Class 14: RNASeq Mini Project

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Background

Here we work through a complete RNASeq analysis project. The input data comes from a knock-down experiment of a HOX gene.

Data Import

Reading the `counts` and `metadata` CSV files

```
metaFile <- "GSE37704_metadata.csv"
countFile <- "GSE37704_featurecounts.csv"
colData = read.csv(metaFile, row.names=1)
countData = read.csv(countFile, row.names=1)
```

Check on data structure

Some book-keeping is required as there looks to be a mis-match between metadata and counts columns.

Looks like we need to get rid of the first "length" column of our `counts` object.

```
countData <- read.csv(countFile, row.names = 1)
countData[] <- lapply(countData, function(x) as.numeric(as.character(x)))
cleancounts <- as.matrix(countData[, -1])
nonzero_counts <- cleancounts[rowSums(cleancounts) > 0, ]
head(nonzero_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
```

DESeq Analysis

Load the package

```
library(DESeq2)
```

Setup DESeq

```
dds = DESeqDataSetFromMatrix(countData=nonzero_counts,
                             colData=colData,
                             design=~condition)
```

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

Run DESeq

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

Get results

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

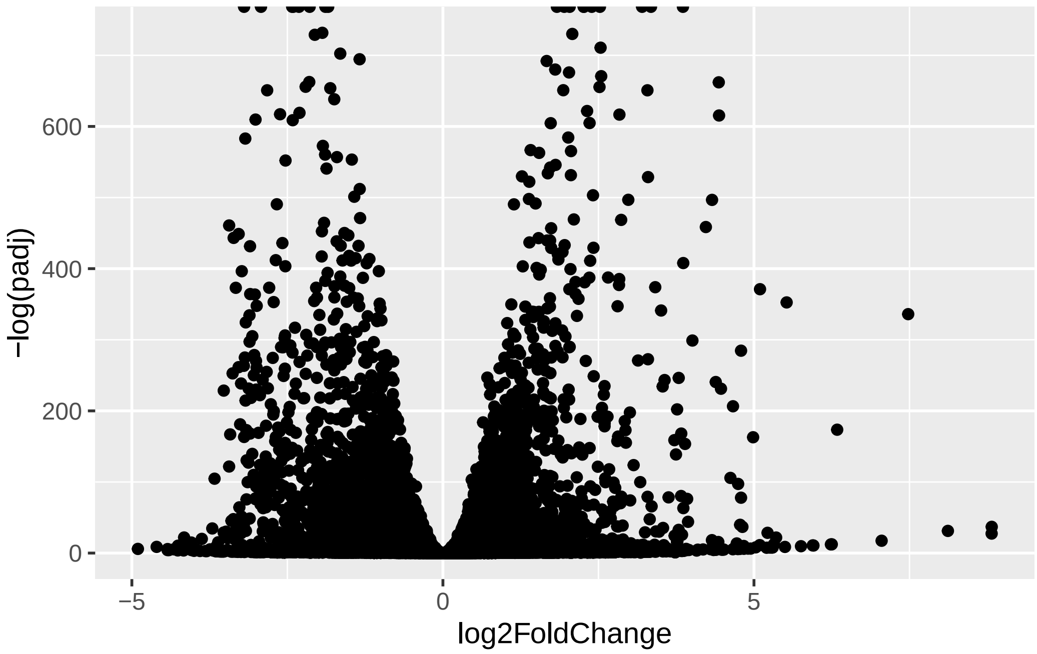
Data Visualization

Volcano Plot

```
library(ggplot2)

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

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

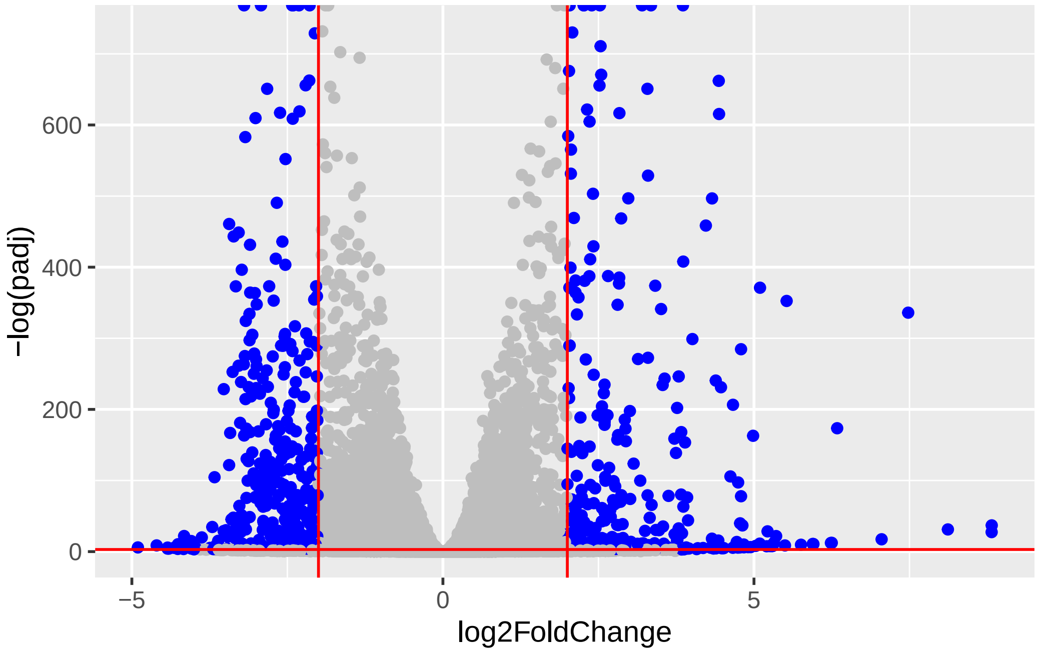


Add threshold lines for fold-change and P-valye and color our subset of genes that make these threshold cut-offs in the plot.

```r
mycols <- rep("gray", nrow(res))
mycols[abs(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 Annotation

Add gene symbols and entrez ids

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

```r
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
```

```r
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
```

```r
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
```

```r
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 ..
```

Let's Reorder results by adjusted p-value and save as CSV file:

```
res <- res[order(res$padj), ]

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

Pathway Analysis

KEGG pathways

- run this in console: `BiocManager::install( c("pathview", "gage", "gageData") )` *

Run gage analysis w/ KEGG

```
library(gage)
library(gageData)
library(pathview)
```

We need a named vector of fold-change value as input for gage.

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

```
     1266      54855       1465       2034       2150       6659 
-2.422719  3.201955 -2.313738 -1.888019  3.344508  2.392288 
```

```
data("kegg.sets.hs")
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

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

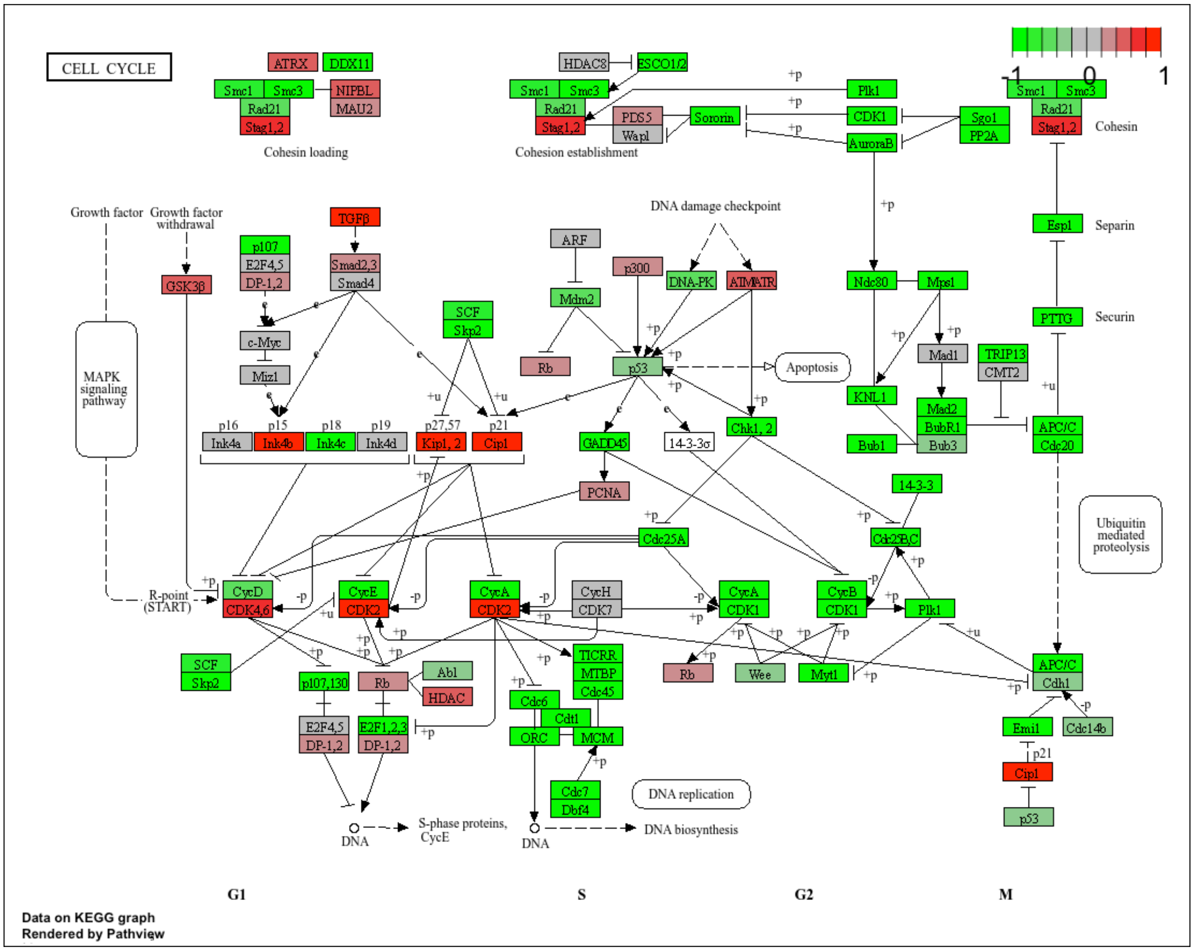
```
                            p.geomean stat.mean        p.val       q.val
hsa04110 Cell cycle      8.995727e-06 -4.378644 8.995727e-06 0.001889103
hsa03030 DNA replication 9.424076e-05 -3.951803 9.424076e-05 0.009841047
                         set.size         exp1
hsa04110 Cell cycle           121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

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

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

```
Info: Working in directory /Users/lourdyakoob/Desktop/Class 14
```

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

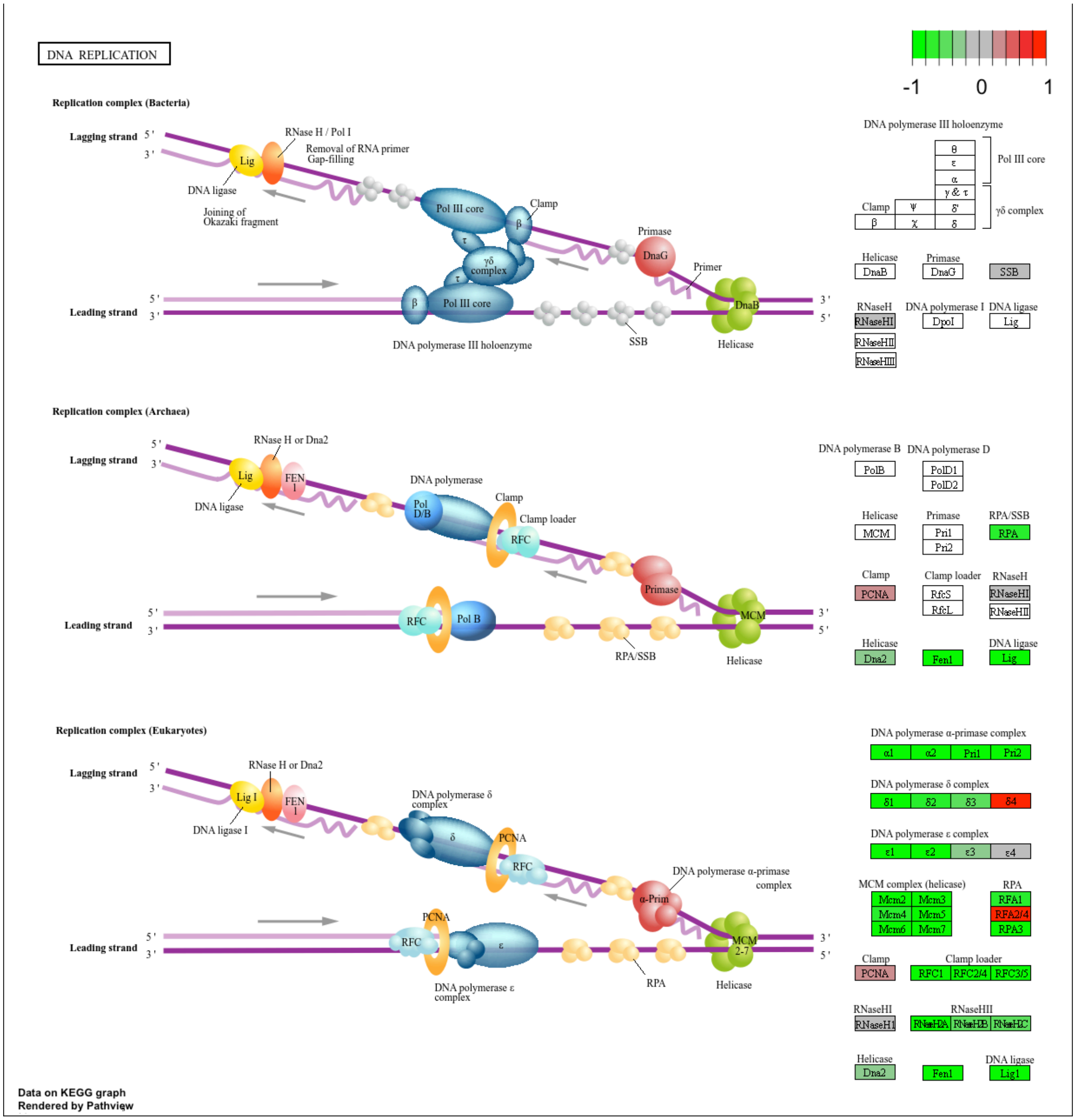


```r
pathview(pathway.id = "hsa03030", gene.data = foldchanges)
```

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

Info: Working in directory /Users/lourdyakoob/Desktop/Class 14

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



GO terms

Same analysis but using GO genesets rather than KEGG.

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

```r
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       0.1951953      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                       0.1967577      426 1.925222e-04
GO:0060562 epithelial tube morphogenesis  0.3565320      257 5.932837e-04
GO:0035295 tube development               0.3565320      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                        3.565432 3.565432
GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
GO:0035295 tube development                3.253665 3.253665
```

```
head(gobpres$less, 4)
```

```
                                            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
                                                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
```

Reactome

Lots of folkslike the reactome web interface. You can also run this as an R fucntion but lets look at the website first https://reactome.org/

The website wants a text file with one gene symbol per line of the genes ypu want to map to the pathways.

```
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"
```

and write out to a file:

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

Save Our Results

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