

Class 14: RNA-Seq analysis mini-project

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##Data Import

```
library(DESeq2)
```

```
colData <- read.csv("GSE37704_metadata.csv", 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("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				

##DESeq setup >Q. Complete the code below to remove the troublesome first column from countData

```
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

Q. Complete the code below to filter countData to exclude genes (i.e. rows) where we have 0 read count across all samples (i.e. columns). Tip: What will rowSums() of countData return and how could you use it in this context?

```
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

Q. How many genes do we still have left

```
nrow(countData)
```

```
[1] 15975
```

##DESeq analysis

```
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)
```

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, contrast=c("condition", "hoxa1_kd", "control_sirna"))
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

```

Q. Call the `summary()` function on your results to get a sense of how many genes are up or down-regulated at the default 0.1 p-value cutoff.

```
summary(res)
```

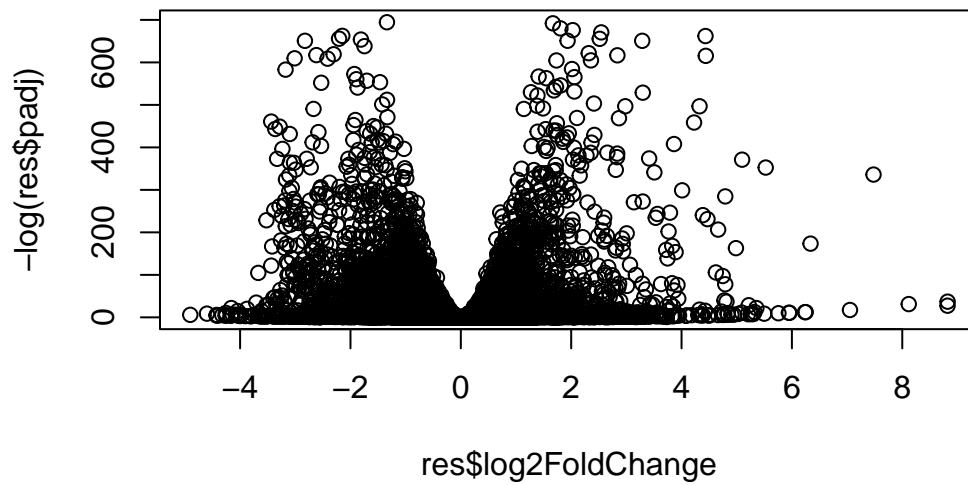
```

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

```

##Result visualization

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



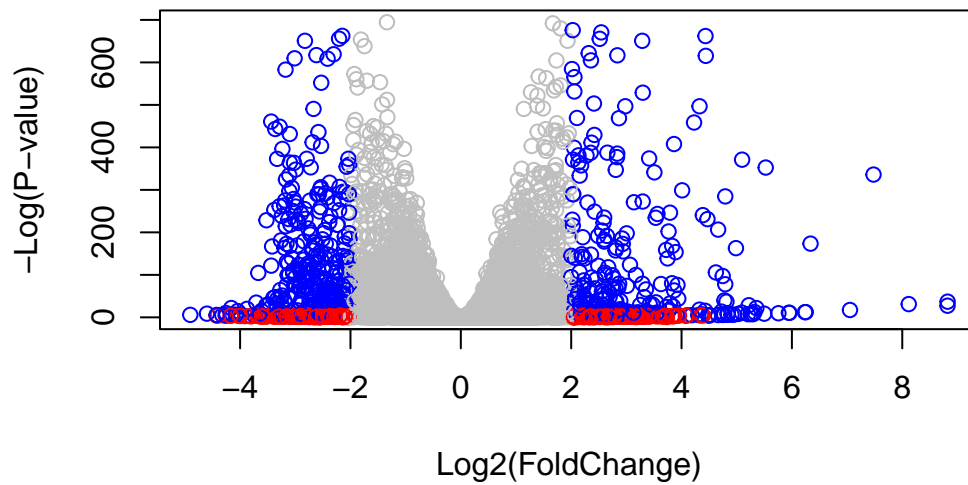
Q. Improve this plot by completing the below code, which adds color and axis labels

```
# 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-"
```



##Add Annotation

Q. Use the mapIDs() function multiple times to add SYMBOL, ENTREZID and GENENAME annotation to our results by completing the code below.

```
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(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	1.43989e-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	0.5215599	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 ..	

Q. Finally for this section let's reorder these results by adjusted p-value and save them to a CSV file in your current project directory.

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

##Pathway analysis

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

```
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)

```

```

      1266      54855      1465      51232      2034      2317
-2.422719  3.201955 -2.313738 -2.059631 -1.888019 -1.649792

```

```
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	1.375901e-03	-3.028500	1.375901e-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	0.073840037	144	1.375901e-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 /Users/sophiawang/Downloads/BIMM 143/Class 14: RNA-Seq analysis m

Info: Writing image file hsa04110.pathview.png


```
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	1.925222e-04	3.565432	1.925222e-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	0.1967577	426	1.925222e-04
G0:0060562	epithelial tube morphogenesis	0.3565320	257	5.932837e-04
G0:0035295	tube development	0.3565320	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	3.565432	3.565432
G0:0060562	epithelial tube morphogenesis	3.261376	3.261376

G0:0035295 tube development

3.253665 3.253665

##Reactome Analysis

```
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=
```

Q: What pathway has the most significant “Entities p-value”? Do the most significant pathways listed match your previous KEGG results? What factors could cause differences between the two methods?

Cell cycle. The most significant pathway matches with KEGG result. Reactome goes into more detailed pathways such as “cell cycle, mitotic”.

