Warning: package 'AnnotationDbi' was built under R version 4.2.1
```

```
Attaching package: 'AnnotationDbi'

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

    select
```

```
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"
```

The main function we will use from the **AnnotationDbi** package is called **mapIds()**. We can use the **mapIds()** function to add individual columns to our results table.

```
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 2 more times to add the Entrez ID and UniProt accession and GENENAME a new columsn 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")
```

```
'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, ..
```

You 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.

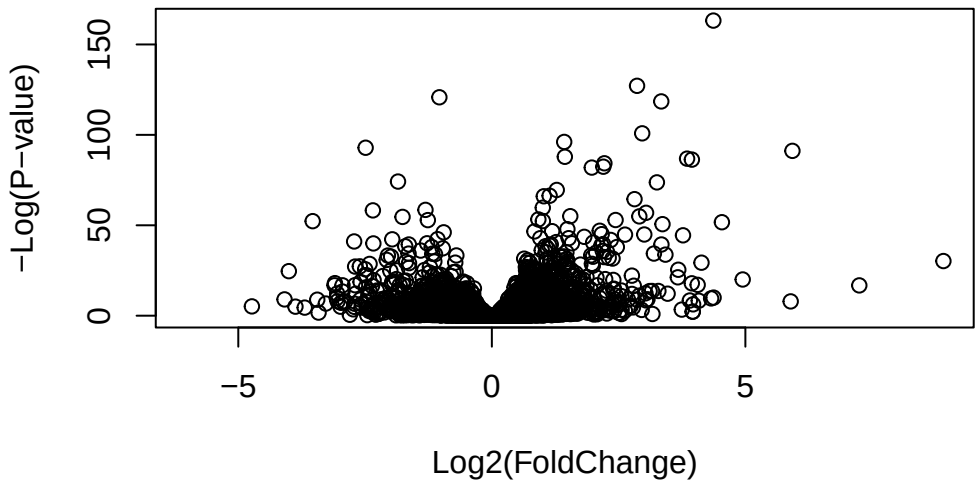
```
write.csv(res[ord, ], "deseq_results.csv")
```

Data Visualization

Volcano plots

Volcano plots are frequently used to highlight the proportion of genes that are both significantly regulated and display a high fold change.

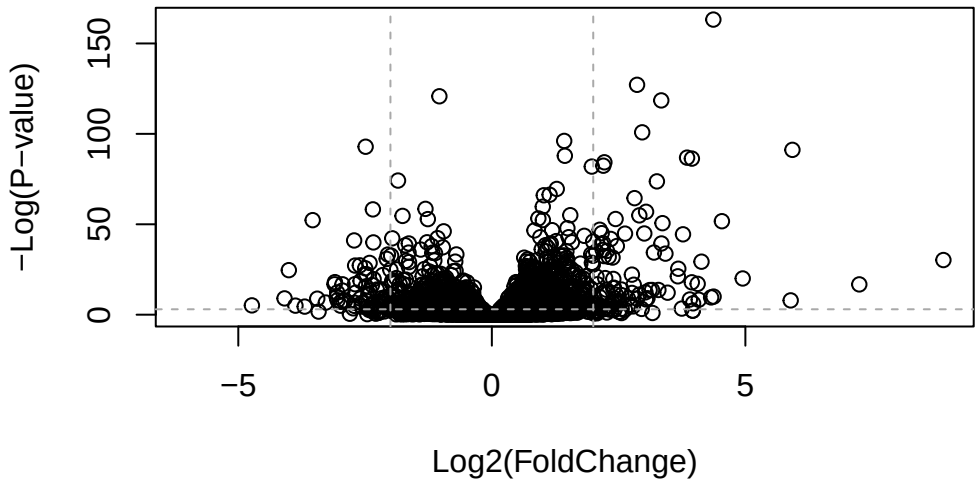
```
plot( res$log2FoldChange,  -log(res$padj),
      xlab="Log2(FoldChange)",
      ylab="-Log(P-value)")
```



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)
```



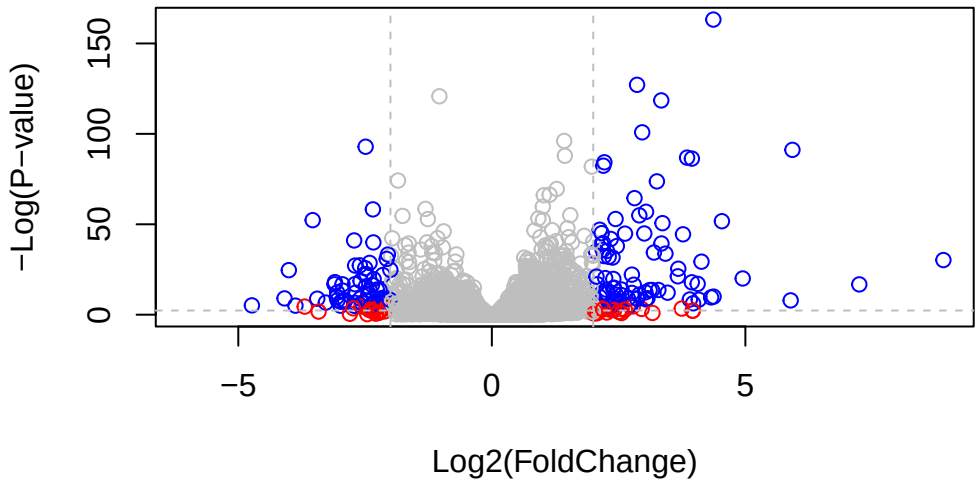
To color the points we will setup a custom color vector indicating transcripts with large fold change and significant differences between conditions:

```
# 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)
```



You can use the **EnhancedVolcano** bioconductor package for more customization.

```
BiocManager::install("Enhanced Volcano")
```

```
Bioconductor version 3.16 (BiocManager 1.30.19), R 4.2.0 (2022-04-22)

Installing package(s) 'Enhanced Volcano'

Warning: package 'Enhanced Volcano' is not available for Bioconductor version '3.16'

A version of this package for your version of R might be available elsewhere,
see the ideas at
https://cran.r-project.org/doc/manuals/r-patched/R-admin.html#Installing-packages

Installation paths not writeable, unable to update packages
  path: /Library/Frameworks/R.framework/Versions/4.2/Resources/library
  packages:
    boot, brew, bslib, callr, class, cli, cluster, codetools, colorspace,
    commonmark, cpp11, crayon, curl, desc, devtools, digest, evaluate, fansi,
    farver, foreign, fs, gert, ggplot2, gh, gitcreds, gtable, highr, htmltools,
    httr, isoband, jsonlite, knitr, lifecycle, markdown, MASS, Matrix, mgcv,
    nlme, nnet, openssl, pillar, pkgbuild, pkgload, processx, ps, purrr, rlang,
    rmarkdown, roxygen2, rpart, rstudioapi, rversions, sass, scales, spatial,
    stringi, stringr, survival, sys, testthat, tibble, tinytex, utf8, vctrs,
    viridisLite, whisker, xfun, yaml, zip

Old packages: 'data.table', 'RSQLite'
```

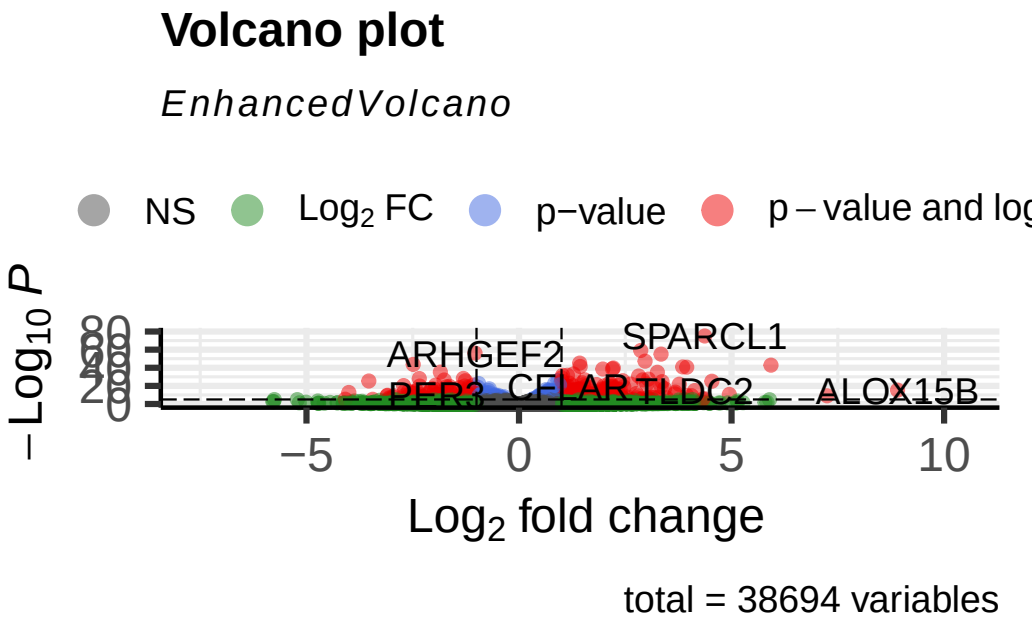
```
library(EnhancedVolcano)
```

```
Warning: package 'EnhancedVolcano' was built under R version 4.2.1
```

```
Loading required package: ggrepel
```

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

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



Pathway analysis

```
library(pathview)
```

```
Warning: package 'pathview' was built under R version 4.2.1
```

```
#############################################################################
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)
```

```
Warning: package 'gage' was built under R version 4.2.1
```

```
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
```

Running the **gage** pathway analysis:

```
# 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
```

Let's try out the **pathview()** function from the pathview package to make a pathway plot with our RNA-Seq expression results shown in color.

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

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

```
Info: Working in directory /Users/audreynguyen/Desktop/BIMM 143/class12
```

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

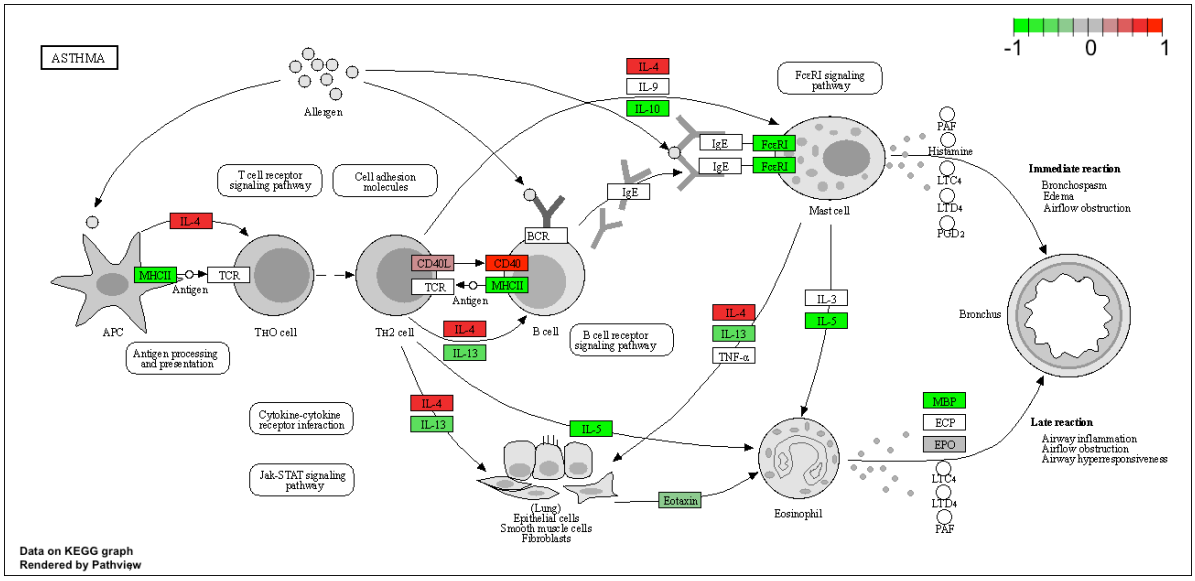


Figure 1: The Asthma pathway with my highlighted differentially expressed genes in color