# Class14

Wanning Cui

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```
library(DESeq2)
```

```
Loading required package: S4Vectors
```

```
Loading required package: stats4
```

```
Loading required package: BiocGenerics
```

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

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

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

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

    anyDuplicated, aperm, append, as.data.frame, basename, cbind,
    colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
```

```
    get, grep, grepl, intersect, is.unsorted, lapply, Map, mapply,
    match, mget, order, paste, pmax, pmax.int, pmin, pmin.int,
    Position, rank, rbind, Reduce, rownames, sapply, setdiff, sort,
    table, tapply, union, unique, unsplit, which.max, which.min

Attaching package: 'S4Vectors'

The following object is masked from 'package:utils':

    findMatches

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

    expand.grid, I, unname

Loading required package: IRanges

Attaching package: 'IRanges'

The following object is masked from 'package:grDevices':

    windows

Loading required package: GenomicRanges

Loading required package: GenomeInfoDb

Loading required package: SummarizedExperiment

Loading required package: MatrixGenerics

Loading required package: matrixStats

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

Attaching package: 'MatrixGenerics'
```

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

    colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
    colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
    colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
    colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
    colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
    colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
    colWeightedMeans, colWeightedMedians, colWeightedSds,
    colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
    rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
    rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
    rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
    rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
    rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
    rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
    rowWeightedSds, rowWeightedVars

Loading required package: Biobase

Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.

Attaching package: 'Biobase'

The following object is masked from 'package:MatrixGenerics':

    rowMedians

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

    anyMissing, rowMedians
```

## Data Import

```
counts <- read.csv("GSE37704_featurecounts.csv",row.names=1)
metadata <- read.csv("GSE37704_metadata.csv")
```

```
head(counts)
```

```
                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
```

```
metadata
```

```
         id     condition
1 SRR493366 control_sirna
2 SRR493367 control_sirna
3 SRR493368 control_sirna
4 SRR493369      hoxa1_kd
5 SRR493370      hoxa1_kd
6 SRR493371      hoxa1_kd
```

I need to get rid of the first `length` column in `counts`:

```
counts <- counts[,-1]
```

## Data Tidying

```
all(colnames(counts) == metadata$id)
```

```
[1] TRUE
```

Remove any genes with zero counts in all samples/columns

Q. How many genes do we have to start with?

```
nrow(counts)
```

```
[1] 19808
```

Find the rowSums() this will be zero for any genes with no count data Find the zero sum genes Remove them before doing our DESeq

```
to.rm.ind<-rowSums(counts)==0
counts <- counts[!to.rm.ind,]
nrow(counts)
```

```
[1] 15975
```

## DESeq setup and analysis

```
dds <- DESeqDataSetFromMatrix(countData=counts,
                              colData=metadata,
                              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)
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 1.43990e-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
                 <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
```

```
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
```

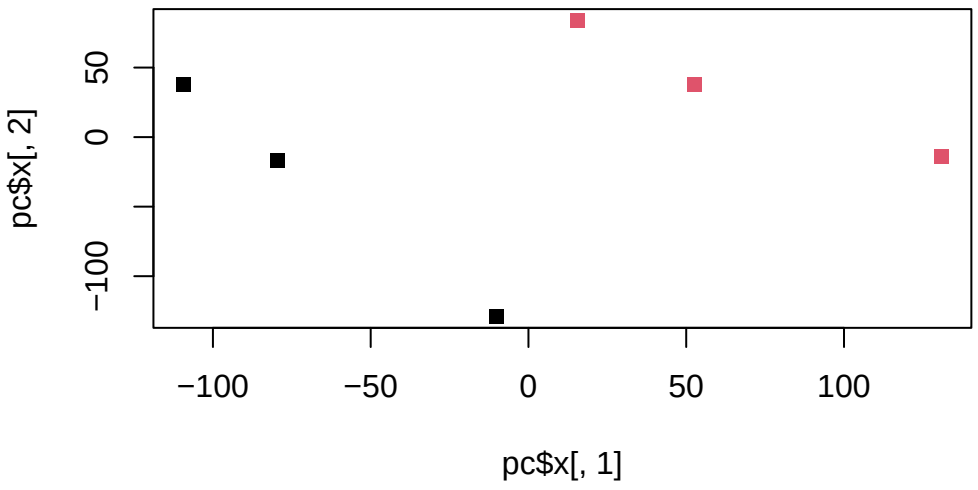
#Side-note: QC with PCA

```
pc<-prcomp(t(counts),scale=T)
```

```
summary(pc)
```

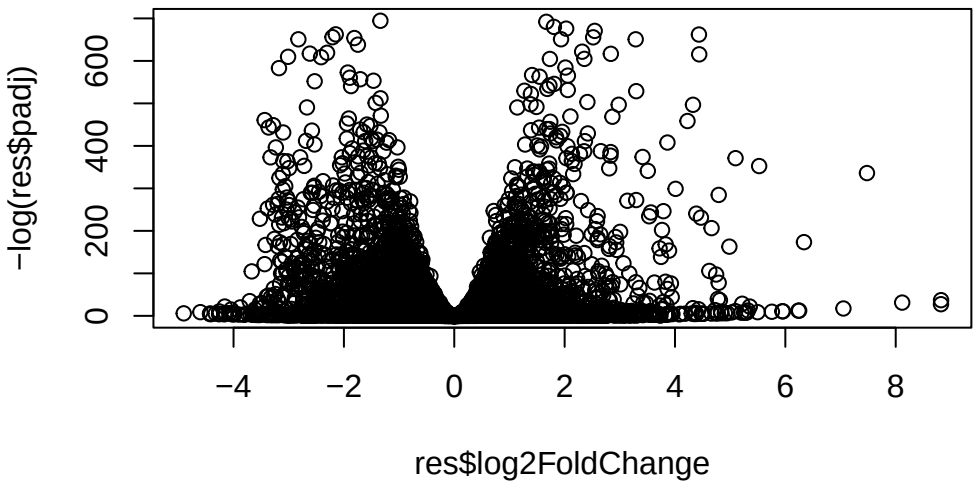
```
Importance of components:
                          PC1     PC2      PC3      PC4      PC5        PC6
Standard deviation     87.7211 73.3196 32.89604 31.15094 29.18417 7.373e-13
Proportion of Variance  0.4817  0.3365  0.06774  0.06074  0.05332 0.000e+00
Cumulative Proportion   0.4817  0.8182  0.88594  0.94668  1.00000 1.000e+00
```

```
plot(pc$x[,1],pc$x[,2],col=as.factor(metadata$condition),pch=15)
```



## Visualization

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




## Adding gene annotation

Let's add some color and annotation data to this plot.

```
library(AnnotationDbi)
```

```
Warning: package 'AnnotationDbi' was built under R version 4.3.2
```

```
library(org.Hs.eg.db)
```

```
columns(org.Hs.eg.db)
```

```
 [1] "ACCNUM"       "ALIAS"        "ENSEMBL"      "ENSEMBLPROT"  "ENSEMBLTRANS"
 [6] "ENTREZID"     "ENZYME"       "EVIDENCE"     "EVIDENCEALL"  "GENENAME"
[11] "GENETYPE"     "GO"           "GOALL"        "IPI"          "MAP"
[16] "OMIM"         "ONTOLOGY"     "ONTOLOGYALL"  "PATH"         "PFAM"
[21] "PMID"         "PROSITE"      "REFSEQ"       "SYMBOL"       "UCSCKG"
[26] "UNIPROT"
```

```
head(row.names(counts))
```

```
[1] "ENSG00000279457" "ENSG00000187634" "ENSG00000188976" "ENSG00000187961"
[5] "ENSG00000187583" "ENSG00000187642"
```

```
res$symbol <- mapIds(org.Hs.eg.db,
                     keys=row.names(counts),
                     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(counts),
                     keytype="ENSEMBL",
                     column="ENTREZID",
                     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 8 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
```

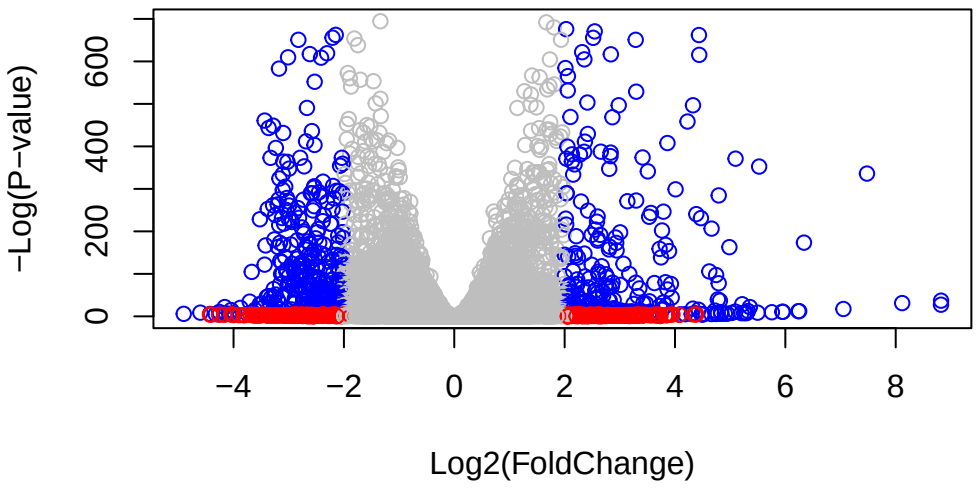
```
ENSG00000188976 1651.1881     -0.6927205 0.0548465 -12.630158 1.43990e-36
ENSG00000187961  209.6379      0.7297556 0.1318599   5.534326 3.12428e-08
ENSG00000187583   47.2551      0.0405765 0.2718928   0.149237 8.81366e-01
ENSG00000187642   11.9798      0.5428105 0.5215598   1.040744 2.97994e-01
                      padj      symbol      entrez
                 <numeric> <character> <character>
ENSG00000279457 6.86555e-01          NA          NA
ENSG00000187634 5.15718e-03      SAMD11      148398
ENSG00000188976 1.76549e-35       NOC2L       26155
ENSG00000187961 1.13413e-07      KLHL17      339451
ENSG00000187583 9.19031e-01     PLEKHN1       84069
ENSG00000187642 4.03379e-01       PERM1       84808
```

```
res = res[order(res$pvalue),]
write.csv(res,"deseq_results.csv")
```

```
mycols <- rep("gray", nrow(res) ) # Make a color vector for all genes
mycols[abs(res$log2FoldChange) > 2 ] <- "red" # Color red the genes with absolute fold cha

# 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(P
```




### Geneset enrichment/pathway analysis

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

The `gage()` function wants a "vector of importance" in our case. Here it will be fold-change values with associated entrez gene names.

```
foldchanges<-res$log2FoldChange
names(foldchanges)<-res$entrez
```

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

```
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                            1.375901e-03 -3.028500
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                            1.375901e-03 0.072234819
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 1.375901e-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 D:/UCSD/R/14
```

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

Have a look at my figure(Figure 1)

```
# top 5 upregulated pathways
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] "hsa04060" "hsa05323" "hsa05146" "hsa05332" "hsa04640"
```

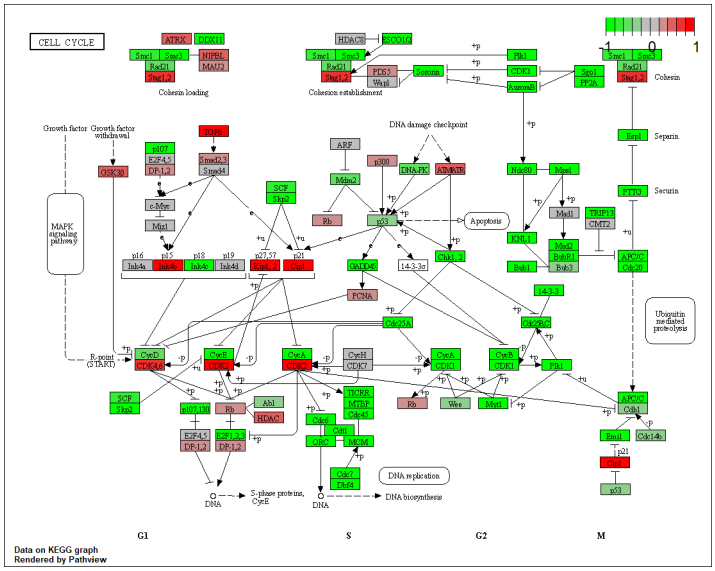



Figure 1: Cell cycle hsa04110

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

```
Info: Downloading xml files for hsakeggresids, 1/1 pathways..

Warning in download.file(xml.url, xml.target, quiet = T): cannot open URL
'https://rest.kegg.jp/get/hsakeggresids/kgml': HTTP status was '400 Bad
Request'

Warning: Download of hsakeggresids xml file failed!
This pathway may not exist!

Info: Downloading png files for hsakeggresids, 1/1 pathways..

Warning: Download of hsakeggresids png file failed!
This pathway may not exist!

Warning: Failed to download KEGG xml/png files, hsakeggresids skipped!
```

## Gene Ontology

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

gobpsets = go.sets.hs[go.subs.hs$BP]

gobpres = gage(foldchanges, gsets=gobpsets)
```

```
head(gobpres$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
```

## Reactome

We will use the online version of Reactome. It wants a list of your genes. We will write this out from R here:

```
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=FALSE)
```

Reactome website: https://reactome.org/PathwayBrowser/#TOOL=AT

Mitotic cell cycle has the most significant "Entities p-value". No, they don't match. There are biases in each analysis.