Class 14: RNASeq mini-project

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##Data Import

```
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 object is masked from 'package:utils':

    findMatches


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
```

```r
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
```

```r
# Import countdata
countData = read.csv(countFile, row.names=1)
head(countData)
```

```
                length SRR493366 SRR493367 SRR493368 SRR493369 SRR493370
ENSG00000186092    918         0         0         0         0         0
ENSG00000279928    718         0         0         0         0         0
ENSG00000279457   1982        23        28        29        29        28
ENSG00000278566    939         0         0         0         0         0
ENSG00000273547    939         0         0         0         0         0
ENSG00000187634   3214       124       123       205       207       212
                SRR493371
ENSG00000186092         0
ENSG00000279928         0
ENSG00000279457        46
ENSG00000278566         0
ENSG00000273547         0
ENSG00000187634       258
```

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

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

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

> Q. Complete the code below to filter countData to exclude genes (i.e. rows) where we have 0 read count across all samples (i.e. columns).
>
> 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.
row_sums <-  rowSums(countData[,-1])
countData_filtered <- countData[row_sums != 0 , ]
head(countData_filtered)
```

```
                SRR493366 SRR493367 SRR493368 SRR493369 SRR493370 SRR493371
```

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

DESeq setup analysis

```r
library(DESeq2)

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

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

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

```
estimating size factors
```

```
estimating dispersions
```

```
gene-wise dispersion estimates
```

```
mean-dispersion relationship
```

```
final dispersion estimates
```

```
fitting model and testing
```

```r
dds
```

```
class: DESeqDataSet
dim: 15879 6
metadata(1): version
assays(4): counts mu H cooks
rownames(15879): ENSG00000279457 ENSG00000187634 ... ENSG00000276345
  ENSG00000271254
rowData names(22): baseMean baseVar ... deviance maxCooks
colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
colData names(2): condition sizeFactor
```

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

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

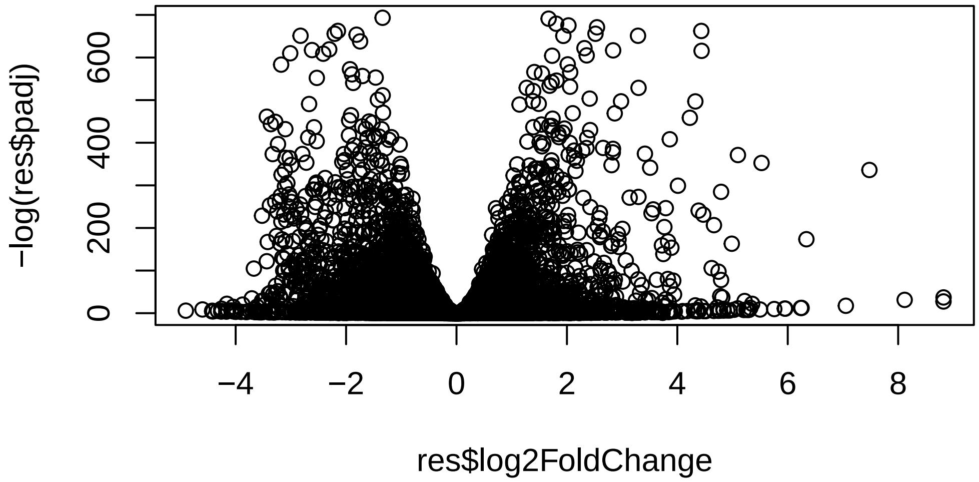
```
summary(res)
```

```

out of 15879 with nonzero total read count
adjusted p-value < 0.1
LFC > 0 (up)       : 4364, 27%
LFC < 0 (down)     : 4418, 28%
outliers [1]       : 0, 0%
low counts [2]     : 1538, 9.7%
(mean count < 1)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results
```

Result visualization

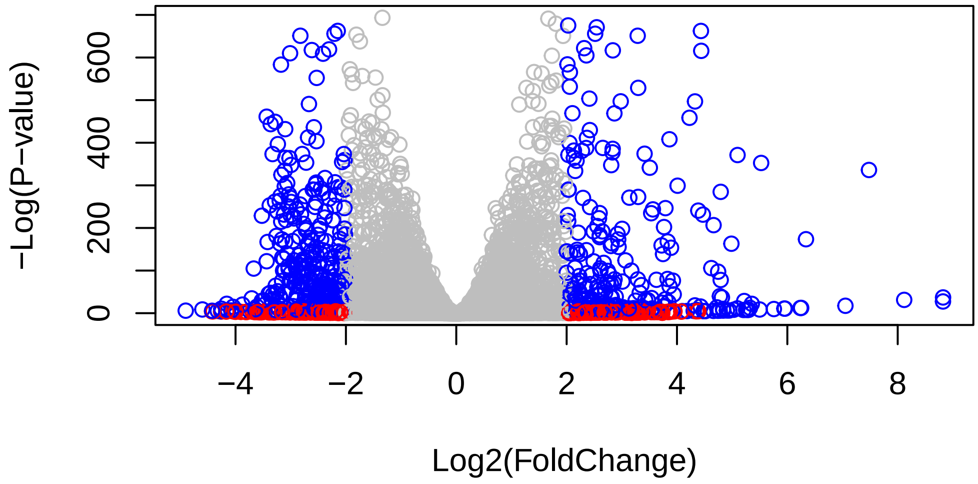
Now we will make a volcano plot

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



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

```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res) )

# Color red the genes with absolute fold change above 2
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

# Color blue those with adjusted p-value less than 0.01
#  and absolute fold change more than 2
inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

plot( res$log2FoldChange, -log(res$padj), col=mycols, xlab="Log2(FoldChange)", ylab="-Log(
```



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

```
library("AnnotationDbi")
```

```
Warning: package 'AnnotationDbi' was built under R version 4.3.2
```

```
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"
```

```r
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
```

```r
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
```

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

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

```r
head(res, 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      0.1792795 0.3245323   0.552424 5.80658e-01
ENSG00000187634  183.229650      0.4264445 0.1401543   3.042680 2.34482e-03
ENSG00000188976 1651.188076     -0.6927208 0.0548418 -12.631264 1.41979e-36
ENSG00000187961  209.637938      0.7297602 0.1317626   5.538448 3.05163e-08
ENSG00000187583   47.255123      0.0405481 0.2716368   0.149273 8.81338e-01
ENSG00000187642   11.979750      0.5427396 0.5210745   1.041578 2.97607e-01
```

```
ENSG00000188290  108.922128      2.0570462 0.1967692  10.454108 1.40312e-25
ENSG00000187608  350.716868      0.2573752 0.1026587   2.507095 1.21728e-02
ENSG00000188157 9128.439422      0.3899088 0.0467420   8.341716 7.32201e-17
ENSG00000237330    0.158192      0.7859552 4.0804729   0.192614 8.47261e-01
                       padj      symbol      entrez                   name
                  <numeric> <character> <character>            <character>
ENSG00000279457 6.77340e-01          NA          NA                     NA
ENSG00000187634 4.97957e-03      SAMD11      148398 sterile alpha motif ..
ENSG00000188976 1.69253e-35       NOC2L       26155 NOC2 like nucleolar ..
ENSG00000187961 1.07765e-07      KLHL17      339451 kelch like family me..
ENSG00000187583 9.16287e-01     PLEKHN1       84069 pleckstrin homology ..
ENSG00000187642 3.94333e-01       PERM1       84808 PPARGC1 and ESRR ind..
ENSG00000188290 1.17812e-24        HES4       57801 hes family bHLH tran..
ENSG00000187608 2.30000e-02       ISG15        9636 ISG15 ubiquitin like..
ENSG00000188157 4.26676e-16        AGRN      375790                  agrin
ENSG00000237330          NA      RNF223      401934 ring finger protein ..
```

Save Results

> Q. Finally for this section let’s reorder these results by adjusted p-value and save them to a CSV file in your current project directory.

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

Geneset enrichment

```r
library(pathview)
```

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

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

```r
library(gage)
```

```r
library(gageData)

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

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

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

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

$`hsa00983 Drug metabolism - other enzymes`
 [1] "10"     "1066"   "10720"  "10941"  "151531" "1548"   "1549"   "1551"  
 [9] "1553"   "1576"   "1577"   "1806"   "1807"   "1890"   "221223" "2990"  
[17] "3251"   "3614"   "3615"   "3704"   "51733"  "54490"  "54575"  "54576" 
[25] "54577"  "54578"  "54579"  "54600"  "54657"  "54658"  "54659"  "54963" 
[33] "574537" "64816"  "7083"   "7084"   "7172"   "7363"   "7364"   "7365"  
[41] "7366"   "7367"   "7371"   "7372"   "7378"   "7498"   "79799"  "83549" 
[49] "8824"   "8833"   "9"      "978"   

$`hsa00230 Purine metabolism`
  [1] "100"    "10201"  "10606"  "10621"  "10622"  "10623"  "107"    "10714" 
  [9] "108"    "10846"  "109"    "111"    "11128"  "11164"  "112"    "113"   
 [17] "114"    "115"    "122481" "122622" "124583" "132"    "158"    "159"   
 [25] "1633"   "171568" "1716"   "196883" "203"    "204"    "205"    "221823"
 [33] "2272"   "22978"  "23649"  "246721" "25885"  "2618"   "26289"  "270"   
 [41] "271"    "27115"  "272"    "2766"   "2977"   "2982"   "2983"   "2984"  
 [49] "2986"   "2987"   "29922"  "3000"   "30833"  "30834"  "318"    "3251"  
 [57] "353"    "3614"   "3615"   "3704"   "377841" "471"    "4830"   "4831"  
 [65] "4832"   "4833"   "4860"   "4881"   "4882"   "4907"   "50484"  "50940" 
 [73] "51082"  "51251"  "51292"  "5136"   "5137"   "5138"   "5139"   "5140"  
 [81] "5141"   "5142"   "5143"   "5144"   "5145"   "5146"   "5147"   "5148"  
 [89] "5149"   "5150"   "5151"   "5152"   "5153"   "5158"   "5167"   "5169"  
 [97] "51728"  "5198"   "5236"   "5313"   "5315"   "53343"  "54107"  "5422"  
[105] "5424"   "5425"   "5426"   "5427"   "5430"   "5431"   "5432"   "5433"  
[113] "5434"   "5435"   "5436"   "5437"   "5438"   "5439"   "5440"   "5441"  
[121] "5471"   "548644" "55276"  "5557"   "5558"   "55703"  "55811"  "55821" 
[129] "5631"   "5634"   "56655"  "56953"  "56985"  "57804"  "58497"  "6240"  
[137] "6241"   "64425"  "646625" "654364" "661"    "7498"   "8382"   "84172" 
[145] "84265"  "84284"  "84618"  "8622"   "8654"   "87178"  "8833"   "9060"  
[153] "9061"   "93034"  "953"    "9533"   "954"    "955"    "956"    "957"   
[161] "9583"   "9615"  
```

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

```
     1266     54855      1465     51232      2034      2317 
-2.422719  3.201955 -2.313737 -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               9.178354e-06 -4.374797 9.178354e-06
hsa03030 DNA replication          8.343481e-05 -3.987549 8.343481e-05
hsa03013 RNA transport            1.337716e-03 -3.037673 1.337716e-03
hsa03440 Homologous recombination 2.833509e-03 -2.881805 2.833509e-03
hsa04114 Oocyte meiosis           3.277977e-03 -2.747404 3.277977e-03
hsa00240 Pyrimidine metabolism    8.211034e-03 -2.421063 8.211034e-03
                                        q.val set.size         exp1
hsa04110 Cell cycle               0.001477715      120 9.178354e-06
hsa03030 DNA replication          0.006716503       36 8.343481e-05
```

```
hsa03013 RNA transport            0.071790767      143 1.337716e-03
hsa03440 Homologous recombination 0.105550857       28 2.833509e-03
hsa04114 Oocyte meiosis           0.105550857      102 3.277977e-03
hsa00240 Pyrimidine metabolism    0.208417400       97 8.211034e-03
```

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

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

Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14

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

```
pathview(gene.data=foldchanges, pathway.id="hsa03030")
```

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

Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14

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

```
pathview(gene.data=foldchanges, pathway.id="hsa03013")
```

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

Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14

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

```
pathview(gene.data=foldchanges, pathway.id="hsa03440")
```

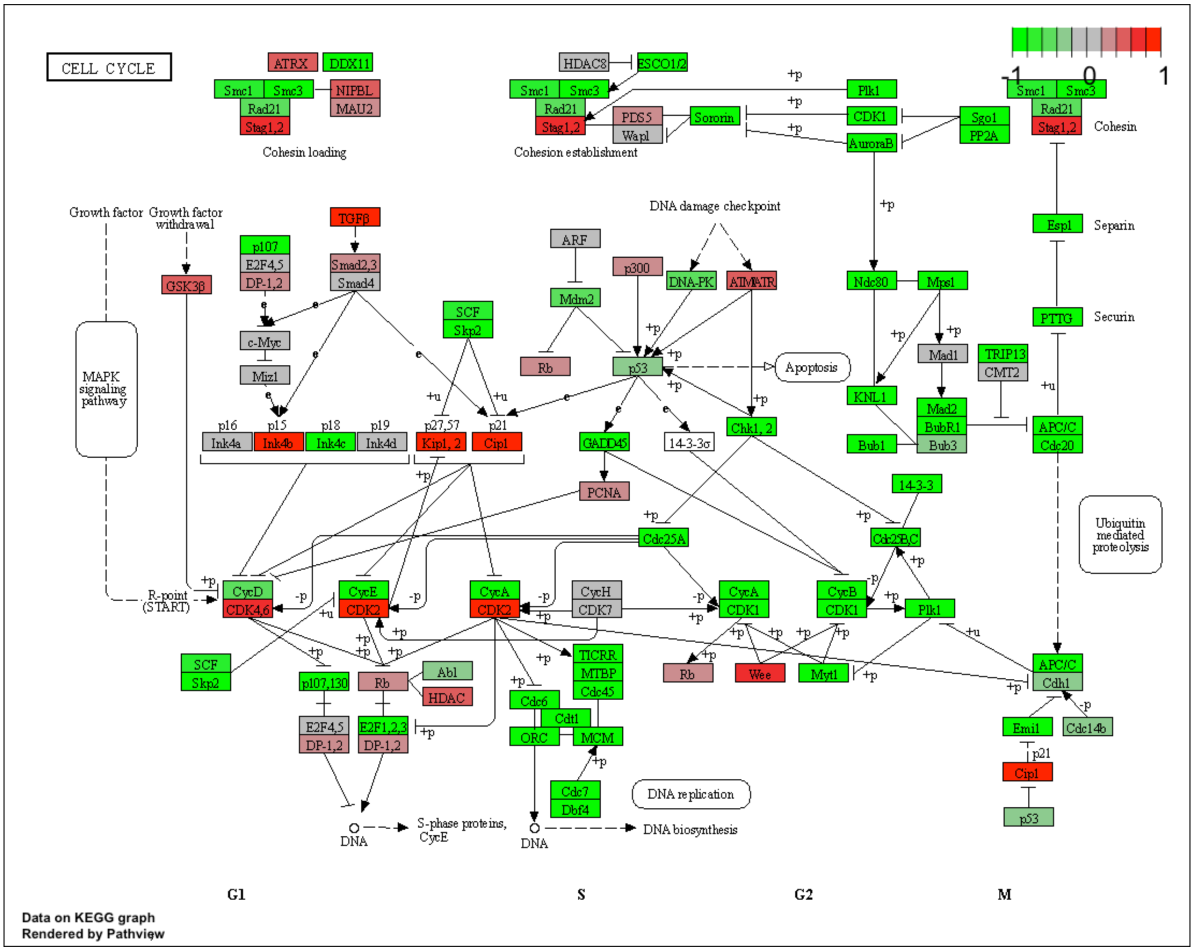
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14

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

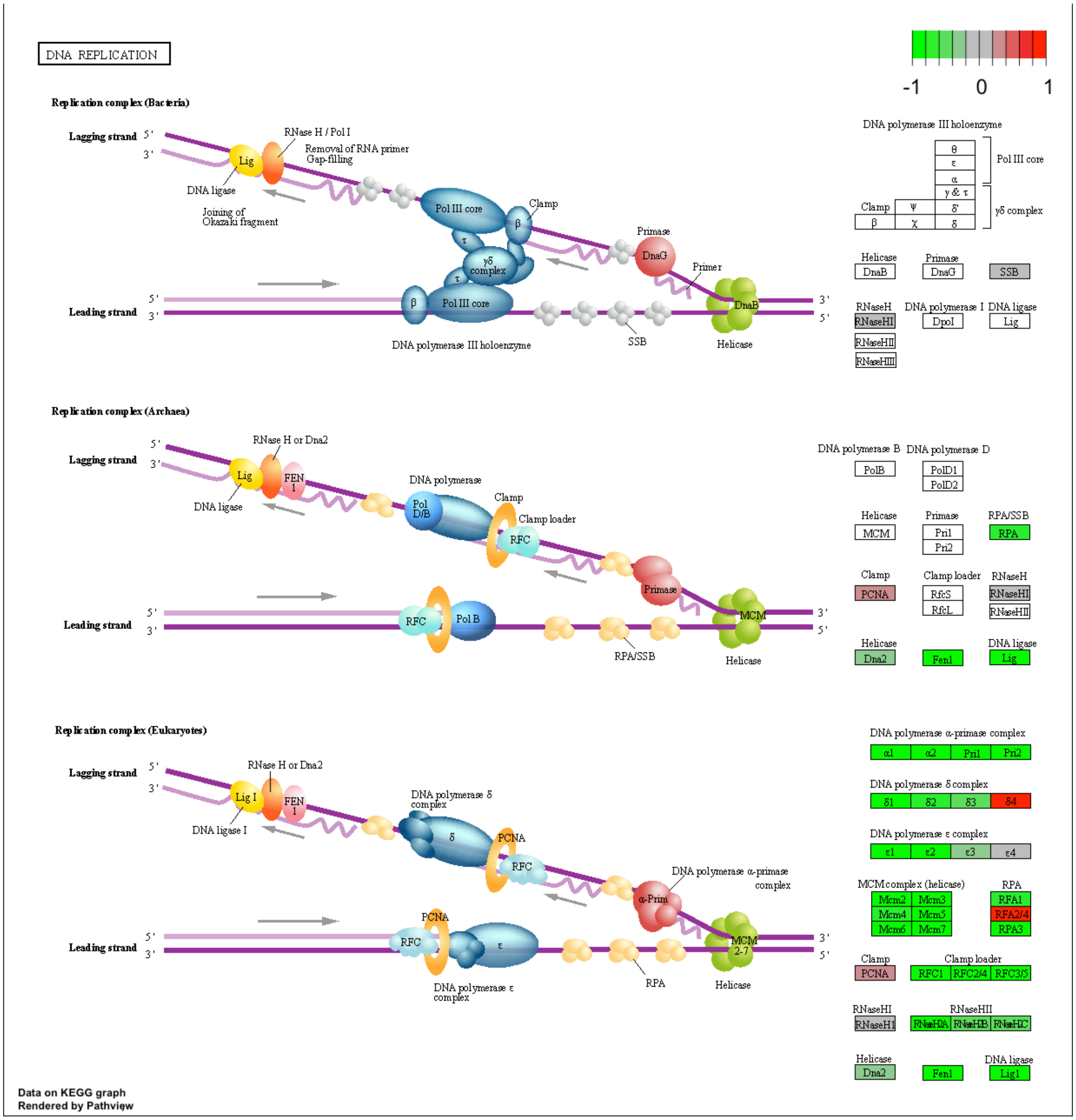
```r
pathview(gene.data=foldchanges, pathway.id="hsa04114")
```

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

Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14

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

```r
pathview(gene.data=foldchanges, pathway.id="hsa00240")
```

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

Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14

Info: Writing image file hsa00240.pathview.png
```


CELL CYCLE
-1 0 1
ATRX
DDX11
Smc1
Smc3
NIPBL
Rad21
MAU2
Stag1,2
Cohesin loading
HDAC8
ESCO1/2
Smc1
Smc3
Rad21
Stag1,2
PDS5
Wapl
Sororin
Cohesion establishment
Plk1
CDK1
AuroraB
Sgo1
PP2A
Smc1
Smc3
Rad21
Stag1,2
Cohesin
Espl
Separin
PTTG
Securin
Growth factor
Growth factor withdrawal
GSK3β
MAPK signaling pathway
TGFβ
p107
E2F4,5
DP-1,2
Smad2,3
Smad4
c-Myc
Miz1
p16
Ink4a
p15
Ink4b
p18
Ink4c
p19
Ink4d
SCF
Skp2
p27,57
Kip1, 2
p21
Cip1
ARF
Mdm2
Rb
p300
DNA damage checkpoint
DNA-PK
ATM/ATR
p53
Apoptosis
GADD45
14-3-3σ
Chk1, 2
PCNA
Ndc80
Mps1
KNL1
Mad1
Mad2
BubR1
Bub3
Bub1
TRIP13
CMT2
APC/C
Cdc20
14-3-3
Cdc25A
Cdc25B,C
Ubiquitin mediated proteolysis
R-point (START)
CycD
CDK4,6
CycE
CDK2
CycA
CDK2
CycH
CDK7
CycA
CDK1
CycB
CDK1
Plk1
SCF
Skp2
p107,130
E2F4,5
DP-1,2
Rb
Abl
HDAC
E2F1,2,3
DP-1,2
TICRR
MTBP
Cdc45
Cdc6
Cdt1
ORC
MCM
Cdc7
Dbf4
Rb
Wee
Myt1
APC/C
Cdh1
Emi1
Cdc14b
p21
Cip1
p53
DNA replication
DNA
S-phase proteins, CycE
DNA
DNA biosynthesis
+p
-p
+u
e
G1
S
G2
M
Data on KEGG graph
Rendered by Pathview



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

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

```
Warning: reconcile groups sharing member nodes!
```

```
     [,1] [,2]
[1,] "9"  "300"
[2,] "9"  "306"
```

```
Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14
```

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

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

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

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

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

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

```
Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14
```

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

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

```
Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14
```

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

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

```
Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14
```

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

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

Info: Working in directory /Users/jesuscalderon/Desktop/BIMM143/Class 14

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/jesuscalderon/Desktop/BIMM143/Class 14

Info: Writing image file hsa04740.pathview.png

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

Gene Ontology

We can do the same style of analysis with GO instread of KEGG 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       0.0001023982  3.775667 0.0001023982
GO:0007610 behavior                       0.0001748323  3.591157 0.0001748323
GO:0002009 morphogenesis of an epithelium 0.0001883739  3.574796 0.0001883739
GO:0048729 tissue morphogenesis           0.0002001781  3.554800 0.0002001781
GO:0035295 tube development               0.0006067279  3.248262 0.0006067279
GO:0060562 epithelial tube morphogenesis  0.0007501480  3.192807 0.0007501480
                                              q.val set.size         exp1
```

```
GO:0007156 homophilic cell adhesion       0.2038813      113 0.0001023982
GO:0007610 behavior                       0.2038813      423 0.0001748323
GO:0002009 morphogenesis of an epithelium 0.2038813      339 0.0001883739
GO:0048729 tissue morphogenesis           0.2038813      424 0.0002001781
GO:0035295 tube development               0.3627158      389 0.0006067279
GO:0060562 epithelial tube morphogenesis  0.3627158      257 0.0007501480

$less
                                             p.geomean stat.mean        p.val
GO:0048285 organelle fission              9.875751e-16 -8.123242 9.875751e-16
GO:0000280 nuclear division               2.876946e-15 -7.993921 2.876946e-15
GO:0007067 mitosis                        2.876946e-15 -7.993921 2.876946e-15
GO:0000087 M phase of mitotic cell cycle 7.812827e-15 -7.853464 7.812827e-15
GO:0007059 chromosome segregation         1.429060e-11 -6.936776 1.429060e-11
GO:0000236 mitotic prometaphase           1.366254e-10 -6.740266 1.366254e-10
                                                 q.val set.size         exp1
GO:0048285 organelle fission              3.906892e-12      375 9.875751e-16
GO:0000280 nuclear division               3.906892e-12      351 2.876946e-15
GO:0007067 mitosis                        3.906892e-12      351 2.876946e-15
GO:0000087 M phase of mitotic cell cycle 7.957364e-12      361 7.812827e-15
GO:0007059 chromosome segregation         1.164398e-08      142 1.429060e-11
GO:0000236 mitotic prometaphase           8.235257e-08       84 1.366254e-10

$stats
                                           stat.mean     exp1
GO:0007156 homophilic cell adhesion         3.775667 3.775667
GO:0007610 behavior                         3.591157 3.591157
GO:0002009 morphogenesis of an epithelium  3.574796 3.574796
GO:0048729 tissue morphogenesis             3.554800 3.554800
GO:0035295 tube development                 3.248262 3.248262
GO:0060562 epithelial tube morphogenesis    3.192807 3.192807
```

Look at our results

```
head(gobpres$less)
```

```
                                             p.geomean stat.mean        p.val
GO:0048285 organelle fission              9.875751e-16 -8.123242 9.875751e-16
GO:0000280 nuclear division               2.876946e-15 -7.993921 2.876946e-15
GO:0007067 mitosis                        2.876946e-15 -7.993921 2.876946e-15
GO:0000087 M phase of mitotic cell cycle 7.812827e-15 -7.853464 7.812827e-15
GO:0007059 chromosome segregation         1.429060e-11 -6.936776 1.429060e-11
```

```
GO:0000236 mitotic prometaphase          1.366254e-10 -6.740266 1.366254e-10
                                                q.val set.size         exp1
GO:0048285 organelle fission             3.906892e-12      375 9.875751e-16
GO:0000280 nuclear division              3.906892e-12      351 2.876946e-15
GO:0007067 mitosis                       3.906892e-12      351 2.876946e-15
GO:0000087 M phase of mitotic cell cycle 7.957364e-12      361 7.812827e-15
GO:0007059 chromosome segregation        1.164398e-08      142 1.429060e-11
GO:0000236 mitotic prometaphase          8.235257e-08       84 1.366254e-10
```

Reactome Analysis

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

```
[1] "Total number of significant genes: 8175"
```

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