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

```
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 metadatarows and counts columns

```
ncol(counts)
```

```
[1] 7
```

```
nrow(metadata)
```

```
[1] 6
```

We need to remove the first column for count

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

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

```
[1] TRUE
```

Remove zero count genes

There are lost 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)
```

```
Warning: package 'matrixStats' was built under R version 4.5.2
```

Setup DESeq

```
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)
```

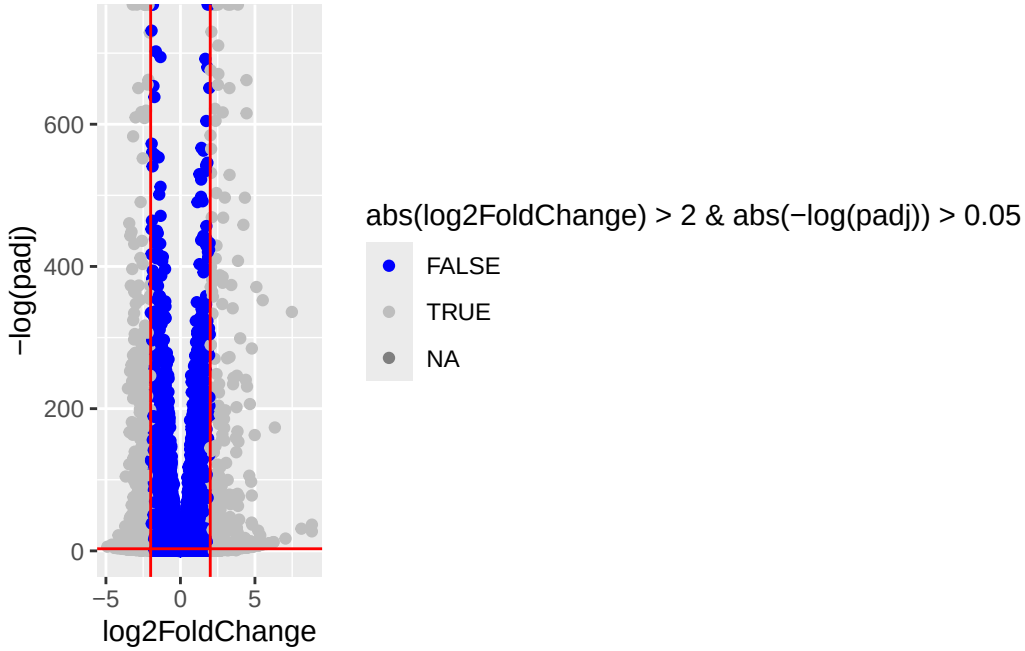
Data Visualization

Volcano plot

```
library(ggplot2)

ggplot(res) +
  aes(log2FoldChange, -log(padj),
      color = abs(log2FoldChange) > 2 & abs(-log(padj)) > 0.05) +
  geom_point() +
  scale_color_manual(values = c("blue", "grey")) +
  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 threshold lines for fold-change and p-value and color our subset of genes that make these threshold cut-offs in the plot.

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
```

Pathway Analysis

KEGG Pathways

Run gage analysis with KEGG

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

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

# Focus on signaling and metabolic pathways only
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]

# Examine the first 3 pathways
head(kegg.sets.hs, 3)
```

```
$`hsa00232 Caffeine metabolism`
[1] "10"   "1544" "1548" "1549" "1553" "7498" "9"

$`hsa00983 Drug metabolism - other enzymes`
 [1] "10"     "1066"   "10720"  "10941"  "151531" "1548"   "1549"   "1551"
 [9] "1553"   "1576"   "1577"   "1806"   "1807"   "1890"   "221223" "2990"
[17] "3251"   "3614"   "3615"   "3704"   "51733"  "54490"  "54575"  "54576"
[25] "54577"  "54578"  "54579"  "54600"  "54657"  "54658"  "54659"  "54963"
[33] "574537" "64816"  "7083"   "7084"   "7172"   "7363"   "7364"   "7365"
[41] "7366"   "7367"   "7371"   "7372"   "7378"   "7498"   "79799"  "83549"
[49] "8824"   "8833"   "9"      "978"

$`hsa00230 Purine metabolism`
  [1] "100"    "10201"  "10606"  "10621"  "10622"  "10623"  "107"    "10714"
```

```
  [9] "108"    "10846"  "109"    "111"    "11128"  "11164"  "112"    "113"
 [17] "114"    "115"    "122481" "122622" "124583" "132"    "158"    "159"
 [25] "1633"   "171568" "1716"   "196883" "203"    "204"    "205"    "221823"
 [33] "2272"   "22978"  "23649"  "246721" "25885"  "2618"   "26289"  "270"
 [41] "271"    "27115"  "272"    "2766"   "2977"   "2982"   "2983"   "2984"
 [49] "2986"   "2987"   "29922"  "3000"   "30833"  "30834"  "318"    "3251"
 [57] "353"    "3614"   "3615"   "3704"   "377841" "471"    "4830"   "4831"
 [65] "4832"   "4833"   "4860"   "4881"   "4882"   "4907"   "50484"  "50940"
 [73] "51082"  "51251"  "51292"  "5136"   "5137"   "5138"   "5139"   "5140"
 [81] "5141"   "5142"   "5143"   "5144"   "5145"   "5146"   "5147"   "5148"
 [89] "5149"   "5150"   "5151"   "5152"   "5153"   "5158"   "5167"   "5169"
 [97] "51728"  "5198"   "5236"   "5313"   "5315"   "53343"  "54107"  "5422"
[105] "5424"   "5425"   "5426"   "5427"   "5430"   "5431"   "5432"   "5433"
[113] "5434"   "5435"   "5436"   "5437"   "5438"   "5439"   "5440"   "5441"
[121] "5471"   "548644" "55276"  "5557"   "5558"   "55703"  "55811"  "55821"
[129] "5631"   "5634"   "56655"  "56953"  "56985"  "57804"  "58497"  "6240"
[137] "6241"   "64425"  "646625" "654364" "661"    "7498"   "8382"   "84172"
[145] "84265"  "84284"  "84618"  "8622"   "8654"   "87178"  "8833"   "9060"
[153] "9061"   "93034"  "953"    "9533"   "954"    "955"    "956"    "957"
[161] "9583"   "9615"
```

We need a named vector of 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
```

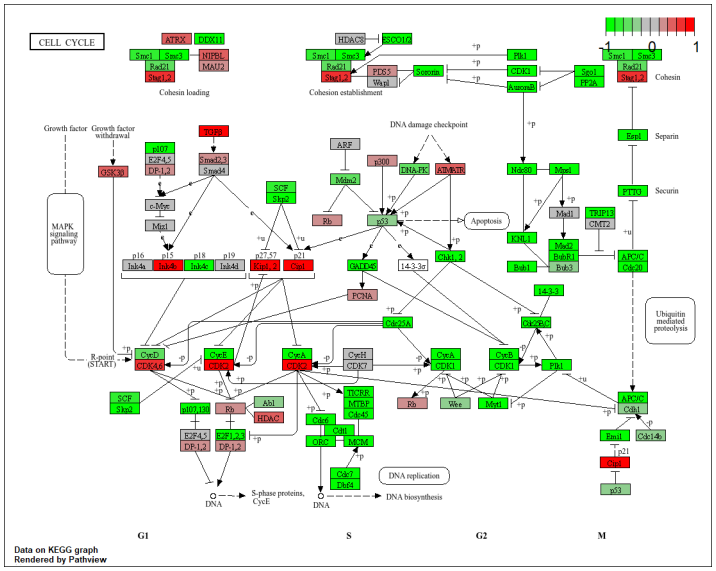
```
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

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

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

```
Info: Working in directory C:/Users/mazon/OneDrive/Documents/BIMM143 Data Sets/class14
```

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



GO terms

Same analysis but using GO terms 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, same.dir=TRUE)
```

```
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 functionbut lets look at the website first. < https://reactome.org/>

The website wants a text file with one gene 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"
```

and write out to a file

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

Save our results

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