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.

```
head(colData)
```

```
             condition
SRR493366 control_sirna
SRR493367 control_sirna
SRR493368 control_sirna
SRR493369      hoxa1_kd
SRR493370      hoxa1_kd
SRR493371      hoxa1_kd
```

```
head(countData)
```

```
                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
```

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

> Q. Complete the code below to remove the troublesome first column from countData

refer to `cleancounts <- countData[, -1]` of the code

```
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

Q. Complete the code below to filter countData to exclude genes (i.e. rows) where we have 0 read count across all samples (i.e. columns).

Tip: What will rowSums() of countData return and how could you use it in this context?

```
filtered_counts <- countData[rowSums(countData) > 0, ]
head(filtered_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

DESeq Analysis

Let's begin by loading the package

```
library(DESeq2)
```

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

Now let's 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
```

Let's run DESeq

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

> Q. Call the summary() function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

Out of the 15,975 genes with nonzero total read count, 4,349 genes (approximately 27%) were up-regulated, and 4,396 genes (approximately 28%) were down-regulated at the default adjusted p-value cutoff of 0.1. The remaining genes either had low counts or were flagged as outliers, providing additional context from DESeq2.

And now we get the results

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

how many genes are up- or down-regulated at the default `alpha = 0.1`

```
summary(res)
```

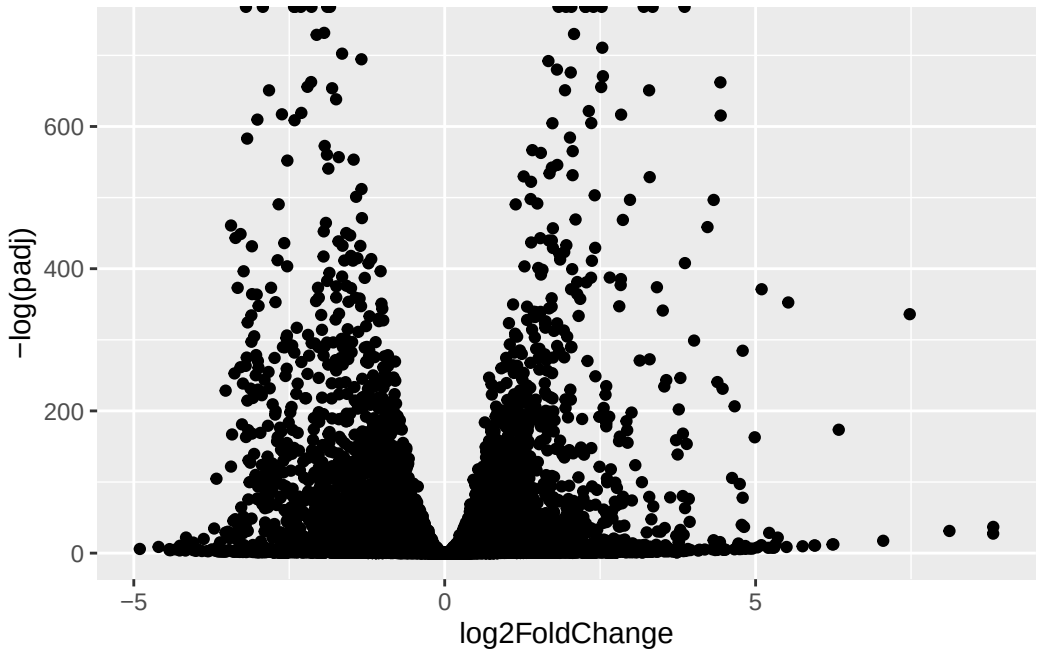
```
out of 15975 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 4349, 27%
LFC < 0 (down)     : 4396, 28%
outliers [1]       : 0, 0%
low counts [2]     : 1237, 7.7%
(mean count < 0)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results
```

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()`).
```

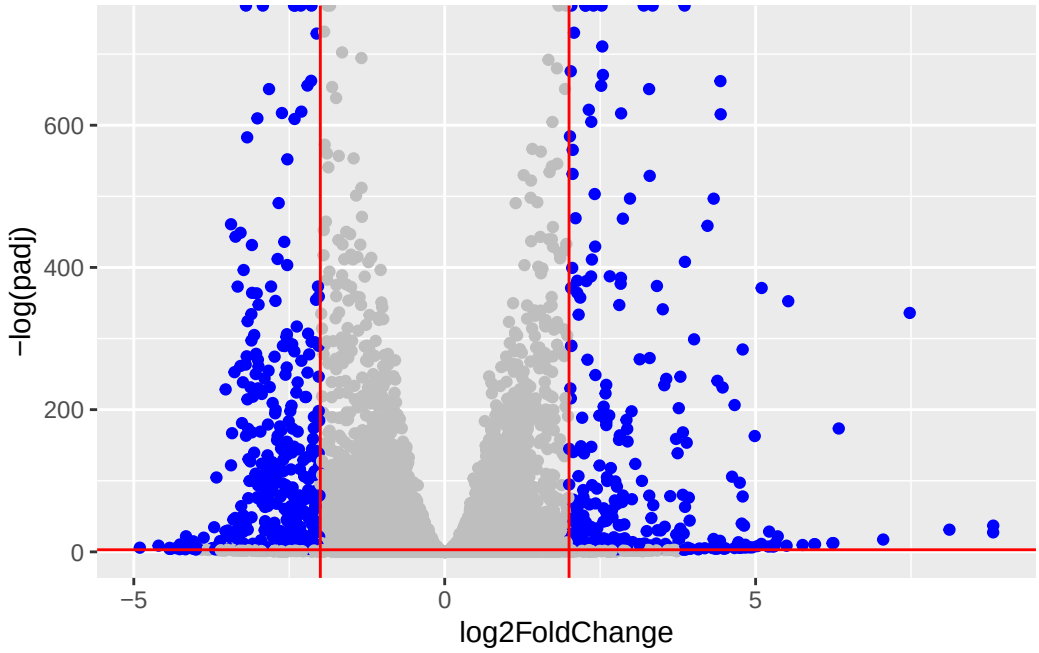


Add threshold lines for fold-change and P-valye and color our subset of genes that make these threshold cut-offs in the plot.

> Q. Improve this plot by completing the below code, which adds color and axis labels

```
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

Let's add gene symbols and entrez ids

let's install the package

```r
if (!requireNamespace("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install("AnnotationDbi")
```

```
Bioconductor version 3.21 (BiocManager 1.30.26), R 4.5.1 (2025-06-13 ucrt)

Warning: package(s) not installed when version(s) same as or greater than current; use
  `force = TRUE` to re-install: 'AnnotationDbi'

Installation paths not writeable, unable to update packages
  path: C:/Program Files/R/R-4.5.1/library
  packages:
    boot, Matrix, mgcv

Old packages: 'BiocManager', 'digest', 'downlit', 'emmeans', 'gert', 'ggplot2',
  'igraph', 'pkgdown', 'promises', 'purrr', 'rbibutils', 'readr', 'reshape2',
  'S7', 'sf', 'stringr', 'testthat', 'xfun', 'xml2'
```

```r
BiocManager::install("org.Hs.eg.db")
```

```
Bioconductor version 3.21 (BiocManager 1.30.26), R 4.5.1 (2025-06-13 ucrt)

Warning: package(s) not installed when version(s) same as or greater than current; use
  `force = TRUE` to re-install: 'org.Hs.eg.db'

Installation paths not writeable, unable to update packages
  path: C:/Program Files/R/R-4.5.1/library
  packages:
    boot, Matrix, mgcv
Old packages: 'BiocManager', 'digest', 'downlit', 'emmeans', 'gert', 'ggplot2',
  'igraph', 'pkgdown', 'promises', 'purrr', 'rbibutils', 'readr', 'reshape2',
  'S7', 'sf', 'stringr', 'testthat', 'xfun', 'xml2'
```

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

Q. Use the mapIDs() function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below.

```
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
```

```
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
```

```
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
```

```
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 ..
```

> Q. Finally for this section let's reorder these results by adjusted p-value and save them to a CSV file in your current project directory.

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

Install the packages for KEGG and GO first

```
if (!requireNamespace("BiocManager", quietly = TRUE))
    install.packages("BiocManager")

BiocManager::install(c("gage", "gageData", "pathview"))
```

```
Bioconductor version 3.21 (BiocManager 1.30.26), R 4.5.1 (2025-06-13 ucrt)

Warning: package(s) not installed when version(s) same as or greater than current; use
  `force = TRUE` to re-install: 'gage' 'gageData' 'pathview'

Installation paths not writeable, unable to update packages
  path: C:/Program Files/R/R-4.5.1/library
  packages:
    boot, Matrix, mgcv

Old packages: 'BiocManager', 'digest', 'downlit', 'emmeans', 'gert', 'ggplot2',
  'igraph', 'pkgdown', 'promises', 'purrr', 'rbibutils', 'readr', 'reshape2',
  'S7', 'sf', 'stringr', 'testthat', 'xfun', 'xml2'
```

```
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
```

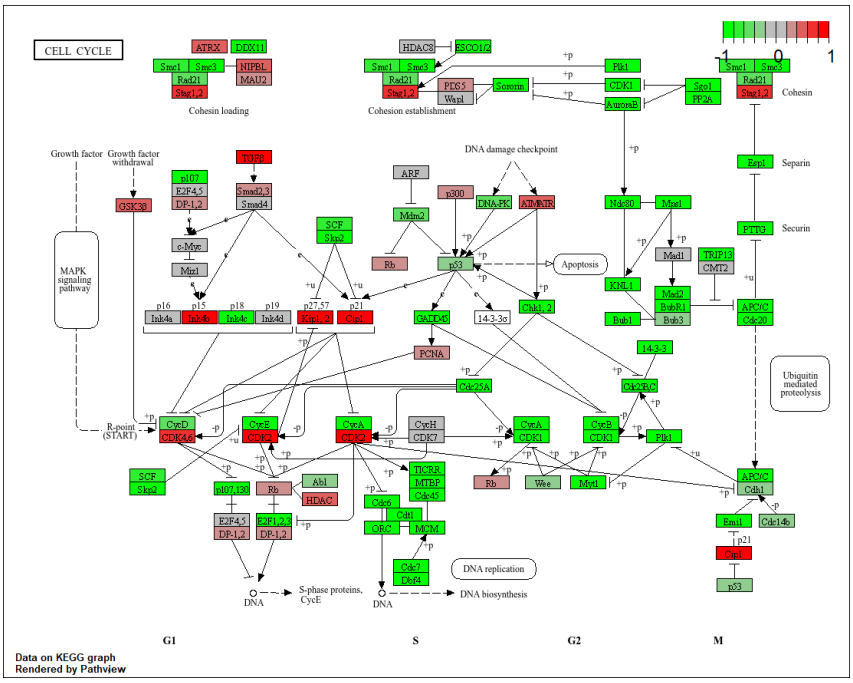
```
pathview(pathway.id = "hsa04110", gene.data = foldchanges)
```

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

```
Info: Working in directory C:/Users/Linda Kubera/Desktop/Bimm 143 Class/Class 14
```

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

```
library(png)
library(grid)

img <- readPNG("hsa04110.pathview.png")
grid::grid.raster(img)
```



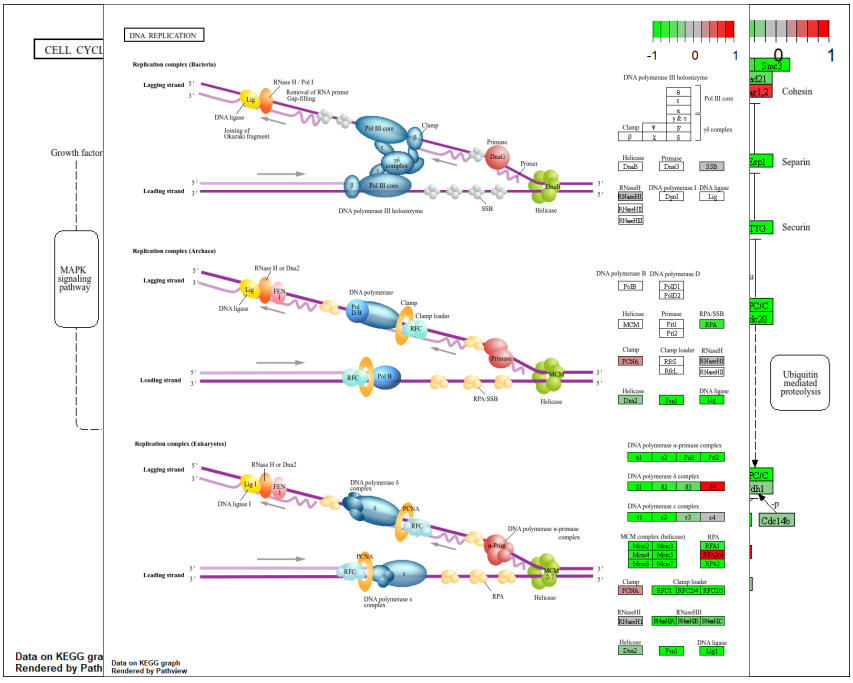

```
library(pathview)
library(png)
library(grid)

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

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

Info: Working in directory C:/Users/Linda Kubera/Desktop/Bimm 143 Class/Class 14

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

```
img1 <- readPNG("hsa04110.pathview.png")
grid::grid.raster(img1)

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

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

Info: Working in directory C:/Users/Linda Kubera/Desktop/Bimm 143 Class/Class 14

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

```
img2 <- readPNG("hsa03030.pathview.png")
grid::grid.raster(img2)
```



Q. Can you do the same procedure as above to plot the pathview figures for the top 5 down-reguled pathways?

The top 5 down-regulated pathways are: hsa00232 (Caffeine metabolism), hsa00983 (Drug metabolism – other enzymes), hsa01100 (Metabolic pathways), hsa00230 (Purine metabolism), and hsa05340 (Primary immunodeficiency). I used `pathview()` to plot these pathways, highlighting down-regulated genes in HOXA1 knockdown versus control.

```
fc <- res$log2FoldChange
names(fc) <- rownames(res)
```

```
keggResults <- gage(fc, gsets = kegg.sets.hs)
```

```
str(keggResults)
```

```
List of 3
 $ greater: num [1:229, 1:6] NA NA NA NA NA NA NA NA NA NA ...
  ..- attr(*, "dimnames")=List of 2
  .. ..$ : chr [1:229] "hsa00232 Caffeine metabolism" "hsa00983 Drug metabolism - other enzyr
  .. ..$ : chr [1:6] "p.geomean" "stat.mean" "p.val" "q.val" ...
 $ less   : num [1:229, 1:6] NA NA NA NA NA NA NA NA NA NA ...
  ..- attr(*, "dimnames")=List of 2
  .. ..$ : chr [1:229] "hsa00232 Caffeine metabolism" "hsa00983 Drug metabolism - other enzyr
  .. ..$ : chr [1:6] "p.geomean" "stat.mean" "p.val" "q.val" ...
 $ stats  : num [1:229, 1:2] NaN NaN NaN NaN NaN NaN NaN NaN NaN NaN ...
  ..- attr(*, "dimnames")=List of 2
  .. ..$ : chr [1:229] "hsa00232 Caffeine metabolism" "hsa00983 Drug metabolism - other enzyr
  .. ..$ : chr [1:2] "stat.mean" "exp1"
```

```
class(keggResults)
```

```
[1] "list"
```

```
str(keggResults)
```

```
List of 3
 $ greater: num [1:229, 1:6] NA NA NA NA NA NA NA NA NA NA ...
  ..- attr(*, "dimnames")=List of 2
  .. ..$ : chr [1:229] "hsa00232 Caffeine metabolism" "hsa00983 Drug metabolism - other enzyr
  .. ..$ : chr [1:6] "p.geomean" "stat.mean" "p.val" "q.val" ...
```

```
 $ less   : num [1:229, 1:6] NA NA NA NA NA NA NA NA NA NA ...
  ..- attr(*, "dimnames")=List of 2
  .. ..$ : chr [1:229] "hsa00232 Caffeine metabolism" "hsa00983 Drug metabolism - other enzy
  .. ..$ : chr [1:6] "p.geomean" "stat.mean" "p.val" "q.val" ...
 $ stats  : num [1:229, 1:2] NaN NaN NaN NaN NaN NaN NaN NaN NaN NaN ...
  ..- attr(*, "dimnames")=List of 2
  .. ..$ : chr [1:229] "hsa00232 Caffeine metabolism" "hsa00983 Drug metabolism - other enzy
  .. ..$ : chr [1:2] "stat.mean" "exp1"
```

```r
# Extract the 'less' (down-regulated) matrix
downMatrix <- keggResults$less

# Convert to a data frame to make it easier to work with
downDF <- as.data.frame(downMatrix)

# Order by p-value (ascending) to get most significant
downDF <- downDF[order(downDF$p.val), ]

# Get the top 5 pathway names
top5_down <- rownames(downDF)[1:5]
top5_down
```

```
[1] "hsa00232 Caffeine metabolism"
[2] "hsa00983 Drug metabolism - other enzymes"
[3] "hsa01100 Metabolic pathways"
[4] "hsa00230 Purine metabolism"
[5] "hsa05340 Primary immunodeficiency"
```

```r
# Get top 5 down-regulated pathways
top5_down <- rownames(keggres$less)[1:5]

# Extract 8-character KEGG IDs
top5_ids <- substr(top5_down, 1, 8)

# Generate Pathview plots
library(pathview)
pathview(gene.data = foldchanges, pathway.id = top5_ids, species = "hsa")
```

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

Info: Working in directory C:/Users/Linda Kubera/Desktop/Bimm 143 Class/Class 14
```

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

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

Info: Working in directory C:/Users/Linda Kubera/Desktop/Bimm 143 Class/Class 14

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory C:/Users/Linda Kubera/Desktop/Bimm 143 Class/Class 14

Info: Writing image file hsa05130.pathview.png

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

Info: Working in directory C:/Users/Linda Kubera/Desktop/Bimm 143 Class/Class 14

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory C:/Users/Linda Kubera/Desktop/Bimm 143 Class/Class 14

Info: Writing image file hsa03440.pathview.png
```

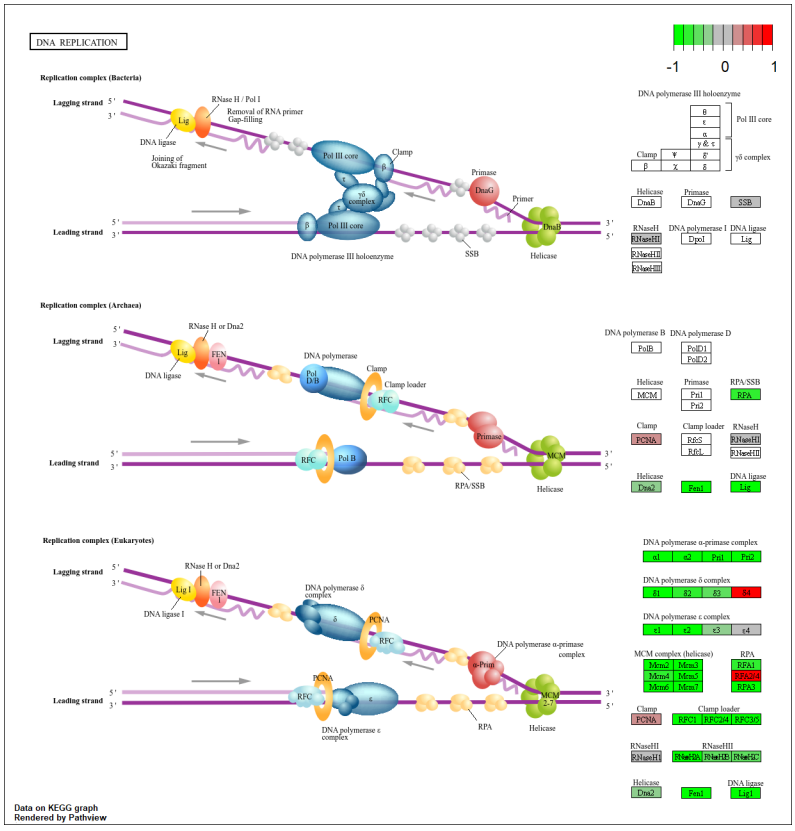
```
setwd("C:/Users/Linda Kubera/Desktop/Bimm 143 Class/Class 14")
```

```
list.files(pattern = "\\.png$")
```

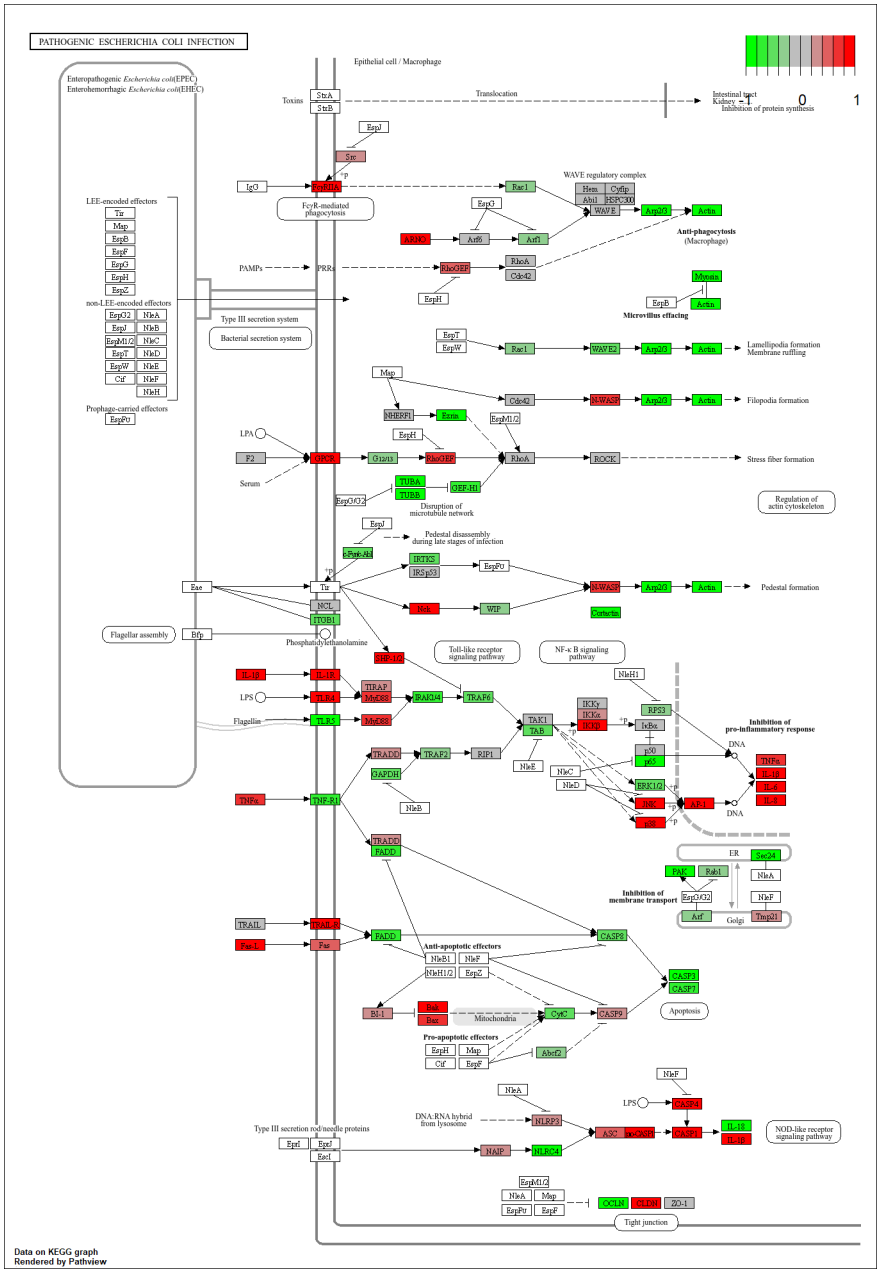
```
 [1] "hsa03013.pathview.png" "hsa03013.png"          "hsa03030.pathview.png"
 [4] "hsa03030.png"          "hsa03440.pathview.png" "hsa03440.png"
 [7] "hsa04110.pathview.png" "hsa04110.png"          "hsa05130.pathview.png"
[10] "hsa05130.png"
```

```
pathway_files <- c("hsa03030.pathview.png",
                   "hsa05130.pathview.png",
                   "hsa03013.pathview.png",
                   "hsa03440.pathview.png")
```

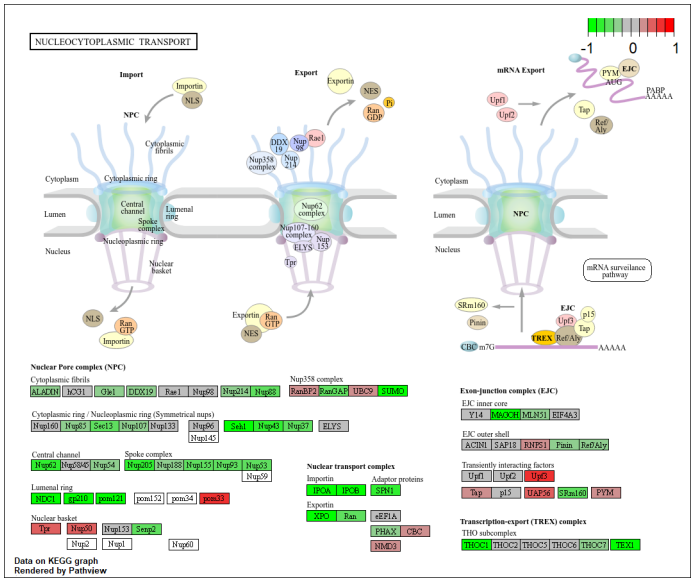
```
knitr::include_graphics("hsa03030.pathview.png")
```



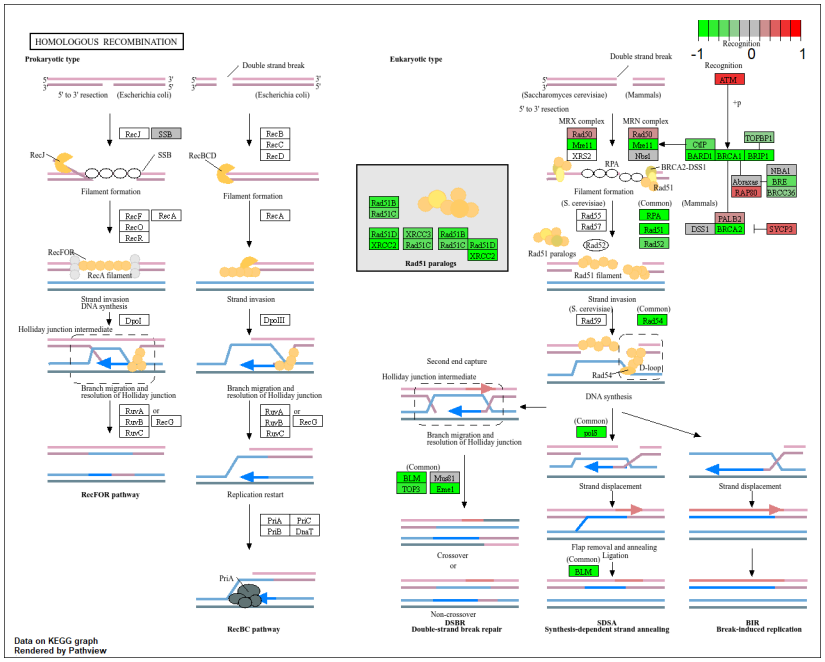

```
knitr::include_graphics("hsa05130.pathview.png")
```




```
knitr::include_graphics("hsa03013.pathview.png")
```




```
knitr::include_graphics("hsa03440.pathview.png")
```



GO terms

Same analysis but using GO genesets rather than KEGG.

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


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=
```

Section 5. GO online (OPTIONAL)

> Q: What pathway has the most significant "Entities p-value"? Do the most significant pathways listed match your previous KEGG results? What factors could cause differences between the two methods?

```
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                     1.375901e-03 -3.028500
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                     1.375901e-03 0.072234819
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 1.375901e-03
hsa03440 Homologous recombination                28 3.066756e-03
```

The pathway with the most significant "Entities p-value" is **hsa04110: Cell cycle** (p-value 8.99e-6).
This matches the top pathways observed in our previous KEGG analysis / differs from the previous KEGG results.

Possible reasons for differences include differences in statistical methods between GAGE and standard KEGG enrichment, variation in gene set coverage, multiple testing corrections, and differences in input data filtering or preprocessing.

Save Our Results

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