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

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

Check on data structure

```
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
```

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

```
ncol(counts)
```

```
[1] 7
```

```
nrow(metadata)
```

```
[1] 6
```

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

```
cleancounts <- counts[, -1]
```

```
colnames(cleancounts)
```

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

```
metadata$id
```

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

Remove the zero count genes

There are lots of genes with zero counts. We can remove these from further analysis

```
head(cleancounts)
```

```
                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
```

```
to.keep.inds <- rowSums(cleancounts) > 0
nonzero_counts <- cleancounts[to.keep.inds,]
```

DESeq analysis

Load the package

```
library(DESeq2)
```

Setup DESeq object

```
dds <- DESeqDataSetFromMatrix(countData= nonzero_counts,
                              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
```

Get 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
```

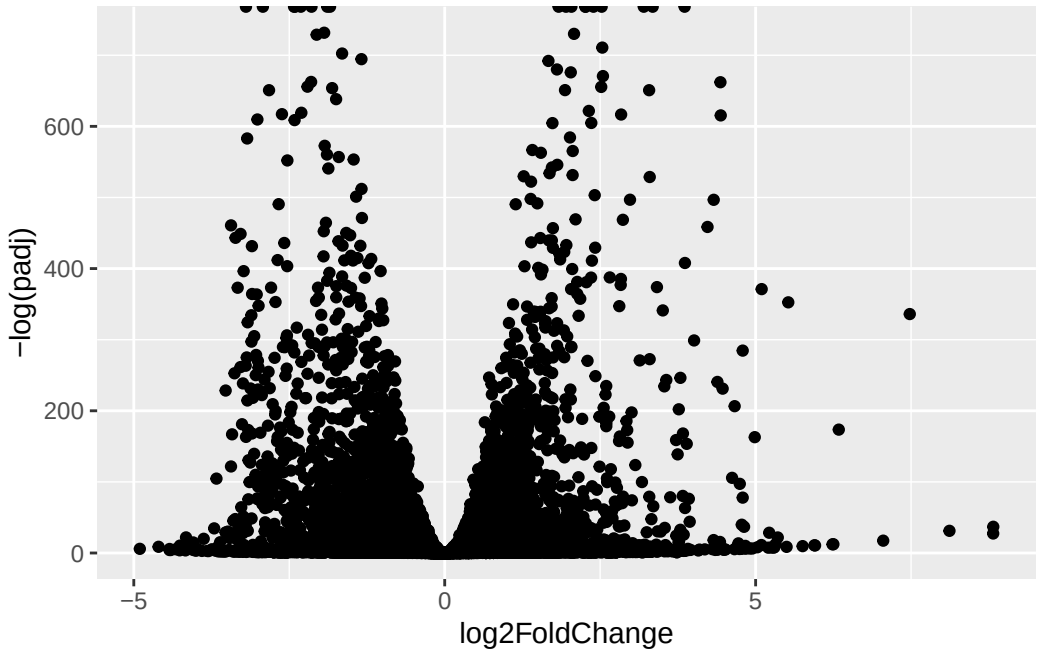
Data Visualization

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()`).
```



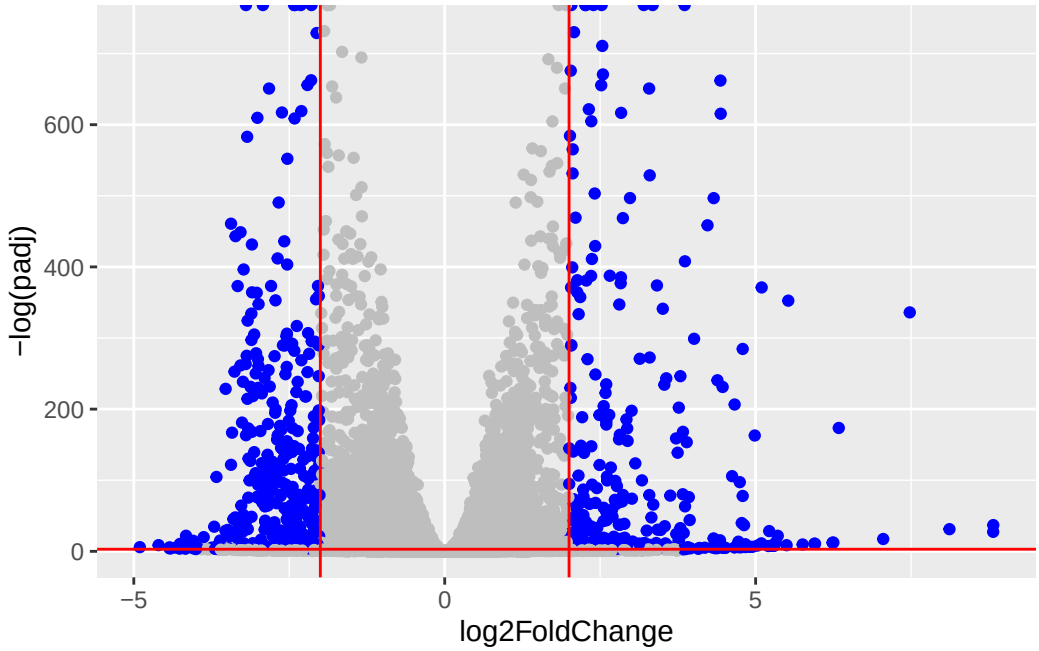
Add threshold lines for fold-change and P-value and color to our subset of genes that make these threshold cut-offs in the plot

```
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

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

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

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

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

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

Pathways analysis

Run gage analysis with KEGG

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

We need a named vector and fold-change values as input for gage

```
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
head(foldchanges)
```

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

```
data(kegg.sets.hs)

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

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

```
                                           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
                                              p.val       q.val
```

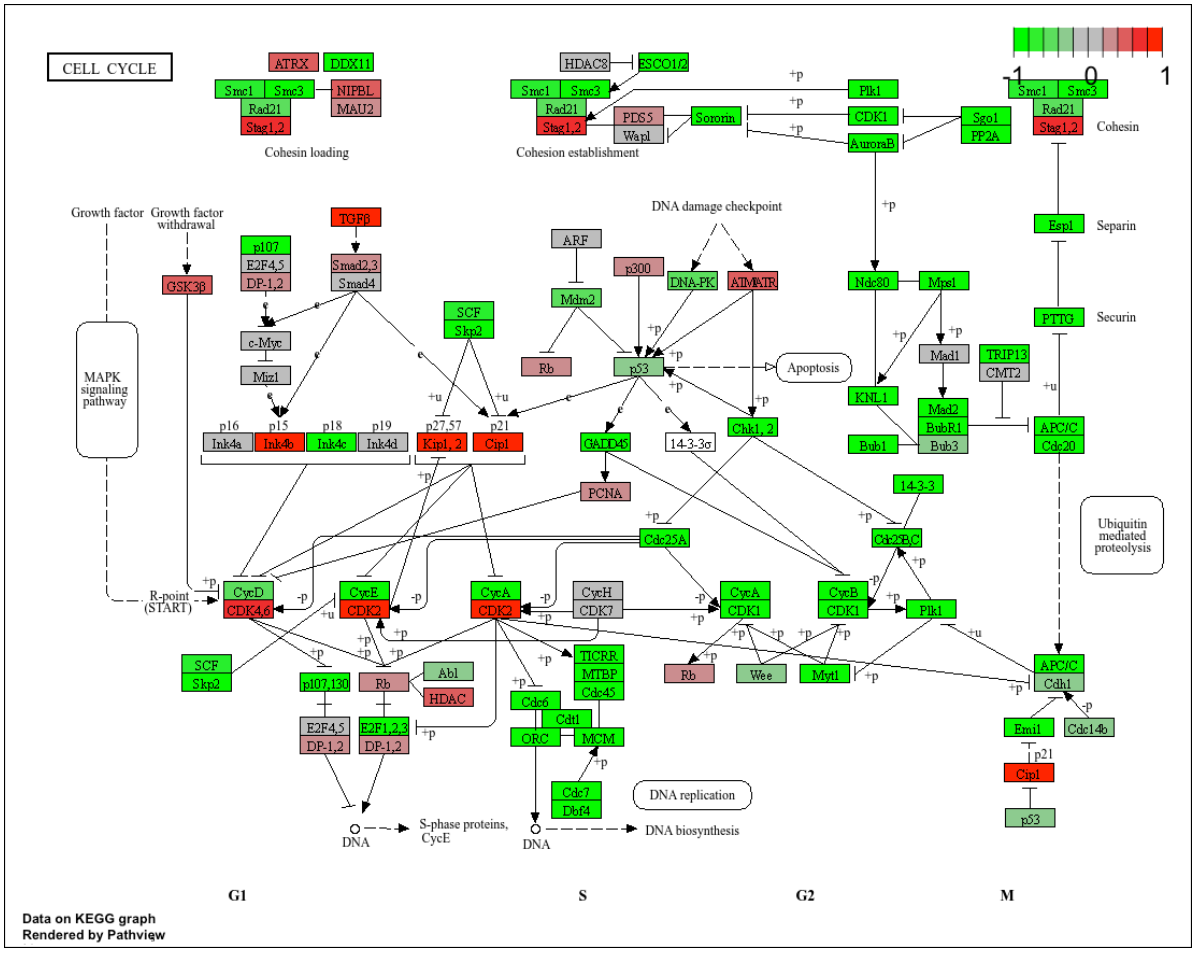
```
hsa04110 Cell cycle                                 8.995727e-06 0.001889103
hsa03030 DNA replication                            9.424076e-05 0.009841047
hsa05130 Pathogenic Escherichia coli infection      1.405864e-04 0.009841047
hsa03013 RNA transport                              1.246882e-03 0.065461279
hsa03440 Homologous recombination                   3.066756e-03 0.128803765
                                                    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
```

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

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

```
Info: Working in directory /Users/charlizemolitor/Desktop/class04/Class 14
```

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

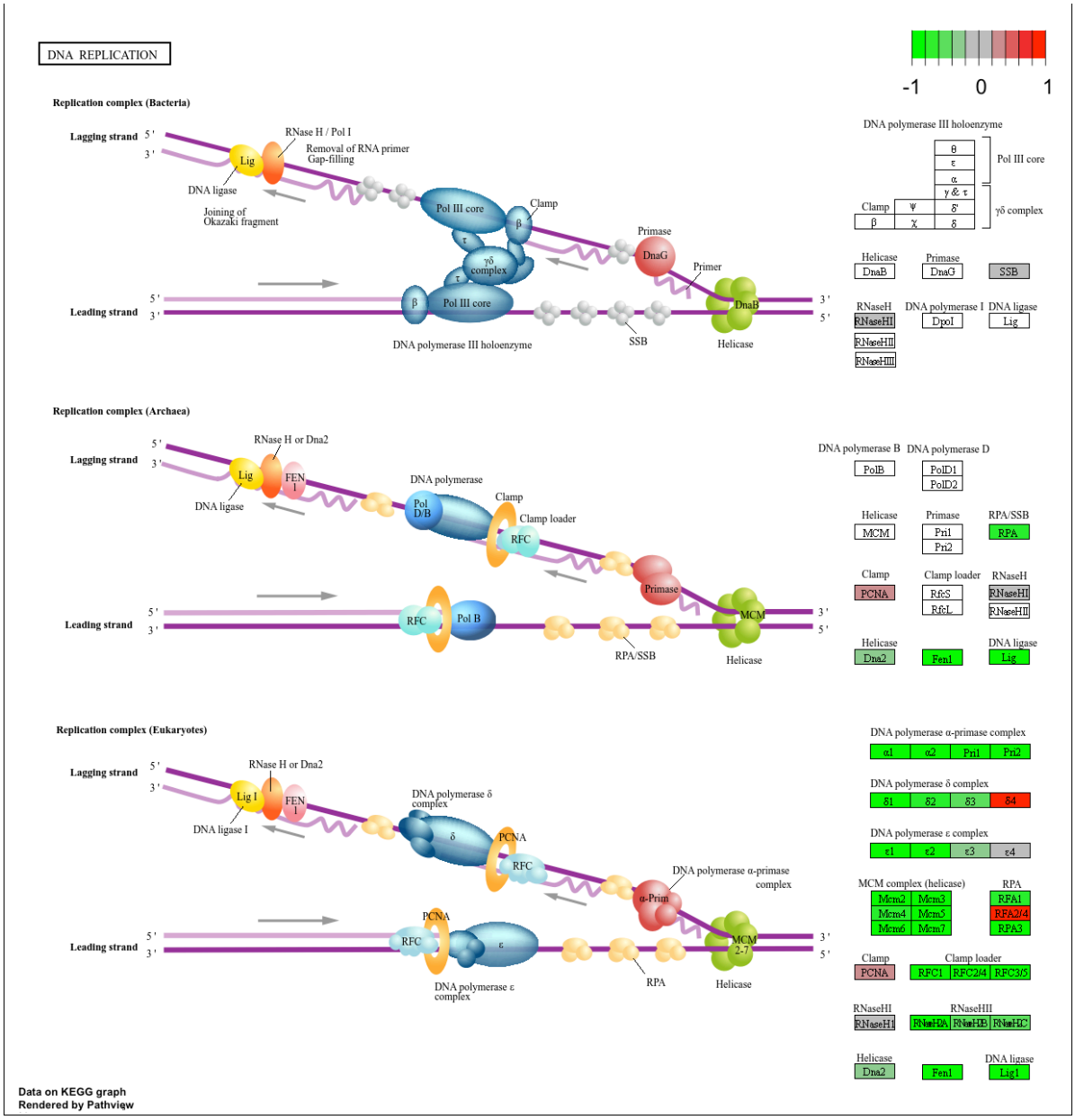



```
pathview(pathway.id = "hsa03030", gene.data = foldchanges )
```

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

Info: Working in directory /Users/charlizemolitor/Desktop/class04/Class 14

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



GO terms

Same analysis but using GO genesets rathe than KEGG

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

```r
# Focus on Biological Process subset of GO
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
```

Reactome

Lots of folks like the reactome web interface. You can also run this as an R function but lets look at the website first < https://reactome.org/user/guide >

The website wants a text file with one gee symbol per line of the genes you want to map to pathways

```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj),] $symbol
head(sig_genes)
```

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

```
#res$symbol
```

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")
```