Lab 14 DESeq2 Mini Project

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Section 1: DESEq Setup and analysis

Data Import

Download count data `GSE37704_metadata.csv` and metadata `GSE37704_featurecounts.csv`. Then load `DESeq2`.

```
library("DESeq2")
```

```
Loading required package: S4Vectors
```

```
Loading required package: stats4
```

```
Loading required package: BiocGenerics
```

```

Attaching package: 'BiocGenerics'
```

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

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

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

    anyDuplicated, 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

Warning: package 'matrixStats' was built under R version 4.3.2


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
metadata <- "GSE37704_metadata.csv"
counts <- "GSE37704_featurecounts.csv"
```

```
# Import metadata
colData = read.csv(metadata, 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(counts, 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

```
# 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).

```
# Filter counts where there are 0 read count across all samples.
countData = countData[rowSums(countData) != 0, ]
head(countData)
```

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

Running DESeq2

```
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, contrast=c("condition", "hoxa1_kd", "control_sirna"))
```

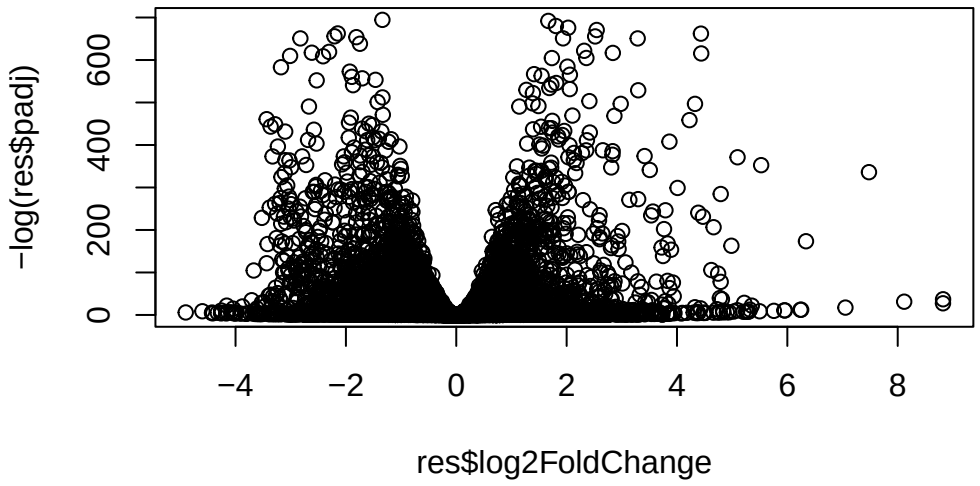
Q. Call the summary() function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```
summary(res)
```

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

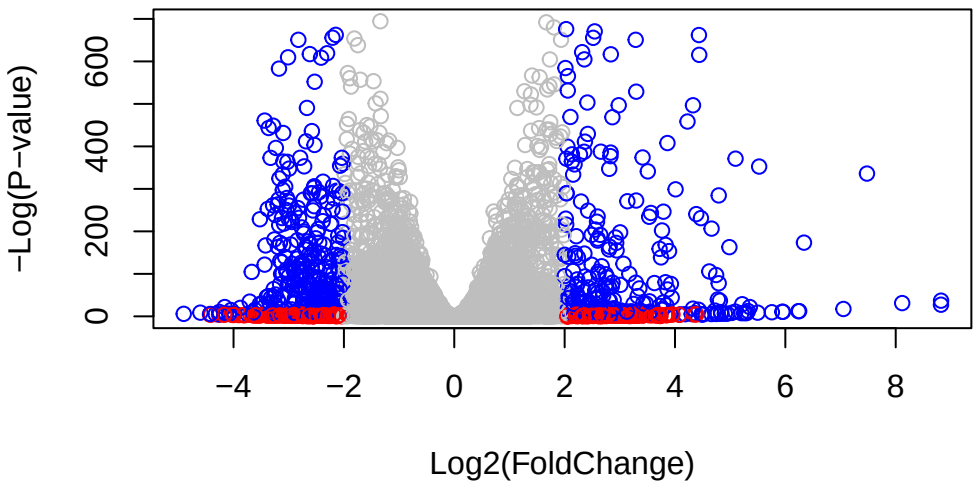
Volcano Plot:

```
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 <- (abs(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

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"
```

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

Section 2: Pathway Analysis

> 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")
```

KEGG Pathways

Run `BiocManager::install( c("pathview", "gage", "gageData"))` in R console: Load Pathway, Gage, and 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)
```

```
[1] -2.422719  3.201955 -2.313738 -2.059631 -1.888019 -1.649792
```

Run Gage Pathway Analysis:

```
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

```
attributes(keggres)
```

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

```
head(keggres$less)
```

```
                                        p.geomean stat.mean p.val q.val
hsa00232 Caffeine metabolism                   NA       NaN    NA    NA
hsa00983 Drug metabolism - other enzymes       NA       NaN    NA    NA
hsa00230 Purine metabolism                     NA       NaN    NA    NA
hsa04514 Cell adhesion molecules (CAMs)        NA       NaN    NA    NA
hsa04010 MAPK signaling pathway                NA       NaN    NA    NA
hsa04012 ErbB signaling pathway                NA       NaN    NA    NA
                                        set.size exp1
hsa00232 Caffeine metabolism                   0   NA
hsa00983 Drug metabolism - other enzymes       0   NA
hsa00230 Purine metabolism                     0   NA
hsa04514 Cell adhesion molecules (CAMs)        0   NA
hsa04010 MAPK signaling pathway                0   NA
hsa04012 ErbB signaling pathway                0   NA
```

View Pathway:

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

```
Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.
```

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

```
Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14
```

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

Change display:

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

```
Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.
```

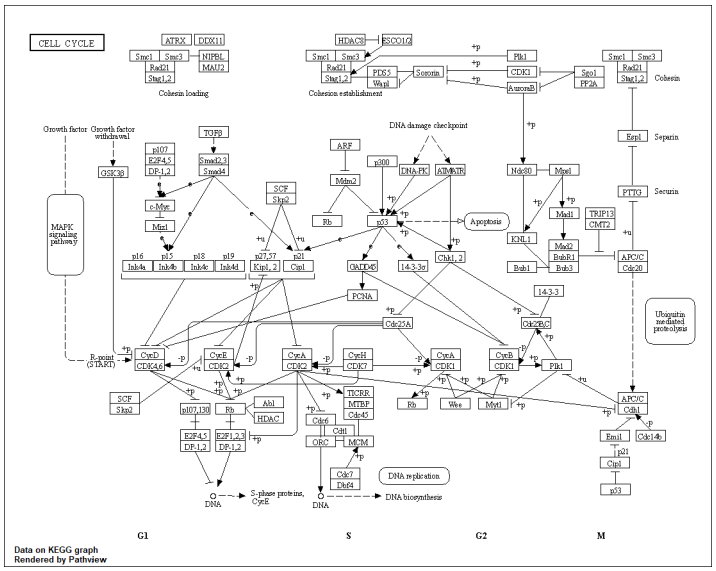
```
'select()' returned 1:1 mapping between keys and columns
```

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

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

```
Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14
```

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




```
# Examine top 5 upregulated pathways:
keggrespathways <- rownames(keggres$greater)[1:5]

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

```
[1] "hsa00232" "hsa00983" "hsa00230" "hsa04514" "hsa04010"
```

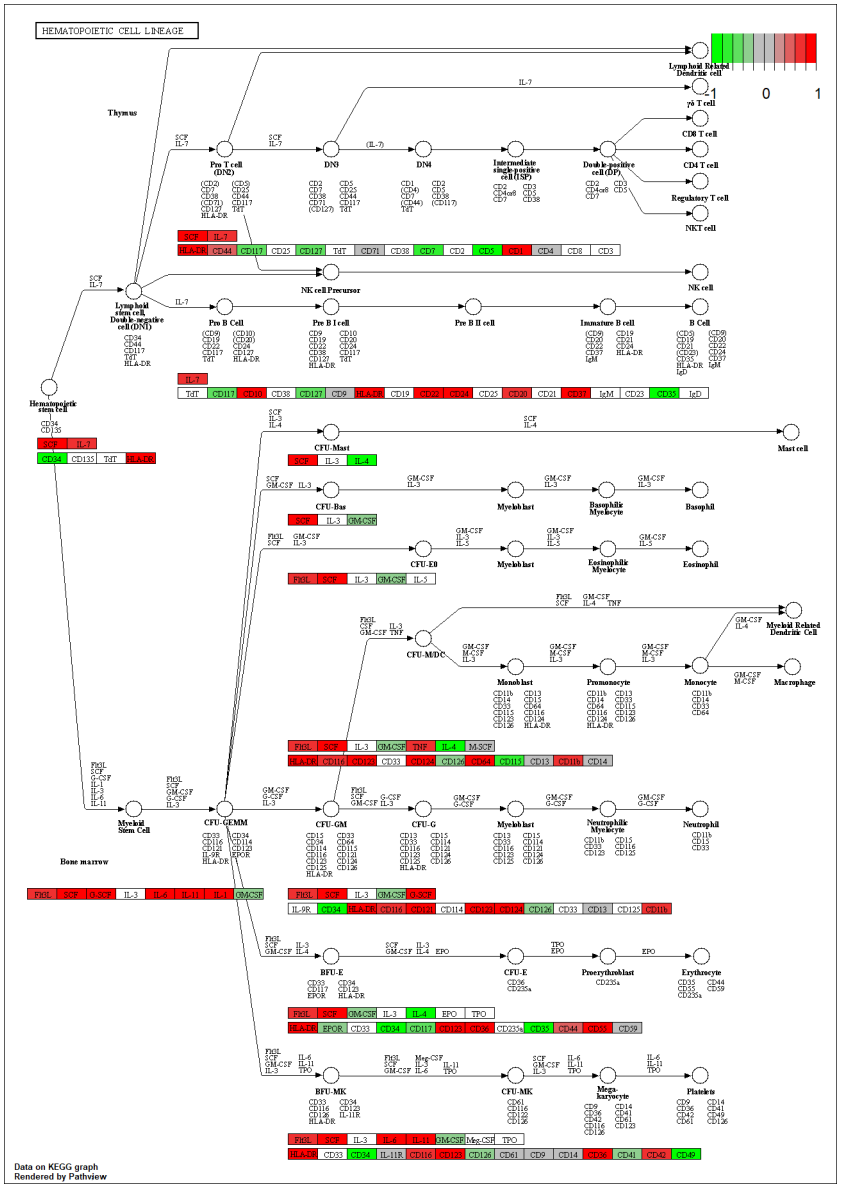
Pathway for top 5 pathways:

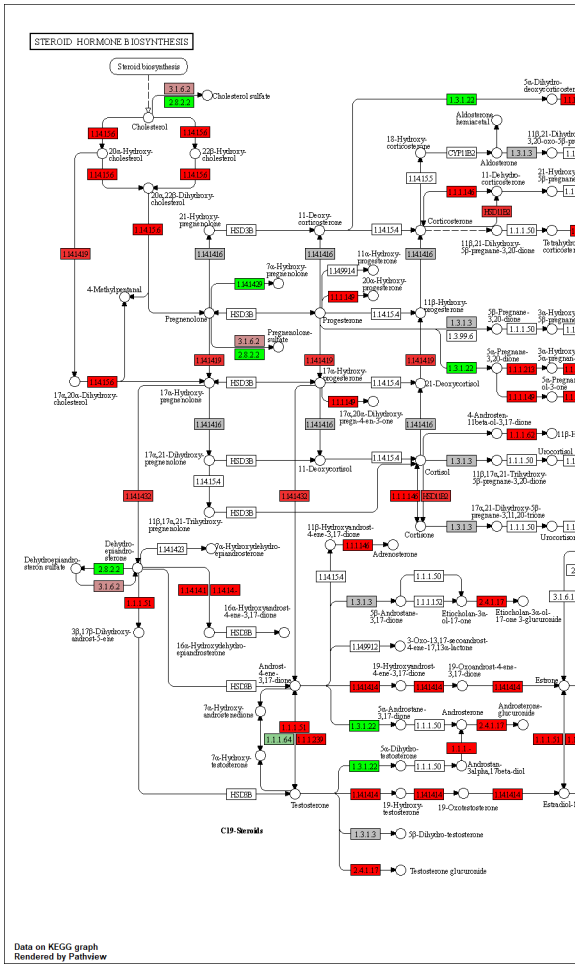
```
pathview(gene.data=foldchanges, pathway.id=keggresids, species="hsa")
```

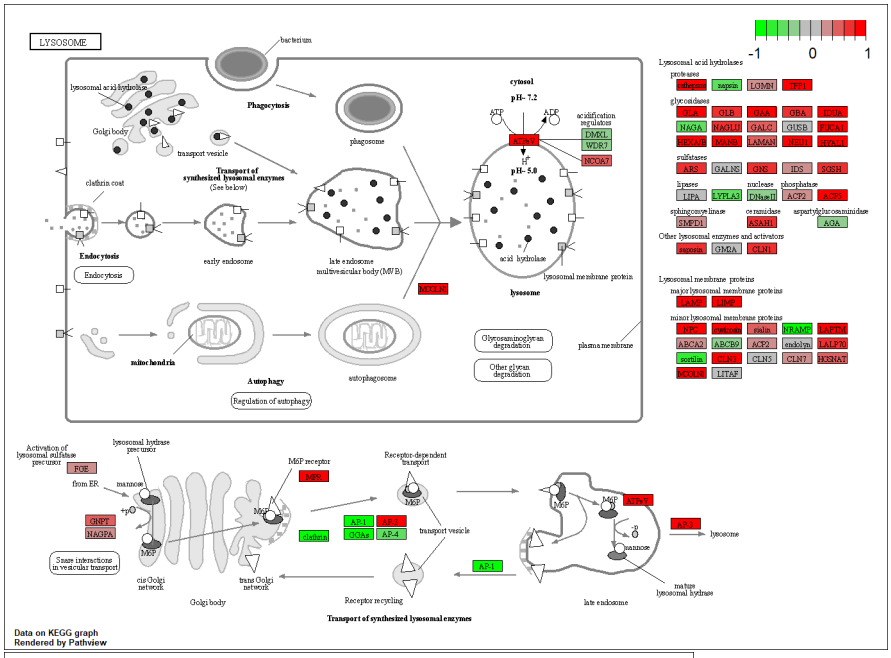
```
Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.
```

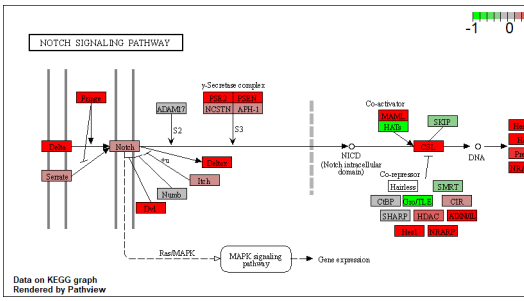
```
'select()' returned 1:1 mapping between keys and columns

Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14

Info: Writing image file hsa00232.pathview.png

Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.

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

Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14

Info: Writing image file hsa00983.pathview.png

Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.

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

Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14

Info: Writing image file hsa00230.pathview.png

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)
```

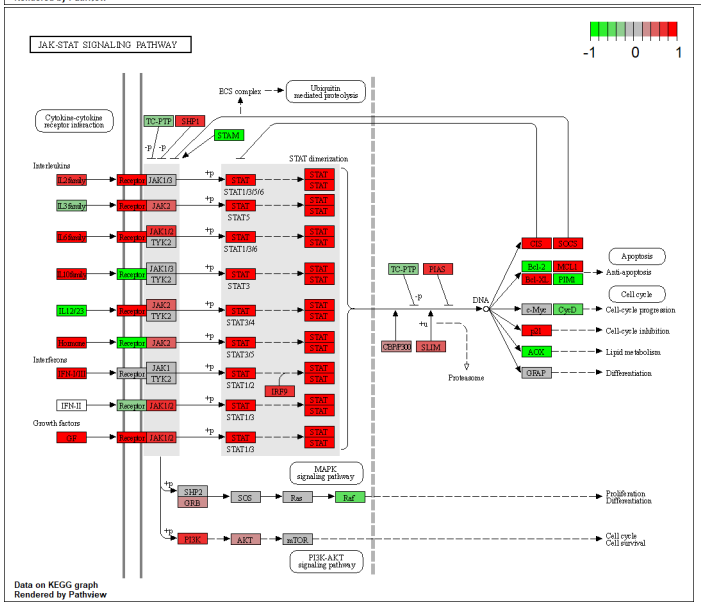
```
Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.

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

Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14

Info: Writing image file hsa04514.pathview.png

Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.

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

Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14

Info: Writing image file hsa04010.pathview.png
```


HEMATOPOIETIC CELL LINEAGE
Data on KEGG graph
Rendered by Pathview


STEROID HORMONE BIOSYNTHESIS
Data on KEGG graph
Rendered by Pathview







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

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

```
Warning: None of the genes or compounds mapped to the pathway!
```

```
Argument gene.idtype or cpd.idtype may be wrong.

'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/zidar/OneDrive/Desktop/BIMM 143/class 14

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?

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

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

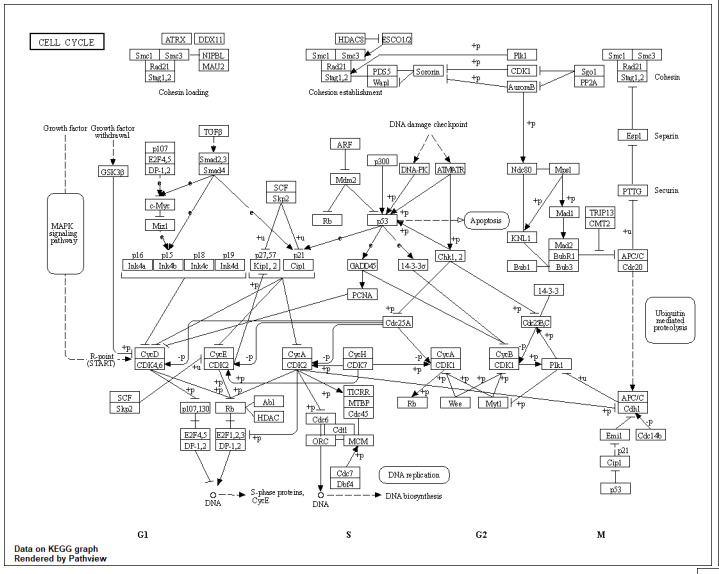
```
[1] "hsa00232" "hsa00983" "hsa00230" "hsa04514" "hsa04010"
```

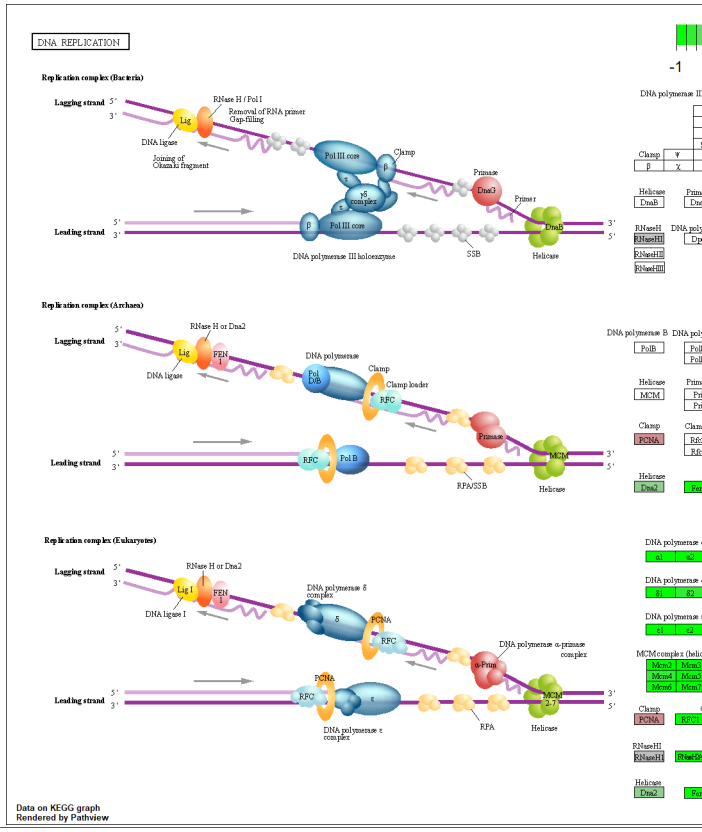
```
pathview(gene.data=foldchanges, pathway.id=keggresids.less, species="hsa")
```

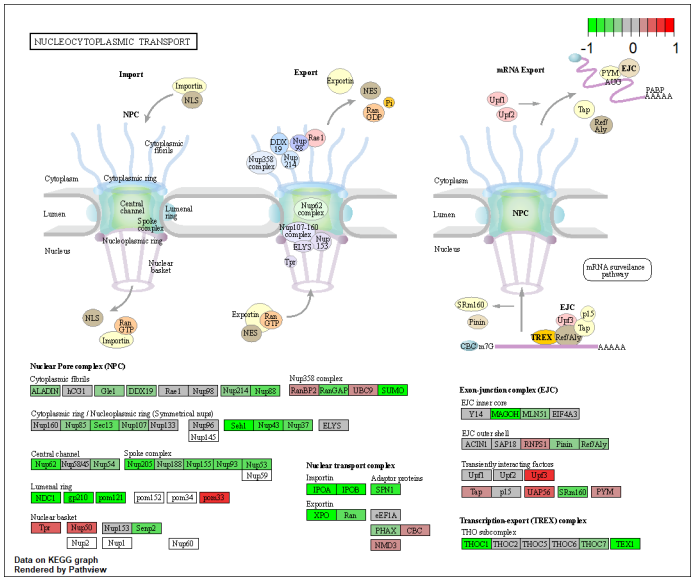
```
Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.

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

Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14

Info: Writing image file hsa00232.pathview.png
```

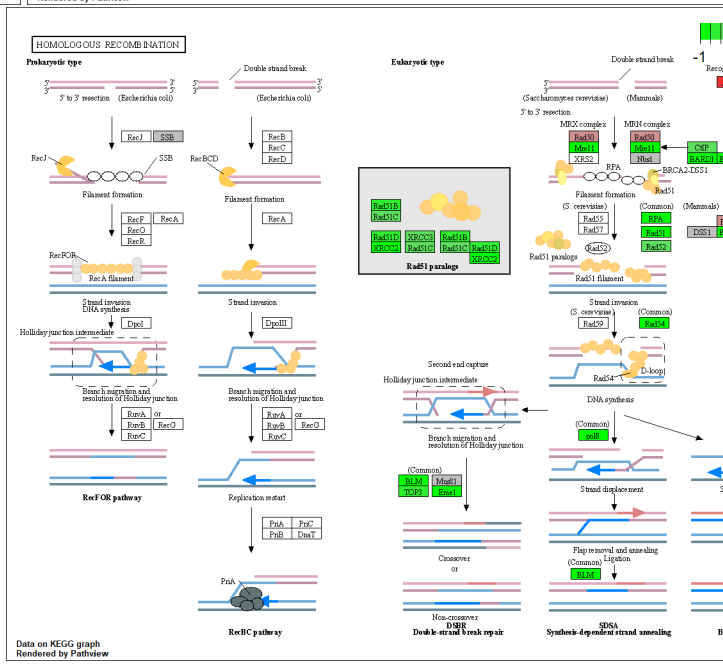
```
Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.

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

Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14

Info: Writing image file hsa00983.pathview.png

Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.

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

Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14

Info: Writing image file hsa00230.pathview.png

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
```

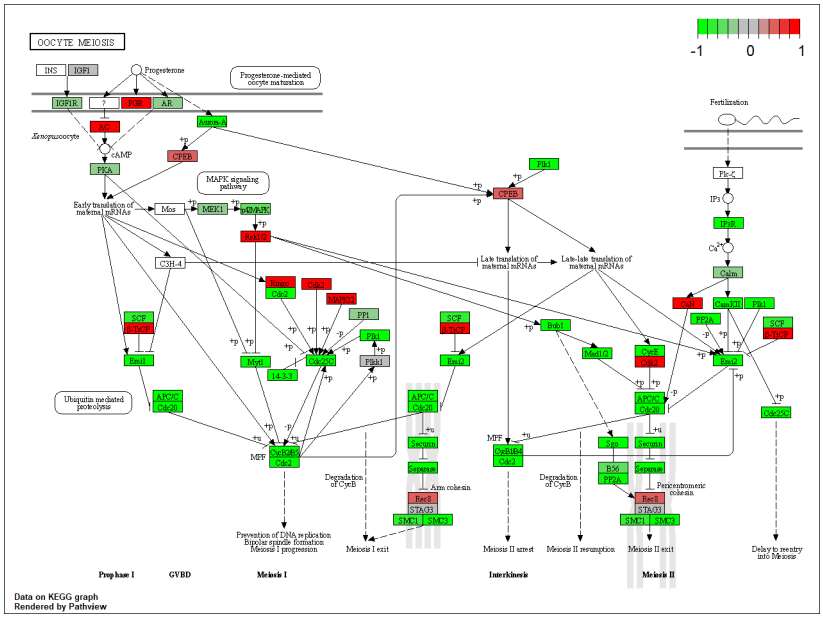
```
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)

Warning in cbind(blk.ind, j): number of rows of result is not a multiple of
vector length (arg 2)


Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.

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

Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14

Info: Writing image file hsa04514.pathview.png

Warning: None of the genes or compounds mapped to the pathway!
Argument gene.idtype or cpd.idtype may be wrong.

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

Info: Working in directory C:/Users/zidar/OneDrive/Desktop/BIMM 143/class 14

Info: Writing image file hsa04010.pathview.png
```


CELL CYCLE
Cohesin loading
Cohesin establishment
Cohesin
Growth factor
Growth factor withdrawal
MAPK signaling pathway
DNA damage checkpoint
Apoptosis
Separin
Securin
Ubiquitin mediated proteolysis
R-point (START)
S-phase proteins, CycE
DNA replication
DNA biosynthesis
G1
S
G2
M
Data on KEGG graph
Rendered by Pathview


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


NUCLEOCYTOPLASMIC TRANSPORT
Import
Export
mRNA Export
NPC
Cytoplasm
Lumen
Nucleus
Nuclear Pore complex (NPC)
Exon-junction complex (EJC)
Nuclear transport complex
Transcription-export (TREX) complex
Data on KEGG graph
Rendered by Pathview


HOMOLOGOUS RECOMBINATION
Prokaryotic type
Eukaryotic type
RecFOR pathway
RecBC pathway
Rad51 paralogs
DSBR
Double-strand break repair
SDSA
Synthesis-dependent strand annealing
Data on KEGG graph
Rendered by Pathview



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 q.val
GO:0000002 mitochondrial genome maintenance             NA       NaN    NA    NA
GO:0000003 reproduction                                 NA       NaN    NA    NA
GO:0000012 single strand break repair                   NA       NaN    NA    NA
GO:0000018 regulation of DNA recombination              NA       NaN    NA    NA
GO:0000019 regulation of mitotic recombination          NA       NaN    NA    NA
GO:0000022 mitotic spindle elongation                   NA       NaN    NA    NA
                                                 set.size exp1
GO:0000002 mitochondrial genome maintenance             0   NA
GO:0000003 reproduction                                 0   NA
```

```
GO:0000012 single strand break repair                0   NA
GO:0000018 regulation of DNA recombination           0   NA
GO:0000019 regulation of mitotic recombination       0   NA
GO:0000022 mitotic spindle elongation                0   NA

$less
                                          p.geomean stat.mean p.val q.val
GO:0000002 mitochondrial genome maintenance          NA       NaN    NA    NA
GO:0000003 reproduction                              NA       NaN    NA    NA
GO:0000012 single strand break repair                NA       NaN    NA    NA
GO:0000018 regulation of DNA recombination           NA       NaN    NA    NA
GO:0000019 regulation of mitotic recombination       NA       NaN    NA    NA
GO:0000022 mitotic spindle elongation                NA       NaN    NA    NA
                                          set.size exp1
GO:0000002 mitochondrial genome maintenance          0   NA
GO:0000003 reproduction                              0   NA
GO:0000012 single strand break repair                0   NA
GO:0000018 regulation of DNA recombination           0   NA
GO:0000019 regulation of mitotic recombination       0   NA
GO:0000022 mitotic spindle elongation                0   NA

$stats
                                          stat.mean exp1
GO:0000002 mitochondrial genome maintenance         NaN   NA
GO:0000003 reproduction                             NaN   NA
GO:0000012 single strand break repair               NaN   NA
GO:0000018 regulation of DNA recombination          NaN   NA
GO:0000019 regulation of mitotic recombination      NaN   NA
GO:0000022 mitotic spindle elongation               NaN   NA
```

Section 4. Reactome Analysis

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

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

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?

The Cell Cycle Mitotic. No, the pathways listed matched are different. Reactome consists of molecules and pathways within those molecules. KEGG is a different database that consists of information about gene/location interaction within pathways.