class13

##background

##data import

```
url1 <- "https://bioboot.github.io/bggn213_F25/class-material/GSE37704_featurecounts.csv"
url2 <- "https://bioboot.github.io/bggn213_F25/class-material/GSE37704_metadata.csv"

counts <- read.csv(url1, row.names = 1)
metadata <- read.csv(url2)
```

```
dim(counts)
```

```
[1] 19808     7
```

```
metadata
```

```
         id     condition
1 SRR493366 control_sirna
2 SRR493367 control_sirna
3 SRR493368 control_sirna
4 SRR493369      hoxa1_kd
5 SRR493370      hoxa1_kd
6 SRR493371      hoxa1_kd
```

```
head(counts)
```

```
                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
```

setup DESeq

```r
library(DESeq2)
```

```
Warning: package 'DESeq2' was built under R version 4.3.3
```

```
Loading required package: S4Vectors
```

```
Warning: package 'S4Vectors' was built under R version 4.3.2
```

```
Loading required package: stats4
```

```
Loading required package: BiocGenerics
```

```
Warning: package 'BiocGenerics' was built under R version 4.3.1
```

```

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


Warning: package 'IRanges' was built under R version 4.3.1



Attaching package: 'IRanges'


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

    windows


Loading required package: GenomicRanges


Warning: package 'GenomicRanges' was built under R version 4.3.1


Loading required package: GenomeInfoDb


Warning: package 'GenomeInfoDb' was built under R version 4.3.3
```

```
Loading required package: SummarizedExperiment

Warning: package 'SummarizedExperiment' was built under R version 4.3.1

Loading required package: MatrixGenerics

Warning: package 'MatrixGenerics' was built under R version 4.3.1

Loading required package: matrixStats

Warning: package 'matrixStats' was built under R version 4.3.3


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

Warning: package 'Biobase' was built under R version 4.3.1
```

```
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
```

remove first col of counts

```
counts <- counts[,-1]
head(counts)
```

```
                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
```

```
if (all(colnames(counts) == metadata$id) == FALSE){
  message("check colnames in counts matches metadata")
  exit
}
```

Filter out 0 counts

```
zero_count_genes <- rowSums(counts) == 0

filtered_counts <- counts[!zero_count_genes, ]
```

##run DESeq

```
dds <- DESeqDataSetFromMatrix(countData=filtered_counts,
                              colData=metadata,
                              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
```

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

```
head(res)
```

```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 6 rows and 6 columns
                 baseMean log2FoldChange     lfcSE       stat      pvalue
                <numeric>      <numeric> <numeric>  <numeric>   <numeric>
ENSG00000279457   29.9136      0.1792571 0.3248216   0.551863 5.81042e-01
ENSG00000187634  183.2296      0.4264571 0.1402658   3.040350 2.36304e-03
ENSG00000188976 1651.1881     -0.6927205 0.0548465 -12.630158 1.43990e-36
ENSG00000187961  209.6379      0.7297556 0.1318599   5.534326 3.12428e-08
ENSG00000187583   47.2551      0.0405765 0.2718928   0.149237 8.81366e-01
ENSG00000187642   11.9798      0.5428105 0.5215598   1.040744 2.97994e-01
                       padj
```

```
                  <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76549e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
ENSG00000187642 4.03379e-01
```

##add

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

```
Loading required package: AnnotationDbi
```

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

```r
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
```

```r
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
```

Visualize it!

```r
library(ggplot2)
library(ggrepel)
```

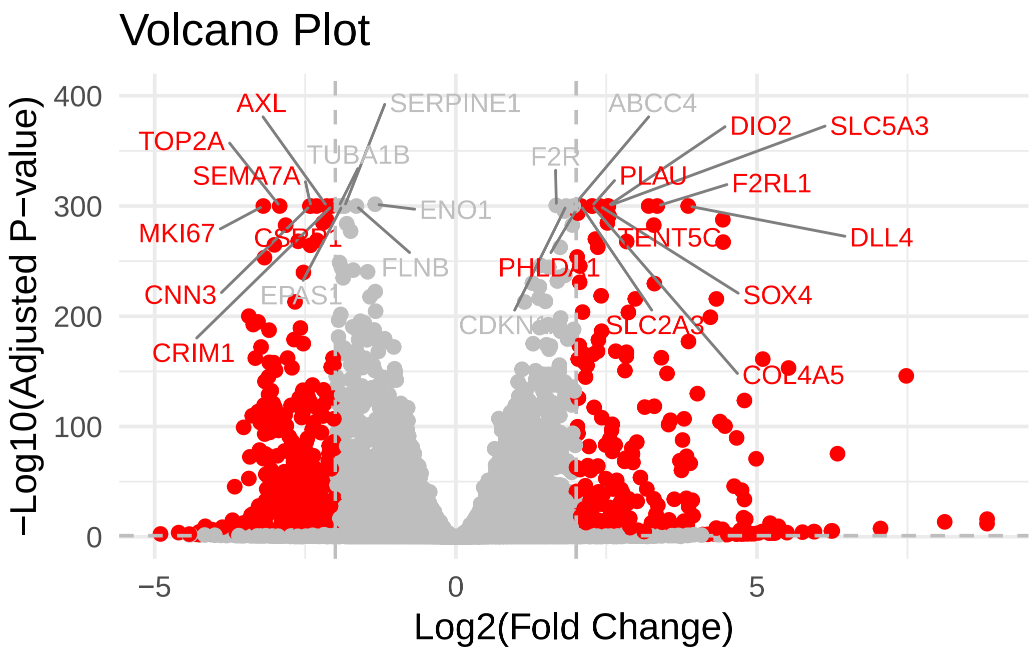
```
Warning: package 'ggrepel' was built under R version 4.3.3
```

```r
res$color <- "gray"
res$color[(res$padj < 0.01) & (abs(res$log2FoldChange) > 2)] <- "red"
res$padj[res$padj == 0] <- 1e-300
res$yval <- -log10(res$padj)

top25 <- res[order(res$padj), ][1:25, ]

ggplot(res, aes(x = log2FoldChange, y = yval, color = color)) +
  geom_point() +
  geom_text_repel(
    data = top25,
    aes(label = symbol),
    size = 3.5,
    max.overlaps = Inf,
    box.padding = 0.5,
    segment.color = "gray50"
  ) +
  scale_color_identity() +
  geom_vline(xintercept = c(-2, 2), linetype = "dashed", color = "gray") +
  geom_hline(yintercept = -log10(0.1), linetype = "dashed", color = "gray") +
  labs(
    x = "Log2(Fold Change)",
    y = "-Log10(Adjusted P-value)",
    title = "Volcano Plot"
  ) +
  coord_cartesian(ylim = c(0, 400)) +
  theme_minimal(base_size = 14)
```

```
Warning: Removed 1237 rows containing missing values or values outside the scale range
(`geom_point()`).
```



There was some crazy high log10(p-values), so I had to cap them to keep them in the plot

saving data

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

##pathway analysis

```
library(gage)
```

```
Warning: package 'gage' was built under R version 4.3.1
```

```
library(gageData)

data(kegg.sets.hs)
data(sigmet.idx.hs)
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]
```

```
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
keggres = gage(foldchanges, gsets=kegg.sets.hs)
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
```

```
head(keggres$greater)
```

```
                                        p.geomean stat.mean       p.val
hsa04640 Hematopoietic cell lineage   0.002822776  2.833362 0.002822776
hsa04630 Jak-STAT signaling pathway   0.005202070  2.585673 0.005202070
hsa00140 Steroid hormone biosynthesis 0.007255099  2.526744 0.007255099
hsa04142 Lysosome                     0.010107392  2.338364 0.010107392
hsa04330 Notch signaling pathway      0.018747253  2.111725 0.018747253
hsa04916 Melanogenesis                0.019399766  2.081927 0.019399766
                                          q.val set.size        exp1
hsa04640 Hematopoietic cell lineage   0.3893570       55 0.002822776
hsa04630 Jak-STAT signaling pathway   0.3893570      109 0.005202070
hsa00140 Steroid hormone biosynthesis 0.3893570       31 0.007255099
hsa04142 Lysosome                     0.4068225      118 0.010107392
hsa04330 Notch signaling pathway      0.4391731       46 0.018747253
hsa04916 Melanogenesis                0.4391731       90 0.019399766
```

```
library(pathview)
```

```
Warning: package 'pathview' was built under R version 4.3.1
```

```
##############################################################################
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).
##############################################################################
```

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

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

```
Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12
```

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

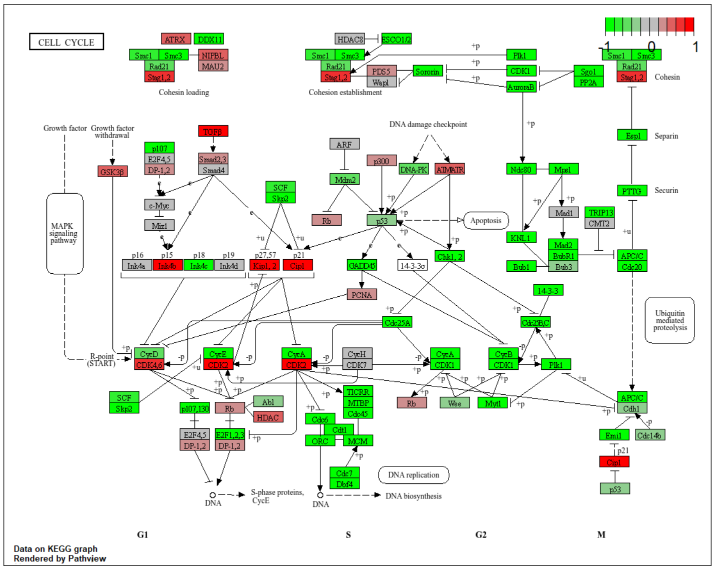


Figure 1: pathway

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

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

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

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

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

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

Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12

Info: Writing image file hsa04142.pathview.png

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

Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12

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

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

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

Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12

Info: Writing image file hsa03440.pathview.png

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

Info: Working in directory C:/Users/marik/OneDrive/Documents/MW lab/Bioinfo class/class12

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

##GO

```r
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
```

##reactome

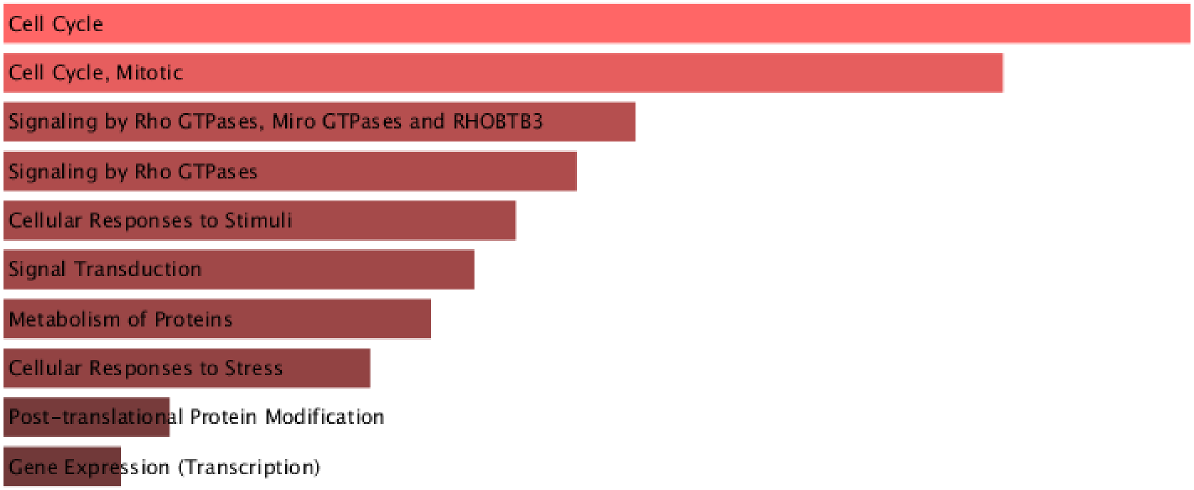
```r
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
print(paste("Total number of significant genes:", length(sig_genes)))
```

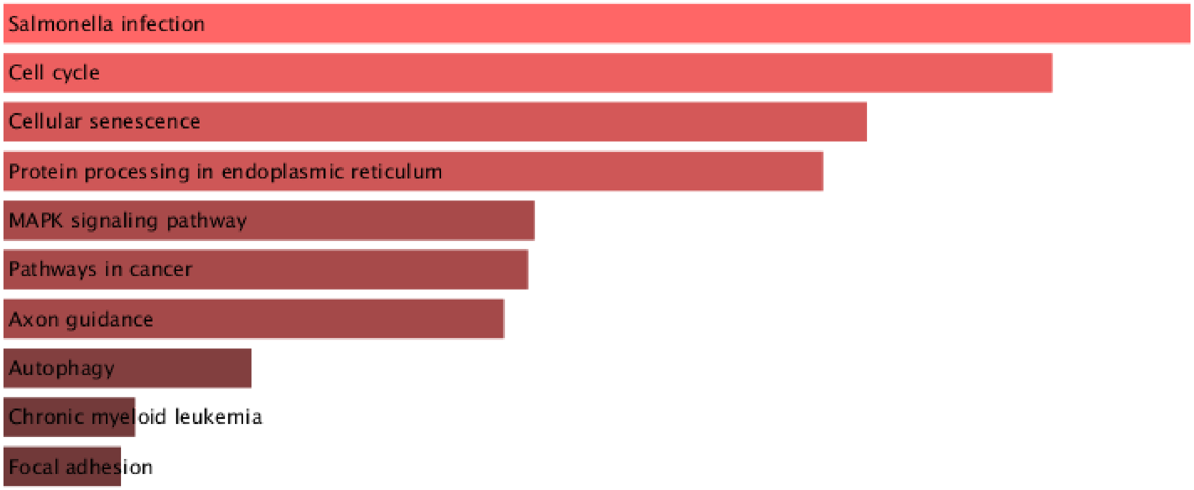
```
[1] "Total number of significant genes: 8147"
```

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

```r
sig_entrez <- res[res$padj <= 0.05 & !is.na(res$padj), "entrez"]
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quote=
```

Also tried enrichR for some interesting pathways!




Salmonella infection
Cell cycle
Cellular senescence
Protein processing in endoplasmic reticulum
MAPK signaling pathway
Pathways in cancer
Axon guidance
Autophagy
Chronic myeloid leukemia
Focal adhesion