Class 14: Pathway Analysis from RNA-Seq Results

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Data Import

Reading in the data from .csv files.

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

```
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)
```

Data Exploration

Remove the first column from countData

```
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

Q. How do we remove all the zero-sum rows?

```
non.zero.inds <- rowSums(countData) > 0
non.zero.counts <- countData[non.zero.inds, ]
nrow((non.zero.counts))
```

```
[1] 15975
```

15,975 non-zero genes remain.

```
head(non.zero.counts)
```

	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

Setup for DESeq

```
library(DESeq2)
```

DESeq Analysis

```
dds <- DESeqDataSetFromMatrix(countData = non.zero.counts,
                              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
```

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

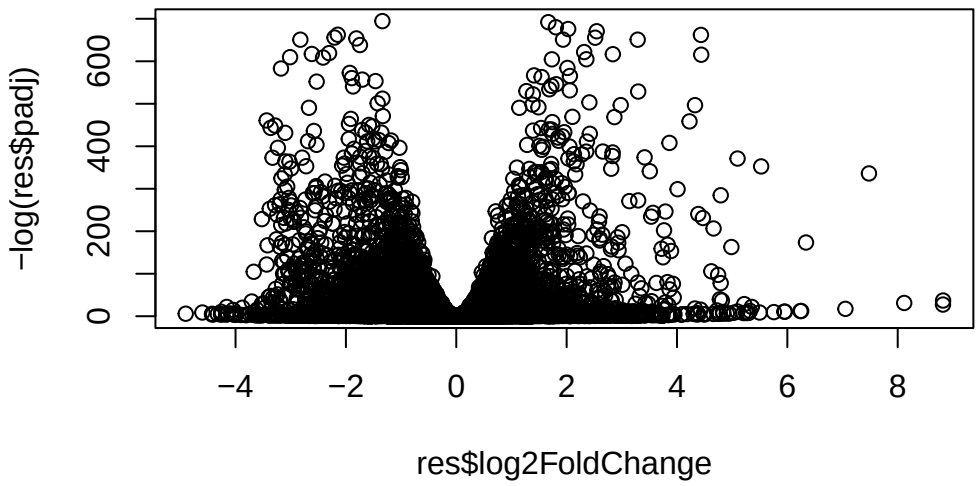
```
head(res)
```

```
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 1.43989e-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 0.5215599   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
```

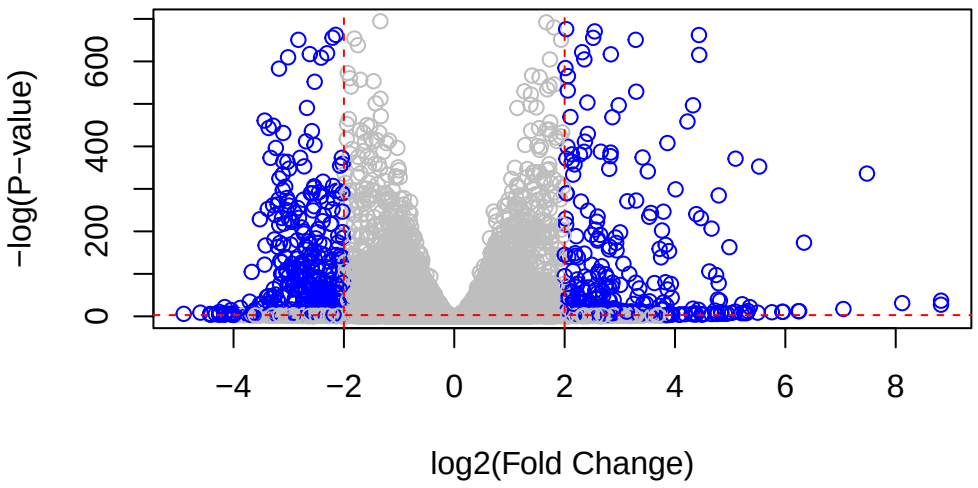
Result extraction and visualization

Using base R

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




```
mycols <- rep("gray", nrow(res))
mycols[abs(res$log2FoldChange)>2] <- "blue"
mycols[res$padj > 0.05] <- "gray"
plot(res$log2FoldChange, -log(res$padj), col=mycols, ylab = "-log(P-value)", xlab = "log2(
abline(v=-2, col="red", lty=2)
abline(v=2, col="red", lty=2)
abline(h=-log(0.05), col="red", lty=2)
```



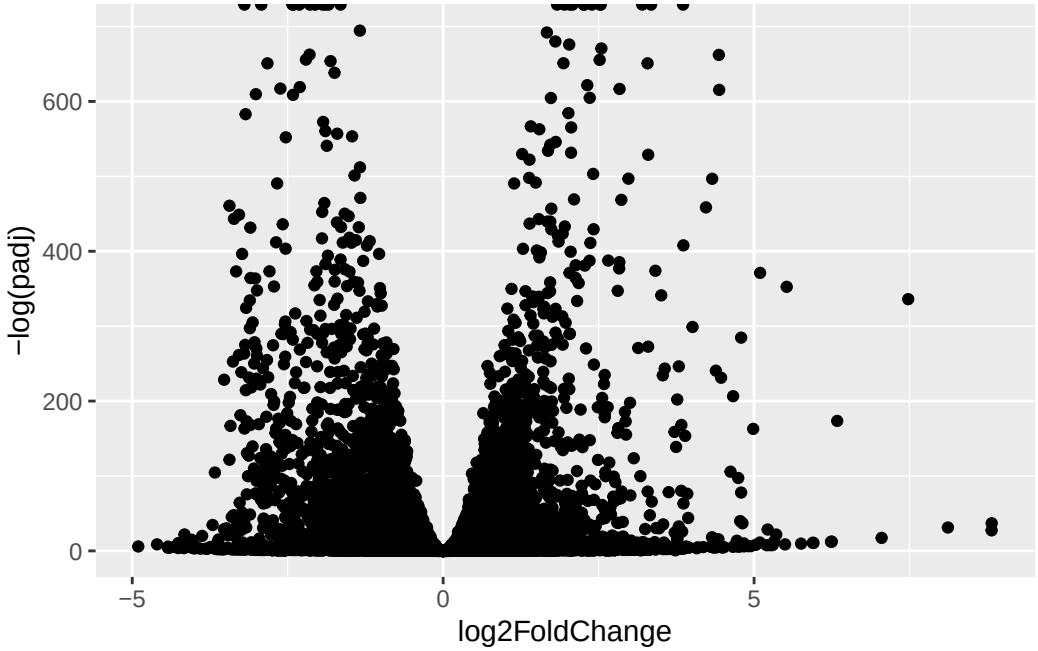
Using ggplot

```
library(ggplot2)
```

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

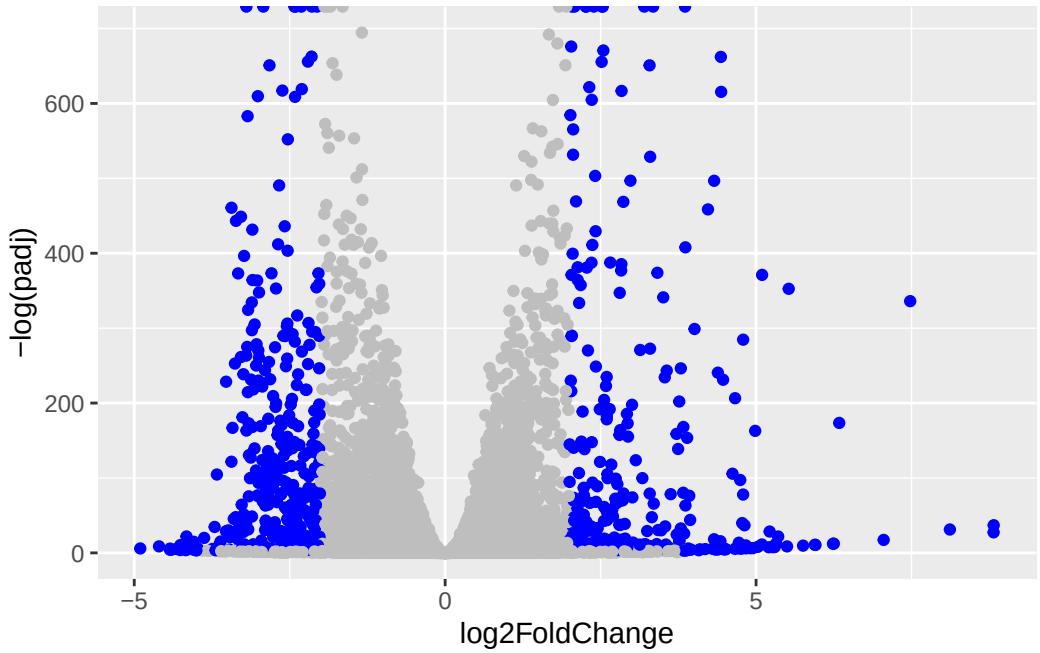
```
ggplot(df) +
  aes (log2FoldChange, -log(padj)) +
  geom_point()
```

```
Warning: Removed 1237 rows containing missing values (`geom_point()`).
```




```
ggplot(df) +
  aes (log2FoldChange, -log(padj)) +
  geom_point(col=mycols)
```

```
Warning: Removed 1237 rows containing missing values (`geom_point()`).
```




```
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
```

Pathway analysis

We have a list of genes/proteins of interest, and we have the quantitative data for each gene/protein (fold change, p-value, spectral counts, presence/absence). In order to prepare a proper pathway, we need to translate one set of IDs into another.

Load up some packages we will need.

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

What are the databases we can translate to?

```
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"
```

Annotate the genes with symbol and Entrez IDs

We can now use these "columns" with the `mapIds()` function to translate between database identifiers.

```
res$symbol <- mapIds(org.Hs.eg.db,
                     keys=row.names(res),
                     keytype="ENSEMBL",
                     column="SYMBOL")
```

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

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

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

```
res$genename <- mapIds(org.Hs.eg.db,
                       keys=row.names(res),
                       keytype="ENSEMBL",
                       column="GENENAME")
```

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

```
res
```

```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 15975 rows and 9 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 1.43989e-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
...                   ...            ...       ...        ...         ...
ENSG00000273748  35.30265       0.674387  0.303666   2.220817 2.63633e-02
ENSG00000278817   2.42302      -0.388988  1.130394  -0.344117 7.30758e-01
ENSG00000278384   1.10180       0.332991  1.660261   0.200565 8.41039e-01
ENSG00000276345  73.64496      -0.356181  0.207716  -1.714752 8.63908e-02
ENSG00000271254 181.59590      -0.609667  0.141320  -4.314071 1.60276e-05
                      padj       symbol      entrez                genename
                 <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 ..
...                      ...          ...         ...                     ...
ENSG00000273748 4.79091e-02           NA          NA                      NA
ENSG00000278817 8.09772e-01        DGCR6        8214 DiGeorge syndrome cr..
ENSG00000278384 8.92654e-01           NA          NA                      NA
ENSG00000276345 1.39762e-01       MRPL23        6150 mitochondrial riboso..
ENSG00000271254 4.53648e-05 LOC102724250   102724250 neuroblastoma breakp..
```

KEGG and GO Analysis

Install and load some packages: "pathview", "gage", and "gageData"

```
#|message: false
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)
```

gage function wants as its input: a vector of fold changes with names of the genes in a format that matches either database/geneset we are going to use.

```
foldchanges <- res$log2FoldChange
names(foldchanges) <- res$entrez
head(foldchanges)
```

```
      <NA>      148398       26155      339451       84069       84808
0.17925708  0.42645712 -0.69272046  0.72975561  0.04057653  0.54281049
```

At this point, we can remove things we don't want with the `rm()` function.

```
data("kegg.sets.hs")
data("sigmet.idx.hs")
```

Focus on signaling and metabolic pathways only

```
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]
```

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

```
      <NA>      148398       26155      339451       84069       84808
0.17925708  0.42645712 -0.69272046  0.72975561  0.04057653  0.54281049
```

```
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              1.246882e-03 -3.059466 1.246882e-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              0.066915974      144 1.246882e-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
```

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

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

Info: Working in directory /Users/rennyng/Desktop/BGGN213/class14

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

Gene Ontology (GO)

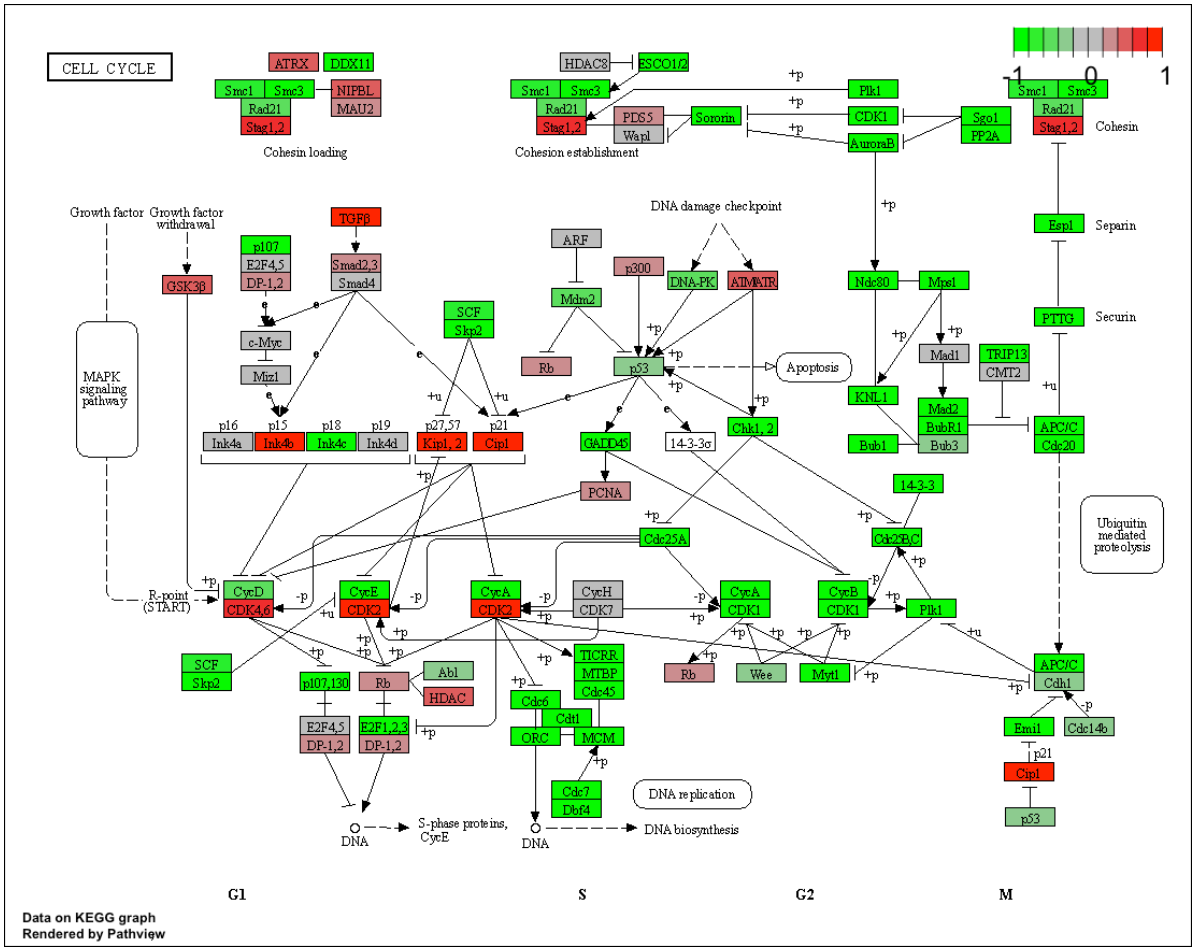


Figure 1: Cell cycle pathway from KEGG

```
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
```

```
head(gobpres$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
```

This gives us a list of GO terms (in contrast to the figures produced by KEGG)

Reactome

Reactome is meant to give both the written lists and also the visuals.

Here we want to implement some criteria:

- ABS (Log2FC) $> +2$
- PADJ < 0.05

```
inds <- (abs(res$log2FoldChange) > 2) & (res$padj < 0.05)
mygenes <- res$symbol[inds]
```

```
cat(head(mygenes), sep = "\n")
```

```
HES4
HES2
DRAXIN
CDA
RUNX3
AUNIP
```

```
write.table(mygenes,
            file="mygenes.txt",
            quote = FALSE,
            row.names = FALSE,
            col.names = FALSE)
```

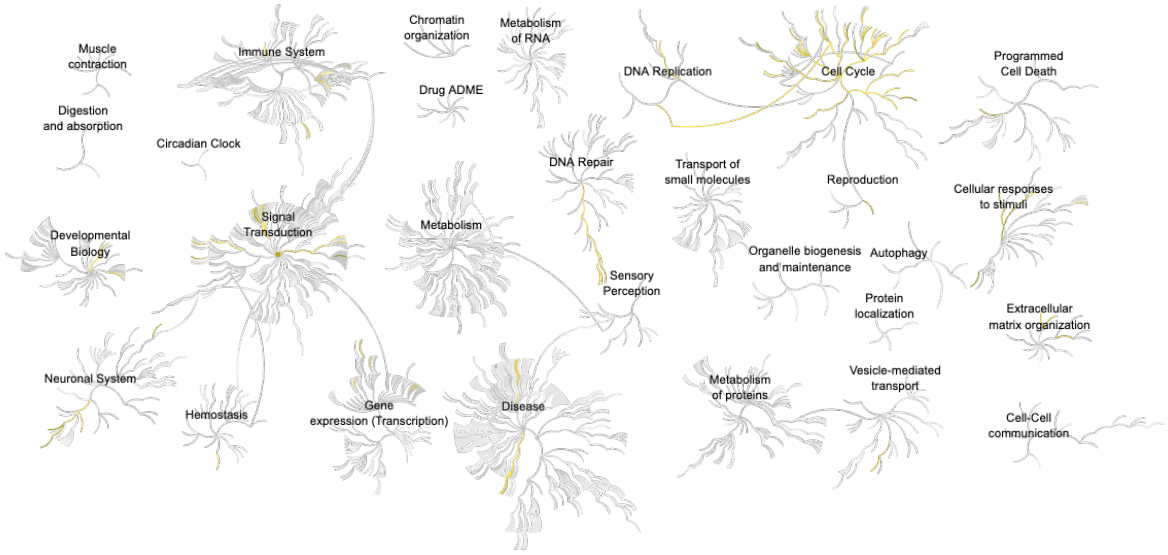


Figure 2: Cell cycle pathway from KEGG

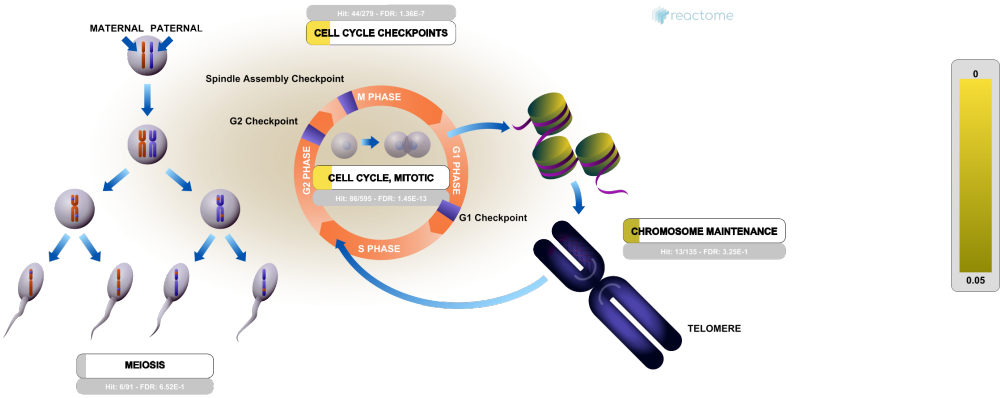


Figure 3: Example Figure from GO

Save our results

```
write.csv(res,file="myresults.csv")
```