Class 14 DESeq2 Mini Project

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About our Input Data

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

Section 1. Differential Expression Analysis

```
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 object is masked from 'package:utils':

    findMatches

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

    expand.grid, I, unname

Loading required package: IRanges

Loading required package: GenomicRanges

Loading required package: GenomeInfoDb

Loading required package: SummarizedExperiment

Loading required package: MatrixGenerics

Loading required package: matrixStats


Attaching package: 'MatrixGenerics'
```

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

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

Loading required package: Biobase

Welcome to Bioconductor

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


Attaching package: 'Biobase'

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

    rowMedians

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

    anyMissing, rowMedians
```

```r
metaFile <- "GSE37704_metadata.csv"
countFile <- "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
```

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

This looks better but there are lots of zero entries in there so let's get rid of them as we have no data for these.

> 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 samples.
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

Running DESeq2

Nice now lets setup the DESeqDataSet object required for the **DESeq()** function and then run the DESeq pipeline.

```
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
```

```
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
```

Next, get results for the HoxA1 knockdown versus control siRNA (remember that these were labeled as "**hoxa1_kd**" and "**control_sirna**" in our original `colData` metaFile input to DESeq, you can check this above and by running `resultsNames(dds)` command).

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

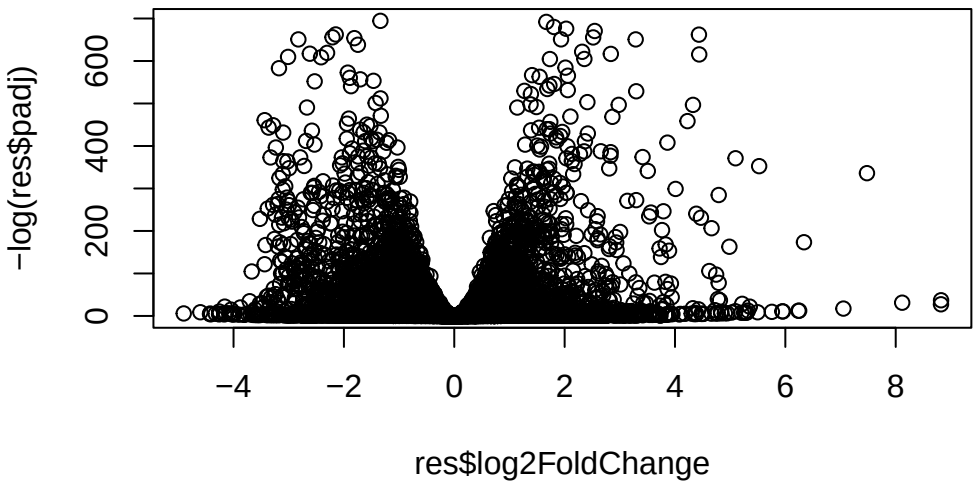
Q. Call the **summary()** function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```
summary(res)
```

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

Volcano Plot

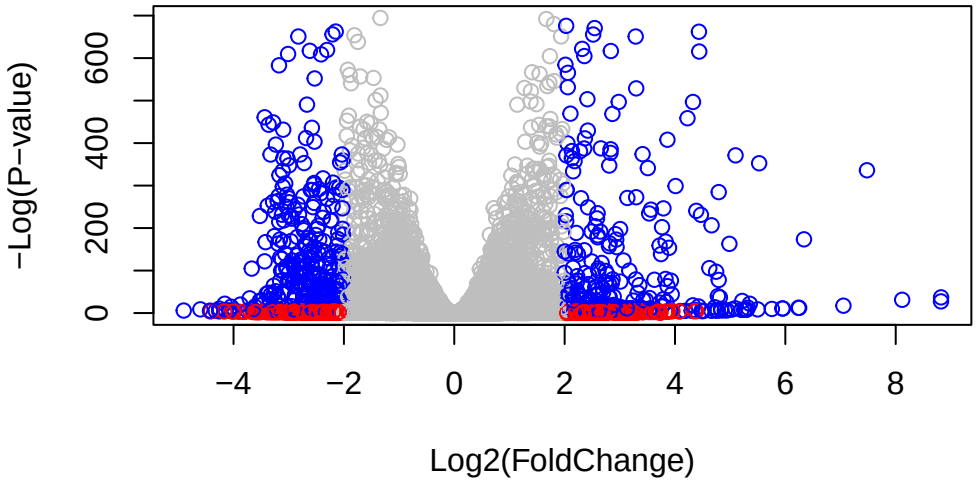
```
plot( res$log2FoldChange, -log(res$padj) )
```



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

plot( res$log2FoldChange, -log(res$padj), col=mycols, xlab="Log2(FoldChange)", ylab="-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 1.43989e-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 0.5215599   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.

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

Section 2. Pathway Analysis

KEGG pathways

```
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
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"
```

```
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)
```

```
attributes(keggres)
```

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

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

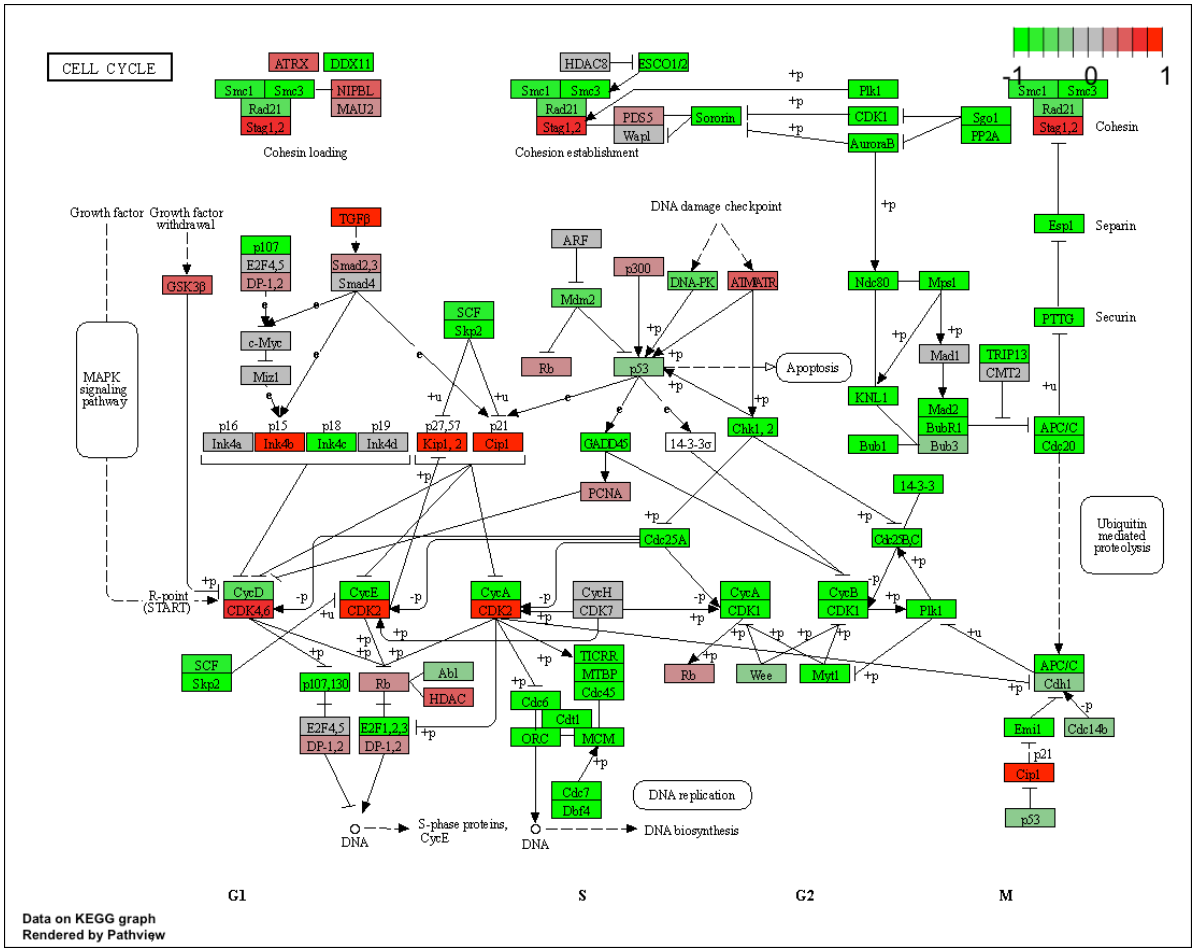
```
                                      p.geomean stat.mean        p.val
hsa04110 Cell cycle                   8.995727e-06 -4.378644 8.995727e-06
hsa03030 DNA replication              9.424076e-05 -3.951803 9.424076e-05
hsa03013 RNA transport                1.375901e-03 -3.028500 1.375901e-03
hsa03440 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
```

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

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

```
Info: Working in directory /Users/francesnicolealfonso/BIMM143/class14
```

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



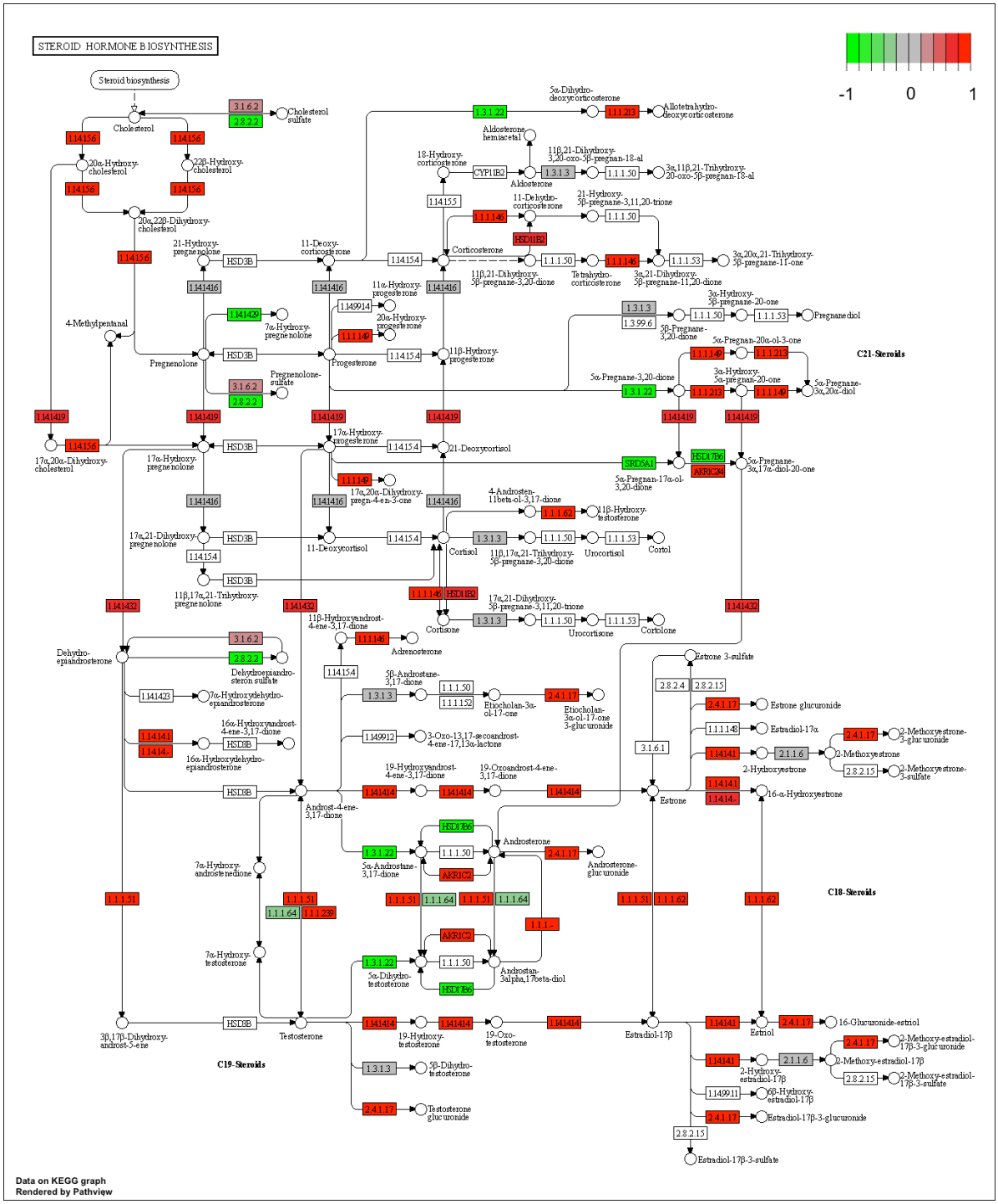

```
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$greater)[1:5]

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

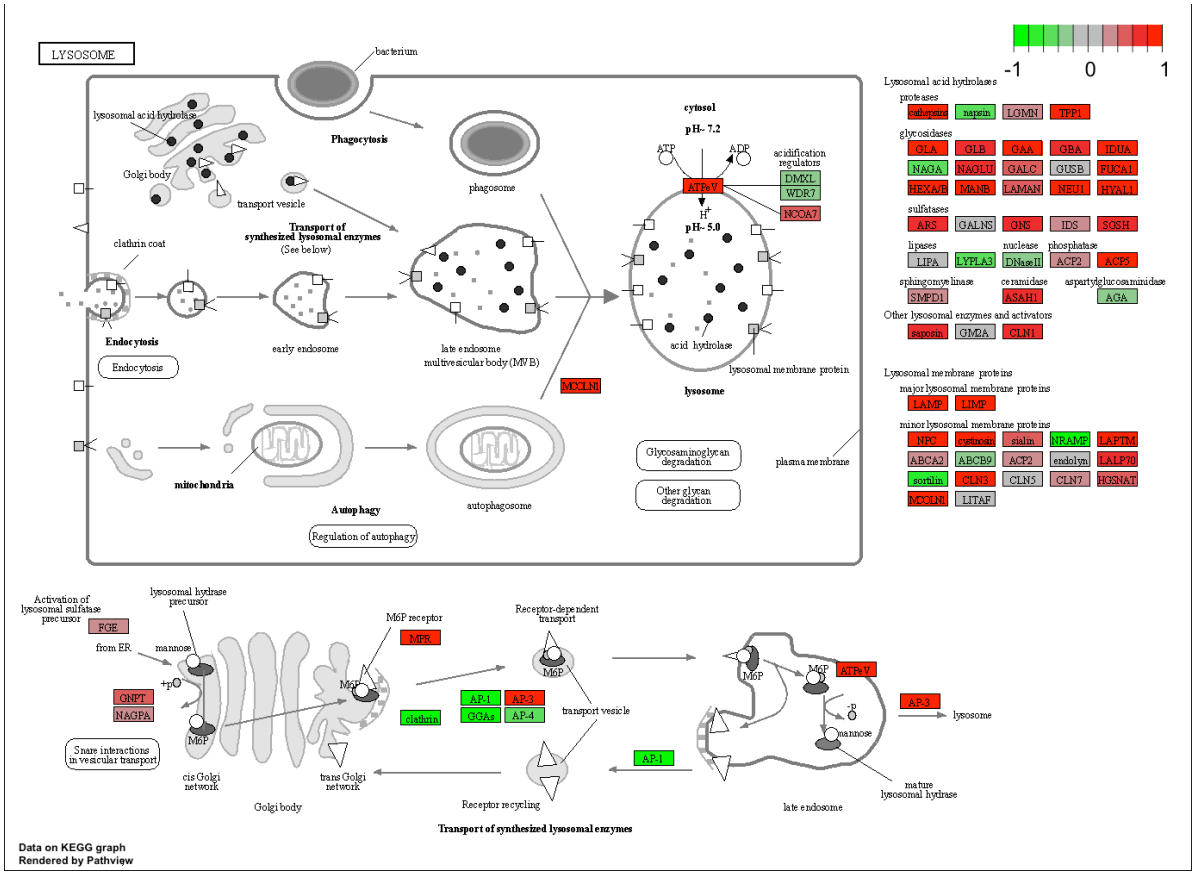
```
[1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```

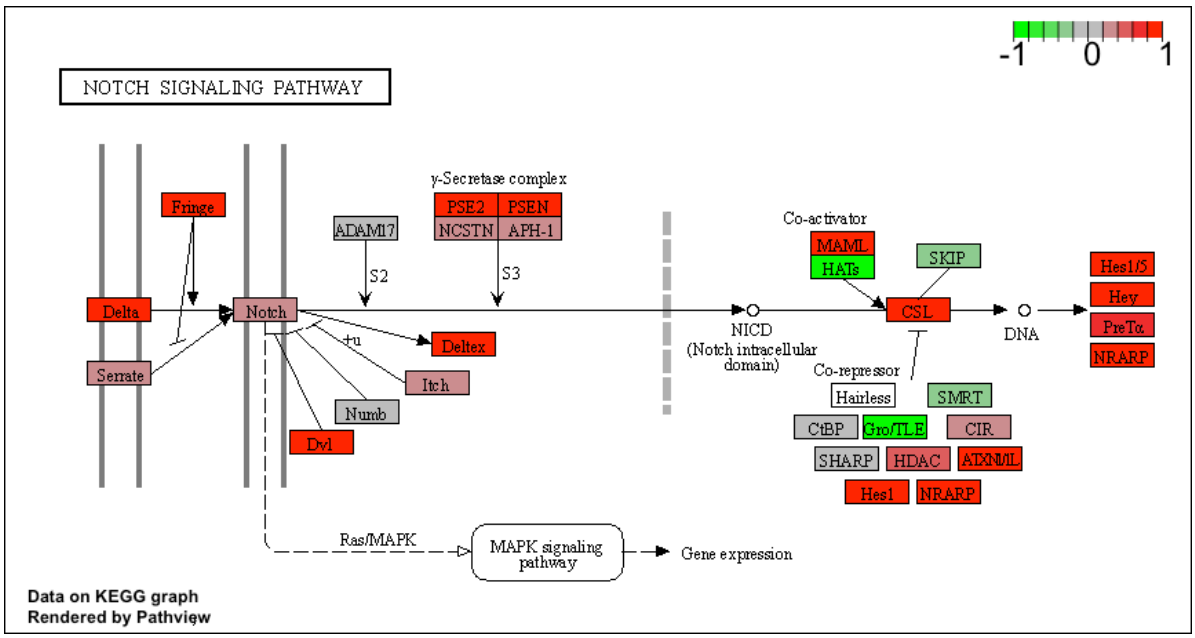
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

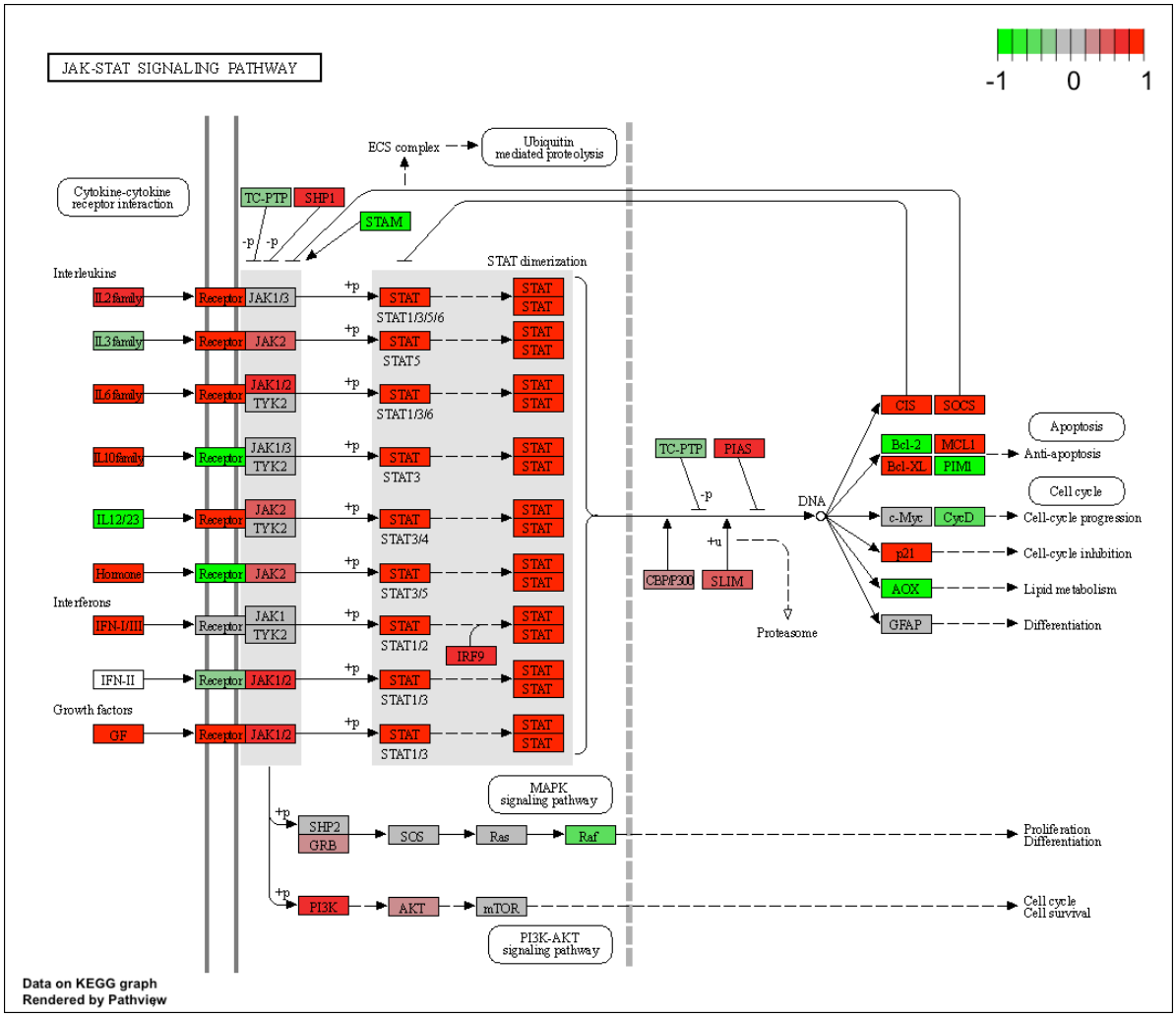
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/francesnicolealfonso/BIMM143/class14
```

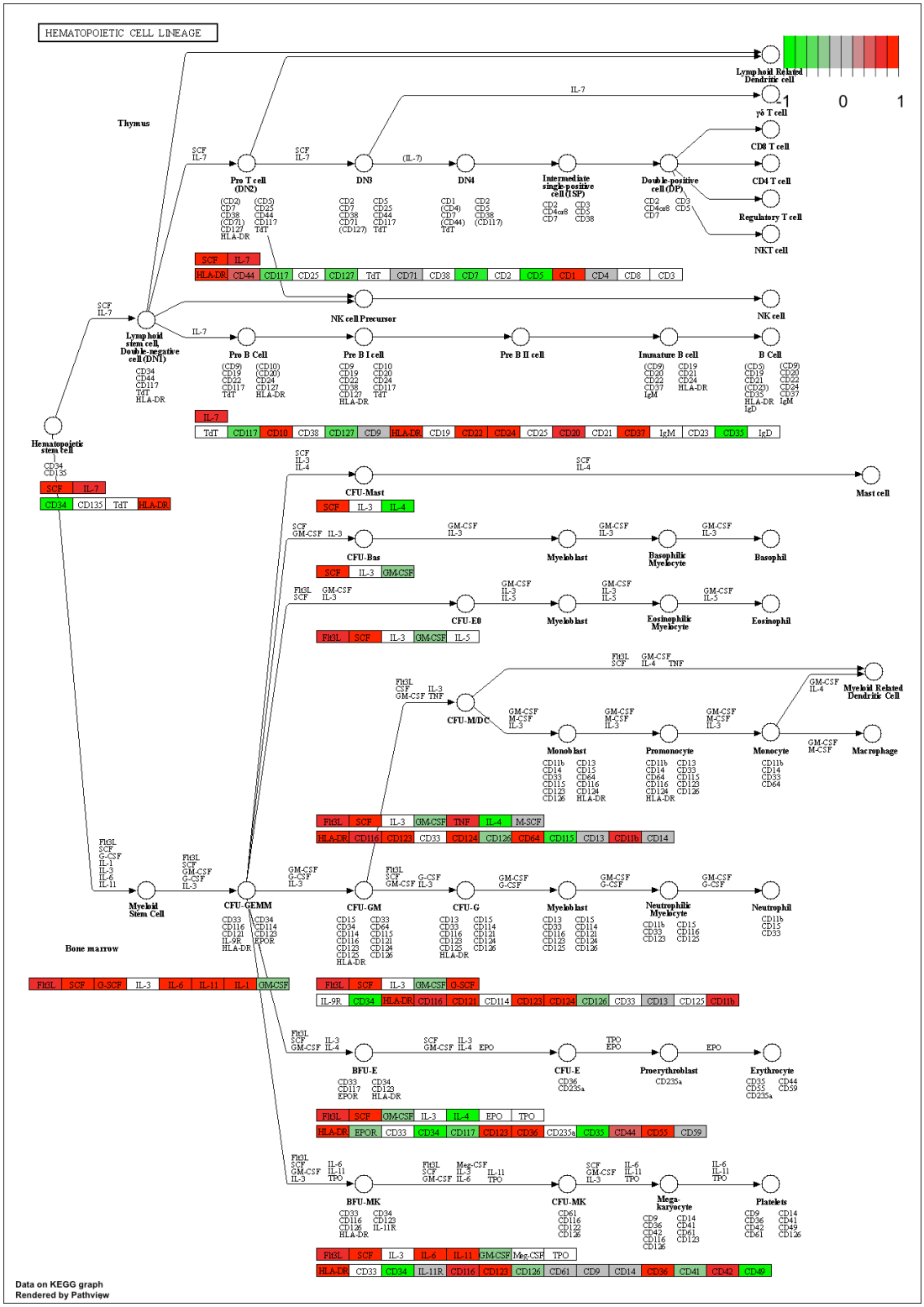
```
Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory /Users/francesnicolealfonso/BIMM143/class14

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory /Users/francesnicolealfonso/BIMM143/class14

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory /Users/francesnicolealfonso/BIMM143/class14

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/francesnicolealfonso/BIMM143/class14

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


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


LYSOSOME
bacterium
lysosomal acid hydrolase
Golgi body
transport vesicle
Phagocytosis
phagosome
cytosol
pH~ 7.2
ATP
ADP
acidification regulators
DMXL
WDR7
NCOA7
ATPeV
H+
pH~ 5.0
Transport of synthesized lysosomal enzymes
(See below)
clathrin coat
Endocytosis
Endocytosis
early endosome
late endosome
multivesicular body (MVB)
MCOLN1
acid hydrolase
lysosomal membrane protein
lysosome
mitochondria
Autophagy
autophagosome
Regulation of autophagy
Glycosaminoglycan degradation
Other glycan degradation
plasma membrane
-1
0
1
Lysosomal acid hydrolases
proteases
cathepsin
napsin
LGMN
TPP1
glycosidases
GLA
GLB
GAA
GBA
IDUA
NAGA
NAGLU
GALC
GUSB
FUCA1
HEXA/B
MANB
LAMAN
NEU1
HYAL1
sulfatases
ARS
GALNS
GNS
IDS
SGSH
lipases
nuclease
phosphatase
LIPA
LYPLA3
DNaseII
ACP2
ACP5
sphingomyelinase
ceramidase
aspartylglucosaminidase
SMPD1
ASAH1
AGA
Other lysosomal enzymes and activators
saposin
GM2A
CLN1
Lysosomal membrane proteins
major lysosomal membrane proteins
LAMP
LIMP
minor lysosomal membrane proteins
NPC
cystinosin
sialin
NRAMP
LAPTM
ABCA2
ABCB9
ACP2
endolyn
LALP70
sortilin
CLN3
CLN5
CLN7
HGSNAT
MCOLN1
LITAF
Activation of lysosomal sulfatase precursor
FGE
lysosomal hydrase precursor
from ER
mannose
+pO
GNPT
NAGPA
M6P
M6P receptor
MPR
Receptor-dependent transport
M6P
clathrin
AP-1
GGAs
AP-3
AP-4
transport vesicle
Snare interactions in vesicular transport
cis Golgi network
trans Golgi network
Golgi body
Receptor recycling
AP-1
M6P
M6P
ATPeV
-p
mannose
AP-3
lysosome
mature lysosomal hydrase
late endosome
Transport of synthesized lysosomal enzymes
Data on KEGG graph
Rendered by Pathview


-1
0
1
NOTCH SIGNALING PATHWAY
Fringe
Delta
Serrate
Notch
ADAM17
S2
γ-Secretase complex
PSE2
PSEN
NCSTN
APH-1
S3
+u
Deltex
Itch
Numb
Dvl
NICD
(Notch intracellular domain)
Co-activator
MAML
HATs
SKIP
CSL
DNA
Hes1/5
Hey
PreTα
NRARP
Co-repressor
Hairless
SMRT
CtBP
Gro/TLE
CIR
SHARP
HDAC
ATXN1L
Hes1
NRARP
Ras/MAPK
MAPK signaling pathway
Gene expression
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
STAT dimerization
Interleukins
IL2family
IL3family
IL6family
IL10family
IL12/23
Hormone
Interferons
IFN-I/III
IFN-II
Growth factors
GF
Receptor
JAK1/3
JAK2
JAK1/2
TYK2
JAK1
STAT
STAT1/3/5/6
STAT5
STAT1/3/6
STAT3
STAT3/4
STAT3/5
STAT1/2
STAT1/3
IRF9
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
PIAS
DNA
CBP/P300
SLIM
Proteasome
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


HEMATOPOIETIC CELL LINEAGE
Lymphoid Related Dendritic cell
-1
0
1
Thymus
IL-7
γδ T cell
CD8 T cell
CD4 T cell
Regulatory T cell
NKT cell
NK cell
Pre T cell (DN2)
DN3
DN4
Intermediate single-positive cell (ISP)
Double-positive cell (DP)
SCF
IL-7
(IL-7)
NK cell Precursor
Lymphoid stem cell, Double-negative cell (DN1)
Pro B Cell
Pre B I cell
Pre B II cell
Immature B cell
B Cell
Hematopoietic stem cell
CD34
CD135
TdT
HLA-DR
CD44
CD117
CD25
CD127
CD71
CD38
CD7
CD2
CD5
CD1
CD4
CD8
CD3
CD10
CD19
CD22
CD24
CD20
CD21
CD37
IgM
CD23
CD35
IgD
CFU-Mast
Mast cell
CFU-Bas
Myeloblast
Basophilic Myelocyte
Basophil
CFU-E0
Eosinophilic Myelocyte
Eosinophil
CFU-M/DC
Monoblast
Promonocyte
Monocyte
Myeloid Related Dendritic Cell
Macrophage
Myeloid Stem Cell
CFU-GEMM
CFU-GM
CFU-G
Neutrophilic Myelocyte
Neutrophil
Bone marrow
BFU-E
CFU-E
Proerythroblast
Erythrocyte
BFU-MK
CFU-MK
Mega-karyocyte
Platelets
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?

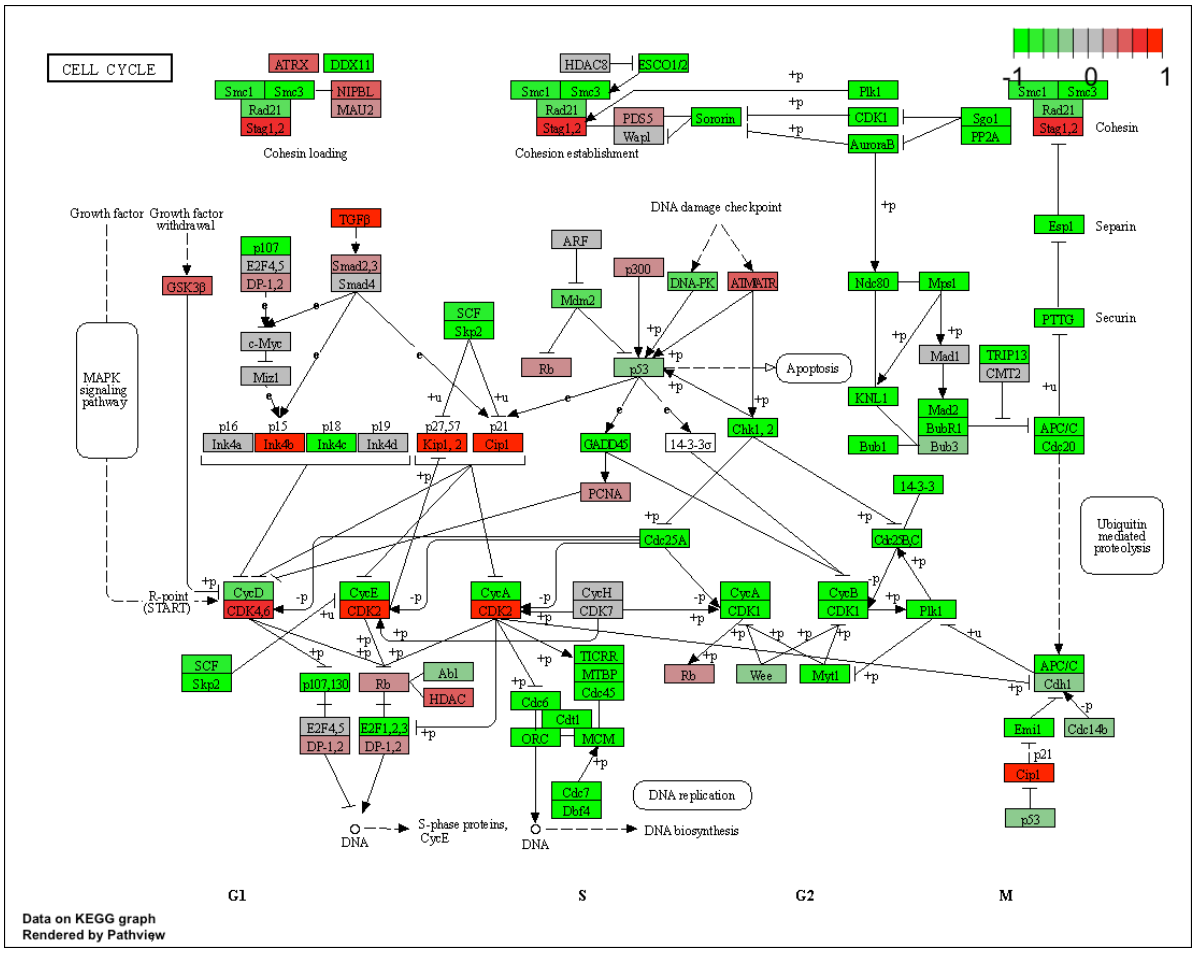
```
keggrespathways <- rownames(keggres$less)[1:5]

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

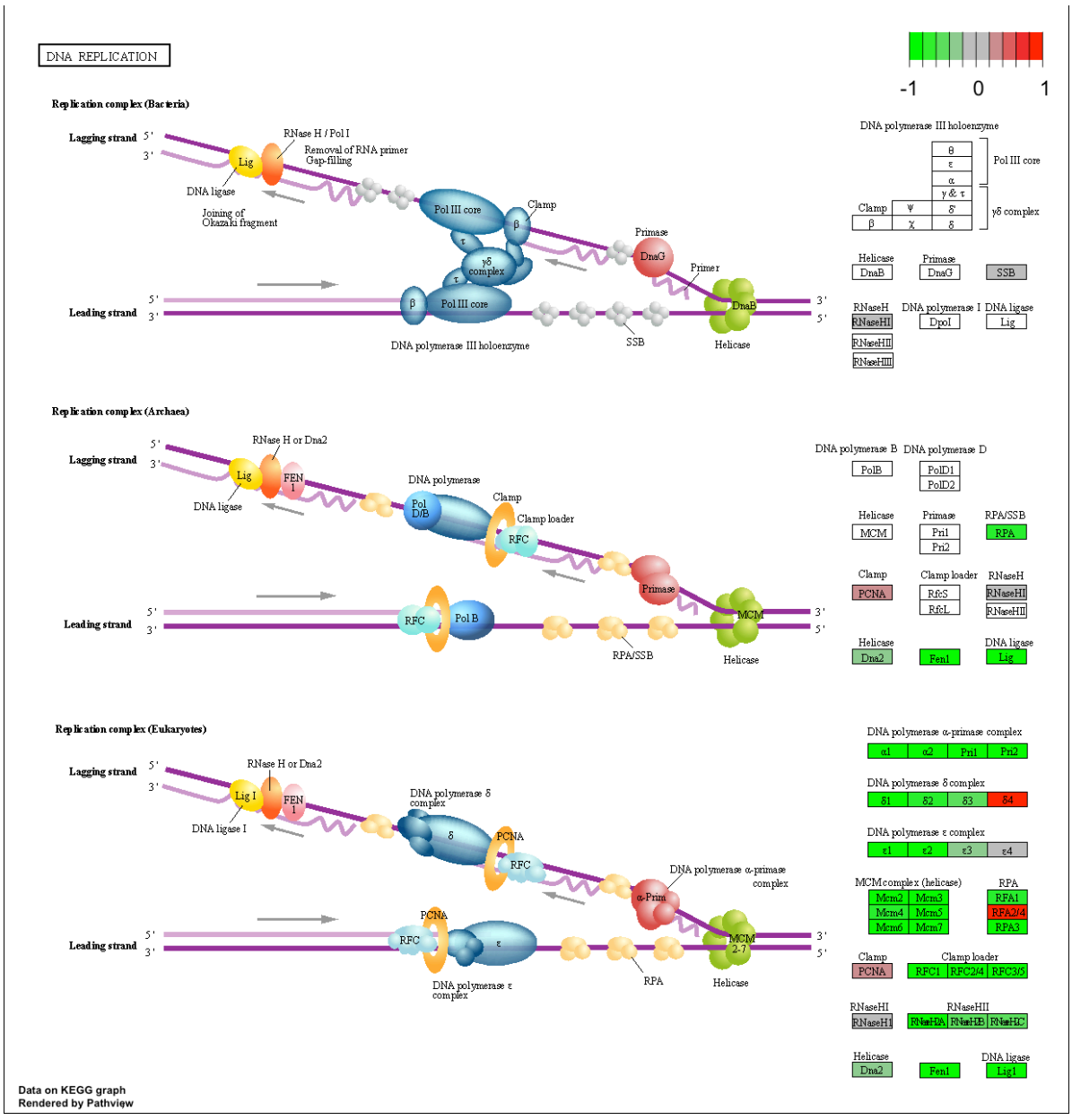
```
[1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

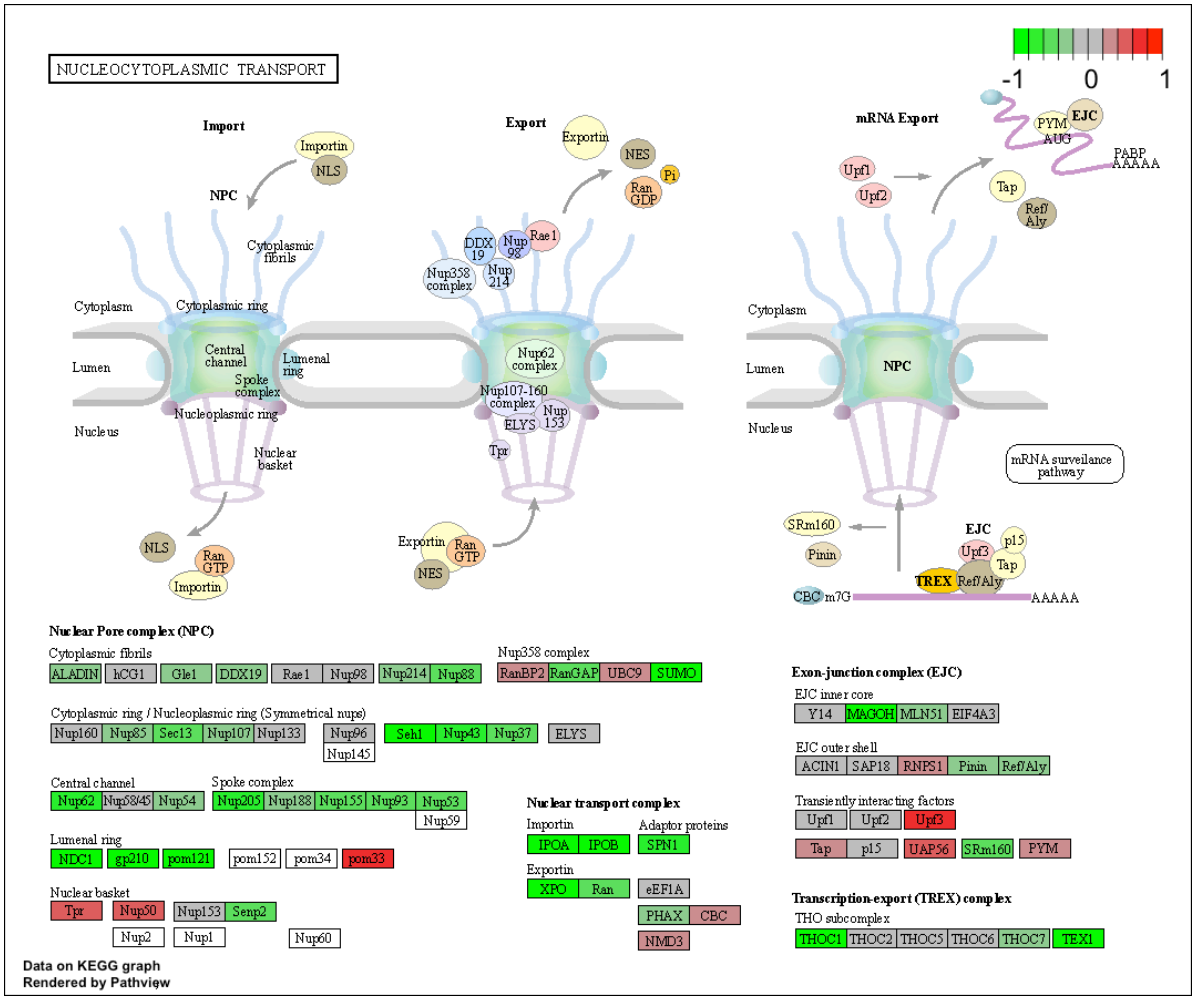
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

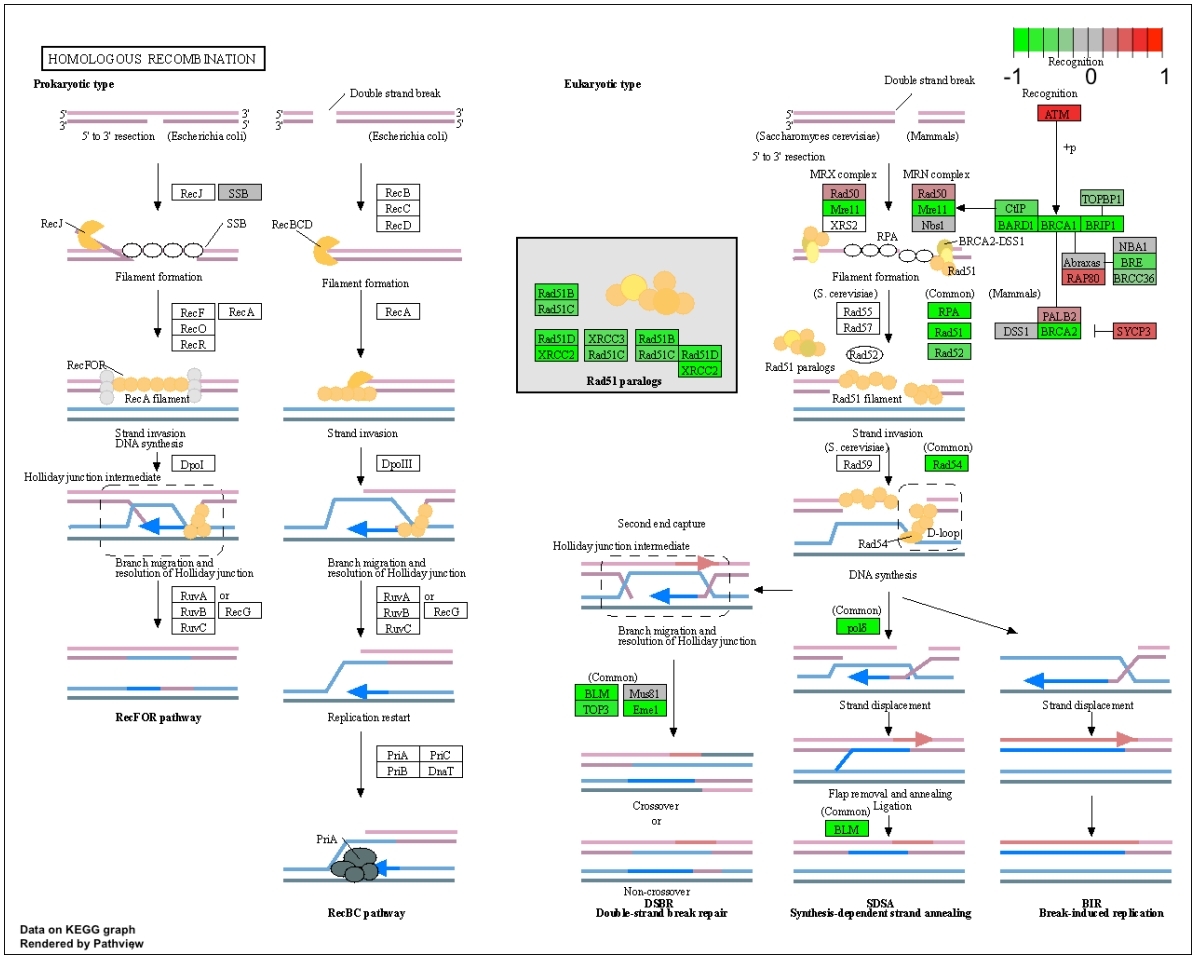
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/francesnicolealfonso/BIMM143/class14

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory /Users/francesnicolealfonso/BIMM143/class14

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/francesnicolealfonso/BIMM143/class14

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory /Users/francesnicolealfonso/BIMM143/class14

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

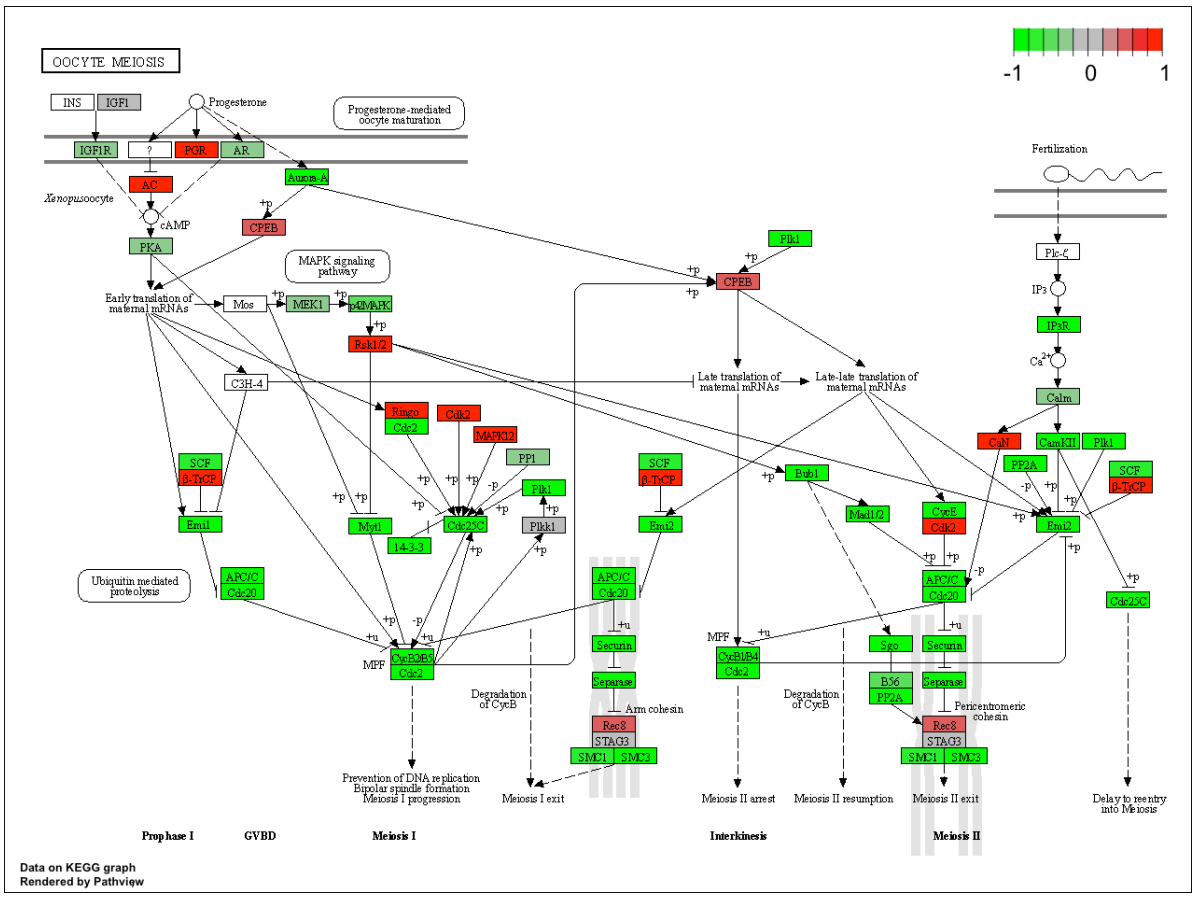
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/francesnicolealfonso/BIMM143/class14

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




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'
γδ complex
β
χ
δ
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
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
RNaseHI
RNaseHII
Helicase
Dna2
Fen1
DNA ligase
Lig
Replication complex (Eukaryotes)
RNase H or Dna2
Lig I
FEN
1
DNA ligase I
DNA polymerase δ
complex
δ
PCNA
RFC
DNA polymerase α-primase
complex
α-Prim
MCM
2-7
RPA
Helicase
DNA polymerase ε
complex
ε
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
RFA3
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
Lumenal ring
Lumen
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


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



Section 3. Gene Ontology (GO)

```
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                       1.925222e-04  3.565432 1.925222e-04
GO:0060562 epithelial tube morphogenesis  5.932837e-04  3.261376 5.932837e-04
GO:0035295 tube development               5.953254e-04  3.253665 5.953254e-04
                                              q.val set.size         exp1
GO:0007156 homophilic cell adhesion       0.1952430      113 8.519724e-05
GO:0002009 morphogenesis of an epithelium 0.1952430      339 1.396681e-04
GO:0048729 tissue morphogenesis           0.1952430      424 1.432451e-04
GO:0007610 behavior                       0.1968058      426 1.925222e-04
GO:0060562 epithelial tube morphogenesis  0.3566193      257 5.932837e-04
GO:0035295 tube development               0.3566193      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             5.843127e-12      376 1.536227e-15
GO:0000280 nuclear division              5.843127e-12      352 4.286961e-15
GO:0007067 mitosis                       5.843127e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.195965e-11      362 1.169934e-14
GO:0007059 chromosome segregation        1.659009e-08      142 2.028624e-11
GO:0000236 mitotic prometaphase          1.178690e-07       84 1.729553e-10

$stats
                                          stat.mean     exp1
GO:0007156 homophilic cell adhesion        3.824205 3.824205
GO:0002009 morphogenesis of an epithelium  3.653886 3.653886
GO:0048729 tissue morphogenesis            3.643242 3.643242
GO:0007610 behavior                        3.565432 3.565432
GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
GO:0035295 tube development                3.253665 3.253665
```

Section 4. Reactome Analysis

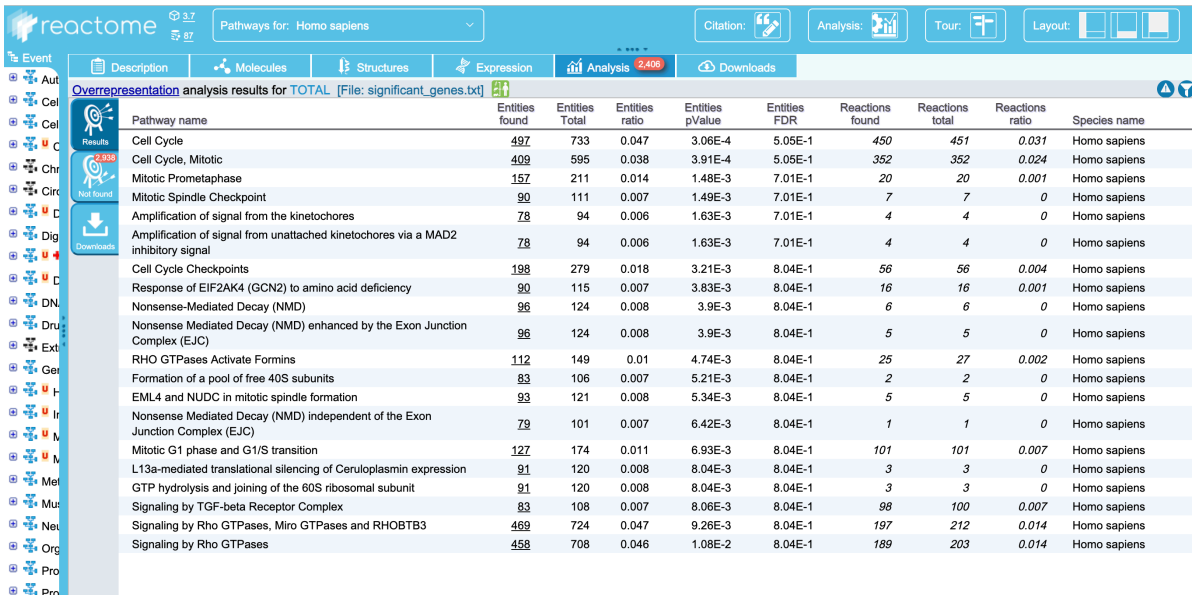
```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
print(paste("Total number of significant genes:", length(sig_genes)))
```

```
[1] "Total number of significant genes: 8147"
```

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

Then, to perform pathway analysis online go to the Reactome website (https://reactome.org/PathwayBrowser/#TOOL=AT). Select "choose file" to upload your significant gene list. Then, select the parameters "Project to Humans", then click "Analyze".

> 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?
>
> The most significant pathway is "Cell Cycle". The most significant pathways do relatively match our previous KESS results as it only matches the top result $less, but not $greater.



Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio	Species name
Cell Cycle	497	733	0.047	3.06E-4	5.05E-1	450	451	0.031	Homo sapiens
Cell Cycle, Mitotic	409	595	0.038	3.91E-4	5.05E-1	352	352	0.024	Homo sapiens
Mitotic Prometaphase	157	211	0.014	1.48E-3	7.01E-1	20	20	0.001	Homo sapiens
Mitotic Spindle Checkpoint	90	111	0.007	1.49E-3	7.01E-1	7	7	0	Homo sapiens
Amplification of signal from the kinetochores	78	94	0.006	1.63E-3	7.01E-1	4	4	0	Homo sapiens
Amplification of signal from unattached kinetochores via a MAD2 inhibitory signal	78	94	0.006	1.63E-3	7.01E-1	4	4	0	Homo sapiens
Cell Cycle Checkpoints	198	279	0.018	3.21E-3	8.04E-1	56	56	0.004	Homo sapiens
Response of EIF2AK4 (GCN2) to amino acid deficiency	90	115	0.007	3.83E-3	8.04E-1	16	16	0.001	Homo sapiens
Nonsense-Mediated Decay (NMD)	96	124	0.008	3.9E-3	8.04E-1	6	6	0	Homo sapiens
Nonsense Mediated Decay (NMD) enhanced by the Exon Junction Complex (EJC)	96	124	0.008	3.9E-3	8.04E-1	5	5	0	Homo sapiens
RHO GTPases Activate Formins	112	149	0.01	4.74E-3	8.04E-1	25	27	0.002	Homo sapiens
Formation of a pool of free 40S subunits	83	106	0.007	5.21E-3	8.04E-1	2	2	0	Homo sapiens
EML4 and NUDC in mitotic spindle formation	93	121	0.008	5.34E-3	8.04E-1	5	5	0	Homo sapiens
Nonsense Mediated Decay (NMD) independent of the Exon Junction Complex (EJC)	79	101	0.007	6.42E-3	8.04E-1	1	1	0	Homo sapiens
Mitotic G1 phase and G1/S transition	127	174	0.011	6.93E-3	8.04E-1	101	101	0.007	Homo sapiens
L13a-mediated translational silencing of Ceruloplasmin expression	91	120	0.008	8.04E-3	8.04E-1	3	3	0	Homo sapiens
GTP hydrolysis and joining of the 60S ribosomal subunit	91	120	0.008	8.04E-3	8.04E-1	3	3	0	Homo sapiens
Signaling by TGF-beta Receptor Complex	83	108	0.007	8.06E-3	8.04E-1	98	100	0.007	Homo sapiens
Signaling by Rho GTPases, Miro GTPases and RHOBTB3	469	724	0.047	9.26E-3	8.04E-1	197	212	0.014	Homo sapiens
Signaling by Rho GTPases	458	708	0.046	1.08E-2	8.04E-1	189	203	0.014	Homo sapiens