LAB_13

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Table of contents

Section 1. Differential Expression Analysis

library

```
library(DESeq2)
```

```
Loading required package: S4Vectors

Loading required package: stats4

Loading required package: BiocGenerics

Attaching package: 'BiocGenerics'

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

    IQR, mad, sd, var, xtabs

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

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

Attaching package: 'S4Vectors'

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

    expand.grid, I, unname

Loading required package: IRanges

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 4.2.2

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
```

load files

```
metaFile <- "data/GSE37704_metadata.csv"
countFile <- "data/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
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.

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

```
sum(rowSums(countData)==0)
```

```
[1] 0
```

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
```

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

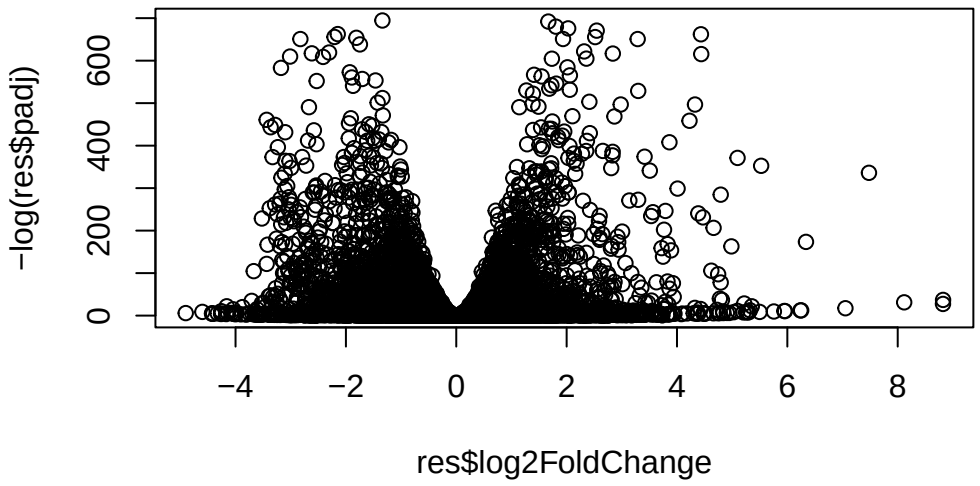
```
#summary(res)
```

Volcono plot

```
#Codes for Enhanced Volcano Plot

#library(EnhancedVolcano)
#x <- as.data.frame(res)
#
#EnhancedVolcano(x,
```

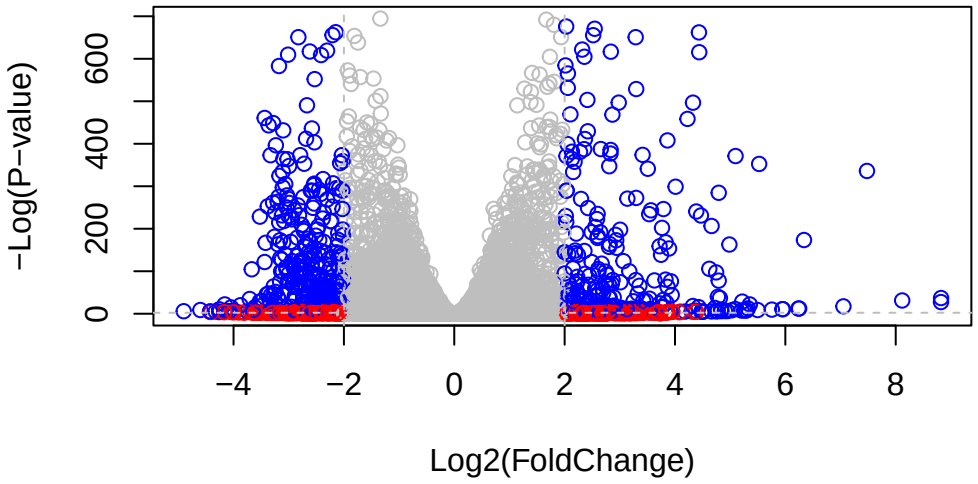
```
#    lab = x$symbol,
#    x = 'log2FoldChange',
#    y = 'pvalue')
```

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




```
# 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(

#cut-off lines
abline(v=c(-2,2), col="gray", lty=2)
```

```r
abline(h=-log(0.1), col="gray", lty=2)
```



Adding gene annotation

```r
# first install the library in console
# BiocManager::install(c("AnnotationDbi", "org.Hs.eg.db"))
library("AnnotationDbi")
library("org.Hs.eg.db")
```

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

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

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

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

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

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

```
  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 9 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      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..
```

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")
```

Section 2. Pathway Analysis

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

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

load packages

```
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"
```

The main gage() function requires a named vector of fold changes, where the names of the values are the Entrez gene IDs.

Note that we used the mapIDs() function above to obtain Entrez gene IDs (stored in $res entrez) and we have the fold change results from DESeq2 analysis (stored in res log2FoldChange).

```
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
```

run the gage pathway analysis

```
# 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
```

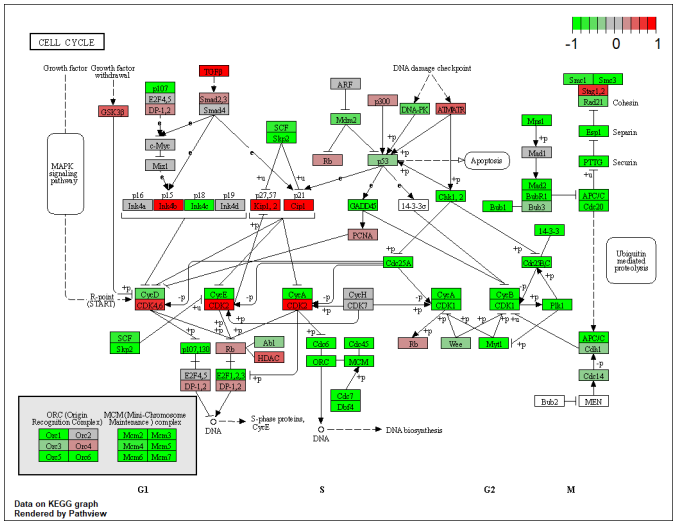
```
hsa03013 RNA transport                 1.375901e-03 -3.028500 1.375901e-03
hsa03440 Homologous recombination      3.066756e-03 -2.852899 3.066756e-03
hsa04114 Oocyte meiosis                3.784520e-03 -2.698128 3.784520e-03
hsa00010 Glycolysis / Gluconeogenesis  8.961413e-03 -2.405398 8.961413e-03
                                             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 C:/Users/zhang/Desktop/BIMM 143 - Bioinformatics Lab/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
```

```
Info: Working in directory C:/Users/zhang/Desktop/BIMM 143 - Bioinformatics Lab/Class_13
```

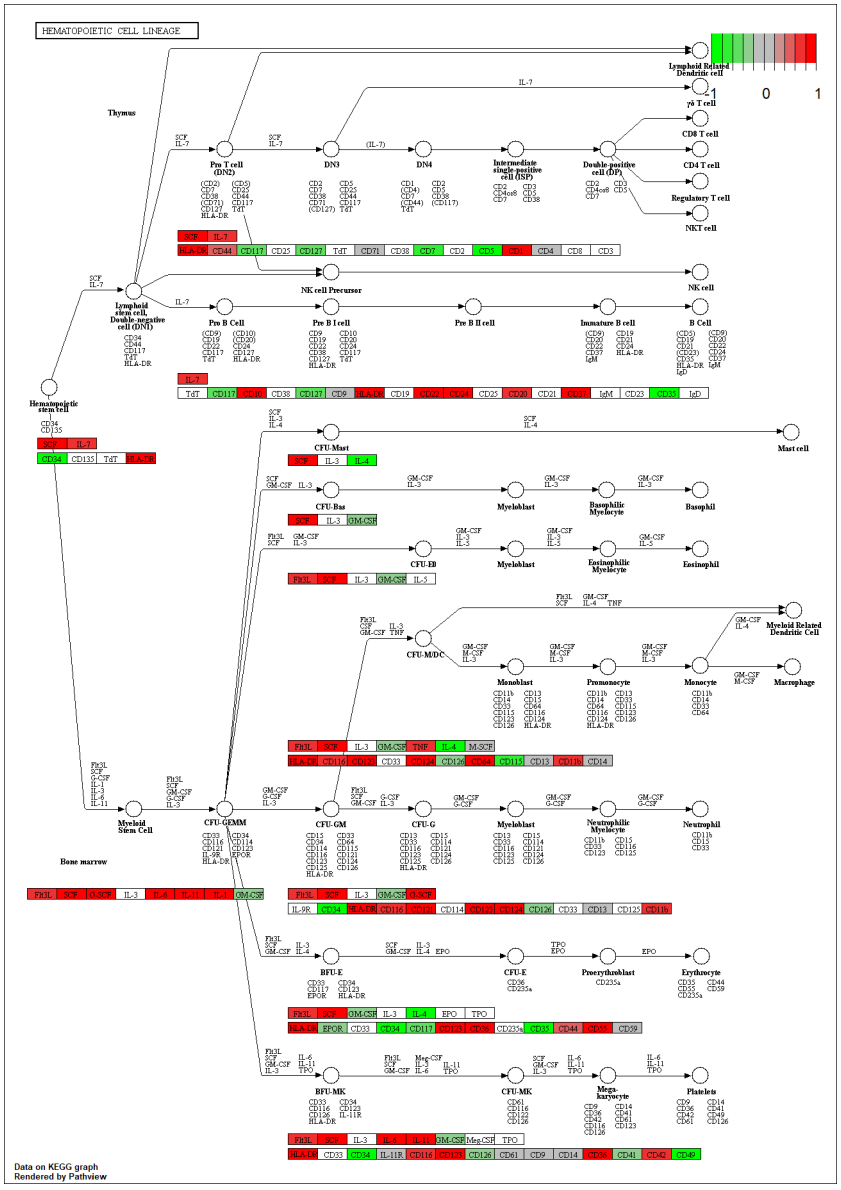
```
Info: Writing image file hsa04110.pathview.pdf
```

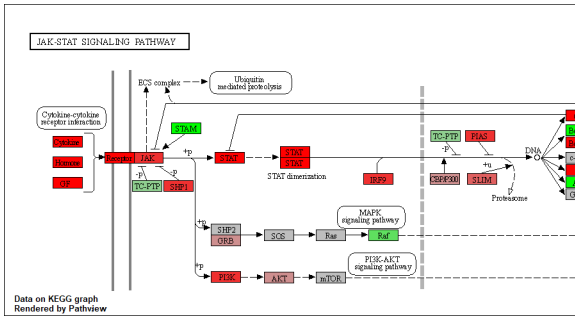
see files

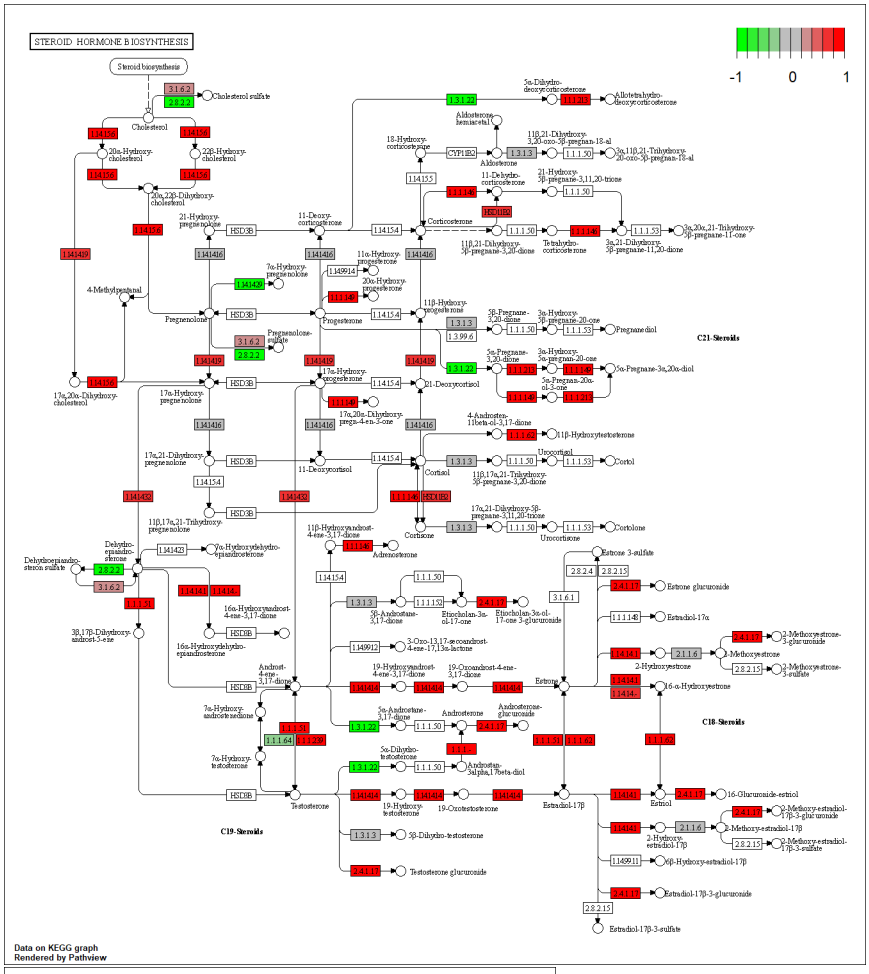
```
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$greater)[1:5]

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

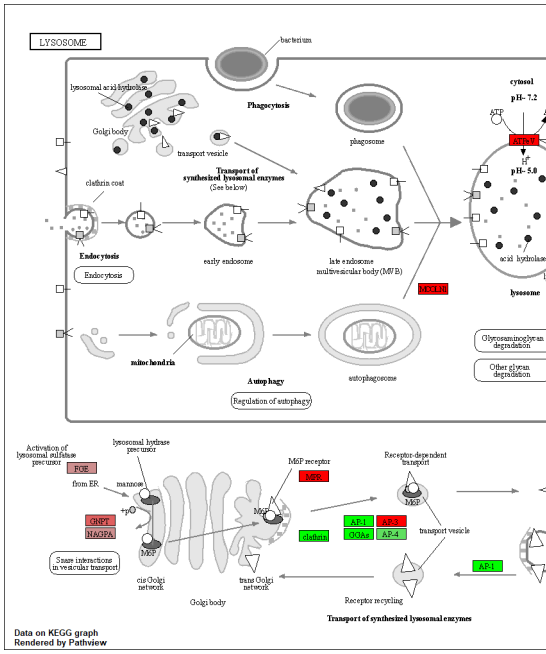
```
[1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```

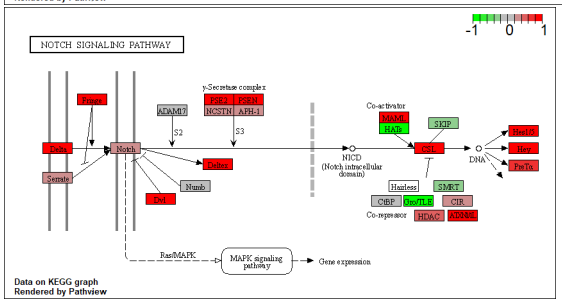
```
## KEGG pathways

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


HEMATOPOIETIC CELL LINEAGE
Lymphoid Related Dendritic cell
Thymus
γδ T cell
CD8 T cell
CD4 T cell
Regulatory T cell
NKT cell
Pro T cell (DN2)
DN3
DN4
Intermediate single positive cell (ISP)
Double-positive cell (DP)
NK cell Precursor
NK cell
Lymphoid stem cell, Double-negative cell (DN1)
Pre B Cell
Pre B I cell
Pre B II cell
Immature B cell
B Cell
Hematopoietic stem cell
CFU-Mast
Mast cell
CFU-Bas
Myeloblast
Basophilic Myelocyte
Basophil
CFU-Eo
Eosinophilic Myelocyte
Eosinophil
CFU-M/DC
Monoblast
Promonocyte
Monocyte
Myeloid Related Dendritic Cell
Macrophage
Myeloid Stem Cell
CFU-GEMM
CFU-GM
CFU-G
Neutrophil
Bone marrow
BFU-E
CFU-E
Proerythroblast
Erythrocyte
BFU-MK
CFU-MK
Mega-karyocyte
Platelets
Data on KEGG graph
Rendered by Pathview


JAK-STAT SIGNALING PATHWAY
Ubiquitin mediated proteolysis
Cytokine-cytokine receptor interaction
Cytokine
Receptor
JAK
STAT
STAT dimerization
Data on KEGG graph
Rendered by Pathview


STEROID HORMONE BIOSYNTHESIS
-1 0 1
Steroid biosynthesis
Cholesterol
Pregnenolone
Progesterone
Corticosterone
Cortisol
Cortisone
Aldosterone
11-Deoxycorticosterone
11-Deoxycortisol
Androstenedione
Testosterone
Estradiol-17β
Estrone
Dehydroepiandrosterone
Androsterone
Pregnanediol
Pregnanetriol
Tetrahydrocorticosterone
Tetrahydrocortisol
Cortolone
Estrone 3-sulfate
Dehydroepiandrosterone sulfate
Androsterone glucuronide
Testosterone glucuronide
Estradiol-17β-3-glucuronide
Estradiol-17β-3-sulfate
C21-Steroids
C19-Steroids
C18-Steroids
HSD3B
HSD17B
CYP11B2
CYP11B1
CYP17A1
SRD5A1
SRD5A2
AKR1C4
HSD11B1
HSD11B2
Data on KEGG graph
Rendered by Pathview


LYSOSOME
bacterium
lysosomal acid hydrolase
Golgi body
transport vesicle
Phagocytosis
phagosome
cytosol
pH- 7.2
ATP
ATP
pH- 5.0
acid hydrolase
lysosome
clathrin coat
Transport of synthesized lysosomal enzymes
(See below)
Endocytosis
Endocytosis
early endosome
late endosome
multivesicular body (MVB)
MCOLN
mitochondria
Autophagy
Regulation of autophagy
autophagosome
Glycosaminoglycan degradation
Other glycan degradation
Activation of lysosomal sulfatase precursor
FGE
from ER
lysosomal hydrase precursor
mannose
+H2O
GNPT
NAGPA
M6P receptor
MSR
M6P
Receptor-dependent transport
MPR
AP-1
AP-3
GGAs
AP-4
clathrin
transport vesicle
AP-1
Snare interactions in vesicular transport
cis Golgi network
trans Golgi network
Golgi body
Receptor recycling
Transport of synthesized lysosomal enzymes
Data on KEGG graph
Rendered by Pathview

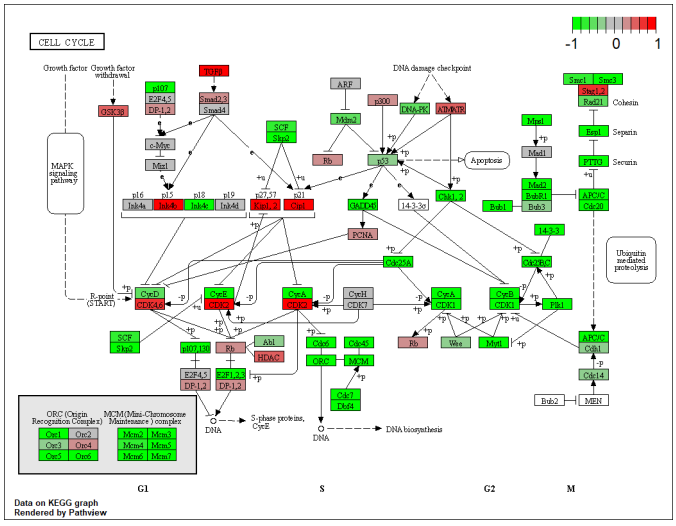

NOTCH SIGNALING PATHWAY
-1 0 1
Fringe
Delta
Serrate
Notch
ADAM17
γ-Secretase complex
PSEN1
PSEN2
NCSTN
APH-1
S2
S3
Deltex
Numb
Fbw7
NICD
(Notch intracellular domain)
Co-activator
MAML
HAT
SKIP
CSL
DNA
Hes/Hey
FrzTa
Hairless
SMRT
CBF
Su(H)
CIR
Co-repressor
HDAC
CtBP/RB
Ras/MAPK
MAPK signaling pathway
Gene expression
Data on KEGG graph
Rendered by Pathview

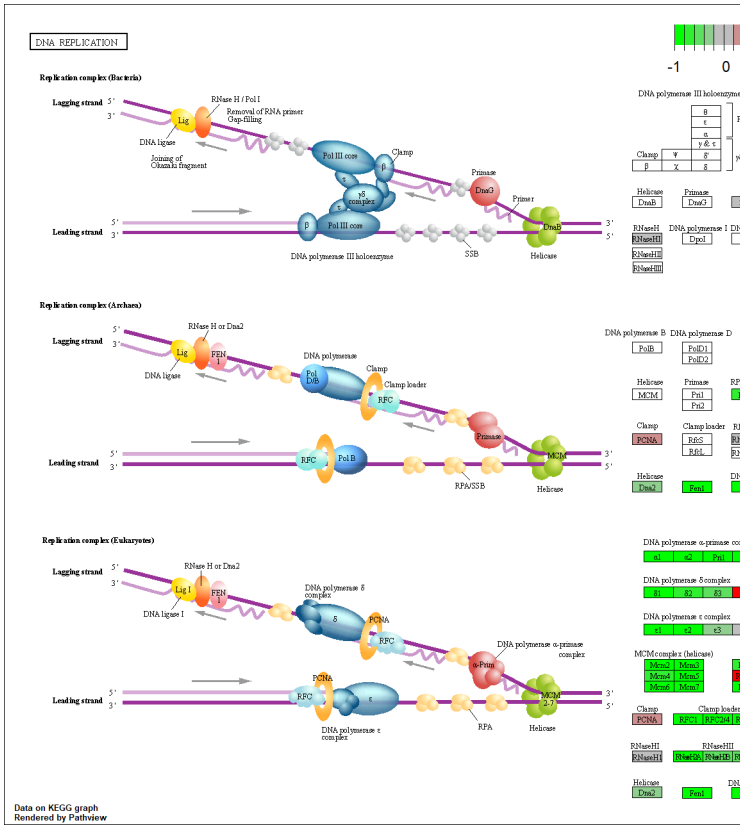
Q. Can you do the same procedure as above to plot the pathview figures for the top 5 down-reguled pathways?

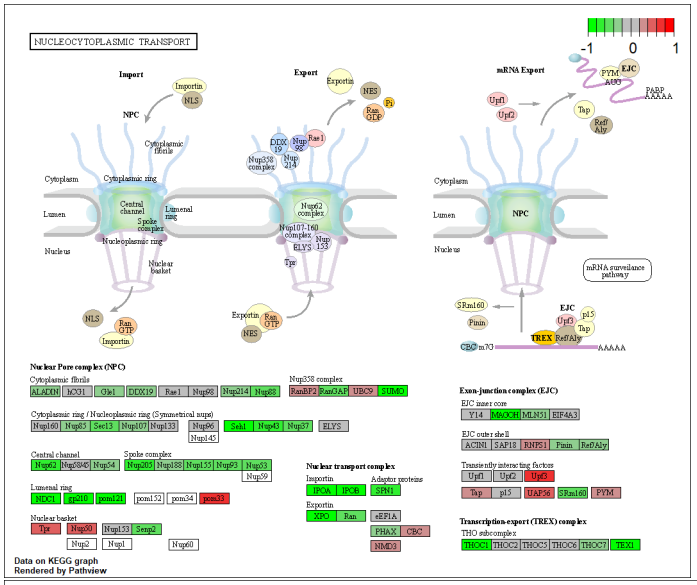
```
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$less)[1:5]

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

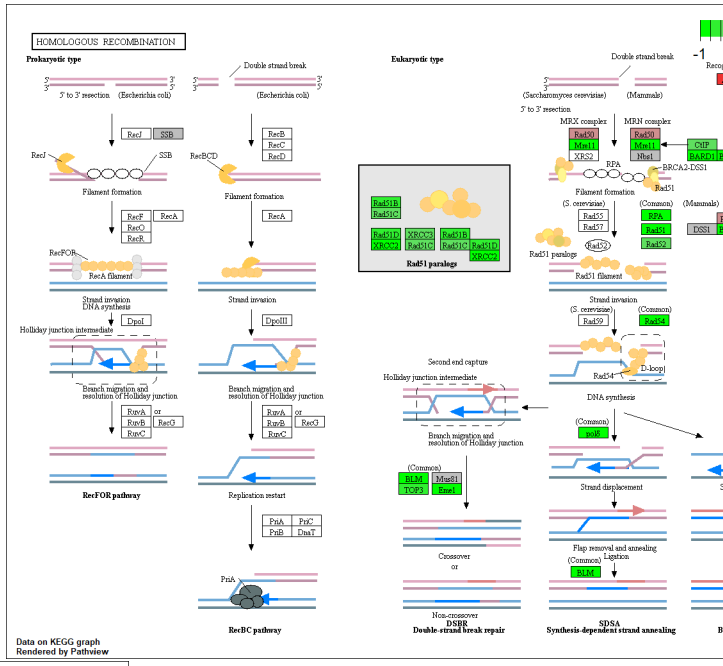
```
[1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

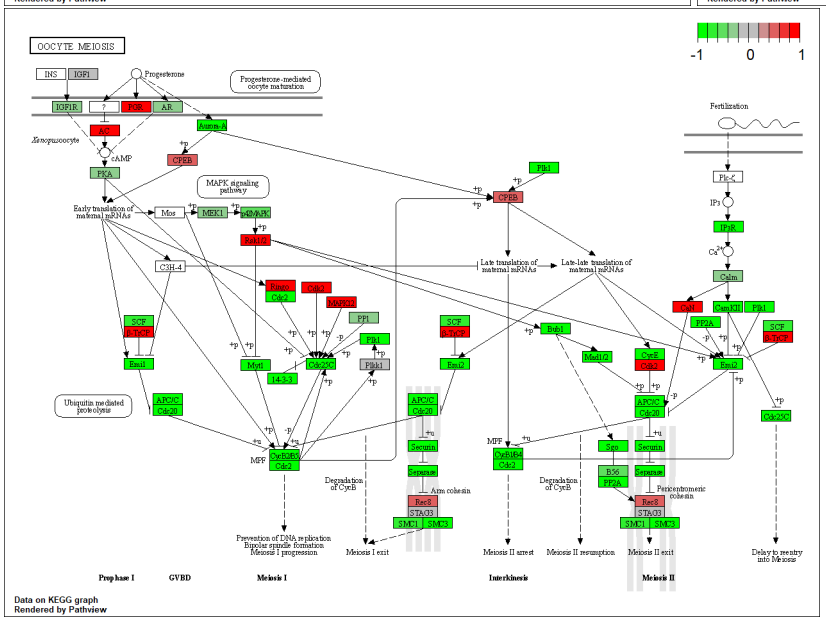
```
## KEGG pathways

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






NUCLEOCYTOPLASMIC TRANSPORT
Import
Export
mRNA Export
NPC
Nuclear Pore complex (NPC)
Nup358 complex
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


OOCYTE MEIOSIS
Progesterone-mediated oocyte maturation
MAPK signaling pathway
Ubiquitin mediated proteolysis
Prophase I
GVBD
Meiosis I
Interkinesis
Meiosis II
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
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
```

Section 4. Reactome Analysis

Reactome is database consisting of biological molecules and their relation to pathways and processes. Reactome, such as many other tools, has an online software available (https://reactome.org/) and R package available (https://bioconductor.org/packages/release/bioc/html/Reacto

First, Using R, output the list of significant genes at the 0.05 level as a plain text file:

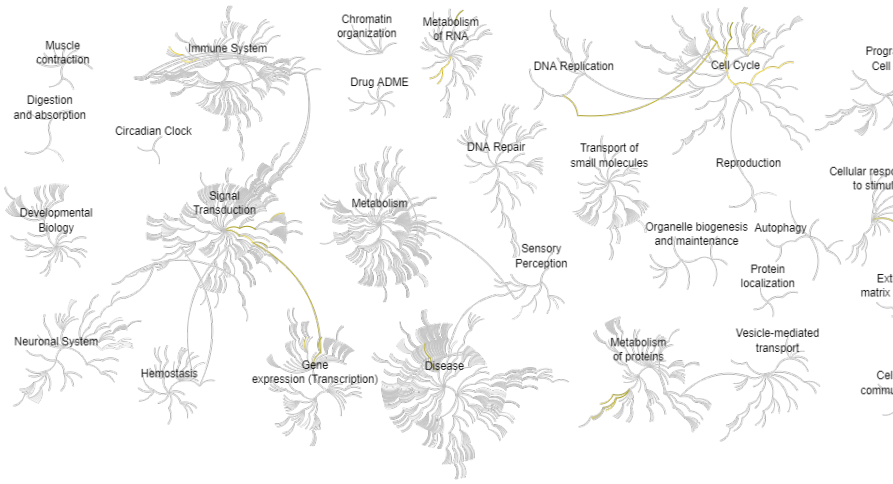
```r
sig_genes <- res[ (res$padj <= 0.05 & !is.na(res$padj) ), "symbol"]

print(paste("Total number of significant genes:", length(sig_genes)))
```

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

"Total number of significant genes: 8147"

```r
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/#T* 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?

Pathway name	Entities found	Entities Total	Entities ratio	Entities pValue	Entities FDR	Reactions found	Reactions total	Reactions ratio
Endosomal/Vacuolar pathway	76	82	0.005	1.67E-4	4.21E-1	4	4	0
Antigen Presentation: Folding, assembly and peptide loading of class I MHC	89	108	0.007	1.81E-3	8.05E-1	15	16	0.001
Cell Cycle, Mitotic	409	596	0.039	1.83E-3	8.05E-1	352	352	0.025
Cell Cycle	495	734	0.048	2.29E-3	8.05E-1	449	451	0.032
Mitotic Spindle Checkpoint	89	111	0.007	3.74E-3	8.05E-1	7	7	0

The *Endosomal/Vacuolar pathway* has the most significant entities p-value. The other method returned the *homophilic cell adhesion pathway* as the most significant. Both KEGG and Reactome covers same number of genes (example for human ~7000). KEGG is a collection of biological information compiled from published material a curated database, where as REACTOME is a open source and open access pathway database (*credit to: https://reactome.org/docs/training/Pathways_&_Networks_Overview.pdf*, Nov. 8, 2022).

Section 5. GO online (OPTIONAL)

Gene Set Gene Ontology (GO) Enrichment is a method to determine over-represented or under-represented GO terms for a given set of genes. GO terms are formal structured controlled vocabularies (ontologies) for gene products in terms of their biological function. The goal of this analysis is to determine the biological process the given set of genes are associated with.

To perform Gene Set GO Enrichment online go to the website http://www.geneontology.org/page/go-enrichment-analysis. Paste your significant gene list from section 4. Then, select "biological process" and "homo sapiens", and click submit.

##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?

Session Information

The sessionInfo() prints version information about R and any attached packages. It's a good practice to always run this command at the end of your R session and record it for the sake of reproducibility in the future.

```
sessionInfo()
```

```
R version 4.2.1 (2022-06-23 ucrt)
Platform: x86_64-w64-mingw32/x64 (64-bit)
Running under: Windows 10 x64 (build 22000)

Matrix products: default

locale:
[1] LC_COLLATE=English_United States.utf8
[2] LC_CTYPE=English_United States.utf8
[3] LC_MONETARY=English_United States.utf8
[4] LC_NUMERIC=C
[5] LC_TIME=English_United States.utf8

attached base packages:
[1] stats4    stats     graphics  grDevices utils     datasets  methods
[8] base

other attached packages:
 [1] gageData_2.34.0             gage_2.46.1
 [3] pathview_1.36.1             org.Hs.eg.db_3.15.0
 [5] AnnotationDbi_1.58.0        DESeq2_1.36.0
 [7] SummarizedExperiment_1.26.1 Biobase_2.56.0
 [9] MatrixGenerics_1.8.1        matrixStats_0.62.0
[11] GenomicRanges_1.48.0        GenomeInfoDb_1.32.4
[13] IRanges_2.30.1              S4Vectors_0.34.0
[15] BiocGenerics_0.42.0

loaded via a namespace (and not attached):
 [1] httr_1.4.4             bit64_4.0.5            jsonlite_1.8.3
 [4] splines_4.2.1          assertthat_0.2.1       blob_1.2.3
 [7] GenomeInfoDbData_1.2.8 yaml_2.3.6             pillar_1.8.1
[10] RSQLite_2.2.18         lattice_0.20-45        glue_1.6.2
[13] digest_0.6.30          RColorBrewer_1.1-3     XVector_0.36.0
[16] colorspace_2.0-3       htmltools_0.5.3        Matrix_1.5-1
```

```
[19] XML_3.99-0.12          pkgconfig_2.0.3        genefilter_1.78.0
[22] zlibbioc_1.42.0        GO.db_3.15.0           xtable_1.8-4
[25] scales_1.2.1           BiocParallel_1.30.4    tibble_3.1.8
[28] annotate_1.74.0        KEGGREST_1.36.3        generics_0.1.3
[31] ggplot2_3.3.6          cachem_1.0.6           cli_3.4.1
[34] survival_3.4-0         magrittr_2.0.3         crayon_1.5.2
[37] KEGGgraph_1.56.0       memoise_2.0.1          evaluate_0.17
[40] fansi_1.0.3            graph_1.74.0           tools_4.2.1
[43] lifecycle_1.0.3        stringr_1.4.1          munsell_0.5.0
[46] locfit_1.5-9.6         DelayedArray_0.22.0    Biostrings_2.64.1
[49] compiler_4.2.1         rlang_1.0.6            grid_4.2.1
[52] RCurl_1.98-1.9         rstudioapi_0.14        bitops_1.0-7
[55] rmarkdown_2.17         gtable_0.3.1           codetools_0.2-18
[58] DBI_1.1.3              R6_2.5.1               knitr_1.40
[61] dplyr_1.0.10           fastmap_1.1.0          bit_4.0.4
[64] utf8_1.2.2             Rgraphviz_2.40.0       stringi_1.7.8
[67] parallel_4.2.1         Rcpp_1.0.9             vctrs_0.5.0
[70] geneplotter_1.74.0     png_0.1-7              tidyselect_1.2.0
[73] xfun_0.34
```