Class_14_lab

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Pathway Analysis from RNA-Seq Results ## Background Working through a complete RNASeq analysis project. The input data is coming from a KO experiment of a HOX gene.

Data Import

> Q. Complete the code below to remove the troublesome first column from count-Data

```
library(DESeq2)
```

```
Loading required package: S4Vectors
```

```
Loading required package: stats4
```

```
Loading required package: BiocGenerics
```

```
Loading required package: generics
```

```
Attaching package: 'generics'

The following objects are masked from 'package:base':

    as.difftime, as.factor, as.ordered, intersect, is.element, setdiff,
    setequal, union


Attaching package: 'BiocGenerics'

The following objects are masked from 'package:stats':

    IQR, mad, sd, var, xtabs

The following objects are masked from 'package:base':

    anyDuplicated, aperm, append, as.data.frame, basename, cbind,
    colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
    get, grep, grepl, is.unsorted, lapply, Map, mapply, match, mget,
    order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
    rbind, Reduce, rownames, sapply, saveRDS, table, tapply, unique,
    unsplit, which.max, which.min


Attaching package: 'S4Vectors'

The following object is masked from 'package:utils':

    findMatches

The following objects are masked from 'package:base':

    expand.grid, I, unname

Loading required package: IRanges

Loading required package: GenomicRanges

Loading required package: GenomeInfoDb
```

```
Loading required package: SummarizedExperiment

Loading required package: MatrixGenerics

Loading required package: matrixStats


Attaching package: 'MatrixGenerics'

The following objects are masked from 'package:matrixStats':

    colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
    colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
    colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
    colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
    colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
    colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
    colWeightedMeans, colWeightedMedians, colWeightedSds,
    colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
    rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
    rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
    rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
    rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
    rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
    rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
    rowWeightedSds, rowWeightedVars

Loading required package: Biobase

Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.


Attaching package: 'Biobase'

The following object is masked from 'package:MatrixGenerics':

    rowMedians
```

```
The following objects are masked from 'package:matrixStats':

    anyMissing, rowMedians
```

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

metaData <- read.csv("GSE37704_metadata.csv", row.names = 1)

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
```

Need the rows of metaData to match exactly to columns of countData. countData has a lenght column that is extra.

```
PrettyCount <-  countData[,-1]
```

Removing Zero Count genes

> 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).

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

DESeq analysis

```
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
```

```
dds = DESeq(dds)

dds
```

```
class: DESeqDataSet
dim: 15975 6
metadata(1): version
assays(4): counts mu H cooks
rownames(15975): ENSG00000279457 ENSG00000187634 ... ENSG00000276345
  ENSG00000271254
rowData names(22): baseMean baseVar ... deviance maxCooks
colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
colData names(2): condition sizeFactor
```

```
res = results(dds, contrast=c("condition", "hoxa1_kd", "control_sirna"))
```

> 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.

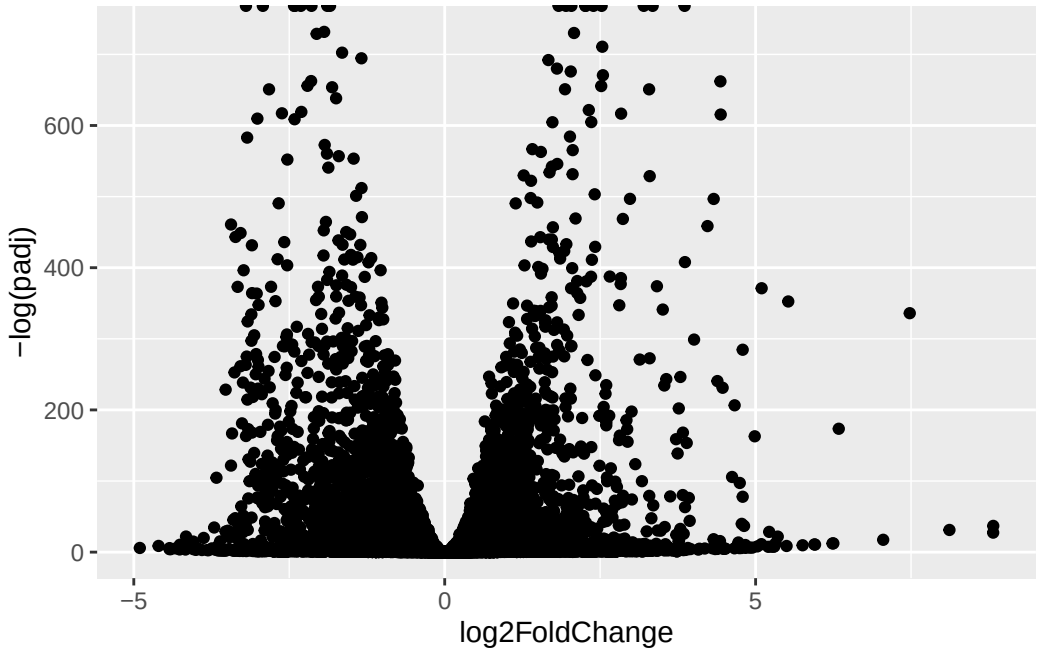
```
summary(res)
```

```
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

```
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()`).
```



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

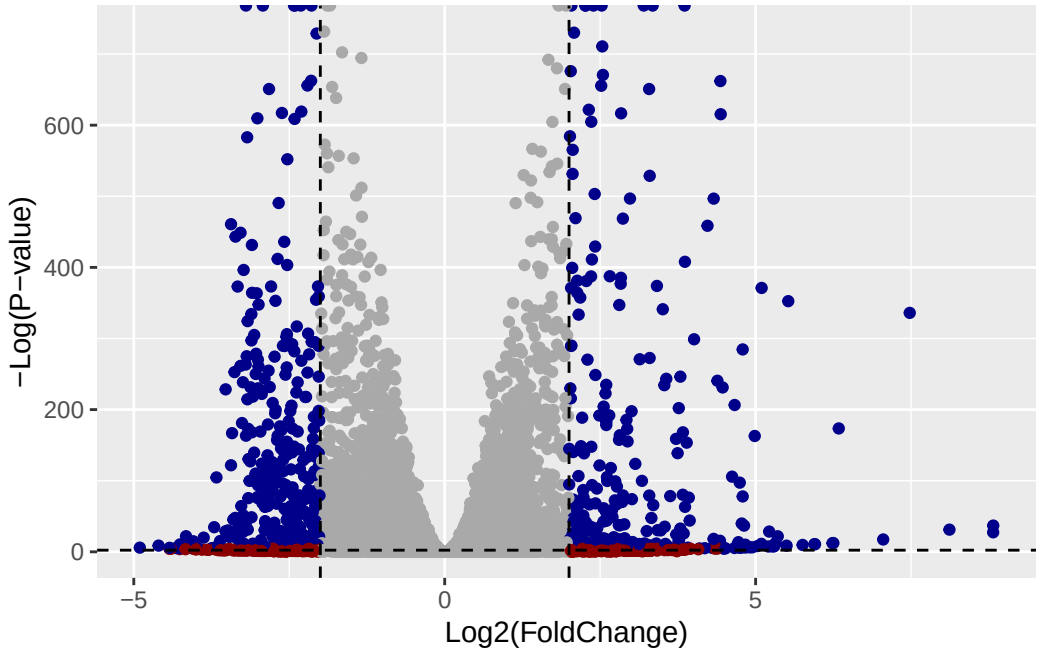
```
mycols <- rep("darkgrey", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ]  <- "darkred"

inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "darkblue"

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

```
  geom_point(col=mycols) +
 labs(y="-Log(P-value)", x="Log2(FoldChange)") +
 geom_vline(xintercept = c(-2, 2), colour = "black", linetype = "dashed") +
    geom_hline(yintercept = -log(0.1), colour = "black", linetype = "dashed")
```

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



Add Annotation

Q. 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(keys=row.names(res),
                    keytype="ENSEMBL",
                    x = org.Hs.eg.db,
                    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 0.3248215   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      symbol      entrez                    name
                  <numeric> <character> <character>             <character>
ENSG00000279457 6.86555e-01          NA          NA                      NA
ENSG00000187634 5.15718e-03      SAMD11      148398 sterile alpha motif ..
ENSG00000188976 1.76553e-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 4.21970e-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.

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

Pathway Analysis with KEGG

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

```
foldC <- res$log2FoldChange
names(foldC) <- res$entrez
head(foldC)
```

```
     1266     54855      1465      2034      2150      6659
-2.422719  3.201955 -2.313738 -1.888019  3.344508  2.392288
```

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

keggres = gage(foldC, gsets=kegg.sets.hs)

attributes(keggres)
```

```
$names
[1] "greater" "less"    "stats"
```

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

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

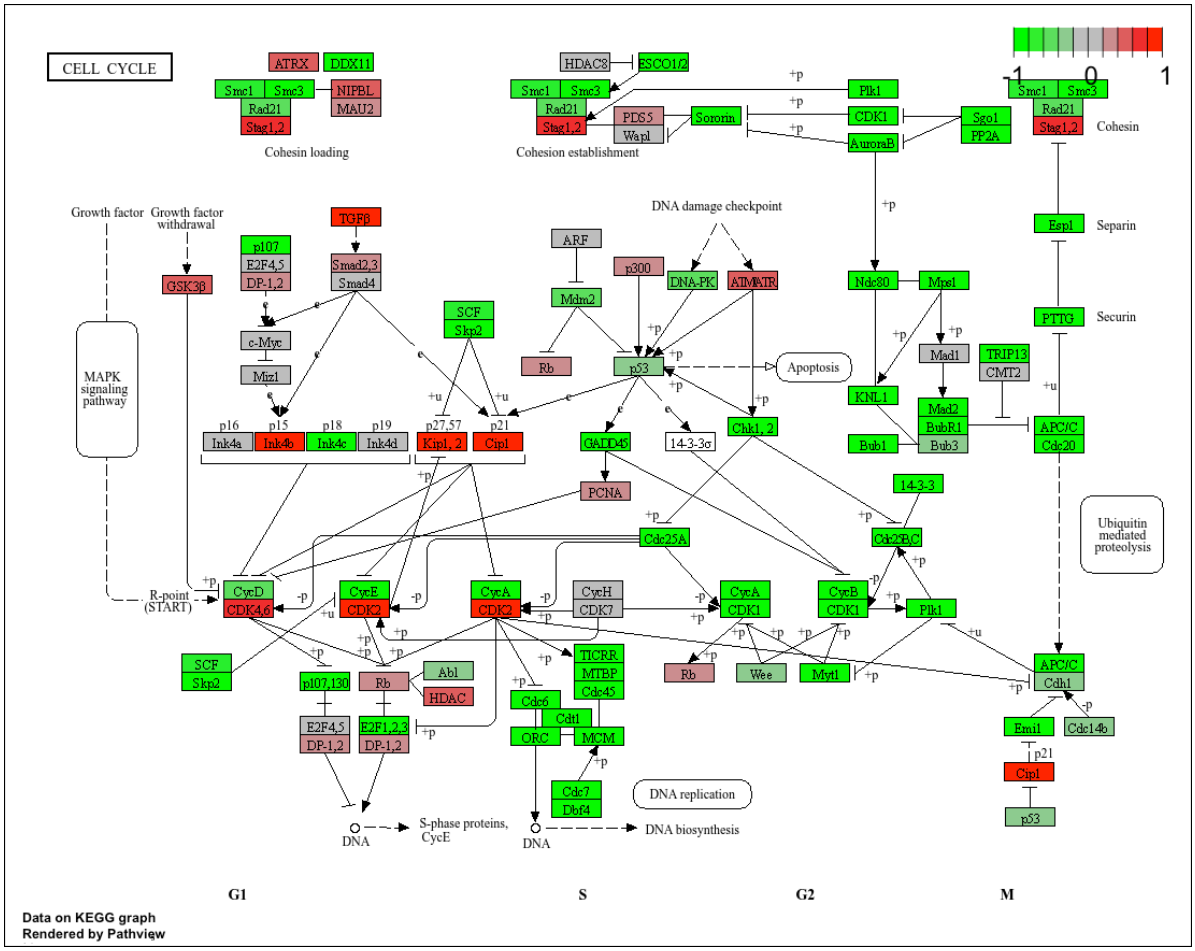
```
                                              p.geomean stat.mean
hsa04110 Cell cycle                         8.995727e-06 -4.378644
hsa03030 DNA replication                    9.424076e-05 -3.951803
hsa05130 Pathogenic Escherichia coli infection 1.405864e-04 -3.765330
hsa03013 RNA transport    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

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

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

Info: Working in directory /Users/tsterns/Desktop/BIMM 143/R_Codes/Class_14
```

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

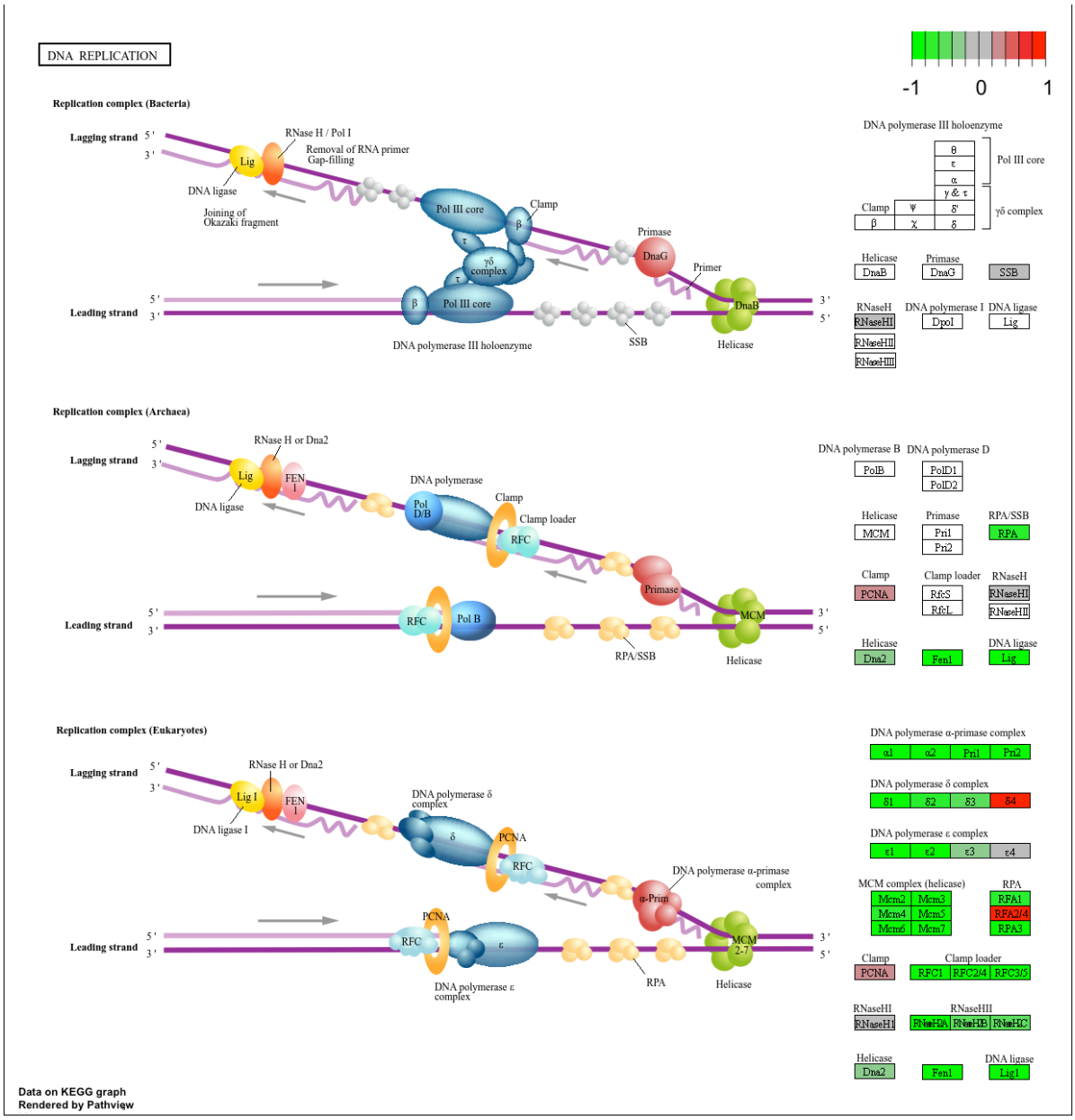



```
pathview(pathway.id = "hsa03030", gene.data = foldC)
```

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

```
Info: Working in directory /Users/tsterns/Desktop/BIMM 143/R_Codes/Class_14
```

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

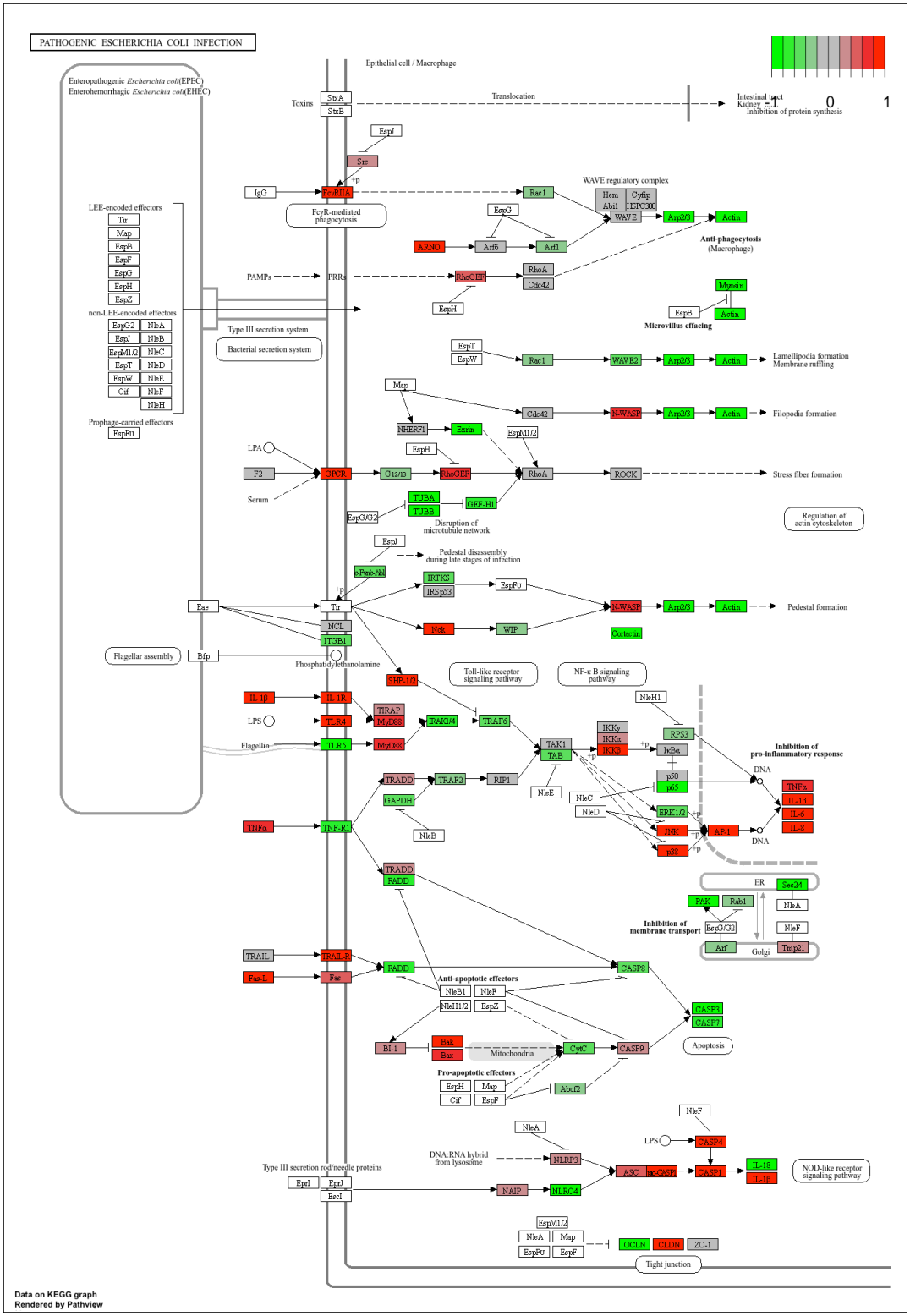



```
pathview(pathway.id = "hsa05130", gene.data = foldC)
```

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

```
Info: Working in directory /Users/tsterns/Desktop/BIMM 143/R_Codes/Class_14
```

```
Info: Writing image file hsa05130.pathview.png
```

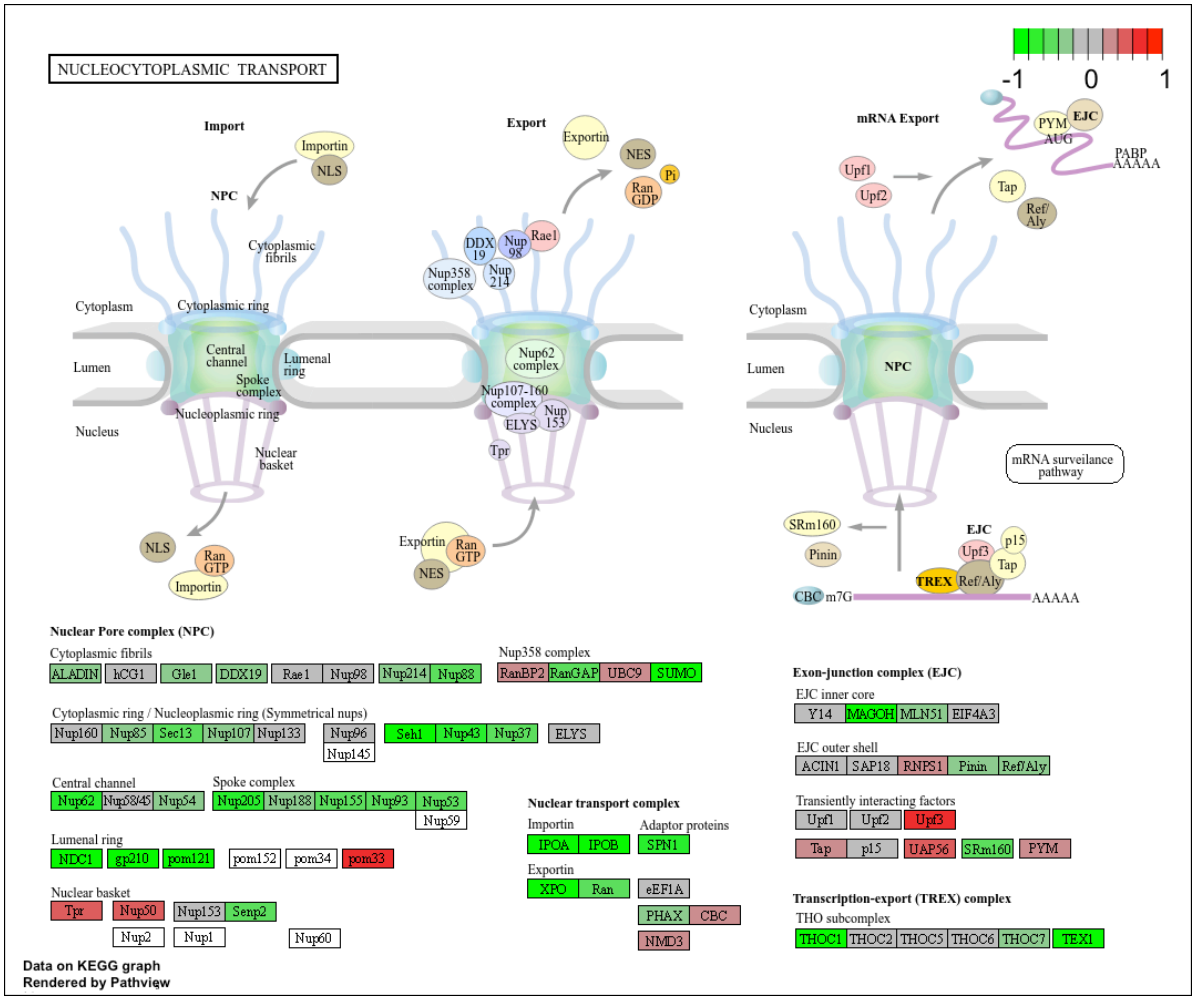

PATHOGENIC ESCHERICHIA COLI INFECTION
Epithelial cell / Macrophage
Enteropathogenic Escherichia coli(EPEC)
Enterohemorrhagic Escherichia coli(EHEC)
Toxins
StxA
StxB
Translocation
Intestinal tract
Kidney
Inhibition of protein synthesis
EspJ
Src
IgG
FcgRIIA
FcγR-mediated phagocytosis
Rac1
WAVE regulatory 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
LPA
F2
Serum
GPCR
G12/13
RhoGEF
RhoA
ROCK
Stress fiber formation
TUBA
TUBB
GEF-H1
EspG/O2
Disruption of microtubule network
Regulation of actin cytoskeleton
EspJ
Pedestal disassembly during late stages of infection
c-Port-Abl
IRTKS
IRSp53
EspFU
Eae
Tir
NCL
ITGB1
N-WASP
Arp2/3
Actin
Pedestal formation
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
LPS
TLR4
TRAP
MyD88
IRAK1/4
TRAF6
Flagellin
TLR5
MyD88
TAK1
TAB
IKKγ
IKKα
IKKβ
RPS3
IκBα
p50
p65
Inhibition of pro-inflammatory response
DNA
TNFα
IL-1β
IL-6
IL-8
ERK1/2
JNK
p38
AP-1
DNA
TRADD
TRAF2
RIP1
GAPDH
NleB
NleE
NleC
NleD
TNFα
TNF-R1
TRADD
FADD
ER
Sec24
PAK
Rab1
NleA
Inhibition of membrane transport
EspG/O2
NleF
Arf
Golgi
Tmp21
TRAIL
TRAIL-R
Fas-L
Fas
FADD
CASP8
Anti-apoptotic effectors
NleB1
NleF
NleH1/2
EspZ
CASP3
CASP7
Bcl-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
proCASP1
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

```
pathview(pathway.id = "hsa03013", gene.data = foldC)
```

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

```
Info: Working in directory /Users/tsterns/Desktop/BIMM 143/R_Codes/Class_14
```

```
Info: Writing image file hsa03013.pathview.png
```

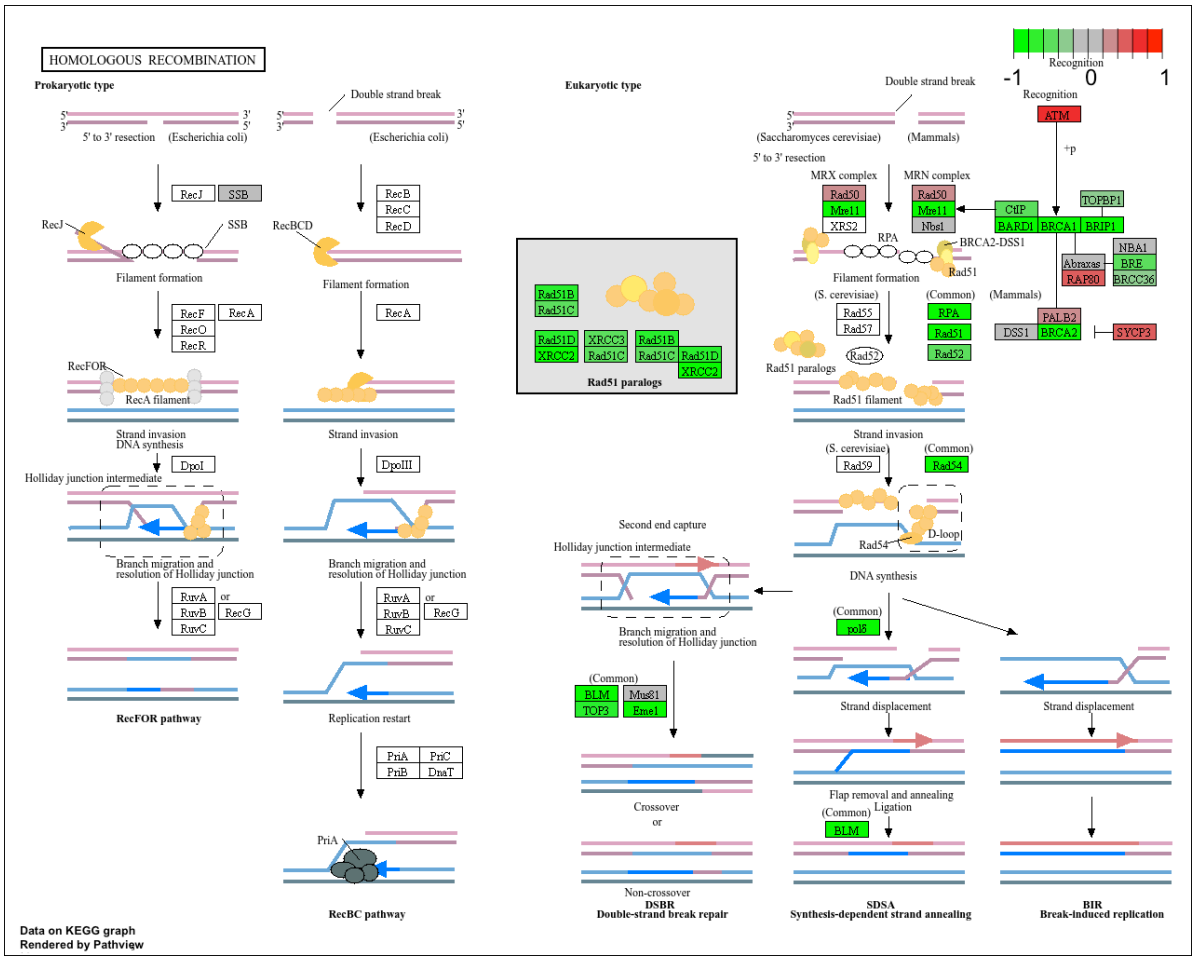



```
pathview(pathway.id = "hsa03440", gene.data = foldC)
```

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

```
Info: Working in directory /Users/tsterns/Desktop/BIMM 143/R_Codes/Class_14
```

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



Pathway analysis with GO

Same analysis but using GO genesets

```
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(foldC, 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
```

> 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?

Homophilic cell adhesion has the largest p-value using the GO database whereas in KEGG it was cell cycle. The main reason why there is a difference is because the two databases are using different reference data.

Reactome

Available as a web interface https://reactome.org/, but also can be loading into R.

The website wants a text file with one gene symbol per line.

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

Saving our Results

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