

Class 14: RNAseq Mini Project

Farnam (PID: A17628539)

Run a complete RNAseq analysis workflow from counts to enriched genesets...

Data Import

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

Data Exploration

```
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

```
head(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

Check if the coData and CountData match up

```
metadata$id
```

```
[1] "SRR493366" "SRR493367" "SRR493368" "SRR493369" "SRR493370" "SRR493371"
```

```
colnames(counts)
```

```
[1] "length"      "SRR493366" "SRR493367" "SRR493368" "SRR493369" "SRR493370"
[7] "SRR493371"
```

```
# RM first column
countData <- counts[,-1]
```

Now check if the metadata and count data columns all match up:

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

```
[1] TRUE
```

We need to remove all the zero count genes.

```
head(countData, 3)
```

	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

To identify these zero count genes we can sum across the rows and check if the sum is more than zero.

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

DESeq setup and analysis

```
library(DESeq2)

dds <- DESeqDataSetFromMatrix(countData = nonzero.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

Result extraction

```
res <- results(dds)
head(res)
```

log2 fold change (MLE): condition hoxa1 kd vs control sirna

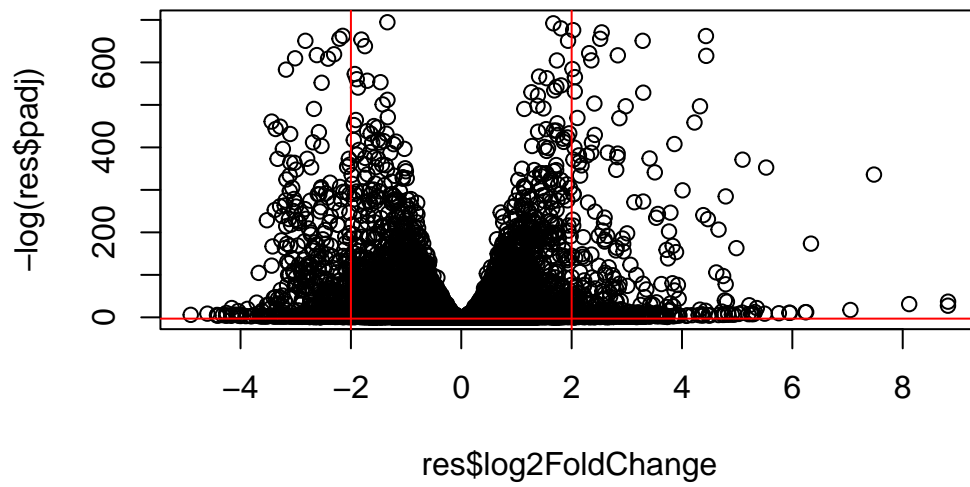
Wald test p-value: condition hoxa1 kd vs control sirna

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	1.43989e-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				

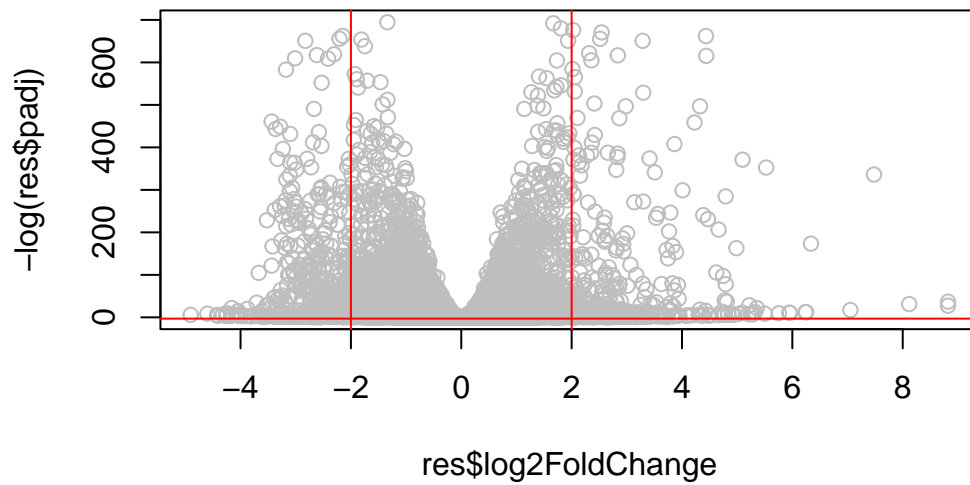
volcano plot

```
plot( res$log2FoldChange, -log(res$padj))
abline(v=c(-2,2), col= "red")
abline(h=log(0.05), col="red")
```



Setup a wee color vector for this plot

```
mycols <- rep("gray", nrow(res))  
plot( res$log2FoldChange, -log(res$padj), col= mycols)  
abline(v=c(-2,2), col= "red")  
abline(h=log(0.05), col="red")
```



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

Gene annotation

```
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(x = org.Hs.eg.db,
                    keys=rownames(nonzero.counts),
                    keytype="ENSEMBL",
                    column="SYMBOL")
```

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

```
res$entrez <- mapIds(x = org.Hs.eg.db,
                    keys=rownames(nonzero.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	1.43989e-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	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)
```

```
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
```

Now we get the result:

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

```
attributes(keggres)
```

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

```
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

Let's look at one of these pathways: hsa04110

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

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

Info: Working in directory /Users/farnamtavakoli/Downloads/School Documents/UCSD Classes/Win

Info: Writing image file hsa04110.pathview.png

Using gene ontology (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
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	0.1952430	113	8.519724e-05	
GO:0002009 morphogenesis of an epithelium	0.1952430	339	1.396681e-04	

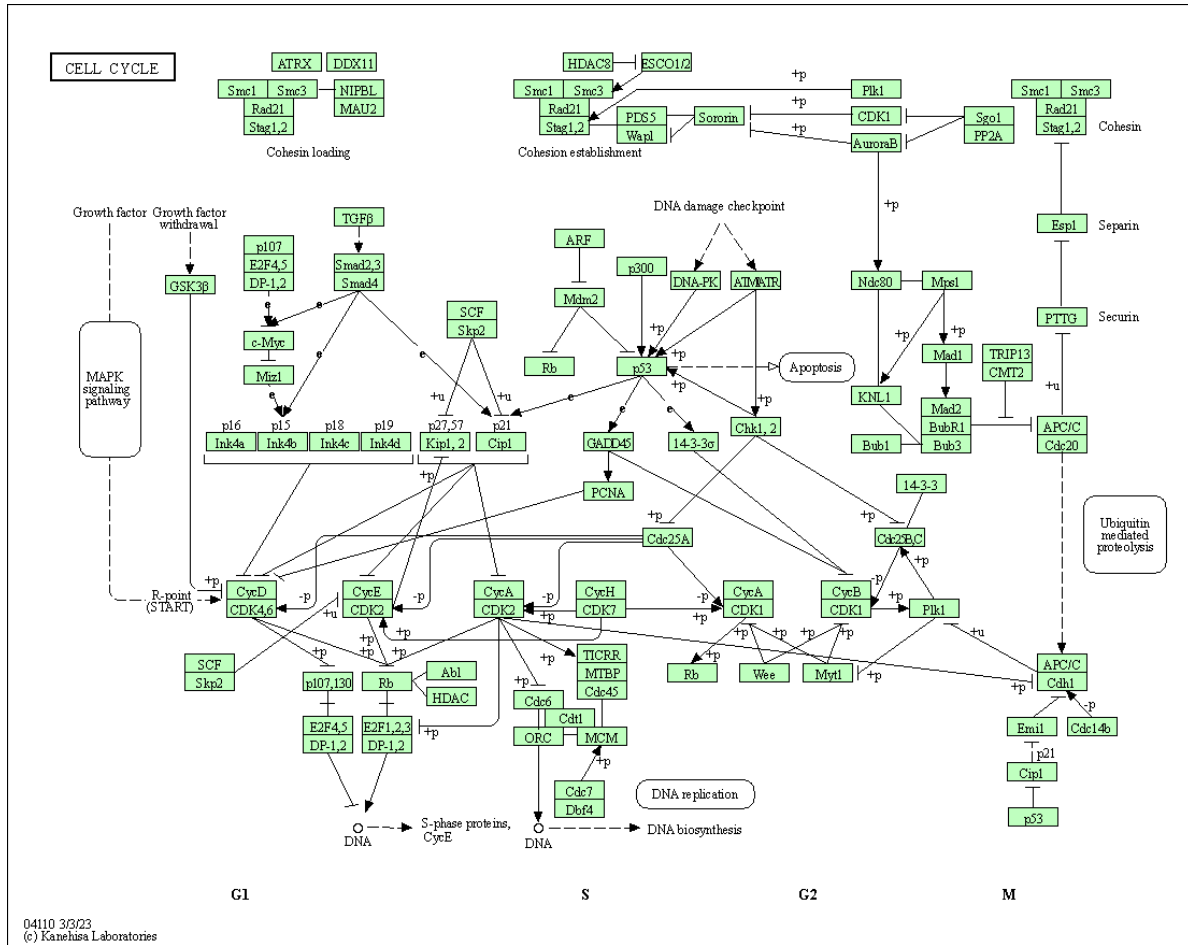


Figure 1: The KEGG cell-cycle pathway with our DEGs highlighted

G0:0048729	tissue morphogenesis	0.1952430	424	1.432451e-04
G0:0007610	behavior	0.1968058	426	1.925222e-04
G0:0060562	epithelial tube morphogenesis	0.3566193	257	5.932837e-04
G0:0035295	tube development	0.3566193	391	5.953254e-04

\$less

		p.geomean	stat.mean	p.val
G0:0048285	organelle fission	1.536227e-15	-8.063910	1.536227e-15
G0:0000280	nuclear division	4.286961e-15	-7.939217	4.286961e-15
G0:0007067	mitosis	4.286961e-15	-7.939217	4.286961e-15
G0:0000087	M phase of mitotic cell cycle	1.169934e-14	-7.797496	1.169934e-14
G0:0007059	chromosome segregation	2.028624e-11	-6.878340	2.028624e-11
G0:0000236	mitotic prometaphase	1.729553e-10	-6.695966	1.729553e-10

		q.val	set.size	expl
G0:0048285	organelle fission	5.843127e-12	376	1.536227e-15
G0:0000280	nuclear division	5.843127e-12	352	4.286961e-15
G0:0007067	mitosis	5.843127e-12	352	4.286961e-15
G0:0000087	M phase of mitotic cell cycle	1.195965e-11	362	1.169934e-14
G0:0007059	chromosome segregation	1.659009e-08	142	2.028624e-11
G0:0000236	mitotic prometaphase	1.178690e-07	84	1.729553e-10

\$stats

		stat.mean	expl
G0:0007156	homophilic cell adhesion	3.824205	3.824205
G0:0002009	morphogenesis of an epithelium	3.653886	3.653886
G0:0048729	tissue morphogenesis	3.643242	3.643242
G0:0007610	behavior	3.565432	3.565432
G0:0060562	epithelial tube morphogenesis	3.261376	3.261376
G0:0035295	tube development	3.253665	3.253665

Reactome Analysis

We can use reactome via an R Package or use their relatively new website interface. Let's try to use the later.

It wants a list of our most interesting i.e. significant) genes in gene SYMBOL format.

```
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"
```

We will write our wee file so we can use them on the website:

```
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quo
```

an example figure from reactome online:

