Class 13: Mini-Project RNA-Seq

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Section 1. Differential Expression Analysis

```r
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

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

Warning: replacing previous import 'S4Arrays::read_block' by
'DelayedArray::read_block' when loading 'SummarizedExperiment'
```

```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
```

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

```r
# 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
```

4

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).

```
remove <- rowSums(countData) == 0
countData <- countData[!remove, ]
```

```
to_keep <- rowSums(countData)>0
countData <- countData[to_keep, ]
```

Running DESeq2

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

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

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

estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing

```
dds
```

```
class: DESeqDataSet
dim: 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
```

```r
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.

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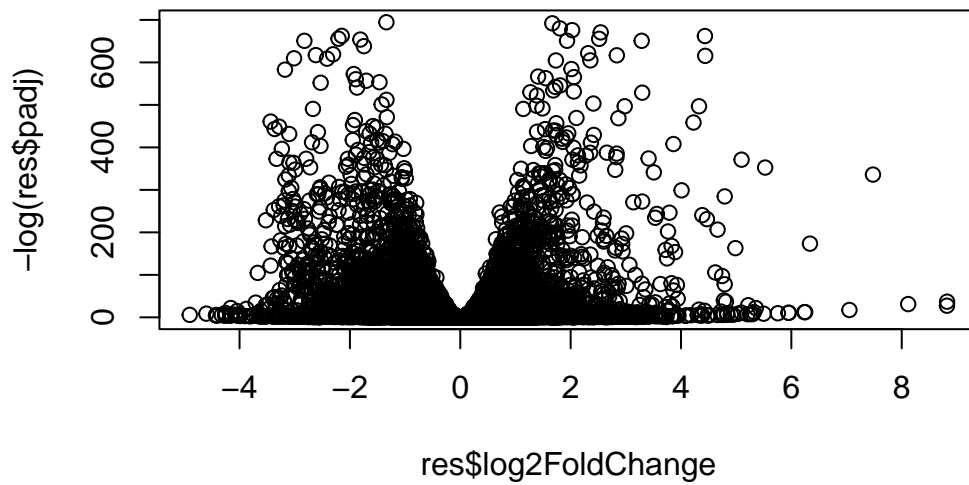
```r
plot( res$log2FoldChange, -log(res$padj) )
```

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(
```

Adding gene annotation

Q5. Use the **mapIDs()** function multiple times to add SYMBOL, ENTREZID and GENE-NAME 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"
```

```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      0.1792571  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 ..
```

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$padj), ]
write.csv(res, file = "deseq_results.csv")
```

Section 2. Pathway Analysis

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

```
Bioconductor version 3.17 (BiocManager 1.30.20), R 4.3.0 (2023-04-21 ucrt)
```

```
Warning: package(s) not installed when version(s) same as or greater than current; use
  `force = TRUE` to re-install: 'pathview' 'gage' 'gageData'
```

```
Installation paths not writeable, unable to update packages
  path: C:/Program Files/R/R-4.3.0/library
  packages:
    class, KernSmooth, MASS, Matrix, nnet
```

```
Old packages: 'BiocParallel', 'cachem', 'DelayedArray', 'DT', 'emmeans',
  'evaluate', 'knitr', 'markdown', 'RcppArmadillo', 'rlang', 'sass', 'sys'
```

```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"
```

```r
  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
```

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

```r
  attributes(keggres)
```

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

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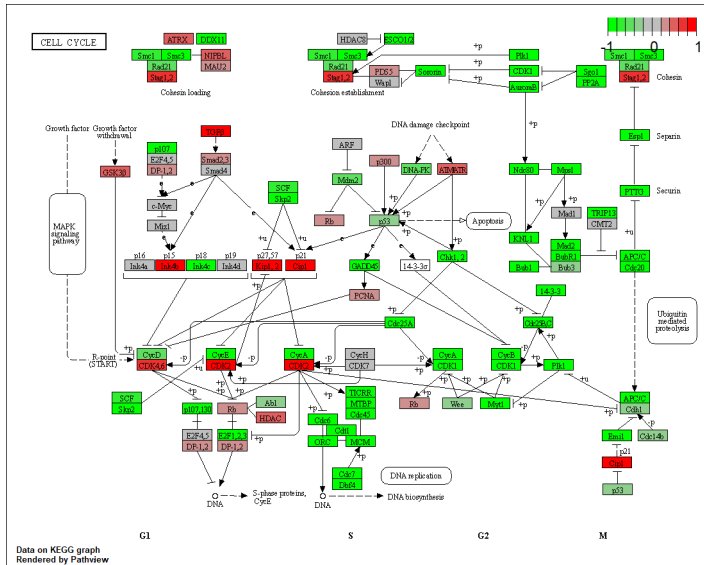
```r
pathview(gene.data=foldchanges, pathway.id="hsa04110")
```

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

```
Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13
```

```
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

Warning: reconcile groups sharing member nodes!

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

Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13

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" "hsa04330"

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

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

Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13

Info: Writing image file hsa04640.pathview.png

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

Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13

Info: Writing image file hsa00140.pathview.png

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

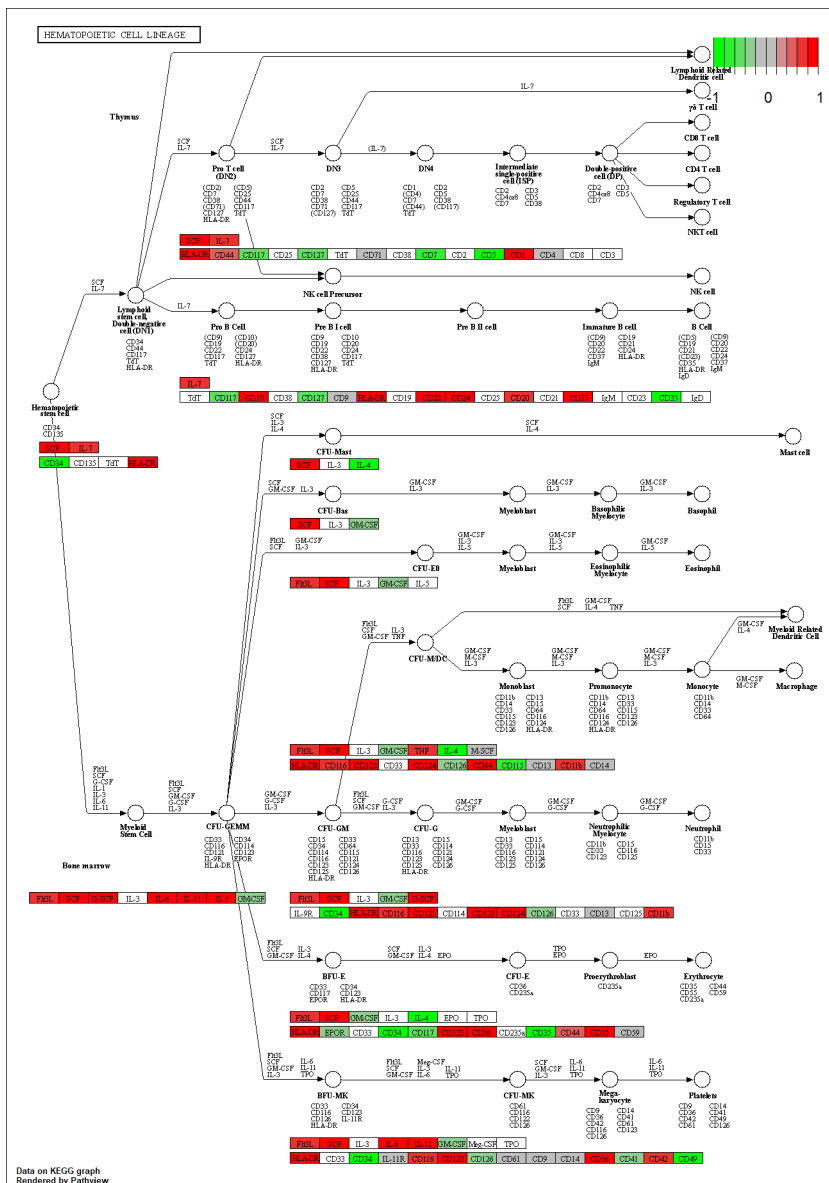Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13

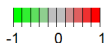Info: Writing image file hsa04142.pathview.png

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

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

Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13

Info: Writing image file hsa04330.pathview.png

HEMATOPOIETIC CELL LINEAGE

Data on KEGG graph
Rendered by Pathview

STEROID HORMONE BIOSYNTHESIS

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

```
keggrespathways <- rownames(keggres$less)[1:5]

keggresids <- substr(keggrespathways, start = 1, stop = 8)

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

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

Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13

Info: Writing image file hsa04110.pathview.png

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

Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13

Info: Writing image file hsa03030.pathview.png

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

Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13

Info: Writing image file hsa03013.pathview.png

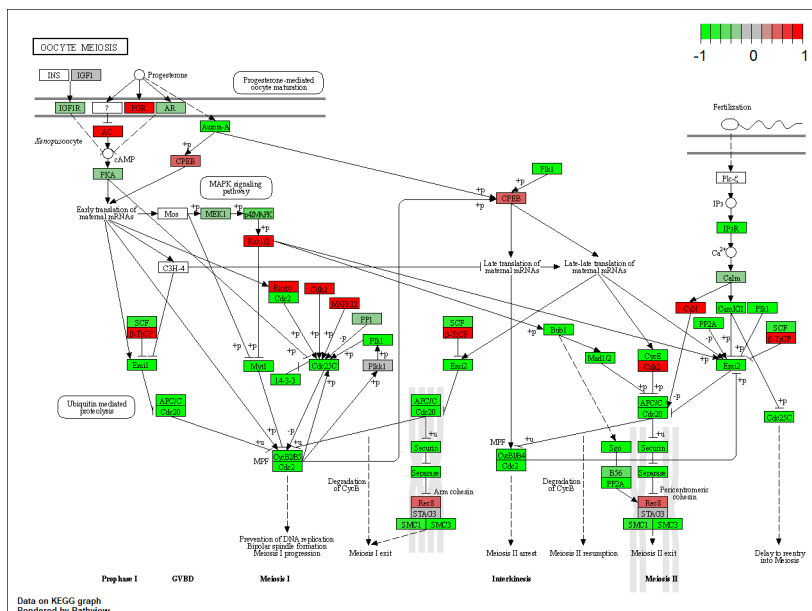'select()' returned 1:1 mapping between keys and columns

Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13
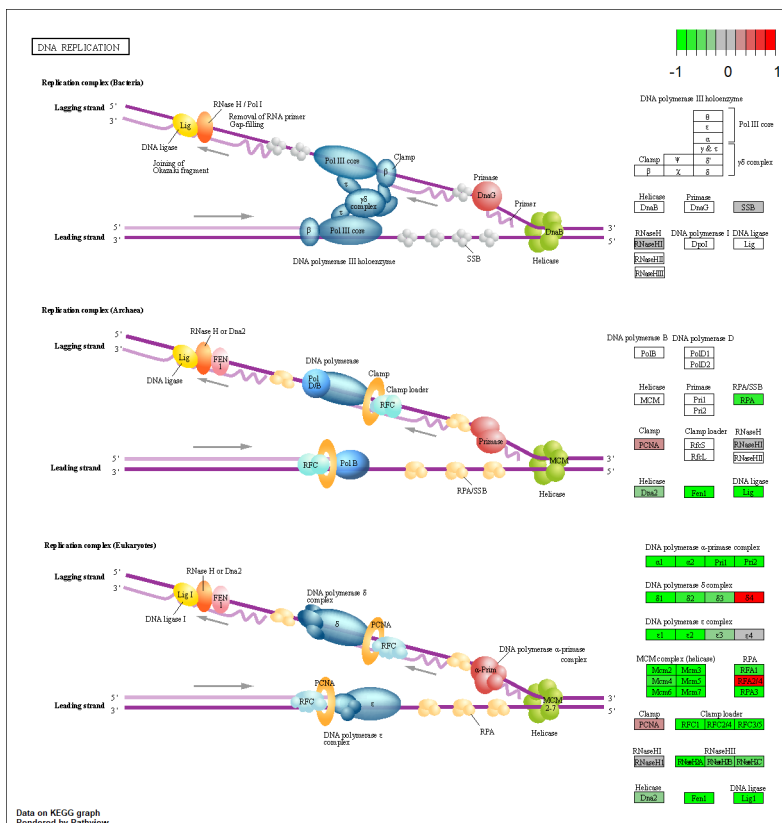
Info: Writing image file hsa03440.pathview.png
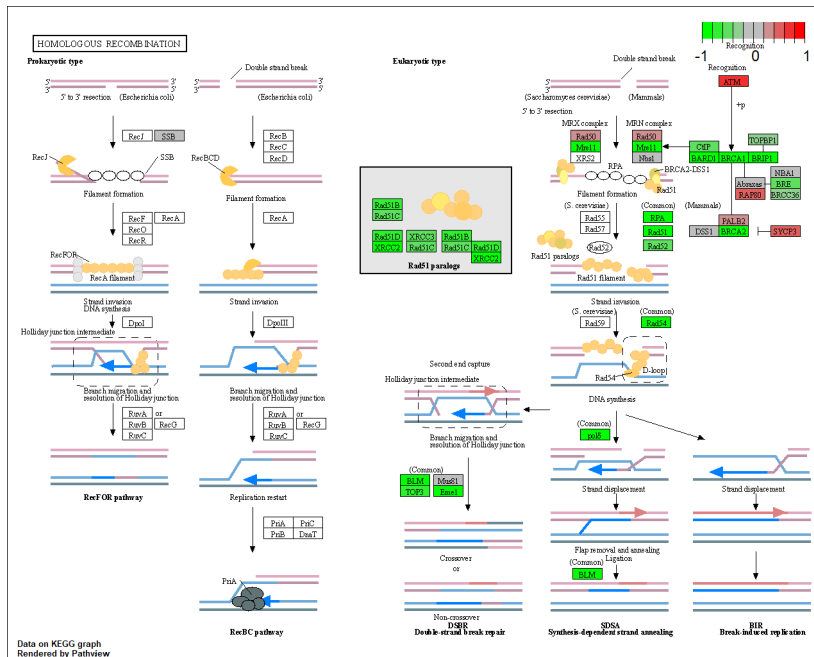
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory C:/Users/parsa/OneDrive/Desktop/Class 13/Class 13

Info: Writing image file hsa04114.pathview.png

Section 3. Gene Ontology (GO)

```
data(go.sets.hs)
data(go.subs.hs)

# Focus on Biological Process subset of GO
gobpsets = go.sets.hs[go.subs.hs$BP]

gobpres = gage(foldchanges, gsets=gobpsets, same.dir=TRUE)

lapply(gobpres, head)
```

$greater

	p.geomean	stat.mean	p.val
GO:0007156 homophilic cell adhesion	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	1.925222e-04	3.565432	1.925222e-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                         0.1967577          426 1.925222e-04
GO:0060562 epithelial tube morphogenesis  0.3565320            257 5.932837e-04
GO:0035295 tube development                  0.3565320          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                         3.565432 3.565432
GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
GO:0035295 tube development                 3.253665 3.253665
```

Section 4. 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: 8147"
```

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

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?

Cell cycle Mitotic

No it does not match

Different gene sets and database