Class14: RNASeq Mini-Project

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Table of contents

Here we run through a complete RNASeq analysis from counts to pathways and biological insights.

Data Import

```r
countData = read.csv("GSE37704_featurecounts.csv", row.names=1)
colData = read.csv("GSE37704_metadata.csv", row.names=1)
```

Wee peak:

```r
head(colData)
```

```
              condition
SRR493366 control_sirna
SRR493367 control_sirna
SRR493368 control_sirna
SRR493369      hoxa1_kd
SRR493370      hoxa1_kd
SRR493371      hoxa1_kd
```

```
#countData[,-1]
```

```
countData <- countData[,-1]
```

Remove zero count genes

Filter count data where you have 0 read count acrooss all samples.

```
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
```

```
to.keep.inds <- rowSums(countData) > 0
countData <- countData[to.keep.inds,]
```

Setup for DESeq

```
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, 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
```

```
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
```

Running DESeq

```
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)

head(dds)
```

```
class: DESeqDataSet
dim: 6 6
metadata(1): version
assays(4): counts mu H cooks
rownames(6): ENSG00000279457 ENSG00000187634 ... ENSG00000187583
  ENSG00000187642
rowData names(22): baseMean baseVar ... deviance maxCooks
colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
colData names(2): condition sizeFactor
```

```r
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>
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 1.43989e-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 0.5215599   1.040744 2.97994e-01
                       padj
                  <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76549e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
ENSG00000187642 4.03379e-01
```

Save results to data

```r
write.csv(res, file = "myresults.csv")
```

Add gene annotation data (gene names etc.)

```r
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$entrez <- mapIds(org.Hs.eg.db,
                     keys=rownames(res),
                     keytype="ENSEMBL",
                     column="ENTREZID")
```

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

```
res$symbol <- mapIds(org.Hs.eg.db,
                     keys=rownames(res),
                     keytype="ENSEMBL",
                     column="SYMBOL")
```

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

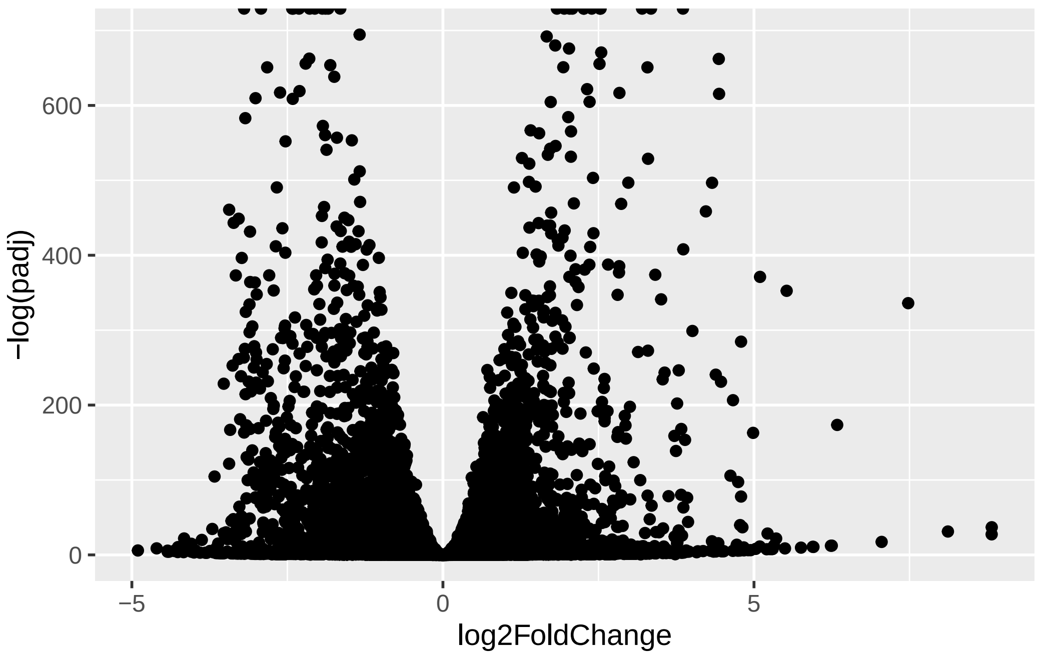
Results visualization

```
library(ggplot2)

data <- as.data.frame(res)

ggplot(data) +
  aes(log2FoldChange, -log(padj))+
  geom_point()
```

```
Warning: Removed 1237 rows containing missing values or values outside the scale range
(`geom_point()`).
```



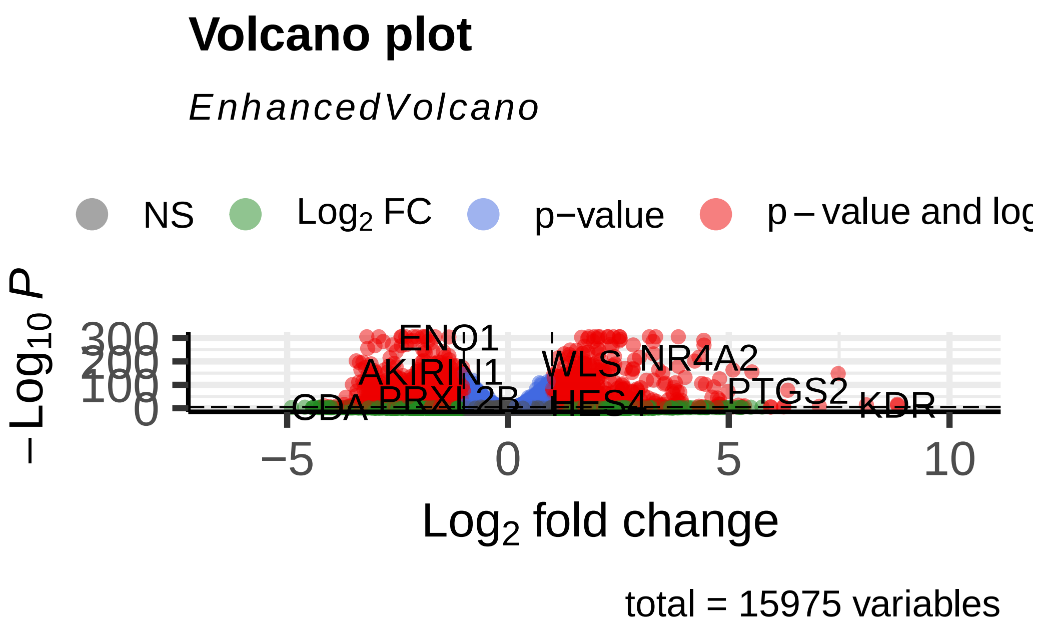
```r
library(EnhancedVolcano)
```

```
Loading required package: ggrepel
```

```r
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...
```



Save our results

```
write.csv(res, file="myresults_annotated.csv")
```

Pathway analysis (KEGG, GO, Reactome)

```
library(gage)
library(gageData)
library(pathview)
```

Fold change vector with ENTREZ ID names

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

```
       <NA>      148398       26155      339451       84069       84808 
 0.17925708  0.42645712 -0.69272046  0.72975561  0.04057653  0.54281049 
```

KEGG

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

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

Look at the first few down (less) pathways

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

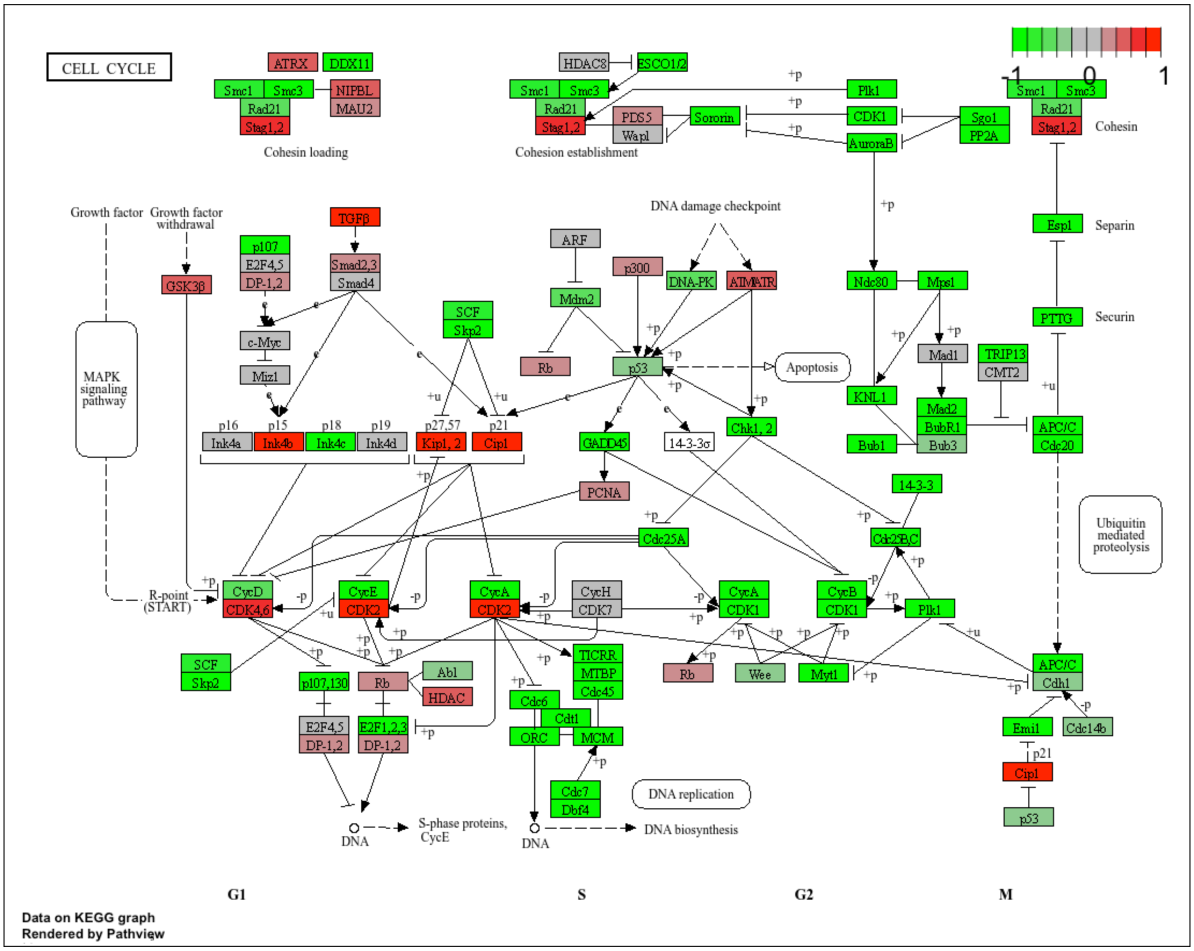
```
                                                  p.geomean stat.mean
hsa04110 Cell cycle                            8.995727e-06 -4.378644
hsa03030 DNA replication                       9.424076e-05 -3.951803
hsa05130 Pathogenic Escherichia coli infection 1.405864e-04 -3.765330
hsa03013 RNA transport                         1.246882e-03 -3.059466
hsa03440 Homologous recombination              3.066756e-03 -2.852899
hsa04114 Oocyte meiosis                        3.784520e-03 -2.698128
                                                      p.val       q.val
hsa04110 Cell cycle                            8.995727e-06 0.001889103
hsa03030 DNA replication                       9.424076e-05 0.009841047
hsa05130 Pathogenic Escherichia coli infection 1.405864e-04 0.009841047
hsa03013 RNA transport                         1.246882e-03 0.065461279
hsa03440 Homologous recombination              3.066756e-03 0.128803765
hsa04114 Oocyte meiosis                        3.784520e-03 0.132458191
                                               set.size         exp1
hsa04110 Cell cycle                                 121 8.995727e-06
hsa03030 DNA replication                             36 9.424076e-05
hsa05130 Pathogenic Escherichia coli infection       53 1.405864e-04
hsa03013 RNA transport                              144 1.246882e-03
hsa03440 Homologous recombination                    28 3.066756e-03
hsa04114 Oocyte meiosis                             102 3.784520e-03
```

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

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

```
Info: Working in directory /Users/christinexie/Desktop/BIMM143/wk7/class14/class14
```

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



Section3: Gene Ontology (GO)

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

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

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

lapply(gobpres, head)
```

```
$greater
                                             p.geomean stat.mean        p.val
GO:0007156 homophilic cell adhesion       8.519724e-05  3.824205 8.519724e-05
GO:0002009 morphogenesis of an epithelium 1.396681e-04  3.653886 1.396681e-04
GO:0048729 tissue morphogenesis           1.432451e-04  3.643242 1.432451e-04
GO:0007610 behavior                       1.925222e-04  3.565432 1.925222e-04
GO:0060562 epithelial tube morphogenesis  5.932837e-04  3.261376 5.932837e-04
GO:0035295 tube development               5.953254e-04  3.253665 5.953254e-04
                                              q.val set.size         exp1
GO:0007156 homophilic cell adhesion       0.1951953      113 8.519724e-05
GO:0002009 morphogenesis of an epithelium 0.1951953      339 1.396681e-04
GO:0048729 tissue morphogenesis           0.1951953      424 1.432451e-04
GO:0007610 behavior                       0.1967577      426 1.925222e-04
GO:0060562 epithelial tube morphogenesis  0.3565320      257 5.932837e-04
GO:0035295 tube development               0.3565320      391 5.953254e-04

$less
                                           p.geomean stat.mean        p.val
GO:0048285 organelle fission            1.536227e-15 -8.063910 1.536227e-15
GO:0000280 nuclear division             4.286961e-15 -7.939217 4.286961e-15
GO:0007067 mitosis                      4.286961e-15 -7.939217 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.169934e-14 -7.797496 1.169934e-14
GO:0007059 chromosome segregation       2.028624e-11 -6.878340 2.028624e-11
GO:0000236 mitotic prometaphase         1.729553e-10 -6.695966 1.729553e-10
                                               q.val set.size         exp1
GO:0048285 organelle fission            5.841698e-12      376 1.536227e-15
GO:0000280 nuclear division             5.841698e-12      352 4.286961e-15
GO:0007067 mitosis                      5.841698e-12      352 4.286961e-15
GO:0000087 M phase of mitotic cell cycle 1.195672e-11      362 1.169934e-14
GO:0007059 chromosome segregation       1.658603e-08      142 2.028624e-11
GO:0000236 mitotic prometaphase         1.178402e-07       84 1.729553e-10

$stats
                                          stat.mean     exp1
GO:0007156 homophilic cell adhesion        3.824205 3.824205
GO:0002009 morphogenesis of an epithelium  3.653886 3.653886
GO:0048729 tissue morphogenesis            3.643242 3.643242
GO:0007610 behavior                        3.565432 3.565432
GO:0060562 epithelial tube morphogenesis   3.261376 3.261376
GO:0035295 tube development                3.253665 3.253665
```

Section4: 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, quote=
```