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

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

Check on data structure

```r
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 mismatch between metadata 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"
```

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

```
[1] TRUE TRUE TRUE TRUE TRUE TRUE
```

> Q1. Complete the code below to remove the troublesome first column from countData

```
# Note we need to remove the odd first $length col
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
```

##Remove zero count genes

There are lots of genes with zero counts

> Q2. 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).

```
countData = countData[rowSums(countData) > 0, ]
```

```
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
```

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

DESeq analysis

Load the package

```r
library(DESeq2)
```

Set up DESeq

```r
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

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

Get results

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

```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 15975 rows and 6 columns
                 baseMean log2FoldChange     lfcSE       stat      pvalue
                <numeric>      <numeric> <numeric>  <numeric>   <numeric>
ENSG00000279457   29.9136      0.1792571 0.3248215   0.551863 5.81042e-01
ENSG00000187634  183.2296      0.4264571 0.1402658   3.040350 2.36304e-03
ENSG00000188976 1651.1881     -0.6927205 0.0548465 -12.630156 1.43993e-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
...                   ...            ...       ...        ...         ...
ENSG00000273748  35.30265       0.674387  0.303666   2.220817 2.63633e-02
ENSG00000278817   2.42302      -0.388988  1.130394  -0.344118 7.30758e-01
ENSG00000278384   1.10180       0.332991  1.660261   0.200565 8.41039e-01
ENSG00000276345  73.64496      -0.356181  0.207716  -1.714752 8.63908e-02
ENSG00000271254 181.59590      -0.609667  0.141320  -4.314071 1.60276e-05
                       padj
                  <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76553e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
...                     ...
ENSG00000273748 4.79091e-02
ENSG00000278817 8.09772e-01
ENSG00000278384 8.92654e-01
ENSG00000276345 1.39761e-01
ENSG00000271254 4.53647e-05
```

> Q3. 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.

```r
summary(res)
```

```
out of 15975 with nonzero total read count
adjusted p-value < 0.1
```

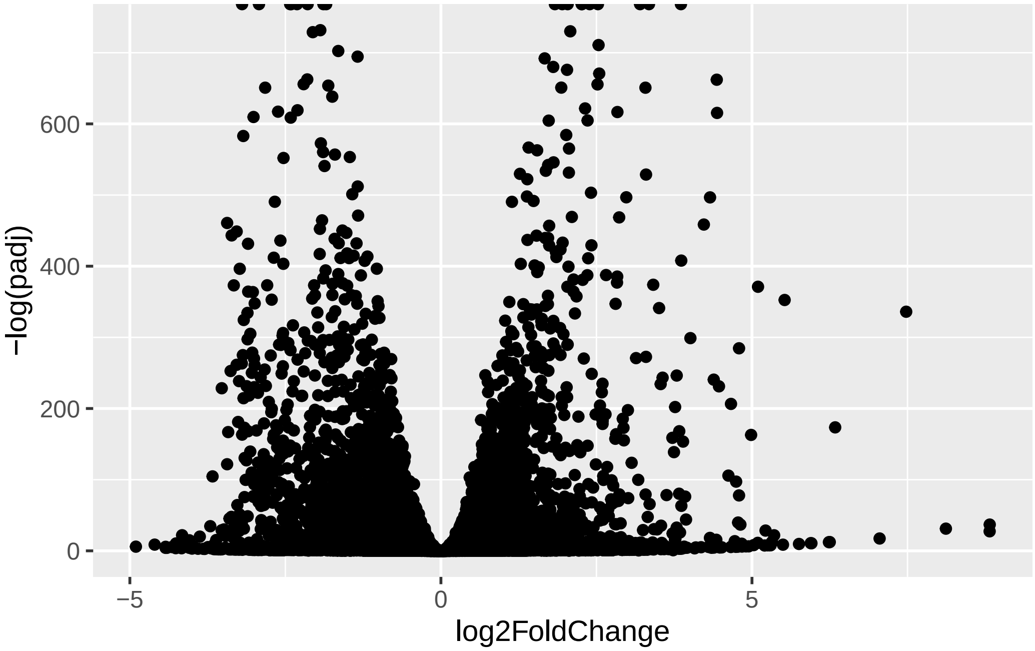
```
LFC > 0 (up)       : 4349, 27%
LFC < 0 (down)     : 4396, 28%
outliers [1]       : 0, 0%
low counts [2]     : 1237, 7.7%
(mean count < 0)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results
```

Data visualization

Volcano plot

```
library(ggplot2)

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

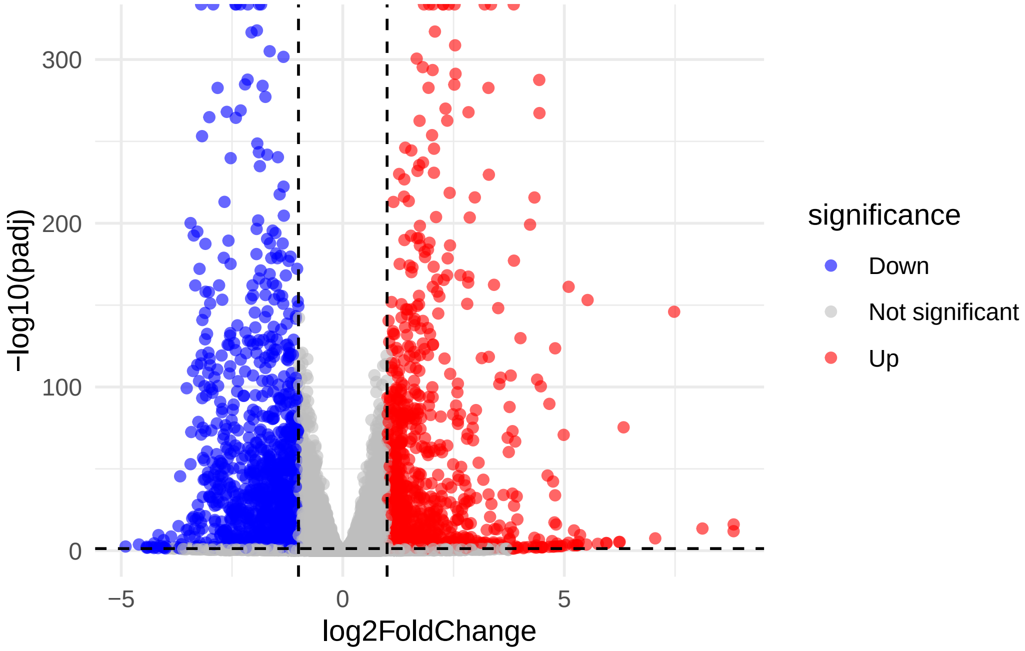
```
Warning: Removed 1237 rows containing missing values or values outside the scale range
(`geom_point()`).
```



Add threshhold lines for fold-change and p-Value and color our subset of genes

```
res$significance <- "Not significant"
res$significance[res$padj < 0.05 & res$log2FoldChange > 1]  <- "Up"
res$significance[res$padj < 0.05 & res$log2FoldChange < -1] <- "Down"

ggplot(res, aes(x = log2FoldChange, y = -log10(padj), color = significance)) +
  geom_point(alpha = 0.6) +
  geom_vline(xintercept = c(-1, 1), linetype = "dashed") +
  geom_hline(yintercept = -log10(0.05), linetype = "dashed") +
  scale_color_manual(values = c("blue", "grey", "red")) +
  theme_minimal()
```

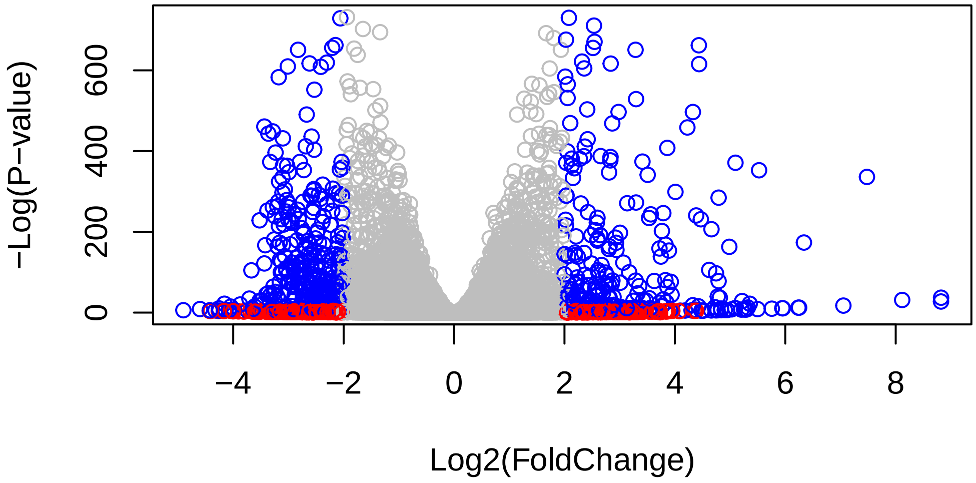
```
Warning: Removed 1237 rows containing missing values or values outside the scale range
(`geom_point()`).
```



Q4. Improve this plot by completing the below code, which adds color and axis labels

```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res))

# Color red the genes with absolute fold change above 2
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"
```

```
# Color blue those with adjusted p-value less than 0.01
# and absolute fold change more than 2
inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2)
mycols[ inds ] <- "blue"

plot(
  res$log2FoldChange,
  -log(res$padj),
  col = mycols,
  xlab = "Log2(FoldChange)",
  ylab = "-Log(P-value)"
)
```



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
```

> Q5. Use the mapIDs() function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below.

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

columns(org.Hs.eg.db)
```

```
 [1] "ACCNUM"       "ALIAS"        "ENSEMBL"      "ENSEMBLPROT"  "ENSEMBLTRANS"
 [6] "ENTREZID"     "ENZYME"       "EVIDENCE"     "EVIDENCEALL"  "GENENAME"
[11] "GENETYPE"     "GO"           "GOALL"        "IPI"          "MAP"
[16] "OMIM"         "ONTOLOGY"     "ONTOLOGYALL"  "PATH"         "PFAM"
[21] "PMID"         "PROSITE"      "REFSEQ"       "SYMBOL"       "UCSCKG"
[26] "UNIPROT"
```

```
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 10 columns
                  baseMean log2FoldChange     lfcSE       stat      pvalue
                 <numeric>      <numeric> <numeric>  <numeric>   <numeric>
ENSG00000279457   29.913579      0.1792571 0.3248215   0.551863 5.81042e-01
ENSG00000187634  183.229650      0.4264571 0.1402658   3.040350 2.36304e-03
ENSG00000188976 1651.188076     -0.6927205 0.0548465 -12.630156 1.43993e-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 1.51281e-25
ENSG00000187608  350.716868      0.2573837 0.1027266   2.505522 1.22271e-02
ENSG00000188157 9128.439422      0.3899088 0.0467164   8.346302 7.04333e-17
ENSG00000237330    0.158192      0.7859552 4.0804729   0.192614 8.47261e-01
                      padj    significance      symbol      entrez
                 <numeric>     <character> <character> <character>
ENSG00000279457 6.86555e-01 Not significant          NA          NA
ENSG00000187634 5.15718e-03 Not significant      SAMD11      148398
ENSG00000188976 1.76553e-35 Not significant       NOC2L       26155
ENSG00000187961 1.13413e-07 Not significant      KLHL17      339451
ENSG00000187583 9.19031e-01 Not significant     PLEKHN1       84069
```

```
ENSG00000187642 4.03379e-01 Not significant       PERM1       84808
ENSG00000188290 1.30538e-24              Up        HES4       57801
ENSG00000187608 2.37452e-02 Not significant       ISG15        9636
ENSG00000188157 4.21970e-16 Not significant        AGRN      375790
ENSG00000237330          NA Not significant      RNF223      401934
                                  name
                           <character>
ENSG00000279457                     NA
ENSG00000187634 sterile alpha motif ..
ENSG00000188976 NOC2 like nucleolar ..
ENSG00000187961 kelch like family me..
ENSG00000187583 pleckstrin homology ..
ENSG00000187642 PPARGC1 and ESRR ind..
ENSG00000188290 hes family bHLH tran..
ENSG00000187608 ISG15 ubiquitin like..
ENSG00000188157                  agrin
ENSG00000237330 ring finger protein ..
```

> Q6. 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.

```
res = res[order(res$padj), ]
write.csv(res, file = "deseq_results.csv")
```

Pathway Analysis

###Run gage analysis with KEGG

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

We need a name vector for the input of gage

```
foldchanges <- res$log2FoldChange
names(foldchanges) <- res$entrez # We need to use Entrez ID here for KEGG
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, 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
```

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

```
                                                   p.geomean stat.mean
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06  4.358967
hsa05323 Rheumatoid arthritis                   1.809824e-04  3.666793
hsa05146 Amoebiasis                             1.313400e-03  3.052596
hsa05332 Graft-versus-host disease              2.605234e-03  2.948229
hsa04640 Hematopoietic cell lineage             2.822776e-03  2.833362
                                                       p.val       q.val
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06 0.001917519
hsa05323 Rheumatoid arthritis                   1.809824e-04 0.019003147
hsa05146 Amoebiasis                             1.313400e-03 0.091937999
hsa05332 Graft-versus-host disease              2.605234e-03 0.118556573
hsa04640 Hematopoietic cell lineage             2.822776e-03 0.118556573
                                                set.size         exp1
hsa04060 Cytokine-cytokine receptor interaction      177 9.131044e-06
```

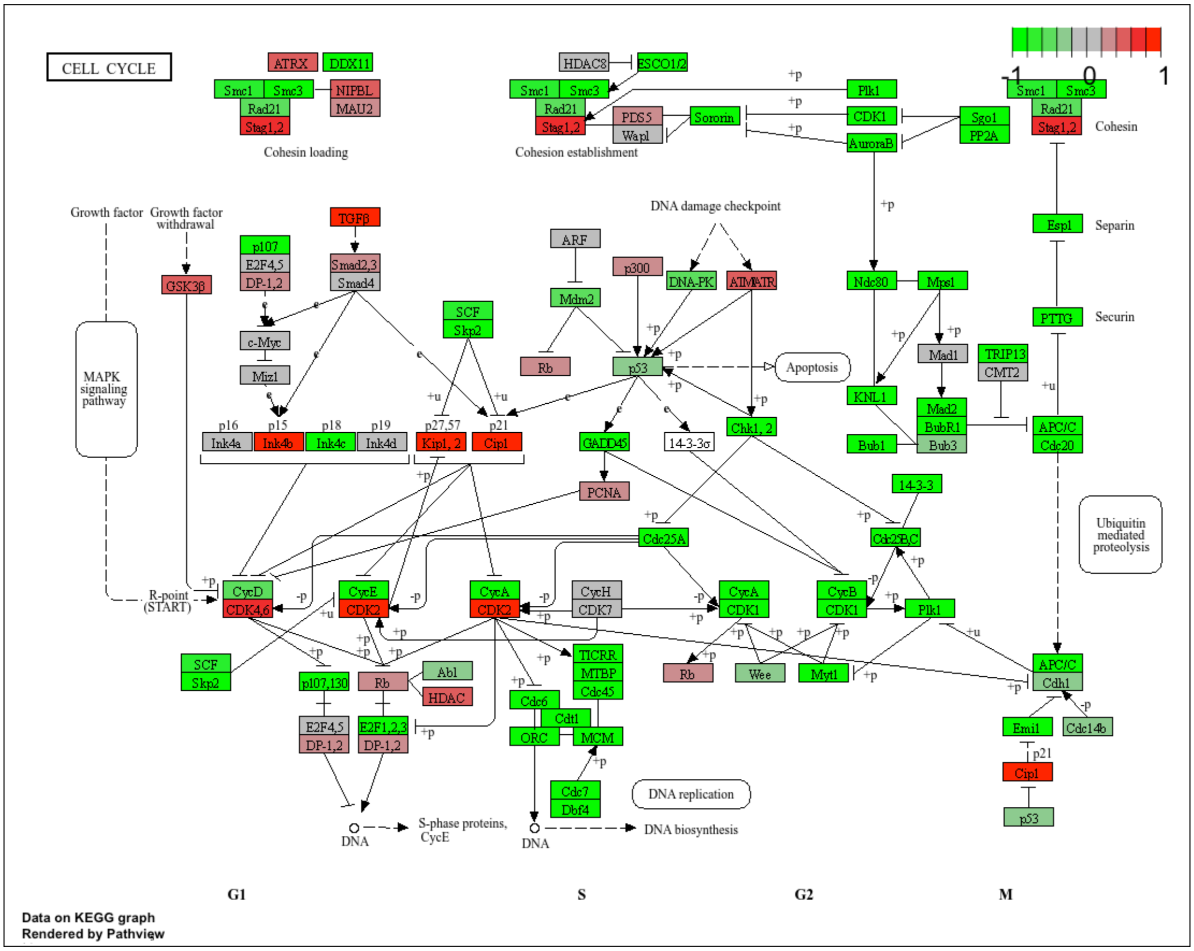
```
hsa05323 Rheumatoid arthritis                         72 1.809824e-04
hsa05146 Amoebiasis                                   94 1.313400e-03
hsa05332 Graft-versus-host disease                    22 2.605234e-03
hsa04640 Hematopoietic cell lineage                   55 2.822776e-03
```

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

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

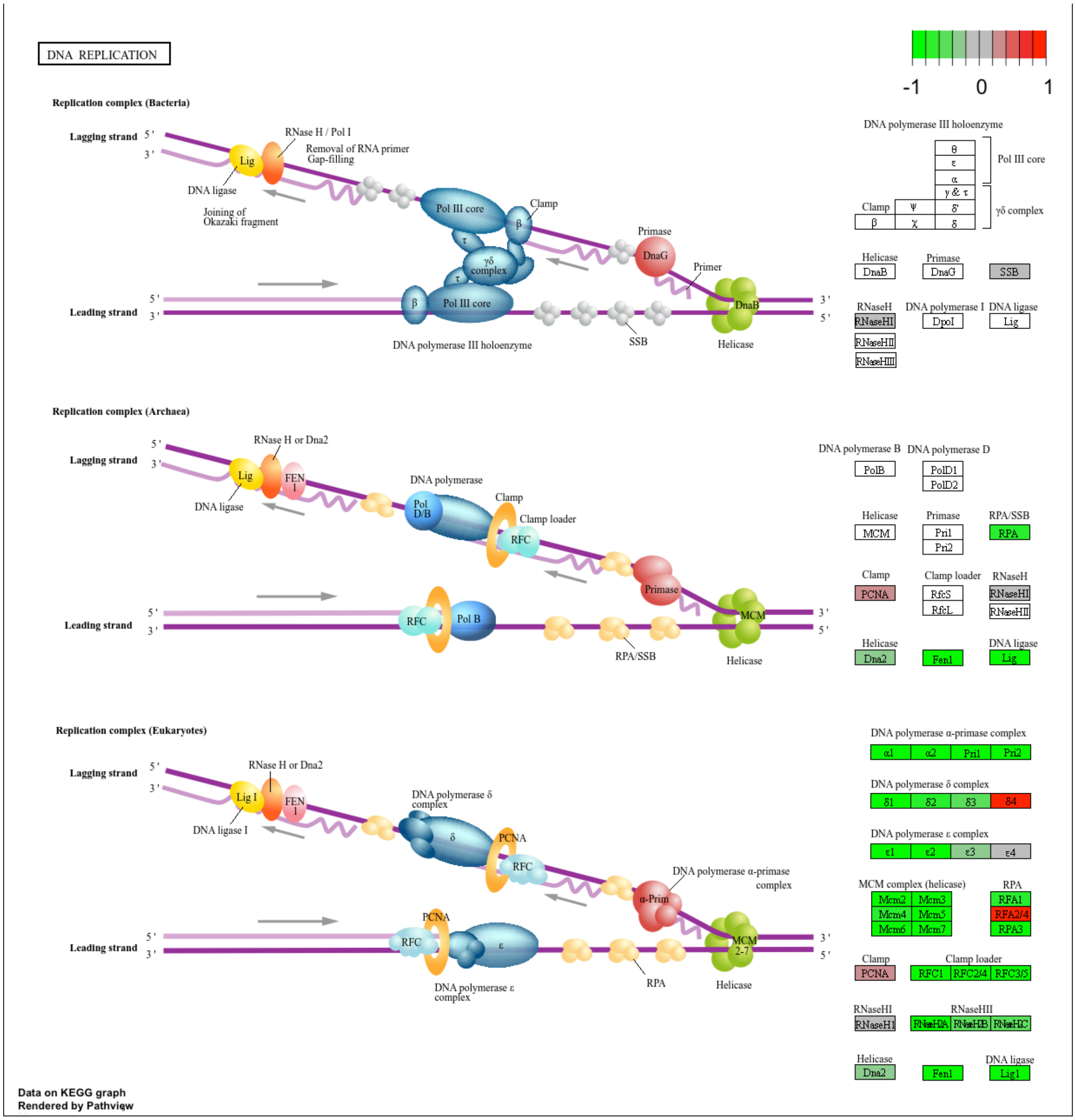
```
Info: Working in directory /Users/apple/Desktop/UCSD/BIMM 143/Class14
```

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



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

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

Info: Working in directory /Users/apple/Desktop/UCSD/BIMM 143/Class14

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



Q7. Can you do the same procedure as above to plot the pathview figures for the top 5 down-reguled pathways?

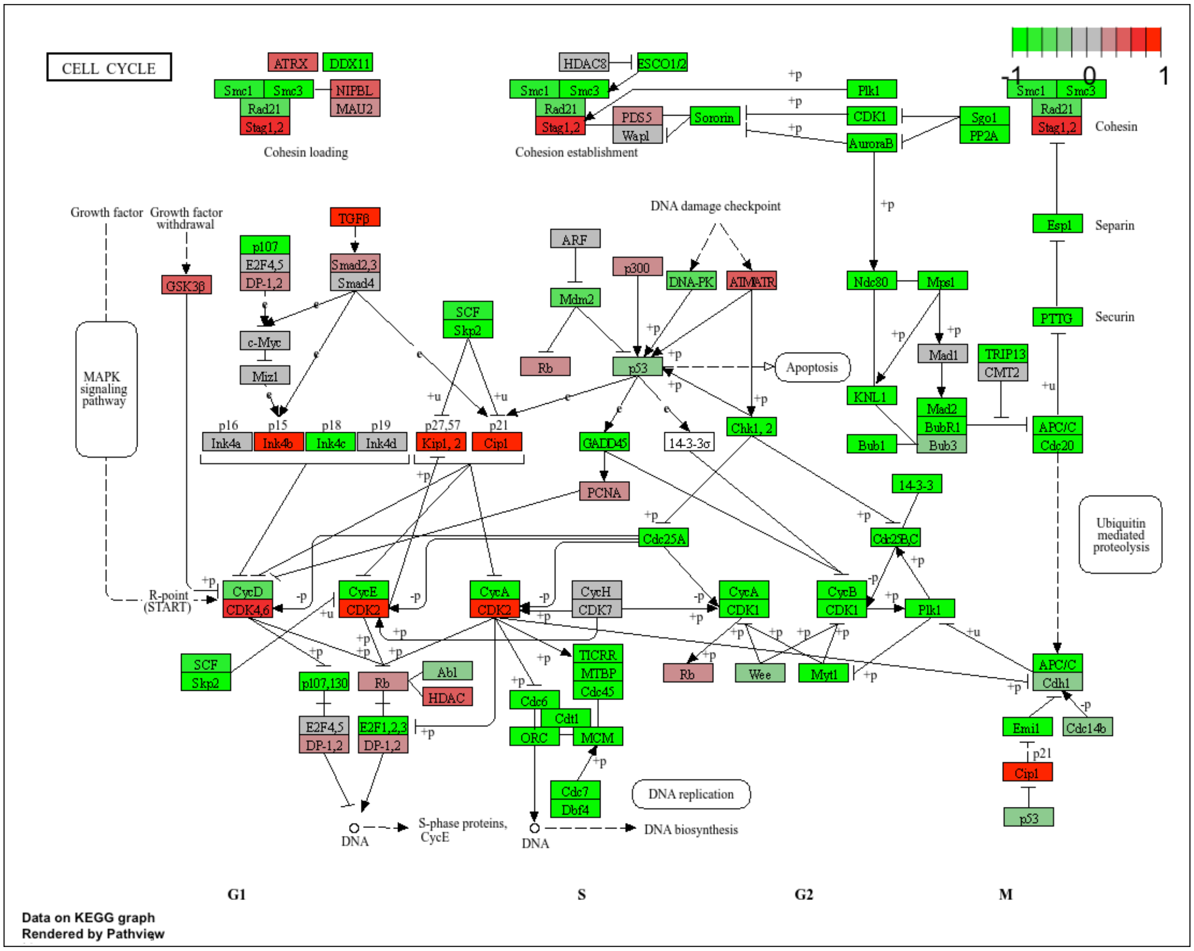
```r
## Focus on top 5 down-regulated pathways
keggrespathways_down <- rownames(keggres$less)[1:5]

## Extract the 8-character KEGG pathway IDs (e.g., "hsa04110")
keggresids_down <- substr(keggrespathways_down, start = 1, stop = 8)

keggresids_down
```

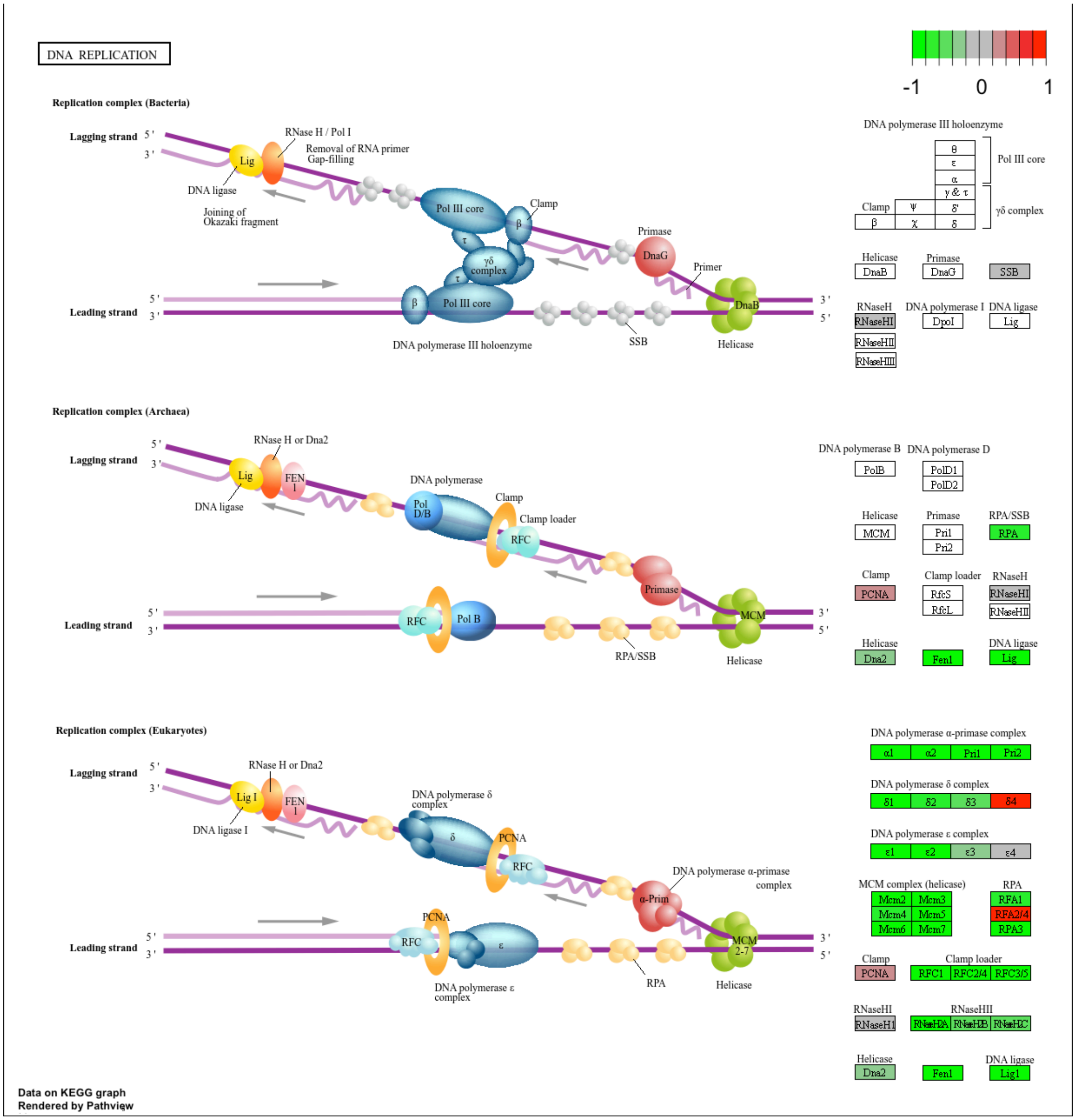
```
[1] "hsa04110" "hsa03030" "hsa05130" "hsa03013" "hsa03440"
```

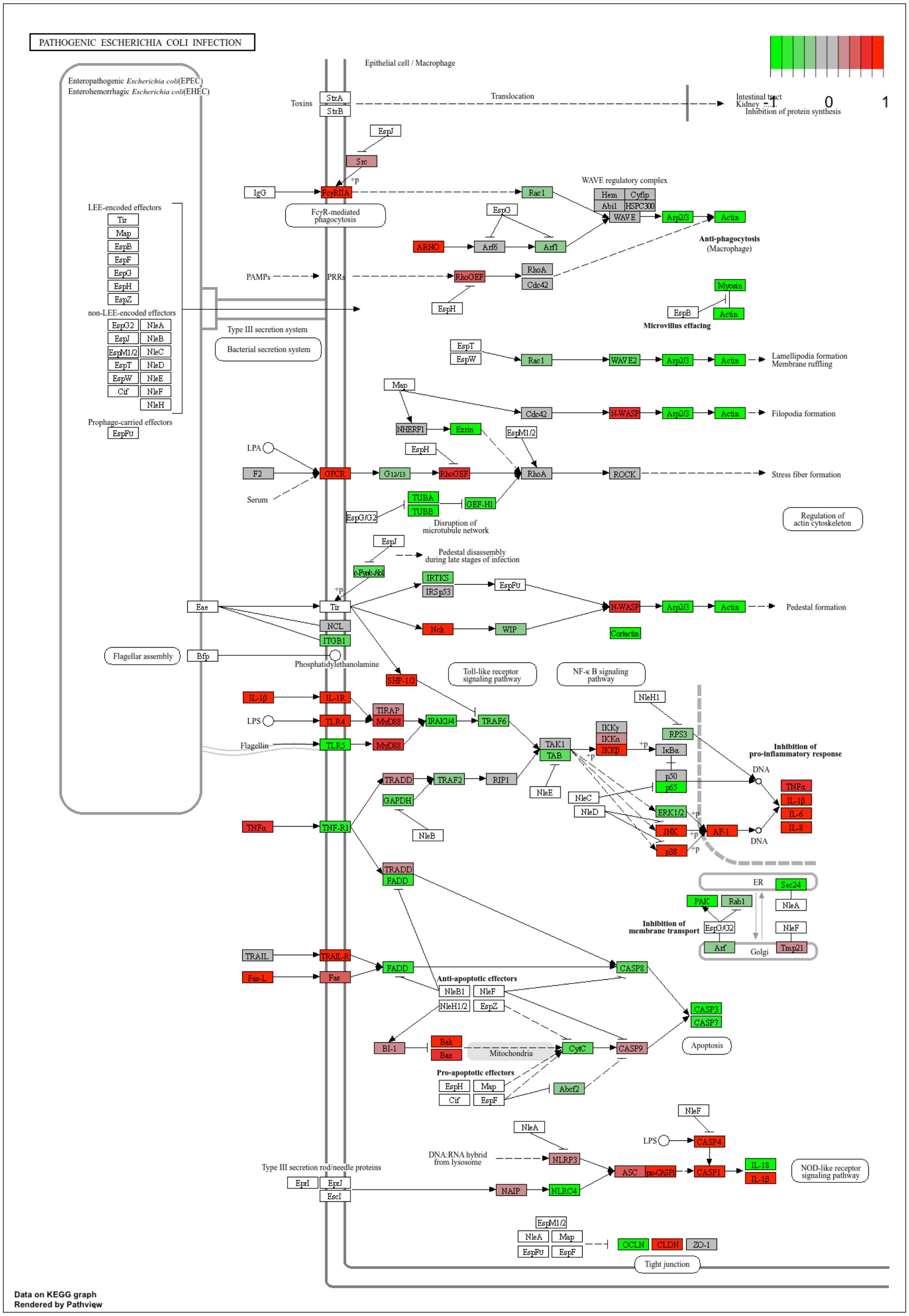
```r
pathview(
    gene.data = foldchanges,
    pathway.id = keggresids_down,
    species = "hsa"
)
```

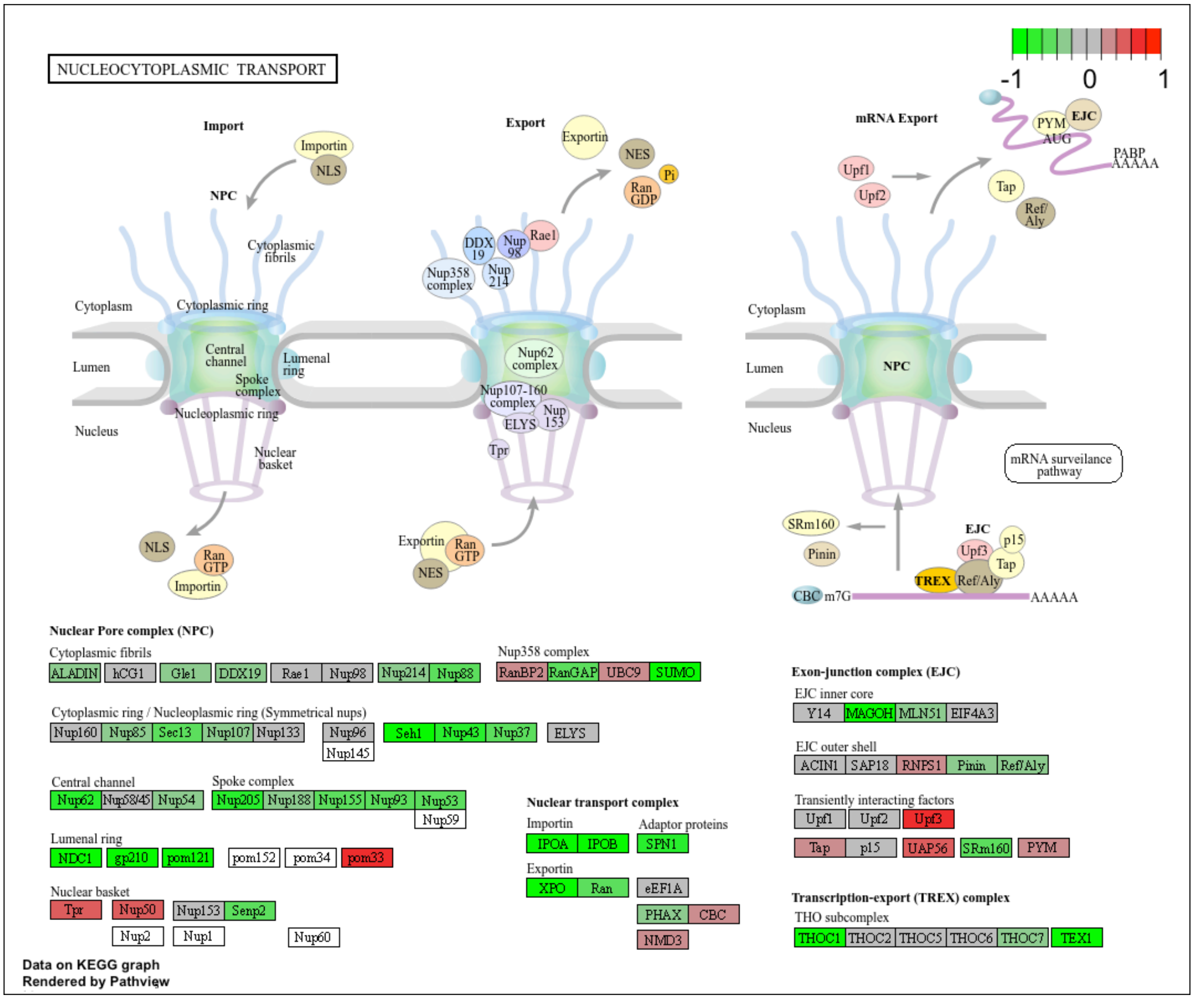
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/apple/Desktop/UCSD/BIMM 143/Class14

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory /Users/apple/Desktop/UCSD/BIMM 143/Class14

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/apple/Desktop/UCSD/BIMM 143/Class14

Info: Writing image file hsa05130.pathview.png

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

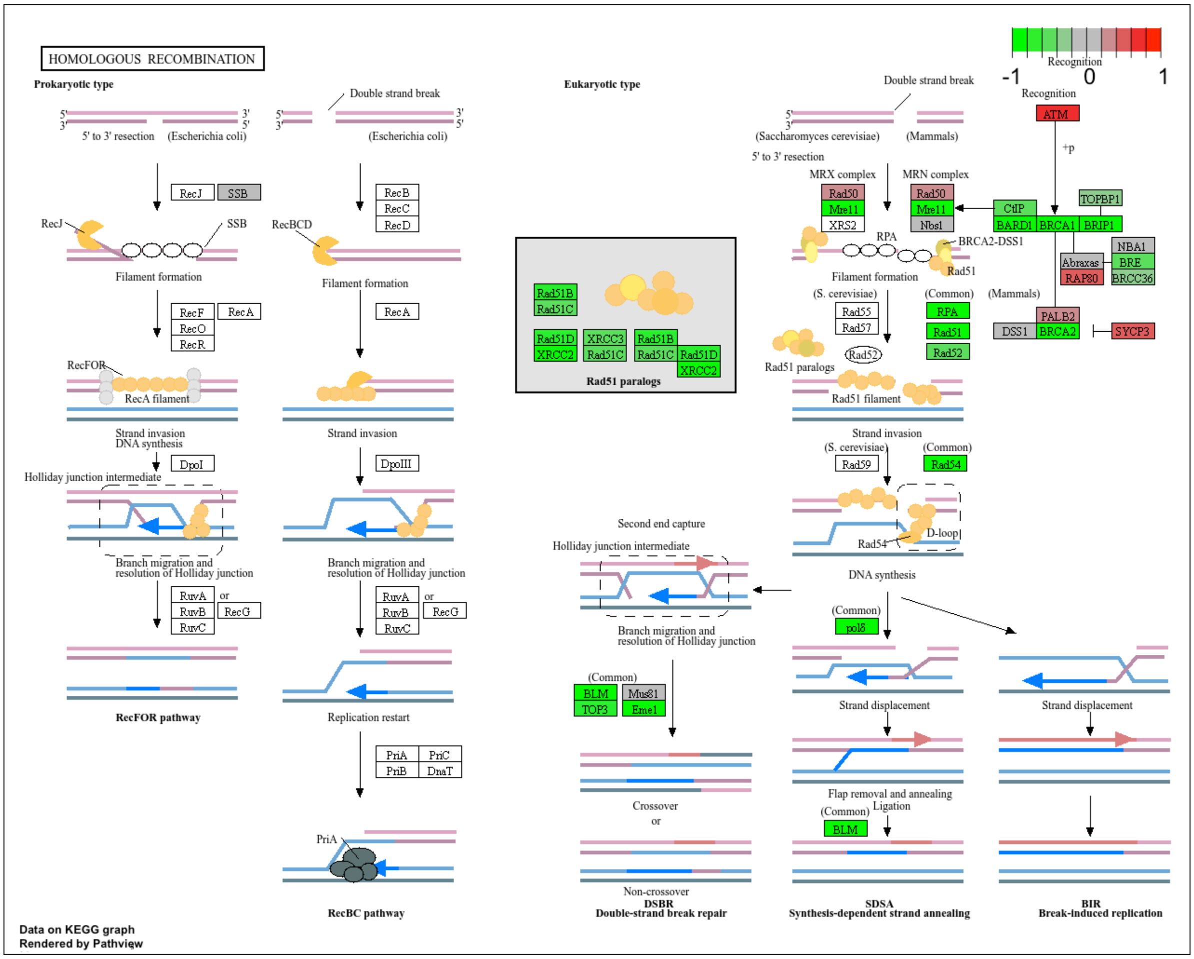
```
Info: Working in directory /Users/apple/Desktop/UCSD/BIMM 143/Class14

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory /Users/apple/Desktop/UCSD/BIMM 143/Class14

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




DNA REPLICATION
-1
0
1
Replication complex (Bacteria)
Lagging strand
5 '
3 '
Lig
RNase H / Pol I
Removal of RNA primer
Gap-filling
DNA ligase
Joining of
Okazaki fragment
Pol III core
Clamp
β
τ
γδ
complex
τ
Primase
DnaG
Primer
Leading strand
5 '
3 '
β
Pol III core
DnaB
3 '
5 '
DNA polymerase III holoenzyme
SSB
Helicase
DNA polymerase III holoenzyme
θ
ε
α
γ & τ
Clamp
ψ
δ'
β
χ
δ
Pol III core
γδ complex
Helicase
DnaB
Primase
DnaG
SSB
RNaseH
RNaseHI
RNaseHII
RNaseHIII
DNA polymerase I
DpoI
DNA ligase
Lig
Replication complex (Archaea)
Lagging strand
5 '
3 '
RNase H or Dna2
Lig
FEN
1
DNA ligase
DNA polymerase
Pol
D/B
Clamp
Clamp loader
RFC
Primase
Leading strand
5 '
3 '
RFC
Pol B
MCM
3 '
5 '
RPA/SSB
Helicase
DNA polymerase B
PolB
DNA polymerase D
PolD1
PolD2
Helicase
MCM
Primase
Pri1
Pri2
RPA/SSB
RPA
Clamp
PCNA
Clamp loader
RfcS
RfcL
RNaseH
RNaseHI
RNaseHII
Helicase
Dna2
Fen1
DNA ligase
Lig
Replication complex (Eukaryotes)
Lagging strand
5 '
3 '
RNase H or Dna2
Lig I
FEN
1
DNA ligase I
DNA polymerase δ
complex
δ
PCNA
RFC
DNA polymerase α-primase
complex
α-Prim
Leading strand
5 '
3 '
PCNA
RFC
ε
MCM
2-7
3 '
5 '
DNA polymerase ε
complex
RPA
Helicase
DNA polymerase α-primase complex
α1
α2
Pri1
Pri2
DNA polymerase δ complex
δ1
δ2
δ3
δ4
DNA polymerase ε complex
ε1
ε2
ε3
ε4
MCM complex (helicase)
Mcm2
Mcm3
Mcm4
Mcm5
Mcm6
Mcm7
RPA
RFA1
RFA2/4
RPA3
Clamp
PCNA
Clamp loader
RFC1
RFC2/4
RFC3/5
RNaseHI
RNaseH1
RNaseHII
RNaseH2A
RNaseH2B
RNaseH2C
Helicase
Dna2
Fen1
DNA ligase
Lig1
Data on KEGG graph
Rendered by Pathview


PATHOGENIC ESCHERICHIA COLI INFECTION
-1
0
1
Epithelial cell / Macrophage
Enteropathogenic Escherichia coli(EPEC)
Enterohemorrhagic Escherichia coli(EHEC)
Toxins
StxA
StxB
Translocation
Intestinal tract
Kidney
Inhibition of protein synthesis
EspJ
Src
+p
IgG
FcγRIIA
FcγR-mediated phagocytosis
Rac1
WAVE regulatory complex
Hem
Cyfip
Abi1
HSPC300
WAVE
Arp2/3
Actin
EspG
ARNO
Arf6
Arf1
Anti-phagocytosis (Macrophage)
LEE-encoded effectors
Tir
Map
EspB
EspF
EspG
EspH
EspZ
non-LEE-encoded effectors
EspG2
NleA
EspJ
NleB
EspM1/2
NleC
EspT
NleD
EspW
NleE
Cif
NleF
NleH
Prophage-carried effectors
EspFu
PAMPs
PRRs
RhoGEF
RhoA
Cdc42
EspH
Myosin
EspB
Actin
Microvillus effacing
Type III secretion system
Bacterial secretion system
EspT
EspW
Rac1
WAVE2
Arp2/3
Actin
Lamellipodia formation
Membrane ruffling
Map
Cdc42
N-WASP
Arp2/3
Actin
Filopodia formation
NHERF1
Ezrin
EspM1/2
EspH
LPA
F2
GPCR
G12/13
RhoGEF
RhoA
ROCK
Stress fiber formation
Serum
TUBA
TUBB
GEF-H1
EspG/G2
Disruption of microtubule network
Regulation of actin cytoskeleton
EspJ
Pedestal disassembly during late stages of infection
c-Fyn/c-Abl
+p
IRTKS
IRSp53
EspFu
N-WASP
Arp2/3
Actin
Pedestal formation
Eae
Tir
NCL
ITGB1
Nck
WIP
Cortactin
Flagellar assembly
Bfp
Phosphatidylethanolamine
SHP-1/2
Toll-like receptor signaling pathway
NF-κ B signaling pathway
NleH1
IL-1β
IL-1R
TIRAP
MyD88
IRAK1/4
TRAF6
LPS
TLR4
IKKγ
IKKα
IKKβ
RPS3
Flagellin
TLR5
MyD88
TAK1
TAB
+p
IκBα
Inhibition of pro-inflammatory response
DNA
TRADD
TRAF2
RIP1
p50
p65
TNFα
IL-1β
IL-6
IL-8
GAPDH
NleE
NleC
NleD
ERK1/2
JNK
AP-1
p38
+p
DNA
TNFα
TNF-R1
NleB
TRADD
FADD
ER
Sec24
PAK
Rab1
NleA
Inhibition of membrane transport
EspG/G2
NleF
Arf
Golgi
Tmp21
TRAIL
TRAIL-R
Fas-L
Fas
FADD
CASP8
Anti-apoptotic effectors
NleB1
NleF
NleH1/2
EspZ
CASP3
CASP7
BI-1
Bak
Bax
Mitochondria
CytC
CASP9
Apoptosis
Pro-apoptotic effectors
EspH
Map
Cif
EspF
Abcf2
NleF
NleA
LPS
CASP4
DNA:RNA hybrid from lysosome
NLRP3
ASC
pro-CASP1
CASP1
IL-18
IL-1β
NOD-like receptor signaling pathway
Type III secretion rod/needle proteins
EprI
EprJ
EscI
NAIP
NLRC4
EspM1/2
NleA
Map
EspFu
EspF
OCLN
CLDN
ZO-1
Tight junction
Data on KEGG graph
Rendered by Pathview


NUCLEOCYTOPLASMIC TRANSPORT
-1
0
1
Import
Importin
NLS
NPC
Cytoplasmic fibrils
Cytoplasm
Cytoplasmic ring
Central channel
Lumenal ring
Lumen
Spoke complex
Nucleoplasmic ring
Nucleus
Nuclear basket
NLS
Ran GTP
Importin
Export
Exportin
NES
Pi
Ran GDP
DDX 19
Nup 98
Rae1
Nup358 complex
Nup 214
Nup62 complex
Nup107-160 complex
ELYS
Nup 153
Tpr
Exportin
Ran GTP
NES
mRNA Export
PYM
EJC
AUG
PABP
AAAAA
Upf1
Upf2
Tap
Ref/ Aly
Cytoplasm
Lumen
NPC
Nucleus
mRNA surveilance pathway
SRm160
Pinin
EJC
p15
Upf3
Tap
TREX
Ref/Aly
CBC
m7G
AAAAA
Nuclear Pore complex (NPC)
Cytoplasmic fibrils
ALADIN
hCG1
Gle1
DDX19
Rae1
Nup98
Nup214
Nup88
Nup358 complex
RanBP2
RanGAP
UBC9
SUMO
Cytoplasmic ring / Nucleoplasmic ring (Symmetrical nups)
Nup160
Nup85
Sec13
Nup107
Nup133
Nup96
Nup145
Seh1
Nup43
Nup37
ELYS
Central channel
Nup62
Nup58/45
Nup54
Spoke complex
Nup205
Nup188
Nup155
Nup93
Nup53
Nup59
Lumenal ring
NDC1
gp210
pom121
pom152
pom34
pom33
Nuclear basket
Tpr
Nup50
Nup153
Senp2
Nup2
Nup1
Nup60
Nuclear transport complex
Importin
IPOA
IPOB
Adaptor proteins
SPN1
Exportin
XPO
Ran
eEF1A
PHAX
CBC
NMD3
Exon-junction complex (EJC)
EJC inner core
Y14
MAGOH
MLN51
EIF4A3
EJC outer shell
ACIN1
SAP18
RNPS1
Pinin
Ref/Aly
Transiently interacting factors
Upf1
Upf2
Upf3
Tap
p15
UAP56
SRm160
PYM
Transcription-export (TREX) complex
THO subcomplex
THOC1
THOC2
THOC5
THOC6
THOC7
TEX1
Data on KEGG graph
Rendered by Pathview



GO terms

Same analysis but using GO geneset 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)

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

We can analyze the reactome using web interfaces or R functions.

The website is https://reactome.org/. It requires a text format withe 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)
```

```
ENSG00000117519 ENSG00000183508 ENSG00000159176 ENSG00000116016 ENSG00000164251
          "CNN3"        "TENT5C"         "CSRP1"         "EPAS1"         "F2RL1"
ENSG00000124766
          "SOX4"
```

Write it our to a file

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

> Q8. 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?

The pathway with the most significant “entities p-value” is “Cell cycle”. Both KEGG and Reactome identify Cell Cycle and DNA replication / mitotic processes as the most significantly enriched. Therefore, the results of KEGG and Reactome largely agree with each other, but there are still certain differences. This can be due to different pathway definitions, curation methods, gene coverage, statistics and classifications between KEGG and Reactome.

Save our results

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