11_09_22_lab

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```
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
```

```
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
```

```
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
```

Q. Complete the code below to remove the troublesome first column from 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
```

```
countData$length <- NULL
countData <- as.matrix(countData)
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).

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 samp
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
```

```
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
```

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

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

```
summary(res)
```

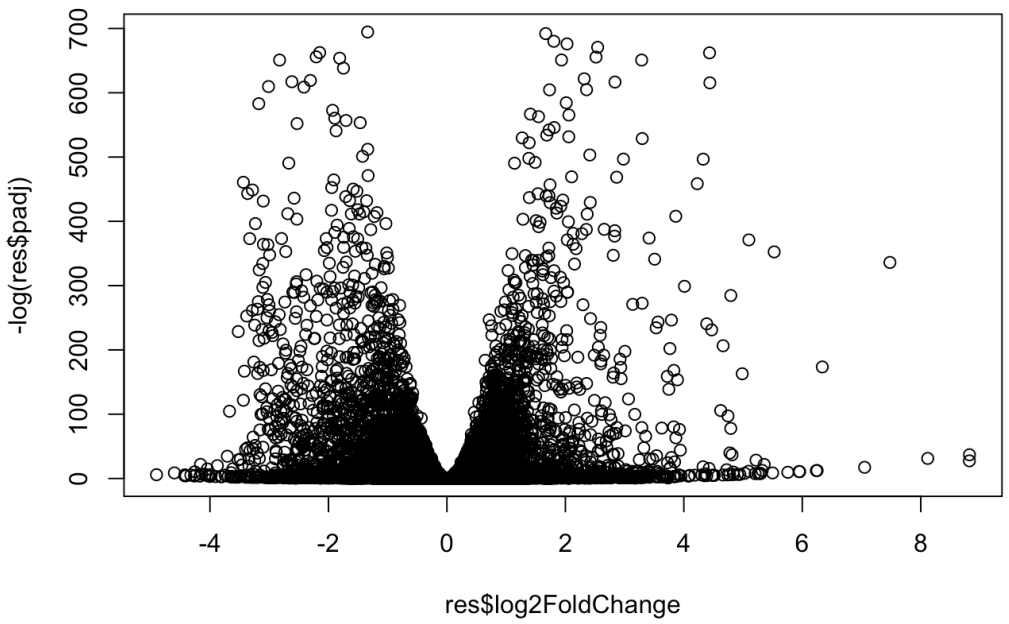
```
out of 15975 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 4349, 27%
LFC < 0 (down)     : 4396, 28%
outliers [1]       : 0, 0%
low counts [2]     : 1237, 7.7%
(mean count < 0)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results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

```
res
```

```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 15975 rows and 6 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
...                  ...           ...       ...        ...
...
ENSG00000273748  35.30265       0.674387  0.303666   2.220817
2.63633e-02
ENSG00000278817   2.42302      -0.388988  1.130394  -0.344117
7.30758e-01
ENSG00000278384   1.10180       0.332991  1.660261   0.200565
8.41039e-01
ENSG00000276345  73.64496      -0.356181  0.207716  -1.714752
8.63908e-02
ENSG00000271254 181.59590      -0.609667  0.141320  -4.314071
1.60276e-05
                      padj
                 <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76549e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
...                   ...
ENSG00000273748 4.79091e-02
ENSG00000278817 8.09772e-01
ENSG00000278384 8.92654e-01
ENSG00000276345 1.39762e-01
ENSG00000271254 4.53648e-05
```

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



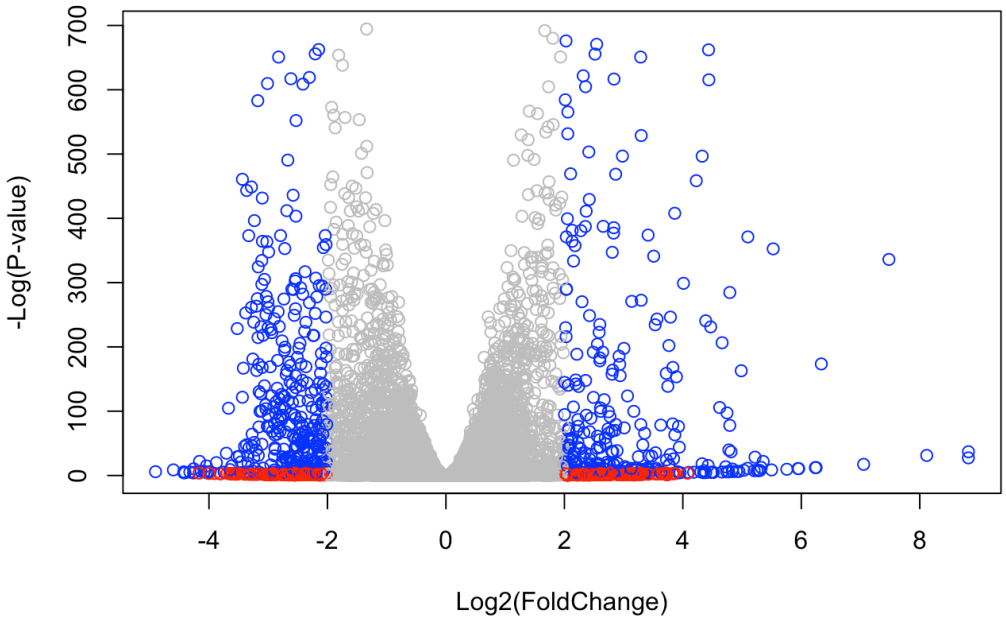
Q. Improve this plot by completing the below code, which adds color and axis labels

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

plot( res$log2FoldChange, -log(res$padj), col=mycols, xlab="Log
```



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

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

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

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

```
res$symbol = mapIds(org.Hs.eg.db,
                    keys=row.names(res),
```

```
                    keytype="ENSEMBL",
                    column="SYMBOL",
                    multiVals="first")
```

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

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

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

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

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

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

```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 10 rows and 9 columns
                   baseMean log2FoldChange     lfcSE
stat      pvalue
                  <numeric>      <numeric> <numeric>
<numeric>   <numeric>
ENSG00000279457   29.913579      0.1792571 0.3248216
0.551863 5.81042e-01
ENSG00000187634  183.229650      0.4264571 0.1402658
3.040350 2.36304e-03
ENSG00000188976 1651.188076     -0.6927205 0.0548465
-12.630158 1.43990e-36
ENSG00000187961  209.637938      0.7297556 0.1318599
5.534326 3.12428e-08
ENSG00000187583   47.255123      0.0405765 0.2718928
0.149237 8.81366e-01
ENSG00000187642   11.979750      0.5428105 0.5215598
1.040744 2.97994e-01
```

```
ENSG00000188290  108.922128      2.0570638 0.1969053
10.446970 1.51282e-25
ENSG00000187608  350.716868      0.2573837 0.1027266
2.505522 1.22271e-02
ENSG00000188157 9128.439422      0.3899088 0.0467163
8.346304 7.04321e-17
ENSG00000237330    0.158192      0.7859552 4.0804729
0.192614 8.47261e-01
                       padj      symbol      entrez
name
                  <numeric> <character> <character>
<character>
ENSG00000279457 6.86555e-01          NA          NA
NA
ENSG00000187634 5.15718e-03      SAMD11      148398 sterile
alpha motif ..
ENSG00000188976 1.76549e-35       NOC2L       26155 NOC2 like
nucleolar ..
ENSG00000187961 1.13413e-07      KLHL17      339451 kelch like
family me..
ENSG00000187583 9.19031e-01     PLEKHN1       84069 pleckstrin
homology ..
ENSG00000187642 4.03379e-01       PERM1       84808 PPARGC1
and ESRR ind..
ENSG00000188290 1.30538e-24        HES4       57801 hes family
bHLH tran..
ENSG00000187608 2.37452e-02       ISG15        9636 ISG15
ubiquitin like..
ENSG00000188157 4.21963e-16        AGRN      375790
agrin
ENSG00000237330          NA      RNF223      401934 ring
finger protein ..
```

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

```
# Run in your R console (i.e. not your Rmarkdown doc!)
#BiocManager::install( c("pathview", "gage", "gageData") )

# For old vesrsions of R only (R < 3.5.0)!
#source("http://bioconductor.org/biocLite.R")
#biocLite( c("pathview", "gage", "gageData") )
```

```
library(pathview)
```

```
##############################################################
################
Pathview is an open source software package distributed under
GNU General
Public License version 3 (GPLv3). Details of GPLv3 is
available at
http://www.gnu.org/licenses/gpl-3.0.html. Particullary, users
are required to
formally cite the original Pathview paper (not just mention
it) in publications
or products. For details, do citation("pathview") within R.

The pathview downloads and uses KEGG data. Non-academic uses
may require a KEGG
license agreement (details at
http://www.kegg.jp/kegg/legal.html).
##############################################################
################
```

```
library(gage)
```

```
library(gageData)

data(kegg.sets.hs)
data(sigmet.idx.hs)

# Focus on signaling and metabolic pathways only
kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]

# Examine the first 3 pathways
head(kegg.sets.hs, 3)
```

```
$`hsa00232 Caffeine metabolism`
[1] "10"   "1544" "1548" "1549" "1553" "7498" "9"

$`hsa00983 Drug metabolism - other enzymes`
 [1] "10"     "1066"   "10720"  "10941"  "151531" "1548"
"1549"   "1551"
 [9] "1553"   "1576"   "1577"   "1806"   "1807"   "1890"
"221223" "2990"
```

```
[17] "3251"   "3614"   "3615"   "3704"   "51733"  "54490"
"54575"  "54576"
[25] "54577"  "54578"  "54579"  "54600"  "54657"  "54658"
"54659"  "54963"
[33] "574537" "64816"  "7083"   "7084"   "7172"   "7363"
"7364"   "7365"
[41] "7366"   "7367"   "7371"   "7372"   "7378"   "7498"
"79799"  "83549"
[49] "8824"   "8833"   "9"      "978"

$`hsa00230 Purine metabolism`
  [1] "100"    "10201"  "10606"  "10621"  "10622"  "10623"
"107"    "10714"
  [9] "108"    "10846"  "109"    "111"    "11128"  "11164"
"112"    "113"
 [17] "114"    "115"    "122481" "122622" "124583" "132"
"158"    "159"
 [25] "1633"   "171568" "1716"   "196883" "203"    "204"
"205"    "221823"
 [33] "2272"   "22978"  "23649"  "246721" "25885"  "2618"
"26289"  "270"
 [41] "271"    "27115"  "272"    "2766"   "2977"   "2982"
"2983"   "2984"
 [49] "2986"   "2987"   "29922"  "3000"   "30833"  "30834"
"318"    "3251"
 [57] "353"    "3614"   "3615"   "3704"   "377841" "471"
"4830"   "4831"
 [65] "4832"   "4833"   "4860"   "4881"   "4882"   "4907"
"50484"  "50940"
 [73] "51082"  "51251"  "51292"  "5136"   "5137"   "5138"
"5139"   "5140"
 [81] "5141"   "5142"   "5143"   "5144"   "5145"   "5146"
"5147"   "5148"
 [89] "5149"   "5150"   "5151"   "5152"   "5153"   "5158"
"5167"   "5169"
 [97] "51728"  "5198"   "5236"   "5313"   "5315"   "53343"
"54107"  "5422"
[105] "5424"   "5425"   "5426"   "5427"   "5430"   "5431"
"5432"   "5433"
[113] "5434"   "5435"   "5436"   "5437"   "5438"   "5439"
"5440"   "5441"
[121] "5471"   "548644" "55276"  "5557"   "5558"   "55703"
"55811"  "55821"
[129] "5631"   "5634"   "56655"  "56953"  "56985"  "57804"
"58497"  "6240"
```

```
[137] "6241"   "64425"  "646625" "654364" "661"    "7498"
"8382"   "84172"
[145] "84265"  "84284"  "84618"  "8622"   "8654"   "87178"
"8833"   "9060"
[153] "9061"   "93034"  "953"    "9533"   "954"    "955"
"956"    "957"
[161] "9583"   "9615"
```

```
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
head(foldchanges)
```

```
     1266     54855      1465     51232      2034      2317
-2.422719  3.201955 -2.313738 -2.059631 -1.888019 -1.649792
```

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

```
attributes(keggres)
```

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

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

```
                                      p.geomean stat.mean
p.val
hsa04110 Cell cycle                 8.995727e-06 -4.378644
8.995727e-06
hsa03030 DNA replication            9.424076e-05 -3.951803
9.424076e-05
hsa03013 RNA transport              1.375901e-03 -3.028500
1.375901e-03
hsa03440 Homologous recombination   3.066756e-03 -2.852899
3.066756e-03
hsa04114 Oocyte meiosis             3.784520e-03 -2.698128
3.784520e-03
hsa00010 Glycolysis / Gluconeogenesis 8.961413e-03 -2.405398
8.961413e-03
                                          q.val set.size
exp1
```

```
hsa04110 Cell cycle                    0.001448312      121
8.995727e-06
hsa03030 DNA replication               0.007586381       36
9.424076e-05
hsa03013 RNA transport                 0.073840037      144
1.375901e-03
hsa03440 Homologous recombination      0.121861535       28
3.066756e-03
hsa04114 Oocyte meiosis                0.121861535      102
3.784520e-03
hsa00010 Glycolysis / Gluconeogenesis 0.212222694       53
8.961413e-03
```

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

```
                                      p.geomean stat.mean
p.val
hsa04640 Hematopoietic cell lineage   0.002822776  2.833362
0.002822776
hsa04630 Jak-STAT signaling pathway   0.005202070  2.585673
0.005202070
hsa00140 Steroid hormone biosynthesis 0.007255099  2.526744
0.007255099
hsa04142 Lysosome                     0.010107392  2.338364
0.010107392
hsa04330 Notch signaling pathway      0.018747253  2.111725
0.018747253
hsa04916 Melanogenesis                0.019399766  2.081927
0.019399766
                                          q.val set.size
exp1
hsa04640 Hematopoietic cell lineage   0.3893570       55
0.002822776
hsa04630 Jak-STAT signaling pathway   0.3893570      109
0.005202070
hsa00140 Steroid hormone biosynthesis 0.3893570       31
0.007255099
hsa04142 Lysosome                     0.4068225      118
0.010107392
hsa04330 Notch signaling pathway      0.4391731       46
0.018747253
hsa04916 Melanogenesis                0.4391731       90
0.019399766
```

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

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

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

```
# A different PDF based output of the same data
pathview(gene.data=foldchanges, pathway.id="hsa04110", kegg.nat
```

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

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

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

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

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

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

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

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

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

Info: Writing image file hsa04142.pathview.png

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

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

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

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 purpose
keggrespathways_down <- rownames(keggres$less)[1:5]

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

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

```
pathview(gene.data=foldchanges, pathway.id=keggresids_down, spe
```

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab
```

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

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

Info: Writing image file hsa03440.pathview.png

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

Info: Working in directory
/Users/andytong/Downloads/Bioinformatics Work/11_09_22_lab

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

```
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
```

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

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

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

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?

On reactome, Endosomal/Vacuolar pathway had the most significant entity p-value entry. The Kegg results compared to GO results seemed to be much more high level. They seemed to be on overall pathways like cell cycle, or replication or glycolysis while GO focused on adhesion, tube development, and other more low level pathways. This could come from being databases of different pathways or different significance algorithms.