Class13

Gregory Jordan

About our Input Data

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

1. Differential Expression Analysis

```
#load in DESeq2
library(DESeq2)
```

```
metaFile <- "https://bioboot.github.io/bimm143_W18/class-material/GSE37704_metadata.csv"
countFile <- "https://bioboot.github.io/bimm143_W18/class-material/GSE37704_featurecounts.

#import the metadata and check it out

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
```

```
# Import countdata
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
```

need to remove our length column to make our count identifiers line up with metadata

Q1. Complete the code below to remove the troublesome first column from countData

Note we need to remove the odd first $length col

```
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
```

Test that our count data is in the right order with metadata now

```
#remember that all will test if every value in the resulting vector (comparing each elemen
all(colnames(countData) == rownames(colData))
```

```
[1] TRUE
```

Lots of 0 counts still here and it is good practice to remove zeroes before we go through other things because 0s will mess with our statistical tests

Q.2 Complete the code below to filter countData to exclude genes (i.e. rows) where we have 0 read count across all samples (i.e. columns).

Tip: What will rowSums() of countData return and how could you use it in this context?

```
# Filter count data where you have 0 read count across all samples.
#keep.inds is a cool feature to keep indices based on a condition
keep.inds <- rowSums(countData) != 0
counts <- countData[keep.inds,]
```

```
nrow(countData)
```

```
[1] 19808
```

```
nrow(counts)
```

```
[1] 15975
```

Running DESeq2

```
#already loaded ddseq at the beginning
#tilda condition for the experiment design
dds <- DESeqDataSetFromMatrix(countData = counts,colData = colData,design = ~condition)
```

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

```r
dds <- DESeq(dds)

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
```

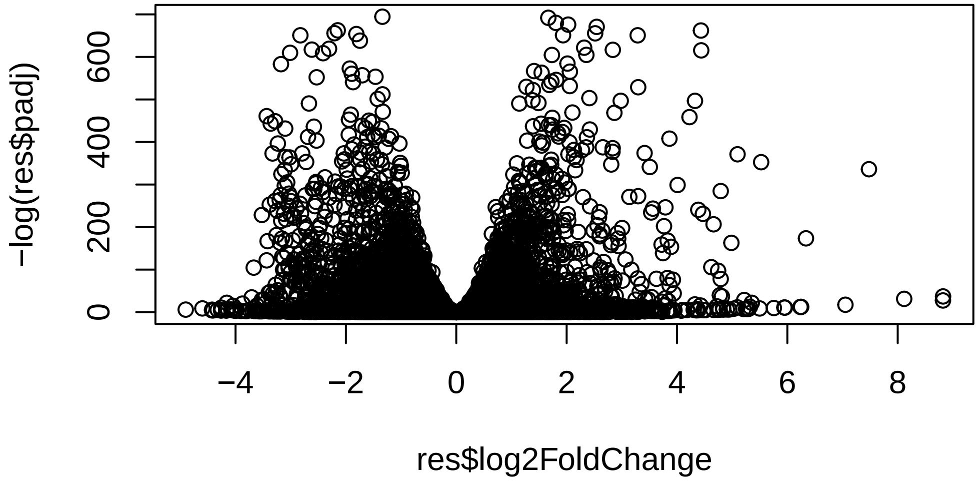
Q3. Call the summary() function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```r
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
```

```
#make volcanoooooo plot
plot(res$log2FoldChange, -log(res$padj) )
```

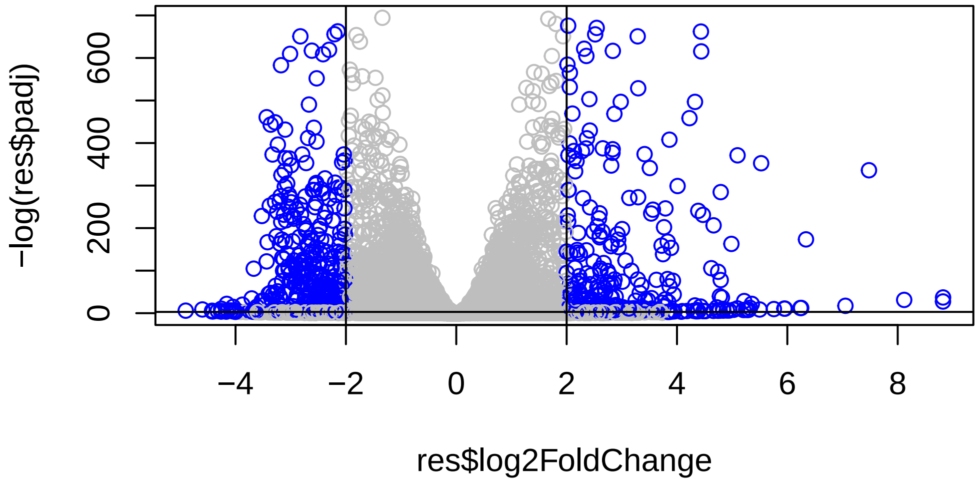


Q4. Improve this plot by completing the below code, which adds color and axis labels

```
# Make a color vector for all genes
mycols <- rep("gray", nrow(counts))
mycols[res$log2FoldChange > 2] <- "blue"
mycols[res$log2FoldChange < -2] <- "blue"
mycols[res$padj > 0.05] <- "gray"

plot(res$log2FoldChange, -log(res$padj), col=mycols)

abline(v=c(-2,+2))
abline(h=-log(0.05))
```



Adding Gene Annotation

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"
```

```
#x = the annotationDb object
#keys = the query essentially
#column = what type you want to convert the keys into. essentailly your key goes into the
#multivals = when there are multiple values that can be returned, return the first value

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.

```r
res.ordered <- res[order(res$padj),]
head(res.ordered)
```

```
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 9 columns
                 baseMean log2FoldChange     lfcSE      stat    pvalue
                <numeric>      <numeric> <numeric> <numeric> <numeric>
ENSG00000117519   4483.63       -2.42272 0.0600016  -40.3776         0
ENSG00000183508   2053.88        3.20196 0.0724172   44.2154         0
ENSG00000159176   5692.46       -2.31374 0.0575534  -40.2016         0
ENSG00000150938   7442.99       -2.05963 0.0538449  -38.2512         0
ENSG00000116016   4423.95       -1.88802 0.0431680  -43.7366         0
ENSG00000136068   3796.13       -1.64979 0.0439354  -37.5504         0
                     padj      symbol      entrez                   name
                <numeric> <character> <character>            <character>
ENSG00000117519         0        CNN3        1266             calponin 3
```

```
ENSG00000183508         0      TENT5C       54855 terminal nucleotidyl..
ENSG00000159176         0       CSRP1        1465 cysteine and glycine..
ENSG00000150938         0       CRIM1       51232 cysteine rich transm..
ENSG00000116016         0       EPAS1        2034 endothelial PAS doma..
ENSG00000136068         0        FLNB        2317              filamin B
```

```
write.csv(x =res.ordered, file="deseq_results.csv")
```

QC with PCA

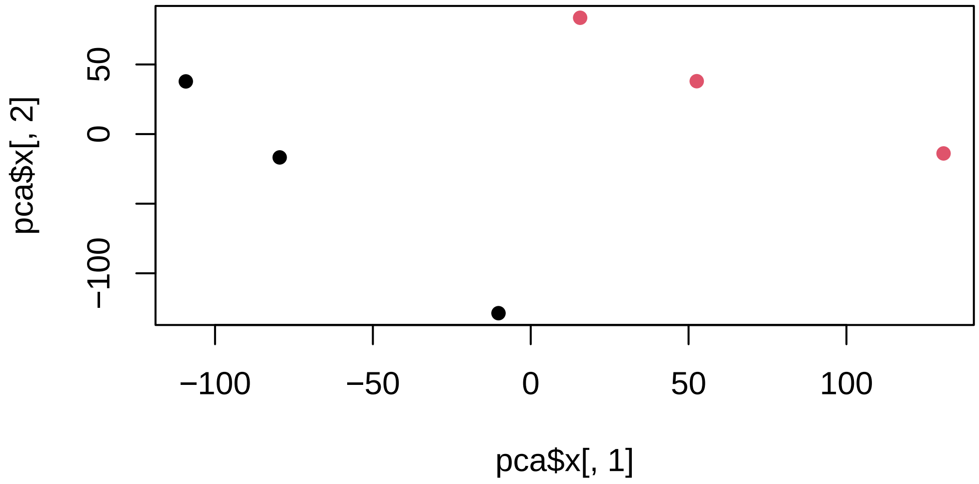
the `prcomp()` function in base R is often used to check principal components contributing to the variability in the counts

```
pca <- prcomp(t(counts),scale=TRUE)
summary(pca)
```

```
Importance of components:
                           PC1     PC2      PC3      PC4      PC5       PC6
Standard deviation     87.7211 73.3196 32.89604 31.15094 29.18417 6.648e-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
```

Our PCA score plot (aka PC1 vs PC2)

```
plot(pca$x[,1],pca$x[,2], col = as.factor(colData$condition),pch=16)
```



```
#this is good. we see the major variance in the dataset being found by PCA is consistent w
```

2. Pathway Analysis

we can use `gage()` with KEGG and GO

```
library(gage)
```

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

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

what `gage()` wants as input is that gector of importance - in our case that will be the log2 fold-change values. this vector should have `names()` that are entrez IDs

```
foldchange <- res$log2FoldChange
names(foldchange) <- res$entrez
#now we have fold change named with entrez ids
```

```
head(foldchange)
```

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

```
#focus on signaling and metabolic pathways only

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

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

```
attributes(keggres)
```

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

```
head(keggres$less,5)
```

```
                                     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
```

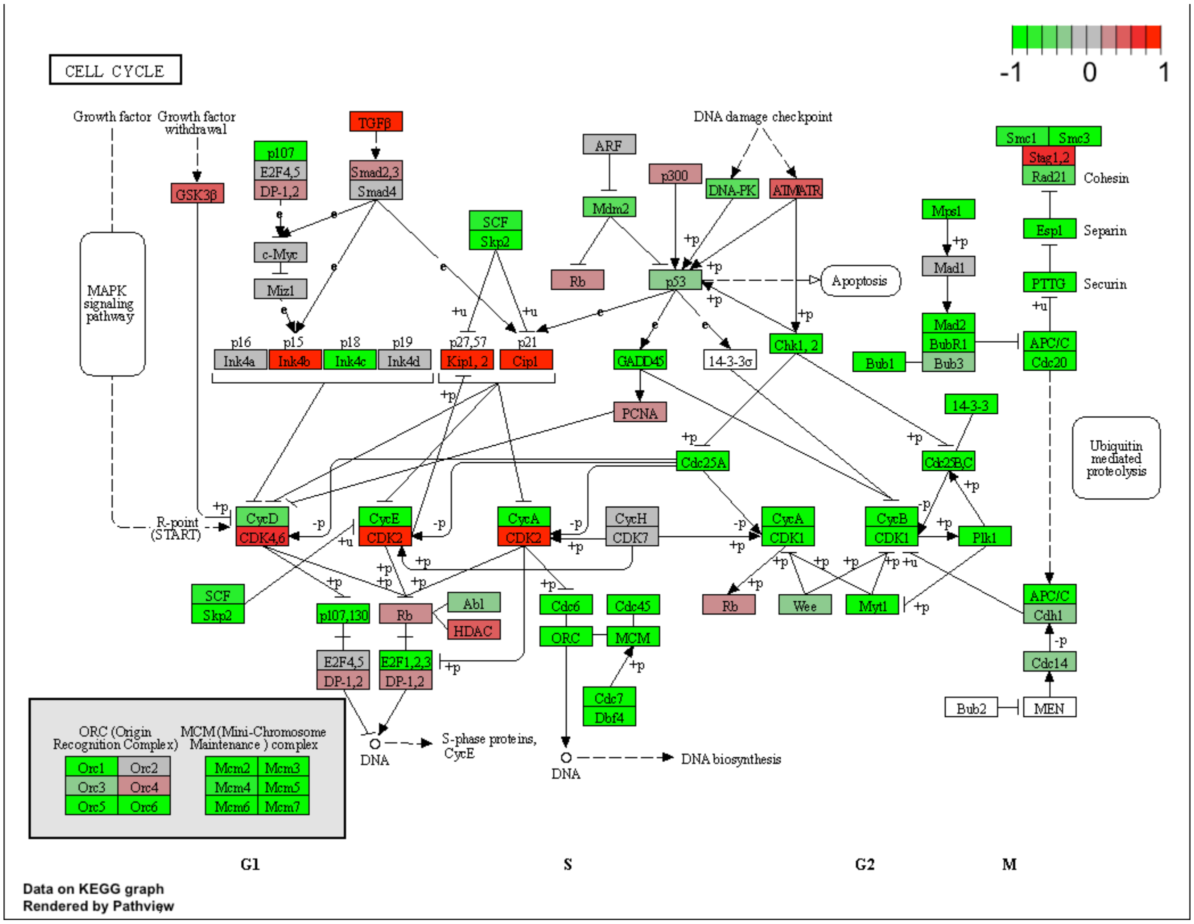
```
hsa03013 RNA transport            1.246882e-03 -3.059466 1.246882e-03
hsa03440 Homologous recombination 3.066756e-03 -2.852899 3.066756e-03
hsa04114 Oocyte meiosis           3.784520e-03 -2.698128 3.784520e-03
                                        q.val set.size         exp1
hsa04110 Cell cycle               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
```

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

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

```
Info: Working in directory /Users/gregoryjordan/Desktop/BGGN213/BGGN 213_R Project/Class13
```

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



# 3.Gene Ontology

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

# Focus on Biological Process subset of GO
gobpsets = go.sets.hs[go.subs.hs$BP]

gobpres = gage(foldchange, 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                       2.195494e-04  3.530241 2.195494e-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       0.1951953      113 8.519724e-05
GO:0002009 morphogenesis of an epithelium 0.1951953      339 1.396681e-04
GO:0048729 tissue morphogenesis           0.1951953      424 1.432451e-04
GO:0007610 behavior                       0.2243795      427 2.195494e-04
GO:0060562 epithelial tube morphogenesis  0.3711390      257 5.932837e-04
GO:0035295 tube development               0.3711390      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             5.841698e-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

$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                        3.530241 3.530241
GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
GO:0035295 tube development                3.253665 3.253665
```

4. Reactome Analysis

```
#get your significant genes
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"
```

```
#make a table of your significant genes
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quo
```

Q7: 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?

Endosomal/Vacuolar pathway is the most significant pathway for KEGG

detection of chemical stimulus involved in sensory perception for GO analysis

These results do not match. I imagine differences could be caused by different search algorithms by the softwares, among different cutoffs used by the different softwares.