class 13

jimmi

Load our data files

```
# Import metadata and take a peak
colData = read.csv("GSE37704_metadata.csv", row.names=1)
head(colData)
```

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

```
# Import countData and take a peak
countDataTmp = read.csv("GSE37704_featurecounts.csv", row.names=1)
head(countDataTmp)
```

```
                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(countDataTmp[,-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
```

Check that my metadata and count data match.

```
rownames(colData) == colnames(countData)
```

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

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).

```
# Filter count data where you have 0 read count across all samples.
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
```

```r
to.keep = rowSums(countData) != 0
countData = countData[to.keep,]

nrow(countData)
```

```
[1] 15975
```

```r
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
```

DESeq analysis

```r
library(DESeq2)
```

```
Loading required package: S4Vectors

Loading required package: stats4

Loading required package: BiocGenerics


Attaching package: 'BiocGenerics'

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

    IQR, mad, sd, var, xtabs
```

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

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


Attaching package: 'S4Vectors'

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

    expand.grid, I, unname

Loading required package: IRanges


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
```

```r
head(colData)
```

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

Setup the object that DESeq needs for analysis with the lovely long-winded function:

Nice now lets setup the DESeqDataSet object required for the `DESeq()` function and then run the DESeq pipeline. This is again similar to our last days 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
```

Run the analysis:

```
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)
```

```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
```

> 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.

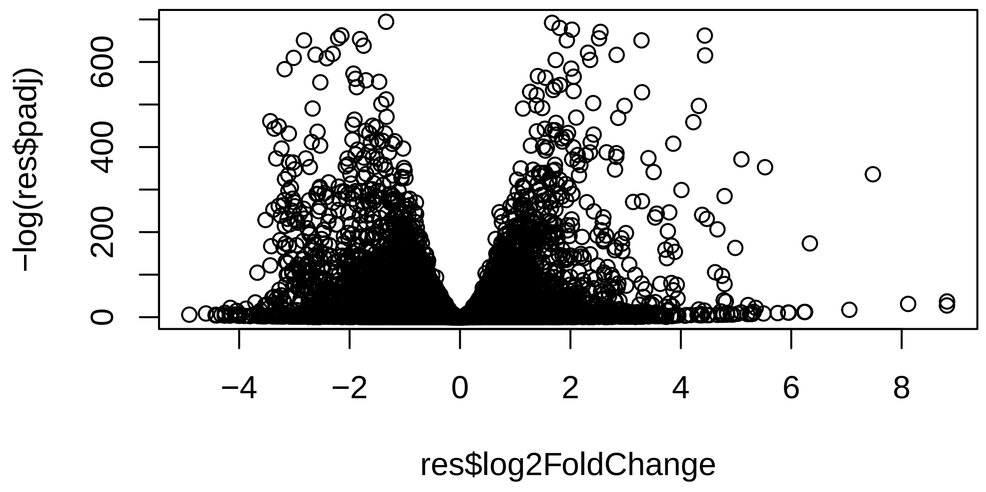
```r
summary(res)
```

```

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
```

Volcano plot

log2 fold change (MLE): condition hoxa1 kd vs control sirna

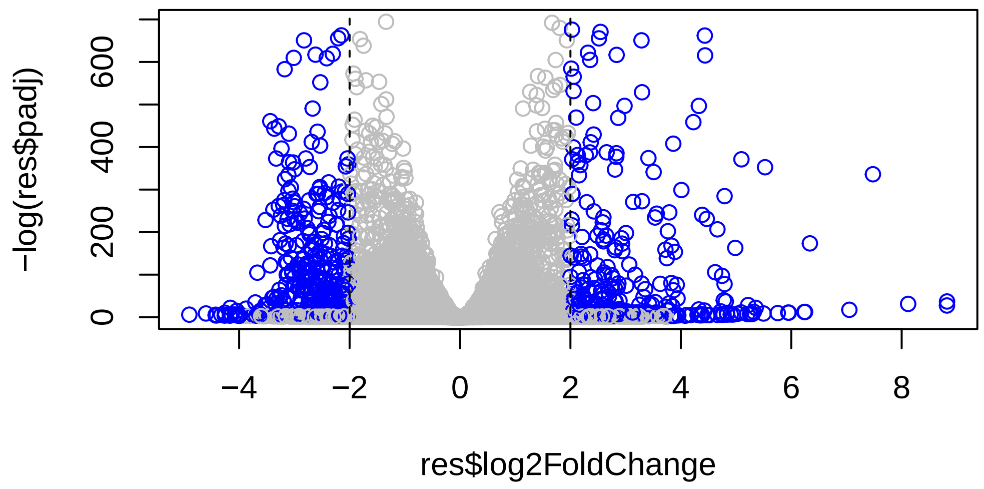
```r
plot( res$log2FoldChange, -log(res$padj) )
```



Q. Improve this plot by completing the below code, which adds color and axis labels

Make some colors to highlight the subset of genes with significant high fold change values

```
mycols = rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2] = "blue"
mycols[ res$padj > 0.05] = "gray"

plot( res$log2FoldChange, -log(res$padj), col=mycols )
abline(v = c(-2,2), lty = 2)
```



```
x = 1:5
x
```

```
[1] 1 2 3 4 5
```

```
mycols = rep("grey", length(x))
mycols[ x > 2] = "blue"
mycols[ x == 5] = "red"

mycols
```

```
[1] "grey" "grey" "blue" "blue" "red"
```

Add gene annotation data

We will load up AnnatationDbi and our Human data package to add gene symbols and entrez IDs to our results object

```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,
                     column = "SYMBOL",
                     keys = rownames(res),
                     keytype = "ENSEMBL")
```

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

```r
res$entrez = mapIds(org.Hs.eg.db,
                     column = "ENTREZID",
                     keys = rownames(res),
                     keytype = "ENSEMBL")
```

```
'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)
```

```
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 9 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      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..
```

> 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")
```

Genset enrichment analysis (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)
```

```r
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.

```
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)
```

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

```
attributes(keggres)
```

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

Lets look at the first few down (less) pathway results:

```r
# 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
```

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.

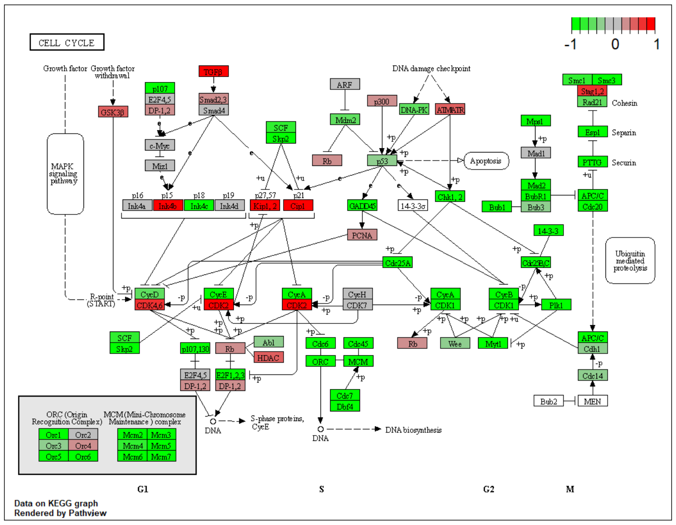
```r
pathview(gene.data=foldchanges, pathway.id="hsa04110")
```

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

```
Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13
```

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

Put this into my document.



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

```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

Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

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.

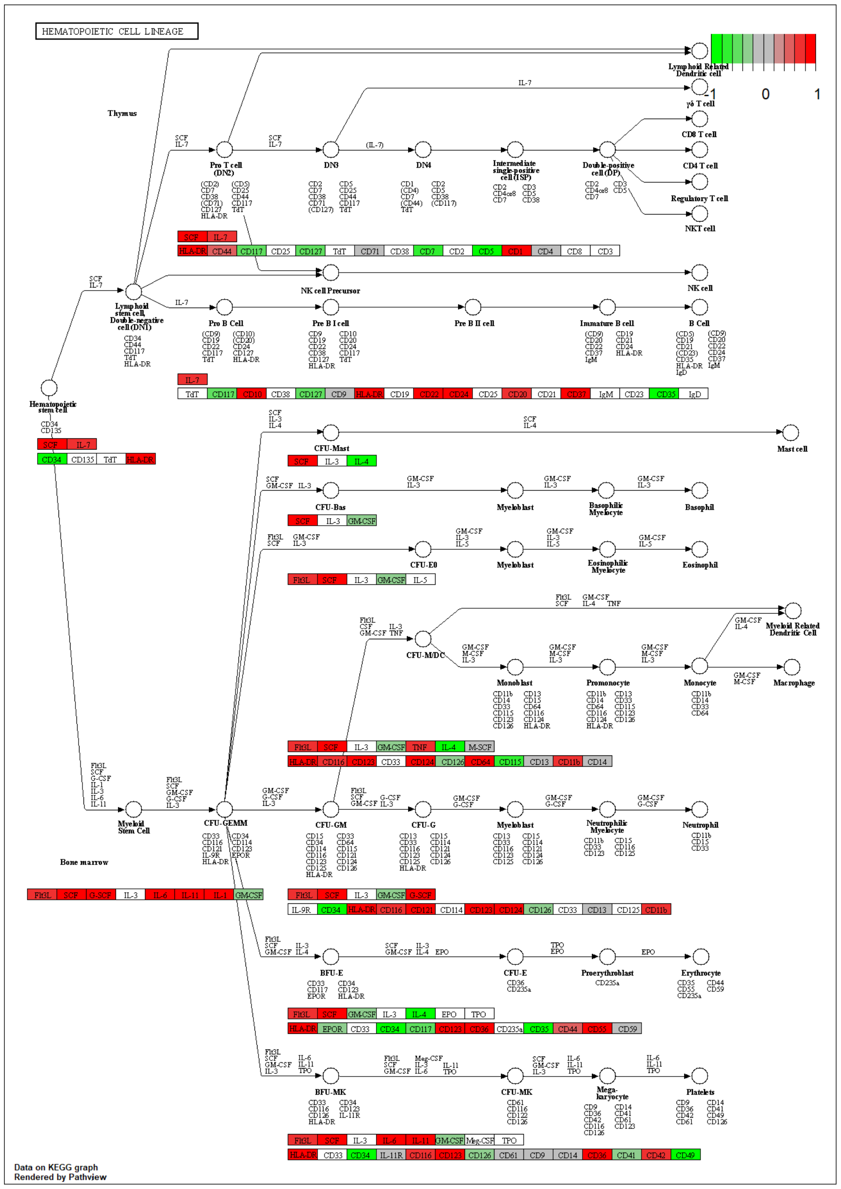
```r
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$greater)[1:5]

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

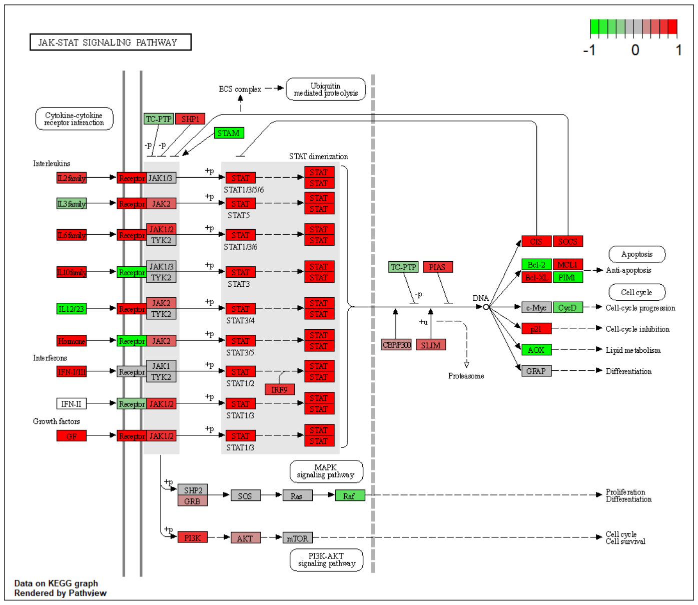
```
[1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```

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

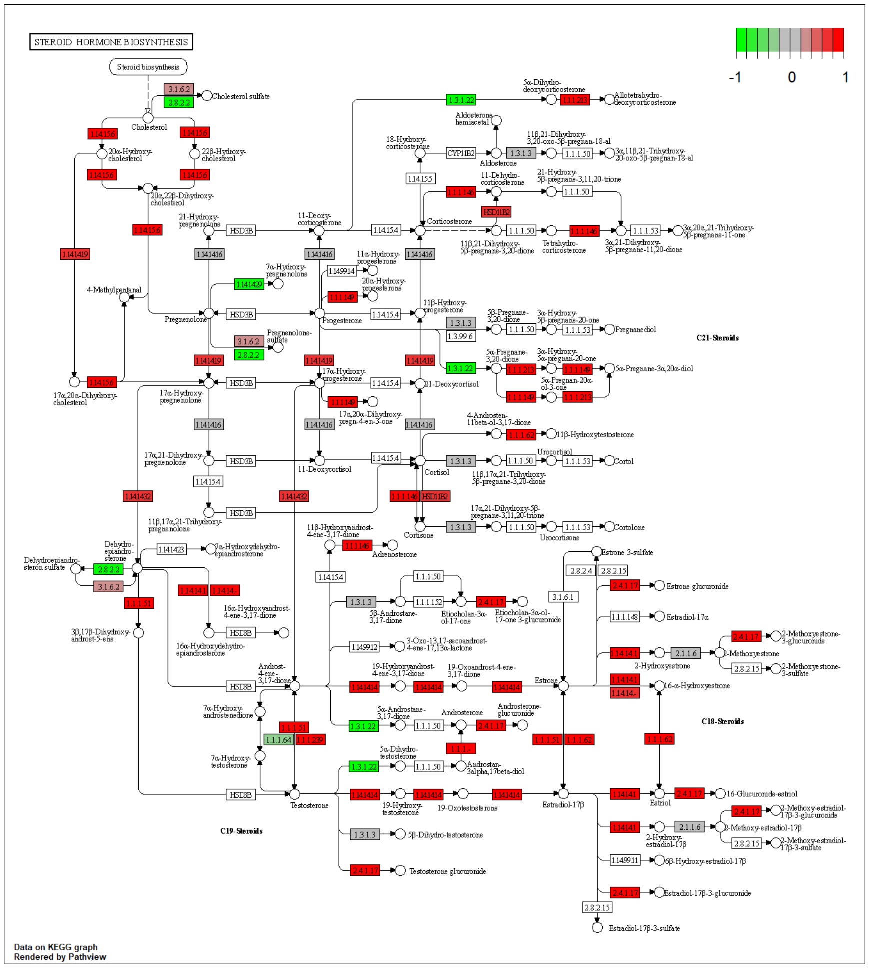
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

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

Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

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 C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

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

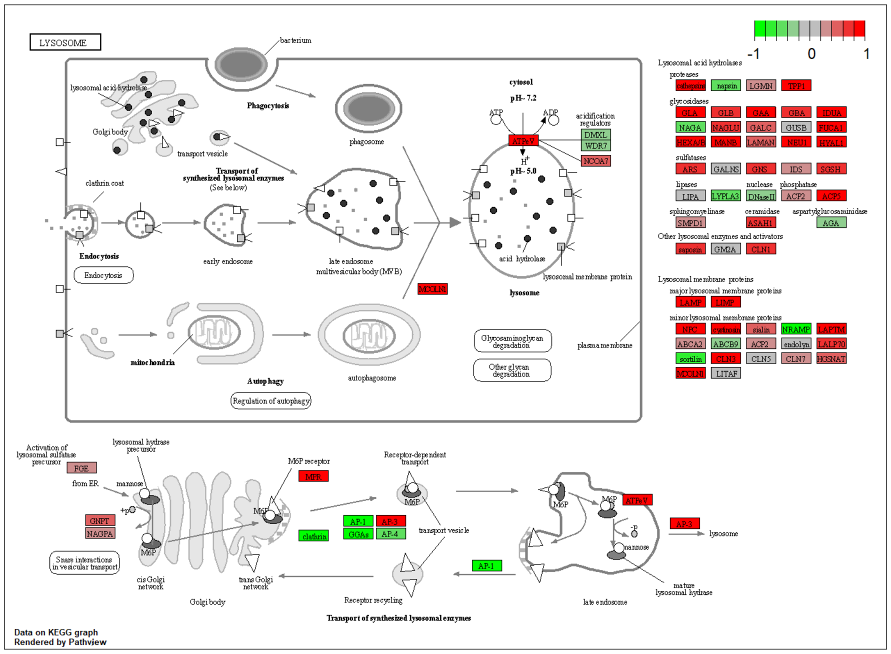

HEMATOPOIETIC CELL LINEAGE
-1
0
1
Thymus
Bone marrow
Hematopoietic stem cell
Lymphoid stem cell, Double-negative cell (DN1)
Pro T cell (DN2)
DN3
DN4
Intermediate single-positive cell (ISP)
Double-positive cell (DP)
Lymphoid Related Dendritic cell
γδ T cell
CD8 T cell
CD4 T cell
Regulatory T cell
NKT 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
Proerythroblast
Erythrocyte
BFU-MK
CFU-MK
Mega-karyocyte
Platelets
Data on KEGG graph
Rendered by Pathview

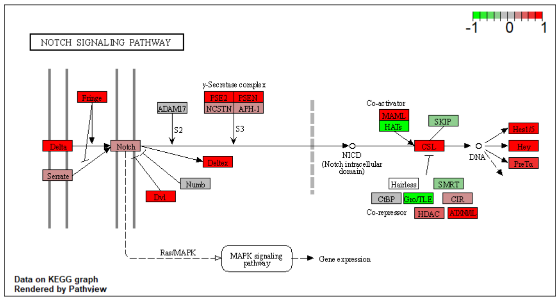


JAK-STAT SIGNALING PATHWAY
-1
0
1
ECS complex
Ubiquitin mediated proteolysis
Cytokine-cytokine receptor interaction
TC-PTP
SHP1
STAM
-p
-p
STAT dimerization
Interleukins
IL2 family
IL3 family
IL6 family
IL10 family
IL12/23
Hormone
Interferons
IFN-I/III
IFN-II
Growth factors
GF
Receptor
JAK1/3
JAK2
JAK1/2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


STEROID HORMONE BIOSYNTHESIS
-1
0
1
Steroid biosynthesis
Cholesterol
Cholesterol sulfate
Pregnenolone
Progesterone
Corticosterone
Aldosterone
Cortisol
Cortisone
Testosterone
Estrone
Estradiol-17β
Estriol
C21-Steroids
C19-Steroids
C18-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?

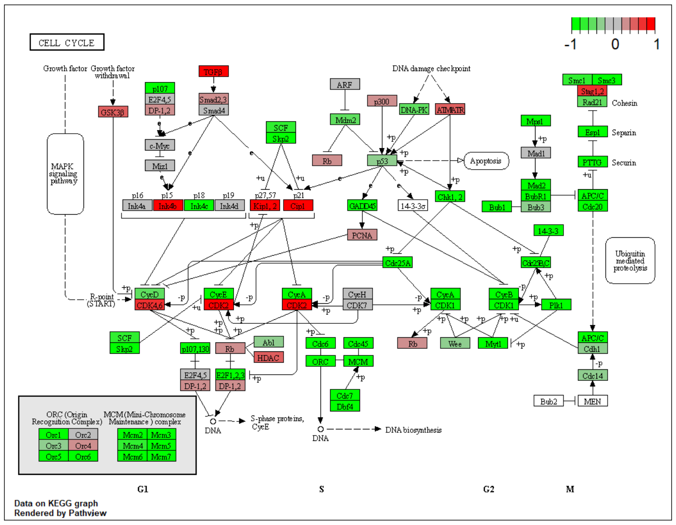
```
keggrespathways <- rownames(keggres$less)[1:5]

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

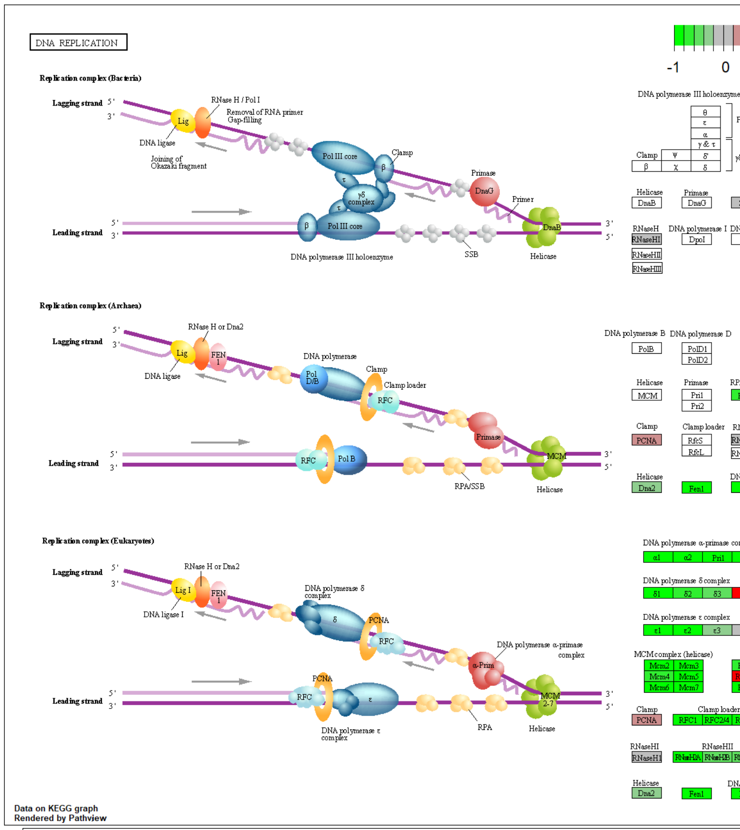
```
[1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

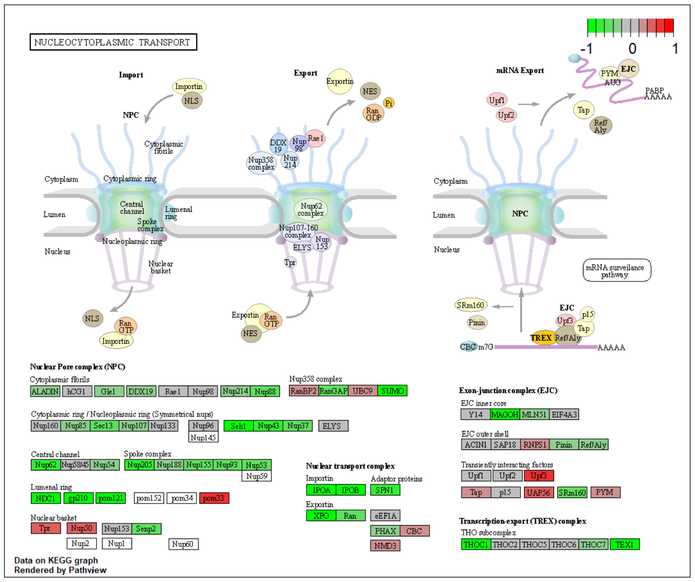
Finally, lets pass these IDs in keggresids to the pathview() function to draw plots for all the top down-regulated 5 pathways.

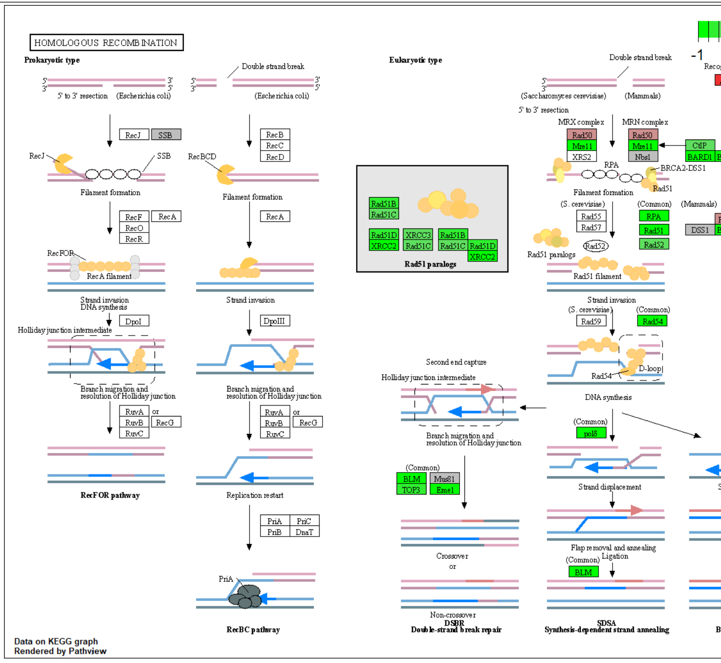
```r
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

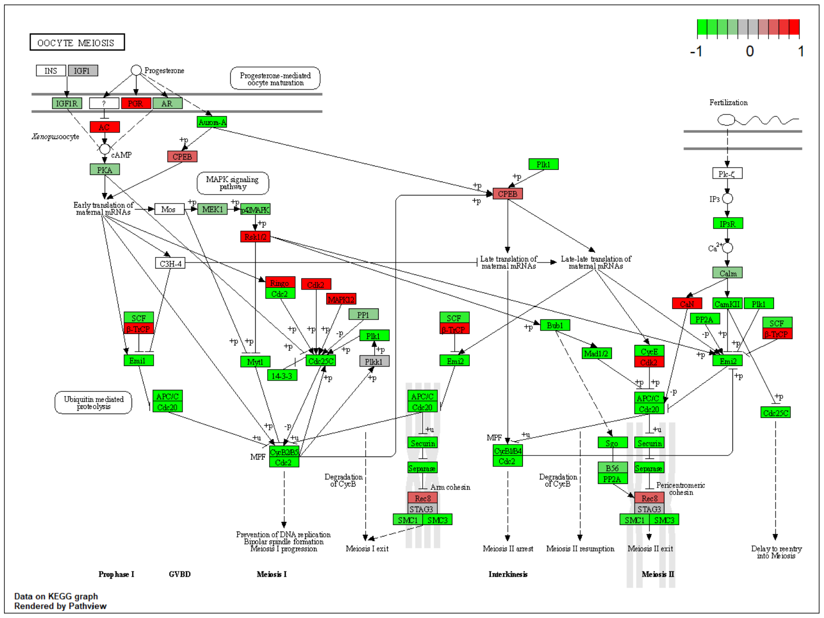
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

Info: Writing image file hsa03440.pathview.png

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

Info: Working in directory C:/Users/jimmi/OneDrive/Desktop/bimm 143/class_13

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


CELL CYCLE
-1 0 1
G1
S
G2
M
Data on KEGG graph
Rendered by Pathview


DNA REPLICATION
-1 0
Replication complex (Bacteria)
Replication complex (Archaea)
Replication complex (Eukaryotes)
Lagging strand
Leading strand
Data on KEGG graph
Rendered by Pathview


NUCLEOCYTOPLASMIC TRANSPORT
-1 0 1
Import
Export
mRNA Export
Nuclear Pore complex (NPC)
Exon-junction complex (EJC)
Nuclear transport complex
Transcription-export (TREX) complex
Data on KEGG graph
Rendered by Pathview


HOMOLOGOUS RECOMBINATION
-1
Prokaryotic type
Eukaryotic type
RecF OR pathway
RecBC pathway
DSBR
Double-strand break repair
SDSA
Synthesis-dependent strand annealing
Data on KEGG graph
Rendered by Pathview



Section 3: Gene Ontology

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)

lapply(gobpres, head)
```

```
$greater
                                            p.geomean stat.mean        p.val
GO:0007156 homophilic cell adhesion       8.519724e-05  3.824205 8.519724e-05
GO:0002009 morphogenesis of an epithelium 1.396681e-04  3.653886 1.396681e-04
GO:0048729 tissue morphogenesis           1.432451e-04  3.643242 1.432451e-04
GO:0007610 behavior                       2.195494e-04  3.530241 2.195494e-04
```

```
GO:0060562 epithelial tube morphogenesis  5.932837e-04  3.261376 5.932837e-04
GO:0035295 tube development               5.953254e-04  3.253665 5.953254e-04
                                              q.val set.size         exp1
GO:0007156 homophilic cell adhesion       0.1951953      113 8.519724e-05
GO:0002009 morphogenesis of an epithelium 0.1951953      339 1.396681e-04
GO:0048729 tissue morphogenesis           0.1951953      424 1.432451e-04
GO:0007610 behavior                       0.2243795      427 2.195494e-04
GO:0060562 epithelial tube morphogenesis  0.3711390      257 5.932837e-04
GO:0035295 tube development               0.3711390      391 5.953254e-04

$less
                                            p.geomean stat.mean        p.val
GO:0048285 organelle fission             1.536227e-15 -8.063910 1.536227e-15
GO:0000280 nuclear division              4.286961e-15 -7.939217 4.286961e-15
GO:0007067 mitosis                       4.286961e-15 -7.939217 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.169934e-14 -7.797496 1.169934e-14
GO:0007059 chromosome segregation        2.028624e-11 -6.878340 2.028624e-11
GO:0000236 mitotic prometaphase          1.729553e-10 -6.695966 1.729553e-10
                                                q.val set.size         exp1
GO:0048285 organelle fission             5.841698e-12      376 1.536227e-15
GO:0000280 nuclear division              5.841698e-12      352 4.286961e-15
GO:0007067 mitosis                       5.841698e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.195672e-11      362 1.169934e-14
GO:0007059 chromosome segregation        1.658603e-08      142 2.028624e-11
GO:0000236 mitotic prometaphase          1.178402e-07       84 1.729553e-10

$stats
                                          stat.mean     exp1
GO:0007156 homophilic cell adhesion        3.824205 3.824205
GO:0002009 morphogenesis of an epithelium  3.653886 3.653886
GO:0048729 tissue morphogenesis            3.643242 3.643242
GO:0007610 behavior                        3.530241 3.530241
GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
GO:0035295 tube development                3.253665 3.253665
```

Section 4: Reactome Analysis

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:

```r
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"
```

```r
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

> 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 pathway with the most significant "Entities p-value" is Endosomal/Vacuolar pathway. The significant pathways did not match because theses platforms possess unique databases, keggs versus reactome.

The `sessionInfo()` prints version information about R and any attached packages. It's a good practice to always run this command at the end of your R session and record it for the sake of reproducibility in the future.

```r
sessionInfo()
```

```
R version 4.2.2 (2022-10-31 ucrt)
Platform: x86_64-w64-mingw32/x64 (64-bit)
Running under: Windows 10 x64 (build 22621)

Matrix products: default

locale:
[1] LC_COLLATE=English_United States.utf8
[2] LC_CTYPE=English_United States.utf8
[3] LC_MONETARY=English_United States.utf8
[4] LC_NUMERIC=C
[5] LC_TIME=English_United States.utf8

attached base packages:
[1] stats4    stats     graphics  grDevices utils     datasets  methods
[8] base

other attached packages:
 [1] gageData_2.36.0             gage_2.48.0
 [3] pathview_1.38.0             org.Hs.eg.db_3.16.0
 [5] AnnotationDbi_1.60.0        DESeq2_1.38.3
 [7] SummarizedExperiment_1.28.0 Biobase_2.58.0
 [9] MatrixGenerics_1.10.0       matrixStats_0.63.0
[11] GenomicRanges_1.50.2        GenomeInfoDb_1.34.9
[13] IRanges_2.32.0              S4Vectors_0.36.1
[15] BiocGenerics_0.44.0

loaded via a namespace (and not attached):
 [1] httr_1.4.4             bit64_4.0.5            jsonlite_1.8.4
 [4] blob_1.2.3             GenomeInfoDbData_1.2.9 yaml_2.3.7
 [7] pillar_1.8.1           RSQLite_2.3.0          lattice_0.20-45
[10] glue_1.6.2             digest_0.6.31          RColorBrewer_1.1-3
[13] XVector_0.38.0         colorspace_2.1-0       htmltools_0.5.4
[16] Matrix_1.5-1           XML_3.99-0.13          pkgconfig_2.0.3
[19] zlibbioc_1.44.0        xtable_1.8-4           GO.db_3.16.0
[22] scales_1.2.1           BiocParallel_1.32.5    tibble_3.1.8
[25] annotate_1.76.0        KEGGREST_1.38.0        generics_0.1.3
[28] ggplot2_3.4.1          cachem_1.0.6           cli_3.6.0
[31] magrittr_2.0.3         crayon_1.5.2           memoise_2.0.1
[34] evaluate_0.20          KEGGgraph_1.58.3       fansi_1.0.4
[37] graph_1.76.0           tools_4.2.2            lifecycle_1.0.3
[40] munsell_0.5.0          locfit_1.5-9.7         DelayedArray_0.23.2
[43] Biostrings_2.66.0      compiler_4.2.2         rlang_1.0.6
[46] grid_4.2.2             RCurl_1.98-1.10        rstudioapi_0.14
[49] bitops_1.0-7           rmarkdown_2.20         gtable_0.3.1
[52] codetools_0.2-18       DBI_1.1.3              R6_2.5.1
[55] knitr_1.42             dplyr_1.1.0            fastmap_1.1.0
[58] bit_4.0.5              utf8_1.2.3             Rgraphviz_2.42.0
[61] parallel_4.2.2         Rcpp_1.0.10            vctrs_0.5.2
[64] geneplotter_1.76.0     png_0.1-8              tidyselect_1.2.0
[67] xfun_0.37
```