Class 14: RNAseq mini project

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Background

The data for for hands-on session comes from GEO entry: GSE37704, which is associated with the following publication:

> Trapnell C, Hendrickson DG, Sauvageau M, Goff L et al. "Differential analysis of gene regulation at transcript resolution with RNA-seq". Nat Biotechnol 2013 Jan;31(1):46-53. PMID: 23222703

The authors report on differential analysis of lung fibroblasts in response to loss of the developmental transcription factor HOXA1.

Data Import

Reading in the counts and metadata

```
counts <- read.csv("GSE37704_featurecounts.csv", row.names=1)
metadata <- read.csv("GSE37704_metadata.csv")
```

Tidy and verify data

> Q1. How many genes ar ein this dataset?

```
nrow(counts)
```

```
[1] 19808
```

> Q2. How many control and knockdown experiments are there?

```
table(metadata$condition)
```

```

control_sirna      hoxa1_kd 
            3             3 
```

> Q3. Does the metadata match the countdata?

```
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
```

```
colnames(counts)
```

```
[1] "length"    "SRR493366" "SRR493367" "SRR493368" "SRR493369" "SRR493370"
[7] "SRR493371"
```

```
metadata$id
```

```
[1] "SRR493366" "SRR493367" "SRR493368" "SRR493369" "SRR493370" "SRR493371"
```

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

```
                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
```

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

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

Remove zero count genes

```
to.keep <- rowSums(newcounts)!=0
countData <- newcounts[to.keep, ]
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
```

PCA quality control

We can use `prcomp()` function.

```
pc <- prcomp( t(countData), scale=T)
summary(pc)
```

```
Importance of components:
                           PC1     PC2      PC3      PC4      PC5       PC6
Standard deviation     87.7211 73.3196 32.89604 31.15094 29.18417 7.373e-13
Proportion of Variance  0.4817  0.3365  0.06774  0.06074  0.05332 0.000e+00
Cumulative Proportion   0.4817  0.8182  0.88594  0.94668  1.00000 1.000e+00
```

Color by “control” = blue, “knockdown”=red

```
metadata$condition
```

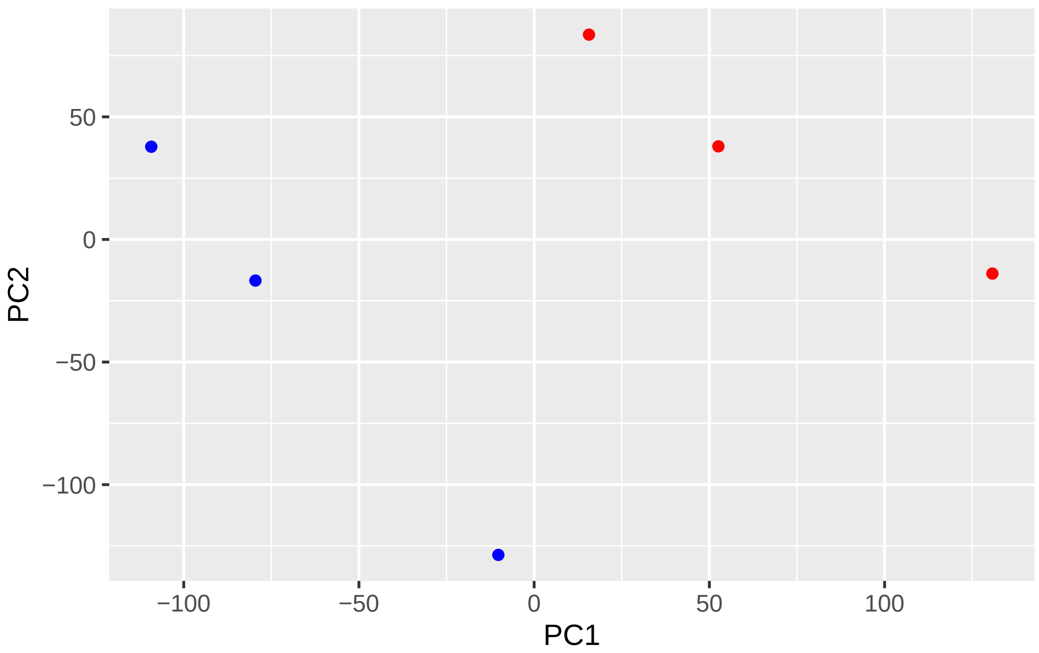
```
[1] "control_sirna" "control_sirna" "control_sirna" "hoxa1_kd"
[5] "hoxa1_kd"      "hoxa1_kd"
```

```
mycols <- c(rep("blue",3), rep("red",3))
mycols
```

```
[1] "blue" "blue" "blue" "red"  "red"  "red"
```

```
library(ggplot2)

ggplot(pc$x)+
  aes(PC1, PC2)+
  geom_point(col=mycols)
```



DESeq analysis

```
library(DESeq2)
```

Set up DESeq input object

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

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

Run DESeq

```
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(3): id condition sizeFactor
```

Extract results

```
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
```

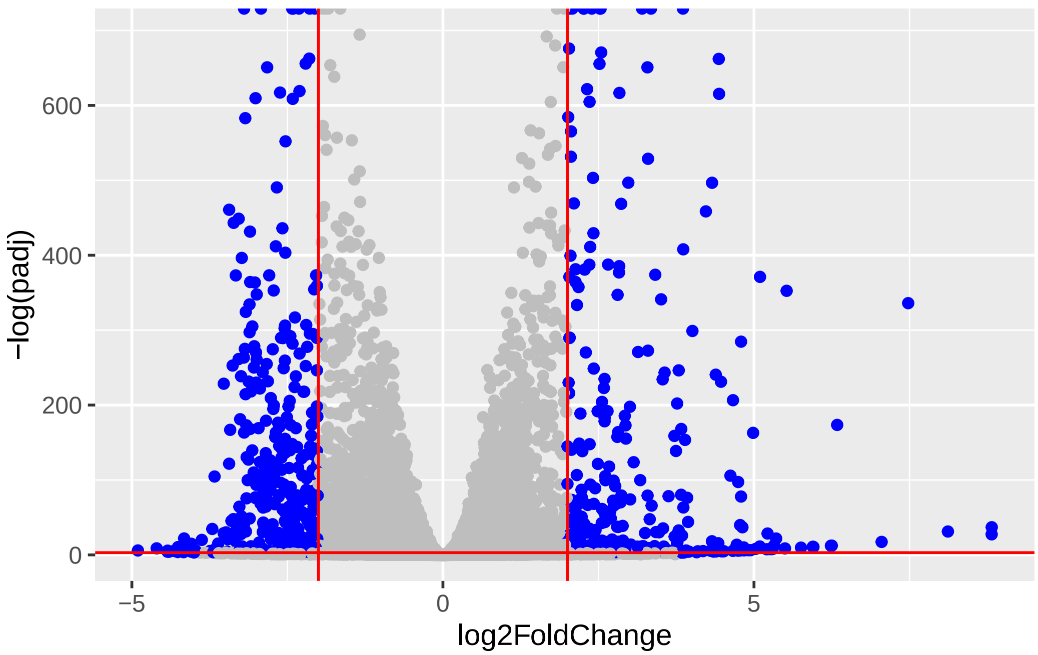
```
ENSG00000187634  183.2296      0.4264571 0.1402658   3.040350 2.36304e-03
ENSG00000188976 1651.1881     -0.6927205 0.0548465 -12.630158 1.43990e-36
ENSG00000187961  209.6379      0.7297556 0.1318599   5.534326 3.12428e-08
ENSG00000187583   47.2551      0.0405765 0.2718928   0.149237 8.81366e-01
ENSG00000187642   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
```

#Volcano plot A plot of log2 fold change vs -log of adjusted p-value with custom colors

```
mycols <- rep("gray", nrow(res))
mycols[res$log2FoldChange>=+2] <- "blue"
mycols[res$log2FoldChange<=-2] <- "blue"
mycols[res$padj>=0.05] <- "gray"
```

```
ggplot(res)+
  aes(log2FoldChange, -log(padj))+
  geom_point(col=mycols)+
  geom_vline(xintercept = c(-2,2), col="red")+
  geom_hline(yintercept = -log(0.05), col="red")
```

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



Add gene annotation

We want to add gene SYMBOL and ENTREZID values to our results object.

```
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"
```

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

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

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

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

Save results

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

Pathway analysis

```r
#|message: false
library(gage)
```

```r
library(gageData)
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).
##############################################################################
```

##KEEG

```
data(kegg.sets.hs)
head(kegg.sets.hs, 1)
```

```
$`hsa00232 Caffeine metabolism`
[1] "10"   "1544" "1548" "1549" "1553" "7498" "9"
```

Make an input vector for `gage()` called `foldchanges` that has `names()` attribute set to ENTREZID.

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

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

```
attributes(keggres)
```

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

```
head(keggres$less, 2)
```

```
                            p.geomean stat.mean        p.val       q.val
hsa04110 Cell cycle      8.995727e-06 -4.378644 8.995727e-06 0.001889103
hsa03030 DNA replication 9.424076e-05 -3.951803 9.424076e-05 0.009841047
                         set.size         exp1
hsa04110 Cell cycle           121 8.995727e-06
hsa03030 DNA replication       36 9.424076e-05
```

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

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

```
Info: Working in directory C:/Users/Bryn Baxter/Documents/BIO213/Class 14

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

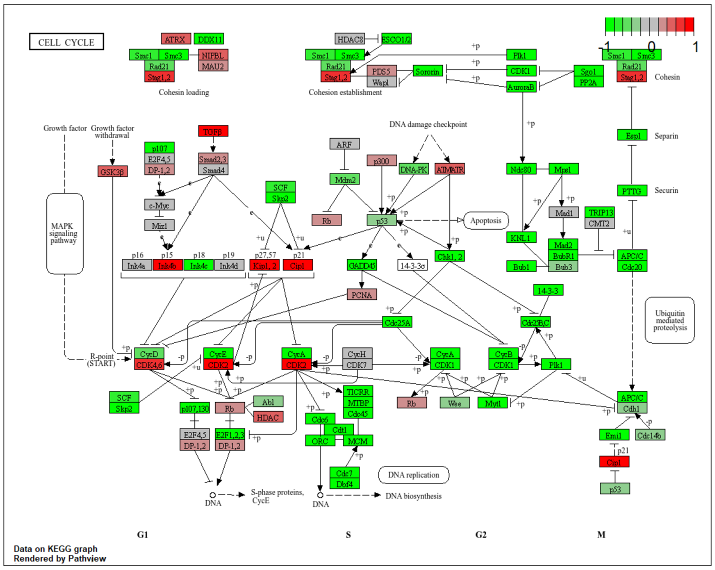


Figure 1: Cell cycle is affected

```r
pathview(foldchanges, pathway.id= "hsa03030" )
```

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

Info: Working in directory C:/Users/Bryn Baxter/Documents/BIO213/Class 14

Info: Writing image file hsa03030.pathview.png
```

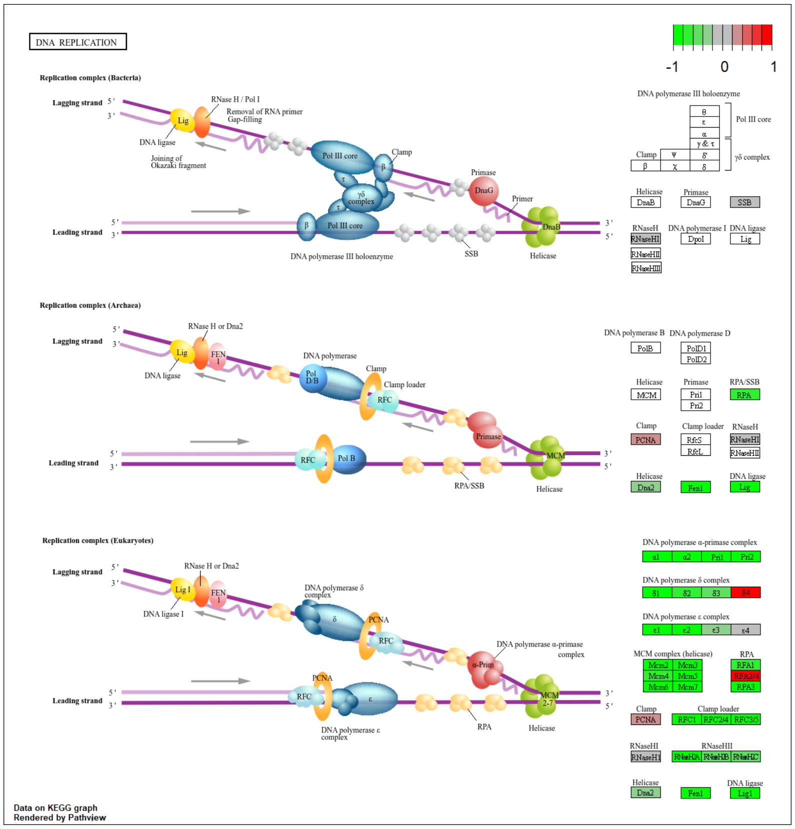


Figure 2: DNA replication is also affected

```
head(keggres$greater, 2)
```

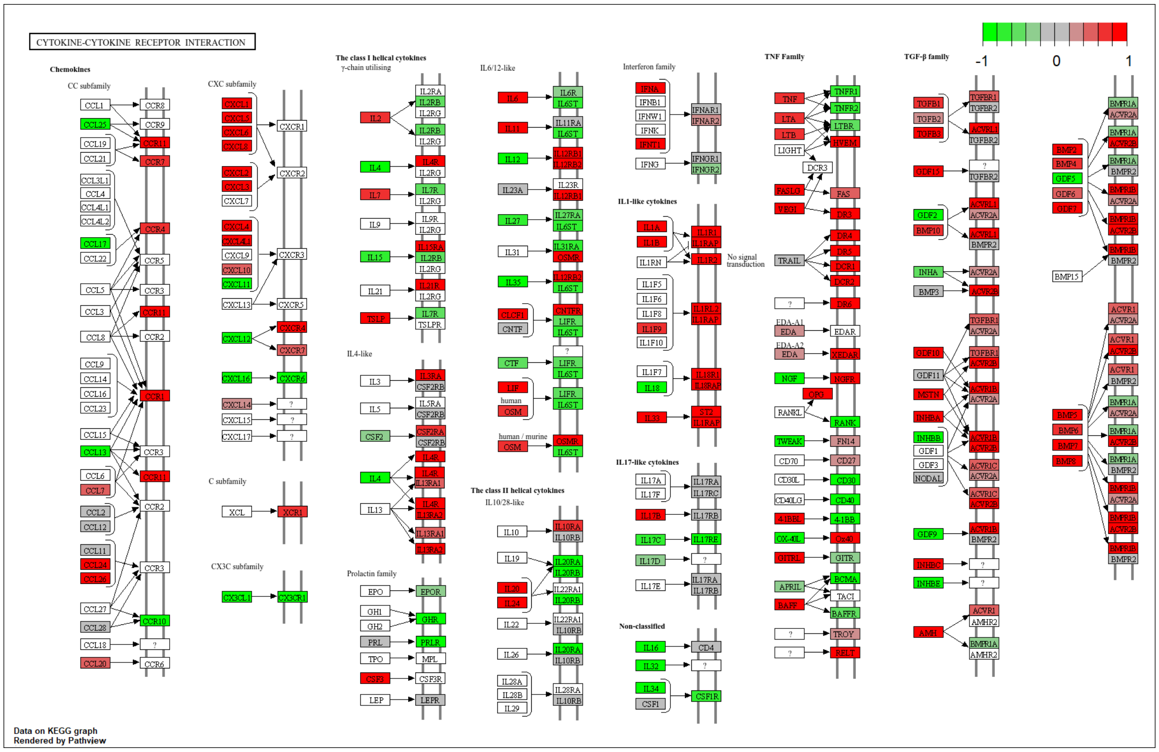
```
                                               p.geomean stat.mean
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06  4.358967
hsa05323 Rheumatoid arthritis                   1.809824e-04  3.666793
                                                   p.val       q.val
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06 0.001917519
hsa05323 Rheumatoid arthritis                   1.809824e-04 0.019003147
                                               set.size         exp1
hsa04060 Cytokine-cytokine receptor interaction      177 9.131044e-06
hsa05323 Rheumatoid arthritis                         72 1.809824e-04
```

```
pathview(foldchanges, pathway.id= "hsa04060")
```

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

```
Info: Working in directory C:/Users/Bryn Baxter/Documents/BIO213/Class 14

Info: Writing image file hsa04060.pathview.png
```

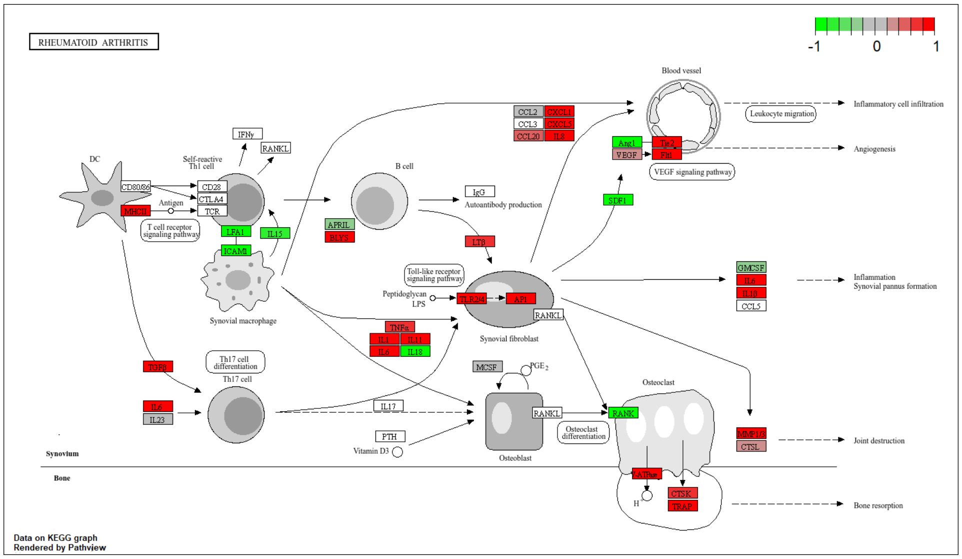


```r
pathview(foldchanges, pathway.id= "hsa05323")
```

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

Info: Working in directory C:/Users/Bryn Baxter/Documents/BIO213/Class 14

Info: Writing image file hsa05323.pathview.png
```



##GO Gene Ontology

```
data(go.sets.hs)
data(go.subs.hs)

# Focus just on GO Biological Process (BP)
gobpsets = go.sets.hs[go.subs.hs$BP]

gobpres = gage(foldchanges, gsets=gobpsets)
```

```
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              5.841698e-12      376 1.536227e-15
GO:0000280 nuclear division               5.841698e-12      352 4.286961e-15
GO:0007067 mitosis                        5.841698e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.195672e-11      362 1.169934e-14
GO:0007059 chromosome segregation         1.658603e-08      142 2.028624e-11
GO:0000236 mitotic prometaphase           1.178402e-07       84 1.729553e-10
```

##Reactome

We can use reactome via R or via their fancy new website interface. The web interaface wants a set of ENTREZ id values for your genes of interest. Lets generate that.

```
inds <- abs(res$log2FoldChange)>=2 &res$padj<=0.05
top.genes <- res$entrez[inds]
```

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