Class 13 : Pathway Analysis from RNA-Seq Results

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Pathway Analysis from RNA-Seq Results

Section 1. Differential Expression Analysis

Here we play with just one, the GAGE package (which stands for Generally Applicable Gene set Enrichment), to do KEGG pathway enrichment analysis on RNA-seq based differential expression results.

Data Imported:

```r
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, aperm, 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
```

```r
# Count Data and Metadata:
metaFile <- "GSE37704_metadata.csv"
countFile <- "GSE37704_featurecounts.csv"
```

Look at each one:

```r
# Import metadata and take a peak
colData = read.csv(metaFile, row.names=1)
head(colData)
```

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

```
# Import countdata
countData = read.csv(countFile, 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
```

> Q. 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
```

> 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?

```
# Filter count data where you have 0 read count across all samp
countData = countData[rowSums(countData) > 0, ]
head(countData)
```

```
                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
```

```
nrow(countData)
```

```
[1] 15975
```

Running DESeq2

```
dds = DESeqDataSetFromMatrix(countData=countData,
                             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
```

Result:

```
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
```

Create results variable to hold results:

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

> 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.

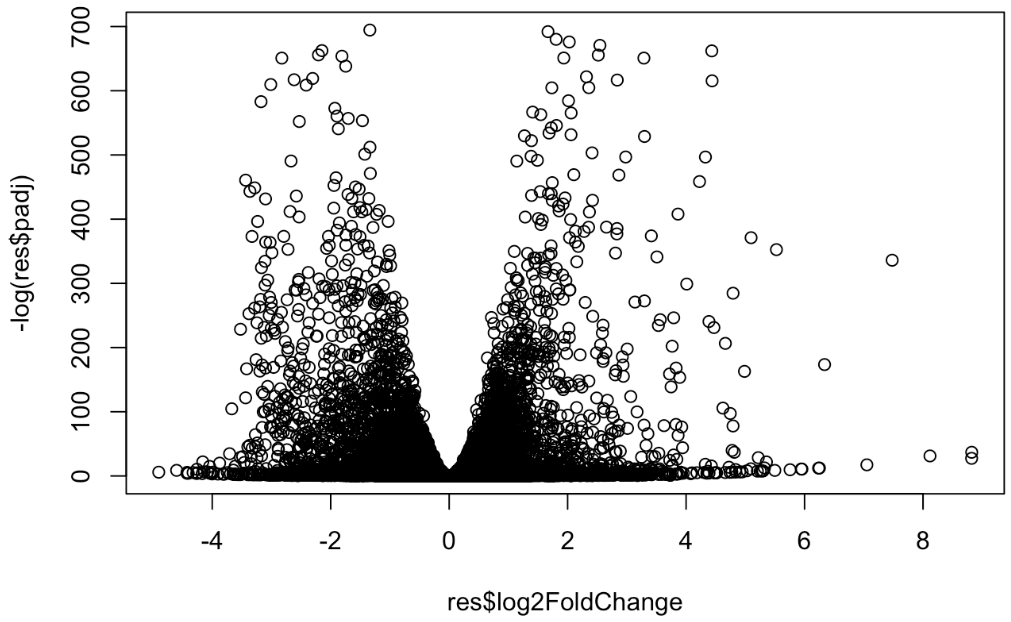
```
summary(res)
```

```
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
```

Volcono plot

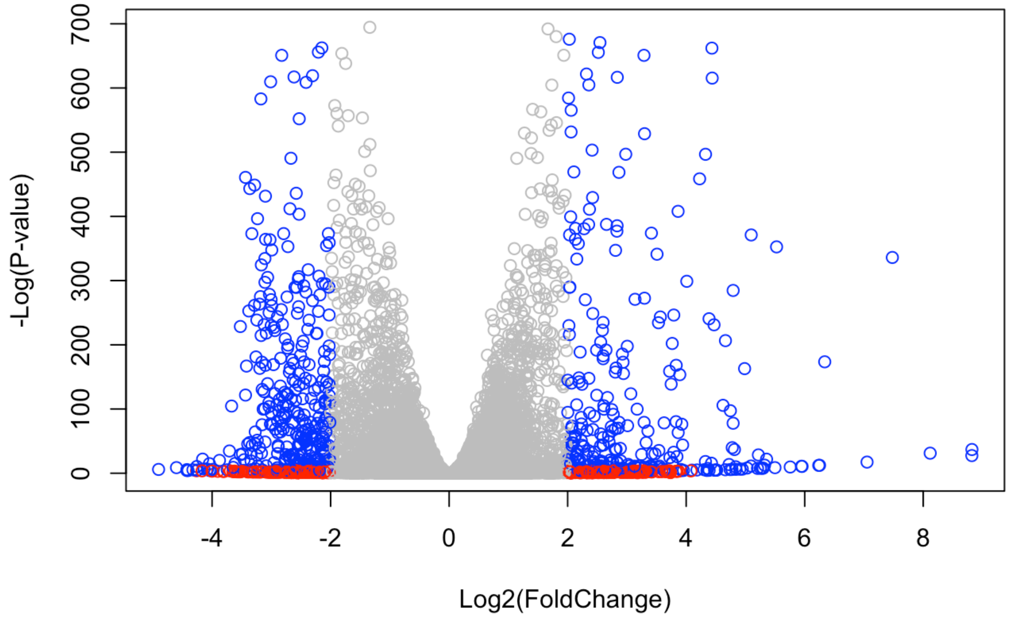
Here is the volcano plot:

```
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 ] <- "red"

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

plot( res$log2FoldChange, -log(res$padj), col=mycols, xlab="Log
```



Adding gene 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(org.Hs.eg.db,
                    keys=row.names(res),
                    keytype="ENSEMBL",
                    column="SYMBOL",
                    multiVals="first")
```

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

```
res$entrez = mapIds(org.Hs.eg.db,
                    keys=row.names(res),
                    keytype="ENSEMBL",
                    column="ENTREZID",
                    multiVals="first")
```

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

```
res$name = mapIds(org.Hs.eg.db,
                    keys=row.names(res),
                    keytype="ENSEMBL",
                    column="GENENAME",
                    multiVals="first")
```

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

```
head(res, 10)
```

```
log2 fold change (MLE): condition hoxa1_kd vs control_sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 10 rows and 9 columns
                   baseMean log2FoldChange     lfcSE
stat      pvalue
                  <numeric>      <numeric> <numeric>
<numeric>   <numeric>
ENSG00000279457   29.913579      0.1792571 0.3248216
0.551863 5.81042e-01
ENSG00000187634  183.229650      0.4264571 0.1402658
3.040350 2.36304e-03
ENSG00000188976 1651.188076     -0.6927205 0.0548465
```

```
-12.630158 1.43990e-36
ENSG00000187961  209.637938      0.7297556 0.1318599
5.534326 3.12428e-08
ENSG00000187583   47.255123      0.0405765 0.2718928
0.149237 8.81366e-01
ENSG00000187642   11.979750      0.5428105 0.5215598
1.040744 2.97994e-01
ENSG00000188290  108.922128      2.0570638 0.1969053
10.446970 1.51282e-25
ENSG00000187608  350.716868      0.2573837 0.1027266
2.505522 1.22271e-02
ENSG00000188157 9128.439422      0.3899088 0.0467163
8.346304 7.04321e-17
ENSG00000237330    0.158192      0.7859552 4.0804729
0.192614 8.47261e-01
                       padj      symbol      entrez
name
                  <numeric> <character> <character>
<character>
ENSG00000279457 6.86555e-01          NA          NA
NA
ENSG00000187634 5.15718e-03      SAMD11      148398 sterile
alpha motif ..
ENSG00000188976 1.76549e-35       NOC2L       26155 NOC2 like
nucleolar ..
ENSG00000187961 1.13413e-07      KLHL17      339451 kelch like
family me..
ENSG00000187583 9.19031e-01     PLEKHN1       84069 pleckstrin
homology ..
ENSG00000187642 4.03379e-01       PERM1       84808 PPARGC1
and ESRR ind..
ENSG00000188290 1.30538e-24        HES4       57801 hes family
bHLH tran..
ENSG00000187608 2.37452e-02       ISG15        9636 ISG15
ubiquitin like..
ENSG00000188157 4.21963e-16        AGRN      375790
agrin
ENSG00000237330          NA      RNF223      401934 ring
finger protein ..
```

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

```
# Least to greatest:
res = res[order(res$pvalue),]
write.csv(res, file="deseq_results.csv")
```

Section 2. Pathway Analysis

We will use the gage package for pathway analysis and the pathview package to draw pathway diagrams.

Package we will use:

```
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).
##############################################################
################
```

```
library(gage)
```

```
library(gageData)

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

# Focus on signaling and metabolic pathways only:
```

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

# Look at 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"  "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"  
```

We used the `mapIDs()` function above to obtain Entrez gene IDs (stored in res $entrez)andwehavethefoldchangeresultsfromDESeq2analysis(storedinres$ log2FoldChange).

```
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 
```

Results:

```
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

What object did `gage()` return?

```
attributes(keggres)
```

```
$names
```

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

Look at first few down(less) pathway results:

```r
head(keggres$less)
```

```
                                         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
hsa00010 Glycolysis / Gluconeogenesis 8.961413e-03 -2.405398
8.961413e-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
hsa00010 Glycolysis / Gluconeogenesis 0.212222694       53
8.961413e-03
```

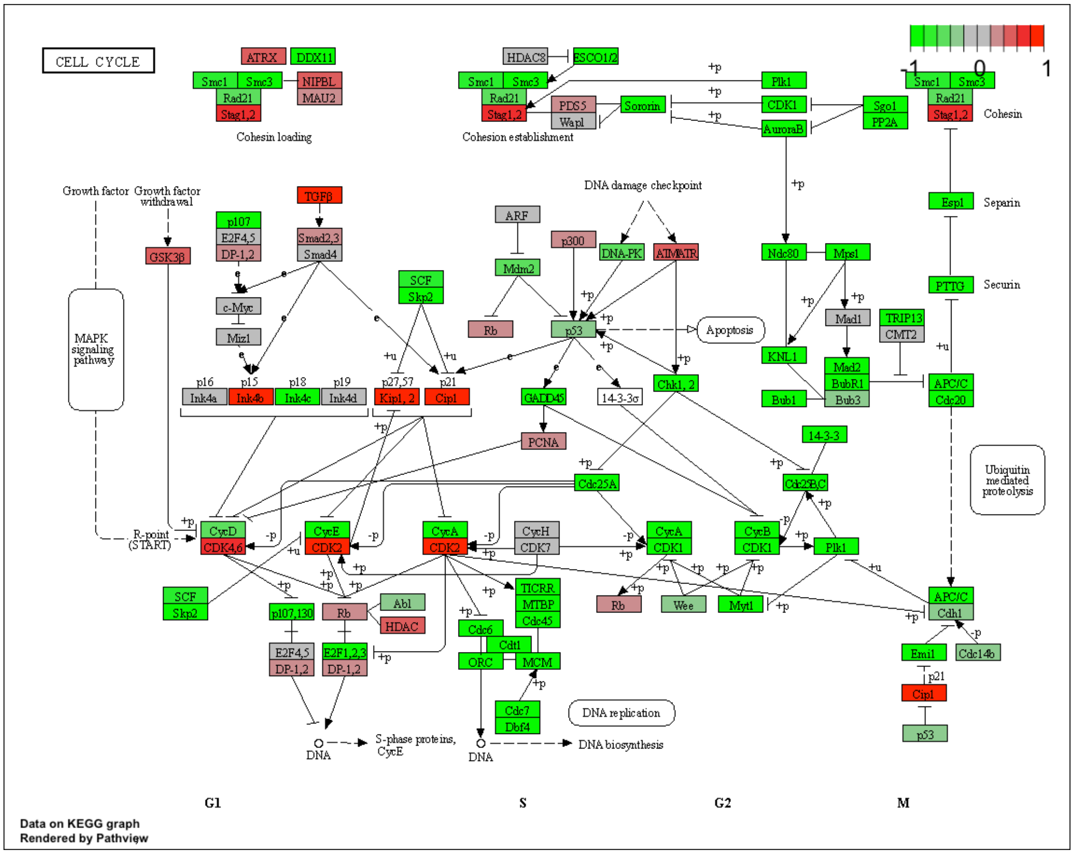
Use the `pathview()` function to make a pathway plot with our RNA-Seq expression results.

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

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

```
Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

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

Here is the pathway:



A different PDF based output of the same data:

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

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

Warning: reconcile groups sharing member nodes!

     [,1] [,2]
[1,] "9"  "300"
[2,] "9"  "306"

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

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

```
## Focus on top 5 upregulated pathways here for demo purposes
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"
```

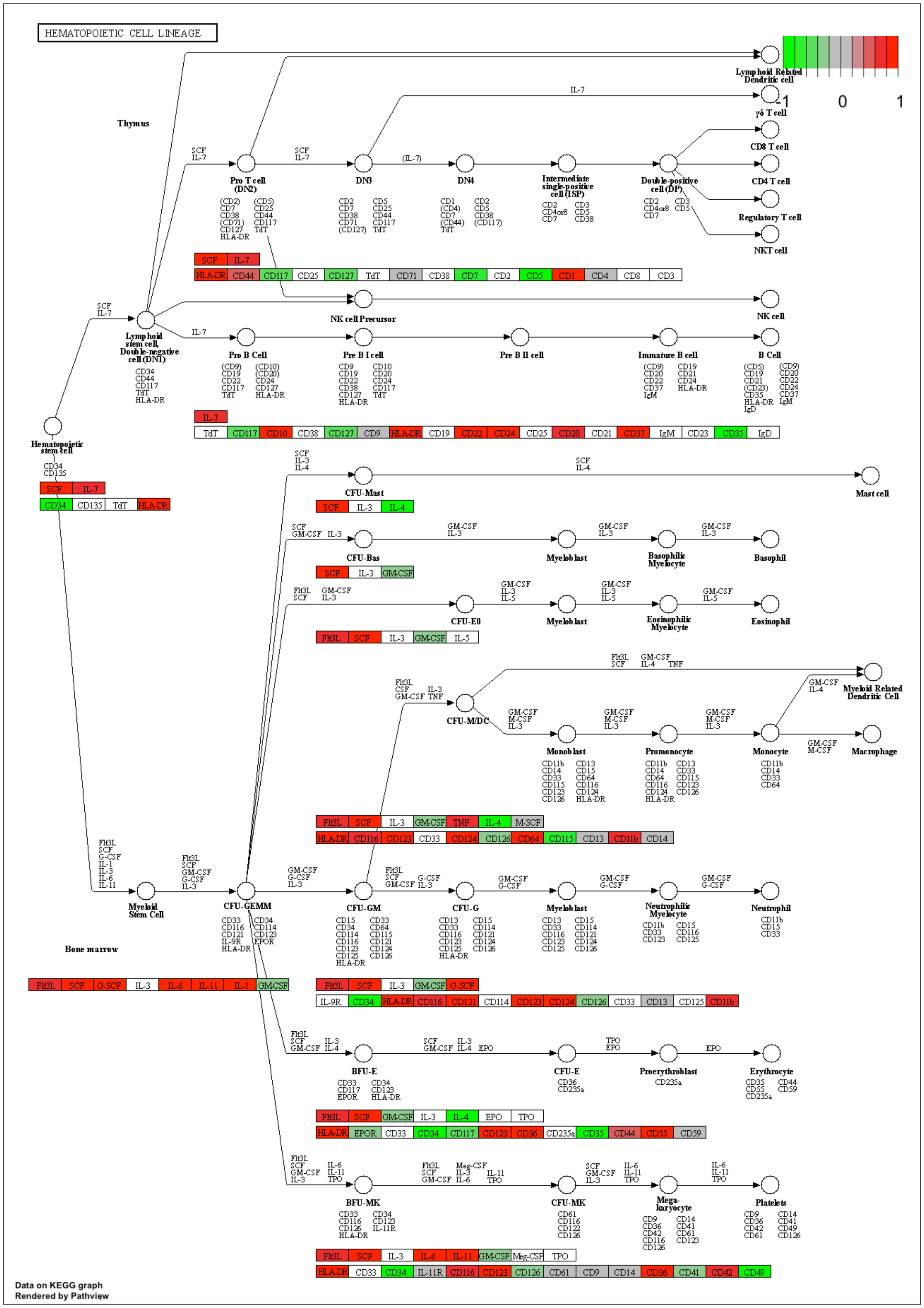
Draw plots for all the top 5 pathways, pass these IDs in keggresids to the pathview() function.

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

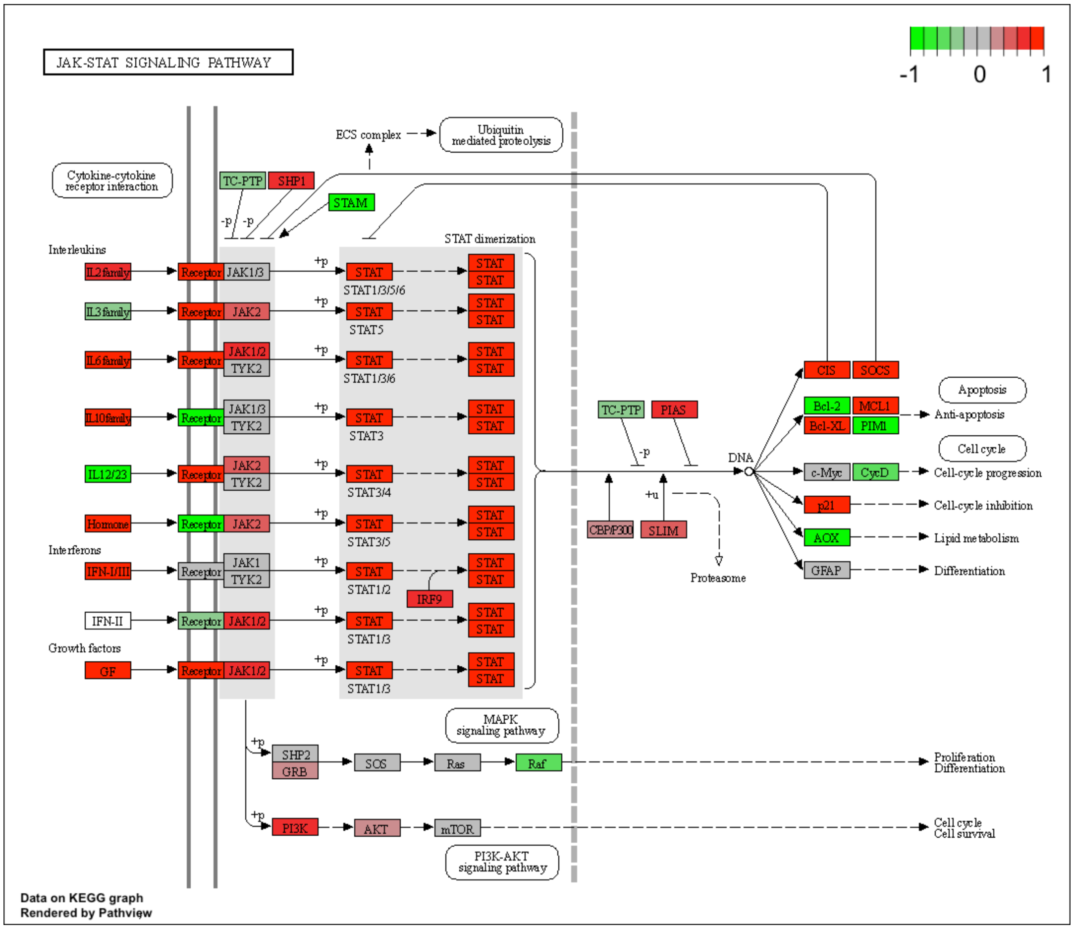
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

Info: Writing image file hsa04142.pathview.png
```

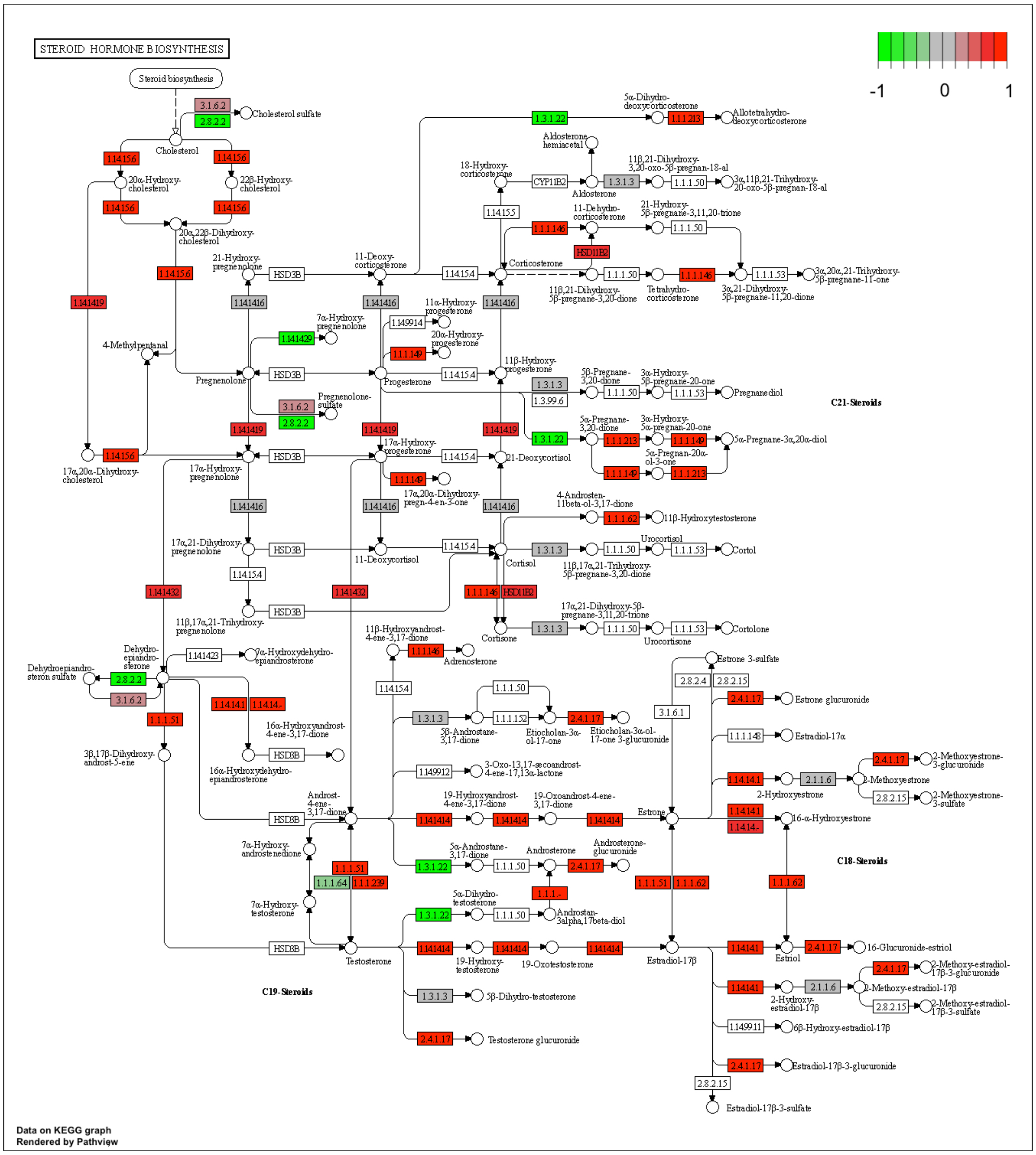
```
Info: some node width is different from others, and hence
adjusted!

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

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

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

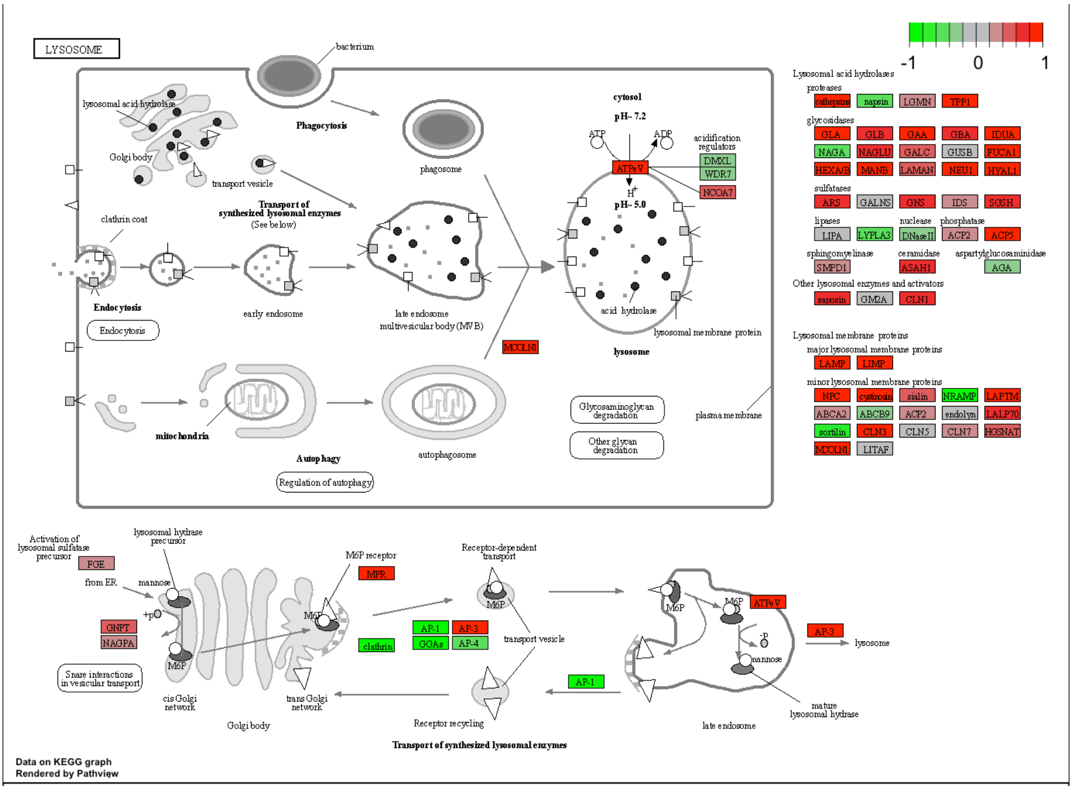
Here are the pathways:

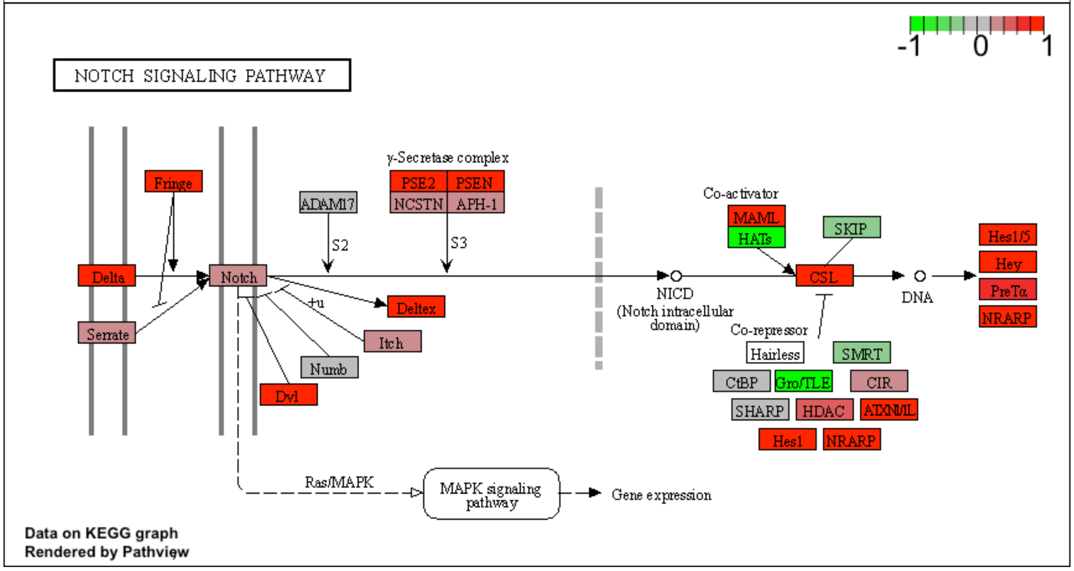

HEMATOPOIETIC CELL LINEAGE
-1
0
1
Thymus
Bone marrow
Hematopoietic stem cell
Lymphoid stem cell, Double-negative cell (DN1)
Pro T cell (DN2)
DN3
DN4
Intermediate single-positive cell (ISP)
Double-positive cell (DP)
Lymphoid Related Dendritic cell
γδ T cell
CD8 T cell
CD4 T cell
Regulatory T cell
NKT cell
NK cell Precursor
NK cell
Pro B Cell
Pre B I cell
Pre B II cell
Immature B cell
B Cell
SCF IL-7
IL-7
(IL-7)
HLA-DR CD44 CD117 CD25 CD127 TdT CD71 CD38 CD7 CD2 CD5 CD1 CD4 CD8 CD3
TdT CD117 CD10 CD38 CD127 CD9 HLA-DR CD19 CD22 CD24 CD25 CD20 CD21 CD37 IgM CD23 CD35 IgD
CD34 CD135 TdT HLA-DR
Myeloid Stem Cell
CFU-GEMM
CFU-Mast
Mast cell
SCF IL-3 IL-4
CFU-Bas
Myeloblast
Basophilic Myelocyte
Basophil
SCF IL-3 GM-CSF
CFU-E0
Eosinophilic Myelocyte
Eosinophil
Flt3L SCF IL-3 GM-CSF IL-5
CFU-M/DC
Monoblast
Promonocyte
Monocyte
Macrophage
Myeloid Related Dendritic Cell
Flt3L SCF IL-3 GM-CSF TNF IL-4 M-SCF
HLA-DR CD116 CD123 CD33 CD124 CD126 CD64 CD115 CD13 CD11b CD14
Flt3L SCF G-SCF IL-3 IL-6 IL-11 IL-1 GM-CSF
CFU-GM
CFU-G
Neutrophilic Myelocyte
Neutrophil
Flt3L SCF IL-3 GM-CSF G-SCF
IL-9R CD34 HLA-DR CD116 CD121 CD114 CD123 CD124 CD126 CD33 CD13 CD125 CD11b
BFU-E
CFU-E
Proerythroblast
Erythrocyte
Flt3L SCF GM-CSF IL-3 IL-4 EPO TPO
HLA-DR EPOR CD33 CD34 CD117 CD123 CD36 CD235a CD35 CD44 CD55 CD59
BFU-MK
CFU-MK
Mega-karyocyte
Platelets
Flt3L SCF IL-3 IL-6 IL-11 GM-CSF Meg-CSF TPO
HLA-DR CD33 CD34 IL-11R CD116 CD123 CD126 CD61 CD9 CD14 CD36 CD41 CD42 CD49
Data on KEGG graph
Rendered by Pathview

]


JAK-STAT SIGNALING PATHWAY
-1
0
1
ECS complex
Ubiquitin mediated proteolysis
Cytokine-cytokine receptor interaction
TC-PTP
SHP1
STAM
-p
-p
STAT dimerization
Interleukins
IL2 family
IL3 family
IL6 family
IL10 family
IL12/23
Hormone
Interferons
IFN-I/III
IFN-II
Growth factors
GF
Receptor
JAK1/3
JAK2
JAK1/2
TYK2
JAK1
+p
STAT
STAT1/3/5/6
STAT5
STAT1/3/6
STAT3
STAT3/4
STAT3/5
STAT1/2
STAT1/3
IRF9
TC-PTP
PIAS
CBP/P300
SLIM
+u
Proteasome
DNA
CIS
SOCS
Bcl-2
MCL1
Bcl-XL
PIM1
Apoptosis
Anti-apoptosis
Cell cycle
c-Myc
CycD
Cell-cycle progression
p21
Cell-cycle inhibition
AOX
Lipid metabolism
GFAP
Differentiation
MAPK signaling pathway
SHP2
GRB
SOS
Ras
Raf
Proliferation
Differentiation
PI3K
AKT
mTOR
Cell cycle
Cell survival
PI3K-AKT signaling pathway
Data on KEGG graph
Rendered by Pathview


STEROID HORMONE BIOSYNTHESIS
Steroid biosynthesis
-1
0
1
3.1.6.2
2.8.2.2
Cholesterol sulfate
Cholesterol
1.14.15.6
20α-Hydroxy-cholesterol
22β-Hydroxy-cholesterol
20α,22β-Dihydroxy-cholesterol
1.14.14.19
4-Methylpentanal
17α,20α-Dihydroxy-cholesterol
21-Hydroxy-pregnenolone
HSD3B
1.14.14.16
7α-Hydroxy-pregnenolone
1.14.14.29
Pregnenolone
Pregnenolone-sulfate
17α-Hydroxy-pregnenolone
17α,21-Dihydroxy-pregnenolone
1.14.15.4
11β,17α,21-Trihydroxy-pregnenolone
1.14.14.32
11-Deoxy-corticosterone
11α-Hydroxy-progesterone
1.14.99.14
20α-Hydroxy-progesterone
1.1.1.149
Progesterone
17α-Hydroxy-progesterone
17α,20α-Dihydroxy-pregn-4-en-3-one
11-Deoxycortisol
18-Hydroxy-corticosterone
1.14.15.5
CYP11B2
Aldosterone
Aldosterone-hemiacetal
1.3.1.3
11β,21-Dihydroxy-3,20-oxo-5β-pregnan-18-al
1.1.1.50
3α,11β,21-Trihydroxy-20-oxo-5β-pregnan-18-al
1.3.1.22
5α-Dihydro-deoxycorticosterone
1.1.1.213
Allotetrahydro-deoxycorticosterone
1.1.1.146
11-Dehydro-corticosterone
21-Hydroxy-5β-pregnane-3,11,20-trione
HSD11B2
Corticosterone
11β,21-Dihydroxy-5β-pregnane-3,20-dione
Tetrahydro-corticosterone
3α,21-Dihydroxy-5β-pregnane-11,20-dione
1.1.1.53
3α,20α,21-Trihydroxy-5β-pregnane-11-one
11β-Hydroxy-progesterone
1.3.99.6
5β-Pregnane-3,20-dione
3α-Hydroxy-5β-pregnane-20-one
Pregnanediol
C21-Steroids
5α-Pregnane-3,20-dione
3α-Hydroxy-5α-pregnan-20-one
5α-Pregnan-20α-ol-3-one
5α-Pregnane-3α,20α-diol
21-Deoxycortisol
4-Androsten-11beta-ol-3,17-dione
1.1.1.62
11β-Hydroxytestosterone
Cortisol
11β,17α,21-Trihydroxy-5β-pregnane-3,20-dione
Urocortisol
Cortol
Cortisone
17α,21-Dihydroxy-5β-pregnane-3,11,20-trione
Urocortisone
Cortolone
Dehydroepiandro-steron sulfate
Dehydro-epiandro-sterone
1.14.14.23
7α-Hydroxydehydro-epiandrosterone
1.1.1.51
1.14.14.1
1.14.14.-
3β,17β-Dihydroxy-androst-5-ene
16α-Hydroxyandrost-4-ene-3,17-dione
16α-Hydroxydehydro-epiandrosterone
11β-Hydroxyandrost-4-ene-3,17-dione
Adrenosterone
Androst-4-ene-3,17-dione
7α-Hydroxy-androstenedione
1.1.1.64
1.1.1.239
7α-Hydroxy-testosterone
Testosterone
C19-Steroids
5β-Androstane-3,17-dione
1.1.1.152
Etiocholan-3α-ol-17-one
2.4.1.17
Etiocholan-3α-ol-17-one 3-glucuronide
1.14.99.12
3-Oxo-13,17-secoandrost-4-ene-17,13α-lactone
19-Hydroxyandrost-4-ene-3,17-dione
1.14.14.14
19-Oxoandrost-4-ene-3,17-dione
5α-Androstane-3,17-dione
Androsterone
Androsterone-glucuronide
1.1.1.-
5α-Dihydro-testosterone
Androstan-3alpha,17beta-diol
19-Hydroxy-testosterone
19-Oxotestosterone
5β-Dihydro-testosterone
Testosterone glucuronide
Estrone 3-sulfate
2.8.2.4
2.8.2.15
3.1.6.1
Estrone glucuronide
1.1.1.148
Estradiol-17α
2-Methoxyestrone-3-glucuronide
2-Hydroxyestrone
2.1.1.6
2-Methoxyestrone
2-Methoxyestrone-3-sulfate
Estrone
16-α-Hydroxyestrone
C18-Steroids
Estradiol-17β
Estriol
16-Glucuronide-estriol
2-Methoxy-estradiol-17β-3-glucuronide
2-Hydroxy-estradiol-17β
2-Methoxy-estradiol-17β
2-Methoxy-estradiol-17β-3-sulfate
1.14.99.11
6β-Hydroxy-estradiol-17β
Estradiol-17β-3-glucuronide
Estradiol-17β-3-sulfate
Data on KEGG graph
Rendered by Pathview





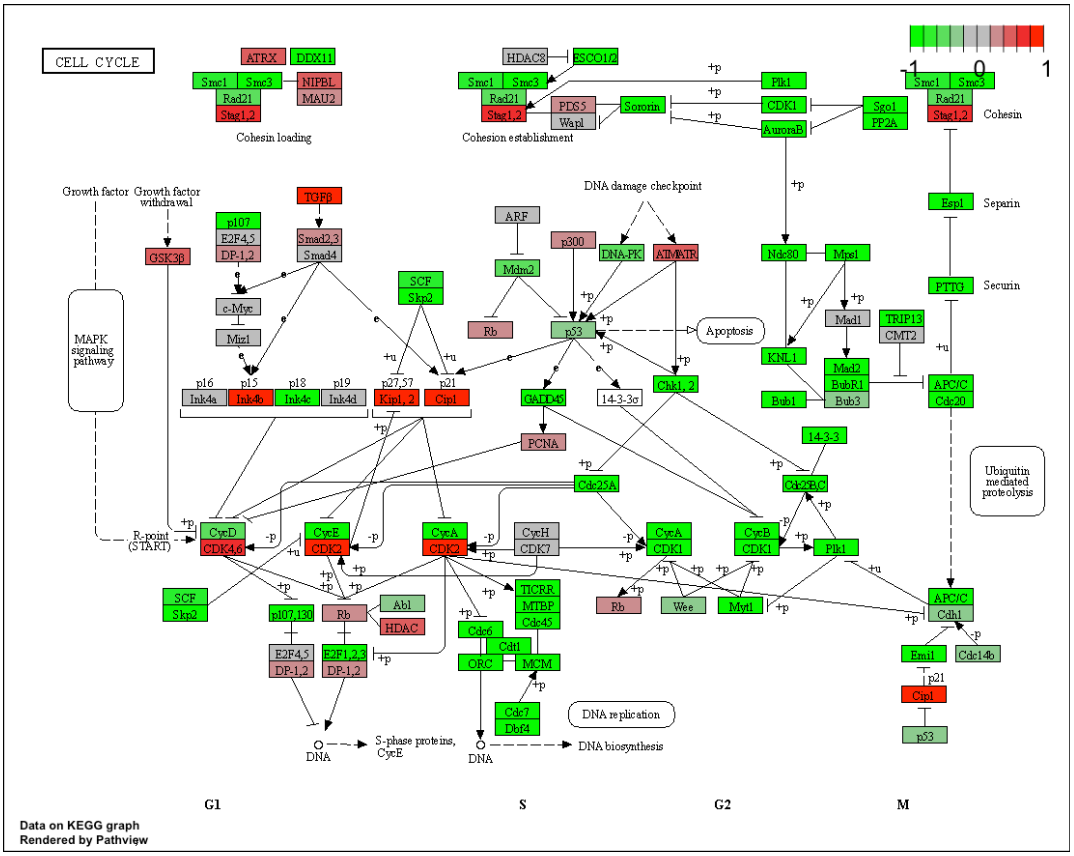
> Q. Can you do the same procedure as above to plot the pathview figures for the top 5 down-reguled pathways?

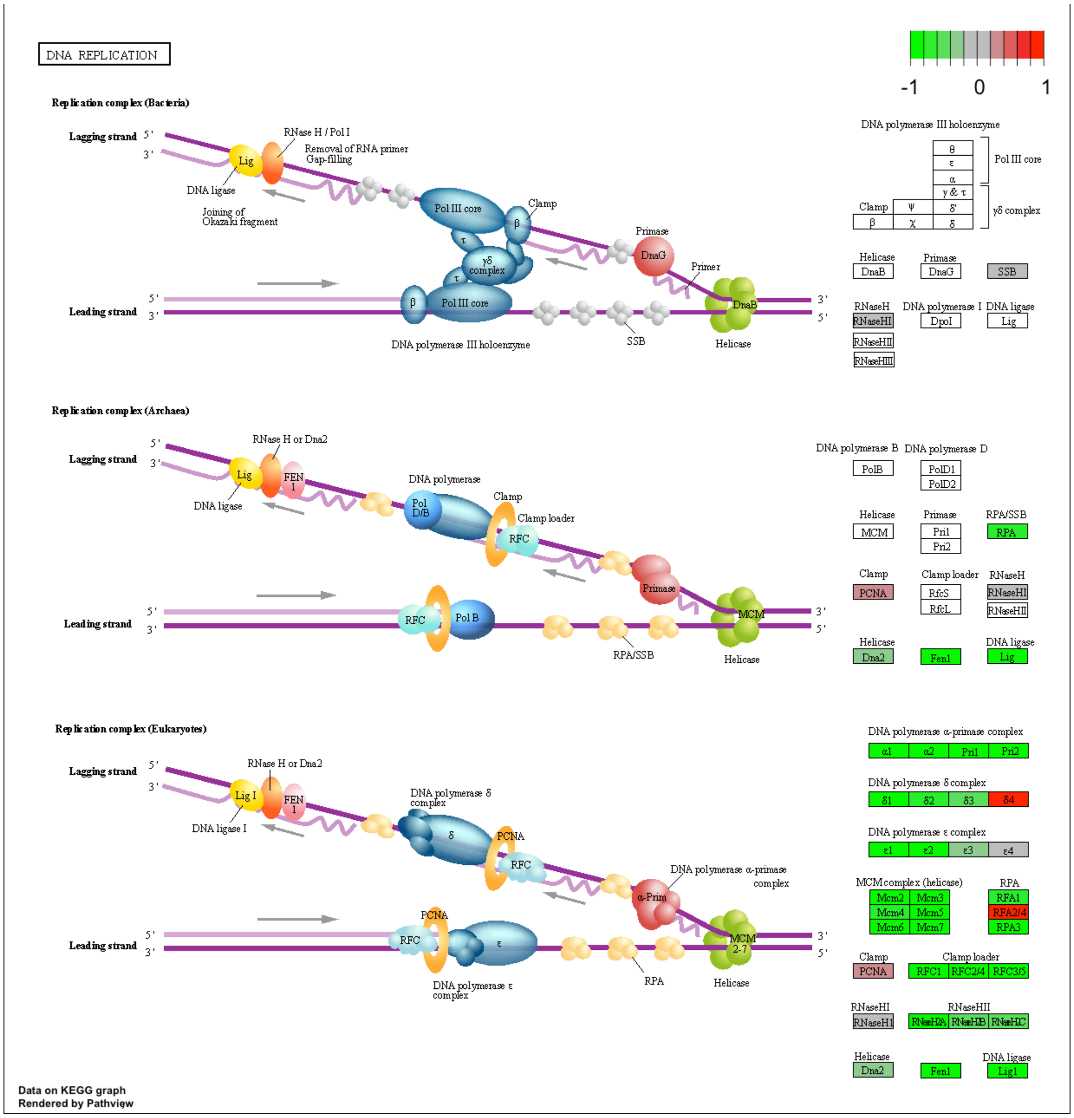
```
## Focus on top 5 downregulated pathways here
keggrespathways <- rownames(keggres$less)[1:5]

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

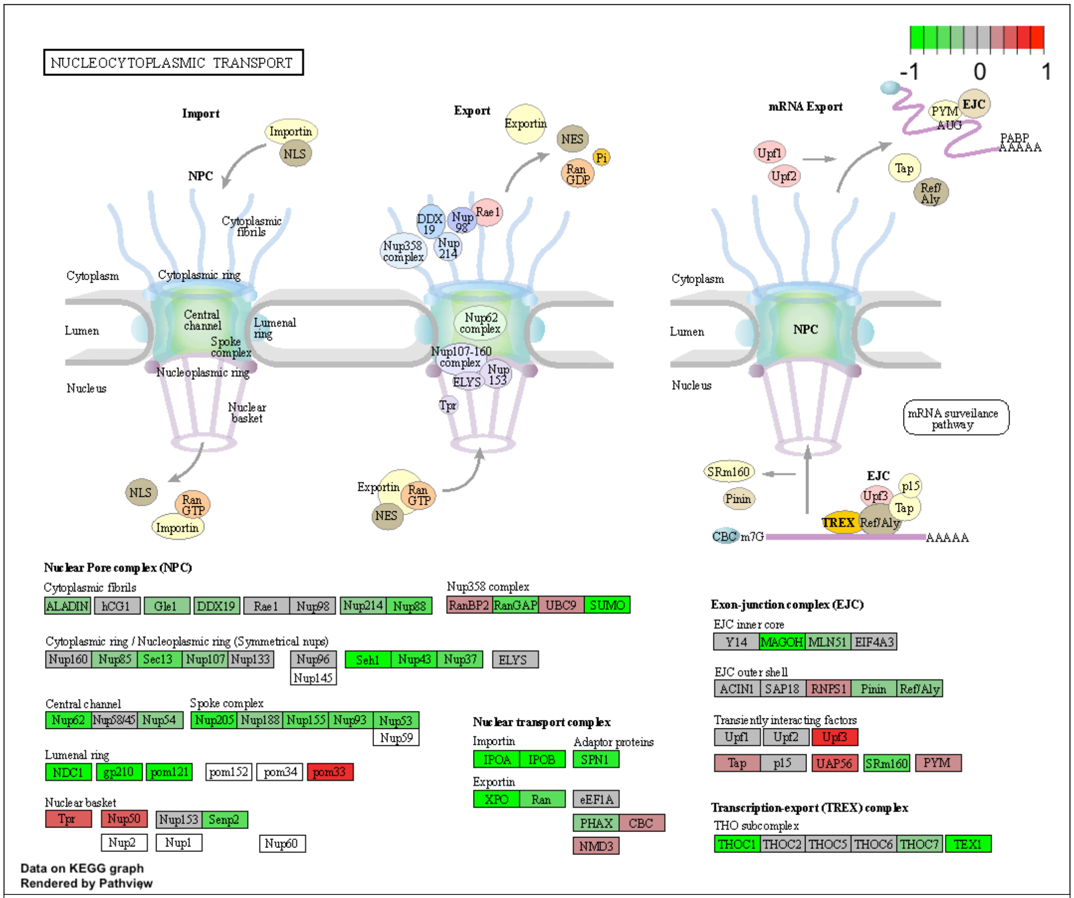
```
[1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

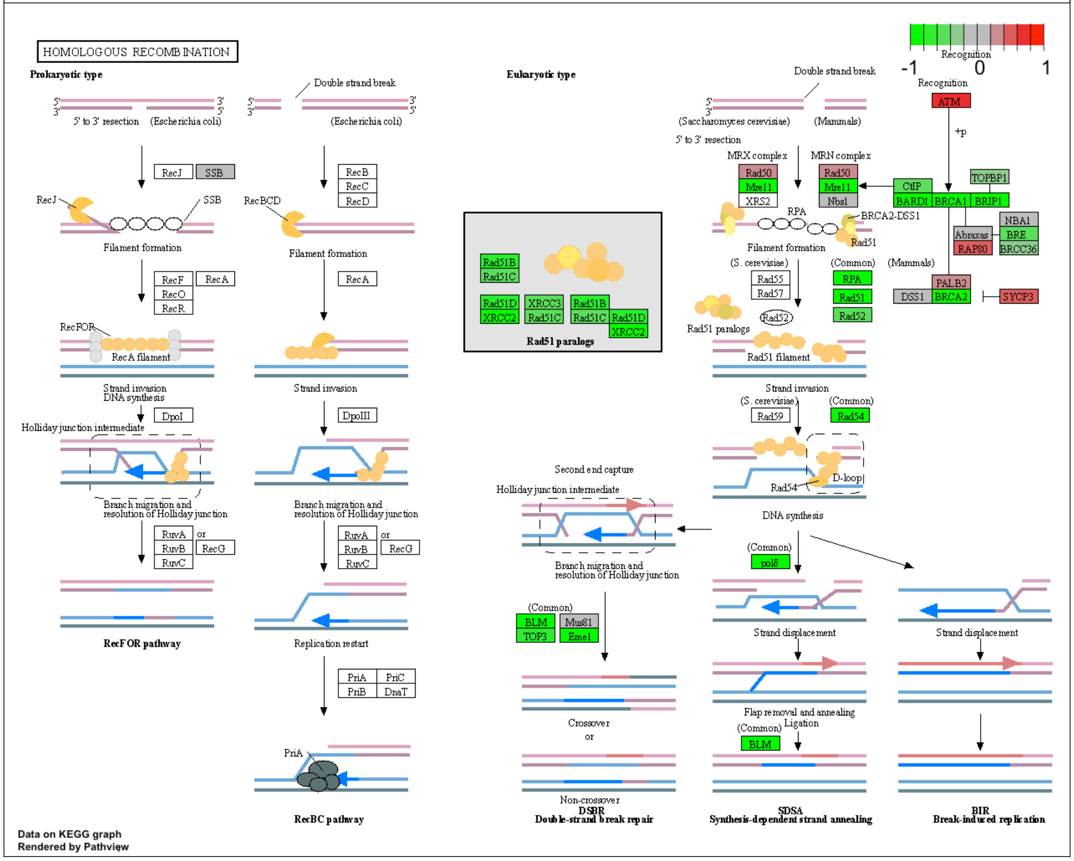
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species=
```

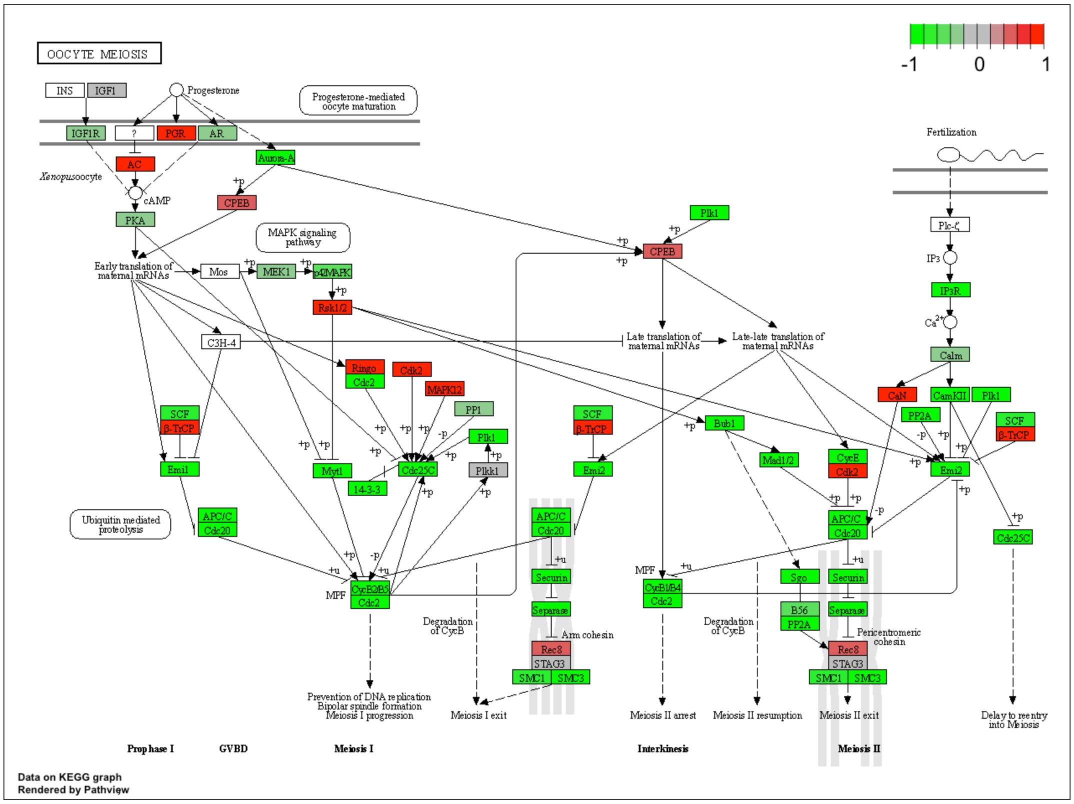
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

Info: Writing image file hsa03440.pathview.png

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

Info: Working in directory
/Users/ltsamaan/Desktop/BIMM143/class13

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


CELL CYCLE
-1 0 1
ATRX
DDX11
Smc1
Smc3
NIPBL
Rad21
MAU2
Stag1,2
Cohesin loading
HDAC8
ESCO1/2
Smc1
Smc3
Rad21
PDS5
Sororin
Stag1,2
Wapl
Cohesion establishment
Plk1
CDK1
AuroraB
Sgo1
PP2A
Smc1
Smc3
Rad21
Stag1,2
Cohesin
Growth factor
Growth factor withdrawal
GSK3β
p107
E2F4,5
DP-1,2
TGFβ
Smad2,3
Smad4
c-Myc
Miz1
SCF
Skp2
ARF
Mdm2
Rb
p300
DNA damage checkpoint
DNA-PK
ATM/ATR
p53
Apoptosis
Ndc80
Mps1
KNL1
Mad1
Mad2
BubR1
Bub1
Bub3
TRIP13
CMT2
Esp1
Separin
PTTG
Securin
APC/C
Cdc20
MAPK signaling pathway
p16
Ink4a
p15
Ink4b
p18
Ink4c
p19
Ink4d
p27,57
Kip1, 2
p21
Cip1
GADD45
14-3-3σ
Chk1, 2
14-3-3
PCNA
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
TICRR
MTBP
Cdc45
Rb
Wee
Myt1
APC/C
Cdh1
E2F4,5
DP-1,2
E2F1,2,3
DP-1,2
Cdc6
Cdt1
ORC
MCM
Cdc7
Dbf4
DNA replication
Emi1
Cdc14b
p21
Cip1
p53
DNA
S-phase proteins, CycE
DNA
DNA biosynthesis
+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'
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
Leading strand
Pol III core
DNA polymerase III holoenzyme
SSB
Helicase
DnaB
DNA polymerase III holoenzyme
θ
ε
α
γ & τ
δ'
δ
Clamp
ψ
β
χ
Pol III core
γδ complex
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
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)
Lig I
DNA ligase I
DNA polymerase δ
complex
δ
PCNA
RFC
DNA polymerase α-primase
complex
α-Prim
MCM
2-7
ε
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
RPA1
RPA2/4
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
Lumen
Central channel
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
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
Cenp2
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


HOMOLOGOUS RECOMBINATION
Recognition
-1 0 1
Prokaryotic type
5' 3' 3' 5'
5' to 3' resection
(Escherichia coli)
Double strand break
(Escherichia coli)
RecJ
SSB
RecB
RecC
RecD
RecJ
SSB
RecBCD
Filament formation
Filament formation
RecF
RecO
RecR
RecA
RecA
RecFOR
RecA filament
Strand invasion DNA synthesis
Strand invasion
DpoI
DpoIII
Holliday junction intermediate
Branch migration and resolution of Holliday junction
Branch migration and resolution of Holliday junction
RuvA
RuvB
RuvC
or
RecG
RuvA
RuvB
RuvC
or
RecG
RecFOR pathway
Replication restart
PriA
PriB
PriC
DnaT
PriA
RecBC pathway
Eukaryotic type
Rad51B
Rad51C
Rad51D
XRCC2
XRCC3
Rad51C
Rad51B
Rad51C
Rad51D
XRCC2
Rad51 paralogs
Double strand break
(Saccharomyces cerevisiae)
(Mammals)
5' to 3' resection
Recognition
ATM
+p
MRX complex
MRN complex
Rad50
Mre11
XRS2
Rad50
Mre11
Nbs1
CtIP
TOPBP1
BARD1
BRCA1
BRIP1
RPA
BRCA2-DSS1
Rad51
NBA1
Abraxas
BRE
RAP80
BRCC36
Filament formation
(S. cerevisiae)
(Common)
(Mammals)
Rad55
Rad57
RPA
Rad51
Rad52
PALB2
DSS1
BRCA2
SYCP3
Rad52
Rad51 paralogs
Rad51 filament
Strand invasion
(S. cerevisiae)
(Common)
Rad59
Rad54
Rad54
D-loop
Second end capture
Holliday junction intermediate
Branch migration and resolution of Holliday junction
DNA synthesis
(Common)
polδ
(Common)
BLM
TOP3
Mus81
Eme1
Strand displacement
Strand displacement
Crossover
or
Non-crossover
Flap removal and annealing
(Common) Ligation
BLM
DSBR
Double-strand break repair
SDSA
Synthesis-dependent strand annealing
BIR
Break-induced replication
Data on KEGG graph
Rendered by Pathview



Section 3. Gene Ontology (GO)

Similar procedure with gene ontology.

```
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                       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
```