

class16: RNASeq Mini Project

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1. Differential Expression Analysis

```
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
```

```
# 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
countData2 <- as.matrix(countData[,-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
```

This looks better but there are lots of zero entries in there so let's get rid of them as we have no data for these.

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).

```
head(countData2)
```

```
##           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
```

```
countsnozero <- countData2[rowSums(countData2) !=0, ]
head(countsnozero)
```

```
##           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
```

2. DESeq Analysis

```
library(DESeq2)
```

```
## Loading required package: S4Vectors
```

```
## Loading required package: stats4
```

```
## Loading required package: BiocGenerics
```

```
## Loading required package: parallel
```

```
##
```

```
## Attaching package: 'BiocGenerics'
```

```
## The following objects are masked from 'package:parallel':
```

```
##
```

```
##   clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,  
##   clusterExport, clusterMap, parApply, parCapply, parLapply,  
##   parLapplyLB, parRapply, parSapply, parSapplyLB
```

```
## 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
```

```
## 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, colAvgPerRowSet, 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, rowAvgPerColSet,
##   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

Setup the object required by DESeq

dds = DESeqDataSetFromMatrix(countData=countData2,
                             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
```

Get our results

```
res <- results(dds)
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 6 columns
```

```
##           baseMean log2FoldChange      lfcSE      stat      pvalue
##           <numeric>      <numeric> <numeric> <numeric> <numeric>
## ENSG00000186092    0.0000           NA       NA       NA       NA
## ENSG00000279928    0.0000           NA       NA       NA       NA
## ENSG00000279457   29.9136    0.179257  0.324822  0.551863 0.58104205
## ENSG00000278566    0.0000           NA       NA       NA       NA
## ENSG00000273547    0.0000           NA       NA       NA       NA
## ENSG00000187634  183.2296    0.426457  0.140266  3.040350 0.00236304
##           padj
##           <numeric>
## ENSG00000186092      NA
## ENSG00000279928      NA
## ENSG00000279457 0.68707978
## ENSG00000278566      NA
## ENSG00000273547      NA
## ENSG00000187634 0.00516278
```

```
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)    : 4393, 27%
## outliers [1]      : 0, 0%
## low counts [2]     : 1221, 7.6%
## (mean count < 0)
## [1] see 'cooksCutoff' argument of ?results
## [2] see 'independentFiltering' argument of ?results
```

3. Annotation

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

We want to add gene symbols, entrez ID's and gene names.

```
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"
```

```
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")
```

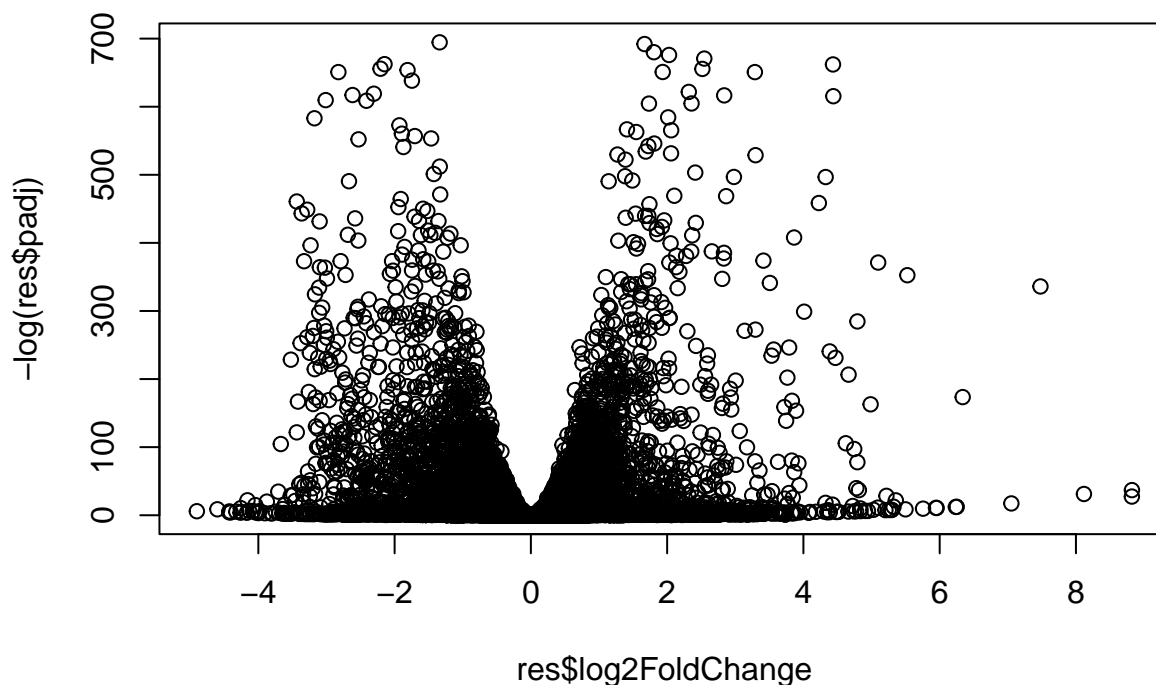
```
## 'select()' returned 1:many mapping between keys and columns
```

```
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>
## ENSG00000186092    0.0000           NA      NA      NA      NA
## ENSG00000279928    0.0000           NA      NA      NA      NA
## ENSG00000279457    29.9136    0.1792571 0.3248216  0.551863 5.81042e-01
## ENSG00000278566    0.0000           NA      NA      NA      NA
## ENSG00000273547    0.0000           NA      NA      NA      NA
## 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>
## ENSG00000186092      NA      OR4F5      79501 olfactory receptor f..
## ENSG00000279928      NA      NA      NA      NA
## ENSG00000279457 6.87080e-01    WASH9P    102723897 WAS protein family h..
## ENSG00000278566      NA      NA      NA      NA
## ENSG00000273547      NA      NA      NA      NA
## ENSG00000187634 5.16278e-03    SAMD11    148398 sterile alpha motif ..
## ENSG00000188976 1.76741e-35    NOC2L      26155 NOC2 like nucleolar ..
## ENSG00000187961 1.13536e-07    KLHL17    339451 kelch like family me..
## ENSG00000187583 9.18988e-01    PLEKHN1     84069 pleckstrin homology ..
## ENSG00000187642 4.03817e-01    PERM1      84808 PPARGC1 and ESRR ind..
```

4. Volcano Plot

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



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

```
library(EnhancedVolcano)
```

```
## Loading required package: ggplot2
```

```
## Loading required package: ggrepel
```

```
## Registered S3 methods overwritten by 'ggalt':
```

```
##   method                      from
##   grid.draw.absoluteGrob      ggplot2
##   grobHeight.absoluteGrob     ggplot2
##   grobWidth.absoluteGrob      ggplot2
##   grobX.absoluteGrob          ggplot2
##   grobY.absoluteGrob          ggplot2
```

```
x <- as.data.frame(res)
```

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

```
## Warning: One or more p-values is 0. Converting to 10^-1 * current lowest non-
## zero p-value...
```

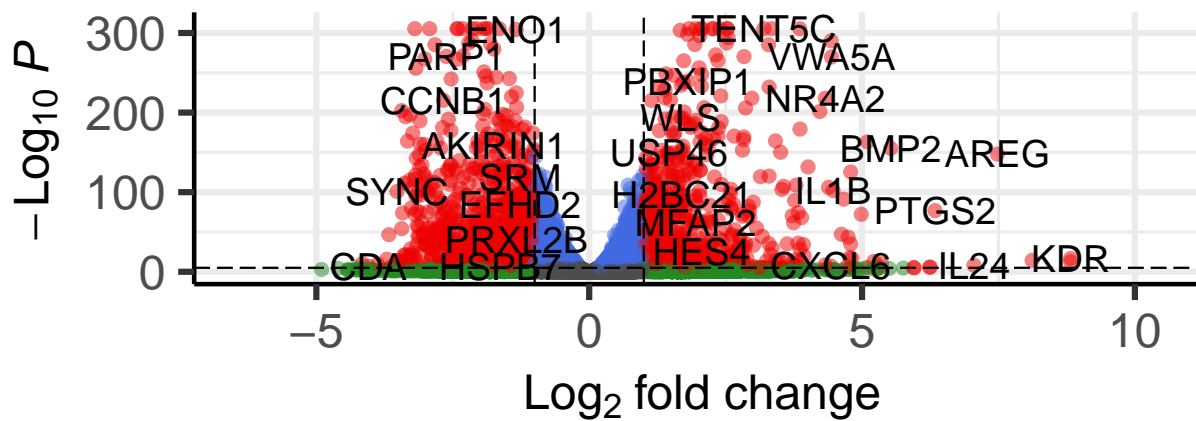


```
## Warning: Ignoring unknown parameters: xlim, ylim
```

Volcano plot

EnhancedVolcano

● NS ● Log_2 FC ● p-value ● p-value and Log_2 FC



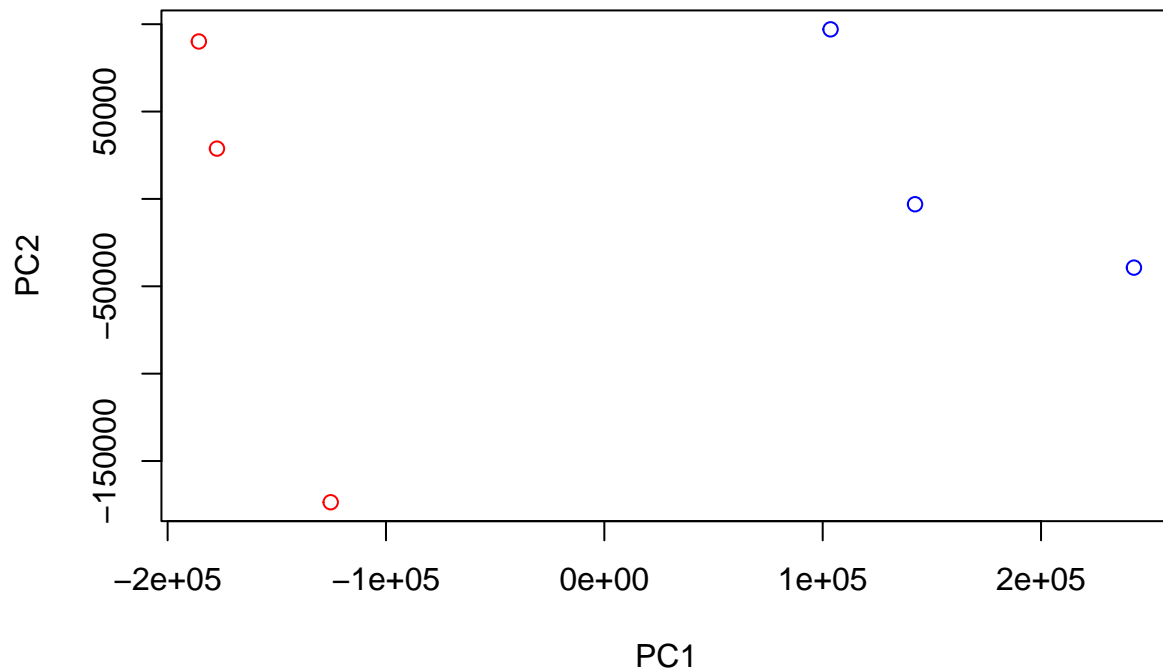
total = 19808 variables

PCA Plot

```
pca <- prcomp(t(countsnozero))
```

```
mycols <- rep(c("red", "blue"), each=3)
```

```
plot(pca$x[,1:2], col=mycols)
```



5. Pathway Analysis

```
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)
```

Focus on the signaling subset of KEGG

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

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

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

```
##      79501      <NA> 102723897      <NA>      <NA>      148398
##      NA      NA 0.1792571      NA      NA 0.4264571
```

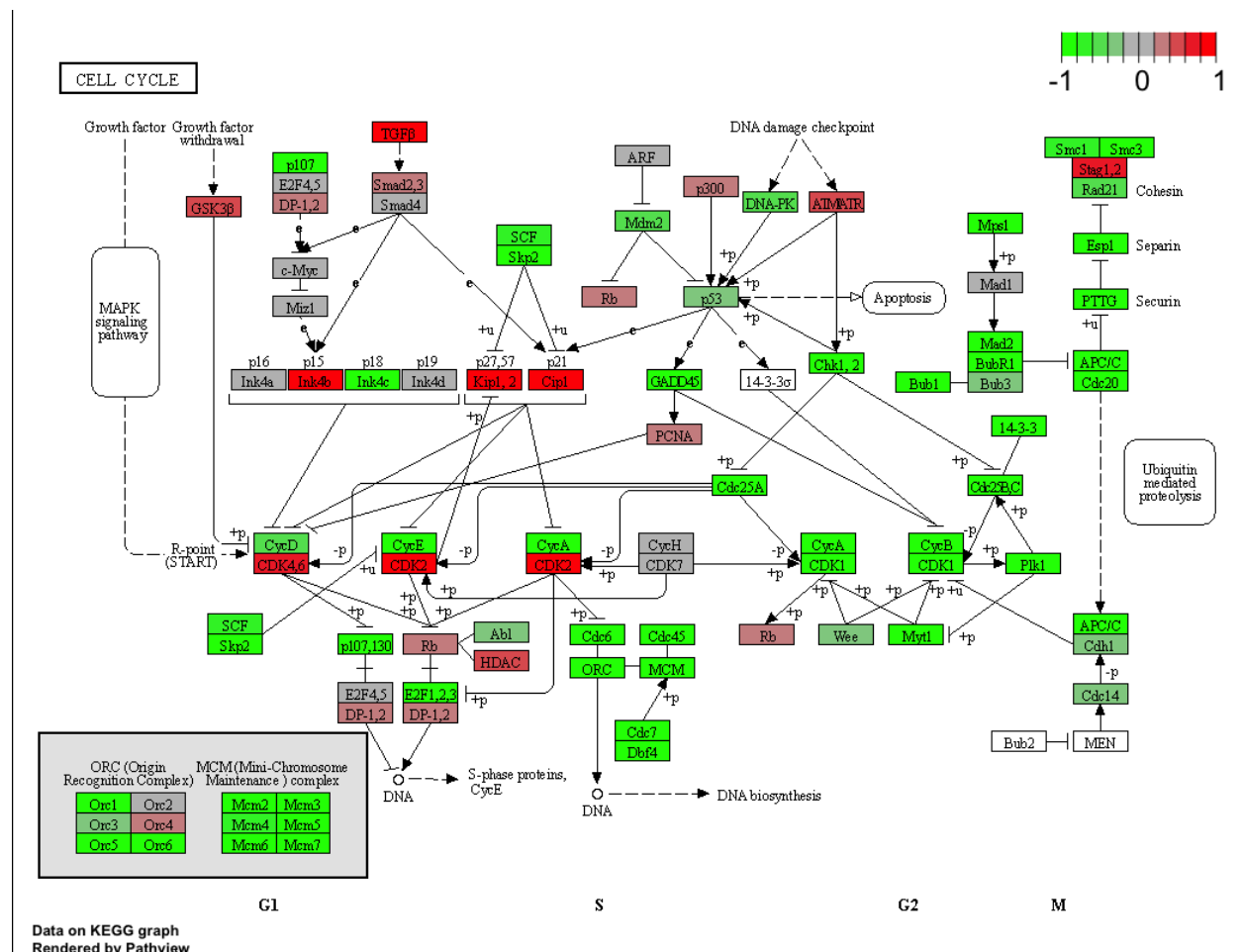
```
keggres <- gage(foldchanges, gsets=kegg.sets.hs)
```

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

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

```
## Info: Working in directory /Users/ethanharding/Desktop/BIMM 143/bimm143_github/class16
```

```
## 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 /Users/ethanharding/Desktop/BIMM 143/bimm143_github/class16

## 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] "hsa04740" "hsa04640" "hsa00140" "hsa04630" "hsa04976"

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

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

## Info: Working in directory /Users/ethanharding/Desktop/BIMM 143/bimm143_github/class16

## Info: Writing image file hsa04740.pathview.png

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

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

## Info: Working in directory /Users/ethanharding/Desktop/BIMM 143/bimm143_github/class16

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

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

## Info: Working in directory /Users/ethanharding/Desktop/BIMM 143/bimm143_github/class16

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

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

## Info: Working in directory /Users/ethanharding/Desktop/BIMM 143/bimm143_github/class16

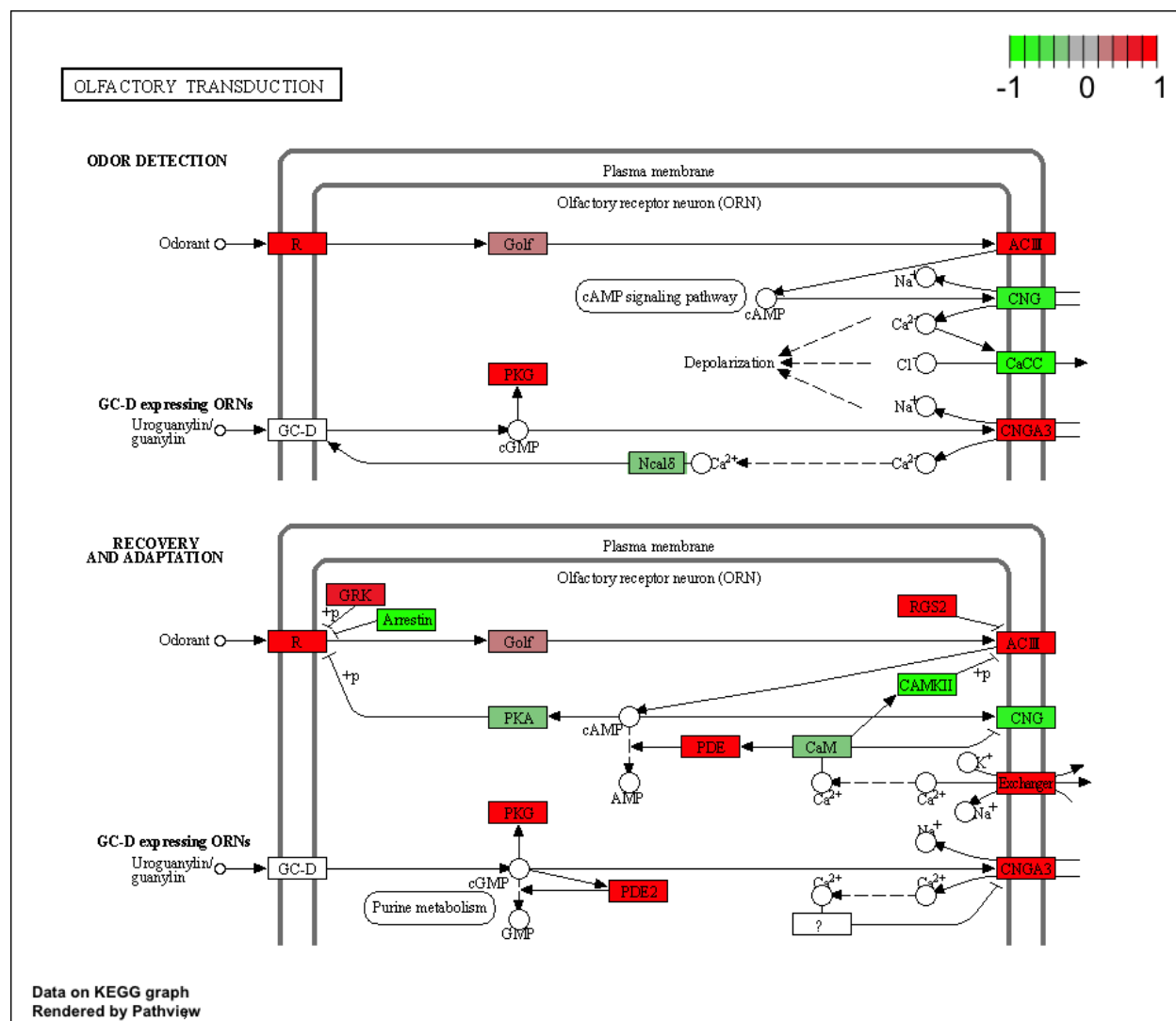
## Info: Writing image file hsa04630.pathview.png

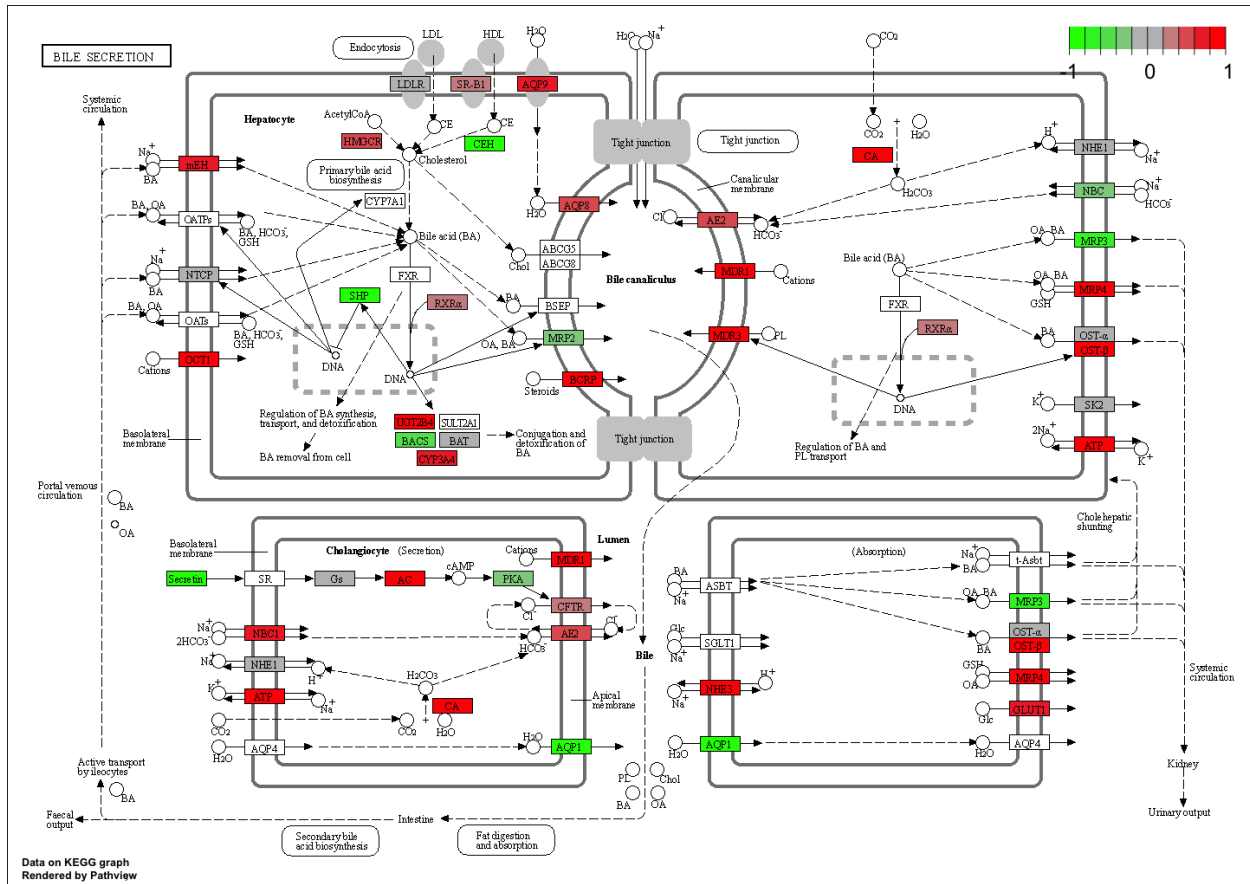
## 'select()' returned 1:1 mapping between keys and columns

```

```
## Info: Writing image file hsa04976.pathview.png
```







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
## G0:0007156 homophilic cell adhesion	1.624062e-05	4.226117	1.624062e-05
## G0:0048729 tissue morphogenesis	5.407952e-05	3.888470	5.407952e-05
## G0:0002009 morphogenesis of an epithelium	5.727599e-05	3.878706	5.727599e-05
## G0:0030855 epithelial cell differentiation	2.053700e-04	3.554776	2.053700e-04
## G0:0060562 epithelial tube morphogenesis	2.927804e-04	3.458463	2.927804e-04
## G0:0048598 embryonic morphogenesis	2.959270e-04	3.446527	2.959270e-04
##	q.val	set.size	exp1
## G0:0007156 homophilic cell adhesion	0.07103646	138	1.624062e-05


```

## G0:0048729 tissue morphogenesis      0.08350839      483 5.407952e-05
## G0:0002009 morphogenesis of an epithelium 0.08350839      382 5.727599e-05
## G0:0030855 epithelial cell differentiation 0.14646752      299 2.053700e-04
## G0:0060562 epithelial tube morphogenesis 0.14646752      289 2.927804e-04
## G0:0048598 embryonic morphogenesis      0.14646752      498 2.959270e-04
##
## $less
##
##          p.geomean stat.mean      p.val
## G0:0048285 organelle fission      6.386337e-16 -8.175381 6.386337e-16
## G0:0000280 nuclear division      1.726380e-15 -8.056666 1.726380e-15
## G0:0007067 mitosis      1.726380e-15 -8.056666 1.726380e-15
## G0:0000087 M phase of mitotic cell cycle 4.593581e-15 -7.919909 4.593581e-15
## G0:0007059 chromosome segregation      9.576332e-12 -6.994852 9.576332e-12
## G0:0051301 cell division      8.718528e-11 -6.455491 8.718528e-11
##
##          q.val set.size      exp1
## G0:0048285 organelle fission      2.517062e-12      386 6.386337e-16
## G0:0000280 nuclear division      2.517062e-12      362 1.726380e-15
## G0:0007067 mitosis      2.517062e-12      362 1.726380e-15
## G0:0000087 M phase of mitotic cell cycle 5.023080e-12      373 4.593581e-15
## G0:0007059 chromosome segregation      8.377375e-09      146 9.576332e-12
## G0:0051301 cell division      6.355807e-08      479 8.718528e-11
##
## $stats
##
##          stat.mean      exp1
## G0:0007156 homophilic cell adhesion      4.226117 4.226117
## G0:0048729 tissue morphogenesis      3.888470 3.888470
## G0:0002009 morphogenesis of an epithelium 3.878706 3.878706
## G0:0030855 epithelial cell differentiation 3.554776 3.554776
## G0:0060562 epithelial tube morphogenesis 3.458463 3.458463
## G0:0048598 embryonic morphogenesis      3.446527 3.446527

```