Class13

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Section 1. Differential Expression Analysis

The countdata and coldata will be downloaded and imported

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

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

```
countData <- read.csv("GSE37704_featurecounts.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
```

```
ENSG00000186092         0
ENSG00000279928         0
ENSG00000279457        46
ENSG00000278566         0
ENSG00000273547         0
ENSG00000187634       258
```

The first unwanted column “length” will be removed in Countdata

```
counts <- countData[,-1]
head(counts)
```

```
                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
```

```
colnames(counts)==rownames(colData)
```

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

DESeq2 is now loaded and used

```
library(DESeq2)
```

```
Loading required package: S4Vectors

Loading required package: stats4

Loading required package: BiocGenerics
```

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


Attaching package: 'S4Vectors'

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
```

```
dds<-DESeqDataSetFromMatrix(countData=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 <- results(dds)
```

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

```
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
```

> 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?

```
to.keep<- rowSums(counts)>0
counts<- counts[to.keep,]
head(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
```

> How many genes do we have left?

```
nrow(counts)
```

```
[1] 15975
```

DESeq2 is now loaded and used

```
library(DESeq2)
```

```
dds<-DESeqDataSetFromMatrix(countData=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 <- results(dds)
```

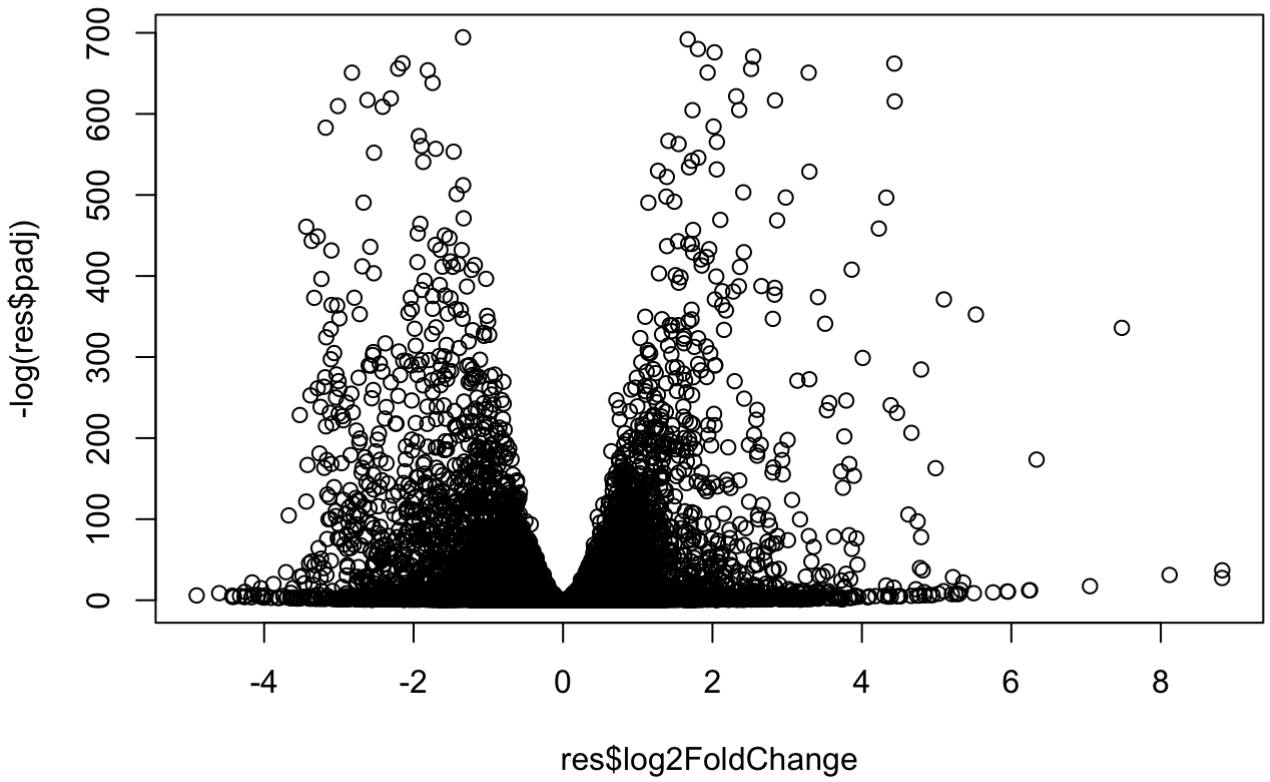
> Q. Call the summary() function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```
summary(res$padj>0.01)
```

```
   Mode   FALSE    TRUE    NA's
logical    7076    7662    1237
```

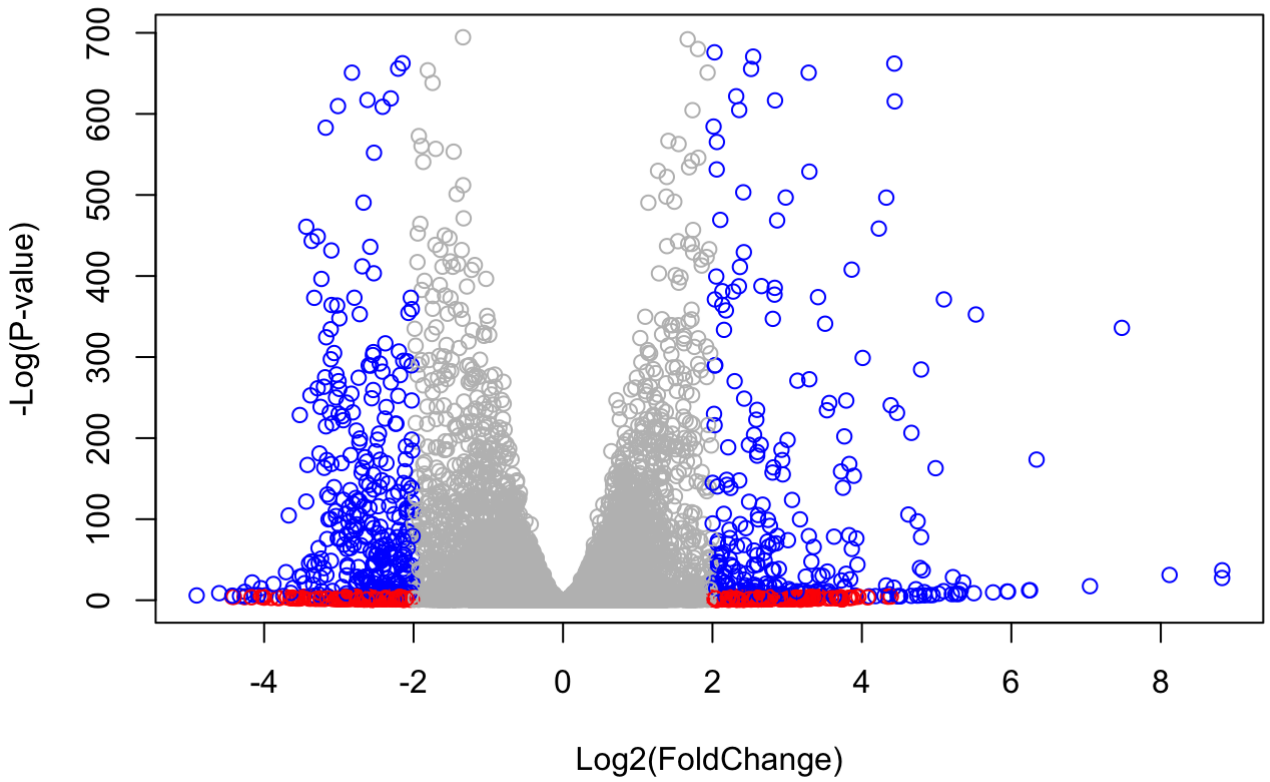
A volcano plot is made

```
res = results(dds, contrast=c("condition", "hoxa1_kd", "control_sirna"))
plot( res$log2FoldChange, -log(res$padj) )
```



> Q. 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$log2FoldChange) > 2 ] <- "blue"
# Color blue those with adjusted p-value less than 0.01
# and absolute fold change more than 2
inds <- (res$padj>0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "red"
plot( res$log2FoldChange, -log(res$padj), col=mycols, xlab="Log2(FoldCha
```



> 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"
```

[20] "UNIPROT"

```
res$symbol = mapIds(org.Hs.eg.db,
                    keys=rownames(res),
                    keytype="ENSEMBL",
                    column="SYMBOL",
                    multiVals="first")
```

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

```
res$entrez = mapIds(org.Hs.eg.db,
                    keys=rownames(res),
                    keytype="ENSEMBL",
                    column="ENTREZID",
                    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 8 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
                <numeric> <character> <character>
ENSG00000279457 6.86555e-01          NA          NA
ENSG00000187634 5.15718e-03      SAMD11      148398
ENSG00000188976 1.76549e-35       NOC2L       26155
ENSG00000187961 1.13413e-07      KLHL17      339451
ENSG00000187583 9.19031e-01     PLEKHN1       84069
ENSG00000187642 4.03379e-01       PERM1       84808
ENSG00000188290 1.30538e-24        HES4       57801
ENSG00000187608 2.37452e-02       ISG15        9636
ENSG00000188157 4.21963e-16        AGRN      375790
ENSG00000237330          NA      RNF223      401934
```

> 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")
```

Section 2. Pathway Analysis

```
library(gage)
```

```
library(gageData)

data(kegg.sets.hs)
data(sigmet.idx.hs)

# Focus on signaling and metabolic pathways only
```

```
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]

# Examine the first 3 pathways
head(kegg.sets.hs, 3)
```

```
$`hsa00232 Caffeine metabolism`
[1] "10"   "1544" "1548" "1549" "1553" "7498" "9"

$`hsa00983 Drug metabolism - other enzymes`
 [1] "10"     "1066"   "10720"  "10941"  "151531" "1548"   "1549"
"1551"
 [9] "1553"   "1576"   "1577"   "1806"   "1807"   "1890"   "221223"
"2990"
[17] "3251"   "3614"   "3615"   "3704"   "51733"  "54490"  "54575"
"54576"
[25] "54577"  "54578"  "54579"  "54600"  "54657"  "54658"  "54659"
"54963"
[33] "574537" "64816"  "7083"   "7084"   "7172"   "7363"   "7364"
"7365"
[41] "7366"   "7367"   "7371"   "7372"   "7378"   "7498"   "79799"
"83549"
[49] "8824"   "8833"   "9"      "978"

$`hsa00230 Purine metabolism`
  [1] "100"    "10201"  "10606"  "10621"  "10622"  "10623"  "107"
"10714"
  [9] "108"    "10846"  "109"    "111"    "11128"  "11164"  "112"
"113"
 [17] "114"    "115"    "122481" "122622" "124583" "132"    "158"
"159"
 [25] "1633"   "171568" "1716"   "196883" "203"    "204"    "205"
"221823"
 [33] "2272"   "22978"  "23649"  "246721" "25885"  "2618"   "26289"
"270"
 [41] "271"    "27115"  "272"    "2766"   "2977"   "2982"   "2983"
"2984"
 [49] "2986"   "2987"   "29922"  "3000"   "30833"  "30834"  "318"
"3251"
 [57] "353"    "3614"   "3615"   "3704"   "377841" "471"    "4830"
"4831"
 [65] "4832"   "4833"   "4860"   "4881"   "4882"   "4907"   "50484"
```

```
 [65] "4632"  "4633"  "4800"  "4681"  "4682"  "4907"  "50484"
"50940"
 [73] "51082"  "51251"  "51292"  "5136"   "5137"   "5138"   "5139"
"5140"
 [81] "5141"   "5142"   "5143"   "5144"   "5145"   "5146"   "5147"
"5148"
 [89] "5149"   "5150"   "5151"   "5152"   "5153"   "5158"   "5167"
"5169"
 [97] "51728"  "5198"   "5236"   "5313"   "5315"   "53343"  "54107"
"5422"
[105] "5424"   "5425"   "5426"   "5427"   "5430"   "5431"   "5432"
"5433"
[113] "5434"   "5435"   "5436"   "5437"   "5438"   "5439"   "5440"
"5441"
[121] "5471"   "548644" "55276"  "5557"   "5558"   "55703"  "55811"
"55821"
[129] "5631"   "5634"   "56655"  "56953"  "56985"  "57804"  "58497"
"6240"
[137] "6241"   "64425"  "646625" "654364" "661"    "7498"   "8382"
"84172"
[145] "84265"  "84284"  "84618"  "8622"   "8654"   "87178"  "8833"
"9060"
[153] "9061"   "93034"  "953"    "9533"   "954"    "955"    "956"
"957"
[161] "9583"   "9615"
```

```
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
head(foldchanges)
```

```
     1266     54855      1465     51232      2034      2317
-2.422719  3.201955 -2.313738 -2.059631 -1.888019 -1.649792
```

```
# Get the results
keggres = gage(foldchanges, gsets=kegg.sets.hs)
head(keggres$less, 5)
```

```
                                    p.geomean stat.mean        p.val
hsa04110 Cell cycle               8.995727e-06 -4.378644 8.995727e-06
hsa03030 DNA replication          9.424076e-05 -3.951803 9.424076e-05
hsa03013 RNA transport            1.375901e-03 -3.028500 1.375901e-03
```

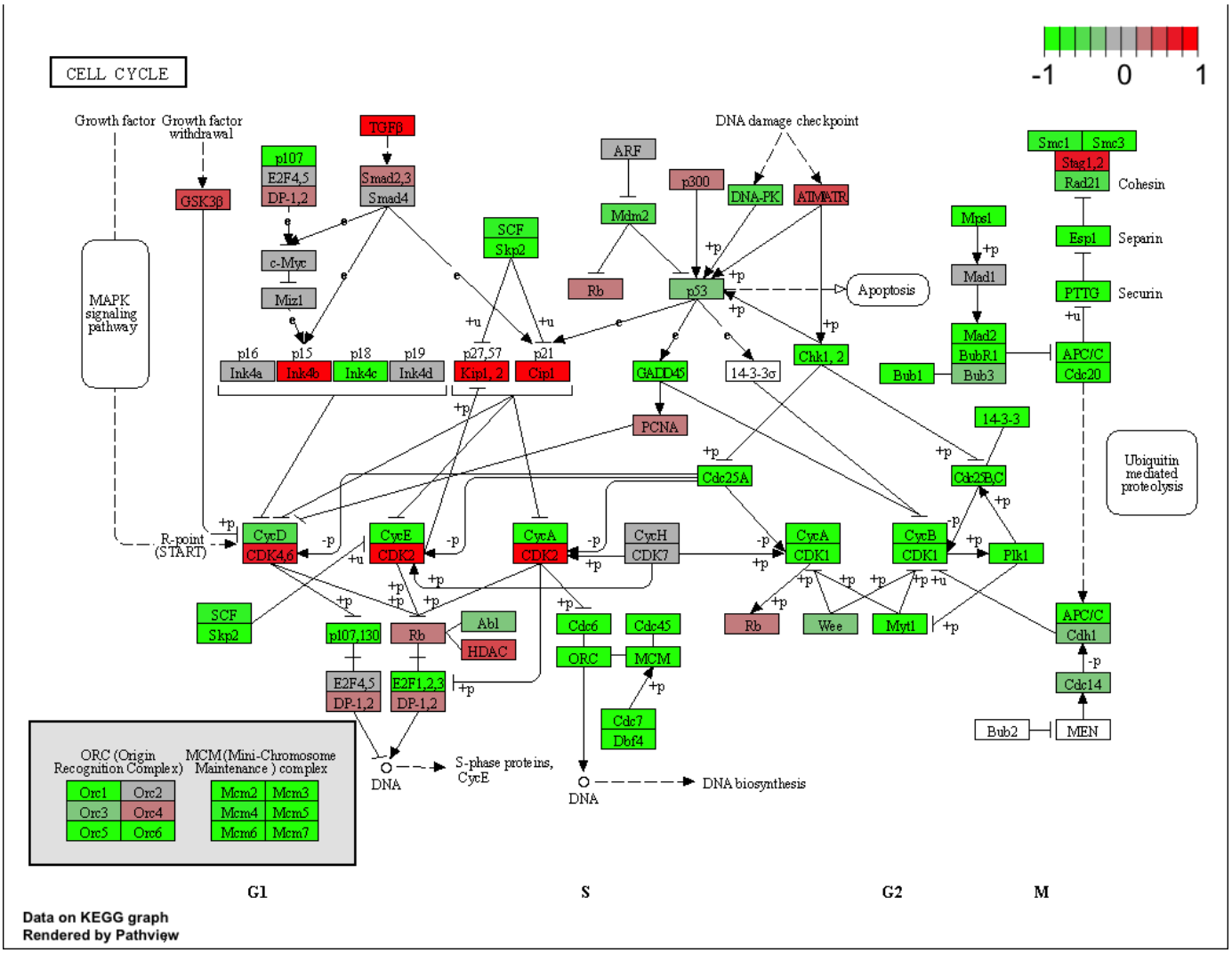
```
hsa03440 Homologous recombination 3.066756e-03 -2.852899 3.066756e-03
hsa04114 Oocyte meiosis           3.784520e-03 -2.698128 3.784520e-03
                                        q.val set.size         exp1
hsa04110 Cell cycle               0.001448312      121 8.995727e-06
hsa03030 DNA replication          0.007586381       36 9.424076e-05
hsa03013 RNA transport            0.073840037      144 1.375901e-03
hsa03440 Homologous recombination 0.121861535       28 3.066756e-03
hsa04114 Oocyte meiosis           0.121861535      102 3.784520e-03
```

```
library(pathview)
```

```
##############################################################################
Pathview is an open source software package distributed under GNU General
Public License version 3 (GPLv3). Details of GPLv3 is available at
http://www.gnu.org/licenses/gpl-3.0.html. Particullary, users are required to
formally cite the original Pathview paper (not just mention it) in publications
or products. For details, do citation("pathview") within R.

The pathview downloads and uses KEGG data. Non-academic uses may require a KEGG
license agreement (details at http://www.kegg.jp/kegg/legal.html).
##############################################################################
```

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

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

Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13

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



image

```
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$greater)[1:5]

# Extract the 8 character long IDs part of each string
keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
```

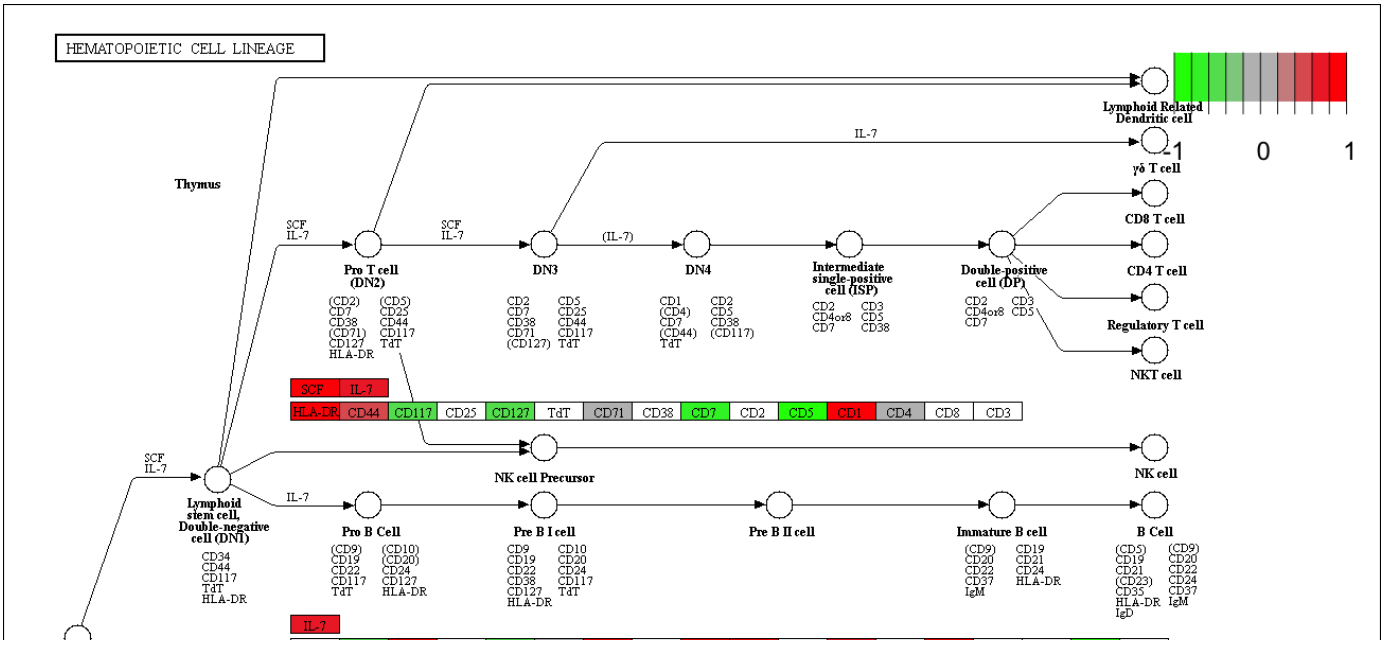
```
[1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```

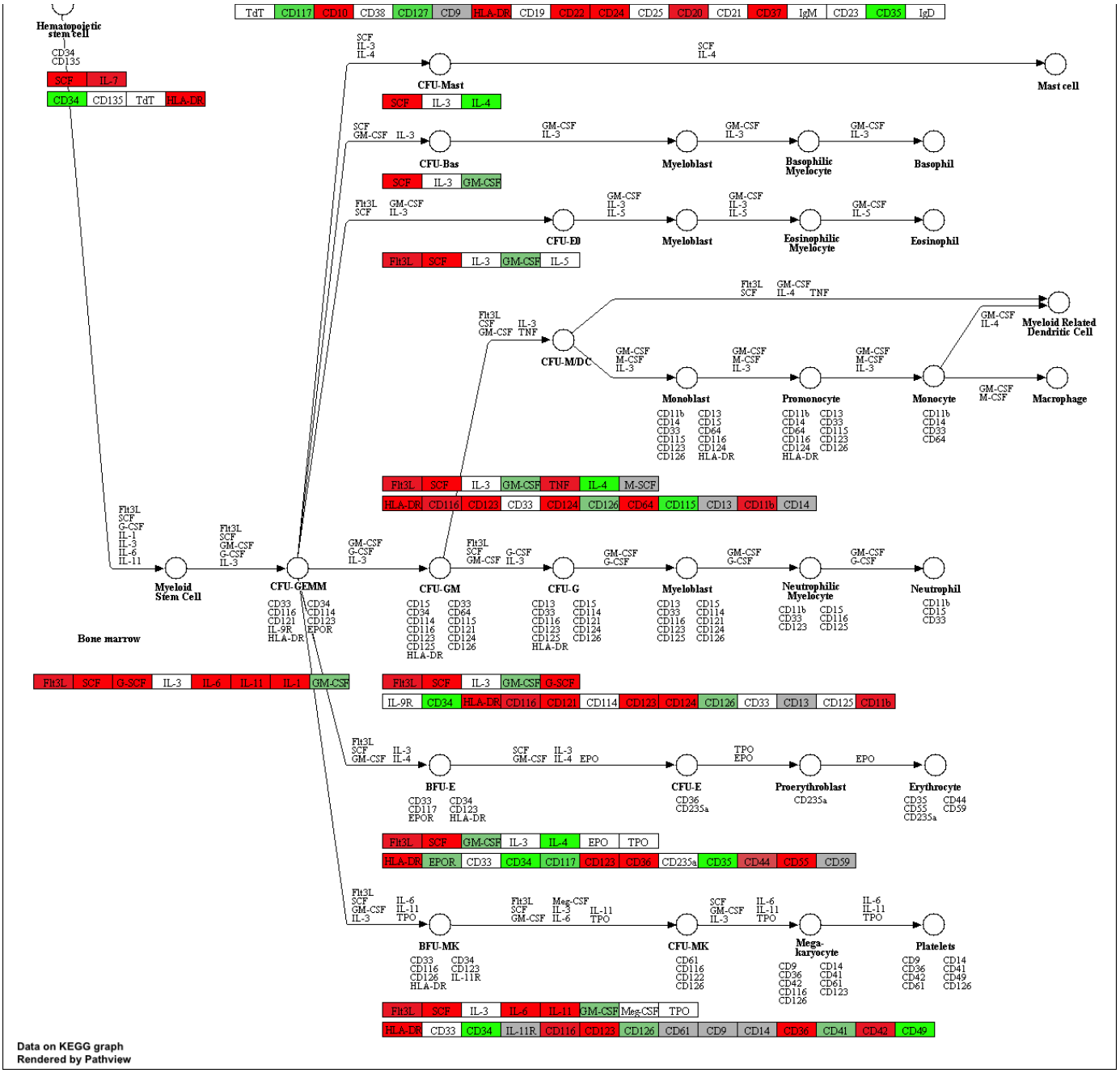
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

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

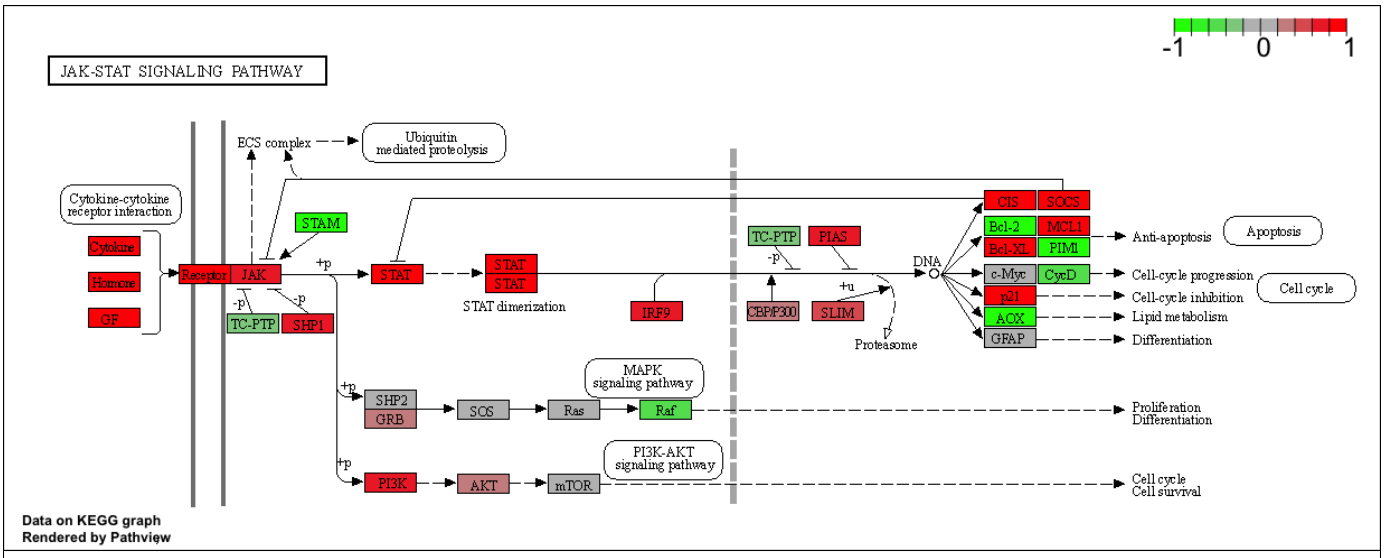
```
Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13
```

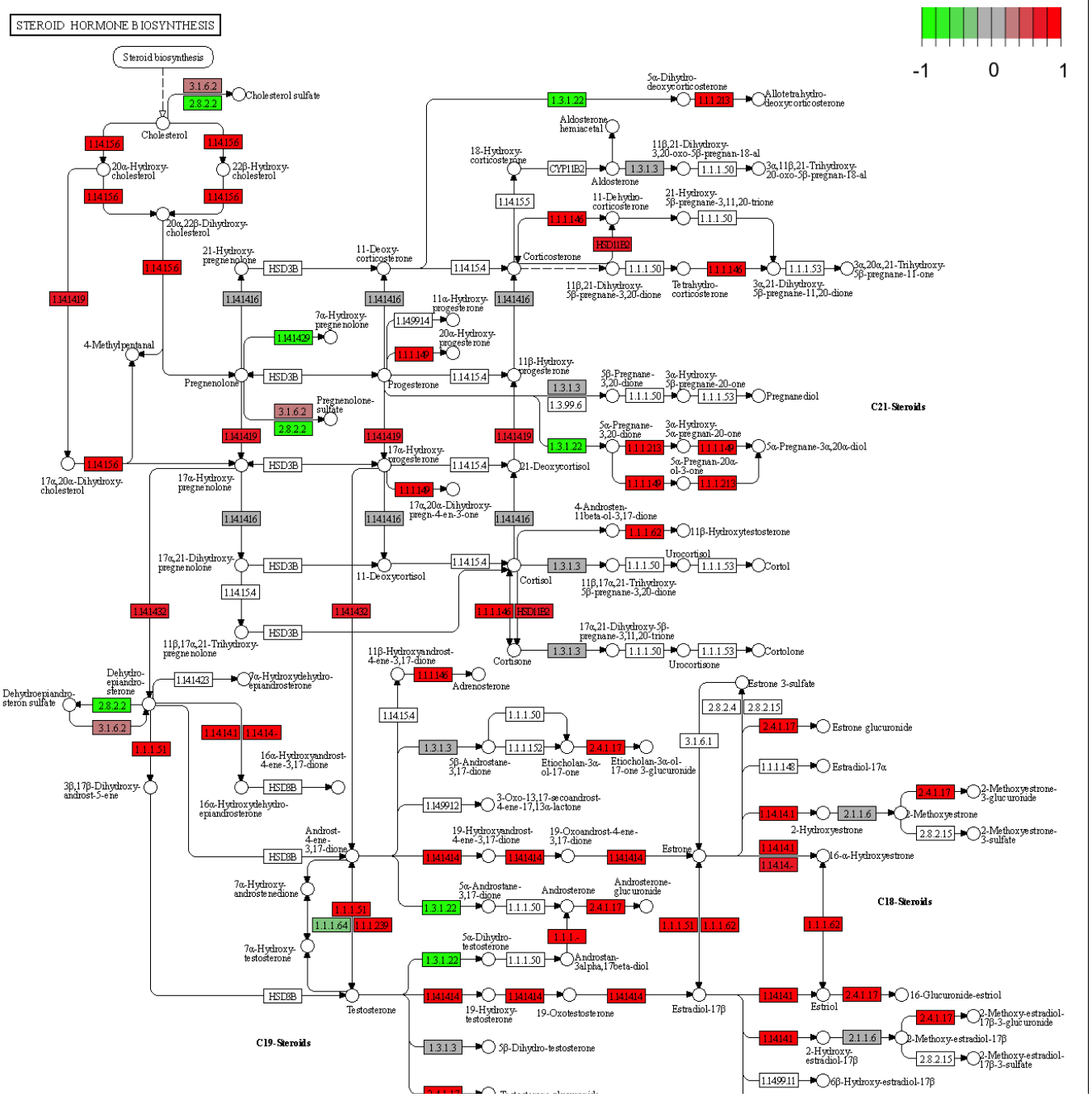
```
Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13

Info: Writing image file hsa04142.pathview.png

Info: some node width is different from others, and hence adjusted!

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

Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13

Info: Writing image file hsa04330.pathview.png
```



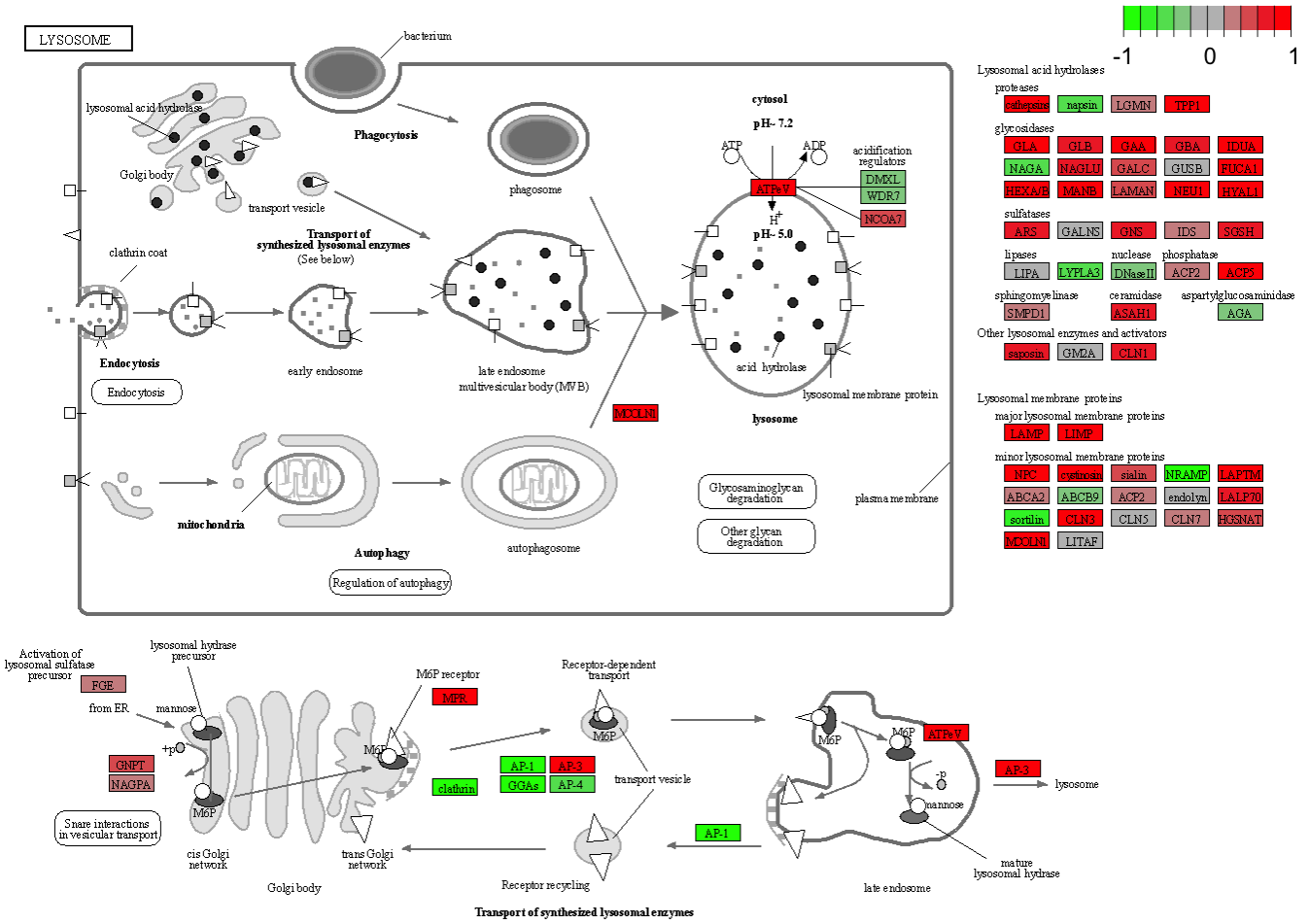

Hematopoietic stem cell
CD34 CD135
SCF IL-7
CD34 CD135 TdT HLA-DR
TdT CD117 CD10 CD38 CD127 CD9 HLA-DR CD19 CD22 CD24 CD25 CD20 CD21 CD37 IgM CD23 CD35 IgD
Myeloid Stem Cell
Bone marrow
CFU-GEMM
CFU-Mast
Mast cell
CFU-Bas
Myeloblast
Basophilic Myelocyte
Basophil
CFU-Eo
Eosinophilic Myelocyte
Eosinophil
CFU-M/DC
Myeloid Related Dendritic Cell
Monoblast
Promonocyte
Monocyte
Macrophage
CFU-GM
CFU-G
Neutrophilic Myelocyte
Neutrophil
BFU-E
CFU-E
Proerythroblast
Erythrocyte
BFU-MK
CFU-MK
Mega-karyocyte
Platelets
Data on KEGG graph
Rendered by Pathview

image

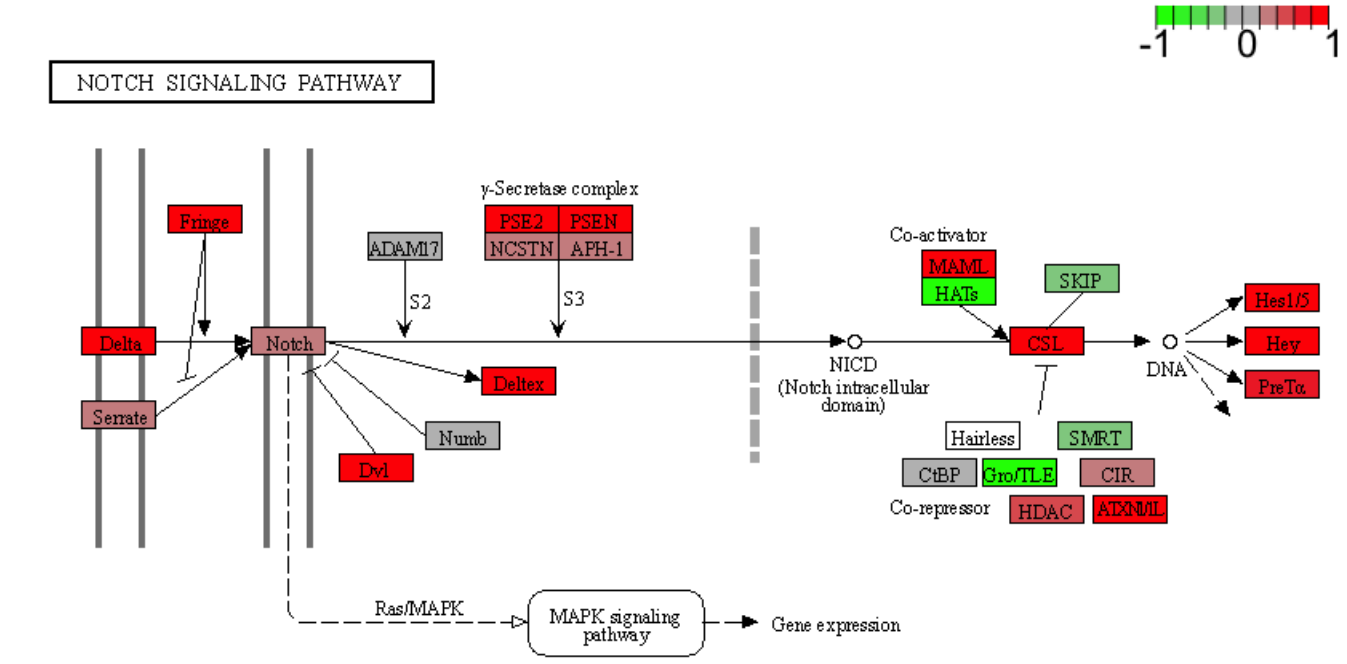

-1
0
1
JAK-STAT SIGNALING PATHWAY
ECS complex
Ubiquitin mediated proteolysis
Cytokine-cytokine receptor interaction
Cytokine
Hormone
GF
STAM
Receptor
JAK
+p
-p
TC-PTP
SHP1
STAT
STAT
STAT
STAT dimerization
IRF9
TC-PTP
PIAS
-p
+u
CBP/P300
SLIM
Proteasome
DNA
CIS
SOCS
Bcl-2
MCL1
Bcl-XL
PIM1
c-Myc
CycD
p21
AOX
GFAP
Anti-apoptosis
Apoptosis
Cell-cycle progression
Cell-cycle inhibition
Lipid metabolism
Differentiation
Cell cycle
MAPK signaling pathway
SHP2
GRB
SOS
Ras
Raf
Proliferation
Differentiation
PI3K-AKT signaling pathway
PI3K
AKT
mTOR
Cell cycle
Cell survival
Data on KEGG graph
Rendered by Pathview


-1
0
1
STEROID HORMONE BIOSYNTHESIS
Steroid biosynthesis
Cholesterol sulfate
Cholesterol
20α-Hydroxy-cholesterol
22β-Hydroxy-cholesterol
20α,22β-Dihydroxy-cholesterol
4-Methylpentanal
Pregnenolone
Pregnenolone-sulfate
17α,20α-Dihydroxy-cholesterol
17α-Hydroxy-pregnenolone
17α,21-Dihydroxy-pregnenolone
11β,17α,21-Trihydroxy-pregnenolone
Dehydroepiandro-sterone sulfate
Dehydro-epiandro-sterone
7α-Hydroxydehydro-epiandrosterone
3β,17β-Dihydroxy-androst-5-ene
16α-Hydroxyandrost-4-ene-3,17-dione
16α-Hydroxydehydro-epiandrosterone
Androst-4-ene-3,17-dione
7α-Hydroxy-androstenedione
7α-Hydroxy-testosterone
Testosterone
C19-Steroids
Progesterone
11-Deoxy-corticosterone
11α-Hydroxy-progesterone
7α-Hydroxy-pregnenolone
20α-Hydroxy-progesterone
11β-Hydroxy-progesterone
17α-Hydroxy-progesterone
17α,20α-Dihydroxy-pregn-4-en-3-one
21-Deoxycortisol
11-Deoxycortisol
Corticosterone
11β,21-Dihydroxy-5β-pregnane-3,20-dione
Tetrahydro-corticosterone
3α,21-Dihydroxy-5β-pregnane-11,20-dione
3α,20α,21-Trihydroxy-5β-pregnane-11-one
Cortisol
Cortisone
11β,17α,21-Trihydroxy-5β-pregnane-3,20-dione
17α,21-Dihydroxy-5β-pregnane-3,11,20-trione
Urocortisol
Cortolone
11β-Hydroxyandrost-4-ene-3,17-dione
Adrenosterone
5α-Androstane-3,17-dione
Androsterone
Androsterone-glucuronide
5α-Dihydro-testosterone
Androstan-3alpha,17beta-diol
19-Hydroxy-testosterone
19-Oxotestosterone
5β-Dihydro-testosterone
5α-Dihydro-deoxycorticosterone
Allotetrahydro-deoxycorticosterone
Aldosterone hemiacetal
18-Hydroxy-corticosterone
Aldosterone
11β,21-Dihydroxy-3,20-oxo-5β-pregnan-18-al
3α,11β,21-Trihydroxy-20-oxo-5β-pregnan-18-al
11-Dehydro-corticosterone
21-Hydroxy-5β-pregnane-3,11,20-trione
5β-Pregnane-3,20-dione
3α-Hydroxy-5β-pregnane-20-one
Pregnanediol
5α-Pregnane-3,20-dione
3α-Hydroxy-5α-pregnan-20-one
5α-Pregnane-3α,20α-diol
5α-Pregnan-20α-ol-3-one
4-Androsten-11beta-ol-3,17-dione
11β-Hydroxytestosterone
3-Oxo-13,17-secoandrost-4-ene-17,13α-lactone
19-Hydroxyandrost-4-ene-3,17-dione
19-Oxoandrost-4-ene-3,17-dione
Estrone
Estrone 3-sulfate
Estrone glucuronide
Estradiol-17α
2-Methoxyestrone-3-glucuronide
2-Methoxyestrone
2-Hydroxyestrone
2-Methoxyestrone-3-sulfate
16-α-Hydroxyestrone
C18-Steroids
Estradiol-17β
Estriol
16-Glucuronide-estriol
2-Methoxy-estradiol-17β-3-glucuronide
2-Methoxy-estradiol-17β
2-Hydroxy-estradiol-17β
2-Methoxy-estradiol-17β-3-sulfate
6β-Hydroxy-estradiol-17β
C21-Steroids

Data on KEGG graph
Rendered by Pathview



Data on KEGG graph
Rendered by Pathview



Data on KEGG graph
Rendered by Pathview

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

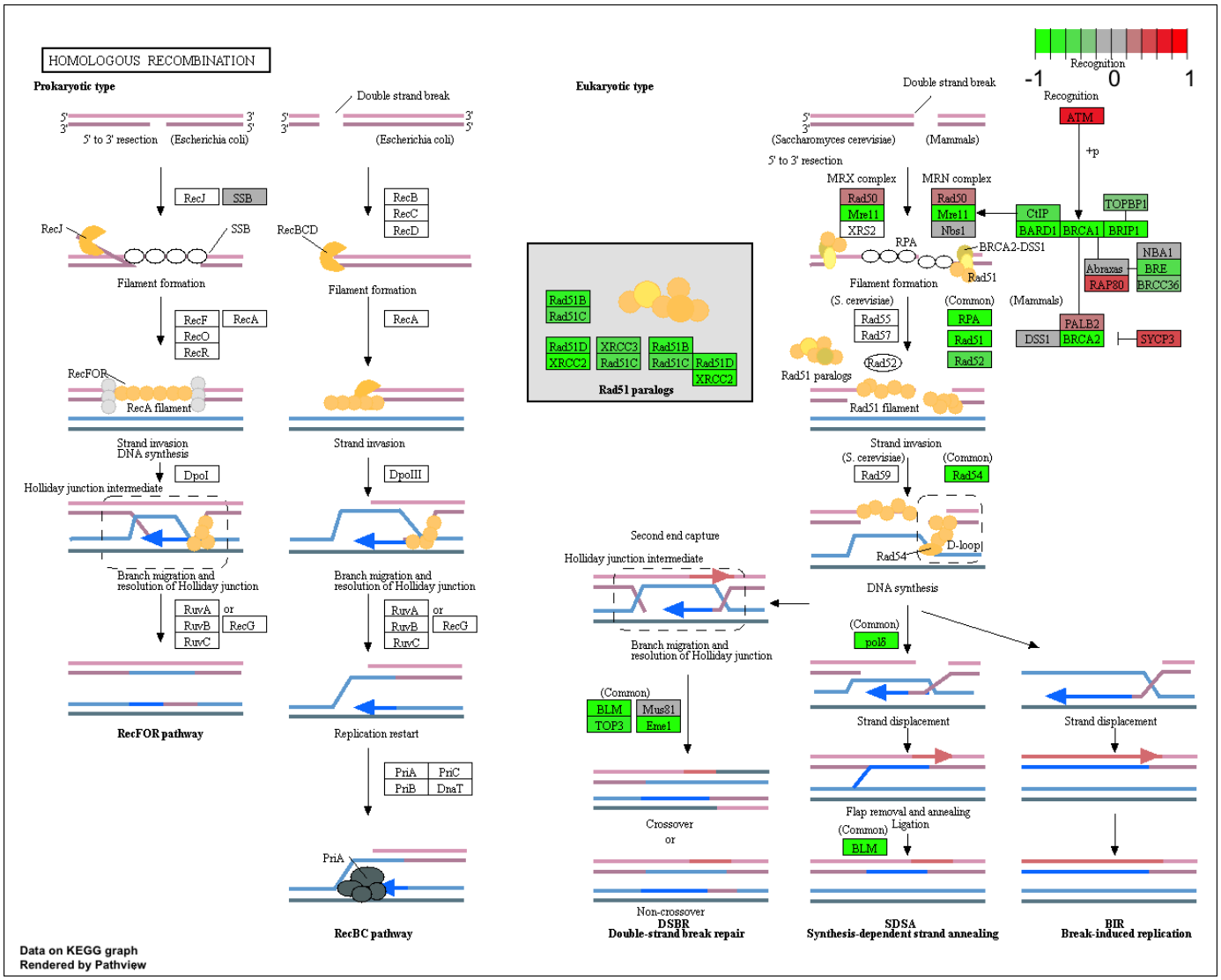
```
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$less)[1:5]
# Extract the 8 character long IDs part of each string
keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
```

```
[1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

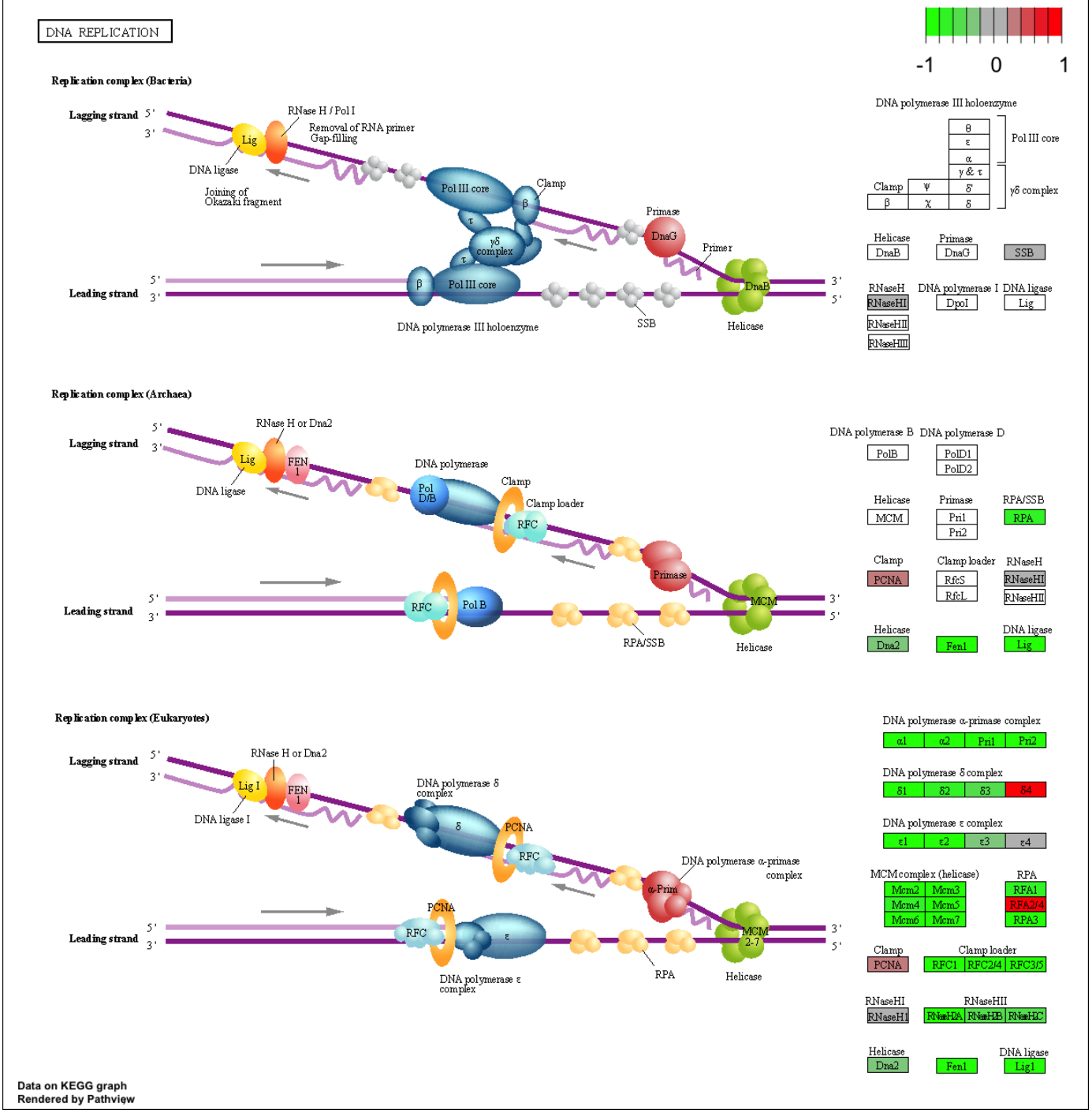
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

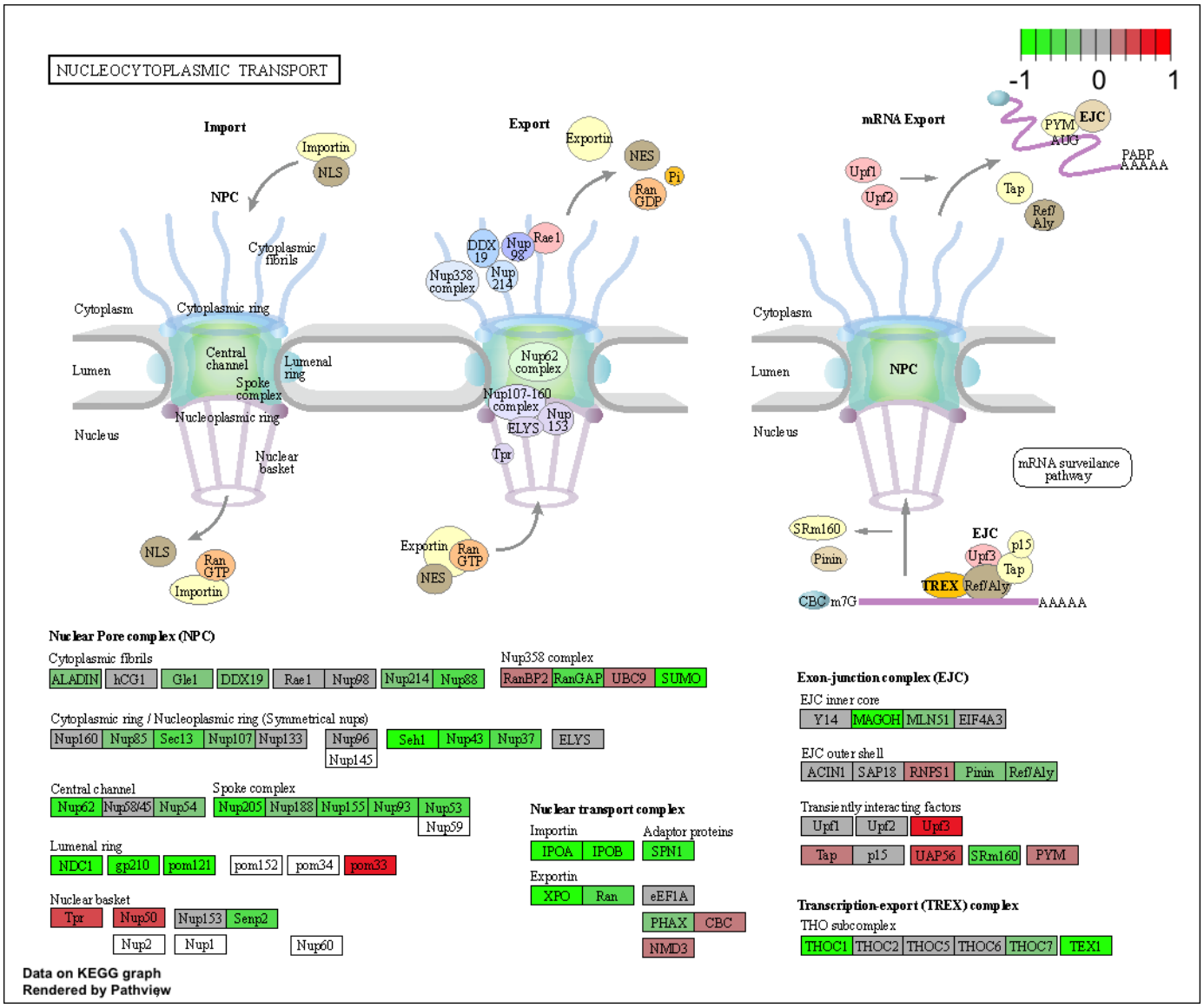
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13

Info: Writing image file hsa03440.pathview.png

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

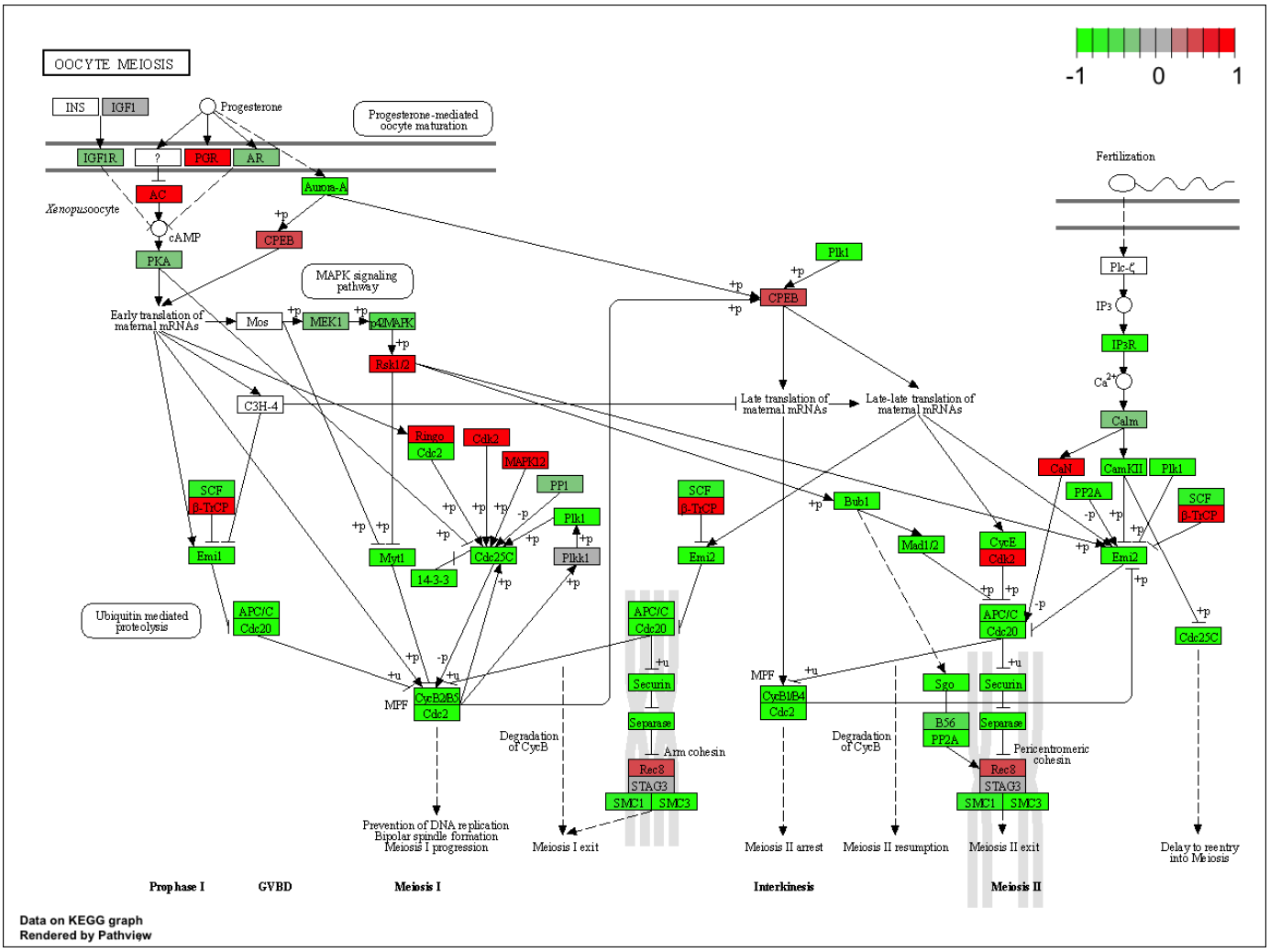
```
Info: Working in directory /Users/ryan/Desktop/BIMM143/Class13

Info: Writing image file hsa04114.pathview.png
```



Section 3. Gene Ontology (GO)


DNA REPLICATION
-1
0
1
Replication complex (Bacteria)
Lagging strand
Leading strand
RNase H / Pol I
Removal of RNA primer
Gap-filling
Lig
DNA ligase
Joining of
Okazaki fragment
Pol III core
Clamp
β
τ
γδ
complex
Primase
DnaG
Primer
DnaB
SSB
Helicase
DNA polymerase III holoenzyme
DNA polymerase III holoenzyme
θ
ε
α
γ & τ
Pol III core
Clamp
ψ
δ'
χ
δ
γδ complex
β
Helicase
DnaB
Primase
DnaG
SSB
RNaseH
RNaseHI
RNaseHII
RNaseHIII
DNA polymerase I
DpoI
DNA ligase
Lig
Replication complex (Archaea)
RNase H or Dna2
FEN
1
DNA polymerase
Pol
D/B
Clamp
Clamp loader
RFC
Primase
MCM
Pol B
RPA/SSB
Helicase
DNA polymerase B
DNA polymerase D
PolB
PolD1
PolD2
Helicase
MCM
Primase
Pri1
Pri2
RPA/SSB
RPA
Clamp
PCNA
Clamp loader
RfcS
RfcL
RNaseH
RNaseHII
RNaseHIII
Helicase
Dna2
Fen1
DNA ligase
Lig
Replication complex (Eukaryotes)
Lig I
DNA ligase I
DNA polymerase δ
complex
δ
PCNA
DNA polymerase α-primase
complex
α-Prim
DNA polymerase ε
complex
ε
RPA
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


NUCLEOCYTOPLASMIC TRANSPORT
-1
0
1
Import
Importin
NLS
NPC
Cytoplasmic fibrils
Cytoplasm
Cytoplasmic ring
Central channel
Lumen
Lumenal ring
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
Nup 153
ELYS
Tpr
Exportin
Ran GTP
NES
mRNA Export
EJC
PYM
AUG
PABP
AAAAA
Upf1
Upf2
Tap
Ref/ Aly
Cytoplasm
Lumen
NPC
Nucleus
mRNA surveilance pathway
SRm160
Pinin
EJC
Upf3
p15
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




```
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
```

```
GO:0048729 tissue morphogenesis           1.432451e-04  3.045242
1.432451e-04
GO:0007610 behavior                       2.195494e-04  3.530241
2.195494e-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                       0.2243795      427 2.195494e-
04
GO:0060562 epithelial tube morphogenesis  0.3711390      257 5.932837e-
04
GO:0035295 tube development               0.3711390      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                        3.530241 3.530241
GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
GO:0035295 tube development                3.253665 3.253665
```

Section 4. Reactome Analysis

```
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"
```

[1] “Total number of significant genes: 8149”

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

> 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?