

Class 13 Lab

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Background

Data Import

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

```
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				

Q1.

```
countData <- as.matrix(countData[,2:7])
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

Q2.

```
# Filter count data where you have 0 read count across all samples.
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

Setup for DESeq

```
library(DESeq2)
```

Warning: package 'matrixStats' was built under R version 4.5.2

Run DEseq

```
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)
```

Results

```
res = results(dds)
```

Q3.

```
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%
(means count < 0)
[1] see 'cooksCutoff' argument of ?results
[2] see 'independentFiltering' argument of ?results

```

Add Annotations

Q5.

```

library("AnnotationDbi")
library("org.Hs.eg.db")

```

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

```

[1] "ACCNUM"        "ALIAS"          "ENSEMBL"        "ENSEMLPROT"    "ENSEMLTRANS"
[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)

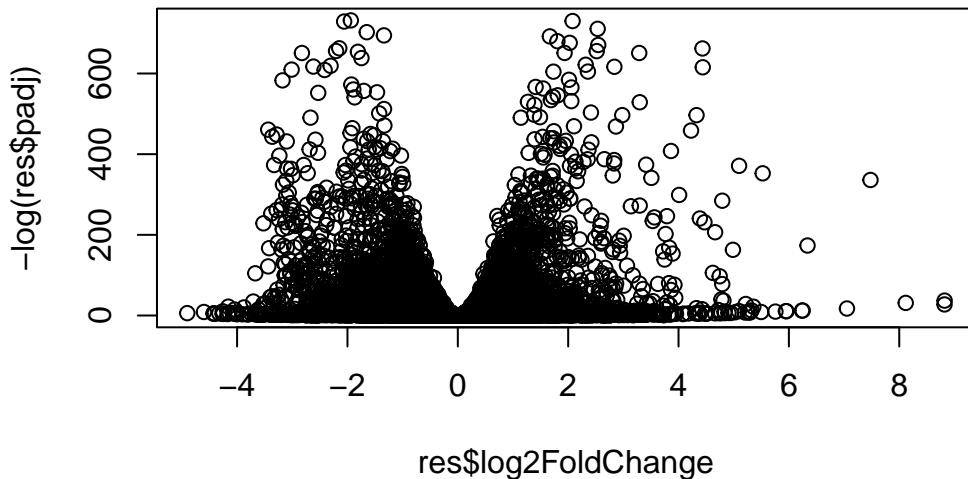
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 10 rows and 9 columns
  baseMean log2FoldChange      lfcSE      stat      pvalue
  <numeric>      <numeric> <numeric> <numeric>    <numeric>
ENSG00000279457   29.913579     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 1.43990e-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  0.5215598  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 ..

Visualization

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



Q4.

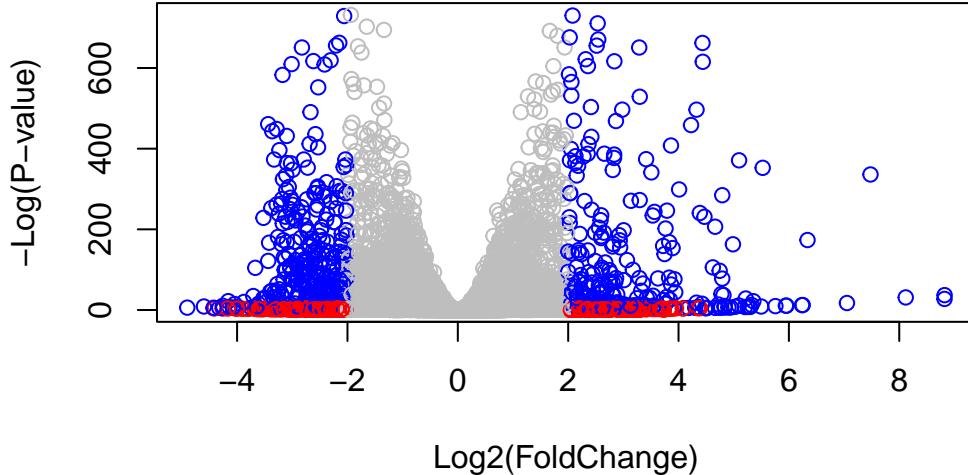
```
# Make a color vector for all genes
mycols <- rep("gray", nrow(res))

# Color red the genes with absolute fold change above 2
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

# 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-
```



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>).

#####

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

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

# Examine the first 3 pathways
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"
```

```
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
```

```
# Get the results
keggres = gage(foldchanges, gsets=kegg.sets.hs)
```

```
attributes(keggres)
```

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

```
# Look at the first few down (less) pathways
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	1.246882e-03	-3.059466	1.246882e-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	0.066915974	144	1.246882e-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

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

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

```
Info: Working in directory C:/Users/ianmk/Documents/Class13Lab
```

```
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
```

```
Warning: reconcile groups sharing member nodes!
```

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

```
Info: Working in directory C:/Users/ianmk/Documents/Class13Lab
```

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

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

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

```
Info: Working in directory C:/Users/ianmk/Documents/Class13Lab
```

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

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

Info: Working in directory C:/Users/ianmk/Documents/Class13Lab

Info: Writing image file hsa04630.pathview.png

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

Info: Working in directory C:/Users/ianmk/Documents/Class13Lab

Info: Writing image file hsa00140.pathview.png

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

Info: Working in directory C:/Users/ianmk/Documents/Class13Lab

Info: Writing image file hsa04142.pathview.png

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

Info: Working in directory C:/Users/ianmk/Documents/Class13Lab

Info: Writing image file hsa04330.pathview.png
```

Q7.

```
## Focus on top 5 upregulated pathways here for demo purposes only
keggrespathways <- rownames(keggres$less)[1:5]

# Extract the 8 character long IDs part of each string
keggresids = substr(keggrespathways, start=1, stop=8)
keggresids
```

```
[1] "hsa04110" "hsa03030" "hsa03013" "hsa03440" "hsa04114"
```

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

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

```
Info: Working in directory C:/Users/ianmk/Documents/Class13Lab
```

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

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

```
Info: Working in directory C:/Users/ianmk/Documents/Class13Lab
```

```
Info: Writing image file hsa03030.pathview.png
```

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

```
Info: Working in directory C:/Users/ianmk/Documents/Class13Lab
```

```
Info: Writing image file hsa03013.pathview.png
```

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

```
Info: Working in directory C:/Users/ianmk/Documents/Class13Lab
```

```
Info: Writing image file hsa03440.pathview.png
```

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

```
Info: Working in directory C:/Users/ianmk/Documents/Class13Lab
```

```
Info: Writing image file hsa04114.pathview.png
```

Save

Q6.

```

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

```

```

save(res, file="my_results.RData")

```

GO Analysis

```

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
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

Reactome

```
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=
```