

Lab_14

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Table of contents

```
library(DESeq2)
```

Load In the Data

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

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

```
#import countdata
```

```
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					

```
countData<- as.matrix(countData[,-1])
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

```
# 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

```
dds = DESeqDataSetFromMatrix(countData=countData,
                              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

```
dds
```

```
class: DESeqDataSet
```

```
dim: 15975 6
```

```
metadata(1): version
```

```
assays(4): counts mu H cooks
```

```
rownames(15975): ENSG00000279457 ENSG00000187634 ... ENSG00000276345
```

```
ENSG00000271254
```

```
rowData names(22): baseMean baseVar ... deviance maxCooks
```

```
colnames(6): SRR493366 SRR493367 ... SRR493370 SRR493371
```

```
colData names(2): condition sizeFactor
```

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

```
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%

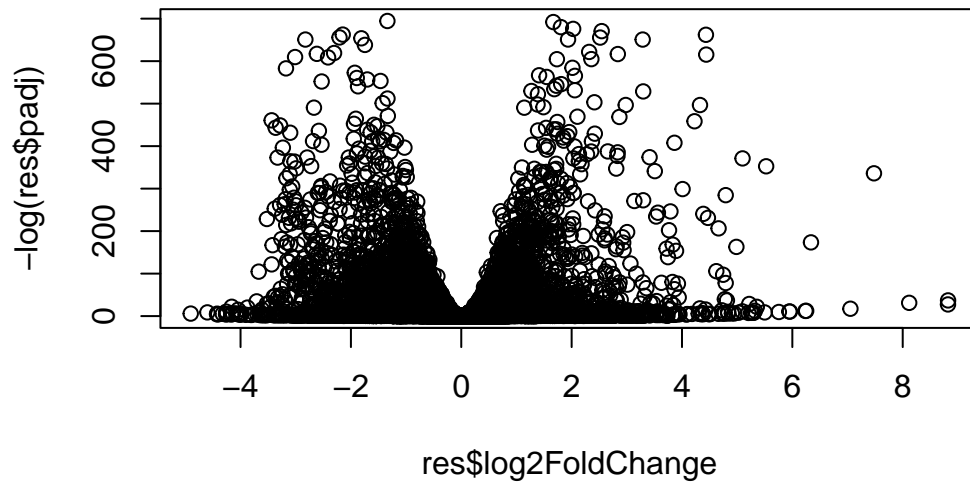
low counts [2] : 1237, 7.7%

(mean count < 0)

[1] see 'cooksCutoff' argument of ?results

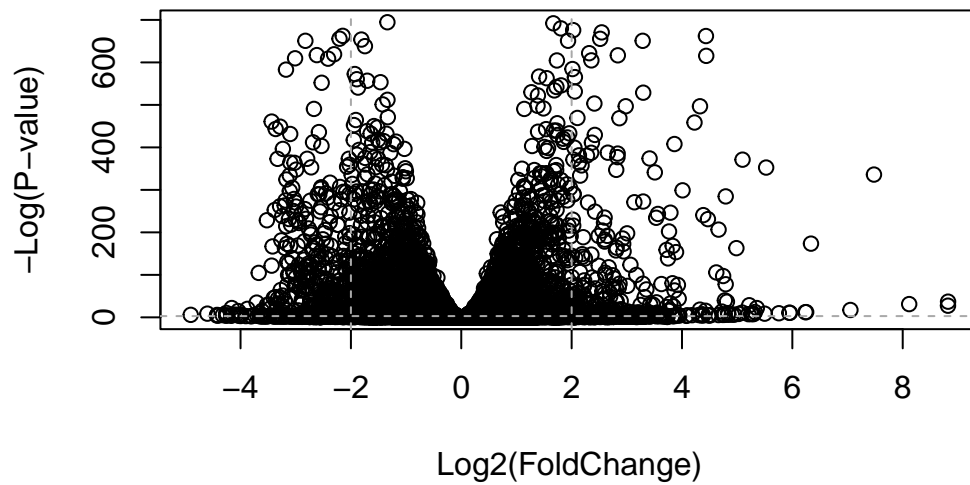
[2] see 'independentFiltering' argument of ?results

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



```
plot( res$log2FoldChange, -log(res$padj),  
      ylab="-Log(P-value)", xlab="Log2(FoldChange)")
```

```
# Add some cut-off lines  
abline(v=c(-2,2), col="darkgray", lty=2)  
abline(h=-log(0.05), col="darkgray", lty=2)
```

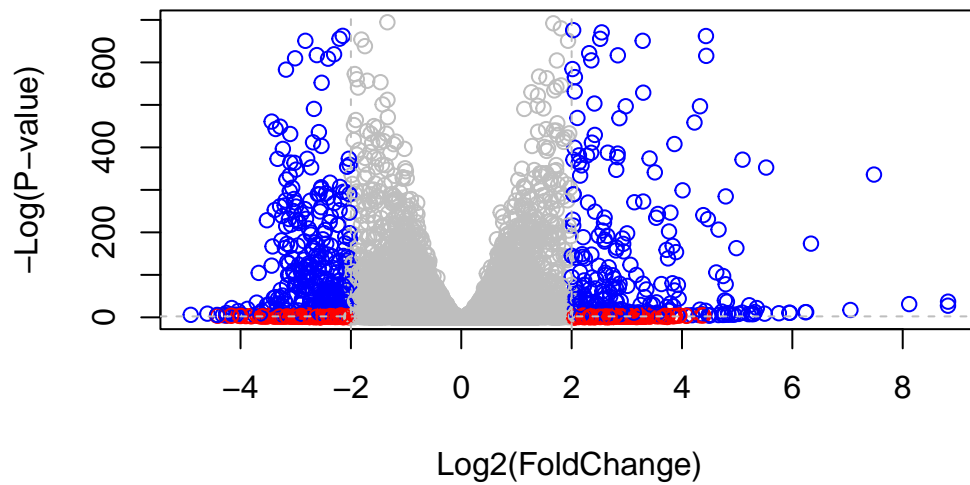


```
mycols <- rep("gray", nrow(res))
mycols[ abs(res$log2FoldChange) > 2 ] <- "red"

inds <- (res$padj < 0.01) & (abs(res$log2FoldChange) > 2 )
mycols[ inds ] <- "blue"

# Volcano plot with custom colors
plot( res$log2FoldChange, -log(res$padj),
      col=mycols, ylab="-Log(P-value)", xlab="Log2(FoldChange)" )

# Cut-off lines
abline(v=c(-2,2), col="gray", lty=2)
abline(h=-log(0.1), col="gray", 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=row.names(countData),
  keytype="ENSEMBL",
  column="SYMBOL",
  multiVals="first")
```

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

```
res$entrezid = mapIds(org.Hs.eg.db,  
                      keys=row.names(countData),  
                      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(countData),  
                  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	entrezid	name	
	<numeric>	<character>	<character>	<character>	
ENSG00000279457	6.86555e-01	WASH9P	102723897	WAS protein family h..	

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 ..

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

The `gag()` function wants a vector of importance in our case here it will be the fold-change values with associated entrez gene names

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

```
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

hsa04110 cell cycle

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

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

Info: Working in directory D:/BGGN213 Intro to Bioinformatics/R projects/Lab_14

Info: Writing image file hsa04110.pathview.png

Picture wasn't showing up, Could not get Pathview to work and only was able to get this resolved at the end of class after an hour of troubleshooting

```
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)

lapply(gobpres, head)
```

\$greater

	p.geomean	stat.mean	p.val
G0:0007156 homophilic cell adhesion	8.519724e-05	3.824205	8.519724e-05
G0:0002009 morphogenesis of an epithelium	1.396681e-04	3.653886	1.396681e-04
G0:0048729 tissue morphogenesis	1.432451e-04	3.643242	1.432451e-04
G0:0007610 behavior	2.195494e-04	3.530241	2.195494e-04
G0:0060562 epithelial tube morphogenesis	5.932837e-04	3.261376	5.932837e-04

G0:0035295	tube development	5.953254e-04	3.253665	5.953254e-04
		q.val	set.size	exp1
G0:0007156	homophilic cell adhesion	0.1951953	113	8.519724e-05
G0:0002009	morphogenesis of an epithelium	0.1951953	339	1.396681e-04
G0:0048729	tissue morphogenesis	0.1951953	424	1.432451e-04
G0:0007610	behavior	0.2243795	427	2.195494e-04
G0:0060562	epithelial tube morphogenesis	0.3711390	257	5.932837e-04
G0:0035295	tube development	0.3711390	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	exp1
G0:0048285	organelle fission	5.841698e-12	376	1.536227e-15
G0:0000280	nuclear division	5.841698e-12	352	4.286961e-15
G0:0007067	mitosis	5.841698e-12	352	4.286961e-15
G0:0000087	M phase of mitotic cell cycle	1.195672e-11	362	1.169934e-14
G0:0007059	chromosome segregation	1.658603e-08	142	2.028624e-11
G0:0000236	mitotic prometaphase	1.178402e-07	84	1.729553e-10

\$stats

		stat.mean	exp1
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	3.530241	3.530241
G0:0060562	epithelial tube morphogenesis	3.261376	3.261376
G0:0035295	tube development	3.253665	3.253665

```
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, quo
```