class14

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Background

In this lab we basically start with RNA-seq data comparing HOXA1 knockdown to control cells, run DESeq2 to figure out which genes are significantly up or down regulated, and then try to make sense of that huge gene list by doing pathway analysis. Instead of just staring at thousands of individual genes, we convert our Ensembl IDs to things like Entrez IDs and gene symbols, make a volcano plot to visualize overall changes, and then use KEGG pathway enrichment (with gage) to see which biological pathways are collectively affected. After that, we use pathview to actually map our fold changes onto real pathway diagrams so we can visually see what's being turned up or down, and we compare those results to GO and Reactome to see if similar biological themes show up across different databases.

Data Import

We have 2 key input files: counts and metadata.

```
library(DESeq2)
```

```
metaFile <- "GSE37704_metadata.csv"
countFile <- "GSE37704_featurecounts.csv"

# Import metadata and take a peek
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
```

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

```
# 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
```

Check and Tidy

> Q1. Complete the code below to remove the troublesome first column from countData

```
countData <- as.matrix(countData[, -1])
head(countData)
```

	SRR493366	SRR493367	SRR493368	SRR493369	SRR493370	SRR493371
ENSG00000186092	0	0	0	0	0	0
ENSG00000279928	0	0	0	0	0	0
ENSG00000279457	23	28	29	29	28	46
ENSG00000278566	0	0	0	0	0	0
ENSG00000273547	0	0	0	0	0	0
ENSG00000187634	124	123	205	207	212	258

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.

> Q2. 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).

```
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

Run DesSeq

Now lets setup the DESeqDataSet object required for the DESeq() function and then run the DESeq pipeline. This is again similar to our last day's hands-on session.

```
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
```

Get results

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).

```
dds <- DESeq(dds)
```

```
estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

```
res = results(dds)
```

> Q3. 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.

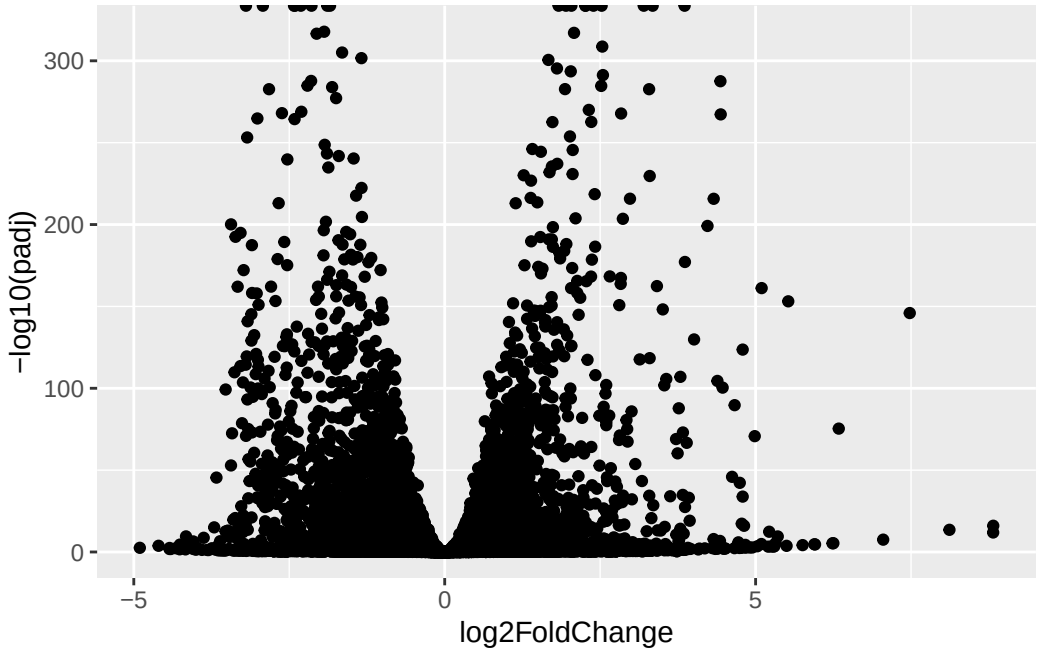
```
summary(res)
```

```
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 Now we will make a volcano plot, a commonly produced visualization from this type of data that we introduced last day. Basically it's a plot of log2 fold change vs -log adjusted p-value.

```
library(ggplot2)

ggplot(res) +
aes(x = log2FoldChange,
y = -log10(padj)) +
geom_point()
```

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

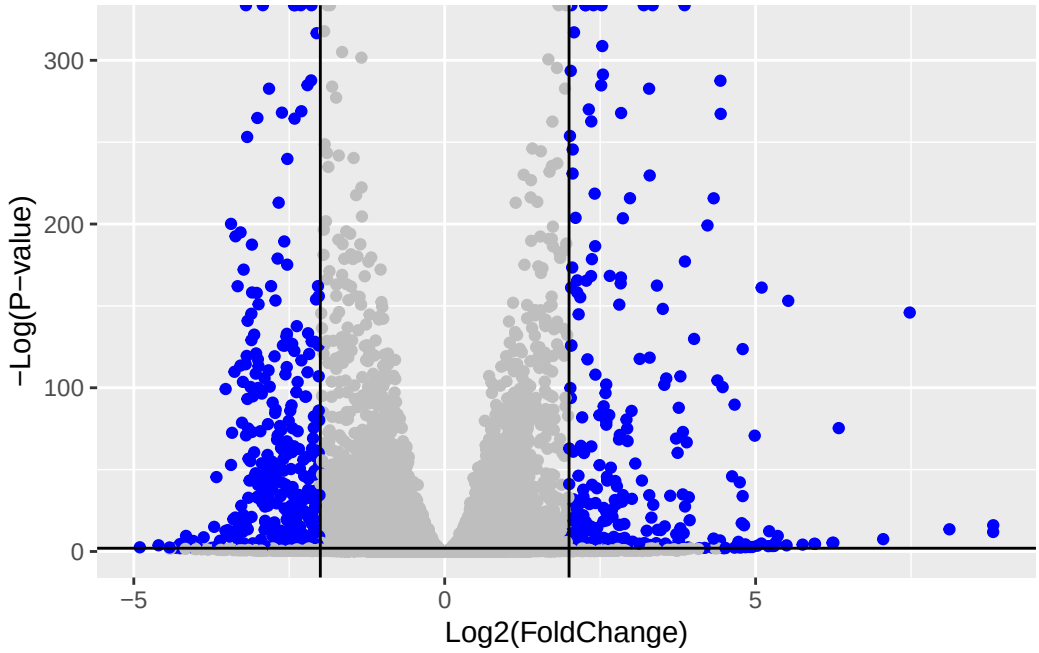


Q4. Improve this plot by completing the below code, which adds color, axis labels and cutoff lines:

```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res))

# Color blue the genes with fold change above 2
mycols[ abs(res$log2FoldChange) > 2 ] <- "blue"
```

```
# Color gray those with adjusted p-value more than 0.01
mycols[ res$padj > 0.01 ] <- "gray"

ggplot(res) +
aes(x = log2FoldChange,
y = -log10(padj)) +
geom_point(color = mycols) +
xlab("Log2(FoldChange)") +
ylab("-Log(P-value)") +
geom_vline(xintercept = c(-2,2)) +
geom_hline(yintercept = -log10(0.01))
```

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



Add Gene Annotation

Since we mapped and counted against the Ensembl annotation, our results only have information about Ensembl gene IDs. However, our pathway analysis downstream will use KEGG pathways, and genes in KEGG pathways are annotated with Entrez gene IDs. So lets add them as we did the last day.

Q5.Use the mapIDs() function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below.

```
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 ..
```

Q6.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$padj),]
write.csv(res, file="deseq_results.csv")
```

Now lets see how pathway analysis can help us make further sense out of this ranked list of differentially expressed genes.

Pathway Analysis

Here we are going to use the gage package for pathway analysis. Once we have a list of enriched pathways, we're going to use the pathview package to draw pathway diagrams, shading the molecules in the pathway by their degree of up/down-regulation.

While there are many freely available tools to do pathway analysis, and some like gage are truly fantastic, many of them are poorly maintained or rarely updated.

```
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"
```

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      2034      2150      6659
-2.422719  3.201955 -2.313738 -1.888019  3.344508  2.392288
```

Now, let's run the gage pathway analysis.

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

Now lets look at the object returned from gage().

```
attributes(keggres)
```

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

It is a list with three elements, "greater", "less" and "stats". You can also see this in your Environment panel/tab window of RStudio or use the R command str(keggres). Like any list we can use the dollar syntax to access a named element, e.g. head(keggres$greater) and head(keggres$less). Lets look at the first few down (less) pathway results:

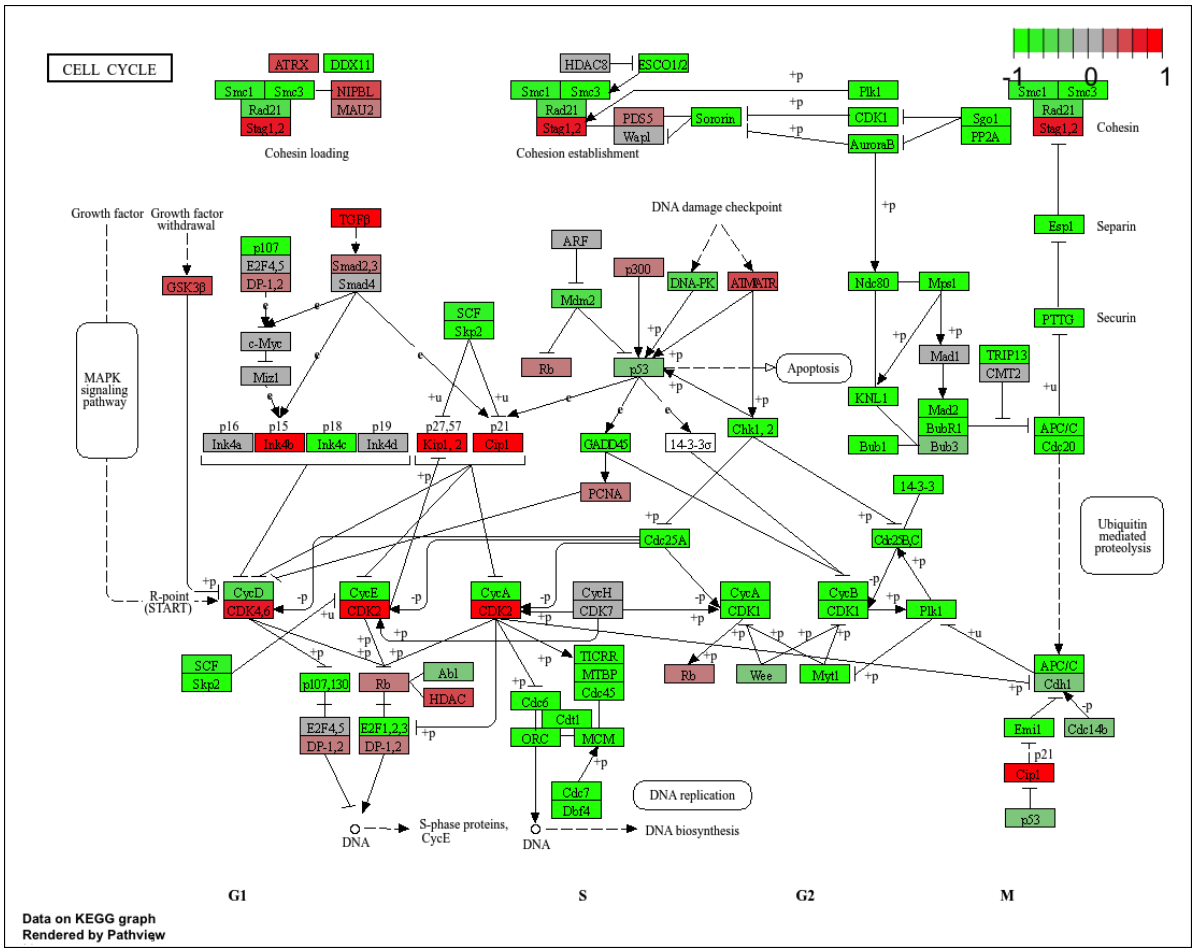
```
# Look at the first few down (less) pathways
head(keggres$less)
```

```
                                      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
```

Each keggres$less and keggres$greater object is data matrix with gene sets as rows sorted by p-value. The top "less/down" pathways is "Cell cycle" with the KEGG pathway identifier hsa04110. Now, let's try out the pathview() function from the pathview package to make a pathway plot with our RNA-Seq expression results shown in color. To begin with lets manually supply a pathway.id (namely the first part of the "hsa04110 Cell cycle") that we could see from the print out above.

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

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

Info: Working in directory /Users/mel/Documents/BIMM143/class14

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



You can play with the other input arguments to pathview() to change the display in various ways including generating a PDF graph. For example:

```
# 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 /Users/mel/Documents/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.

> Q7. Can you do the same procedure as above to plot the pathview figures for the top 5 down-regulated pathways?

```
## 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
```

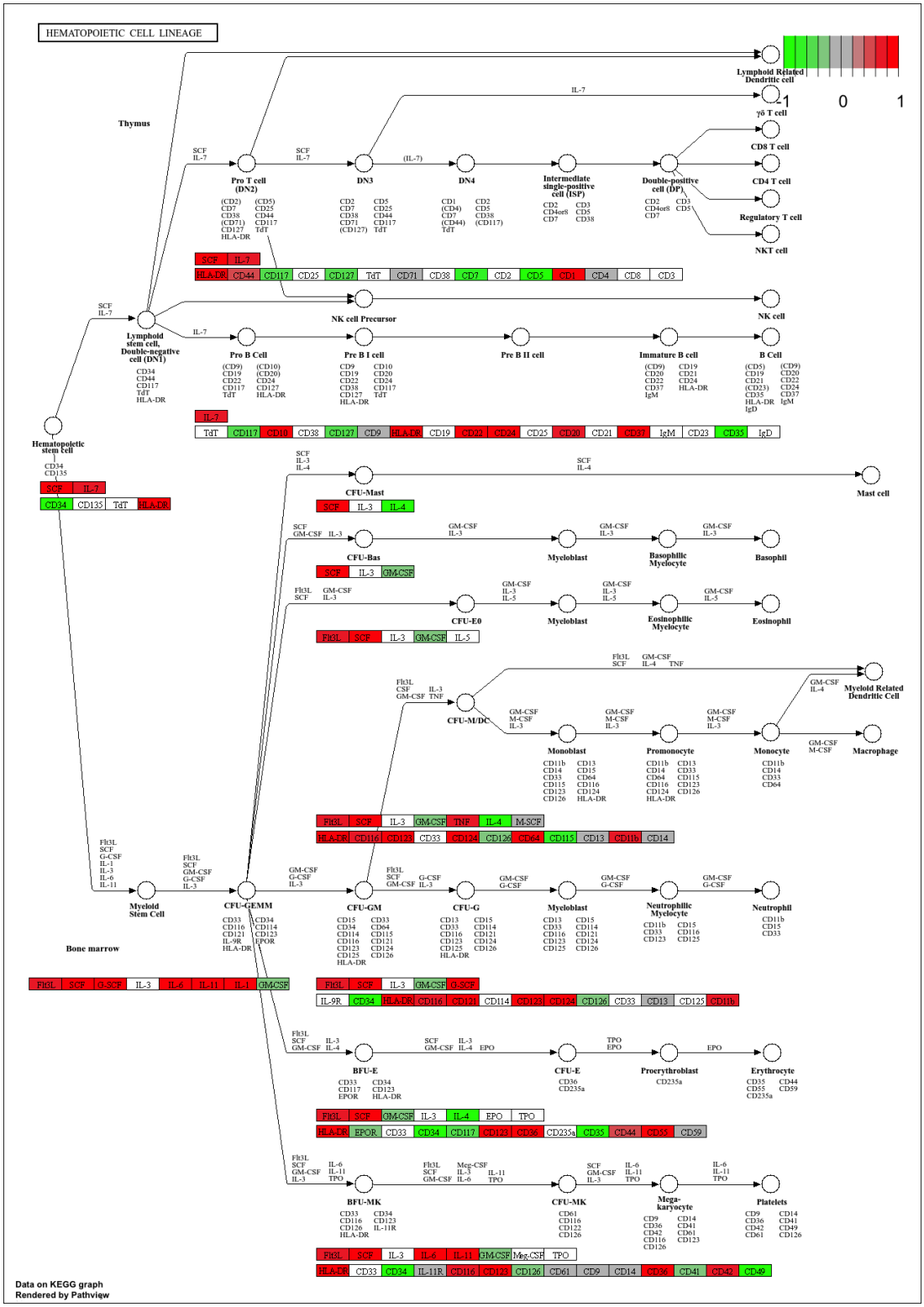
```
[1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```

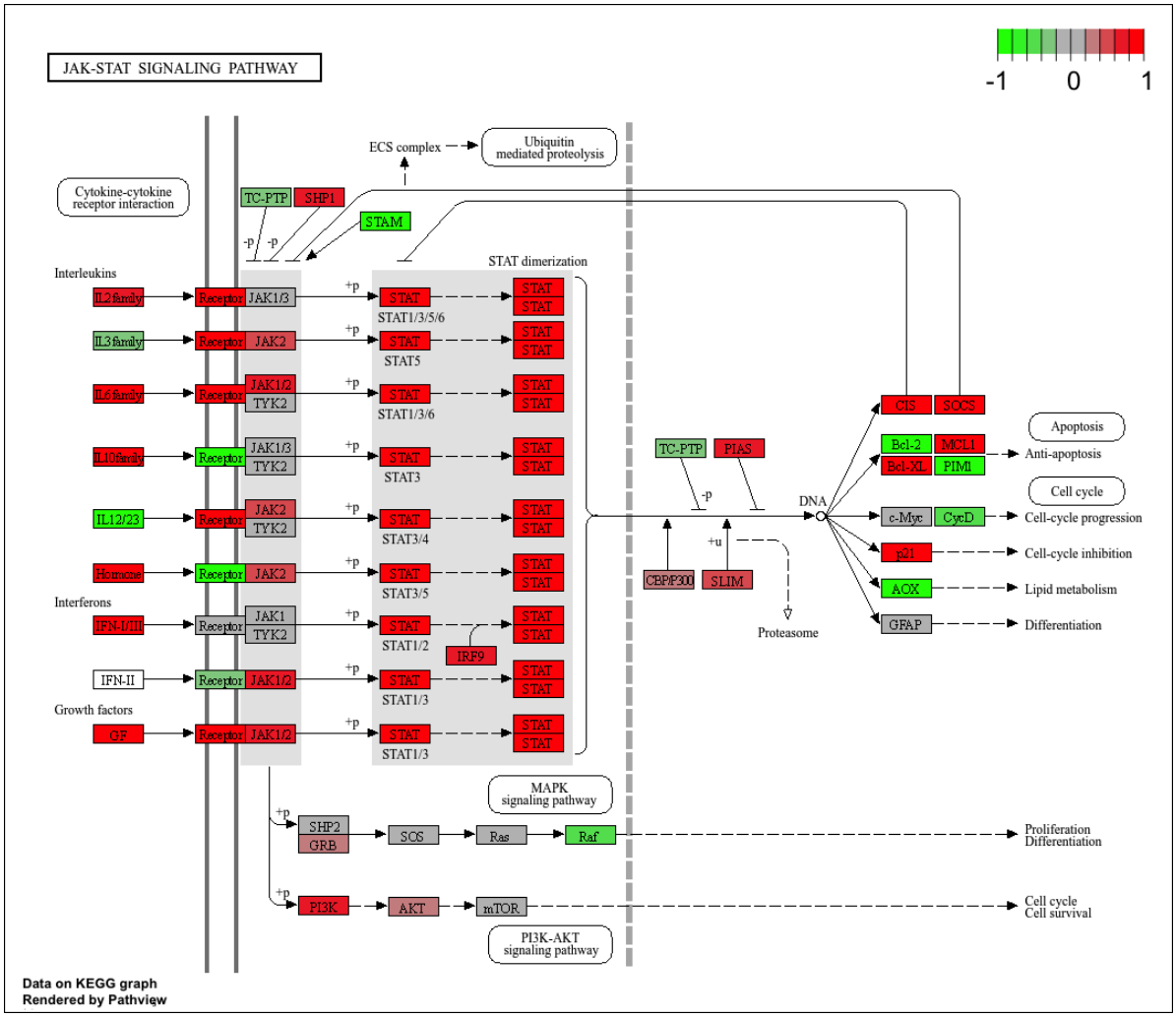
Finally, lets pass these IDs in keggresids to the pathview() function to draw plots for all the top 5 pathways.

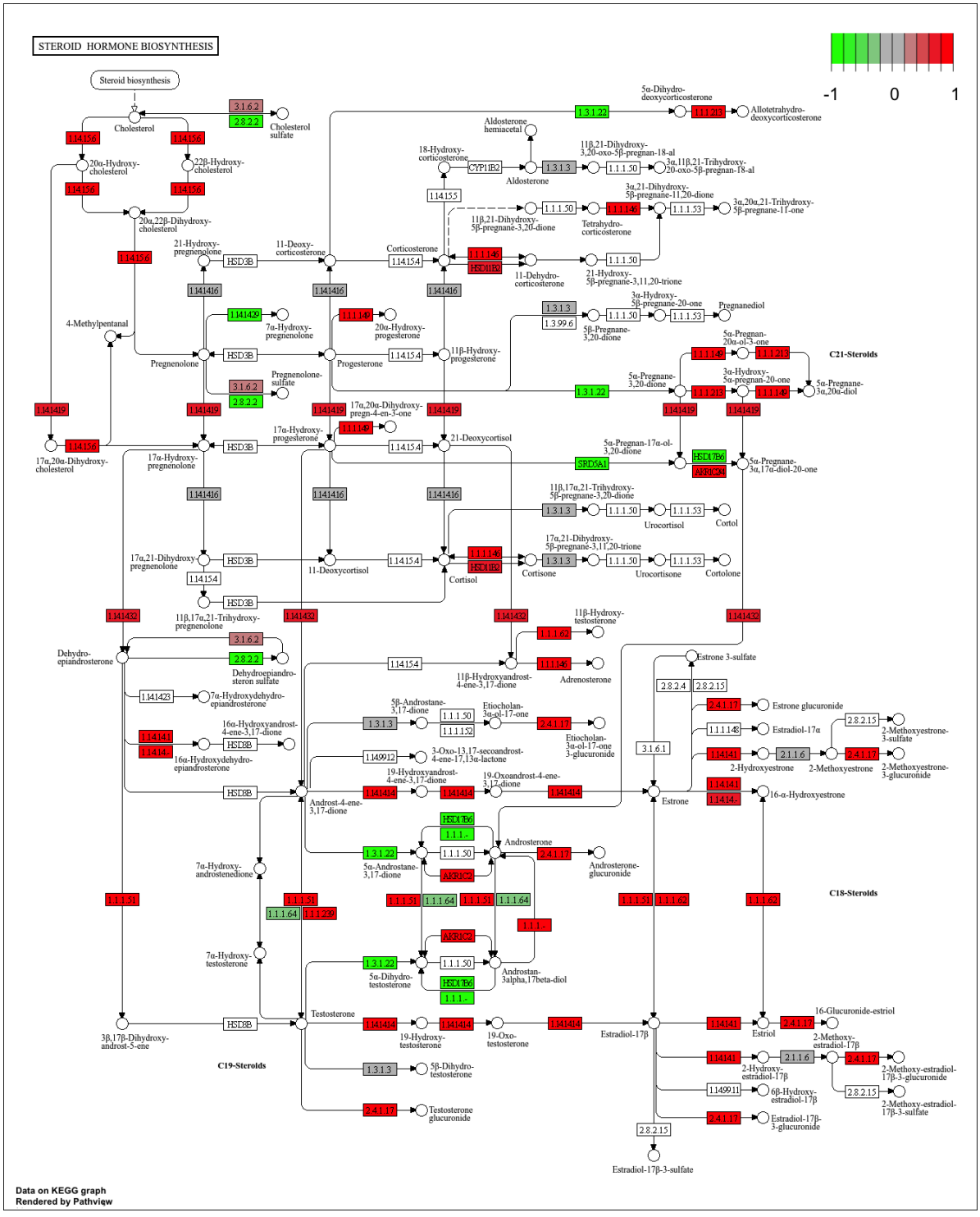
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

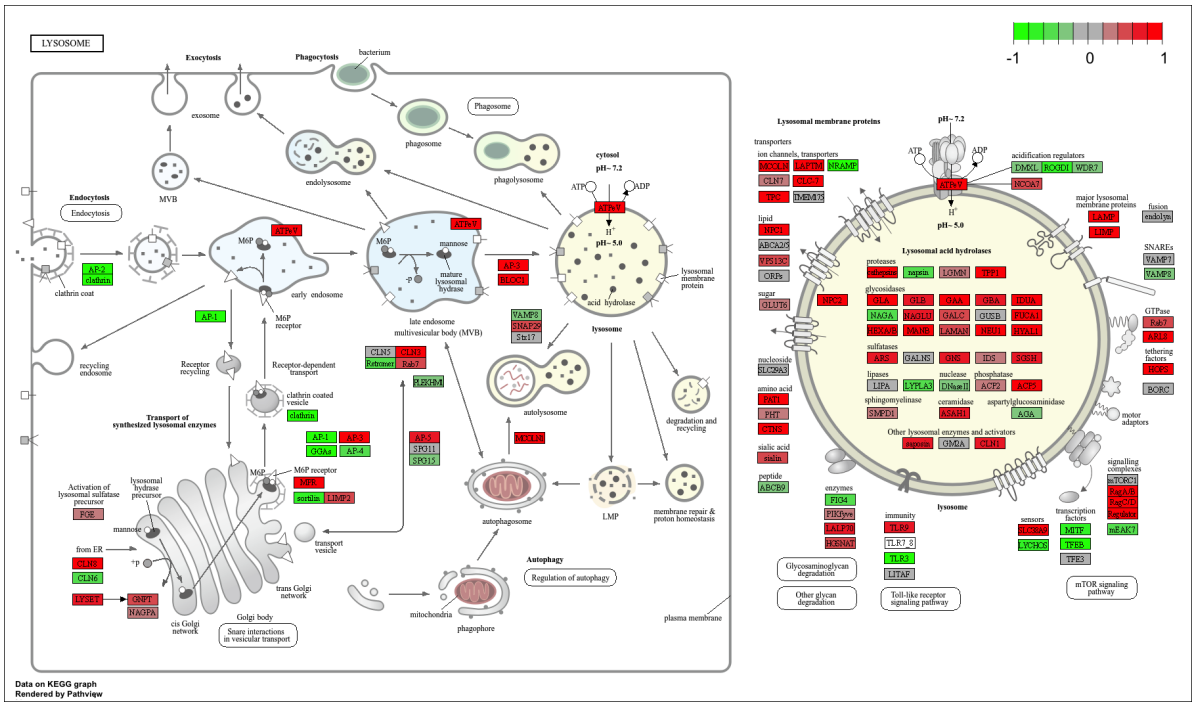
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/mel/Documents/BIMM143/class14

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory /Users/mel/Documents/BIMM143/class14

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory /Users/mel/Documents/BIMM143/class14
```

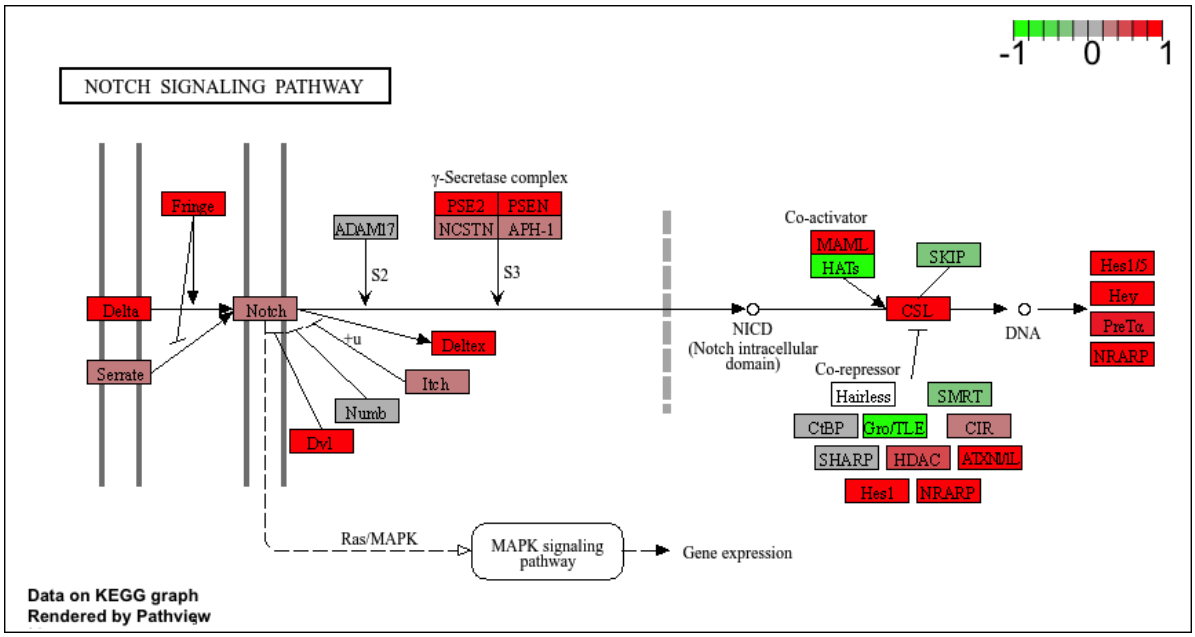
```
Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory /Users/mel/Documents/BIMM143/class14

Info: Writing image file hsa04142.pathview.png

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

Info: Working in directory /Users/mel/Documents/BIMM143/class14

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


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
SCF
IL-7
Pro T cell (DN2)
DN3
DN4
Intermediate single-positive cell (ISP)
Double-positive cell (DP)
(CD2) (CD5) CD7 CD25 CD38 CD44 (CD71) CD117 CD127 TdT HLA-DR
CD2 CD5 CD7 CD25 CD38 CD44 CD71 CD117 (CD127) TdT
CD1 (CD4) CD7 (CD44) TdT
CD2 CD5 CD38 (CD117)
CD2 CD4or8 CD7
CD2 CD3 CD4or8 CD5 CD7
SCF IL-7
HLA-DR CD44 CD117 CD25 CD127 TdT CD71 CD38 CD7 CD2 CD5 CD1 CD4 CD8 CD3
NK cell Precursor
NK cell
Lymphoid stem cell, Double-negative cell (DN1)
Pro B Cell
Pre B I cell
Pre B II cell
Immature B cell
B Cell
CD34 CD44 CD117 TdT HLA-DR
(CD9) (CD10) CD19 (CD20) CD22 CD24 CD117 CD127 TdT HLA-DR
CD9 CD10 CD19 CD20 CD22 CD24 CD38 CD117 CD127 TdT HLA-DR
(CD9) CD19 CD20 CD21 CD22 CD24 CD37 HLA-DR IgM
(CD5) (CD9) CD19 CD20 CD21 CD22 (CD23) CD24 CD35 CD37 HLA-DR IgM IgD
IL-7
TdT CD117 CD10 CD38 CD127 CD9 HLA-DR CD19 CD22 CD24 CD25 CD20 CD21 CD37 IgM CD23 CD35 IgD
Hematopoietic stem cell
CD34 CD135
SCF IL-7
CD34 CD135 TdT HLA-DR
SCF IL-3 IL-4
CFU-Mast
Mast cell
SCF IL-3 IL-4
SCF GM-CSF IL-3
CFU-Bas
Myeloblast
Basophilic Myelocyte
Basophil
GM-CSF IL-3
SCF IL-3 GM-CSF
Flt3L SCF GM-CSF IL-3
CFU-E0
Eosinophilic Myelocyte
Eosinophil
GM-CSF IL-3 IL-5
Flt3L SCF IL-3 GM-CSF IL-5
Flt3L SCF IL-4 TNF
Flt3L CSF GM-CSF TNF IL-3
CFU-M/DC
Myeloid Related Dendritic Cell
Monoblast
Promonocyte
Monocyte
Macrophage
GM-CSF M-CSF IL-3
GM-CSF IL-4
GM-CSF M-CSF
CD11b CD13 CD14 CD15 CD33 CD64 CD115 CD116 CD123 CD124 CD126 HLA-DR
CD11b CD13 CD14 CD33 CD64 CD115 CD116 CD123 CD124 HLA-DR
CD11b CD14 CD33 CD64
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-CSF IL-1 IL-3 IL-6 IL-11
Flt3L SCF GM-CSF G-CSF IL-3
GM-CSF G-CSF IL-3
Flt3L SCF GM-CSF G-CSF IL-3
G-CSF GM-CSF
Myeloid Stem Cell
CFU-GEMM
CFU-GM
CFU-G
Myeloblast
Neutrophilic Myelocyte
Neutrophil
CD33 CD116 CD121 IL-9R HLA-DR
CD34 CD114 CD123 EPOR
CD15 CD33 CD34 CD64 CD114 CD115 CD116 CD121 CD123 CD124 CD125 CD126 HLA-DR
CD13 CD15 CD33 CD114 CD116 CD121 CD123 CD124 CD125 CD126 HLA-DR
CD13 CD15 CD33 CD114 CD116 CD121 CD123 CD124 CD125 CD126
CD11b CD15 CD33 CD115 CD116 CD123 CD125
CD11b CD15 CD33
Bone marrow
Flt3L SCF G-CSF IL-3 IL-6 IL-11 IL-1 GM-CSF
Flt3L SCF IL-3 GM-CSF G-CSF
IL-9R CD34 HLA-DR CD116 CD121 CD114 CD123 CD124 CD126 CD33 CD13 CD125 CD11b
Flt3L SCF GM-CSF IL-3 IL-4
SCF GM-CSF IL-3 IL-4 EPO
TPO EPO
EPO
BFU-E
CFU-E
Procrythroblast
Erythrocyte
CD33 CD117 EPOR CD34 CD123 HLA-DR
CD36 CD235a
CD235a
CD35 CD55 CD235a CD44 CD59
Flt3L SCF GM-CSF IL-3 IL-4 EPO TPO
HLA-DR EPOR CD33 CD34 CD117 CD123 CD36 CD235a CD35 CD44 CD55 CD59
Flt3L SCF GM-CSF IL-3 IL-6 IL-11 TPO
Flt3L SCF GM-CSF IL-3 Meg-CSF IL-6 IL-11 TPO
SCF GM-CSF IL-3 IL-6 IL-11 TPO
IL-6 IL-11 TPO
BFU-MK
CFU-MK
Mega-karyocyte
Platelets
CD33 CD116 CD126 CD34 CD123 IL-11R HLA-DR
CD61 CD116 CD122 CD126
CD9 CD36 CD42 CD116 CD126 CD14 CD41 CD61 CD123
CD9 CD36 CD42 CD61 CD14 CD41 CD49 CD126
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
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
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
TC-PTP
PIAS
DNA
+u
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


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

lapply(gobpres, head)
```

```
$greater
                                           p.geomean stat.mean        p.val
GO:0007156 homophilic cell adhesion       8.519724e-05  3.824205 8.519724e-05
GO:0002009 morphogenesis of an epithelium 1.396681e-04  3.653886 1.396681e-04
GO:0048729 tissue morphogenesis           1.432451e-04  3.643242 1.432451e-04
GO:0007610 behavior                       1.925222e-04  3.565432 1.925222e-04
GO:0060562 epithelial tube morphogenesis  5.932837e-04  3.261376 5.932837e-04
GO:0035295 tube development               5.953254e-04  3.253665 5.953254e-04
                                               q.val set.size         exp1
GO:0007156 homophilic cell adhesion       0.1951953      113 8.519724e-05
GO:0002009 morphogenesis of an epithelium 0.1951953      339 1.396681e-04
GO:0048729 tissue morphogenesis           0.1951953      424 1.432451e-04
GO:0007610 behavior                       0.1967577      426 1.925222e-04
GO:0060562 epithelial tube morphogenesis  0.3565320      257 5.932837e-04
GO:0035295 tube development               0.3565320      391 5.953254e-04

$less
                                             p.geomean stat.mean        p.val
GO:0048285 organelle fission              1.536227e-15 -8.063910 1.536227e-15
GO:0000280 nuclear division               4.286961e-15 -7.939217 4.286961e-15
GO:0007067 mitosis                        4.286961e-15 -7.939217 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.169934e-14 -7.797496 1.169934e-14
GO:0007059 chromosome segregation         2.028624e-11 -6.878340 2.028624e-11
GO:0000236 mitotic prometaphase           1.729553e-10 -6.695966 1.729553e-10
                                                 q.val set.size         exp1
GO:0048285 organelle fission              5.841698e-12      376 1.536227e-15
GO:0000280 nuclear division               5.841698e-12      352 4.286961e-15
GO:0007067 mitosis                        5.841698e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.195672e-11      362 1.169934e-14
GO:0007059 chromosome segregation         1.658603e-08      142 2.028624e-11
```

```
GO:0000236 mitotic prometaphase          1.178402e-07       84 1.729553e-10

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

Reactome Analysis

Reactome is database consisting of biological molecules and their relation to pathways and processes. Reactome, such as many other tools, has an online software available and R package available.

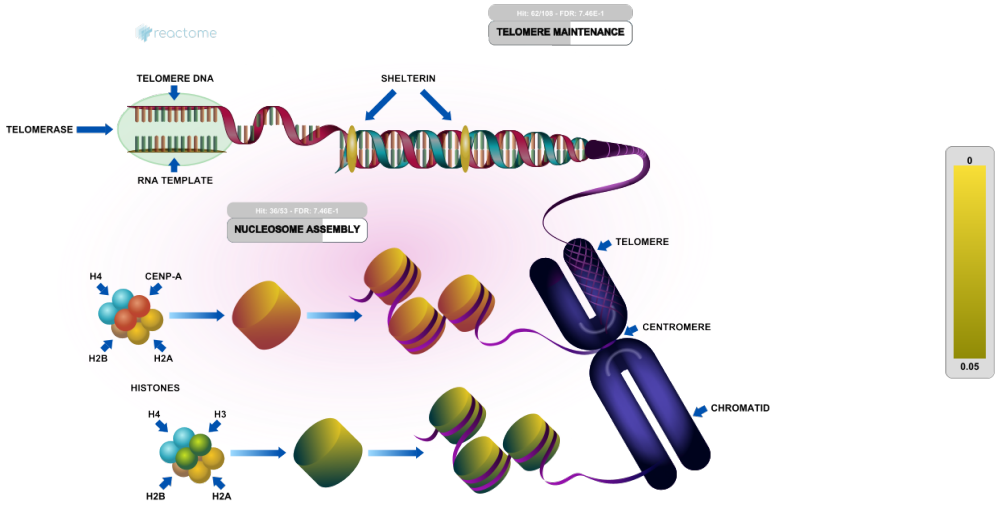
Let's now conduct over-representation enrichment analysis and pathway-topology analysis with Reactome using the previous list of significant genes generated from our differential expression results above.

First, using R, output the list of significant genes at the 0.05 level as a plain text file:

```
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, quote=
```



Q8: 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 cell cycle, Mitotic is the most significant because the p-value 2.15e-5 which do match the previous KEGG results. the different factors could be filtering and mapping which can contribute to the differences between the two.