Class 13: RNAseq mini project

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```
#load the libraries
library(DESeq2)
```

```
Loading required package: S4Vectors
```

```
Loading required package: stats4
```

```
Loading required package: BiocGenerics
```

```

Attaching package: 'BiocGenerics'
```

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

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

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

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

```

Attaching package: 'S4Vectors'
```

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

    expand.grid, I, unname

Loading required package: IRanges

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
```

```
library(gage)
```

```
library(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).
#############################################################################
```

Read the countData and colData

```
#read the input 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 so we can compare how countData match t
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. Does coldata match to countdata?

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

```
[1] TRUE
```

Alternative:

```
# ! reverse T to F, F to T.
if (!all(rownames(colData) == colnames(countData))){
  cat("Wow something is wrong")
}
```

Looks like the coldata match to the countdata.

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?

```
head(countData)
```

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

```r
# 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
```

PCA as a quality control

```r
#do the base R PCA on countData, and we need to take the transpose of countData
pca <- prcomp(t(countData), scale = T)
attributes(pca)
```

```
$names
[1] "sdev"     "rotation" "center"   "scale"    "x"

$class
[1] "prcomp"
```

```r
summary(pca)
```

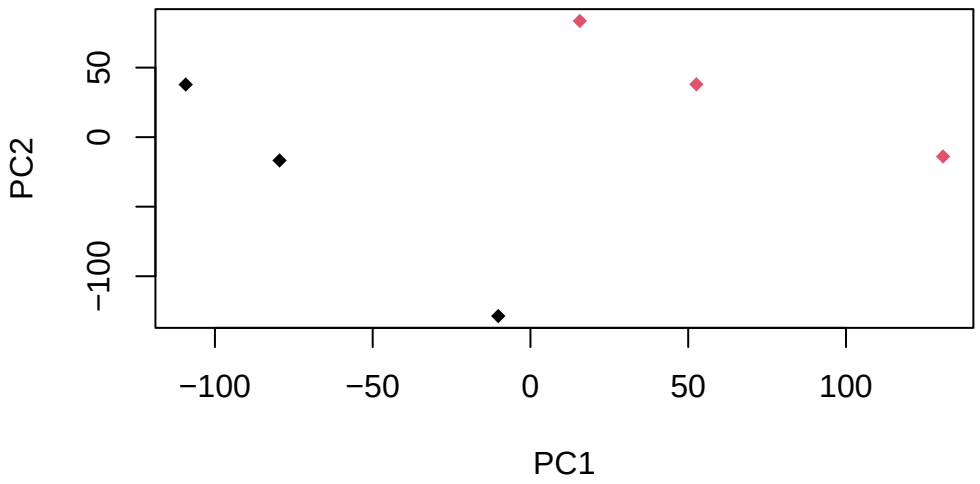
```
Importance of components:
                          PC1     PC2      PC3      PC4      PC5       PC6
Standard deviation     87.7211 73.3196 32.89604 31.15094 29.18417 6.648e-13
Proportion of Variance  0.4817  0.3365  0.06774  0.06074  0.05332 0.000e+00
Cumulative Proportion   0.4817  0.8182  0.88594  0.94668  1.00000 1.000e+00
```

Q. How much variance is captured in the first two PCs?

PC1: 0.4817 PC2: 0.3365 Cumulative: 0.8182

Q. What does your score plot (PC1 vs PC2) plot look like when colored by condition (control/knock-down)?

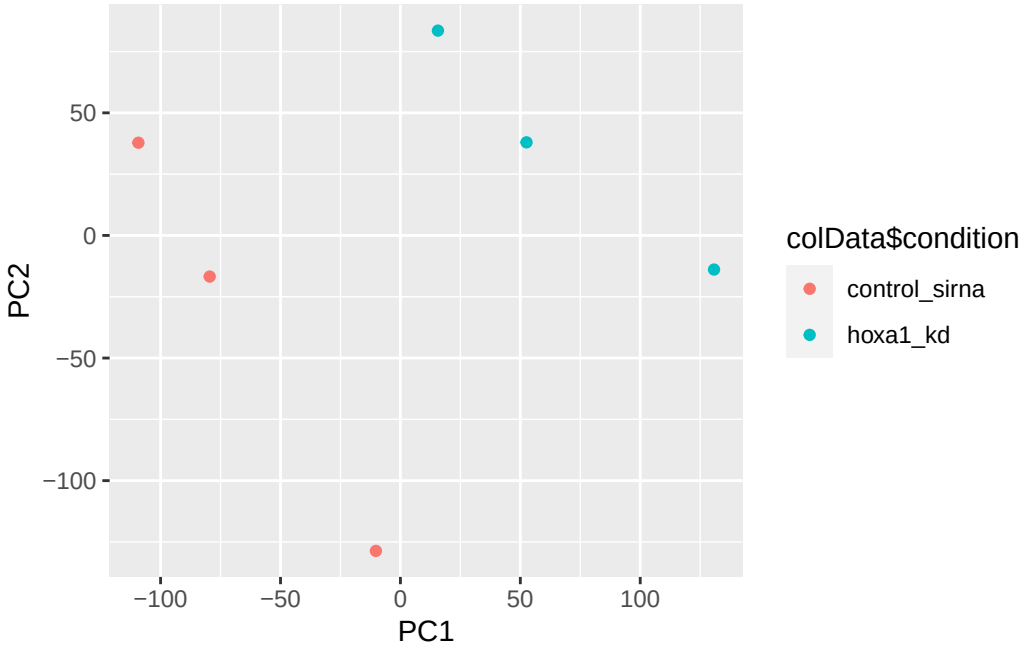
```
plot(pca$x[,1], pca$x[,2], xlab="PC1", ylab="PC2", pch = 18, col=as.factor(colData$conditi
```



ggplot:

```
library(ggplot2)
```

```
x <- as.data.frame(pca$x)

ggplot(x) +
  aes(PC1, PC2, col = colData$condition) +
  geom_point()
```



DESeq analysis

```
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)
head(res)
```

```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 6 rows and 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
ENSG00000187642   11.9798      0.5428105 0.5215598   1.040744 2.97994e-01
                      padj
                 <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76549e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
ENSG00000187642 4.03379e-01
```

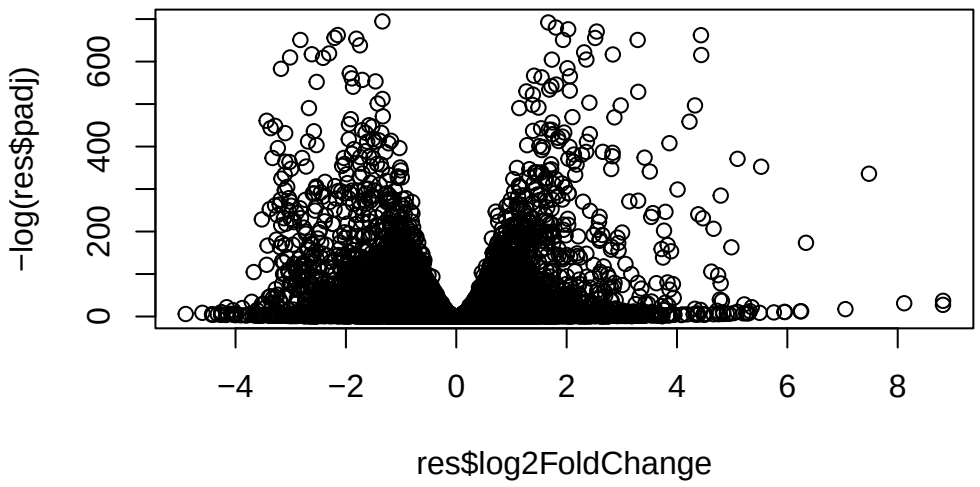
Q. Call the summary() function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```
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
```

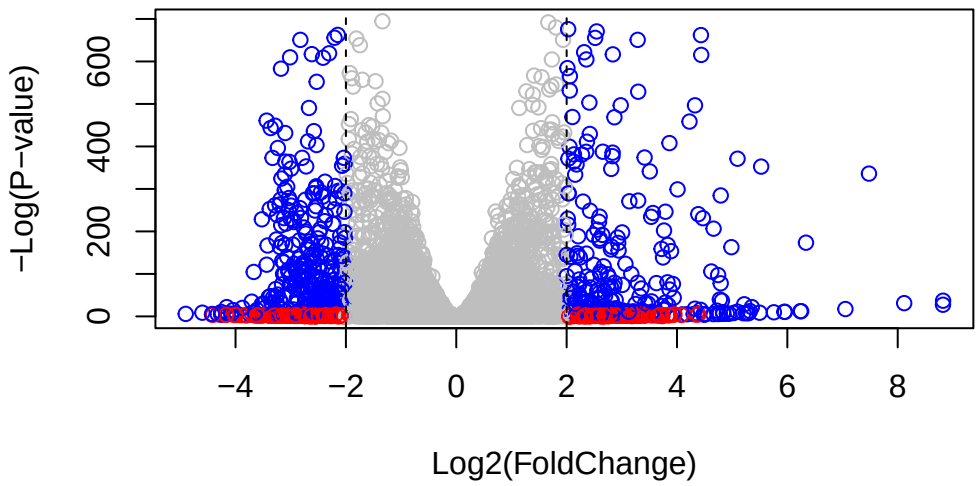
Summary plot

```
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(
abline(v=c(-2,2), lty = 2)
```



#Add 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"
```

```
head(res)
```

```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 6 rows and 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
ENSG00000187642   11.9798      0.5428105 0.5215598   1.040744 2.97994e-01
                      padj
                 <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76549e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
ENSG00000187642 4.03379e-01
```

```
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 ..
```

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")
```

Pathway analysis

```
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"
```

I need to create the input for gage() - a vector of fold change values with entrez IDs as the names().

```
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)
```

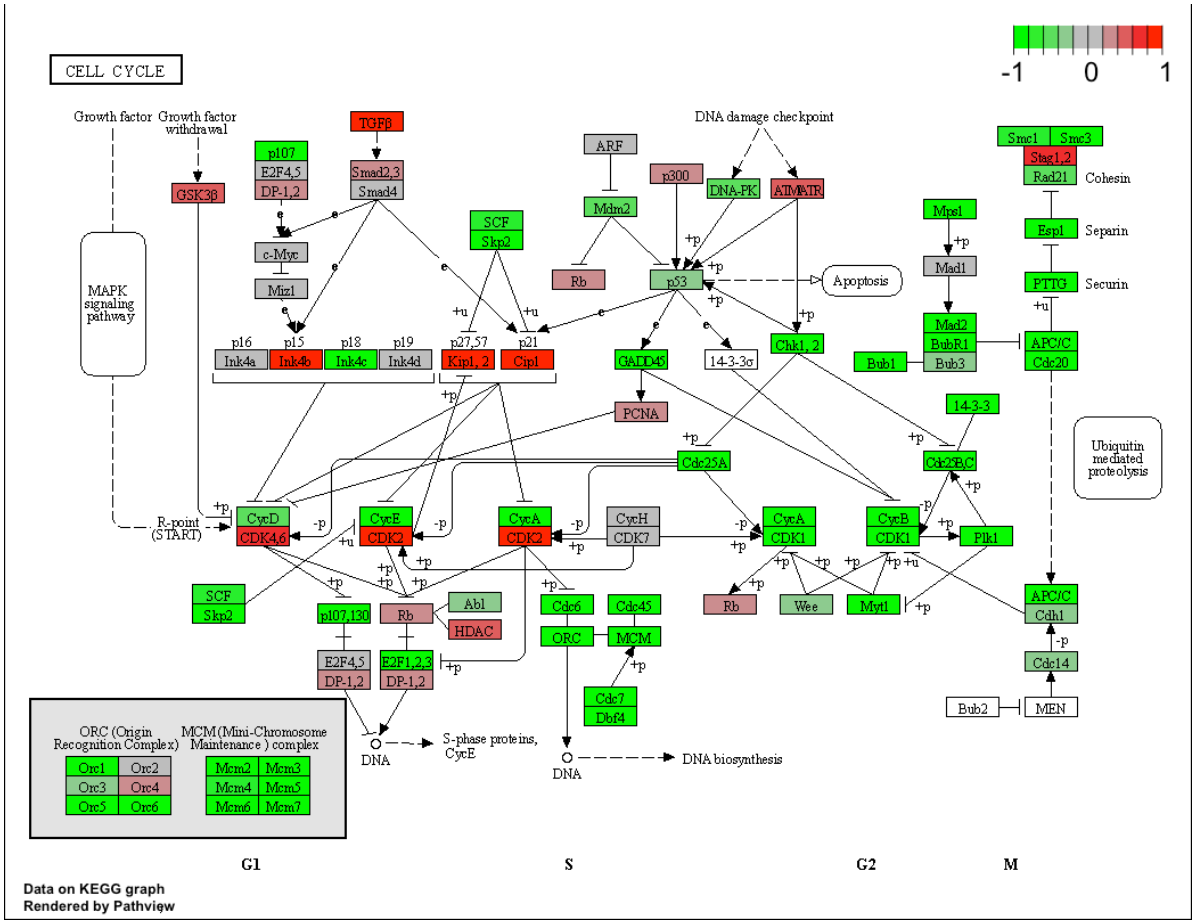
```
                                      p.geomean stat.mean        p.val
hsa04110 Cell cycle                   8.995727e-06 -4.378644 8.995727e-06
hsa03030 DNA replication              9.424076e-05 -3.951803 9.424076e-05
hsa03013 RNA transport                1.375901e-03 -3.028500 1.375901e-03
hsa03440 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
```

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

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

```
Info: Working in directory /Users/wenxitang/Desktop/BGGN 213/Class13
```

```
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 /Users/wenxitang/Desktop/BGGN 213/Class13
```

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

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

```
#top 1 down-regulated already plot
topdown <- substr(rownames(keggres$less)[2:5], start =1, stop = 8)
topdown
```

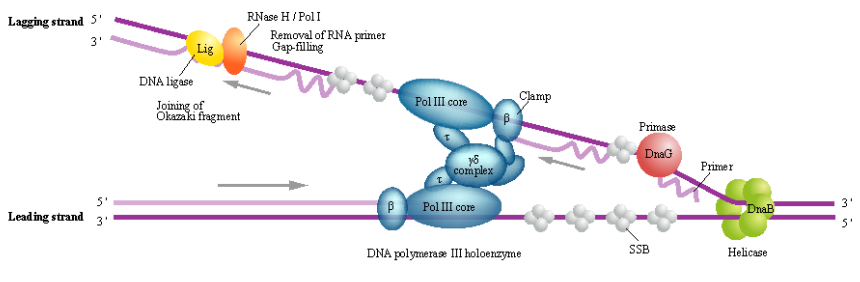
```
[1] "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

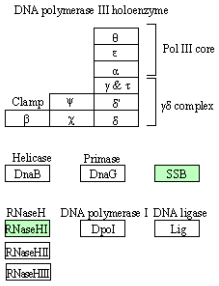
```
pathview(gene.data=foldchanges, pathway.id= topdown, species = "hsa")
```

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

Info: Working in directory /Users/wenxitang/Desktop/BGGN 213/Class13

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory /Users/wenxitang/Desktop/BGGN 213/Class13

Info: Writing image file hsa03013.pathview.png

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

Info: Working in directory /Users/wenxitang/Desktop/BGGN 213/Class13

Info: Writing image file hsa03440.pathview.png

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

Info: Working in directory /Users/wenxitang/Desktop/BGGN 213/Class13

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

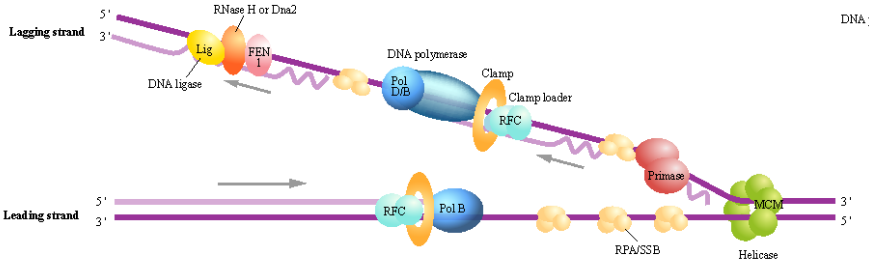
DNA REPLICATION

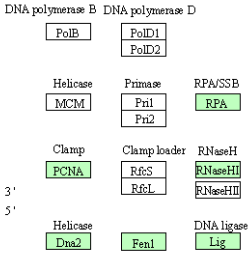
Replication complex (Bacteria)



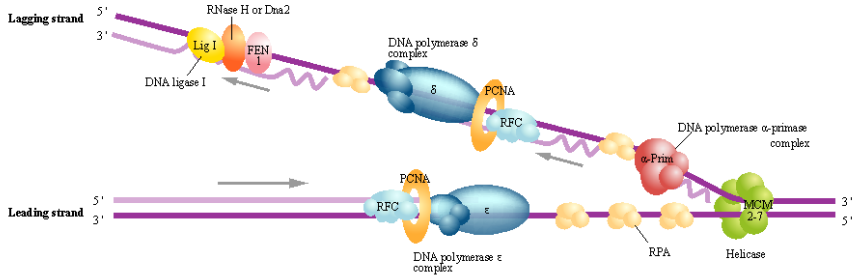


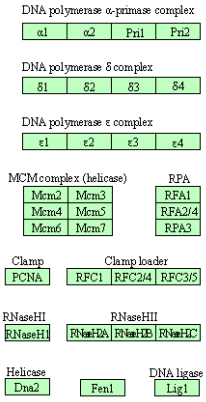
Replication complex (Archaea)



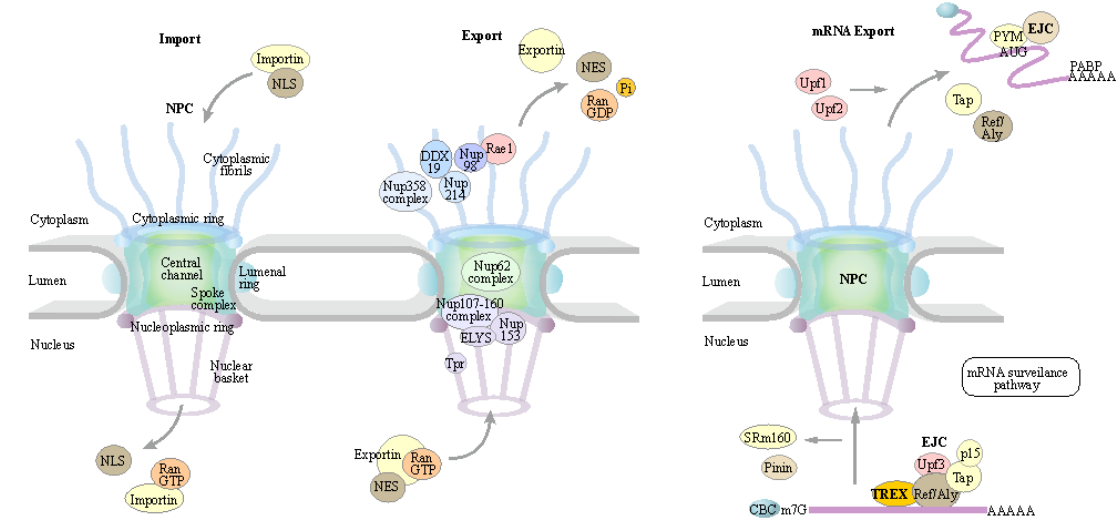


Replication complex (Eukaryotes)





NUCLEOCYTOPLASMIC TRANSPORT



Nuclear Pore complex (NPC)

Cytoplasmic fibrils

ALADIN	hCG1	Gle1	DDX19	Rae1	Nup98	Nup214	Nup88

Nup358 complex

RanBP2	RanGAP	UBC9	SUMO

Cytoplasmic ring / Nucleoplasmic ring (Symmetrical nups)

Nup160	Nup85	Sec13	Nup107	Nup133	Nup96	Seh1	Nup43	Nup37	ELYS
					Nup145				

Central channel

Nup62	Nup58/45	Nup54

Spoke complex

Nup205	Nup188	Nup155	Nup93	Nup53
				Nup59

Lumenal ring

NDC1	gp210	pom121	pom152	pom34	pom33

Nuclear basket

Tpr	Nup50	Nup153	Senp2
	Nup2	Nup1	Nup60

Nuclear transport complex

Importin

IPOA	IPOB

Adaptor proteins

SPN1

Exportin

XPO	Ran

eEF1A

PHAX	CBC

NMD3

Exon-junction complex (EJC)

EJC inner core

Y14	MAGOH	MLN51	EIF4A3

EJC outer shell

ACIN1	SAP18	RNPS1	Pinin	Ref/Aly

Transiently interacting factors

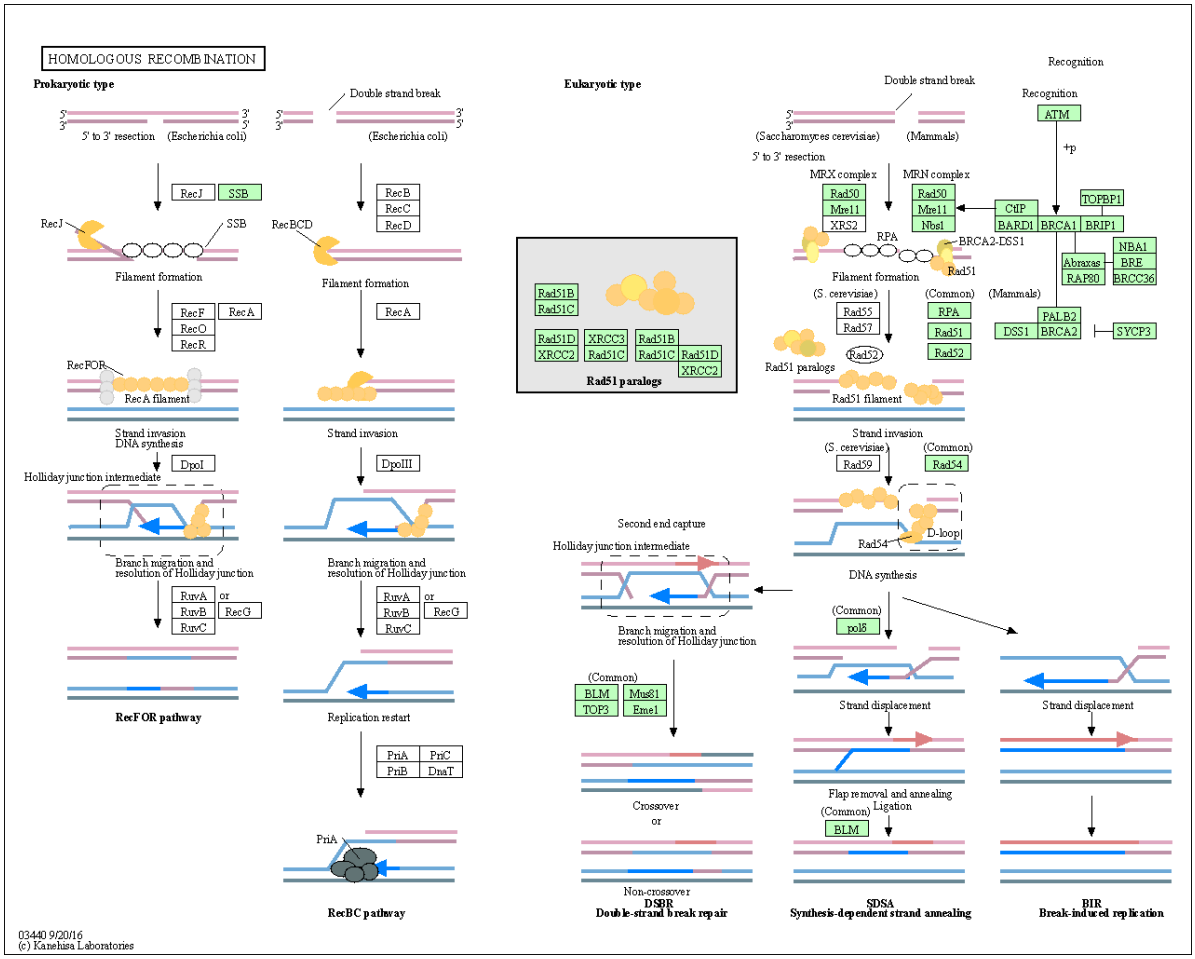
Upf1	Upf2	Upf3

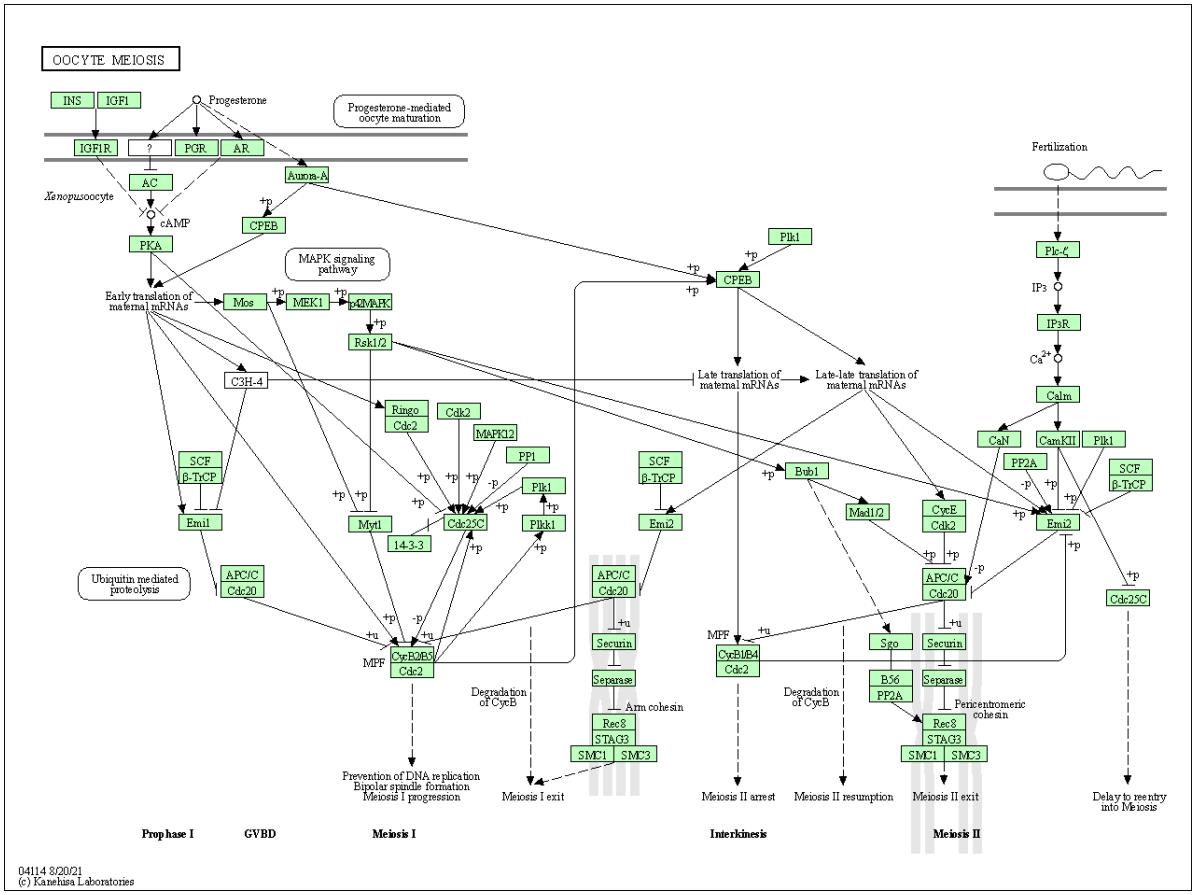
Tap	p15	UAP56	SRm160	PYM

Transcription-export (TREX) complex

THO subcomplex

THOC1	THOC2	THOC5	THOC6	THOC7	TEX1


HOMOLOGOUS RECOMBINATION
Prokaryotic type
Double strand break
5' to 3' resection
(Escherichia coli)
RecJ
SSB
RecBCD
RecF
RecO
RecR
RecA
RecFOR
RecA filament
Filament formation
Strand invasion
DNA synthesis
DpoI
DpoIII
Holliday junction intermediate
Branch migration and resolution of Holliday junction
RuvA
RuvB
RuvC
or
RecG
RecFOR pathway
Replication restart
PriA
PriB
PriC
DnaT
RecBC pathway
Eukaryotic type
(Saccharomyces cerevisiae)
(Mammals)
Recognition
ATM
+p
MRX complex
MRN complex
Rad50
Mre11
XRS2
Nbs1
CtIP
BARD1
BRCA1
BRIP1
TOPBP1
RPA
BRCA2-DSS1
Rad51
NBA1
BRE
Abraxas
RAP80
BRCC36
PALB2
DSS1
SYCP3
(S. cerevisiae)
(Common)
Rad55
Rad57
Rad52
Rad51 paralogs
Rad51 filament
Rad51B
Rad51C
Rad51D
XRCC2
XRCC3
Rad59
Rad54
D-loop
DNA synthesis
pol δ
Second end capture
BLM
TOP3
Mus81
Eme1
Crossover
Non-crossover
Strand displacement
Flap removal and annealing
Ligation
DSBR
Double-strand break repair
SDSA
Synthesis-dependent strand annealing
BIR
Break-induced replication
03440 9/20/16
(c) Kanehisa Laboratories



GO analysis

```
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
```

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: 8147"
```

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

Then, to perform pathway analysis online go to the Reactome website (https://reactome.org/PathwayBrowser/# Select "choose file" to upload your significant gene list. Then, select the parameters "Project to Humans", then click "Analyze".

> 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?

"Endosomal/Vacuolar pathway" has the most significant entities p-value. In KEGG results, the most significant pathway, followed by "cell cycle", "cell cycle mitotic", "antigen representation". In KEGG, it's "Cell cycle", "DNA replication", "RNA transport", etc.

Some pathways match to previous KEGG results but some don't. KEGG has more broad terms/pathways defined but Reactome has more detailed entries down the 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
```

```
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
```