Class14

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##Background

Here we work through a complete RNASeq analysis project. The input data comes from a

##Data Import

Reading the `counts` and `metadata` CSV files

```
library(DESeq2)
metafile <- read.csv("GSE37704_metadata.csv", row.names = 1)
countfile <- "GSE37704_featurecounts.csv"
```

```
# Import countdata
countData = read.csv(countfile, row.names=1)
```

```
countData <- as.matrix(countData[,-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

```
nonzero_counts <- countData[rowSums(countData)>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

Load the package

##DESeq analysis

```
library(DESeq2)
```

Setup DESeq object

```
dds <- DESeqDataSetFromMatrix(countData = nonzero_counts,
                              colData = metafile,
                              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)
```

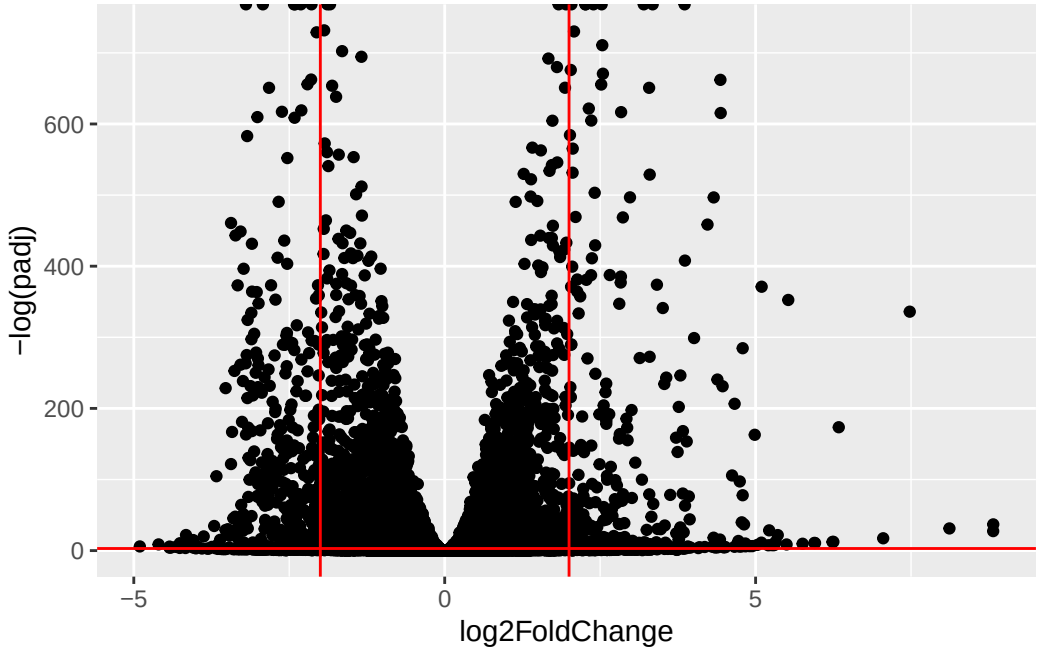
##Data Visualization

Volcano plot

```
library(ggplot2)

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

##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

```
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)
```

```
# Get the results
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

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

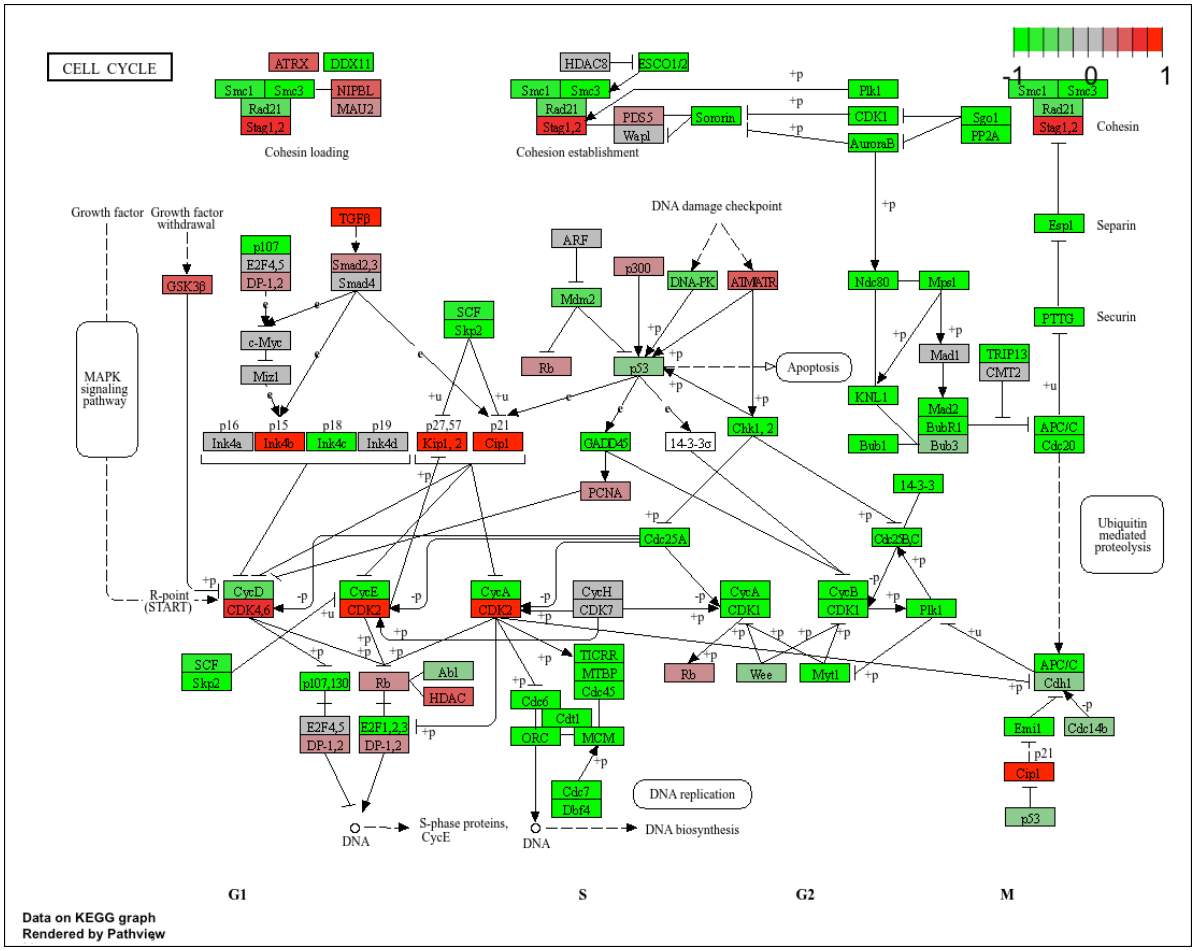
```
                                              p.geomean stat.mean
hsa04110 Cell cycle                         8.995727e-06 -4.378644
hsa03030 DNA replication                    9.424076e-05 -3.951803
hsa05130 Pathogenic Escherichia coli infection 1.405864e-04 -3.765330
hsa03013 RNA transport                      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/danielleweatherwax/Downloads/BIMM/Class15
```

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



GO terms

Same anaysis, but using GO genesets rather than KEGG

```r
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)

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 let's 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"
```

```
#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")
```