Pathway Analaysis from RNA-seq Results

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Section 1. Differential Expression Analysis

We first call DESeq2.

```
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, 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
```

Now we load out datafiles as follows:

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

# Import metadata and take a look at the first 6 rows.
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
```

```
# We import countdata and take a look at the first 6 rows.
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
```

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

```
# Note we need to remove the odd first $length col.
countData <- as.matrix(countData[,-1])
head(countData)
```

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

This looks better but we see that there are lots of zero entries so we 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). Tip: What will rowSums() of countData return and how could you use it in this context?**

```
# Filter count data where you have 0 read count across all samples.
countData = countData[rowSums(countData) > 0,]
head(countData)
```

```
##                 SRR493366 SRR493367 SRR493368 SRR493369 SRR493370 SRR493371
## ENSG00000279457        23        28        29        29        28        46
## ENSG00000187634       124       123       205       207       212       258
## ENSG00000188976      1637      1831      2383      1226      1326      1504
## ENSG00000187961       120       153       180       236       255       357
## ENSG00000187583        24        48        65        44        48        64
## ENSG00000187642         4         9        16        14        16        16
```

Running DEseq2

Now we setup the DESeqDataSet object required for the DESeq() function and then run the DESeq pipeline as follows.

```
dds = DESeqDataSetFromMatrix(countData=countData,
                             colData=colData,
                             design=~condition)
```

```
## Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in
## design formula are characters, converting to factors
```

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

```
## estimating size factors

## estimating dispersions

## gene-wise dispersion estimates

## mean-dispersion relationship

## final dispersion estimates

## fitting model and testing
```

```
dds
```

```
## class: DESeqDataSet
## dim: 15975 6
## metadata(1): version
## assays(4): counts mu H cooks
## rownames(15975): ENSG00000279457 ENSG00000187634 ... ENSG00000276345
##   ENSG00000271254
## rowData names(22): baseMean baseVar ... deviance maxCooks
## colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
## colData names(2): condition sizeFactor
```

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

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

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.

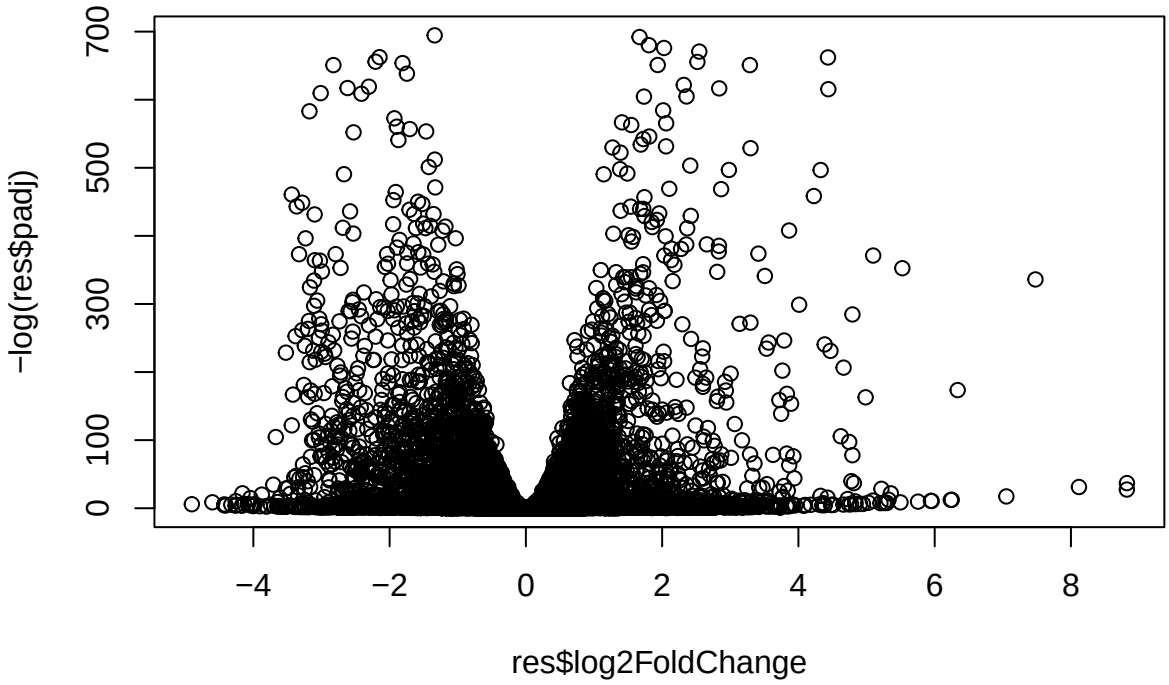
```
summary(res)
```

```
##
## 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

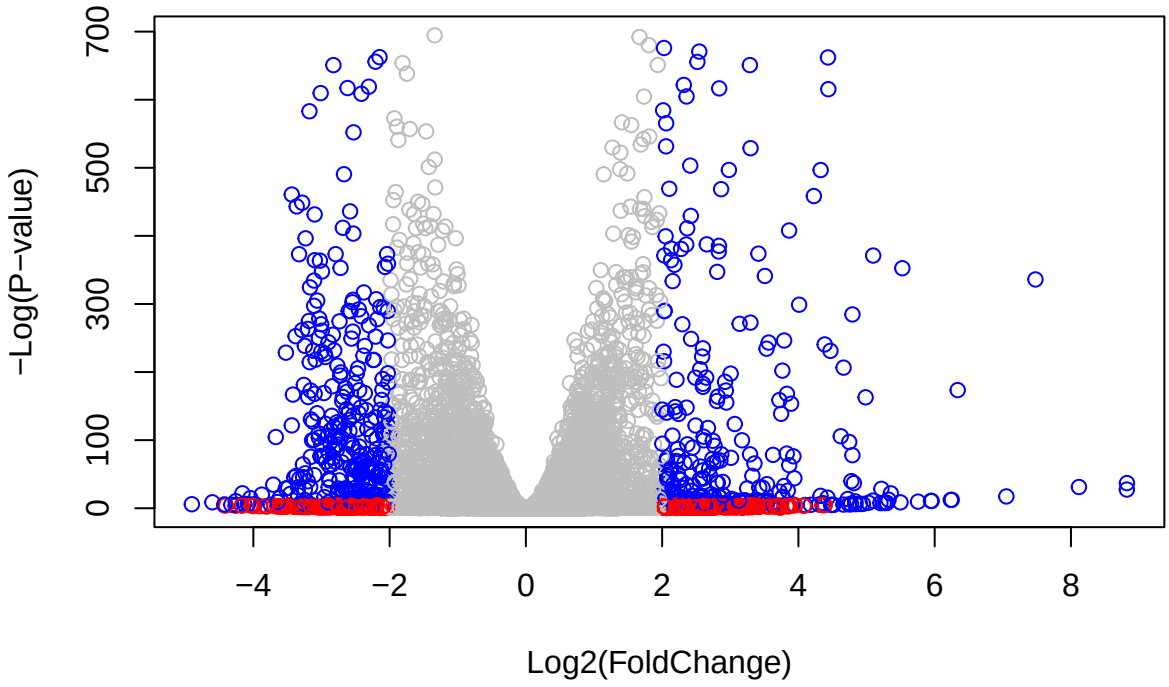
Now we will make a volcano plot, a commonly produced visualization from this type of data that is essentially a plot of log2 fold change vs -log adjusted p-value.

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



Q4. 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

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 we now add them.

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

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

```
##
```

```
columns(org.Hs.eg.db)
```

```
##  [1] "ACCNUM"       "ALIAS"        "ENSEMBL"      "ENSEMBLPROT"  "ENSEMBLTRANS"
##  [6] "ENTREZID"     "ENZYME"       "EVIDENCE"     "EVIDENCEALL"  "GENENAME"
## [11] "GENETYPE"     "GO"           "GOALL"        "IPI"          "MAP"
## [16] "OMIM"         "ONTOLOGY"     "ONTOLOGYALL"  "PATH"         "PFAM"
## [21] "PMID"         "PROSITE"      "REFSEQ"       "SYMBOL"       "UCSCKG"
## [26] "UNIPROT"
```

```
res$symbol = mapIds(org.Hs.eg.db,
                    keys=row.names(res),
                    keytype="ENSEMBL",
                    column="SYMBOL",
                    multiVals="first")
```

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

```
res$entrez = mapIds(org.Hs.eg.db,
                    keys=row.names(res),
                    keytype="ENSEMBL",
                    column="ENTREZID",
                    multiVals="first")
```

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

```
res$name =   mapIds(org.Hs.eg.db,
                    keys=row.names(res),
                    keytype="ENSEMBL",
                    column="GENENAME",
                    multiVals="first")
```

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

```
head(res, 10)
```

```
## log2 fold change (MLE): condition hoxa1_kd vs control_sirna
## Wald test p-value: condition hoxa1 kd vs control sirna
## DataFrame with 10 rows and 9 columns
##                    baseMean log2FoldChange     lfcSE       stat      pvalue
##                   <numeric>      <numeric> <numeric>  <numeric>   <numeric>
## ENSG00000279457   29.913579      0.1792571 0.3248216   0.551863 5.81042e-01
## ENSG00000187634  183.229650      0.4264571 0.1402658   3.040350 2.36304e-03
## ENSG00000188976 1651.188076     -0.6927205 0.0548465 -12.630158 1.43990e-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      WASH9P   102723897 WAS protein family h..
## 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$pvalue),]
write.csv(res, file ="deseq_results.csv")
```

Section 2. 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.

Kegg pathways

We first load the packages and setup the KEGG data-sets we need.

```
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 and we have the fold change results from DESeq2 analysis.

```
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 we run the gage pathway analysis.

```
# We get the results as follows:
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

Now we examine the object returned from gage().

```
attributes(keggres)
```

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

So we see that the result is a list with three elements, “greater”, “less” and “stats”. Now we look at the first few down (less) pathway results:

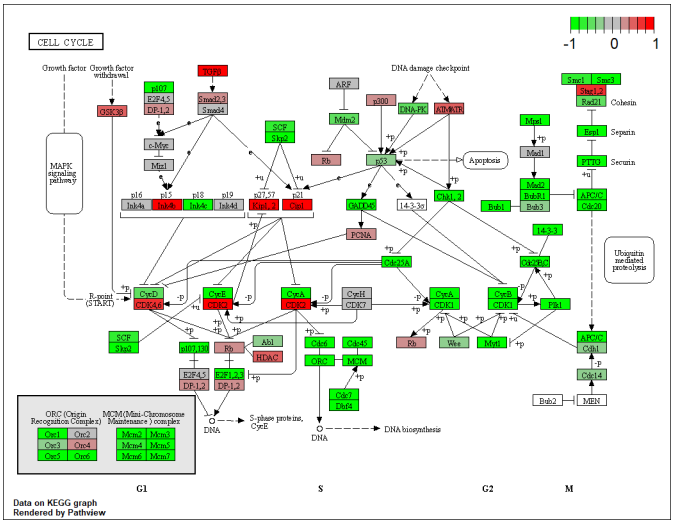
```
# We look at the first few down (less) pathways.
head(keggres$less)
```

```
##                                            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, we use the pathview() function from the pathview package to make a pathway plot with our RNA-Seq expression results shown in color. We start by manually supplying 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")
```

```
## Info: Downloading xml files for hsa04110, 1/1 pathways..

## Info: Downloading png files for hsa04110, 1/1 pathways..

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

## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project

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



We 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
```

```
## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project
```

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

Now, we 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 will use these KEGG pathway IDs for pathview plotting below.

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

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

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

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")
```

```
## Info: Downloading xml files for hsa04640, 1/1 pathways..
```

```
## Info: Downloading png files for hsa04640, 1/1 pathways..
```

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

```
## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project
```

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

```
## Info: Downloading xml files for hsa04630, 1/1 pathways..
```

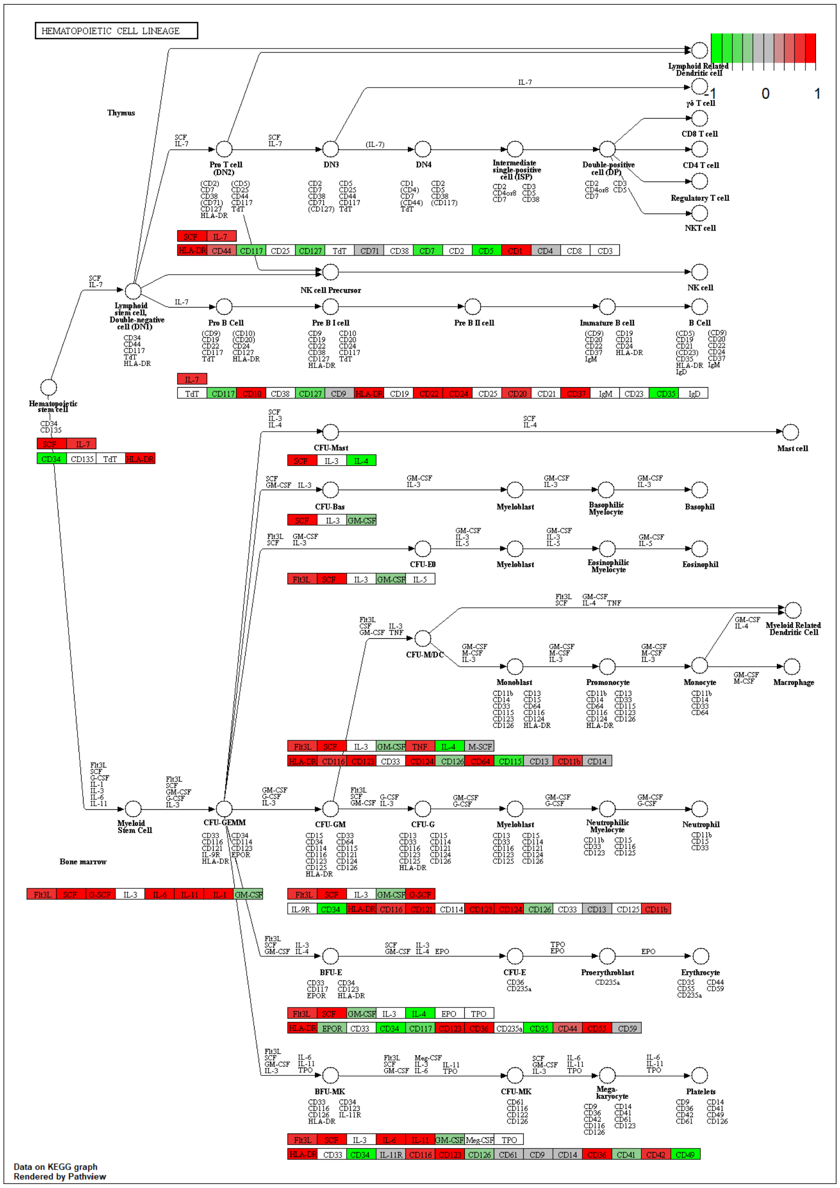
```
## Info: Downloading png files for hsa04630, 1/1 pathways..
```

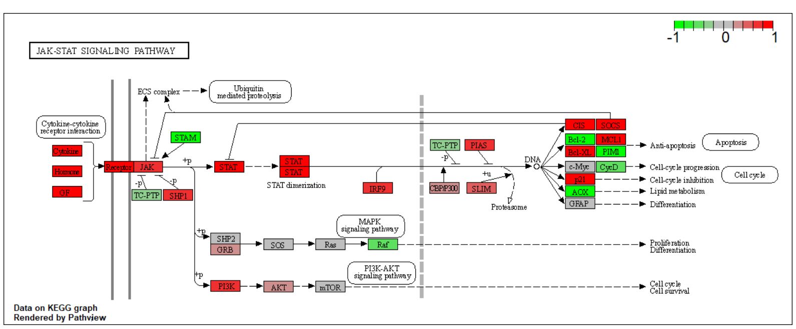
```
## 'select()' returned 1:1 mapping between keys and columns
```

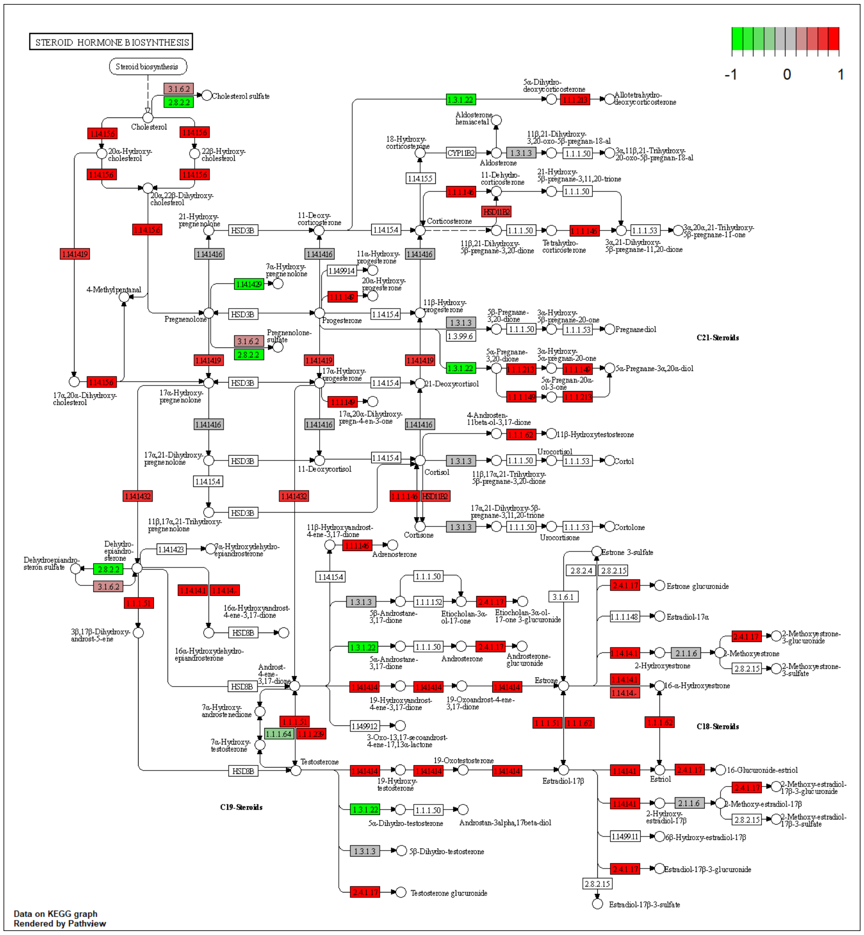
```
## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project
```

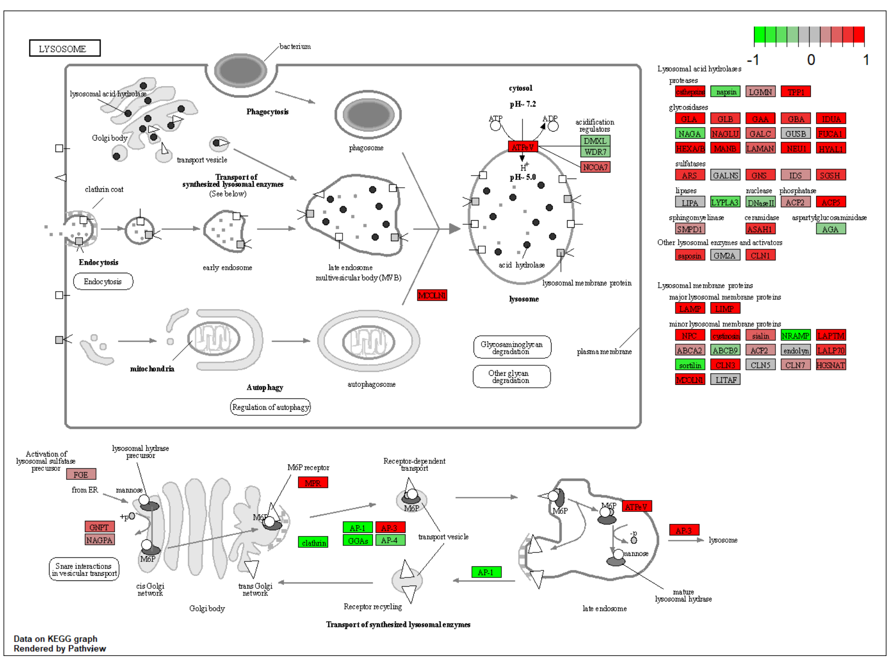
```
## Info: Writing image file hsa04630.pathview.png
```

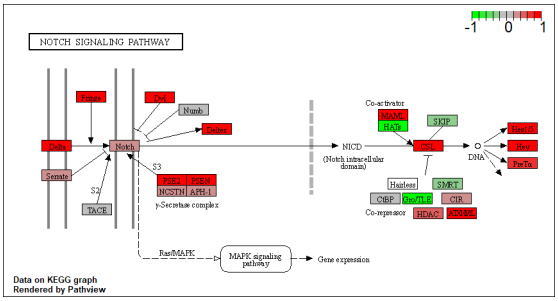
```
## Info: Downloading xml files for hsa00140, 1/1 pathways..

## Info: Downloading png files for hsa00140, 1/1 pathways..

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

## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project

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

## Info: Downloading xml files for hsa04142, 1/1 pathways..

## Info: Downloading png files for hsa04142, 1/1 pathways..

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

## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project

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

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

## Info: Downloading xml files for hsa04330, 1/1 pathways..

## Info: Downloading png files for hsa04330, 1/1 pathways..

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

## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project

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


HEMATOPOIETIC CELL LINEAGE
-1
0
1
Lymphoid Related Dendritic cell
γδ T cell
CD8 T cell
CD4 T cell
Regulatory T cell
NKT cell
Thymus
Pro T cell (DN2)
DN3
DN4
Intermediate single-positive cell (ISP)
Double-positive cell (DP)
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
Hematopoietic stem cell
CFU-Mast
Mast cell
CFU-Bas
Myeloblast
Basophilic Myelocyte
Basophil
CFU-EB
Eosinophilic Myelocyte
Eosinophil
CFU-M/DC
Myeloid Related Dendritic Cell
Monoblast
Promonocyte
Monocyte
Macrophage
Myeloid Stem Cell
CFU-GEMM
CFU-GM
CFU-G
Neutrophilic Myelocyte
Neutrophil
Bone marrow
BFU-E
CFU-E
Proerythroblast
Erythrocyte
BFU-MK
CFU-MK
Mega-karyocyte
Platelets
Data on KEGG graph
Rendered by Pathview


JAK-STAT SIGNALING PATHWAY
-1
0
1
Cytokine-cytokine receptor interaction
Cytokine
Hormone
GF
Receptor
JAK
STAM
STAT
STAT dimerization
ECS complex
Ubiquitin mediated proteolysis
TC-PTP
SHP1
PIAS
IRF9
CBP/P300
SLIM
Proteasome
DNA
Anti-apoptosis
Apoptosis
Cell cycle progression
Cell cycle inhibition
Cell cycle
Lipid metabolism
Differentiation
SHP2
GRB
SOS
Ras
Raf
MAPK signaling pathway
Proliferation
Differentiation
PI3K
AKT
mTOR
PI3K-AKT signaling pathway
Cell cycle
Cell survival
Data on KEGG graph
Rendered by Pathview


STEROID HORMONE BIOSYNTHESIS
-1
0
1
Steroid biosynthesis
Cholesterol
Cholesterol sulfate
Pregnenolone
Progesterone
Aldosterone
Corticosterone
Cortisol
Cortisone
Androstenedione
Testosterone
Estrone
Estradiol-17β
Estriol
C21-Steroids
C19-Steroids
C18-Steroids
Data on KEGG graph
Rendered by Pathview


LYSOSOME
-1
0
1
bacterium
Phagocytosis
phagosome
cytosol
pH~ 7.2
pH~ 5.0
lysosome
Golgi body
transport vesicle
clathrin coat
Endocytosis
early endosome
late endosome
multivesicular body (MVB)
mitochondria
Autophagy
autophagosome
Lysosomal acid hydrolases
Lysosomal membrane proteins
Transport of synthesized lysosomal enzymes
Data on KEGG graph
Rendered by Pathview



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

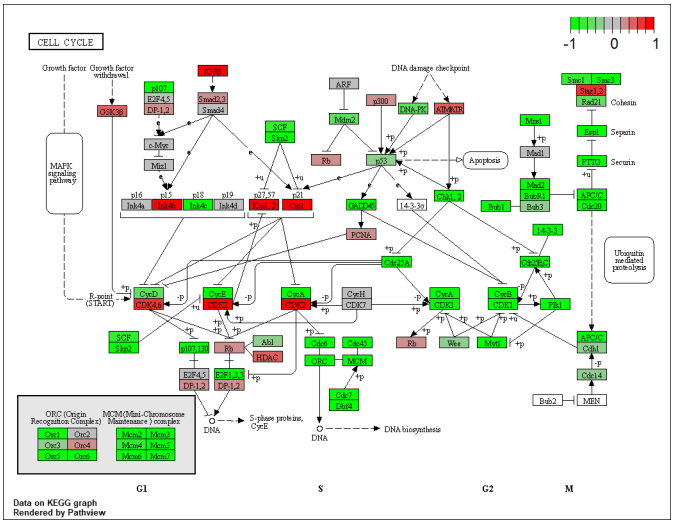
```
## Focus on top 5 downregulated pathways here.
keggrespathways.2 <- rownames(keggres$less)[1:5]

# Extract the 8 character long IDs part of each string.
keggresids.2 = substr(keggrespathways.2, start=1, stop=8)
keggresids.2
```

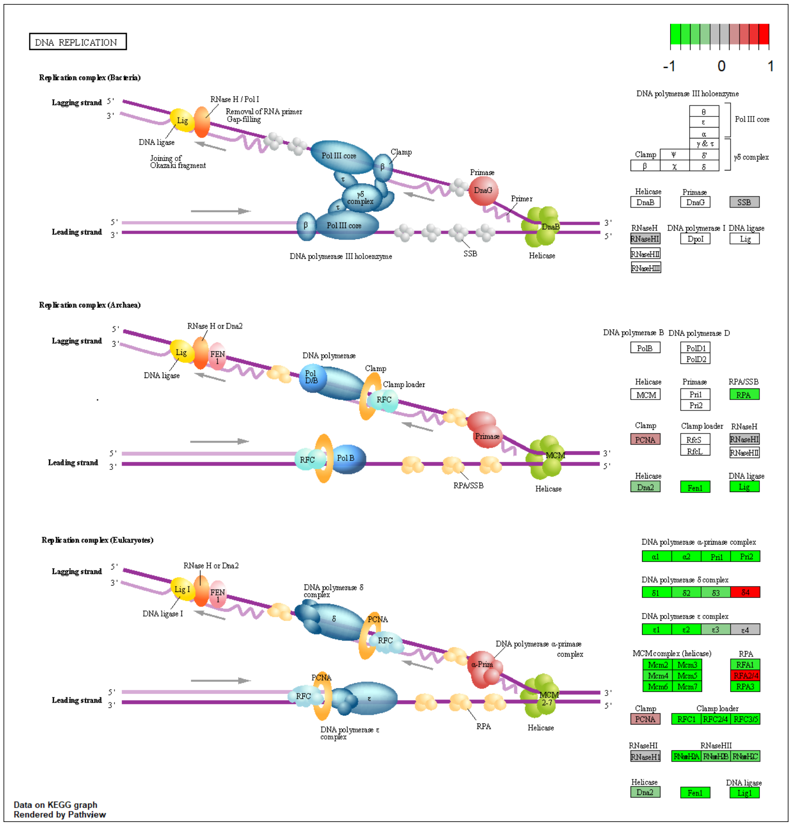
```
## [1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

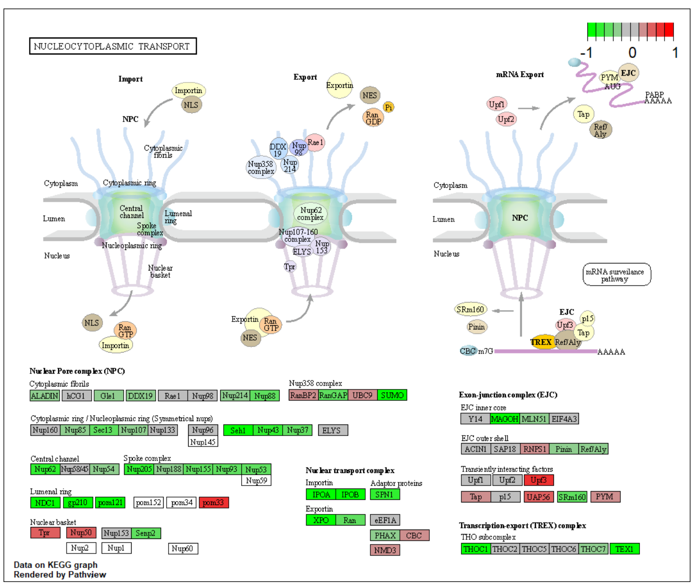
```
pathview(gene.data=foldchanges, pathway.id=keggresids.2, species="hsa")
```

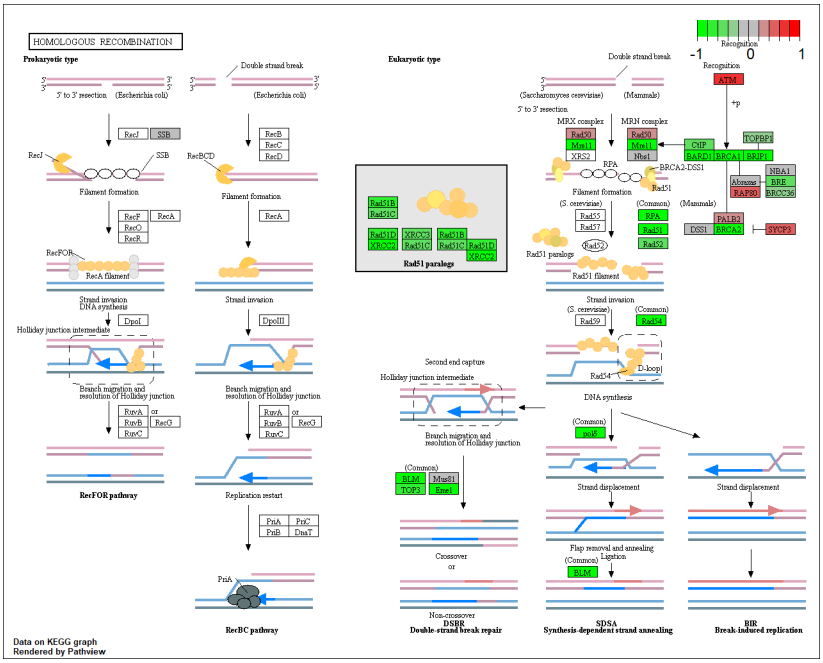
```
## 'select()' returned 1:1 mapping between keys and columns

## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project

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

## Info: Downloading xml files for hsa03030, 1/1 pathways..

## Info: Downloading png files for hsa03030, 1/1 pathways..

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

## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project

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

## Info: Downloading xml files for hsa03013, 1/1 pathways..

## Info: Downloading png files for hsa03013, 1/1 pathways..

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

## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project

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

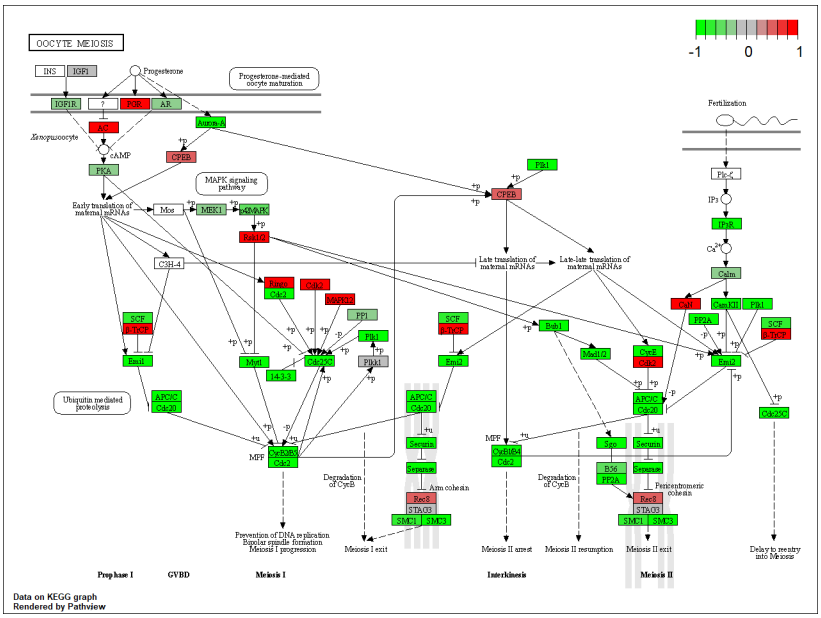
```
## Info: Downloading xml files for hsa03440, 1/1 pathways..

## Info: Downloading png files for hsa03440, 1/1 pathways..

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

## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project

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

## Info: Downloading xml files for hsa04114, 1/1 pathways..

## Info: Downloading png files for hsa04114, 1/1 pathways..

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

## Info: Working in directory C:/Users/Caleb/Desktop/BIMM143 GIT/02-24-22_RNA-Seq_Mini_Project

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




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


NUCLEOCYTOPLASMIC TRANSPORT
-1
0
1
Import
Export
mRNA Export
NPC
Cytoplasm
Lumen
Nucleus
Cytoplasmic fibrils
Cytoplasmic ring
Central channel
Spoke complex
Lumenal ring
Nucleoplasmic ring
Nuclear basket
Nuclear Pore complex (NPC)
Nuclear transport complex
Exon-junction complex (EJC)
Transcription-export (TREX) complex
mRNA surveillance pathway
Data on KEGG graph
Rendered by Pathview





Section 3. 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 (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

Reactome is database consisting of biological molecules and their relation to pathways and processes. We 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=FALSE)
```

We then perform pathway analysis online on the Reactome website.

> **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 pathway that has the most significant "Entities p-value" is the Endosomal/Vacuolar pathway. The most significant pathways listed do not match the previous KEGG results. A factor that could causes difference between the two method is that reactome is a databases that uses biological molecules in their relations to pathways and processes, while KEGG is a database that focuses on the pathways and genes themselves. Thus it is likely that the two methods will differ due to the slightly differing data being drawn from each database. Another factor that could cause this difference is that when we used KEGG, we used kegg.sets.hs[sigmet.idx.hs], which narrows the field to signaling and metabolic pathways only, while reactomes searches against a wide range human related entries in the database.

Section 5. GO online (OPTIONAL)

> **Q9. 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 pathway corresponding to negative regulation of integrin activation. The most significant pathways listed do not match the previous KEGG results. A factor that could cause this is the difference is due to slightl different data being drawn from each database. Another factor that could cause this difference is that when we used KEGG, we used kegg.sets.hs[sigmet.idx.hs], which narrows the field to signaling and metabolic pathways only, while GO appears to search against a much wider range of the human genes in the database.

Session Information

```
sessionInfo()
```

```
## R version 4.1.2 (2021-11-01)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 19044)
##
## 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] stats4    stats     graphics  grDevices utils     datasets  methods
## [8] base
##
## other attached packages:
##  [1] gageData_2.32.0             gage_2.44.0
##  [3] pathview_1.34.0             org.Hs.eg.db_3.14.0
##  [5] AnnotationDbi_1.56.2        DESeq2_1.34.0
##  [7] SummarizedExperiment_1.24.0 Biobase_2.54.0
##  [9] MatrixGenerics_1.6.0        matrixStats_0.61.0
## [11] GenomicRanges_1.46.1        GenomeInfoDb_1.30.1
## [13] IRanges_2.28.0              S4Vectors_0.32.3
## [15] BiocGenerics_0.40.0
##
## loaded via a namespace (and not attached):
##  [1] httr_1.4.2             bit64_4.0.5            splines_4.1.2
##  [4] assertthat_0.2.1       highr_0.9              blob_1.2.2
##  [7] GenomeInfoDbData_1.2.7 yaml_2.2.2             pillar_1.7.0
## [10] RSQLite_2.2.10         lattice_0.20-45        glue_1.6.1
## [13] digest_0.6.29          RColorBrewer_1.1-2     XVector_0.34.0
## [16] colorspace_2.0-2       htmltools_0.5.2        Matrix_1.4-0
## [19] XML_3.99-0.8           pkgconfig_2.0.3        genefilter_1.76.0
## [22] zlibbioc_1.40.0        GO.db_3.14.0           purrr_0.3.4
## [25] xtable_1.8-4           scales_1.1.1           BiocParallel_1.28.3
## [28] tibble_3.1.6           annotate_1.72.0        KEGGREST_1.34.0
## [31] generics_0.1.2         ggplot2_3.3.5          ellipsis_0.3.2
## [34] cachem_1.0.6           cli_3.1.1              survival_3.2-13
## [37] magrittr_2.0.2         crayon_1.5.0           KEGGgraph_1.54.0
## [40] memoise_2.0.1          evaluate_0.14          fansi_1.0.2
## [43] graph_1.72.0           tools_4.1.2            lifecycle_1.0.1
## [46] stringr_1.4.0          locfit_1.5-9.4         munsell_0.5.0
## [49] DelayedArray_0.20.0    Biostrings_2.62.0      compiler_4.1.2
## [52] rlang_1.0.1            grid_4.1.2             RCurl_1.98-1.6
## [55] rstudioapi_0.13        bitops_1.0-7           rmarkdown_2.11
## [58] gtable_0.3.0           DBI_1.1.2              R6_2.5.1
## [61] knitr_1.37             dplyr_1.0.8            fastmap_1.1.0
## [64] bit_4.0.4              utf8_1.2.2             Rgraphviz_2.38.0
## [67] stringi_1.7.6          parallel_4.1.2         Rcpp_1.0.8
## [70] vctrs_0.3.8            geneplotter_1.72.0     png_0.1-7
## [73] tidyselect_1.1.1       xfun_0.29
```