Class 14:Pathway Analysis from RNA-Seq Results

A16442048

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 <- "data/GSE37704_metadata.csv"
countFile <- "data/GSE37704_featurecounts.csv"
```

```r
colData <-  read.csv("GSE37704_metadata.csv", 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
countData <-  read.csv("GSE37704_featurecounts.csv", 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
```

```r
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
```

We need to remove the 0 count genes

```r
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
```

DESeq setup and Analysis

```
#/message: false
library(DESeq2)
```

```
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
```

```
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"))
```

```
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
```

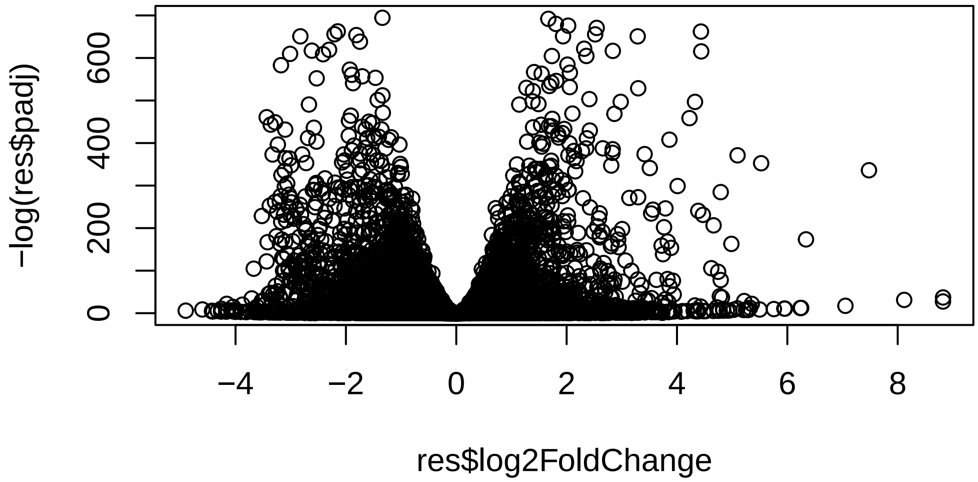
```
res <-  results(dds, contrast=c("condition", "hoxa1_kd", "control_sirna"))
```

```
summary(res)
```

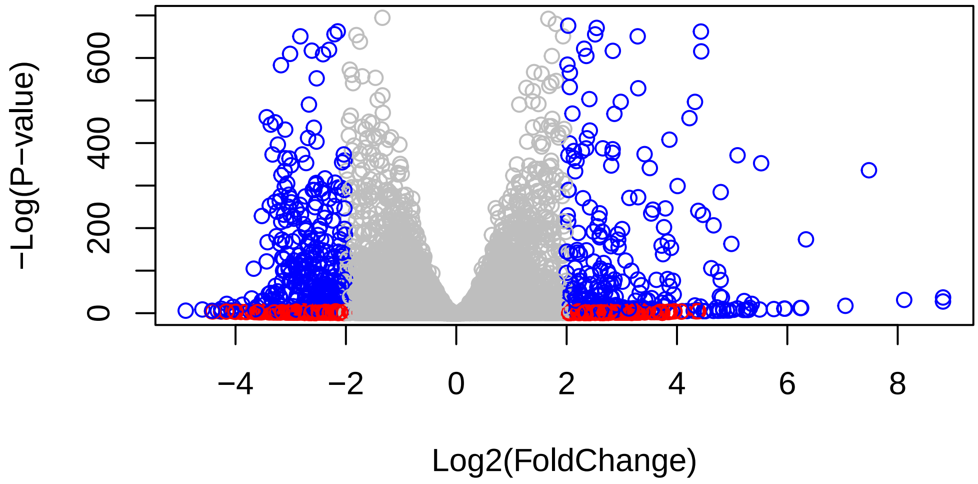
```

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) )
```



```
mycols <- rep("gray", nrow(res) )

mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

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

```
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"     
```

columns(org.Hs.eg.db)

```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 1.43989e-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 0.5215599   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 <-  res[order(res$pvalue),]
write.csv(res, file="deseq_results.csv")
```

KEGG Pathways

```
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)
```

```r
library(gageData)

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

kegg.sets.hs = kegg.sets.hs[sigmet.idx.hs]

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

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

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

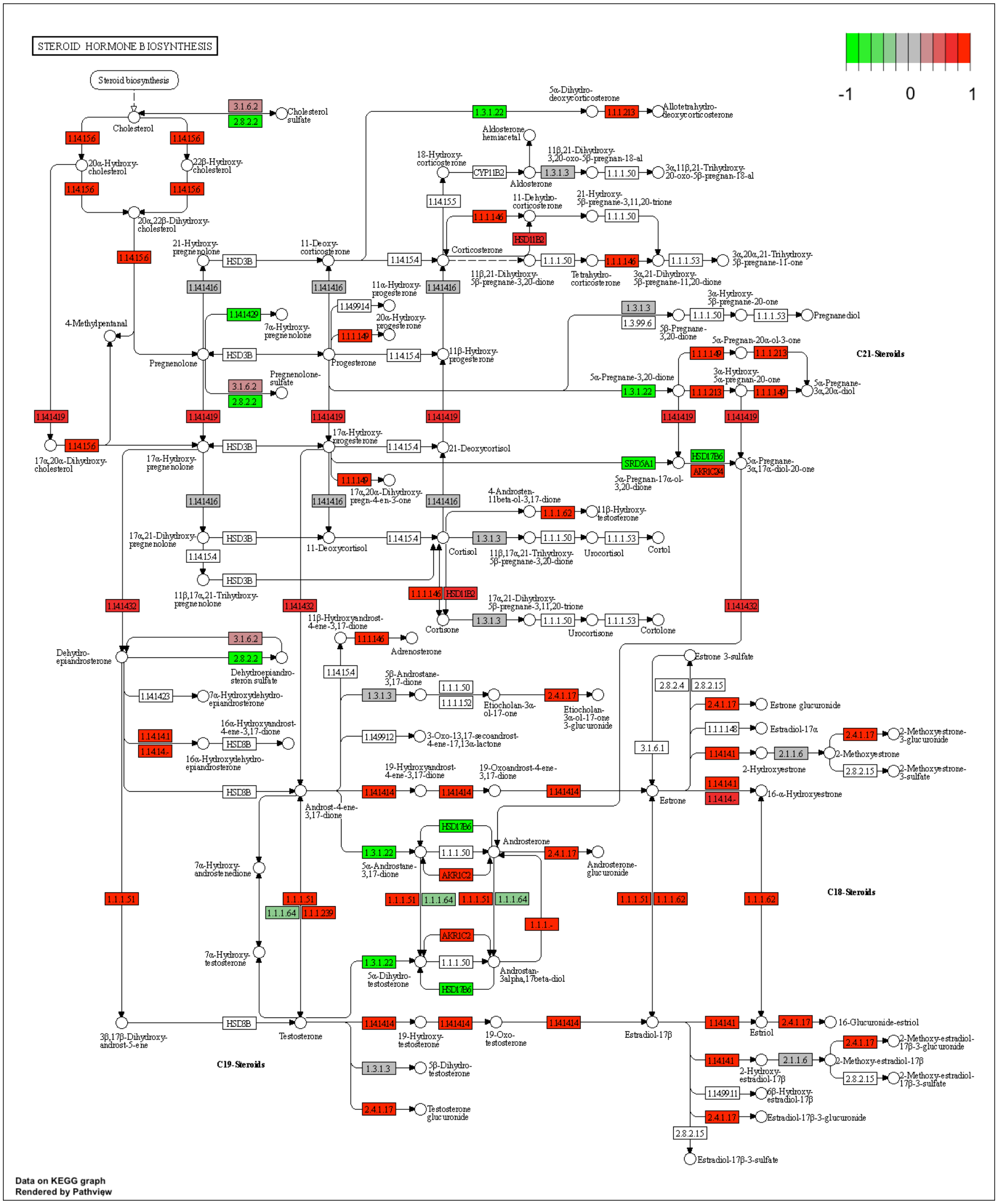
```
                                         p.geomean stat.mean        p.val
hsa04110 Cell cycle                   8.995727e-06 -4.378644 8.995727e-06
hsa03030 DNA replication              9.424076e-05 -3.951803 9.424076e-05
hsa03013 RNA transport                1.375901e-03 -3.028500 1.375901e-03
hsa03440 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 /Users/annmarielacid/Desktop/bimm 143/class14
```

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



```r
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!
```

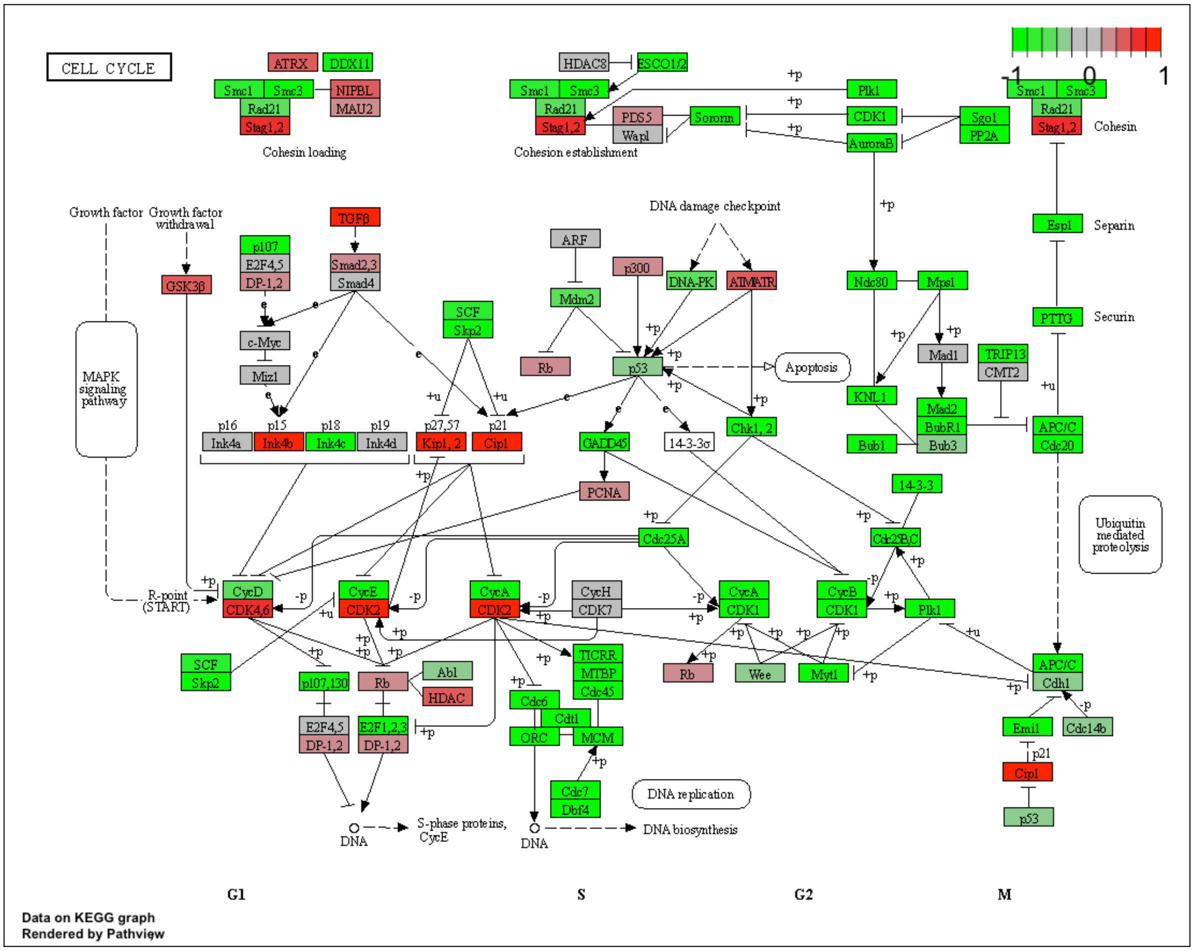
```
     [,1] [,2] 
[1,] "9"  "300"
[2,] "9"  "306"
```

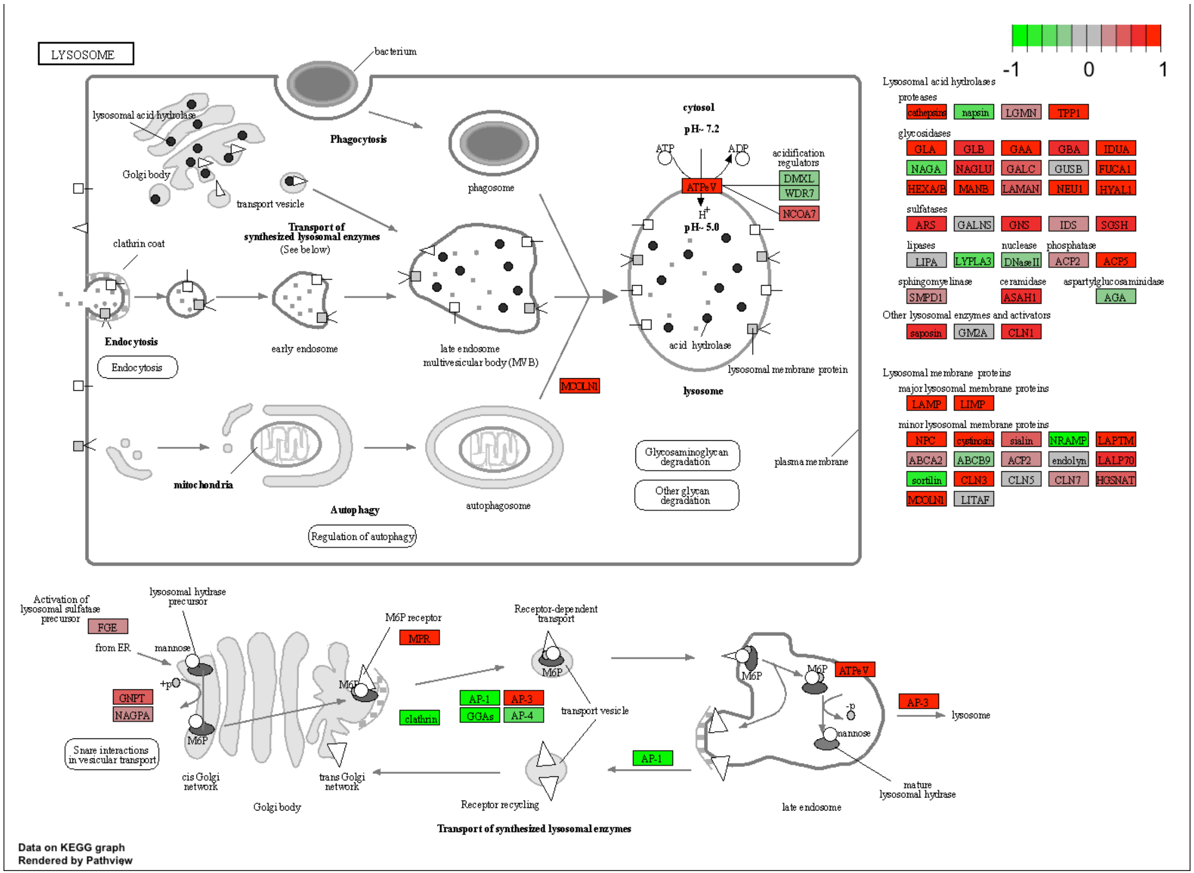
```
Info: Working in directory /Users/annmarielacid/Desktop/bimm 143/class14
```

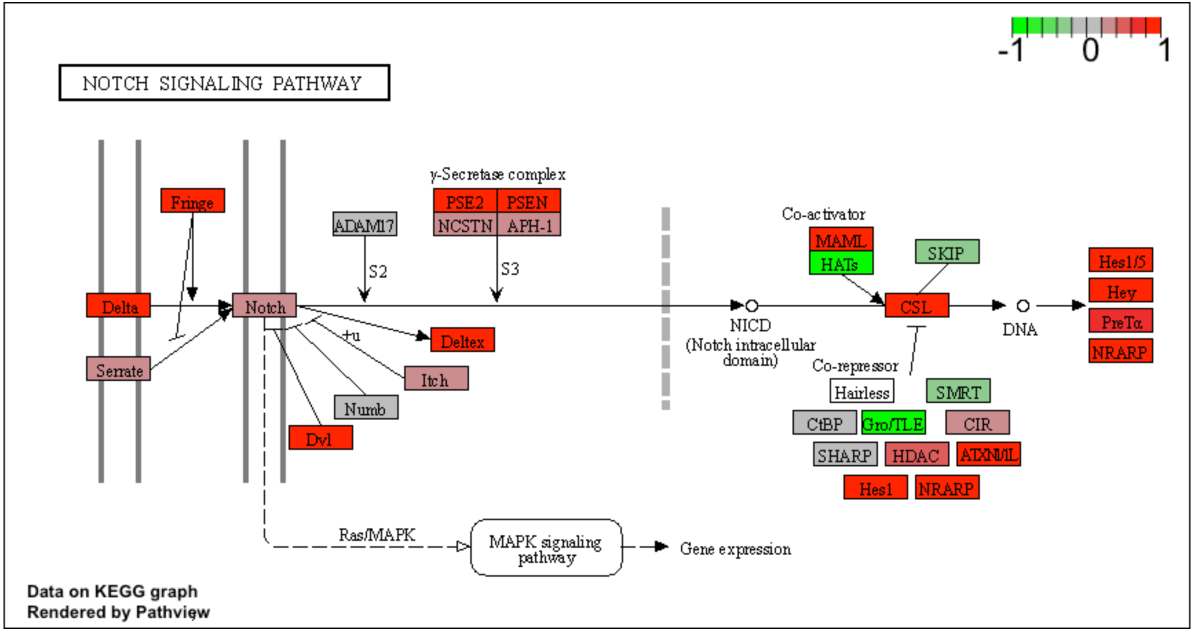
```
Info: Writing image file hsa04110.pathview.pdf
```

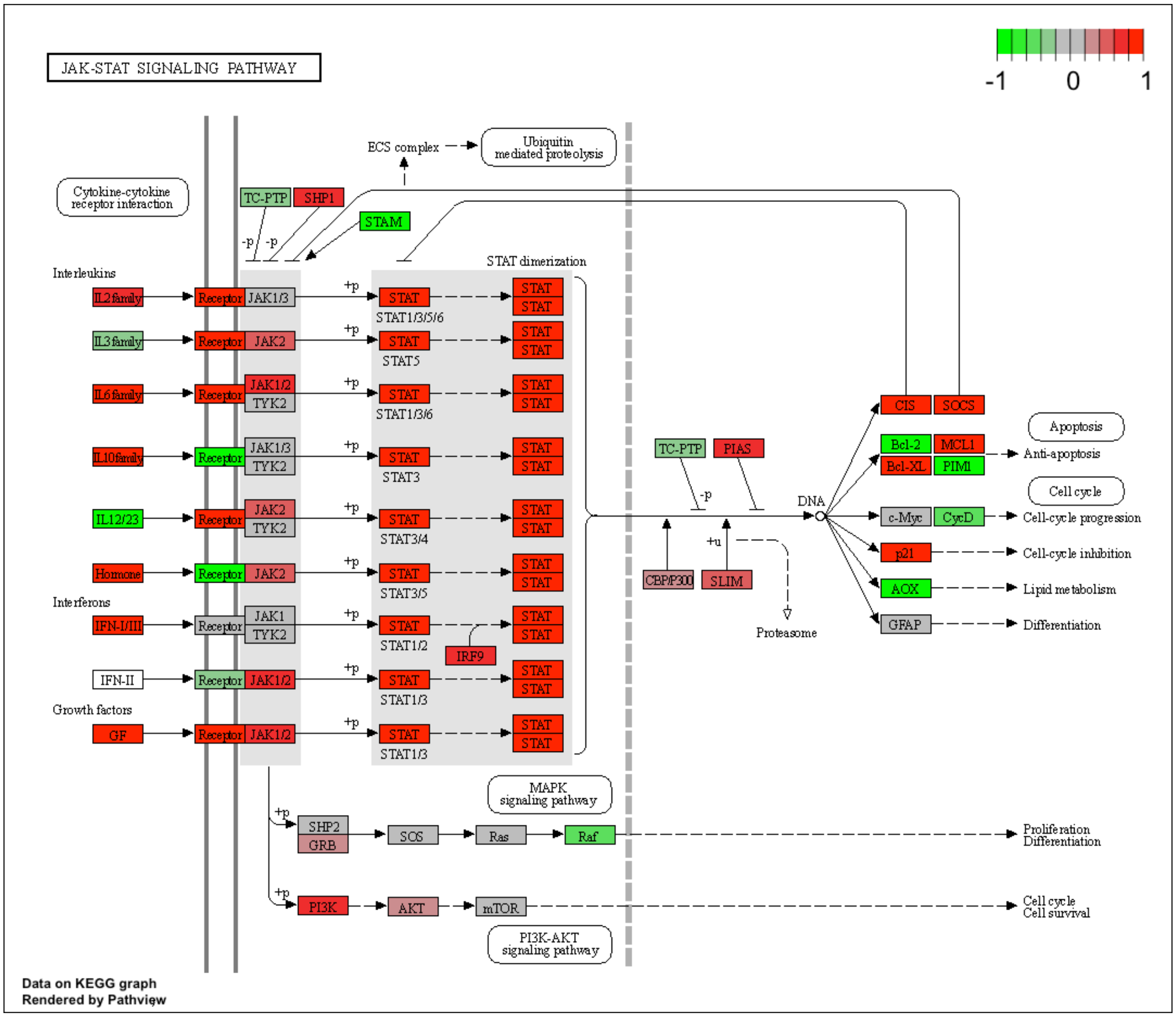
```r
keggrespathways <- rownames(keggres$greater)[1:5]

keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
```

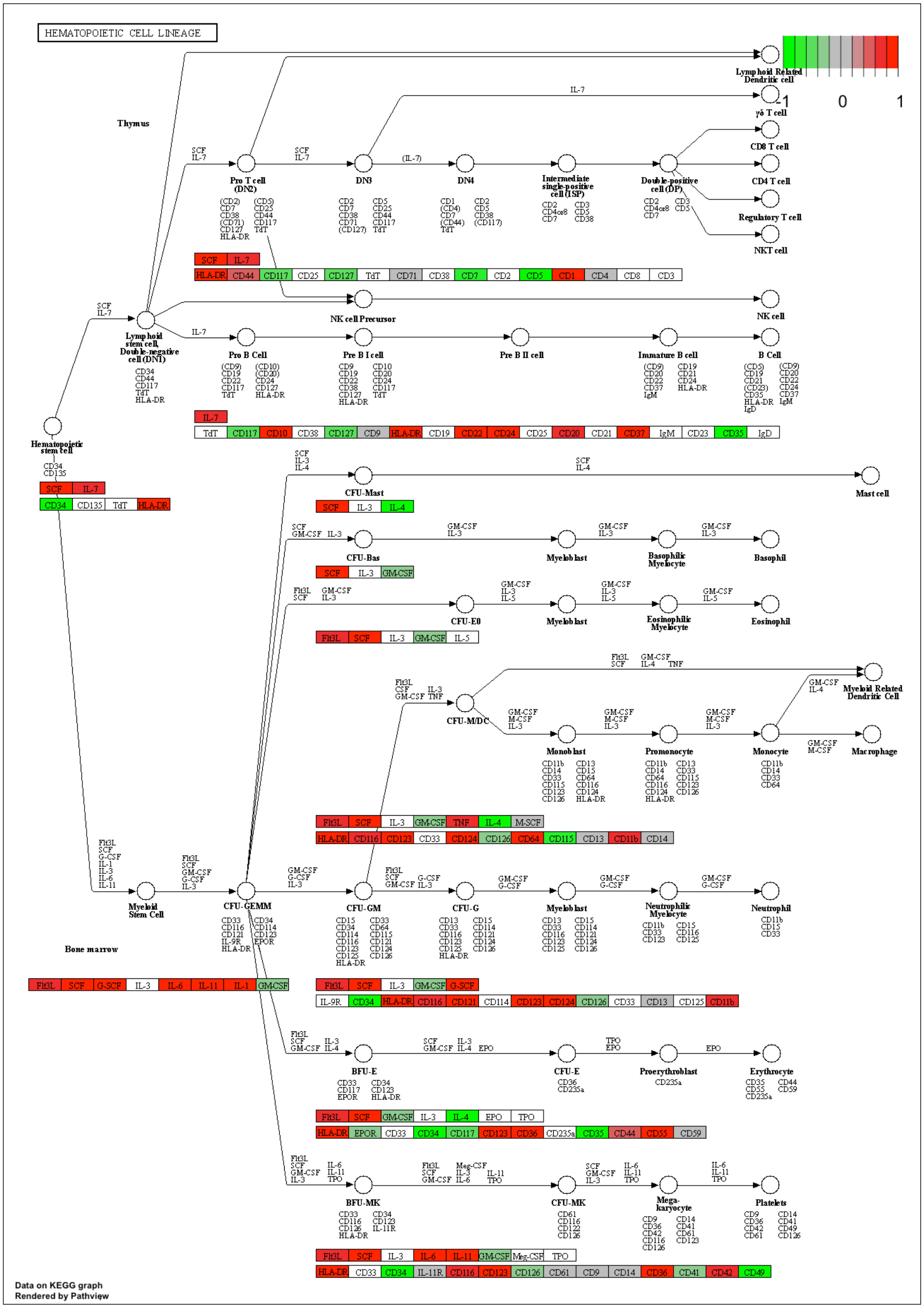
```
[1] "hsa04640" "hsa04630" "hsa00140" "hsa04142" "hsa04330"
```


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


-1
0
1
LYSOSOME
bacterium
lysosomal acid hydrolase
Golgi body
transport vesicle
Phagocytosis
phagosome
cytosol
pH~ 7.2
ATP
ADP
ATPeV
acidification regulators
DMXL
WDR7
NCOA7
H+
pH~ 5.0
clathrin coat
Transport of synthesized lysosomal enzymes
(See below)
Endocytosis
Endocytosis
early endosome
late endosome
multivesicular body (MVB)
acid hydrolase
lysosomal membrane protein
lysosome
MCOLN1
mitochondria
Autophagy
Regulation of autophagy
autophagosome
Glycosaminoglycan degradation
Other glycan degradation
plasma membrane
Lysosomal acid hydrolases
proteases
cathepsins
napsin
LGMN
TPP1
glycosidases
GLA
GLB
GAA
GBA
IDUA
NAGA
NAGLU
GALC
GUSB
FUCA1
HEXA/B
MANB
LAMAN
NEU1
HYAL1
sulfatases
ARS
GALNS
GNS
IDS
SGSH
lipases
LIPA
LYPLA3
nuclease
DNaseII
phosphatase
ACP2
ACP5
sphingomyelinase
SMPD1
ceramidase
ASAH1
aspartylglucosaminidase
AGA
Other lysosomal enzymes and activators
saposin
GM2A
CLN1
Lysosomal membrane proteins
major lysosomal membrane proteins
LAMP
LIMP
minor lysosomal membrane proteins
NPC
cystinosin
sialin
NRAMP
LAPTM
ABCA2
ABCB9
ACP2
endolyn
LALP70
sortilin
CLN3
CLN5
CLN7
HGSNAT
MCOLN1
LITAF
Activation of lysosomal sulfatase precursor
FGE
lysosomal hydrase precursor
from ER
mannose
+p
GNPT
NAGPA
M6P
Snare interactions in vesicular transport
cis Golgi network
M6P
M6P receptor
MPR
clathrin
trans Golgi network
Golgi body
AP-1
AP-3
GGAs
AP-4
Receptor-dependent transport
M6P
transport vesicle
Receptor recycling
AP-1
M6P
M6P
ATPeV
-p
mannose
AP-3
lysosome
mature lysosomal hydrase
late endosome
Transport of synthesized lysosomal enzymes
Data on KEGG graph
Rendered by Pathview


-1
0
1
NOTCH SIGNALING PATHWAY
Fringe
Delta
Serrate
Notch
ADAM17
S2
γ-Secretase complex
PSE2
PSEN
NCSTN
APH-1
S3
Deltex
+u
Itch
Numb
Dvl
NICD
(Notch intracellular domain)
Co-activator
MAML
HATs
SKIP
CSL
Co-repressor
Hairless
SMRT
CtBP
Gro/TLE
CIR
SHARP
HDAC
ATXN1/L
Hes1
NRARP
DNA
Hes1/5
Hey
PreTα
NRARP
Ras/MAPK
MAPK signaling pathway
Gene expression
Data on KEGG graph
Rendered by Pathview


JAK-STAT SIGNALING PATHWAY
-1
0
1
ECS complex
Ubiquitin mediated proteolysis
Cytokine-cytokine receptor interaction
TC-PTP
SHP1
STAM
-p
-p
STAT dimerization
Interleukins
IL2 family
Receptor
JAK1/3
+p
STAT
STAT
STAT
STAT1/3/5/6
IL3 family
Receptor
JAK2
+p
STAT
STAT
STAT
STAT5
IL6 family
Receptor
JAK1/2
TYK2
+p
STAT
STAT
STAT
STAT1/3/6
IL10 family
Receptor
JAK1/3
TYK2
+p
STAT
STAT
STAT
STAT3
IL12/23
Receptor
JAK2
TYK2
+p
STAT
STAT
STAT
STAT3/4
Hormone
Receptor
JAK2
+p
STAT
STAT
STAT
STAT3/5
Interferons
IFN-I/III
Receptor
JAK1
TYK2
+p
STAT
STAT
STAT
STAT1/2
IRF9
IFN-II
Receptor
JAK1/2
+p
STAT
STAT
STAT
STAT1/3
Growth factors
GF
Receptor
JAK1/2
+p
STAT
STAT
STAT
STAT1/3
TC-PTP
PIAS
-p
+u
DNA
CBP/P300
SLIM
Proteasome
CIS
SOCS
Apoptosis
Bcl-2
MCL1
Bcl-XL
PIM1
Anti-apoptosis
Cell cycle
c-Myc
CycD
Cell-cycle progression
p21
Cell-cycle inhibition
AOX
Lipid metabolism
GFAP
Differentiation
MAPK signaling pathway
+p
SHP2
GRB
SOS
Ras
Raf
Proliferation
Differentiation
+p
PI3K
AKT
mTOR
Cell cycle
Cell survival
PI3K-AKT signaling pathway
Data on KEGG graph
Rendered by Pathview



Data on KEGG graph
Rendered by Pathview

Gene Ontology

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

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       0.1952430      113 8.519724e-05
GO:0002009 morphogenesis of an epithelium 0.1952430      339 1.396681e-04
GO:0048729 tissue morphogenesis           0.1952430      424 1.432451e-04
GO:0007610 behavior                       0.1968058      426 1.925222e-04
GO:0060562 epithelial tube morphogenesis  0.3566193      257 5.932837e-04
GO:0035295 tube development               0.3566193      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             5.843127e-12      376 1.536227e-15
GO:0000280 nuclear division              5.843127e-12      352 4.286961e-15
GO:0007067 mitosis                       5.843127e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.195965e-11      362 1.169934e-14
GO:0007059 chromosome segregation        1.659009e-08      142 2.028624e-11
```

```
GO:0000236 mitotic prometaphase          1.178690e-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
```

Reactome Analysis

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

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

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

> 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?

Cell Cycle at 3.06E-4. The cell cycle matches but the others are a little off as reactome lists the rest of the significant pathways as parts of mitosis. From the significant genes txt file I uploaded in reactome, 2938 were not found, which could be the cause of discrepancies.