Lab13

Nate Tran

Reading and Exploring Data

Loading in counts and meta data and confirming that they match.

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

counts <- counts[,meta$id]

all.equal(meta$id, colnames(counts))
```

```
[1] TRUE
```

Excluding zero count genes (genes with 0 counts in EVERY sample)

```r
counts <- counts[!rowSums(counts)==0,]
```

PCA Quality Check

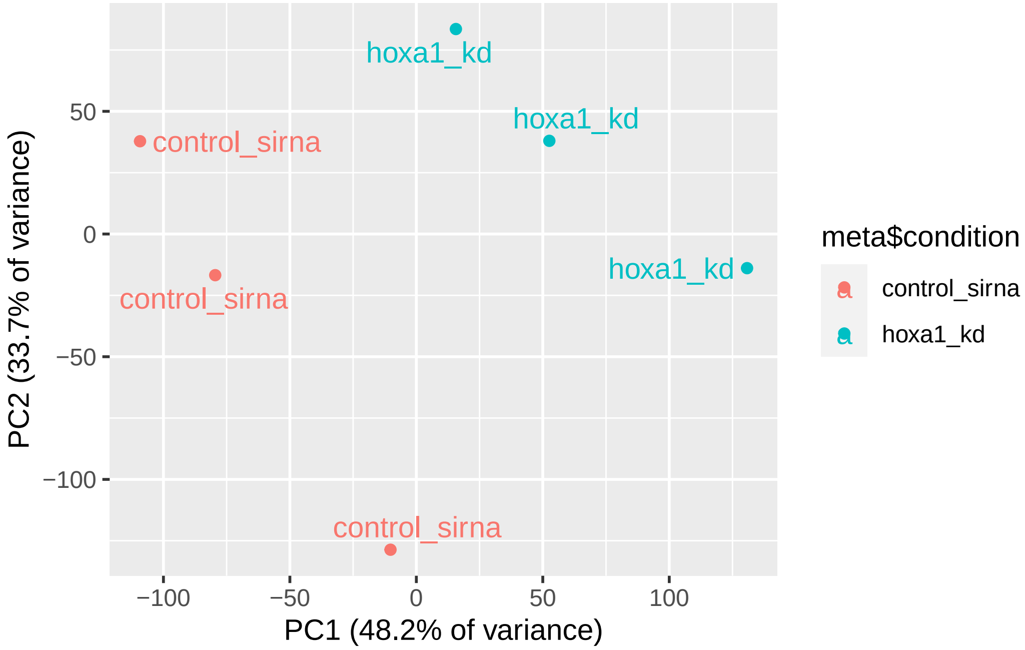
```r
pca <- prcomp(t(counts), scale=T)
summary(pca)
```

```
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
```

Using ggplot to visualize PCA results

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

ggplot(as.data.frame(pca$x)) +
  aes(PC1, PC2, color = meta$condition) +
  geom_point() +
  geom_text_repel(label = meta$condition) +
  xlab("PC1 (48.2% of variance)") +
  ylab("PC2 (33.7% of variance)")
```



There seems to be a clear distinction between controls and HOXA1 KD samples. Great success!

DESeq Analysis

Running DESeq on input counts and meta data

```r
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 objects are masked from 'package:base':

    expand.grid, I, unname


Loading required package: IRanges



Attaching package: 'IRanges'


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

    windows


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(counts, meta, ~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
```

Extracting results from DESeq analysis

```
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
```

Plotting DESeq Results

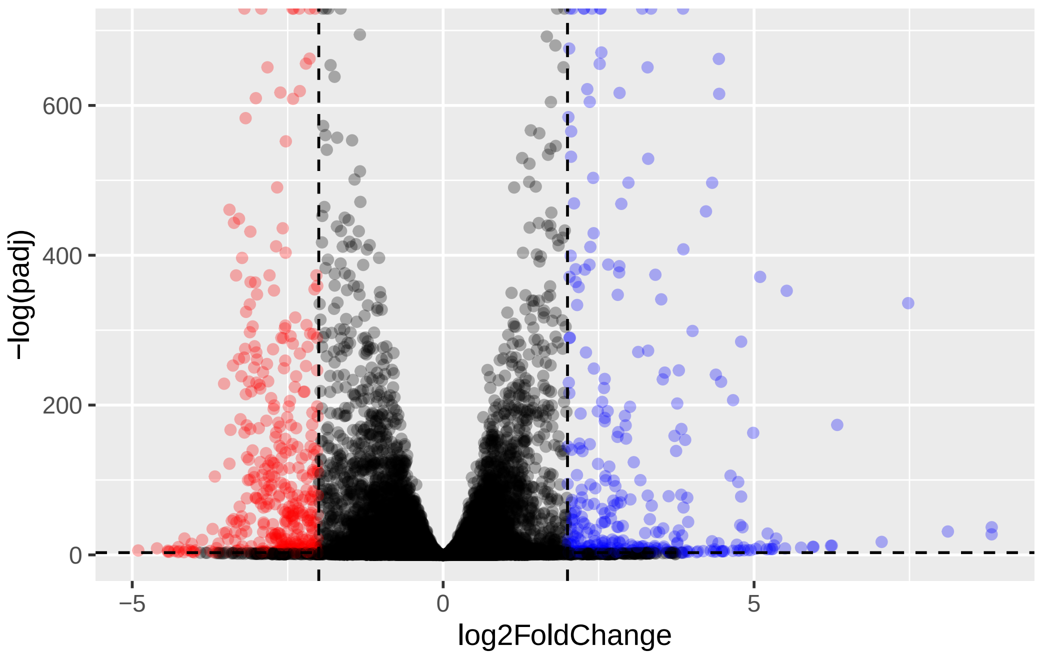
Creating color vector to differentiate upregulated and downregulated genes.

```r
my_colors <- rep("black", nrow(res))
my_colors[res$log2FoldChange > 2 & res$padj < 0.05] <- "blue"
my_colors[res$log2FoldChange < -2 & res$padj < 0.05] <- "red"
```

Plotting results in summary volcano plot.

```r
ggplot(as.data.frame(res)) +
  aes(log2FoldChange, -log(padj)) +
  geom_point(color=my_colors, alpha=0.3) +
  geom_vline(xintercept = c(-2,2), linetype="dashed") +
  geom_hline(yintercept=-log(0.05), linetype="dashed")
```

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



Adding Annotation Data

Loading in annotation data libraries

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

Mapping alternative IDs to entries in DESeq results (gene names, symbols, entrez, uniprot)

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

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

```
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
```

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

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

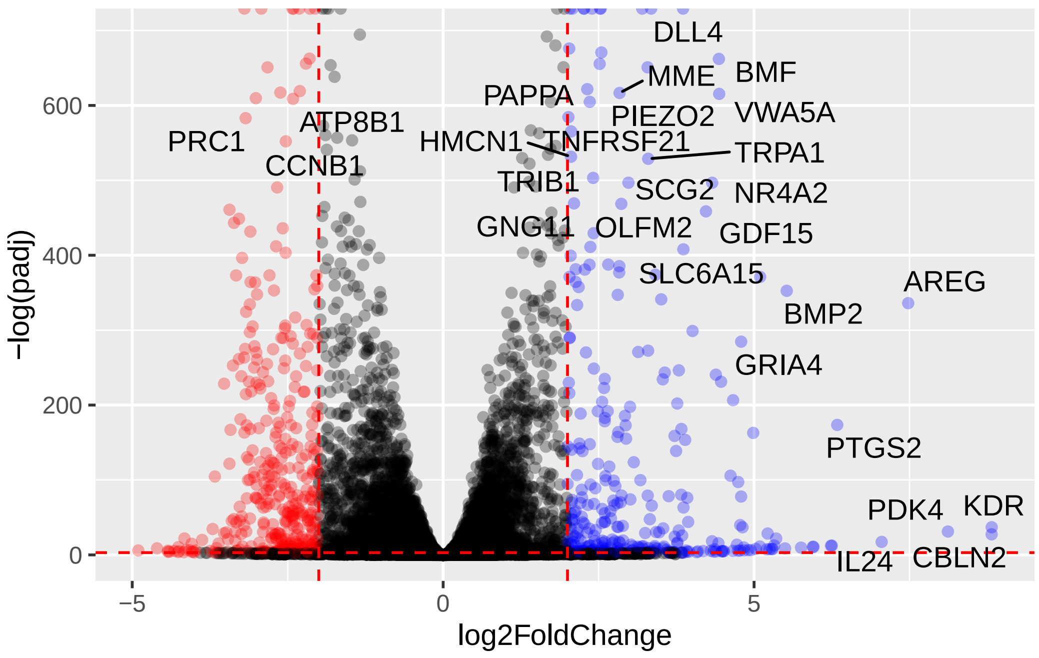
Re-plotting volcano plot with annotation tags

```r
res_df <- as.data.frame(res)
ggplot(as.data.frame(res)) +
  aes(log2FoldChange, -log(padj), label = symbol) +
  geom_point(color=my_colors, alpha=0.3) +
  geom_vline(xintercept = c(-2,2), linetype="dashed", color = "red") +
  geom_hline(yintercept=-log(0.05), linetype="dashed", color = "red") +
  geom_text_repel(data=subset(res_df, (res$log2FoldChange > 2 | res$log2FoldChange < -2) &
```

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

```
Warning: Removed 2 rows containing missing values (`geom_text_repel()`).
```

```
Warning: ggrepel: 652 unlabeled data points (too many overlaps). Consider
increasing max.overlaps
```



Pathway Analysis: KEGG

```
FC <- res_df$log2FoldChange
names(FC) <- res_df$entrez
```

Loading in necessary packages

```
#/ message: false
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).
##############################################################################
```

Using GAGE to do pathway analysis using KEGG database of human pathways/processes

```
data(kegg.sets.hs)

kegg <- gage(FC, gsets=kegg.sets.hs)
head(kegg$less)
```

```
                                                  p.geomean stat.mean
hsa04110 Cell cycle                            8.995727e-06 -4.378644
hsa03030 DNA replication                       9.424076e-05 -3.951803
hsa05130 Pathogenic Escherichia coli infection 1.405864e-04 -3.765330
hsa03013 RNA transport                         1.246882e-03 -3.059466
hsa03440 Homologous recombination              3.066756e-03 -2.852899
hsa04114 Oocyte meiosis                        3.784520e-03 -2.698128
                                                      p.val       q.val
hsa04110 Cell cycle                            8.995727e-06 0.001889103
hsa03030 DNA replication                       9.424076e-05 0.009841047
hsa05130 Pathogenic Escherichia coli infection 1.405864e-04 0.009841047
hsa03013 RNA transport                         1.246882e-03 0.065461279
hsa03440 Homologous recombination              3.066756e-03 0.128803765
hsa04114 Oocyte meiosis                        3.784520e-03 0.132458191
                                               set.size         exp1
hsa04110 Cell cycle                                 121 8.995727e-06
hsa03030 DNA replication                             36 9.424076e-05
hsa05130 Pathogenic Escherichia coli infection       53 1.405864e-04
hsa03013 RNA transport                              144 1.246882e-03
hsa03440 Homologous recombination                    28 3.066756e-03
hsa04114 Oocyte meiosis                             102 3.784520e-03
```

```
head(kegg$greater)
```

```
                                                   p.geomean stat.mean
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06  4.358967
```

```
hsa05323 Rheumatoid arthritis                   1.809824e-04  3.666793
hsa05146 Amoebiasis                             1.313400e-03  3.052596
hsa05332 Graft-versus-host disease              2.605234e-03  2.948229
hsa04640 Hematopoietic cell lineage             2.822776e-03  2.833362
hsa04630 Jak-STAT signaling pathway             5.202070e-03  2.585673
                                                       p.val       q.val
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06 0.001917519
hsa05323 Rheumatoid arthritis                   1.809824e-04 0.019003147
hsa05146 Amoebiasis                             1.313400e-03 0.091937999
hsa05332 Graft-versus-host disease              2.605234e-03 0.118556573
hsa04640 Hematopoietic cell lineage             2.822776e-03 0.118556573
hsa04630 Jak-STAT signaling pathway             5.202070e-03 0.182072434
                                                set.size         exp1
hsa04060 Cytokine-cytokine receptor interaction      177 9.131044e-06
hsa05323 Rheumatoid arthritis                         72 1.809824e-04
hsa05146 Amoebiasis                                   94 1.313400e-03
hsa05332 Graft-versus-host disease                    22 2.605234e-03
hsa04640 Hematopoietic cell lineage                   55 2.822776e-03
hsa04630 Jak-STAT signaling pathway                  109 5.202070e-03
```

Using pathview to visualize some affected pathways e.g. cell cycle and cytokine-cytokine receptor interactions

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

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

```
Info: Working in directory C:/Users/Nate Tran/Documents/RStudioWorkspace/class13
```

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

```
pathview(gene.data=FC, pathway.id="hsa04060")
```

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

```
Info: Working in directory C:/Users/Nate Tran/Documents/RStudioWorkspace/class13
```

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

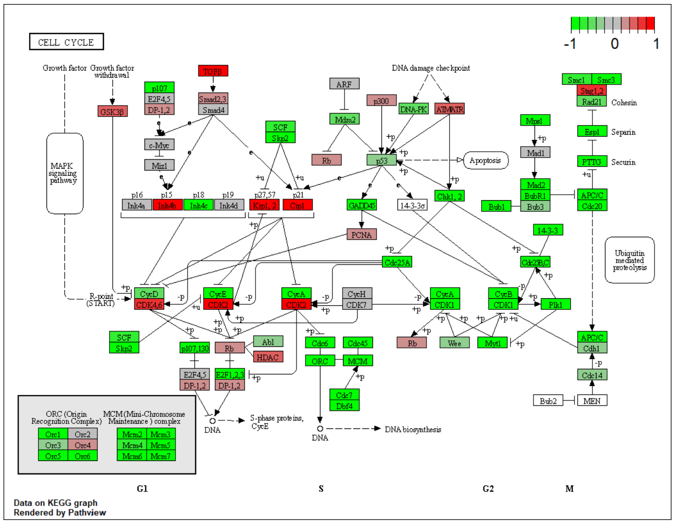


Figure 1: Cell cycle pathway affected by HOXA1 knockdown

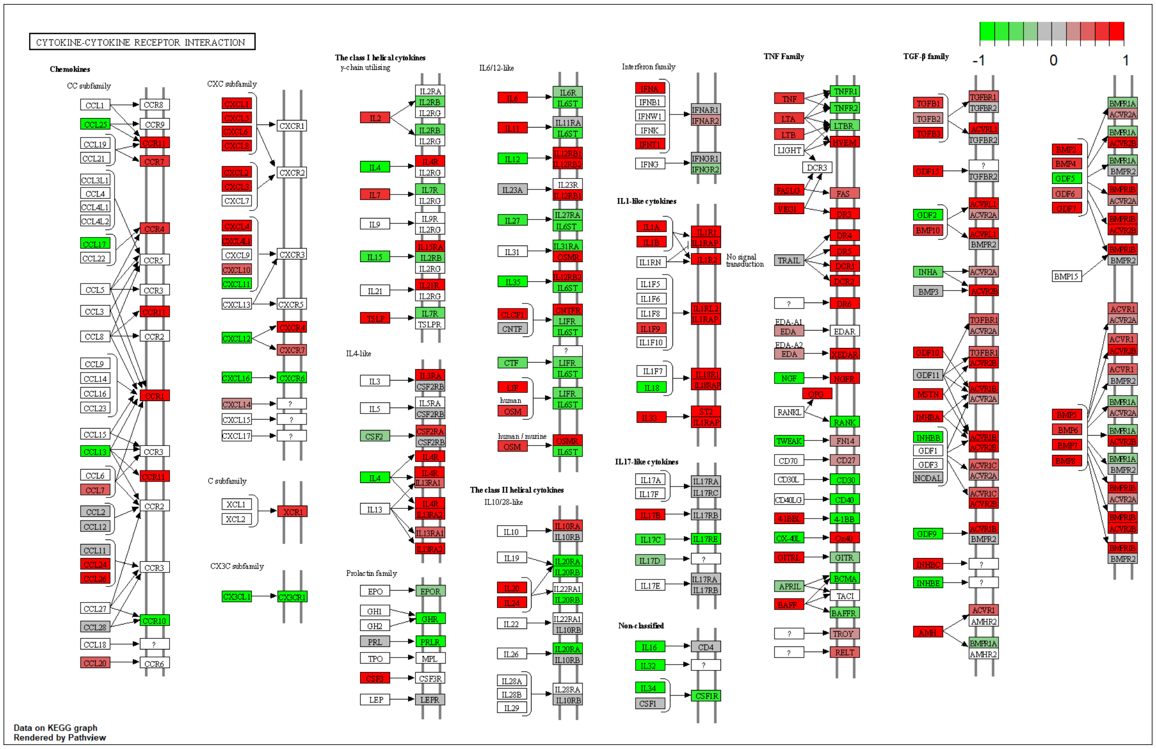


Figure 2: Cytokien-cytokine receptor interactions affected by HOXA1 knockdown

Pathway Analysis: GO

Using GAGE to do pathway analysis using GO database of processes

```
data(go.sets.hs)

go <- gage(FC, gsets=go.sets.hs)
```

Exploring GO analysis

```
head(go$greater)
```

```
GO:0007156 homophilic cell adhesion                                                 8.51
GO:0005125 cytokine activity                                                        1.14
GO:0002009 morphogenesis of an epithelium                                           1.39
GO:0048729 tissue morphogenesis                                                     1.43
GO:0000981 sequence-specific DNA binding RNA polymerase II transcription factor activity 1.93
GO:0007610 behavior                                                                 2.19
                                                                                    stat
GO:0007156 homophilic cell adhesion                                                  3.8
GO:0005125 cytokine activity                                                         3.7
GO:0002009 morphogenesis of an epithelium                                            3.6
GO:0048729 tissue morphogenesis                                                      3.6
GO:0000981 sequence-specific DNA binding RNA polymerase II transcription factor activity  3.5
GO:0007610 behavior                                                                  3.5

GO:0007156 homophilic cell adhesion                                                 8.51
GO:0005125 cytokine activity                                                        1.14
GO:0002009 morphogenesis of an epithelium                                           1.39
GO:0048729 tissue morphogenesis                                                     1.43
GO:0000981 sequence-specific DNA binding RNA polymerase II transcription factor activity 1.93
GO:0007610 behavior                                                                 2.19

GO:0007156 homophilic cell adhesion                                                 0.19
GO:0005125 cytokine activity                                                        0.19
GO:0002009 morphogenesis of an epithelium                                           0.19
GO:0048729 tissue morphogenesis                                                     0.19
GO:0000981 sequence-specific DNA binding RNA polymerase II transcription factor activity 0.19
GO:0007610 behavior                                                                 0.19
                                                                                    set
GO:0007156 homophilic cell adhesion
```

```
GO:0005125 cytokine activity
GO:0002009 morphogenesis of an epithelium
GO:0048729 tissue morphogenesis
GO:0000981 sequence-specific DNA binding RNA polymerase II transcription factor activity
GO:0007610 behavior

GO:0007156 homophilic cell adhesion                                                     8.51
GO:0005125 cytokine activity                                                            1.14
GO:0002009 morphogenesis of an epithelium                                               1.39
GO:0048729 tissue morphogenesis                                                         1.43
GO:0000981 sequence-specific DNA binding RNA polymerase II transcription factor activity 1.93
GO:0007610 behavior                                                                     2.19
```

```r
head(go$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:0000775 chromosome, centromeric region 2.131309e-11 -6.863475 2.131309e-11
                                                 q.val set.size         exp1
GO:0048285 organelle fission              7.732248e-12      376 1.536227e-15
GO:0000280 nuclear division               7.732248e-12      352 4.286961e-15
GO:0007067 mitosis                        7.732248e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle  1.582628e-11      362 1.169934e-14
GO:0007059 chromosome segregation         1.922085e-08      142 2.028624e-11
GO:0000775 chromosome, centromeric region 1.922085e-08      146 2.131309e-11
```

Reactome Analysis

```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"
```

Writing significantly altered genes to table file for export.

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