class 13 - functional annotation

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Read in countData and colData

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

Do the row names of meta match the columns of countData?

```
all(
    rownames(colData) == colnames(countData)
    )
```

```
Warning in rownames(colData) == colnames(countData): longer object length is
not a multiple of shorter object length
```

```
[1] FALSE
```

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

The numrows and numcols are different between meta and countData. This is because countData's first column is not a sample name, but instead referring to the length of the transcript. Let's remove it.

```
countData <- countData[-1]
all(
    rownames(colData) == colnames(countData)
    )
```

```
[1] TRUE
```

```
library(dplyr)
library(ggplot2)
```

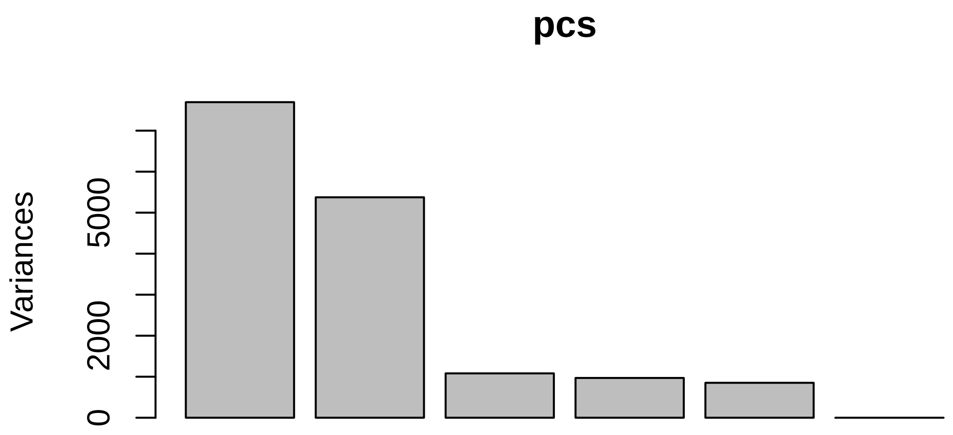
Q. Complete the code below to filter countData to exclude genes (i.e. rows) where we have 0 read count across all samples (i.e. columns).

```
clean.counts <- countData %>% filter_all(
  any_vars(
    . != 0
  )
)

nrow(clean.counts)
```

```
[1] 15975
```

PCA as quality control

```
pcs <- prcomp(t(clean.counts), scale=T)
plot(pcs)
```



```
summary(pcs)
```

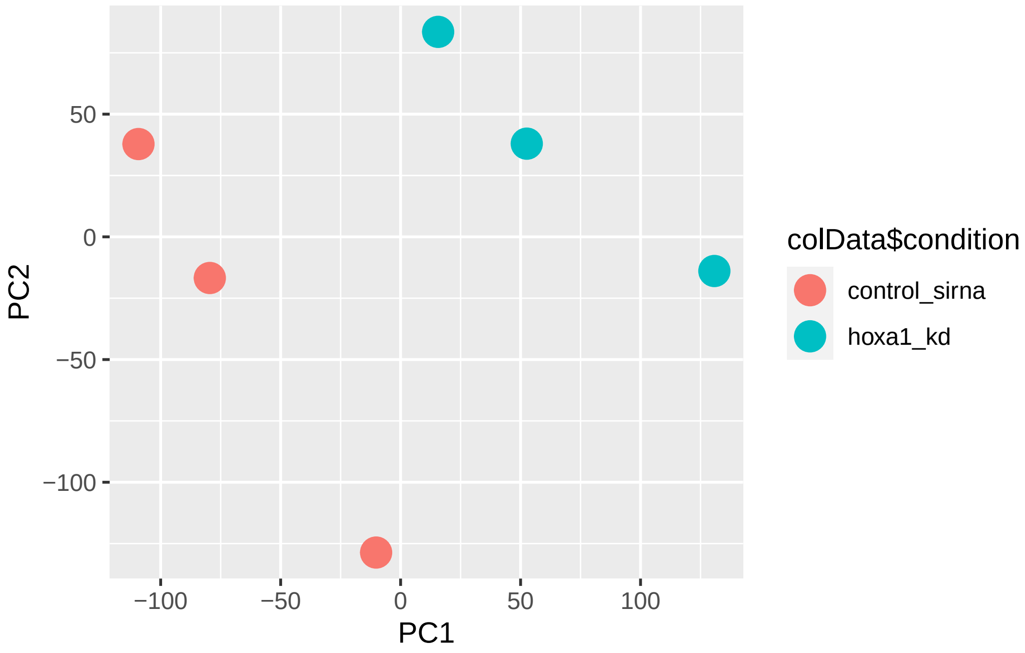
```
Importance of components:
                           PC1     PC2      PC3      PC4      PC5       PC6
Standard deviation     87.7211 73.3196 32.89604 31.15094 29.18417 6.648e-13
Proportion of Variance  0.4817  0.3365  0.06774  0.06074  0.05332 0.000e+00
Cumulative Proportion   0.4817  0.8182  0.88594  0.94668  1.00000 1.000e+00
```

> How much variance is captured by the first two PCs?

About `81.8%` variance captured in the first two components. pretty good.

Let's plot samples in PCA space

```
ggplot(as.data.frame(pcs$x)) +
  aes(x=PC1, y=PC2, col=colData$condition) +
  geom_point(size = 5)
```



DESeq analysis

```
library(DESeq2)
```

```
dds <- DESeqDataSetFromMatrix(countData = clean.counts,
                              colData = colData,
                              design = ~condition)
```

```
Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
design formula are characters, converting to factors
```

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

```
res <- as.data.frame(results(dds))

head(res)
```

```
                  baseMean log2FoldChange      lfcSE        stat       pvalue
ENSG00000279457   29.91358     0.17925708 0.32482157   0.5518632 5.810421e-01
ENSG00000187634  183.22965     0.42645712 0.14026582   3.0403495 2.363037e-03
ENSG00000188976 1651.18808    -0.69272046 0.05484654 -12.6301576 1.439895e-36
ENSG00000187961  209.63794     0.72975561 0.13185990   5.5343255 3.124282e-08
ENSG00000187583   47.25512     0.04057653 0.27189281   0.1492372 8.813664e-01
ENSG00000187642   11.97975     0.54281049 0.52155985   1.0407444 2.979942e-01
```

```
                        padj
ENSG00000279457 6.865548e-01
ENSG00000187634 5.157181e-03
ENSG00000188976 1.765489e-35
ENSG00000187961 1.134130e-07
ENSG00000187583 9.190306e-01
ENSG00000187642 4.033793e-01
```

> 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.

```
DESeq2::summary(res)
```

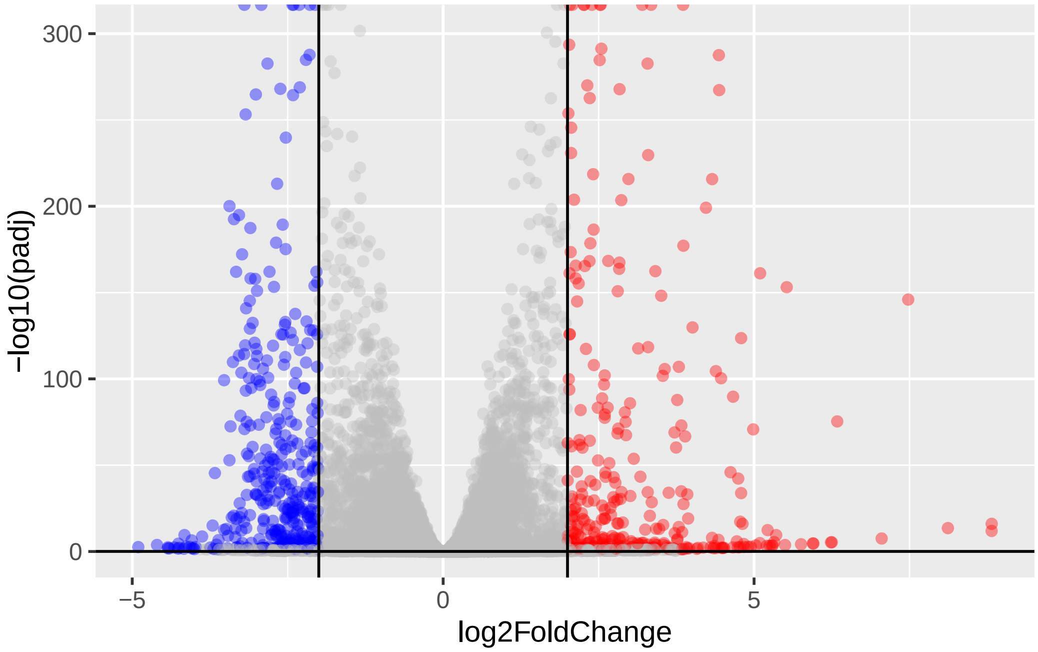
```
    baseMean        log2FoldChange          lfcSE              stat          
 Min.   :     0.1   Min.   :-4.902884   Min.   :0.03163   Min.   :-52.97126  
 1st Qu.:    12.1   1st Qu.:-0.459361   1st Qu.:0.07507   1st Qu.: -2.34434  
 Median :   214.8   Median : 0.008707   Median :0.13108   Median :  0.05028  
 Mean   :  1002.1   Mean   : 0.015164   Mean   :0.60432   Mean   : -0.08060  
 3rd Qu.:   774.3   3rd Qu.: 0.508047   3rd Qu.:0.53867   3rd Qu.:  2.22749  
 Max.   :399481.5   Max.   : 8.822085   Max.   :4.08047   Max.   : 48.42078  
                                                                             
     pvalue             padj       
 Min.   :0.00000   Min.   :0.0000  
 1st Qu.:0.00000   1st Qu.:0.0000  
 Median :0.02204   Median :0.0163  
 Mean   :0.24012   Mean   :0.2300  
 3rd Qu.:0.47941   3rd Qu.:0.4411  
 Max.   :0.99997   Max.   :1.0000  
                   NA's   :1237    
```

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

Summary volcano plot

```
my.colors <- rep("gray", nrow(res))
my.colors[ res$log2FoldChange > 2 & res$padj < 0.05 ] <- "red"
my.colors[ res$log2FoldChange < -2 & res$padj < 0.05 ] <- "blue"

ggplot(as.data.frame(res)) +
  aes(x=log2FoldChange, y=-log10(padj)) +
  geom_point(color = my.colors, alpha = 0.4) +
  geom_hline(yintercept = 0.05) +
  geom_vline(xintercept = c(-2, 2)) +
  ylim(0, NA) +
  labs(ylab = "-log10(Adjusted P-value)",
       xlab = "log2(Fold Change)"
       )
```

```
Warning: Removed 1237 rows containing missing values (`geom_point()`).
```



Add annotations

```
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"
```

> 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)
```

```
                 baseMean log2FoldChange      lfcSE       stat       pvalue
ENSG00000279457  29.91358     0.17925708 0.32482157  0.5518632 5.810421e-01
ENSG00000187634 183.22965     0.42645712 0.14026582  3.0403495 2.363037e-03
```

```
ENSG00000188976 1651.18808    -0.69272046 0.05484654 -12.6301576 1.439895e-36
ENSG00000187961  209.63794     0.72975561 0.13185990   5.5343255 3.124282e-08
ENSG00000187583   47.25512     0.04057653 0.27189281   0.1492372 8.813664e-01
ENSG00000187642   11.97975     0.54281049 0.52155985   1.0407444 2.979942e-01
                        padj  symbol entrez
ENSG00000279457 6.865548e-01    <NA>   <NA>
ENSG00000187634 5.157181e-03  SAMD11 148398
ENSG00000188976 1.765489e-35   NOC2L  26155
ENSG00000187961 1.134130e-07  KLHL17 339451
ENSG00000187583 9.190306e-01 PLEKHN1  84069
ENSG00000187642 4.033793e-01   PERM1  84808
                                                                    name
ENSG00000279457                                                     <NA>
ENSG00000187634                 sterile alpha motif domain containing 11
ENSG00000188976 NOC2 like nucleolar associated transcriptional repressor
ENSG00000187961                              kelch like family member 17
ENSG00000187583                 pleckstrin homology domain containing N1
ENSG00000187642             PPARGC1 and ESRR induced regulator, muscle 1
```

> 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.

```r
res <- res[order(res$pvalue),]
write.csv(res, file = "deseq_results.csv")
```

KEGG, GO

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

I need to create the input for `gage()` - a vector of fold-change values with entrez IDs as the `names()`

```r
fc <- res$log2FoldChange
names(fc) <- res$entrez
```

```r
data(kegg.sets.hs)

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

```r
head(kegg.res$less)
```

```
                                                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
hsa04114 Oocyte meiosis                      3.784520e-03 -2.698128
                                                    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
hsa04114 Oocyte meiosis                      3.784520e-03 0.132458191
                                             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
hsa04114 Oocyte meiosis                           102 3.784520e-03
```

```r
pathview(fc, pathway.id = "hsa04110")
```

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

```
Info: Working in directory /Users/jack/Dropbox/213/class13
```

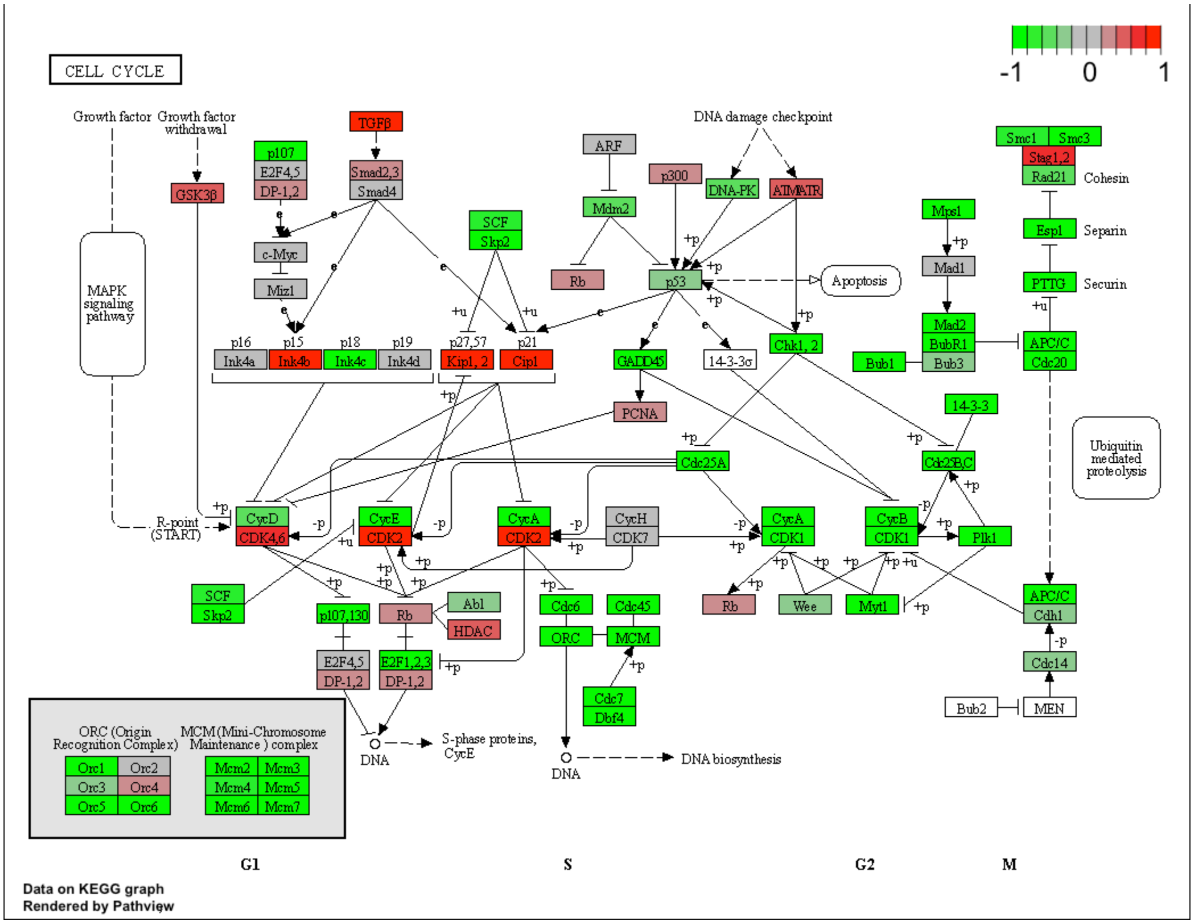
```
Info: Writing image file hsa04110.pathview.png
```

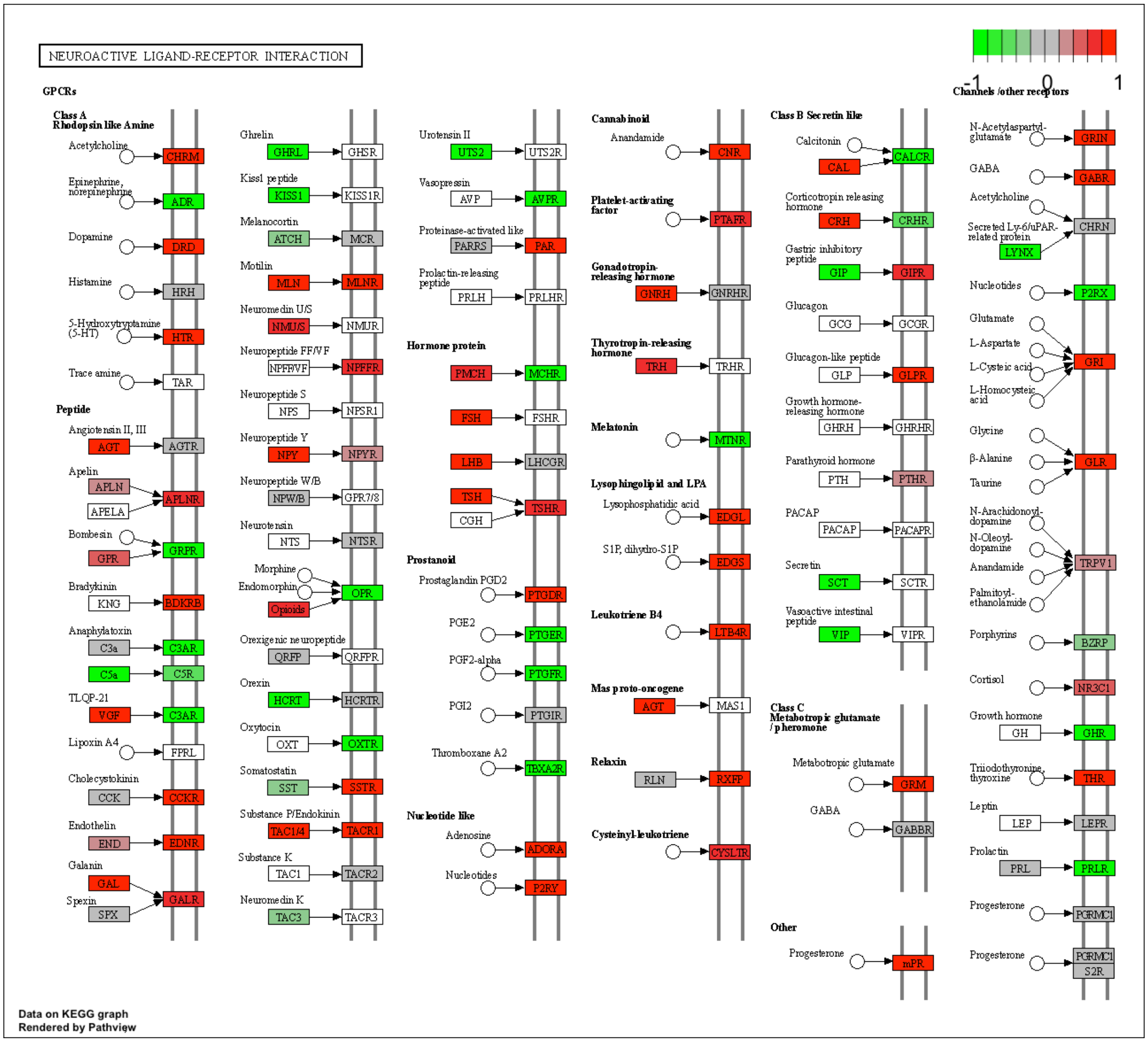
```r
pathview(fc, pathway.id = "hsa04080")
```

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

```
Info: Working in directory /Users/jack/Dropbox/213/class13
```

```
Info: Writing image file hsa04080.pathview.png
```





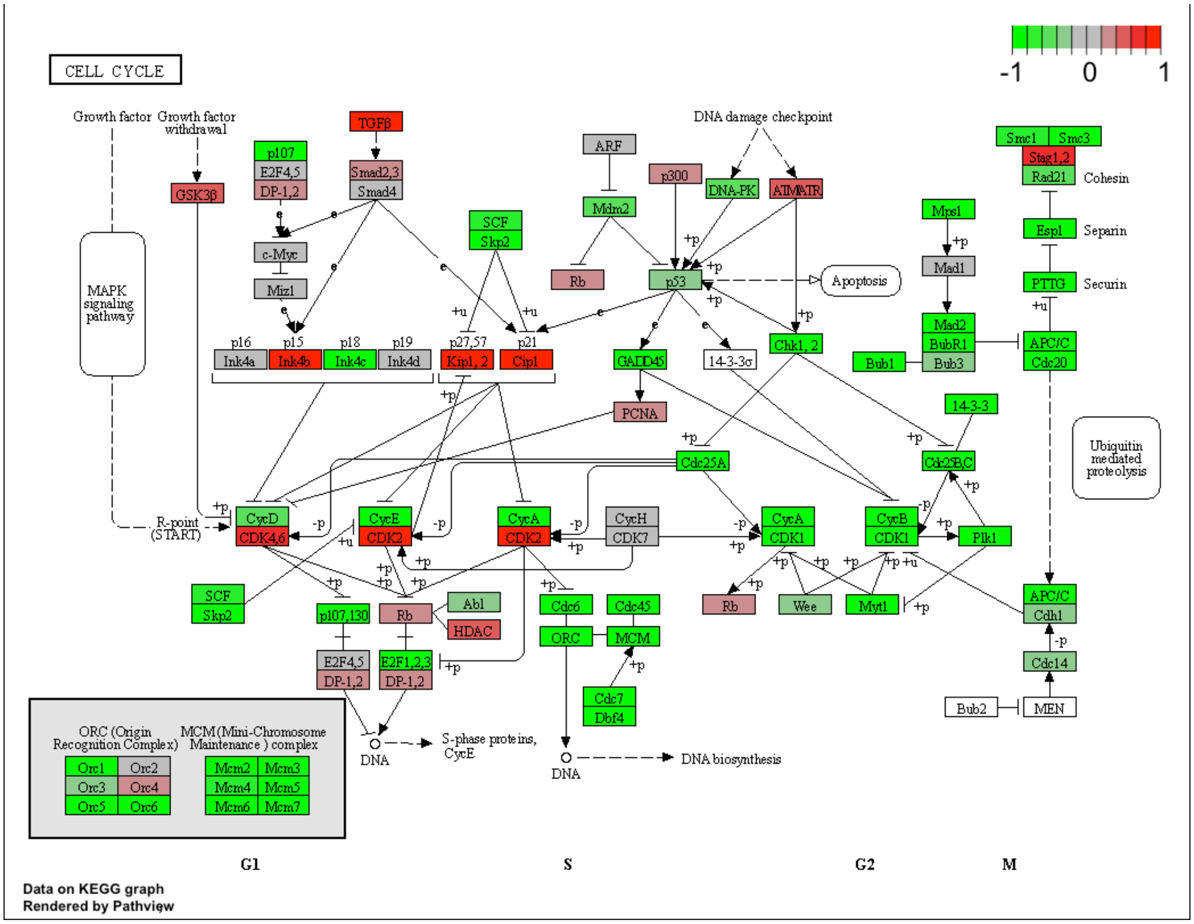
Q. Can you do the same procedure as above to plot the pathview figures for the top 5 down-reguled pathways?

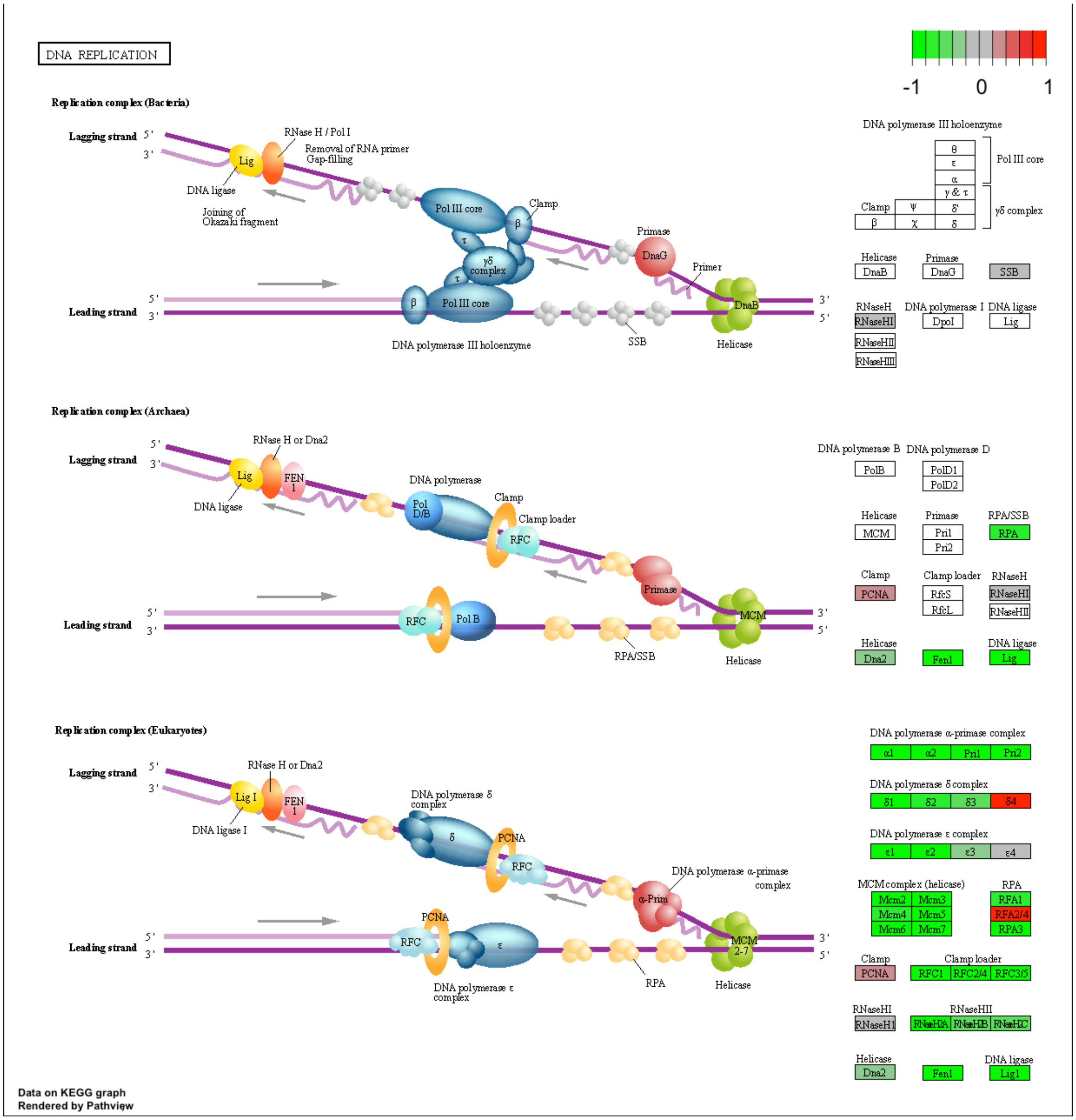
```
kegg.res.pathways <- rownames(kegg.res$less)[1:5]
kegg.res.ids = substr(kegg.res.pathways, start=1, stop=8)
kegg.res.ids
```

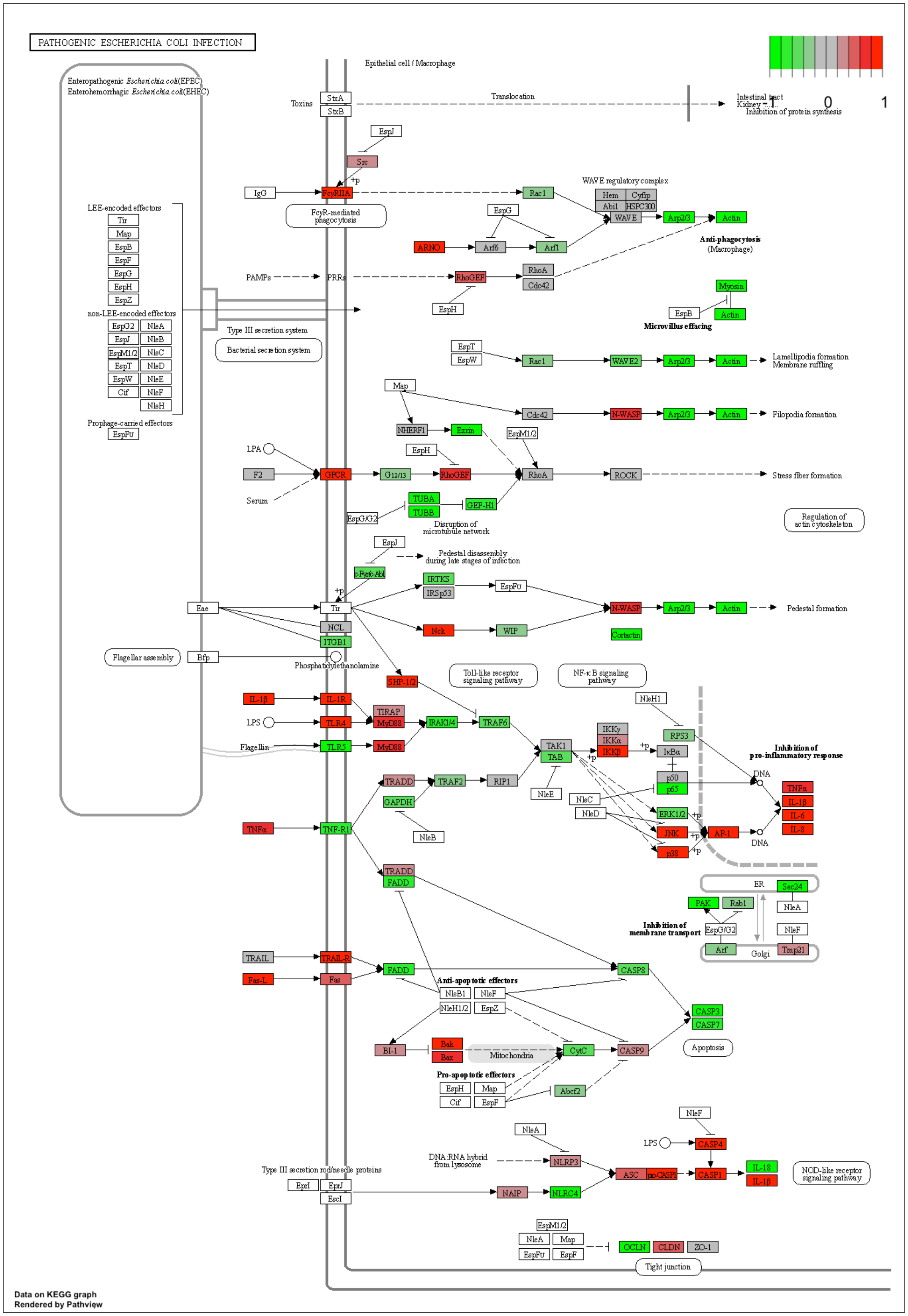
```
[1] "hsa04110" "hsa03030" "hsa05130" "hsa03013" "hsa03440"
```

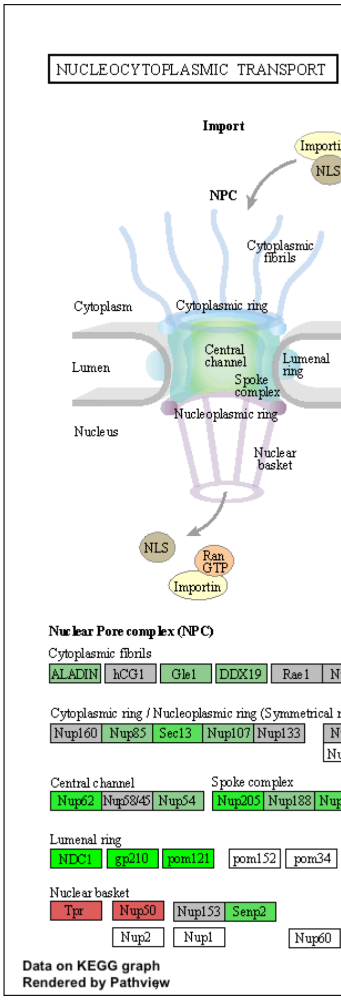
```
pathview(gene.data=fc, pathway.id=kegg.res.ids, species="hsa")
```

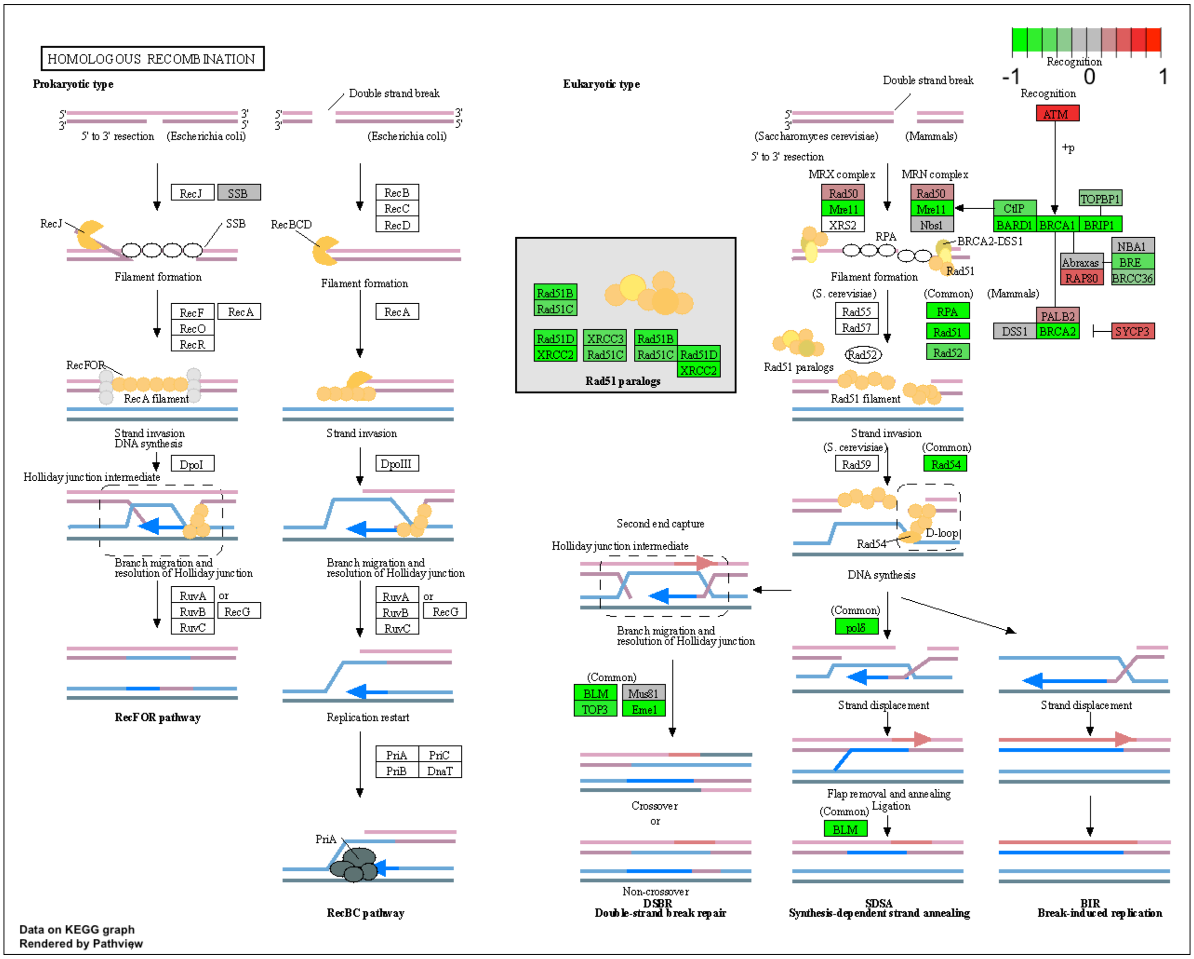
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/jack/Dropbox/213/class13

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory /Users/jack/Dropbox/213/class13

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/jack/Dropbox/213/class13

Info: Writing image file hsa05130.pathview.png

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

Info: Working in directory /Users/jack/Dropbox/213/class13

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory /Users/jack/Dropbox/213/class13

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


CELL CYCLE
-1
0
1
Growth factor
Growth factor withdrawal
GSK3β
MAPK signaling pathway
TGFβ
p107
E2F4,5
DP-1,2
Smad2,3
Smad4
c-Myc
Miz1
SCF
Skp2
ARF
Mdm2
Rb
p300
DNA damage checkpoint
DNA-PK
ATM/ATR
p53
Apoptosis
Mps1
Mad1
Mad2
BubR1
Bub3
Bub1
Smc1
Smc3
Stag1,2
Rad21
Cohesin
Espl
Separin
PTTG
Securin
APC/C
Cdc20
p16
Ink4a
p15
Ink4b
p18
Ink4c
p19
Ink4d
p27,57
Kip1, 2
p21
Cip1
GADD45
14-3-3σ
Chk1, 2
PCNA
14-3-3
Cdc25A
Cdc25B,C
Ubiquitin mediated proteolysis
R-point (START)
CycD
CDK4,6
CycE
CDK2
CycA
CDK2
CycH
CDK7
CycA
CDK1
CycB
CDK1
Plk1
SCF
Skp2
p107,130
Rb
Abl
HDAC
Cdc6
Cdc45
ORC
MCM
Cdc7
Dbf4
Rb
Wee
Myt1
APC/C
Cdh1
Cdc14
Bub2
MEN
E2F4,5
DP-1,2
E2F1,2,3
DP-1,2
DNA
S-phase proteins, CycE
DNA
DNA biosynthesis
ORC (Origin Recognition Complex)
MCM (Mini-Chromosome Maintenance) complex
Orc1
Orc2
Orc3
Orc4
Orc5
Orc6
Mcm2
Mcm3
Mcm4
Mcm5
Mcm6
Mcm7
+p
-p
+u
e
G1
S
G2
M
Data on KEGG graph
Rendered by Pathview


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
5'
3'
Leading strand
β
Pol III core
DnaB
3'
5'
DNA polymerase III holoenzyme
SSB
Helicase
DNA polymerase III holoenzyme
θ
ε
α
γ & τ
Pol III core
Clamp
ψ
δ'
γδ complex
β
χ
δ
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
PCNA
Leading strand
5'
3'
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
Enteropathogenic Escherichia coli (EPEC)
Enterohemorrhagic Escherichia coli (EHEC)
Epithelial cell / Macrophage
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
Anti-phagocytosis (Macrophage)
EspG
ARNO
Arf6
Arf1
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-κB signaling pathway
NleH1
IL-1β
IL-1R
TIRAP
MyD88
LPS
TLR4
IRAK1/4
TRAF6
IKKγ
IKKα
IKKβ
RPS3
Flagellin
TLR5
MyD88
TAK1
TAB
+p
IκBα
Inhibition of pro-inflammatory response
p50
p65
DNA
TNFα
IL-1β
IL-6
IL-8
TRADD
TRAF2
RIP1
GAPDH
NleE
NleC
NleD
ERK1/2
JNK
AP-1
p38
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
Apoptosis
BI-1
Bak
Bax
Mitochondria
CytC
CASP9
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
Import
Importi
NLS
NPC
Cytoplasmic fibrils
Cytoplasm
Cytoplasmic ring
Lumen
Central channel
Lumenal ring
Spoke complex
Nucleoplasmic ring
Nucleus
Nuclear basket
NLS
Ran GTP
Importin
Nuclear Pore complex (NPC)
Cytoplasmic fibrils
ALADIN
hCG1
Gle1
DDX19
Rae1
N
Cytoplasmic ring / Nucleoplasmic ring (Symmetrical r
Nup160
Nup85
Sec13
Nup107
Nup133
N
Nu
Central channel
Spoke complex
Nup62
Nup58/45
Nup54
Nup205
Nup188
Nup
Lumenal ring
NDC1
gp210
pom121
pom152
pom34
Nuclear basket
Tpr
Nup50
Nup153
Senp2
Nup2
Nup1
Nup60
Data on KEGG graph
Rendered by Pathview



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

go.bp.sets = go.sets.hs[go.subs.hs$BP]

go.bp.res = gage(fc, gsets=go.bp.sets, same.dir=TRUE)

head(go.bp.res$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
```

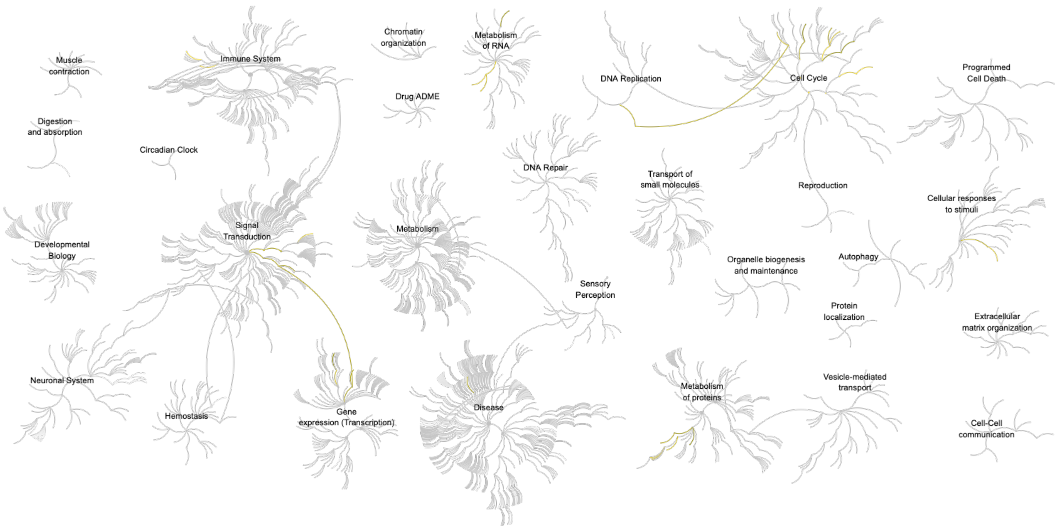
```
GO:0000236 mitotic prometaphase         1.729553e-10 -6.695966 1.729553e-10
                                               q.val set.size         exp1
GO:0048285 organelle fission            5.841698e-12      376 1.536227e-15
GO:0000280 nuclear division             5.841698e-12      352 4.286961e-15
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
```

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

> 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?

```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
write.table(sig_genes,
            file="significant_genes.txt",
            row.names=FALSE,
            col.names=FALSE,
            quote=FALSE
            )
```

Exported to Reactome!



> 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?

Endosomal/Vacuolar pathway. They do roughly match the 2nd hit in the KEGG database: “Lysosome.” The reactome database gene lists could be different from the KEGG gene lists, accounting for differences in enrichment of biological processes from both approaches.