class14

Mason Lew (PID :A17533139)

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Background

The data for for hands-on session comes from GEO entry: GSE37704, which is associated with the following publication:

> Trapnell C, Hendrickson DG, Sauvageau M, Goff L et al. "Differential analysis of gene regulation at transcript resolution with RNA-seq". Nat Biotechnol 2013 Jan;31(1):46-53. PMID: 23222703

The authors report on differential analysis of lung fibroblasts in response to loss of the developmental transcription factor HOXA1. Their results and others indicate that HOXA1 is required for lung fibroblast and HeLa cell cycle progression. In particular their analysis show that "loss of HOXA1 results in significant expression level changes in thousands of individual transcripts, along with isoform switching events in key regulators of the cell cycle". For our session we have used their Sailfish gene-level estimated counts and hence are restricted to protein-coding genes only.

Data Import

Loading our data:

```
metaFile <- "/Users/Mason/Desktop/BIMM143/class13/GSE37704_metadata.csv"
countFile <- "/Users/Mason/Desktop/BIMM143/class13/GSE37704_featurecounts.csv"

# 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
```

Inspect and Tidy data

```r
# 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 How many genes total

```r
nrow(countData)
```

```
[1] 19808
```

Q. Filter and remove 0 data

```r
# Filter count data where you have 0 read count across all samples.
sumdata <- rowSums(countData)

countData = countData[(sumdata != 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
```

Q. how many genes are left

```r
nrow(countData)
```

```
[1] 15975
```

##Setup for DESeq

```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, saveRDS, setdiff,
    table, tapply, union, 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


Attaching package: 'IRanges'
```

```
The following object is masked from 'package:grDevices':

    windows

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
```

##Running DESeq

```r
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
```

```r
dds = DESeq(dds)
```

```
estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

```r
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
```

```r
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.

```r
summary(res)
```

```

out of 15975 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 4349, 27%
LFC < 0 (down)     : 4396, 28%
outliers [1]       : 0, 0%
low counts [2]     : 1237, 7.7%
(mean count < 0)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results
```

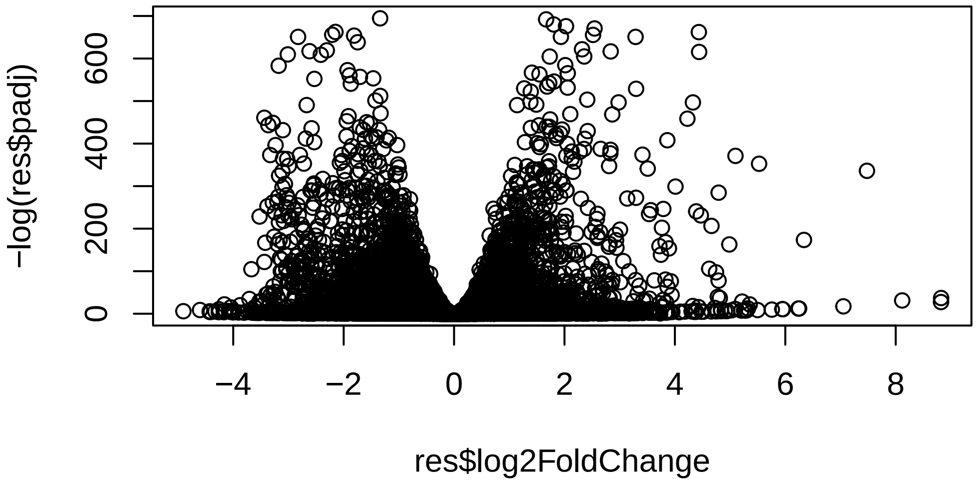
```r
head(res)
```

```
log2 fold change (MLE): condition hoxa1_kd vs control_sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 6 rows and 6 columns
                 baseMean log2FoldChange     lfcSE      stat      pvalue
                <numeric>      <numeric> <numeric> <numeric>   <numeric>
ENSG00000279457   29.9136      0.1792571 0.3248216   0.551863 5.81042e-01
ENSG00000187634  183.2296      0.4264571 0.1402658   3.040350 2.36304e-03
ENSG00000188976 1651.1881     -0.6927205 0.0548465 -12.630158 1.43990e-36
ENSG00000187961  209.6379      0.7297556 0.1318599   5.534326 3.12428e-08
ENSG00000187583   47.2551      0.0405765 0.2718928   0.149237 8.81366e-01
ENSG00000187642   11.9798      0.5428105 0.5215598   1.040744 2.97994e-01
```

```
                        padj
                   <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76549e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
ENSG00000187642 4.03379e-01
```

Volcano plot of results

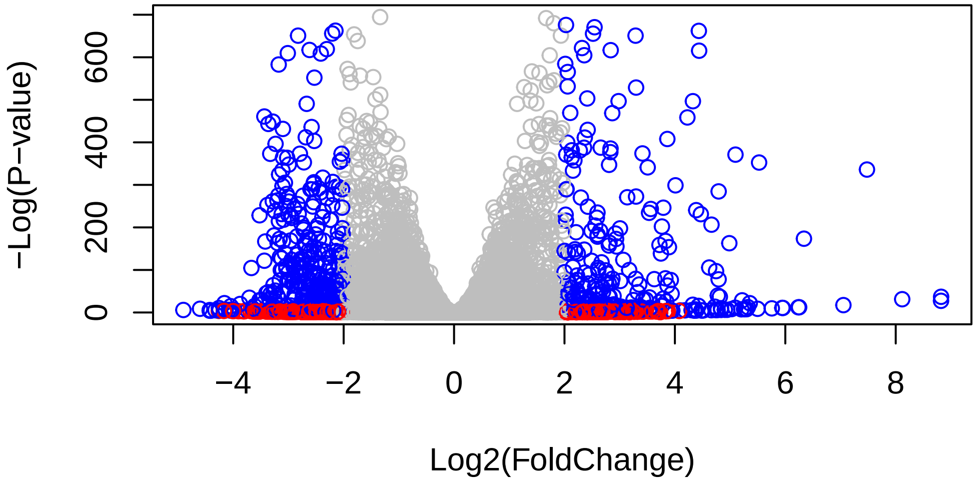
```
plot( res$log2FoldChange, -log(res$padj) )
```



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"
```

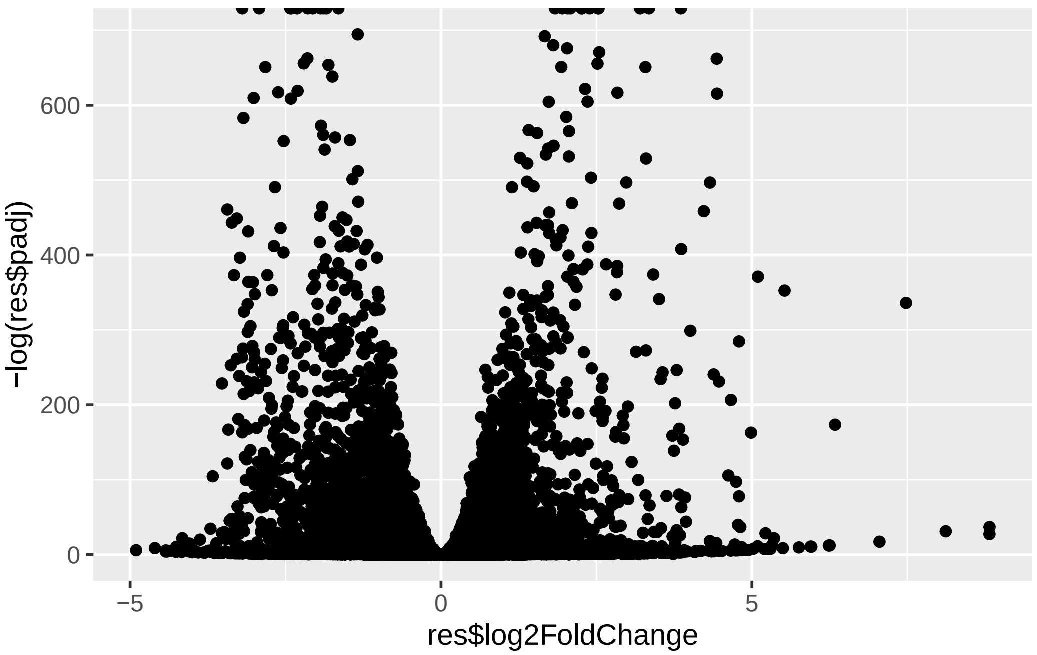
```
# Color blue those with adjusted p-value less than 0.01
#  and absolute fold change more than 2
inds <- (res$pvalue < .01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

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



This time in ggplot2

```
library(ggplot2)

ggplot(res, aes(x= res$log2FoldChange, y = -log(res$padj))) +
         geom_point()
```

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



Gene annotation

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

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

```r
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"
```

```r
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
```

```r
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
```

```r
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
```

```r
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.

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

Pathway analysis

Now we can load the packages and setup the KEGG data-sets we need.

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

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

```r
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"  "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"  
```

The main **gage()** function requires a named vector of fold changes, where the names of the values are the Entrez gene IDs.

Note that we used the mapIDs() function above to obtain Entrez gene IDs (stored in **res$entrez**) and we have the fold change results from DESeq2 analysis (stored in **res$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 
```

Now, let's run the gage pathway analysis.

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

```
attributes(keggres)
```

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

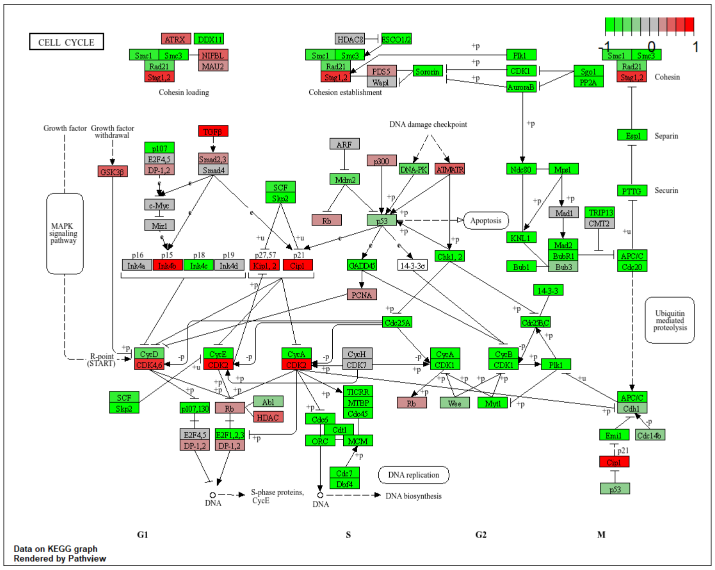
```
# Look at the first few down (less) pathways
head(keggres$less)
```

```
                                         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
```

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

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

Info: Working in directory C:/Users/Mason/Desktop/BIMM143/class14

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



```r
# A different PDF based output of the same data
pathview(gene.data=foldchanges, pathway.id="hsa04110", kegg.native=FALSE)
```

```
'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 C:/Users/Mason/Desktop/BIMM143/class14
```

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

Now, let's process our results a bit more to automagicaly pull out the top 5 upregulated pathways, then further process that just to get the pathway IDs needed by the pathview() function. We'll use these KEGG pathway IDs for pathview plotting below.

```
## 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 C:/Users/Mason/Desktop/BIMM143/class14
```

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

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

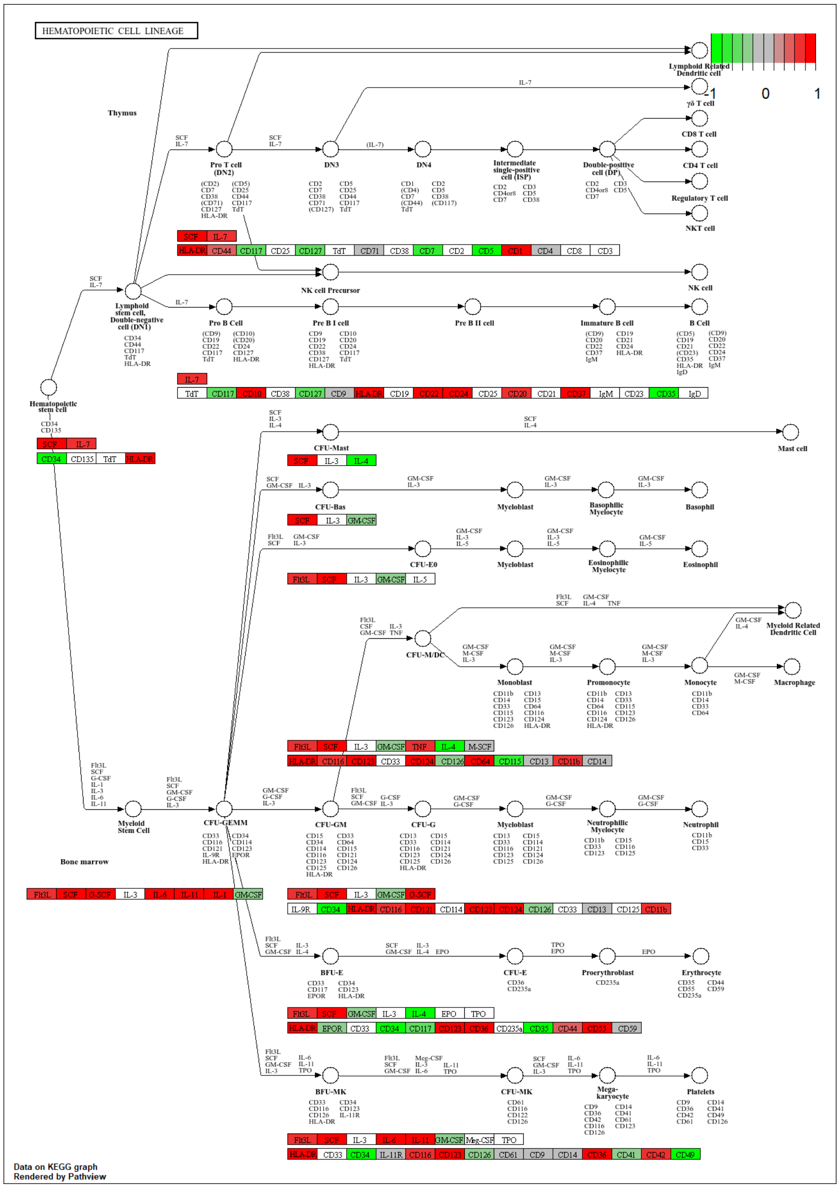
```
Info: Working in directory C:/Users/Mason/Desktop/BIMM143/class14
```

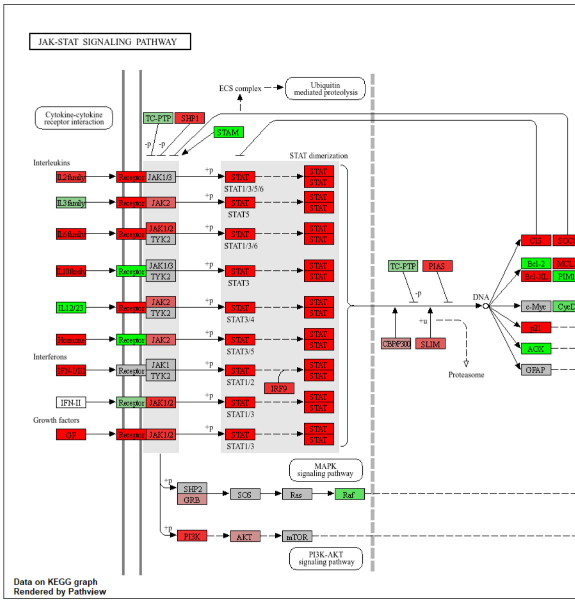
```
Info: Writing image file hsa04630.pathview.png
```

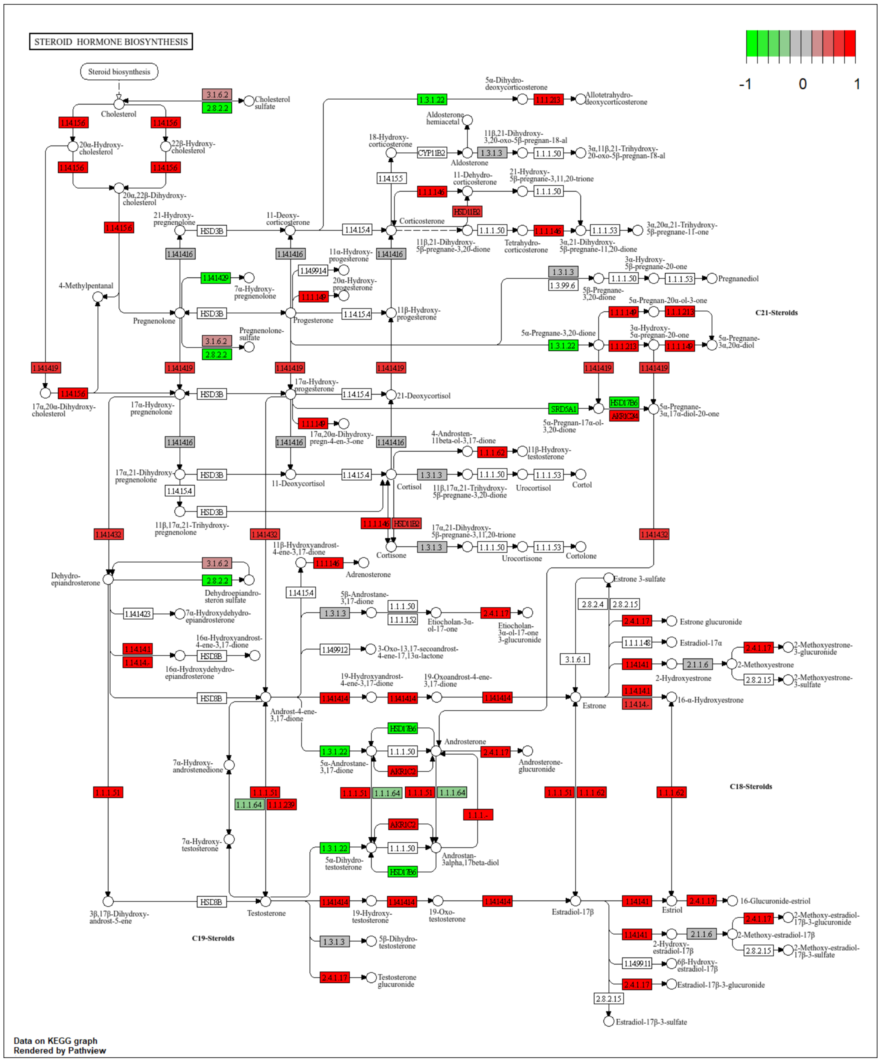
```
'select()' returned 1:1 mapping between keys and columns
```

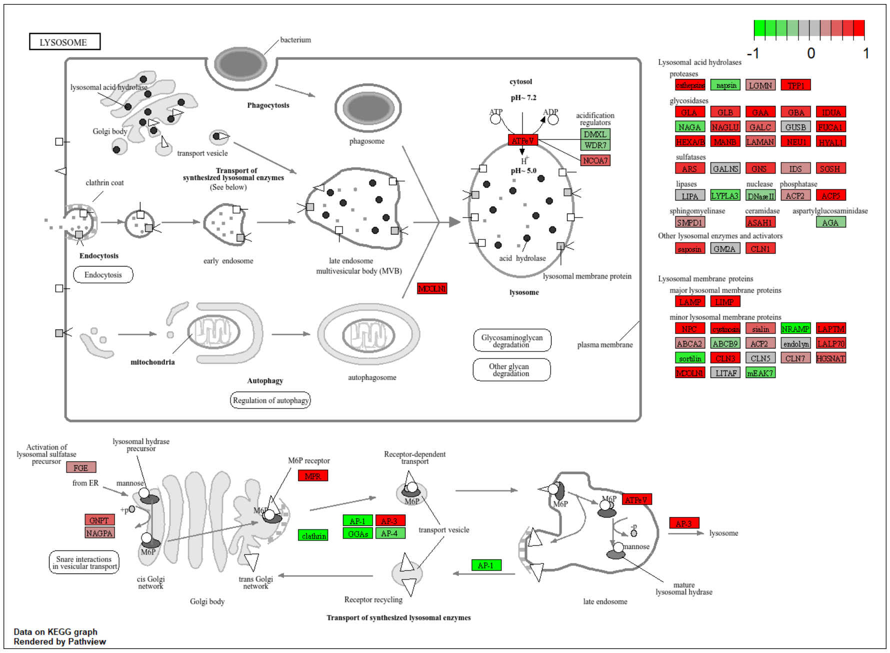
```
Info: Working in directory C:/Users/Mason/Desktop/BIMM143/class14
```

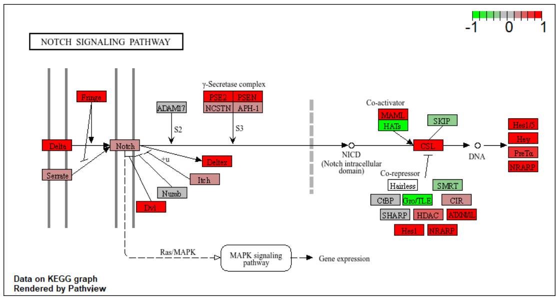
```
Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory C:/Users/Mason/Desktop/BIMM143/class14

Info: Writing image file hsa04142.pathview.png

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

Info: Working in directory C:/Users/Mason/Desktop/BIMM143/class14

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


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
Myeloid Stem Cell
CFU-GEMM
CFU-Mast
Mast cell
CFU-Bas
Myeloblast
Basophilic Myelocyte
Basophil
CFU-E0
Eosinophilic Myelocyte
Eosinophil
CFU-M/DC
Monoblast
Promonocyte
Monocyte
Macrophage
Myeloid Related Dendritic Cell
CFU-GM
CFU-G
Neutrophilic Myelocyte
Neutrophil
BFU-E
CFU-E
Procrythroblast
Erythrocyte
BFU-MK
CFU-MK
Mega-karyocyte
Platelets
SCF
IL-7
IL-3
IL-4
IL-5
IL-6
IL-11
Flt3L
GM-CSF
G-CSF
M-CSF
TNF
EPO
TPO
G-SCF
Meg-CSF
IL-9R
IL-11R
HLA-DR
CD2
CD3
CD4
CD5
CD7
CD8
CD9
CD10
CD13
CD14
CD15
CD16
CD19
CD20
CD21
CD22
CD23
CD24
CD25
CD33
CD34
CD35
CD36
CD37
CD38
CD41
CD42
CD44
CD49
CD59
CD61
CD64
CD71
CD114
CD115
CD116
CD117
CD121
CD123
CD124
CD125
CD126
CD127
CD135
CD11b
CD235a
TdT
IgM
IgD
Data on KEGG graph
Rendered by Pathview


JAK-STAT SIGNALING PATHWAY
Cytokine-cytokine receptor interaction
ECS complex
Ubiquitin mediated proteolysis
TC-PTP
SHP1
STAM
-p
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
STAT dimerization
TC-PTP
PIAS
CBP/P300
SLIM
+u
Proteasome
DNA
CIS
SOC
Bcl-2
Bcl-XL
MCl
PIMI
c-Myc
CycD
p21
AOX
GFAP
SHP2
GRB
SOS
Ras
Raf
MAPK signaling pathway
PI3K
AKT
mTOR
PI3K-AKT signaling pathway
Data on KEGG graph
Rendered by Pathview


STEROID HORMONE BIOSYNTHESIS
-1
0
1
Steroid biosynthesis
Cholesterol
Cholesterol sulfate
3.1.6.2
2.8.2.2
1.14.15.6
20α-Hydroxy-cholesterol
22β-Hydroxy-cholesterol
20α,22β-Dihydroxy-cholesterol
4-Methylpentanal
Pregnenolone
21-Hydroxy-pregnenolone
7α-Hydroxy-pregnenolone
Pregnenolone-sulfate
17α,20α-Dihydroxy-cholesterol
17α-Hydroxy-pregnenolone
17α,21-Dihydroxy-pregnenolone
11β,17α,21-Trihydroxy-pregnenolone
HSD3B
1.14.14.16
1.14.14.19
1.14.14.29
1.14.14.32
1.14.15.4
1.14.15.5
1.14.99.14
1.1.1.149
1.1.1.146
HSD11B2
CYP11B2
11-Deoxy-corticosterone
Corticosterone
18-Hydroxy-corticosterone
Aldosterone
Aldosterone hemiacetal
11-Dehydro-corticosterone
11β,21-Dihydroxy-5β-pregnane-3,20-dione
11β,21-Dihydroxy-3,20-oxo-5β-pregnan-18-al
3α,11β,21-Trihydroxy-20-oxo-5β-pregnan-18-al
21-Hydroxy-5β-pregnane-3,11,20-trione
Tetrahydro-corticosterone
3α,21-Dihydroxy-5β-pregnane-11,20-dione
3α,20α,21-Trihydroxy-5β-pregnane-11-one
5α-Dihydro-deoxycorticosterone
Allotetrahydro-deoxycorticosterone
1.3.1.22
1.1.1.213
1.3.1.3
1.1.1.50
1.1.1.53
1.3.99.6
Progesterone
11α-Hydroxy-progesterone
20α-Hydroxy-progesterone
11β-Hydroxy-progesterone
17α-Hydroxy-progesterone
21-Deoxycortisol
17α,20α-Dihydroxy-pregn-4-en-3-one
11-Deoxycortisol
Cortisol
Cortisone
5β-Pregnane-3,20-dione
3α-Hydroxy-5β-pregnane-20-one
Pregnanediol
5α-Pregnan-20α-ol-3-one
5α-Pregnane-3,20-dione
3α-Hydroxy-5α-pregnan-20-one
5α-Pregnane-3α,20α-diol
SRD5A1
HSD17B6
AKR1C2/4
5α-Pregnan-17α-ol-3,20-dione
5α-Pregnane-3α,17α-diol-20-one
C21-Steroids
4-Androsten-11beta-ol-3,17-dione
11β-Hydroxy-testosterone
1.1.1.62
11β,17α,21-Trihydroxy-5β-pregnane-3,20-dione
Urocortisol
Cortol
17α,21-Dihydroxy-5β-pregnane-3,11,20-trione
Urocortisone
Cortolone
Dehydro-epiandrosterone
Dehydroepiandro-steron sulfate
1.14.14.23
7α-Hydroxydehydro-epiandrosterone
1.14.14.1
1.14.14.-
16α-Hydroxyandrost-4-ene-3,17-dione
16α-Hydroxydehydro-epiandrosterone
11β-Hydroxyandrost-4-ene-3,17-dione
Adrenosterone
5β-Androstane-3,17-dione
1.1.1.152
Etiocholan-3α-ol-17-one
Etiocholan-3α-ol-17-one 3-glucuronide
2.4.1.17
1.14.99.12
3-Oxo-13,17-secoandrost-4-ene-17,13α-lactone
19-Hydroxyandrost-4-ene-3,17-dione
19-Oxoandrost-4-ene-3,17-dione
1.14.14.14
Androst-4-ene-3,17-dione
Estrone
Estrone 3-sulfate
2.8.2.4
2.8.2.15
3.1.6.1
Estrone glucuronide
1.1.1.148
Estradiol-17α
2-Hydroxyestrone
2.1.1.6
2-Methoxyestrone
2-Methoxyestrone-3-glucuronide
2-Methoxyestrone-3-sulfate
16-α-Hydroxyestrone
7α-Hydroxy-androstenedione
5α-Androstane-3,17-dione
Androsterone
Androsterone-glucuronide
AKR1C2
1.1.1.51
1.1.1.64
1.1.1.239
1.1.1.-
C18-Steroids
7α-Hydroxy-testosterone
5α-Dihydro-testosterone
Androstan-3alpha,17beta-diol
3β,17β-Dihydroxy-androst-5-ene
Testosterone
19-Hydroxy-testosterone
19-Oxo-testosterone
Estradiol-17β
Estriol
16-Glucuronide-estriol
2-Hydroxy-estradiol-17β
2-Methoxy-estradiol-17β
2-Methoxy-estradiol-17β-3-glucuronide
2-Methoxy-estradiol-17β-3-sulfate
1.14.99.11
6β-Hydroxy-estradiol-17β
Estradiol-17β-3-glucuronide
Estradiol-17β-3-sulfate
5β-Dihydro-testosterone
Testosterone glucuronide
C19-Steroids
Data on KEGG graph
Rendered by Pathview





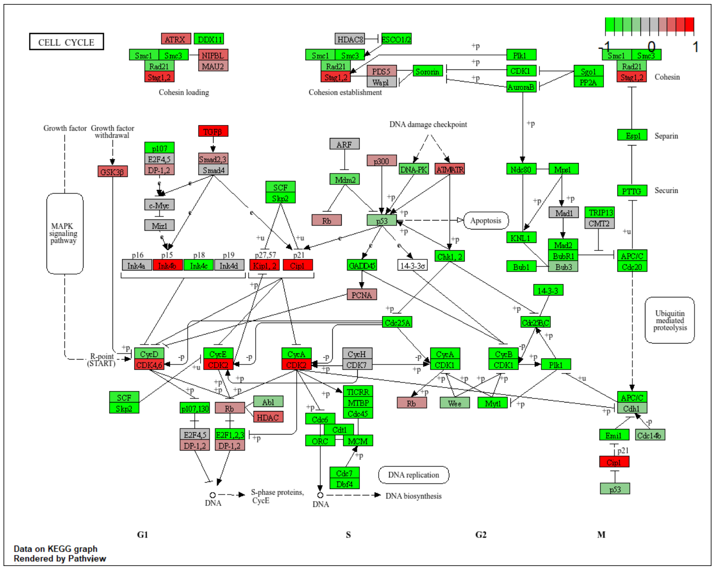
Q. Can you do the same procedure as above to plot the pathview figures for the top 5 down-reguled pathways?

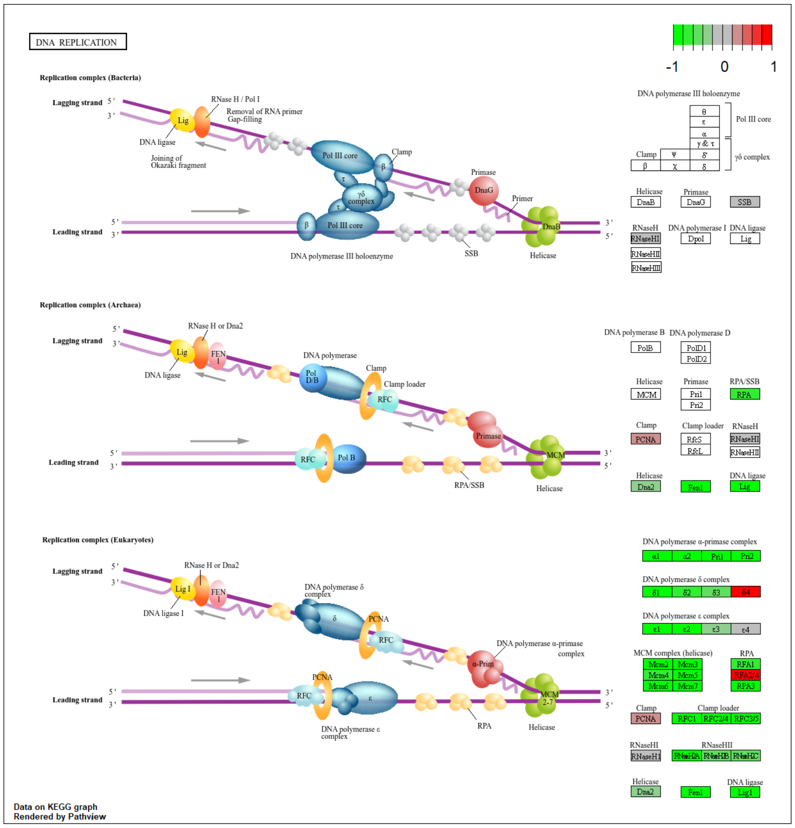
```
## Focus on top 5 downregulated pathways here for demo purposes only
keggdownrespathways <- rownames(keggres$less)[1:5]

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

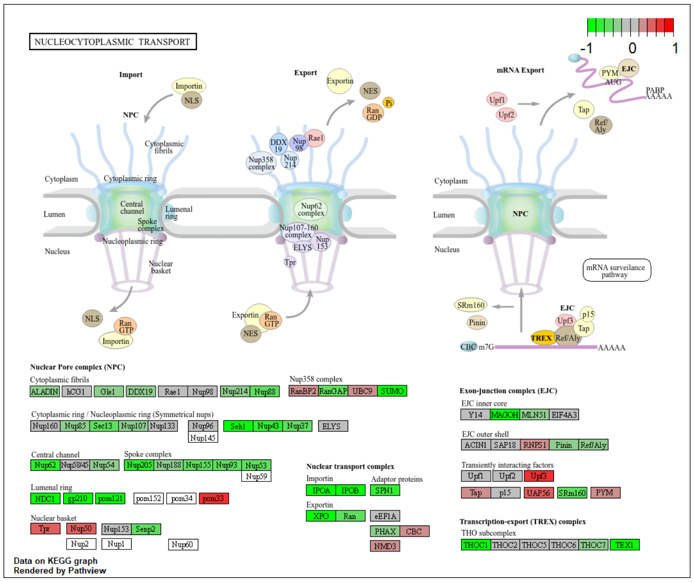
```
[1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

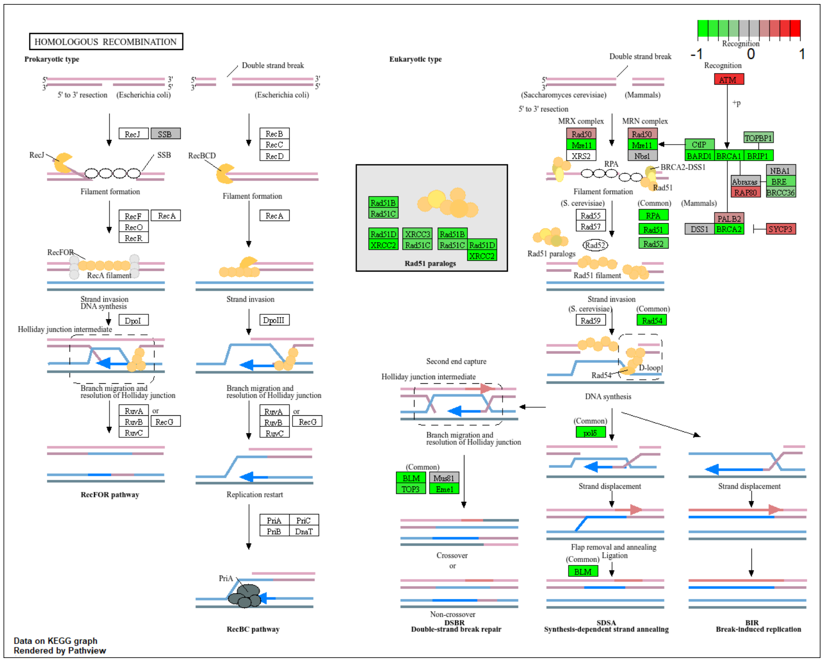
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

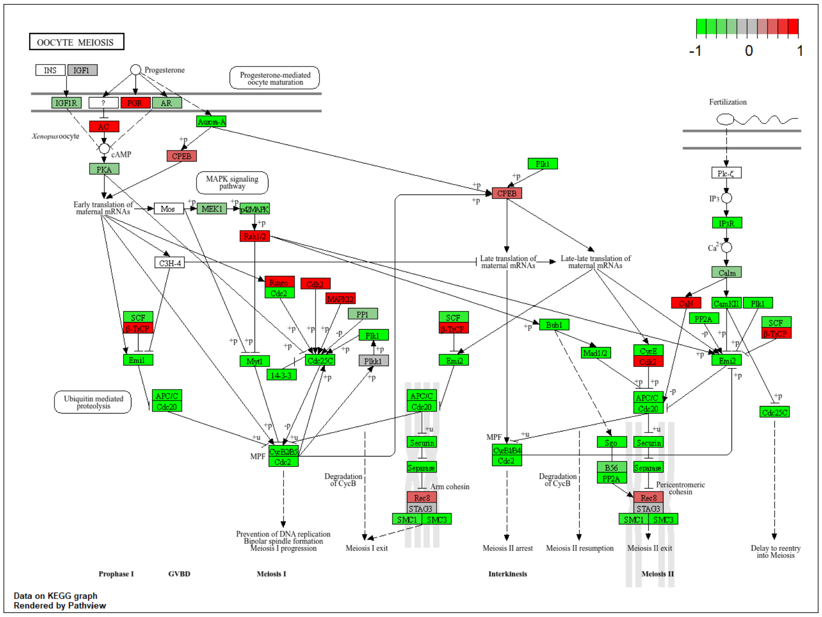
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory C:/Users/Mason/Desktop/BIMM143/class14

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory C:/Users/Mason/Desktop/BIMM143/class14

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory C:/Users/Mason/Desktop/BIMM143/class14

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory C:/Users/Mason/Desktop/BIMM143/class14

Info: Writing image file hsa03440.pathview.png

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

Info: Working in directory C:/Users/Mason/Desktop/BIMM143/class14

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
Stag1,2
PDS5
Wapl
Sororin
Cohesion establishment
Plk1
CDK1
AuroraB
Sgo1
PP2A
Smc1
Smc3
Rad21
Stag1,2
Cohesin
+p
Growth factor
Growth factor withdrawal
GSK3B
MAPK signaling pathway
p107
E2F4,5
DP-1,2
TGFB
Smad2,3
Smad4
c-Myc
Miz1
SCF
Skp2
ARF
Mdm2
Rb
p300
DNA damage checkpoint
DNA-PK
ATM/ATR
p53
Apoptosis
GADD45
14-3-3σ
Chk1,2
Nek80
Mps1
Mad1
TRIP13
CMT2
KNL1
Mad2
BubR1
Bub3
Bub1
Espl
Separin
PTTG
Securin
APC/C
Cdc20
p16
p15
p18
p19
Ink4a
Ink4b
Ink4c
Ink4d
p27,57
Cip1,2
p21
Cip1
PCNA
Cdc25A
14-3-3
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
E2F4,5
DP-1,2
E2F1,2,3
DP-1,2
TICRR
MTBP
Cdc45
Cdc6
Cdt1
ORC
MCM
Cdc7
Dbf4
DNA replication
Rb
Wee
Myt1
APC/C
Cdh1
Emi1
Cdc14b
p21
Cip1
p53
DNA
S-phase proteins, CycE
DNA
DNA biosynthesis
+p
-p
+u
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
DnaB
SSB
Helicase
DNA polymerase III holoenzyme
DNA polymerase III holoenzyme
θ
ε
α
γ & τ
δ'
δ
Ψ
χ
β
Clamp
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
Lig
FEN1
DNA ligase
DNA polymerase
Pol D/B
Clamp
Clamp loader
RFC
Primase
MCM
Helicase
RFC
Pol B
RPA/SSB
DNA polymerase B
PolB
DNA polymerase D
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)
RNase H or Dna2
Lig I
FEN1
DNA ligase I
DNA polymerase δ complex
δ
PCNA
RFC
DNA polymerase α-primase complex
α-Prim
MCM 2-7
Helicase
PCNA
RFC
ε
DNA polymerase ε complex
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
RPA1
RPA2/4
RPA3
Clamp
PCNA
Clamp loader
RFC1
RFC2/4
RFC3/5
RNaseHI
RNaseHI
RNaseHII
RNaseHIIA
RNaseHIIB
RNaseHIIC
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
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


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



##Gene Ontology (GO)

We can also do a similar procedure with gene ontology. Similar to above, `go.sets.hs` has all GO terms. `go.subs.hs` is a named list containing indexes for the BP, CC, and MF ontologies. Let's focus on BP (a.k.a Biological Process) here.

```
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)

head(gobpres$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
```