RNA Seq Mini Proj Class14

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Import Data

We need two things: Counts MetaData (what DESeq calls colData- as it describes the columns in Counts)

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

Start with a sneak peak:

```
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
```

```
head(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
```

We want the columns in `counts` to match the rows in the `metadata`

```
colnames(counts)
```

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

```
metadata$id
```

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

Since `counts` and `metadata` columns do not match, we must remove the `length` column from the `counts` dataset

```
countData <- counts[,-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
```

```
all(colnames(countData) == metadata$id)
```

```
[1] TRUE
```

yay now they match.

Data CleanUp

Must filter out zero counts

It is standard practice to remove any genes/transcripts that we have no data for, ie. zero counts in all columns

```
to.keep.inds <- rowSums(countData) > 0
cleanCounts <- countData[to.keep.inds,]
head(cleanCounts)
```

```
                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)
```

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

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

DESeq

```
dds <- DESeq(dds)
```

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

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

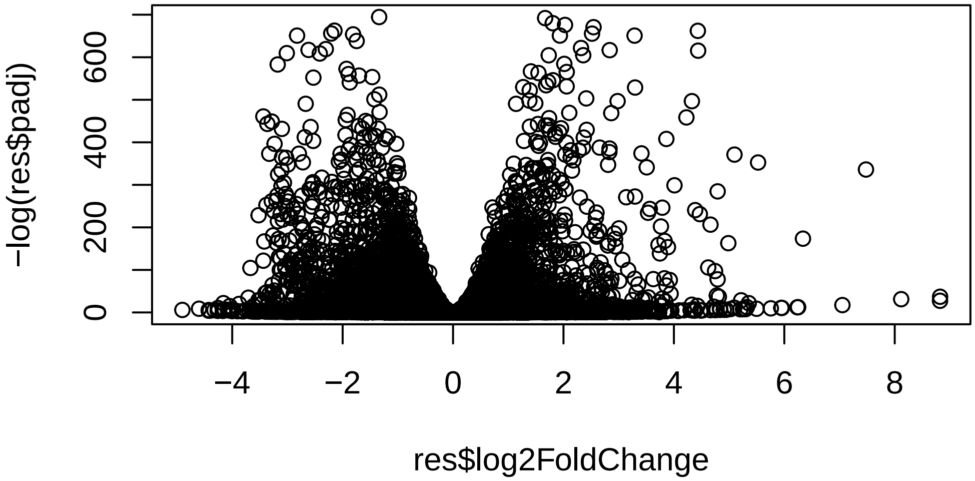
Inspect Results

```r
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
```

Data Viz

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



Pathway Analysis

Annotation of genes

First I need to translate my Ensembl IDs in my `res` object to Entrez and gene symbol formats.

For this I will use the AnnotationDbi package and its `mapIds()` function

Need to know what format stuff is currently in, and what we would like to "translate" them into.

We currently have Ensembl IDs, but to use KEGG, we need to use Entrez IDs.

```
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"
```

Let's map to "SYMBOL," "ENTREZID," and "GENENAME," from our "ENSEMBL" ids.

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

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

```
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 7 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               genename
                  <numeric>            <character>
ENSG00000279457 6.86555e-01                     NA
ENSG00000187634 5.15718e-03 sterile alpha motif ..
ENSG00000188976 1.76549e-35 NOC2 like nucleolar ..
ENSG00000187961 1.13413e-07 kelch like family me..
ENSG00000187583 9.19031e-01 pleckstrin homology ..
ENSG00000187642 4.03379e-01 PPARGC1 and ESRR ind..
```

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

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

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

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

```
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 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
ENSG00000187642   11.9798      0.5428105 0.5215599   1.040744 2.97994e-01
                       padj               genename      symbol    entrezid
                  <numeric>            <character> <character> <character>
ENSG00000279457 6.86555e-01                     NA          NA          NA
ENSG00000187634 5.15718e-03 sterile alpha motif ..      SAMD11      148398
ENSG00000188976 1.76549e-35 NOC2 like nucleolar ..       NOC2L       26155
ENSG00000187961 1.13413e-07 kelch like family me..      KLHL17      339451
ENSG00000187583 9.19031e-01 pleckstrin homology ..     PLEKHN1       84069
ENSG00000187642 4.03379e-01 PPARGC1 and ESRR ind..       PERM1       84808
```

Before going any further lets focus in on a subset of “top” hits.

We can use a starting point log2FC of +/-2 and an adjusted P-value of 0.05

Let’s do the foldchange one first

```r
top.inds <- (abs(res$log2FoldChange) > 2) & (res$padj <= 0.05)
top.inds[is.na(top.inds)] <- FALSE
```

Let's save our "top genes" to a .csv file!

```r
top.genes <- res[top.inds,]
write.csv(top.genes, file="top_geneset.csv")
```

Now we can do some pathway analysis (finally!)

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

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

```r
# focus on signaling and metabolic pathways
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]
```

```r
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrezid
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)
```

```
attributes(keggres)
```

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

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

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

Info: Working in directory /Users/janiec-w/Desktop/Classes/BGGN213 Bioinformatics/pRojects/J

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

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

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

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

```r
head(gores$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
```

```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
head(sig_genes)
```

```
ENSG00000187634 ENSG00000188976 ENSG00000187961 ENSG00000188290 ENSG00000187608
       "SAMD11"         "NOC2L"        "KLHL17"          "HES4"         "ISG15"
ENSG00000188157
         "AGRN"
```

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

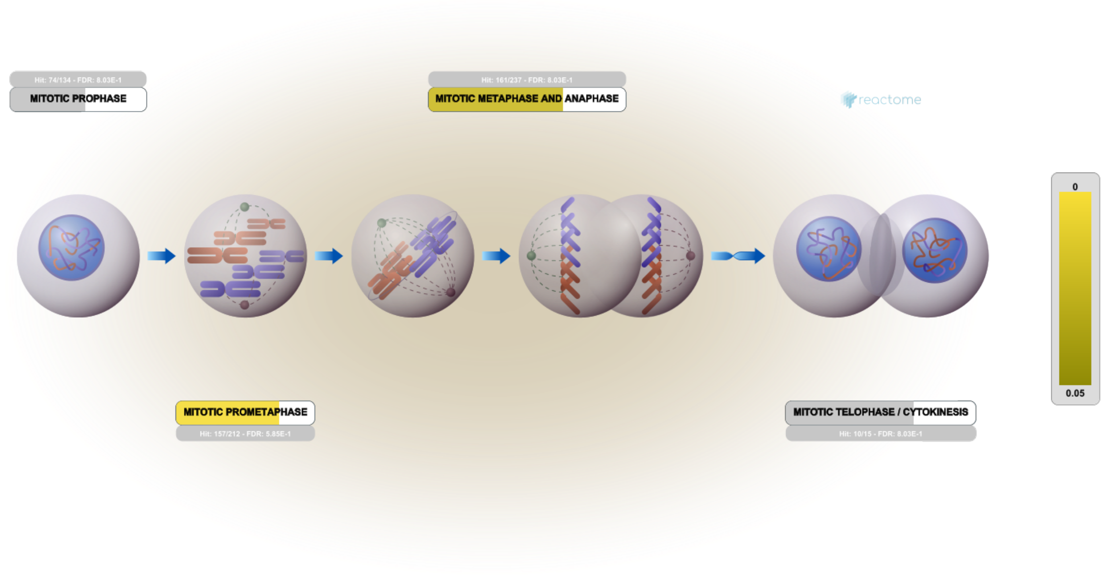

Hit: 74/134 - FDR: 8.03E-1
MITOTIC PROPHASE
Hit: 161/237 - FDR: 8.03E-1
MITOTIC METAPHASE AND ANAPHASE
reactome
0
0.05
MITOTIC PROMETAPHASE
Hit: 157/212 - FDR: 5.85E-1
MITOTIC TELOPHASE / CYTOKINESIS
Hit: 10/15 - FDR: 8.03E-1

Figure 1: Overview