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")
```

```
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
```

```
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"
```

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

```
[1] TRUE
```

Remove zero count genes

There are lots of genes with zero counts. We can remove these from futher 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)
```

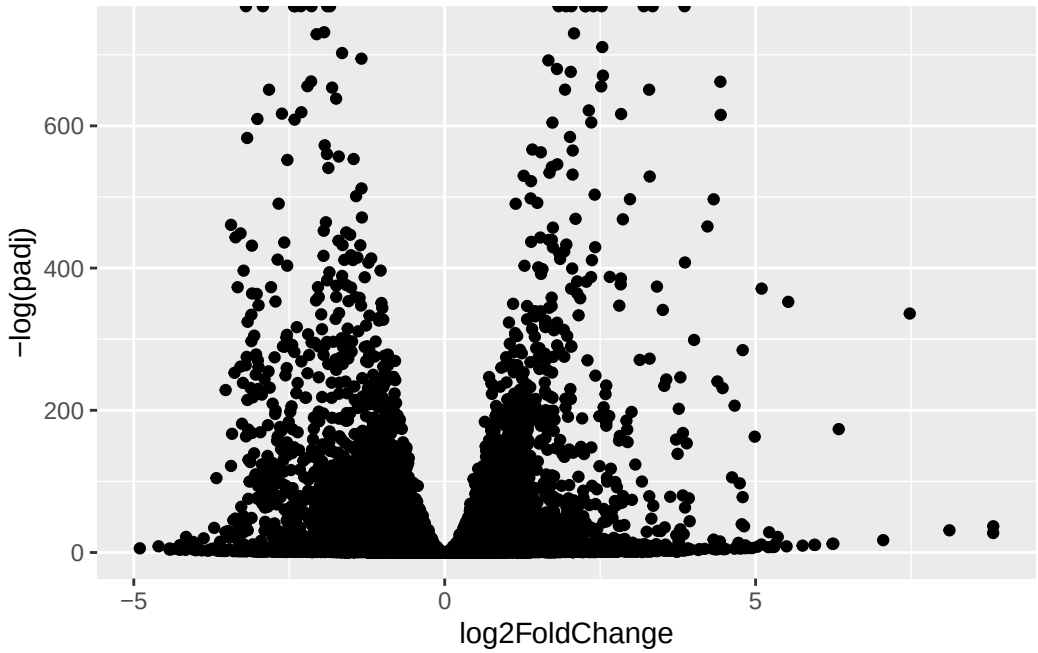
Data Visualization

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()`).
```

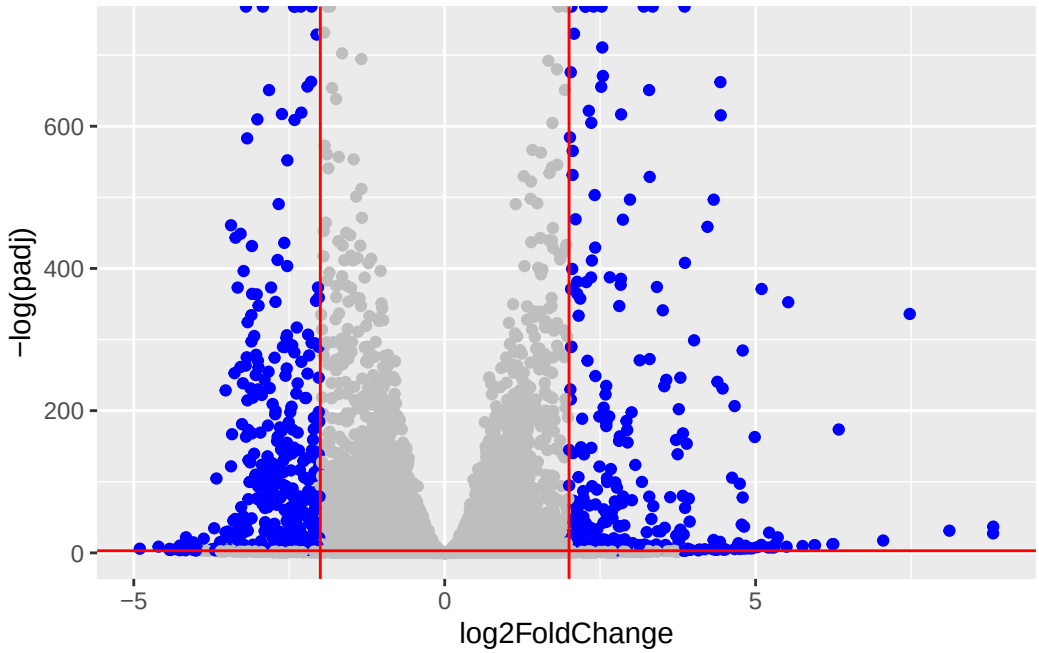


Add threshold lines for fold-change and P-value and color our subset of genes that make these threshold cut-offs in the plot

```
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 symbol 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
```

Pathway Analysis

Run gage analysis

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

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

```
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)
```

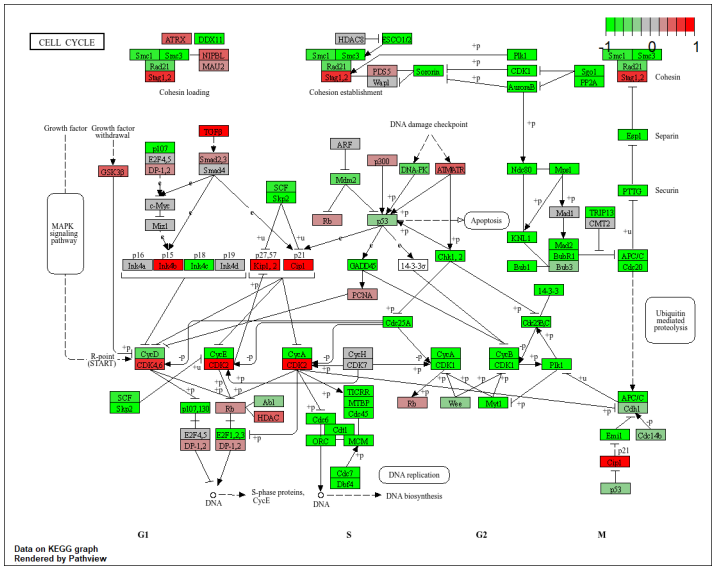
```
                                                    p.geomean stat.mean
hsa04110 Cell cycle                                8.995727e-06 -4.378644
hsa03030 DNA replication                           9.424076e-05 -3.951803
hsa05130 Pathogenic Escherichia coli infection     1.405864e-04 -3.765330
hsa03013 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 C:/Users/berne/Desktop/BIMM 143/class14

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

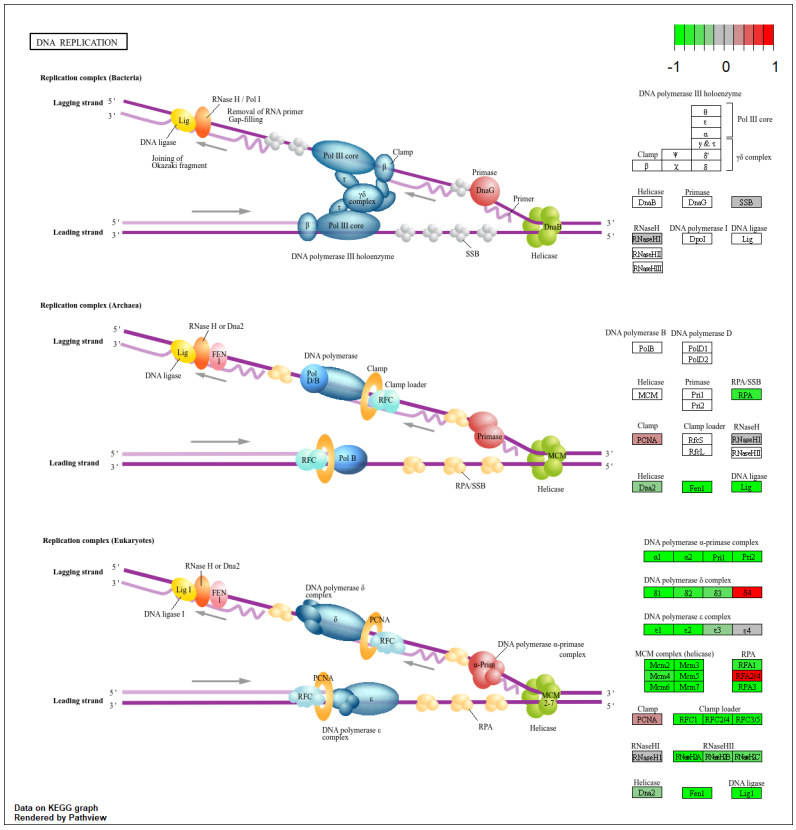


```
pathview(pathway.id = "hsa03030", gene.data=foldchanges)
```

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

```
Info: Working in directory C:/Users/berne/Desktop/BIMM 143/class14
```

```
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)

# Focus on Biological Process subset of GO
```

```
gobpsets = go.sets.hs[go.subs.hs$BP]

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

```
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 folks like the reactome web interface. You can also run this as an R function bu tlets look at the website first. https://reactome.org/

The website wants a text file with one gene symbol per pline 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"
```

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")
```