Class 14: DESeq2 Analysis Mini-Proj

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Finishing up Class 13

OLD CODE

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

```
                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(count)
```

```
[1] 38694
```

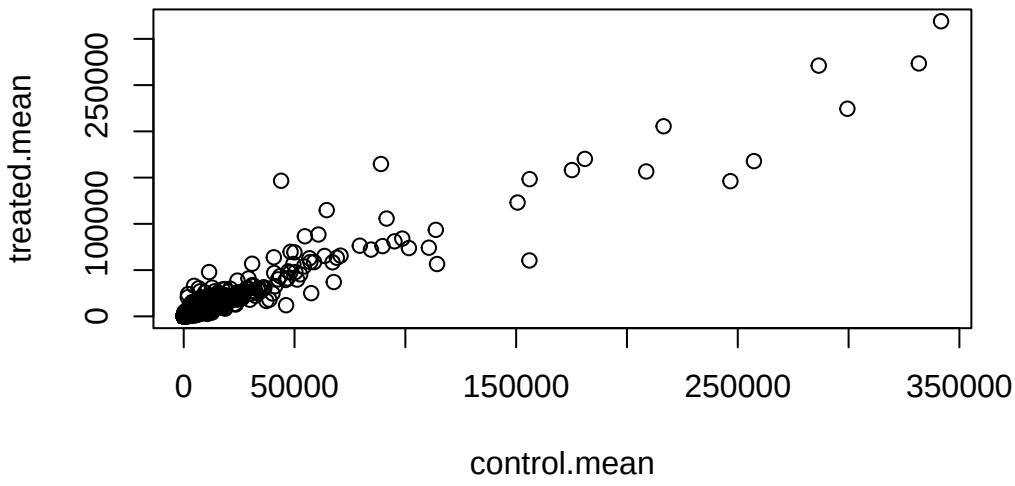
```
table(metadata$dex)
```

```
control treated
      4       4
```

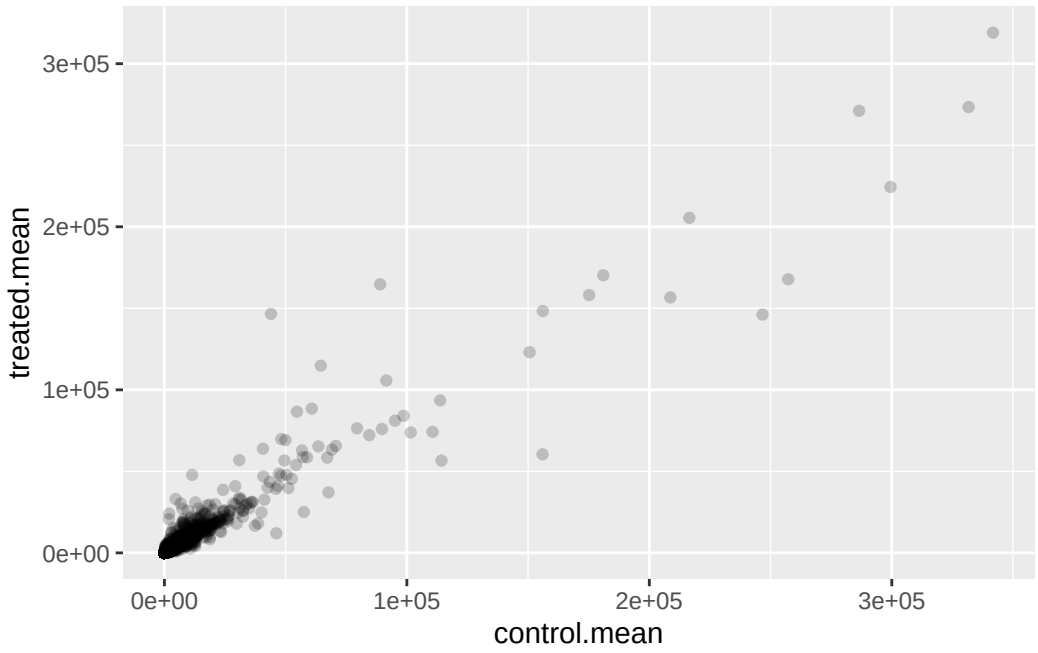
```
control.mean <- rowMeans(count[, metadata$dex == "control"])
treated.mean <- rowMeans(count[, metadata$dex == "treated"])
meancount <- data.frame(control.mean,treated.mean)
head(meancount)
```

```
                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
```

```
plot(meancount)
library(ggplot2)
```



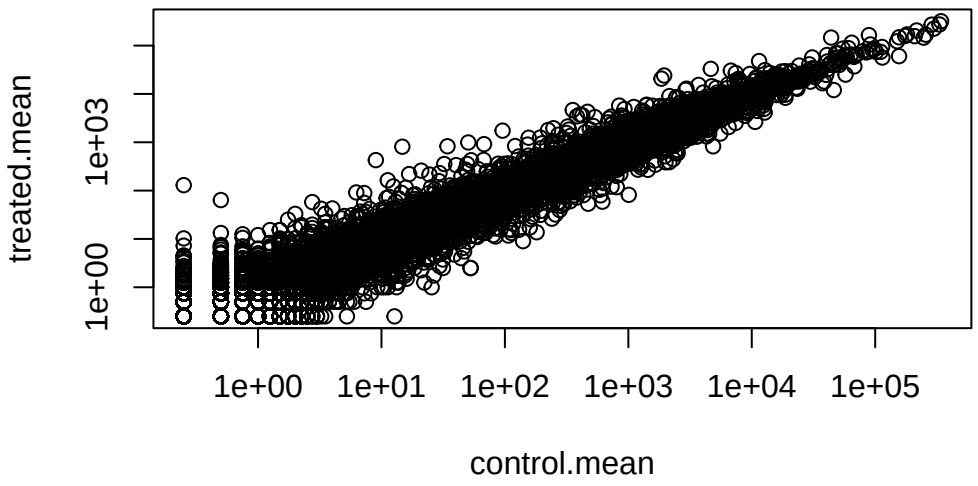

```
ggplot(meancount) +
  aes(control.mean, treated.mean) +
  geom_point(alpha=.2)
```




```
plot(meancount, 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
```




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

```
                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
```

```
to.rm.inds <- rowSums(meancount[,1:2] == 0) > 0
   #meancount[!to.rm.inds,]
mycount <- meancount[-to.rm.inds,]
head(mycount)
```

```
                control.mean treated.mean      log2fc
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
ENSG00000000971      5219.00      6687.50  0.35769358
```

```
dim(mycount)
```

```
[1] 38693     3
```

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

```
dds <- DESeqDataSetFromMatrix(countData = count,
                              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 <- DESeq(dds)
```

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

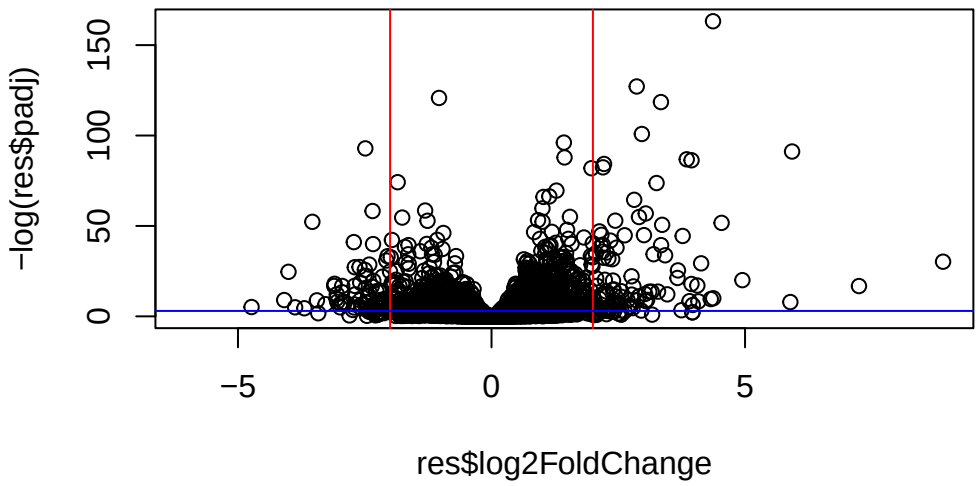
```
final dispersion estimates

fitting model and testing
```

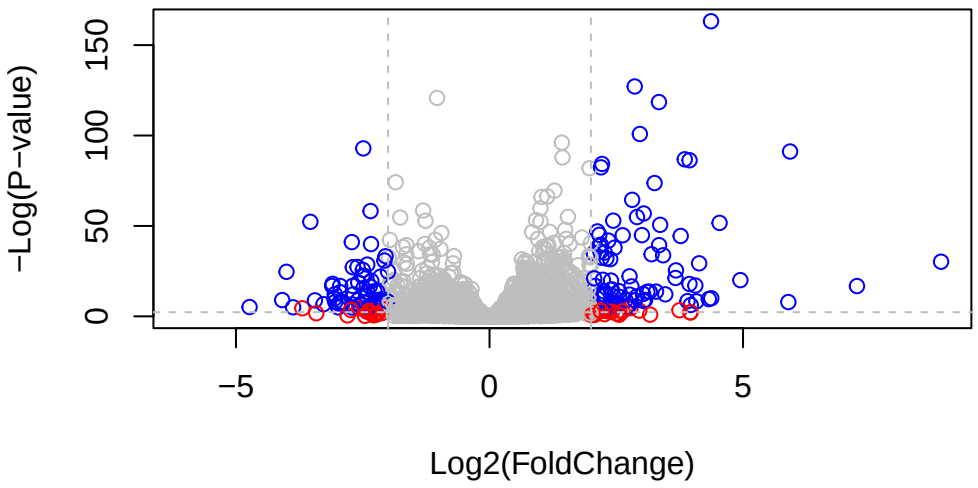
```
res <- results(dds)
head(res)
```

```
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
```

```
plot(res$log2FoldChange, -log(res$padj))
   abline(v=2, col="red") #everything to the right up regulated
   abline(v=-2,col="red") #everything to the left down regulated
   abline(h=-log(.05), col="blue")
```




```
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)
```



NEW CODE

Section 8: Adding Annotation Data

Installing packages I need

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

```
Warning: package 'AnnotationDbi' was built under R version 4.3.2
```

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

Gettting a list of all available key types to map between

```
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.

```
  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       FIRRM       55732  A0A024R922
ENSG00000000938        NA         FGR        2268      P09769
                           genename
                        <character>
ENSG00000000003          tetraspanin 6
ENSG00000000005            tenomodulin
ENSG00000000419 dolichyl-phosphate m..
ENSG00000000457 SCY1 like pseudokina..
ENSG00000000460 FIGNL1 interacting r..
ENSG00000000938 FGR proto-oncogene, ..
```

Ordering results by 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
```

Writing results to new CSV

```
write.csv(res[ord,], "deseq_results2.csv")
```

10: 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)
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
```

```
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

```
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
```

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

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

```
Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq
```

```
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?

Yes, yes i can

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

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

```
Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq
```

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

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

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

```
Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq
```

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

OPTIONAL: Plotting counts for genes of interest

Finding GeneID for CRISPLD2

```
i <- grep("CRISPLD2", res$symbol)
res[i,]
```

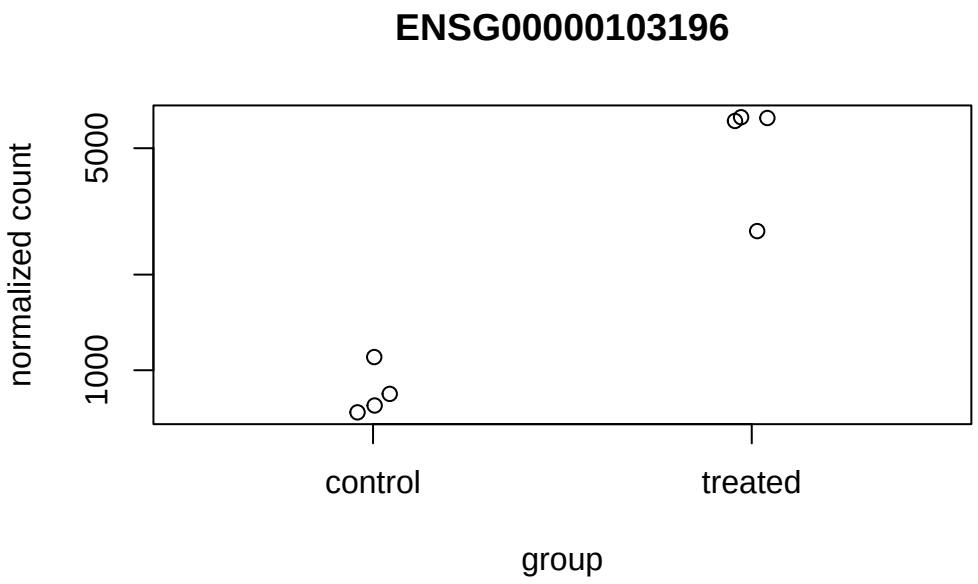
```
log2 fold change (MLE): dex treated vs control
Wald test p-value: dex treated vs control
DataFrame with 1 row and 10 columns
                baseMean log2FoldChange     lfcSE      stat      pvalue
               <numeric>      <numeric> <numeric> <numeric>   <numeric>
ENSG00000103196   3096.16        2.62603  0.267444   9.81899 9.32747e-23
                     padj      symbol      entrez     uniprot
                  <numeric> <character> <character> <character>
ENSG00000103196 3.36344e-20    CRISPLD2       83716  A0A140VK80
                            genename
                         <character>
ENSG00000103196 cysteine rich secret..
```

```
rownames(res[i,])
```

```
[1] "ENSG00000103196"
```

Plotting the counts

```
plotCounts(dds, gene="ENSG00000103196", intgroup="dex")
```



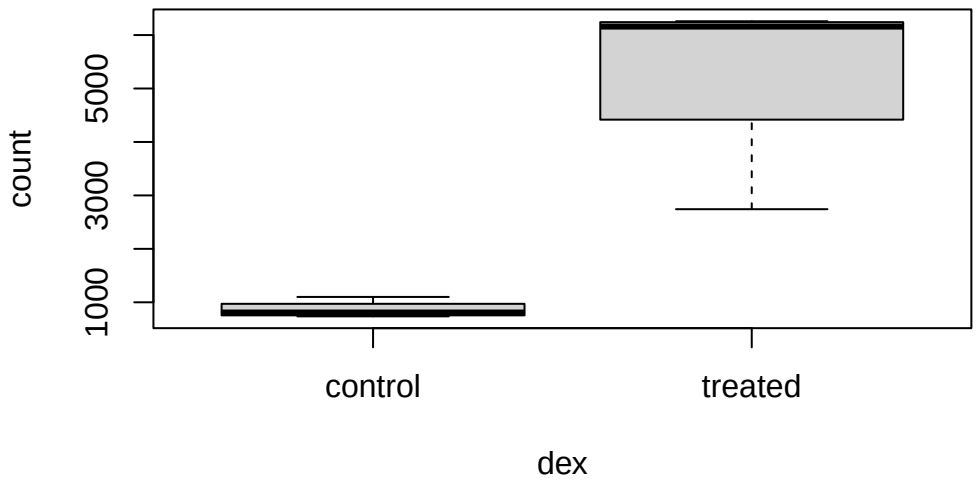
Getting the data instead

```
d <- plotCounts(dds, gene="ENSG00000103196", intgroup="dex", returnData=TRUE)
head(d)
```

```
               count     dex
SRR1039508  774.5002 control
SRR1039509 6258.7915 treated
SRR1039512 1100.2741 control
SRR1039513 6093.0324 treated
SRR1039516  736.9483 control
SRR1039517 2742.1908 treated
```

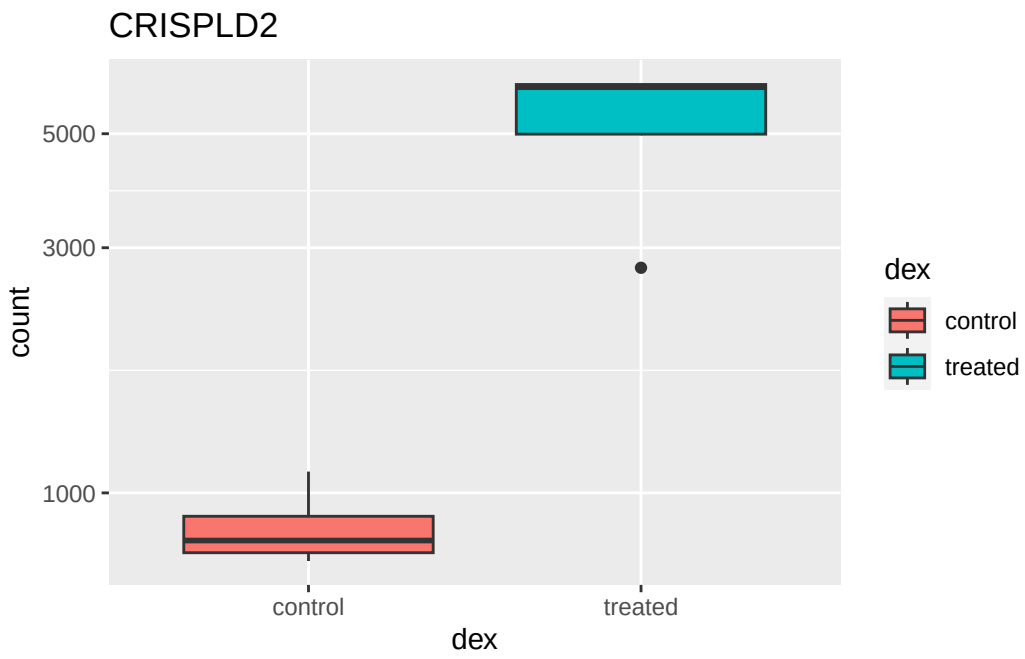
Making a boxplot

```
boxplot(count ~ dex , data=d)
```



New and improved boxplot w/ ggplot2

```
library(ggplot2)
ggplot(d, aes(dex, count, fill=dex)) +
  geom_boxplot() +
  scale_y_log10() +
  ggtitle("CRISPLD2")
```



Class 14:RNA-Seq Analysis Mini-Project

Section 1: Differential Expression Analysis

```
library(DESeq2)
```

Downloading data...

```
metaFile <- "Data/GSE37704_metadata.csv"
countFile <- "Data/GSE37704_featurecounts.csv"
```

Takin' a peek, so to speak

```
colData = read.csv(metaFile, row.names=1)
head(colData)
```

```
             condition
SRR493366 control_sirna
SRR493367 control_sirna
SRR493368 control_sirna
```

```
SRR493369      hoxa1_kd
SRR493370      hoxa1_kd
SRR493371      hoxa1_kd
```

```
countData = read.csv(countFile, row.names=1)
head(countData)
```

```
                length SRR493366 SRR493367 SRR493368 SRR493369 SRR493370
ENSG00000186092    918         0         0         0         0         0
ENSG00000279928    718         0         0         0         0         0
ENSG00000279457   1982        23        28        29        29        28
ENSG00000278566    939         0         0         0         0         0
ENSG00000273547    939         0         0         0         0         0
ENSG00000187634   3214       124       123       205       207       212
                SRR493371
ENSG00000186092         0
ENSG00000279928         0
ENSG00000279457        46
ENSG00000278566         0
ENSG00000273547         0
ENSG00000187634       258
```

Q1. Align the columns

```
countData <- as.matrix(countData[,-1])
head(countData)
```

```
                SRR493366 SRR493367 SRR493368 SRR493369 SRR493370 SRR493371
ENSG00000186092         0         0         0         0         0         0
ENSG00000279928         0         0         0         0         0         0
ENSG00000279457        23        28        29        29        28        46
ENSG00000278566         0         0         0         0         0         0
ENSG00000273547         0         0         0         0         0         0
ENSG00000187634       124       123       205       207       212       258
```

Q2. Exclude gene samples w/ no data

```
countData = countData[-which(rowSums(countData) == 0), ]
head(countData)
```

```
                SRR493366 SRR493367 SRR493368 SRR493369 SRR493370 SRR493371
ENSG00000279457        23        28        29        29        28        46
ENSG00000187634       124       123       205       207       212       258
ENSG00000188976      1637      1831      2383      1226      1326      1504
ENSG00000187961       120       153       180       236       255       357
ENSG00000187583        24        48        65        44        48        64
ENSG00000187642         4         9        16        14        16        16
```

```
dds = DESeqDataSetFromMatrix(countData=countData,
                             colData=colData,
                             design=~condition)
```

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

```
dds = DESeq(dds)
```

```
estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

```
dds
```

```
class: DESeqDataSet
dim: 15975 6
metadata(1): version
assays(4): counts mu H cooks
rownames(15975): ENSG00000279457 ENSG00000187634 ... ENSG00000276345
  ENSG00000271254
rowData names(22): baseMean baseVar ... deviance maxCooks
colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
colData names(2): condition sizeFactor
```

```
res = results(dds, contrast=c("condition", "hoxa1_kd", "control_sirna"))
```

Q3. Use summary function on results

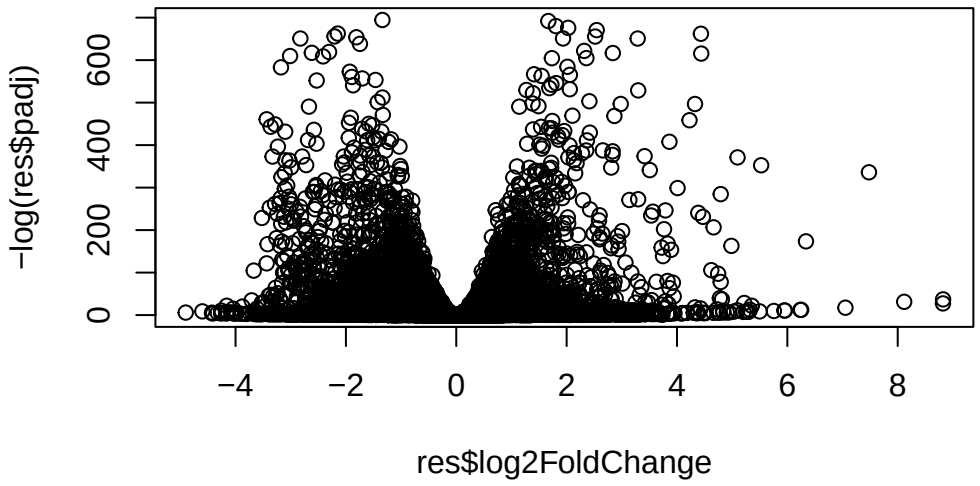
Summarizing results

```
summary(res)
```

```
out of 15975 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 4349, 27%
LFC < 0 (down)     : 4396, 28%
outliers [1]       : 0, 0%
low counts [2]     : 1237, 7.7%
(mean count < 0)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results
```

Making a volcano plot

```
plot( res$log2FoldChange, -log(res$padj) )
```



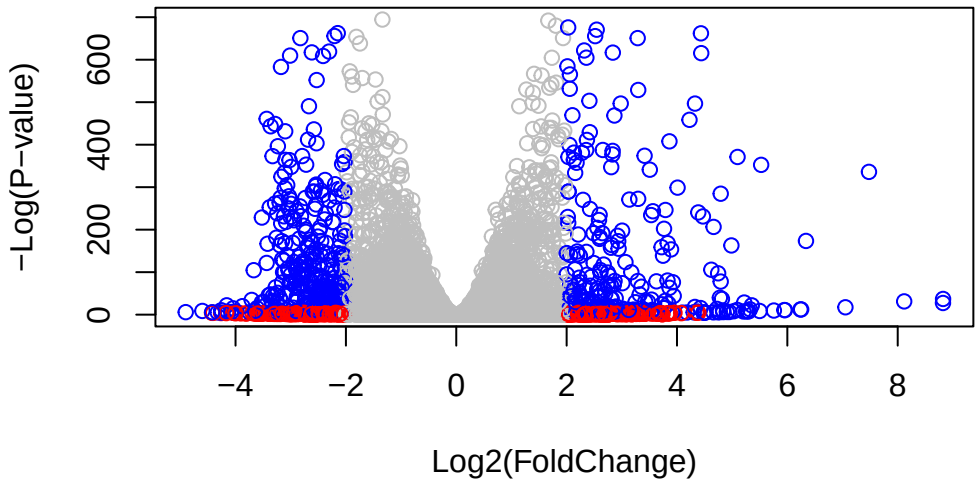
Q4. Improve plot by adding color + axis labels

```
mycols <- rep("gray", nrow(res) )

mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

inds <- (res$padj < .01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

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



Q5. Use the mapIDs() function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below

```
library("AnnotationDbi")
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),
                    keytype="ENSEMBL",
                    column="SYMBOL",
                    multiVals="first")
```

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

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

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

```
res$name =   mapIds(org.Hs.eg.db,
                    keys=row.names(res),
                    keytype= "ENSEMBL",
                    column="GENENAME",
                    multiVals="first")
```

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

```
head(res, 10)
```

```
log2 fold change (MLE): condition hoxa1_kd vs control_sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 10 rows and 9 columns
                  baseMean log2FoldChange     lfcSE       stat      pvalue
                 <numeric>      <numeric> <numeric>  <numeric>   <numeric>
ENSG00000279457   29.913579      0.1792571 0.3248216   0.551863 5.81042e-01
ENSG00000187634  183.229650      0.4264571 0.1402658   3.040350 2.36304e-03
```

```
ENSG00000188976 1651.188076     -0.6927205 0.0548465 -12.630158 1.43990e-36
ENSG00000187961  209.637938      0.7297556 0.1318599   5.534326 3.12428e-08
ENSG00000187583   47.255123      0.0405765 0.2718928   0.149237 8.81366e-01
ENSG00000187642   11.979750      0.5428105 0.5215598   1.040744 2.97994e-01
ENSG00000188290  108.922128      2.0570638 0.1969053  10.446970 1.51282e-25
ENSG00000187608  350.716868      0.2573837 0.1027266   2.505522 1.22271e-02
ENSG00000188157 9128.439422      0.3899088 0.0467163   8.346304 7.04321e-17
ENSG00000237330    0.158192      0.7859552 4.0804729   0.192614 8.47261e-01
                       padj      symbol      entrez                   name
                  <numeric> <character> <character>            <character>
ENSG00000279457 6.86555e-01          NA          NA                     NA
ENSG00000187634 5.15718e-03      SAMD11      148398 sterile alpha motif ..
ENSG00000188976 1.76549e-35       NOC2L       26155 NOC2 like nucleolar ..
ENSG00000187961 1.13413e-07      KLHL17      339451 kelch like family me..
ENSG00000187583 9.19031e-01     PLEKHN1       84069 pleckstrin homology ..
ENSG00000187642 4.03379e-01       PERM1       84808 PPARGC1 and ESRR ind..
ENSG00000188290 1.30538e-24        HES4       57801 hes family bHLH tran..
ENSG00000187608 2.37452e-02       ISG15        9636 ISG15 ubiquitin like..
ENSG00000188157 4.21963e-16        AGRN      375790                  agrin
ENSG00000237330          NA      RNF223      401934 ring finger protein ..
```

Q6. Finally for this section let's reorder these results by adjusted p-value and save them to a CSV file in your current project directory.

```
res = res[order(res$pvalue),]
write.csv(res, file ="deseq_results.csv")
```

Section 2: Pathway Analysis

```
library(pathview)
```

```
library(gage)
library(gageData)
data(kegg.sets.hs)
data(sigmet.idx.hs)
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]
head(kegg.sets.hs, 3)
```

```
$`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"

$`hsa00230 Purine metabolism`
  [1] "100"    "10201"  "10606"  "10621"  "10622"  "10623"  "107"    "10714"
  [9] "108"    "10846"  "109"    "111"    "11128"  "11164"  "112"    "113"
 [17] "114"    "115"    "122481" "122622" "124583" "132"    "158"    "159"
 [25] "1633"   "171568" "1716"   "196883" "203"    "204"    "205"    "221823"
 [33] "2272"   "22978"  "23649"  "246721" "25885"  "2618"   "26289"  "270"
 [41] "271"    "27115"  "272"    "2766"   "2977"   "2982"   "2983"   "2984"
 [49] "2986"   "2987"   "29922"  "3000"   "30833"  "30834"  "318"    "3251"
 [57] "353"    "3614"   "3615"   "3704"   "377841" "471"    "4830"   "4831"
 [65] "4832"   "4833"   "4860"   "4881"   "4882"   "4907"   "50484"  "50940"
 [73] "51082"  "51251"  "51292"  "5136"   "5137"   "5138"   "5139"   "5140"
 [81] "5141"   "5142"   "5143"   "5144"   "5145"   "5146"   "5147"   "5148"
 [89] "5149"   "5150"   "5151"   "5152"   "5153"   "5158"   "5167"   "5169"
 [97] "51728"  "5198"   "5236"   "5313"   "5315"   "53343"  "54107"  "5422"
[105] "5424"   "5425"   "5426"   "5427"   "5430"   "5431"   "5432"   "5433"
[113] "5434"   "5435"   "5436"   "5437"   "5438"   "5439"   "5440"   "5441"
[121] "5471"   "548644" "55276"  "5557"   "5558"   "55703"  "55811"  "55821"
[129] "5631"   "5634"   "56655"  "56953"  "56985"  "57804"  "58497"  "6240"
[137] "6241"   "64425"  "646625" "654364" "661"    "7498"   "8382"   "84172"
[145] "84265"  "84284"  "84618"  "8622"   "8654"   "87178"  "8833"   "9060"
[153] "9061"   "93034"  "953"    "9533"   "954"    "955"    "956"    "957"
[161] "9583"   "9615"
```

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

```
     1266     54855      1465     51232      2034      2317
-2.422719  3.201955 -2.313738 -2.059631 -1.888019 -1.649792
```

Runnin' gage pathway analysis

```
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

```
attributes(keggres)
```

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

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

```
                                       p.geomean stat.mean        p.val
hsa04110 Cell cycle                    8.995727e-06 -4.378644 8.995727e-06
hsa03030 DNA replication               9.424076e-05 -3.951803 9.424076e-05
hsa03013 RNA transport                 1.375901e-03 -3.028500 1.375901e-03
hsa03440 Homologous recombination      3.066756e-03 -2.852899 3.066756e-03
hsa04114 Oocyte meiosis                3.784520e-03 -2.698128 3.784520e-03
hsa00010 Glycolysis / Gluconeogenesis  8.961413e-03 -2.405398 8.961413e-03
                                            q.val set.size         exp1
hsa04110 Cell cycle                    0.001448312      121 8.995727e-06
hsa03030 DNA replication               0.007586381       36 9.424076e-05
hsa03013 RNA transport                 0.073840037      144 1.375901e-03
hsa03440 Homologous recombination      0.121861535       28 3.066756e-03
hsa04114 Oocyte meiosis                0.121861535      102 3.784520e-03
hsa00010 Glycolysis / Gluconeogenesis  0.212222694       53 8.961413e-03
```

Using `pathview()`

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

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

```
Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq
```

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

Focusing on top 5 upregulated pathways

```
keggrespathways <- rownames(keggres$greater)[1:5]
keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
```

```
[1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```

Making the mega `pathview()`

```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

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

Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq

Info: Writing image file hsa04142.pathview.png

Info: some node width is different from others, and hence adjusted!
```

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

Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq

Info: Writing image file hsa04330.pathview.png
```

> Q7. Can you do the same procedure as above to plot the pathview figures for the top 5 down-reguled pathways?

Yes

```
keggrespathways2 <- rownames(keggres$less)[1:5]
keggresids2 = substr(keggrespathways2, start=1, stop=8)
keggresids2
```

```
[1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

```
pathview(gene.data=foldchanges, pathway.id=keggresids2, species="hsa")
```

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

Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq

Info: Writing image file hsa03013.pathview.png
```

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

Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq

Info: Writing image file hsa03440.pathview.png

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

Info: Working in directory /Users/Nate/Desktop/Fall Quarter 2023 /BIMM 143/Class 14: RNA seq

Info: Writing image file hsa04114.pathview.png
```

Section 3: Gene Ontology

```
data(go.sets.hs)
data(go.subs.hs)
gobpsets = go.sets.hs[go.subs.hs$BP]
gobpres = gage(foldchanges, gsets=gobpsets, same.dir=TRUE)
lapply(gobpres, head)
```

```
$greater
                                           p.geomean stat.mean        p.val
GO:0007156 homophilic cell adhesion       8.519724e-05  3.824205 8.519724e-05
GO:0002009 morphogenesis of an epithelium 1.396681e-04  3.653886 1.396681e-04
GO:0048729 tissue morphogenesis           1.432451e-04  3.643242 1.432451e-04
GO:0007610 behavior                       1.925222e-04  3.565432 1.925222e-04
GO:0060562 epithelial tube morphogenesis  5.932837e-04  3.261376 5.932837e-04
GO:0035295 tube development               5.953254e-04  3.253665 5.953254e-04
                                               q.val set.size         exp1
GO:0007156 homophilic cell adhesion       0.1952430      113 8.519724e-05
GO:0002009 morphogenesis of an epithelium 0.1952430      339 1.396681e-04
GO:0048729 tissue morphogenesis           0.1952430      424 1.432451e-04
GO:0007610 behavior                       0.1968058      426 1.925222e-04
GO:0060562 epithelial tube morphogenesis  0.3566193      257 5.932837e-04
GO:0035295 tube development               0.3566193      391 5.953254e-04

$less
                                             p.geomean stat.mean        p.val
```

```
GO:0048285 organelle fission             1.536227e-15 -8.063910 1.536227e-15
GO:0000280 nuclear division              4.286961e-15 -7.939217 4.286961e-15
GO:0007067 mitosis                       4.286961e-15 -7.939217 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.169934e-14 -7.797496 1.169934e-14
GO:0007059 chromosome segregation        2.028624e-11 -6.878340 2.028624e-11
GO:0000236 mitotic prometaphase          1.729553e-10 -6.695966 1.729553e-10
                                                q.val set.size         exp1
GO:0048285 organelle fission             5.843127e-12      376 1.536227e-15
GO:0000280 nuclear division              5.843127e-12      352 4.286961e-15
GO:0007067 mitosis                       5.843127e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.195965e-11      362 1.169934e-14
GO:0007059 chromosome segregation        1.659009e-08      142 2.028624e-11
GO:0000236 mitotic prometaphase          1.178690e-07       84 1.729553e-10

$stats
                                          stat.mean     exp1
GO:0007156 homophilic cell adhesion        3.824205 3.824205
GO:0002009 morphogenesis of an epithelium  3.653886 3.653886
GO:0048729 tissue morphogenesis            3.643242 3.643242
GO:0007610 behavior                        3.565432 3.565432
GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
GO:0035295 tube development                3.253665 3.253665
```

Section 4: Reactome Analysis

Listing the significant genes at the 0.05 level as plain text file

```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
print(paste("Total number of significant genes:", length(sig_genes)))
```

```
[1] "Total number of significant genes: 8147"
```

```
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quo
```

> Q8. What pathway has the most significant "Entities p-value"? Do the most significant pathways listed match your previous KEGG results? What factors could cause differences between the two methods?

Mitotic cell cycle has the most significant "Entities p-value". The most significant pathways listed don't match my previous KEGG results. I think the methodology of the KEGG system versus the Reactome system causes the difference in results.

Section 5: GO Online

> Q9. What pathway has the most significant "Entities p-value"? Do the most significant pathways listed match your previous KEGG results? What factors could cause differences between the two methods?

The pathway with the most significant "Entities p-value" is "GO biological process complete". These results definitely don't match up to my KEGG results. I'm not exactly sure what cause the difference because I wasn't in class, so I don't completely understand whats happening. But, I'm going to take a stab in the dark and chalk it up to methodology differences between KEGG and Panther.