Class 13: RNASeq Mini Project

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Read the countData and colData

```
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
```

```
colData <- read.csv("GSE37704_metadata.csv")
head(colData)
```

```
         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
```

Q. Do these match - No so lets fix them

```
countData <- countData[,-1]
#countData[, colData$id]
```

```
colnames(countData) == colData$id
```

```
[1] TRUE TRUE TRUE TRUE TRUE TRUE
```

Remove zero count genes

```
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

```
counts <- countData[rowSums(countData[])>0,]
head(counts)
```

	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

Q. How many genes do we have left?

```
nrow(counts)
```

```
[1] 15975
```

PCA as quality control

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

```
Importance of components:
                            PC1     PC2      PC3      PC4      PC5       PC6
Standard deviation     87.7211 73.3196 32.89604 31.15094 29.18417 6.648e-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
```

Q. How much variance is captured in the first two PCs?

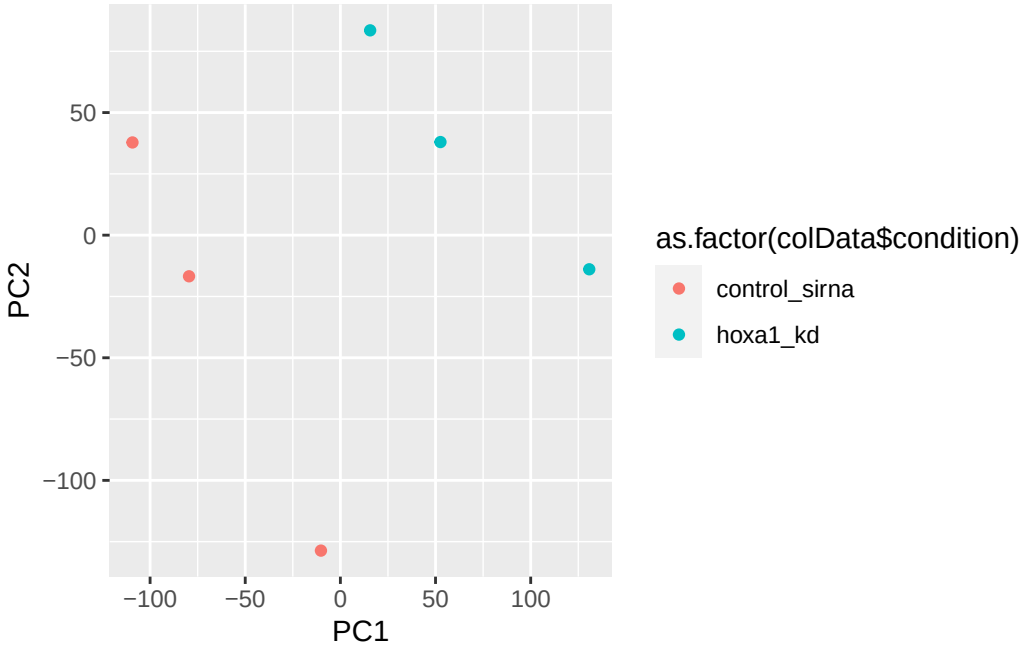
81.82%

Q. What does your score plot (PC1 vs PC2) look like when colored by condition (control/lockdown)

```
library(ggplot2)

x <- as.data.frame(pc$x)

ggplot(x) +
  aes(PC1, PC2, col = as.factor(colData$condition)) +
  geom_point()
```



DeSeq Analysis

```
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 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 = counts,
                              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)
res
```

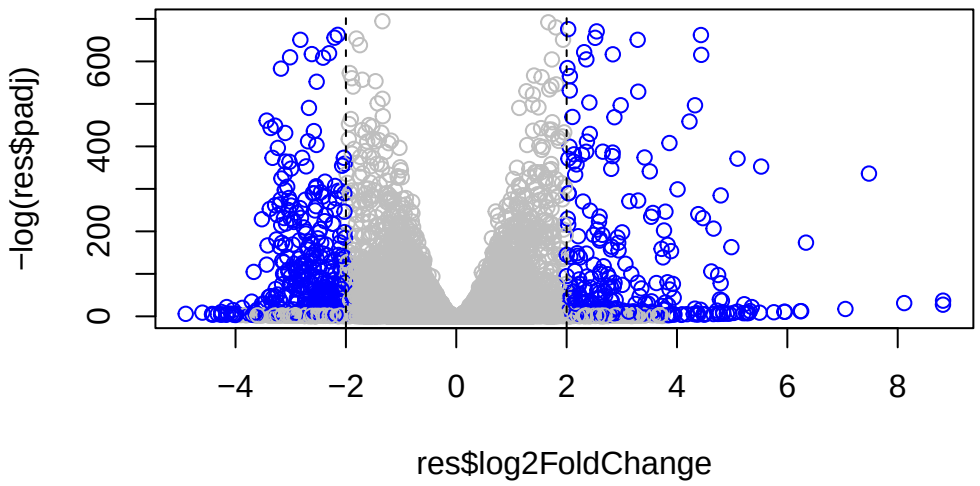
```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 15975 rows and 6 columns
                 baseMean log2FoldChange     lfcSE       stat      pvalue
                <numeric>      <numeric> <numeric>  <numeric>   <numeric>
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
...                  ...            ...       ...        ...          ...
ENSG00000273748  35.30265       0.674387  0.303666   2.220817 2.63633e-02
ENSG00000278817   2.42302      -0.388988  1.130394  -0.344117 7.30758e-01
ENSG00000278384   1.10180       0.332991  1.660261   0.200565 8.41039e-01
ENSG00000276345  73.64496      -0.356181  0.207716  -1.714752 8.63908e-02
ENSG00000271254 181.59590      -0.609667  0.141320  -4.314071 1.60276e-05
                      padj
                 <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76549e-35
ENSG00000187961 1.13413e-07
ENSG00000187583 9.19031e-01
...                   ...
ENSG00000273748 4.79091e-02
ENSG00000278817 8.09772e-01
ENSG00000278384 8.92654e-01
ENSG00000276345 1.39762e-01
ENSG00000271254 4.53648e-05
```

Summary plot

```
mycols <- rep("gray", nrow(res))
mycols[abs(res$log2FoldChange) > 2] <- "blue"
mycols[res$padj > 0.05] <- "gray"

plot( res$log2FoldChange, -log(res$padj), col = mycols )
abline(v = c(-2,2), lty=2)
```




```
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 = rownames(counts),
                     keytype = "ENSEMBL",
                     column = "SYMBOL")
```

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

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

```
'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
```

Pathway analysis

```
library(gage)
```

```
library(gageData)
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).
#############################################################################
```

Need to create the input for gage() - a vector of fold change values with entrez IDs as the names()

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

```
data(kegg.sets.hs)

keggres <- gage(foldchange, 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                1.246882e-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(foldchange, pathway.id = "hsa04110")
```

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

```
Info: Working in directory /Users/suzanneenos/Documents/bioinformatics/class13
```

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

```
pathview(gene.data=foldchange, pathway.id="hsa04110", kegg.native=FALSE)
```

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

```
Info: Working in directory /Users/suzanneenos/Documents/bioinformatics/class13
```

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

Gene Ontology

```
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(foldchange, gsets=gobpsets, same.dir=TRUE)

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             5.841698e-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
```

Write out a text file of our genes for reactions

```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quo
```