RNA-Seq Analysis Mini-Project

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11/21/2021

#Section 1 Differential Expression Analysis

```
library(DESeq2)
```

```
## Loading required package: S4Vectors

## Loading required package: stats4

## Loading required package: BiocGenerics

## Loading required package: parallel

##
## Attaching package: 'BiocGenerics'

## The following objects are masked from 'package:parallel':
##
##     clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
##     clusterExport, clusterMap, parApply, parCapply, parLapply,
##     parLapplyLB, parRapply, parSapply, parSapplyLB

## The following objects are masked from 'package:stats':
##
##     IQR, mad, sd, var, xtabs

## The following objects are masked from 'package:base':
##
##     anyDuplicated, 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
```

Loading our files

```
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
```

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.
countData = countData[rowSums(countData) > 1, ]
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

```
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: 15280 6
## metadata(1): version
## assays(4): counts mu H cooks
## rownames(15280): ENSG00000279457 ENSG00000187634 ... ENSG00000276345
##   ENSG00000271254
## rowData names(22): baseMean baseVar ... deviance maxCooks
## colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
## colData names(2): condition sizeFactor
```

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

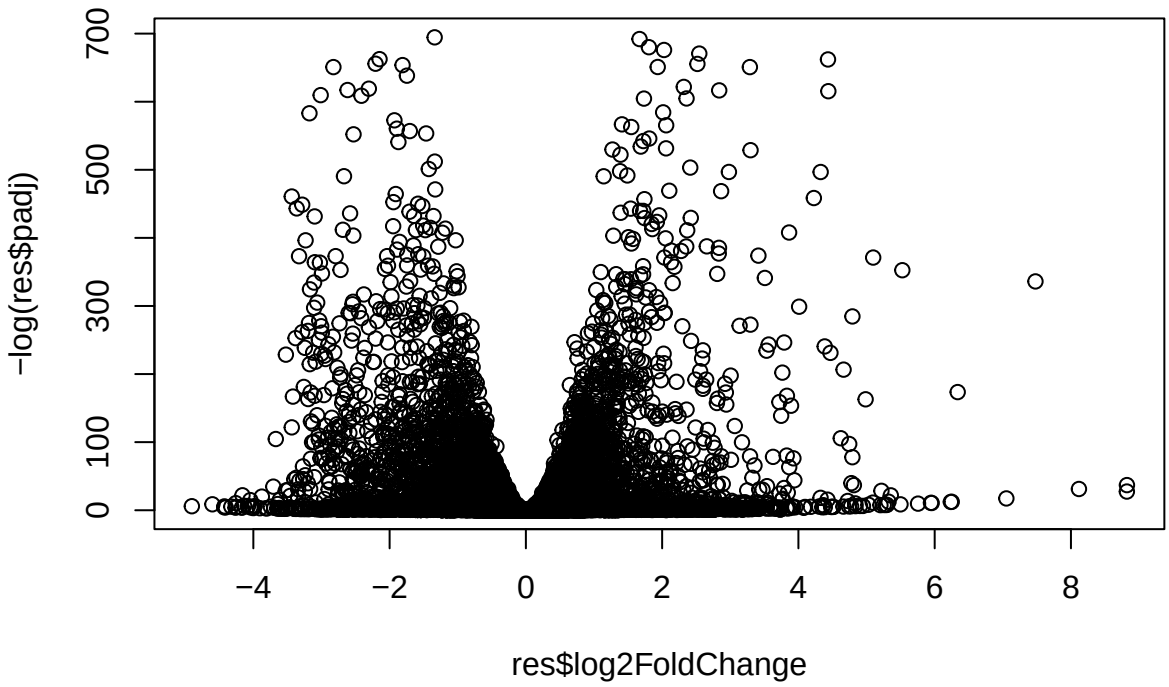
Q. Call the summary() function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```
summary(res)
```

```
##
## out of 15280 with nonzero total read count
## adjusted p-value < 0.1
## LFC > 0 (up)       : 4351, 28%
## LFC < 0 (down)     : 4399, 29%
## outliers [1]       : 0, 0%
## low counts [2]     : 590, 3.9%
## (mean count < 1)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

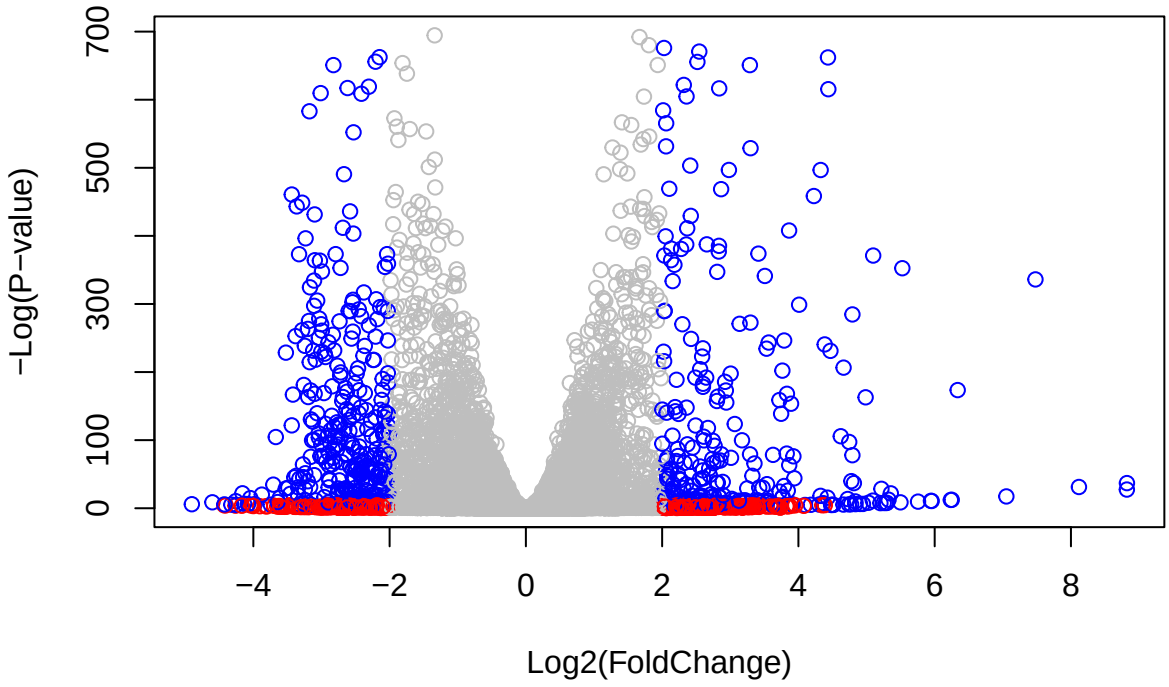
Volcano Plot

```
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(P-value)" )
```



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   29.9136      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
## ENSG00000188290  108.9221      2.0570638 0.1969053  10.446970 1.51282e-25
## ENSG00000187608  350.7169      0.2573837 0.1027266   2.505522 1.22271e-02
## ENSG00000188157 9128.4394      0.3899088 0.0467163   8.346304 7.04321e-17
## ENSG00000131591  156.4791      0.1965923 0.1456109   1.350121 1.76977e-01
##                       padj      symbol      entrez                  name
##                  <numeric> <character> <character>           <character>
## ENSG00000279457 6.85033e-01      WASH9P   102723897 WAS protein family h..
## ENSG00000187634 5.14039e-03      SAMD11      148398 sterile alpha motif ..
## ENSG00000188976 1.75974e-35       NOC2L       26155 NOC2 like nucleolar ..
## ENSG00000187961 1.13044e-07      KLHL17      339451 kelch like family me..
## ENSG00000187583 9.19159e-01     PLEKHN1       84069 pleckstrin homology ..
## ENSG00000187642 4.02066e-01       PERM1       84808 PPARGC1 and ESRR ind..
## ENSG00000188290 1.30113e-24        HES4       57801 hes family bHLH tran..
## ENSG00000187608 2.36679e-02       ISG15        9636 ISG15 ubiquitin like..
## ENSG00000188157 4.20589e-16        AGRN      375790                 agrin
## ENSG00000131591 2.60893e-01    C1orf159       54991 chromosome 1 open re..
```

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")
```

#Section 2 Pathway Analysis

```
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                 1.003993e-05 -4.353454 1.003993e-05
## hsa03030 DNA replication            8.909558e-05 -3.968611 8.909558e-05
## hsa03013 RNA transport              1.470985e-03 -3.007794 1.470985e-03
## hsa04114 Oocyte meiosis             1.946905e-03 -2.921710 1.946905e-03
## hsa03440 Homologous recombination   2.941989e-03 -2.868141 2.941989e-03
## hsa00010 Glycolysis / Gluconeogenesis 6.059196e-03 -2.558327 6.059196e-03
##                                          q.val set.size         exp1
## hsa04110 Cell cycle                 0.001606390      120 1.003993e-05
## hsa03030 DNA replication            0.007127646       36 8.909558e-05
## hsa03013 RNA transport              0.077876201      143 1.470985e-03
## hsa04114 Oocyte meiosis             0.077876201       99 1.946905e-03
## hsa03440 Homologous recombination   0.094143663       28 2.941989e-03
## hsa00010 Glycolysis / Gluconeogenesis 0.161578551       48 6.059196e-03
```

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

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

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

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

```
# 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:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

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

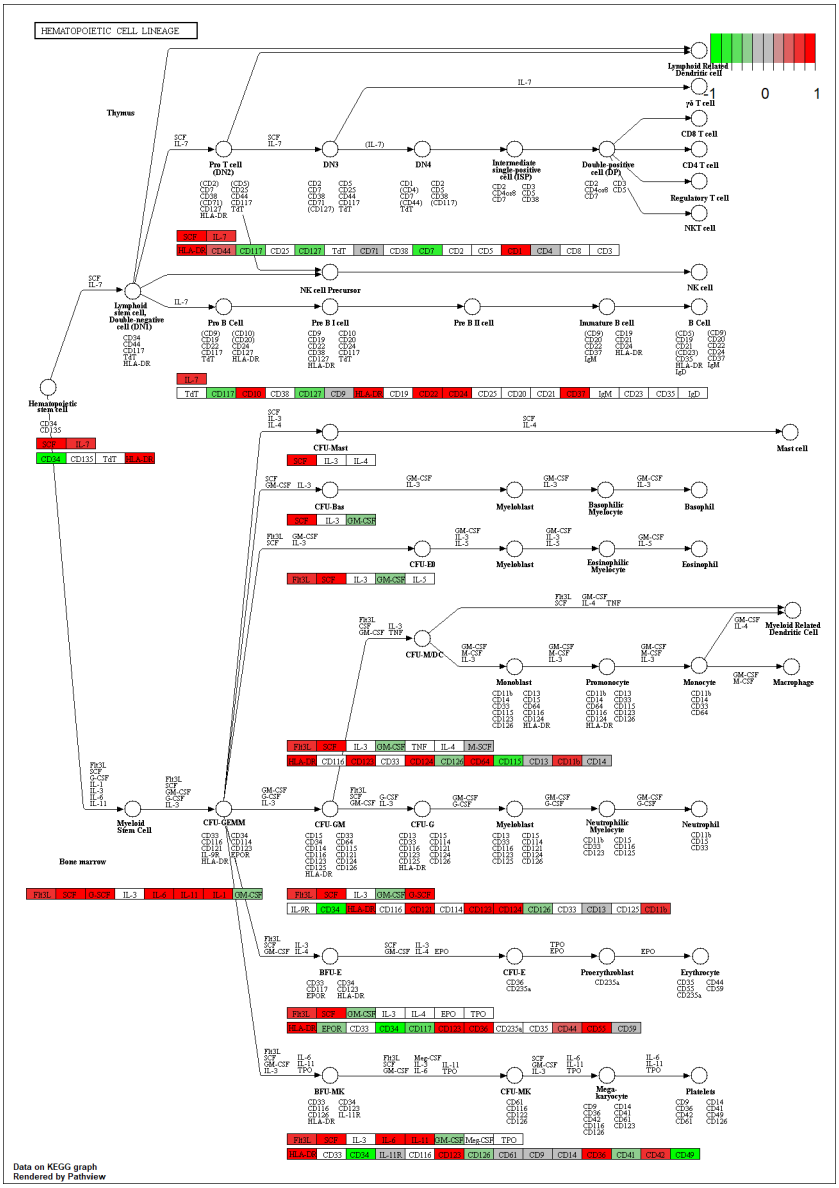
```
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$greater)[1:5]

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

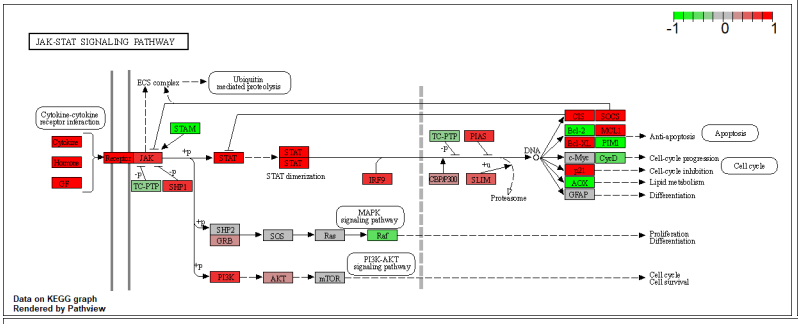
```
## [1] "hsa04640" "hsa04630" "hsa04142" "hsa00140" "hsa04740"
```

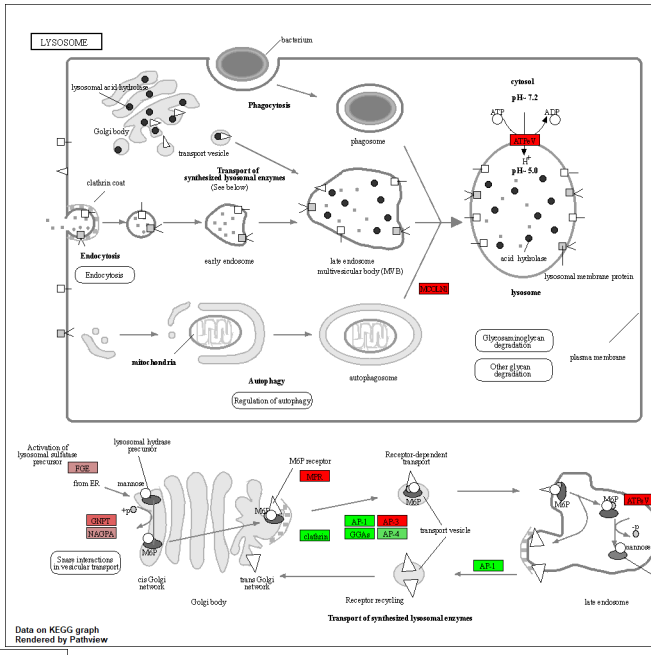
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

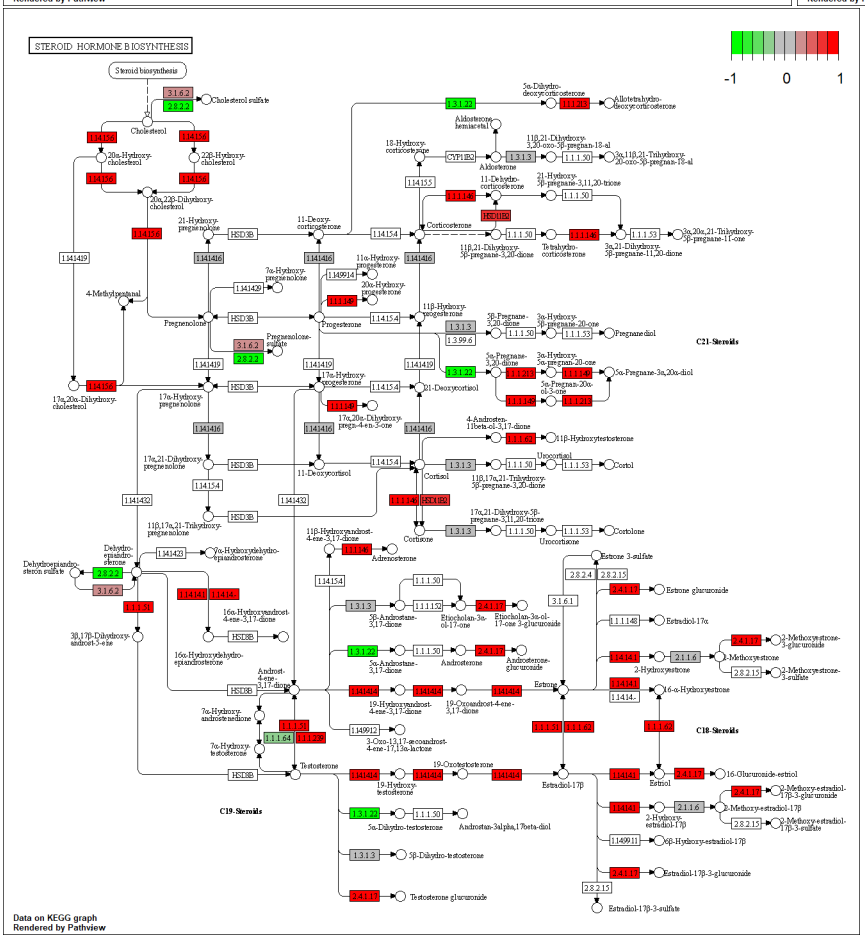
```
## 'select()' returned 1:1 mapping between keys and columns

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

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

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

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

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

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

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

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

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

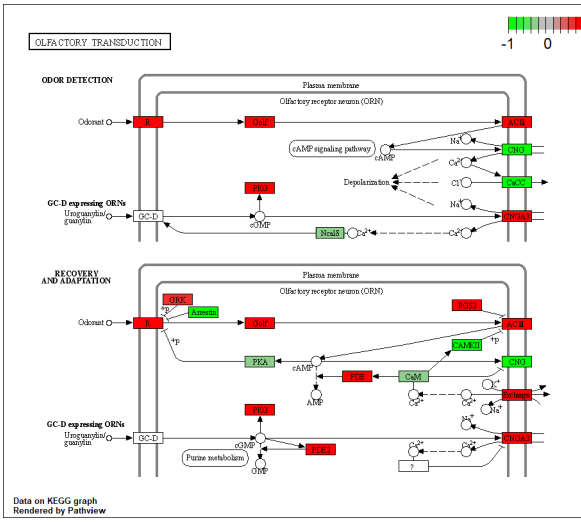
```
## 'select()' returned 1:1 mapping between keys and columns

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

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

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

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

## Info: Writing image file hsa04740.pathview.png

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











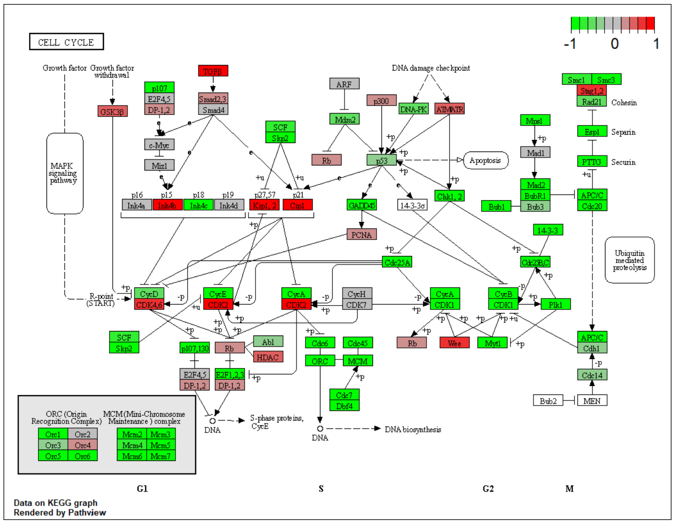
Q. Can you do the same procedure as above to plot the pathview figures for the top 5 down-reguled pathways?

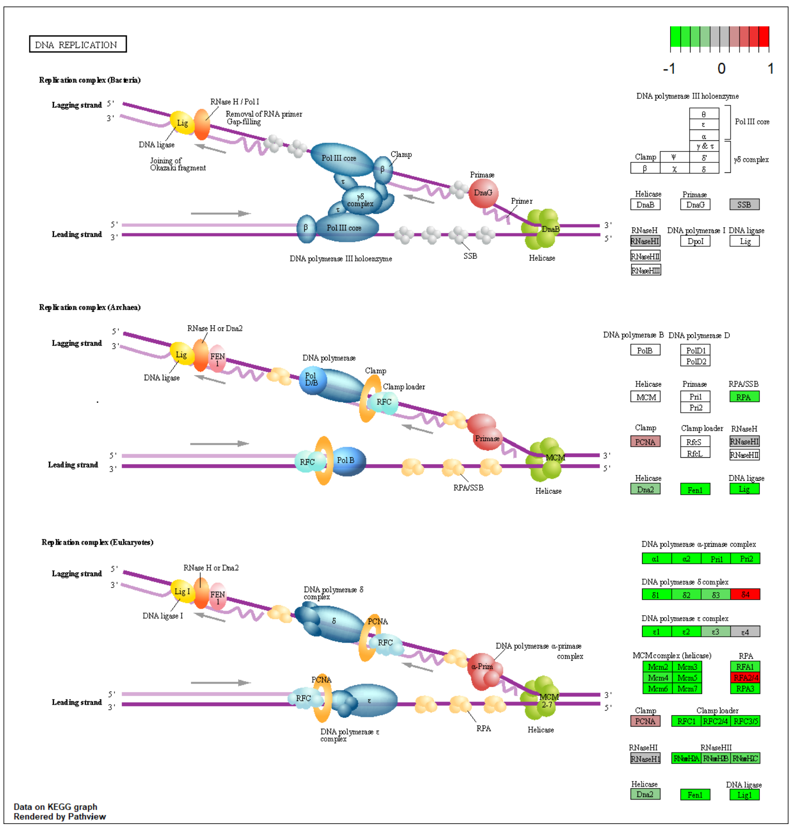
```
## Focus on top 5 downregulated pathways here for demo purposes only
keggrespathwaysdr <- rownames(keggres$less)[1:5]

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

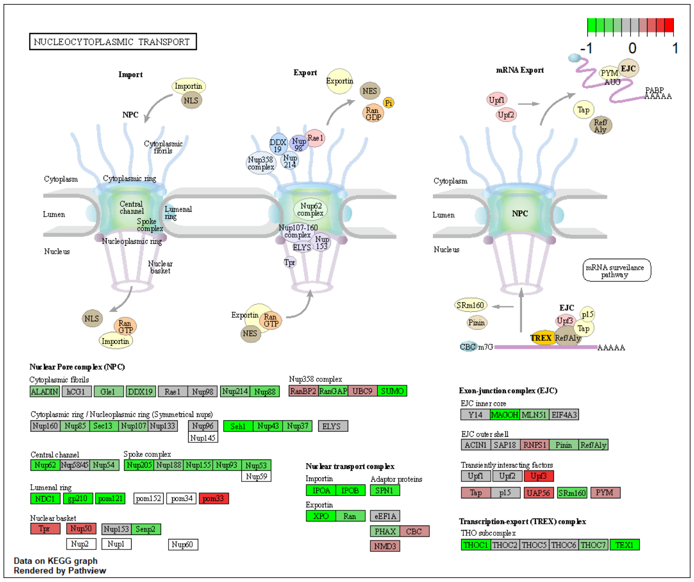
```
## [1] "hsa04110" "hsa03030" "hsa03013" "hsa04114" "hsa03440"
```

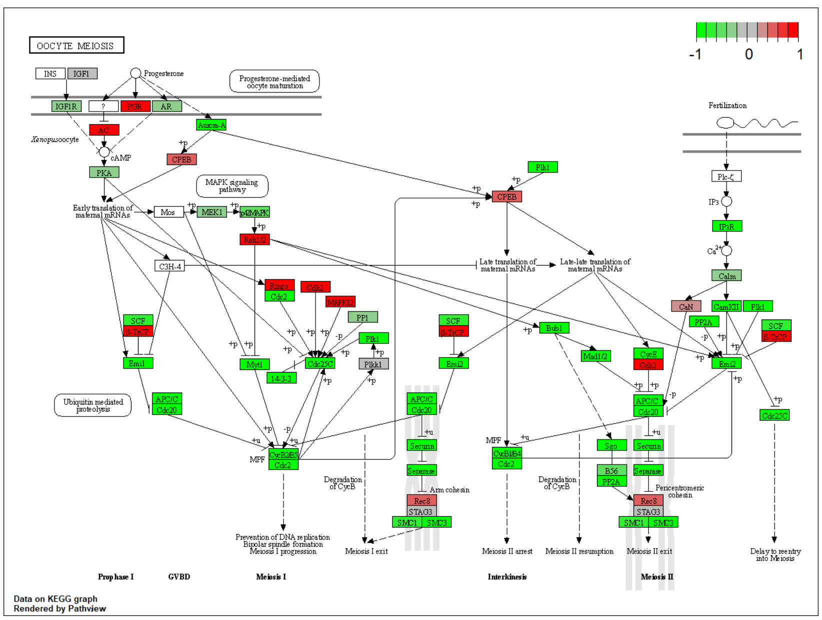
```
pathview(gene.data=foldchanges, pathway.id=keggresidsdr, species="hsa")
```

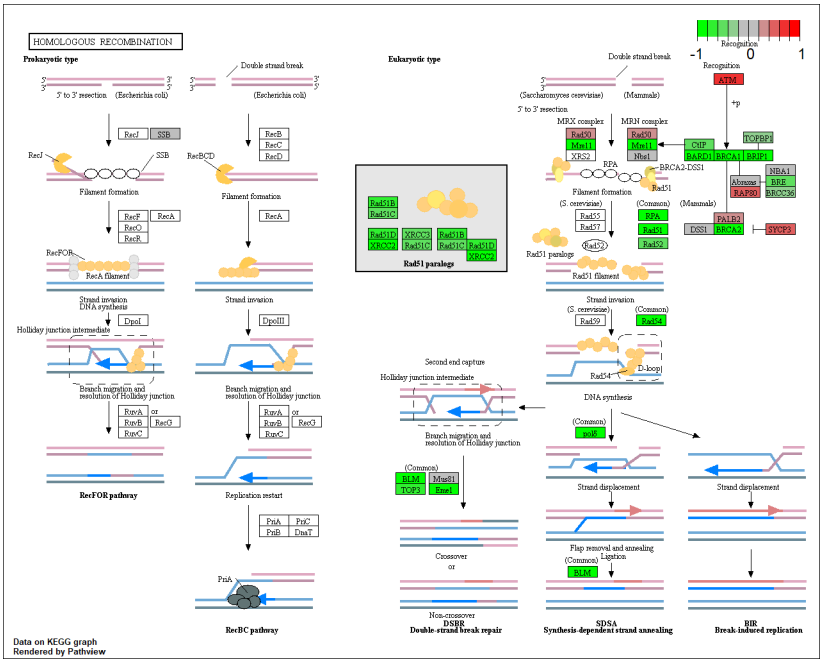
```
## 'select()' returned 1:1 mapping between keys and columns

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

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

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

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

## Info: Writing image file hsa03030.pathview.png

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

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

## Info: Writing image file hsa03013.pathview.png

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

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

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

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

## Info: Working in directory C:/bimm143_github/Pathway Analysis from RNA-Seq Results Extra Credit

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


CELL CYCLE
-1
0
1
Growth factor
Growth factor withdrawal
DNA damage checkpoint
MAPK signaling pathway
Apoptosis
Ubiquitin mediated proteolysis
Cohesin
Separin
Securin
R-point (START)
ORC (Origin Recognition Complex)
MCM (Mini-Chromosome Maintenance) complex
S-phase proteins, CycE
DNA
DNA biosynthesis
G1
S
G2
M
Data on KEGG graph
Rendered by Pathview


DNA REPLICATION
-1
0
1
Replication complex (Bacteria)
Lagging strand
Leading strand
RNase H / Pol I
Removal of RNA primer
Gap-filling
DNA ligase
Joining of Okazaki fragment
Pol III core
Clamp
Primase
Primer
SSB
Helicase
DNA polymerase III holoenzyme
Replication complex (Archaea)
RNase H or Dna2
DNA polymerase
Clamp loader
RPA/SSB
Replication complex (Eukaryotes)
DNA ligase I
DNA polymerase δ complex
DNA polymerase α-primase complex
DNA polymerase ε complex
MCM complex (helicase)
RPA
Data on KEGG graph
Rendered by Pathview


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


OOCYTE MEIOSIS
-1 0 1
Progesterone
Progesterone-mediated oocyte maturation
MAPK signaling pathway
Ubiquitin mediated proteolysis
Fertilization
Prophase I
GVBD
Meiosis I
Interkinesis
Meiosis II
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       4.892477e-05  3.971899 4.892477e-05
## GO:0060429 epithelium development         6.727546e-05  3.834595 6.727546e-05
## GO:0007610 behavior                       1.988039e-04  3.557821 1.988039e-04
## GO:0048729 tissue morphogenesis           2.470962e-04  3.498983 2.470962e-04
## GO:0002009 morphogenesis of an epithelium 3.227439e-04  3.429317 3.227439e-04
## GO:0016337 cell-cell adhesion             8.195506e-04  3.163057 8.195506e-04
##                                                q.val set.size         exp1
## GO:0007156 homophilic cell adhesion       0.1337436      107 4.892477e-05
## GO:0060429 epithelium development         0.1337436      478 6.727546e-05
## GO:0007610 behavior                       0.2456136      403 1.988039e-04
## GO:0048729 tissue morphogenesis           0.2456136      403 2.470962e-04
## GO:0002009 morphogenesis of an epithelium 0.2566460      326 3.227439e-04
## GO:0016337 cell-cell adhesion             0.3782658      318 8.195506e-04
##
## $less
##                                               p.geomean stat.mean        p.val
## GO:0000279 M phase                          1.475361e-16 -8.323749 1.475361e-16
## GO:0048285 organelle fission                7.498413e-16 -8.160305 7.498413e-16
```

```
## GO:0000280 nuclear division              2.135098e-15 -8.034814 2.135098e-15
## GO:0007067 mitosis                       2.135098e-15 -8.034814 2.135098e-15
## GO:0000087 M phase of mitotic cell cycle 5.927567e-15 -7.891758 5.927567e-15
## GO:0007059 chromosome segregation        1.055918e-11 -6.988373 1.055918e-11
##                                                 q.val set.size         exp1
## GO:0000279 M phase                       5.866036e-13      492 1.475361e-16
## GO:0048285 organelle fission             1.490684e-12      373 7.498413e-16
## GO:0000280 nuclear division              2.122288e-12      349 2.135098e-15
## GO:0007067 mitosis                       2.122288e-12      349 2.135098e-15
## GO:0000087 M phase of mitotic cell cycle 4.713601e-12      359 5.927567e-15
## GO:0007059 chromosome segregation        6.997217e-09      141 1.055918e-11
##
## $stats
##                                           stat.mean     exp1
## GO:0007156 homophilic cell adhesion        3.971899 3.971899
## GO:0060429 epithelium development          3.834595 3.834595
## GO:0007610 behavior                        3.557821 3.557821
## GO:0048729 tissue morphogenesis            3.498983 3.498983
## GO:0002009 morphogenesis of an epithelium  3.429317 3.429317
## GO:0016337 cell-cell adhesion              3.163057 3.163057
```

#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: 8149"
```

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

> 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 the endosomal/vacuolar pathway with the value being 8.61E-4. This value does not match my previous KEGG result. I think this can be a result of different representations of the same biological pathway. This can lead to different results that are statistically significant.

```
sessionInfo()
```

```
## R version 4.1.1 (2021-08-10)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19043)
##
## Matrix products: default
##
## locale:
## [1] LC_COLLATE=English_United States.1252
## [2] LC_CTYPE=English_United States.1252
## [3] LC_MONETARY=English_United States.1252
```

```
## [4] LC_NUMERIC=C
## [5] LC_TIME=English_United States.1252
##
## attached base packages:
## [1] parallel  stats4    stats     graphics  grDevices utils     datasets
## [8] methods   base
##
## other attached packages:
##  [1] gageData_2.30.0             gage_2.42.0
##  [3] pathview_1.32.0             org.Hs.eg.db_3.13.0
##  [5] AnnotationDbi_1.54.1        DESeq2_1.32.0
##  [7] SummarizedExperiment_1.22.0 Biobase_2.52.0
##  [9] MatrixGenerics_1.4.3        matrixStats_0.61.0
## [11] GenomicRanges_1.44.0        GenomeInfoDb_1.28.4
## [13] IRanges_2.26.0              S4Vectors_0.30.2
## [15] BiocGenerics_0.38.0
##
## loaded via a namespace (and not attached):
##  [1] httr_1.4.2             bit64_4.0.5            splines_4.1.1
##  [4] highr_0.9              blob_1.2.2             GenomeInfoDbData_1.2.6
##  [7] yaml_2.2.1             pillar_1.6.3           RSQLite_2.2.8
## [10] lattice_0.20-44        glue_1.4.2             digest_0.6.28
## [13] RColorBrewer_1.1-2     XVector_0.32.0         colorspace_2.0-2
## [16] htmltools_0.5.2        Matrix_1.3-4           XML_3.99-0.8
## [19] pkgconfig_2.0.3        genefilter_1.74.1      zlibbioc_1.38.0
## [22] GO.db_3.13.0           purrr_0.3.4            xtable_1.8-4
## [25] scales_1.1.1           BiocParallel_1.26.2    tibble_3.1.5
## [28] annotate_1.70.0        KEGGREST_1.32.0        generics_0.1.1
## [31] ggplot2_3.3.5          ellipsis_0.3.2         cachem_1.0.6
## [34] survival_3.2-11        magrittr_2.0.1         crayon_1.4.1
## [37] KEGGgraph_1.52.0       memoise_2.0.0          evaluate_0.14
## [40] fansi_0.5.0            graph_1.70.0           tools_4.1.1
## [43] lifecycle_1.0.1        stringr_1.4.0          munsell_0.5.0
## [46] locfit_1.5-9.4         DelayedArray_0.18.0    Biostrings_2.60.2
## [49] compiler_4.1.1         rlang_0.4.11           grid_4.1.1
## [52] RCurl_1.98-1.5         bitops_1.0-7           rmarkdown_2.11
## [55] gtable_0.3.0           DBI_1.1.1              R6_2.5.1
## [58] knitr_1.36             dplyr_1.0.7            fastmap_1.1.0
## [61] bit_4.0.4              utf8_1.2.2             Rgraphviz_2.36.0
## [64] stringi_1.7.5          Rcpp_1.0.7             vctrs_0.3.8
## [67] geneplotter_1.70.0     png_0.1-7              tidyselect_1.1.1
## [70] xfun_0.26
```

#GO Online

> 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 p-value was 6.34E-60 found in the detection of chemical stimulus involved in sensory perception pathways. This does not match with my previous KEGG results. This may be a result of coming from two different data bases.