Class 14

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Background

Today we will complete an RNASeq analysis from counts to pathways.

We will work with data on differential analysis of lung fibroblasts in response to loss of the developmental transcription factor HOXA1.

Data Import

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

A wee peak

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 correspondence of the columns of counts and the rows of metadata

```
colnames(counts)
```

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

[7] "SRR493371"

metadata\$id

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

We need to remove the first "length" column from our counts

```
countData <- counts[,-1]
colnames(countData) == metadata$id</pre>
```

[1] TRUE TRUE TRUE TRUE TRUE TRUE

Q. How many genes do we have?

nrow(countData)

[1] 19808

DESeq Setup

library(DESeq2)

Warning in DESeqDataSet(se, design = design, ignoreRank): some variables in design formula are characters, converting to factors

DESeq Analysis

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)</pre>

And a wee peak:

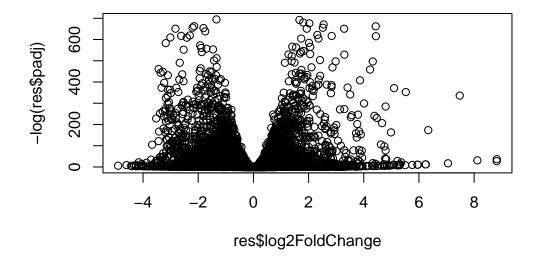
head(res)

 $\log 2$ 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

Data Tame with 6 10% and 6 columns					
	baseMean	log2FoldChange	lfcSE	stat	pvalue
	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>	<numeric></numeric>
ENSG00000186092	0.0000	NA	NA	NA	NA
ENSG00000279928	0.0000	NA	NA	NA	NA
ENSG00000279457	29.9136	0.179257	0.324822	0.551863	0.58104205
ENSG00000278566	0.0000	NA	NA	NA	NA
ENSG00000273547	0.0000	NA	NA	NA	NA
ENSG00000187634	183.2296	0.426457	0.140266	3.040350	0.00236304
	padj				
	<numeric></numeric>	•			
ENSG00000186092	NA	L			
ENSG00000279928	NA	L			
ENSG00000279457	0.68707978	3			
ENSG00000278566	NA	L			
ENSG00000273547	NA	L			
ENSG00000187634	0.00516278	3			

Result visualization

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



Add some color

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

```
[1]
                                  6.870798e-01
               NA
                              NA
                                                          NA
                                                                        NA
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                   1.767405e-35
                                  1.135361e-07
                                                9.189880e-01
                                                              4.038172e-01
[11]
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                                  5.093091e-01
                                                7.096740e-15
                                                              4.506618e-08
               NA
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                                                2.531300e-17
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                                                                        NA
```

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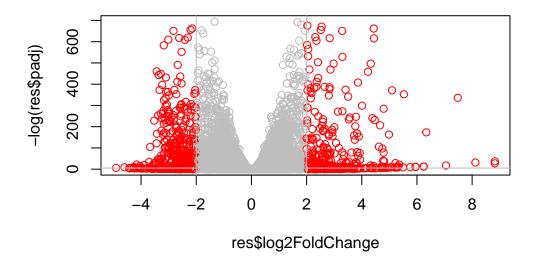
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plot(res$log2FoldChange, -log(res$padj), col=mycols)
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plot(res$log2FoldChange, -log(res$padj), col=mycols)
abline(v=c(-2,2), col="gray")
abline(h=-log(0.005), col="gray")
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Add Annotation

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

```
columns(org.Hs.eg.db)
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res$symbol <- mapIds(org.Hs.eg.db,</pre>
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'select()' returned 1:many mapping between keys and columns

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

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

```
head(res, 10)
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 $\log 2$ 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

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ENSG00000278566	0.0000	NA	NA	NA	NA
ENSG00000273547	0.0000	NA	NA	NA	NA
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
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ENSG00000279928	N.	A NA	NA		JA.
ENSG00000279457	6.87080e-0	1 NA	NA		JA.
ENSG00000278566	N.	A NA	NA		JA
ENSG00000273547	N.	A NA	NA	. 1	JA

ENSG00000187634	5.16278e-03	SAMD11	148398	148398
ENSG00000188976	1.76740e-35	NOC2L	26155	26155
ENSG00000187961	1.13536e-07	KLHL17	339451	339451
ENSG00000187583	9.18988e-01	PLEKHN1	84069	84069
ENSG00000187642	4.03817e-01	PERM1	84808	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).

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

```
foldchanges = res$log2FoldChange
names(foldchanges) = res$entrez
head(foldchanges)
```

```
79501 <NA> <NA> <NA> <NA> 148398
NA NA 0.1792571 NA NA 0.4264571
```

```
keggres = gage(foldchanges, gsets=kegg.sets.hs)
attributes(keggres)
```

\$names

[1] "greater" "less" "stats"

head(keggres\$less, 3)

				p.geomean	stat.mean
hsa04110	Cell cycle			7.077982e-06	-4.432593
hsa03030	DNA replication			9.424076e-05	-3.951803
hsa05130	Pathogenic Escherichia	coli	${\tt infection}$	1.076420e-04	-3.835716
				p.val	q.val
hsa04110	Cell cycle			7.077982e-06	0.001507610
hsa03030	DNA replication			9.424076e-05	0.007642585
hsa05130	Pathogenic Escherichia	coli	${\tt infection}$	1.076420e-04	0.007642585
				set.size	exp1
hsa04110	Cell cycle			124 7.07	77982e-06
hsa03030	DNA replication			36 9.42	24076e-05
hsa05130	Pathogenic Escherichia	coli	${\tt infection}$	55 1.07	76420e-04

head(keggres\$greater, 3)

			p.geomean	stat.mean
hsa04740	Olfactory transduction		6.512007e-08	5.345472
hsa04060	Cytokine-cytokine receptor in	nteraction	8.703597e-08	5.313429
hsa05323	Rheumatoid arthritis		4.392802e-05	4.030693
			p.val	q.val
hsa04740	Olfactory transduction		6.512007e-08	9.269331e-06
hsa04060	Cytokine-cytokine receptor in	nteraction	8.703597e-08	9.269331e-06
hsa05323	Rheumatoid arthritis		4.392802e-05	3.118889e-03
			set.size	exp1
hsa04740	Olfactory transduction		354 6.51	12007e-08
hsa04060	Cytokine-cytokine receptor in	nteraction	263 8.70)3597e-08
hsa05323	Rheumatoid arthritis		87 4.39	92802e-05

Generate pathway figures

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

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

Info: Working in directory /Users/noellim/Desktop/BIMM143/class14

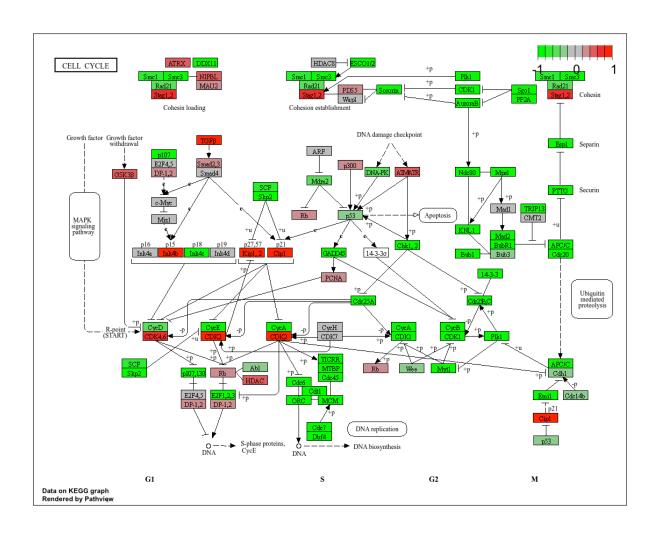
Info: Writing image file hsa04110.pathview.png

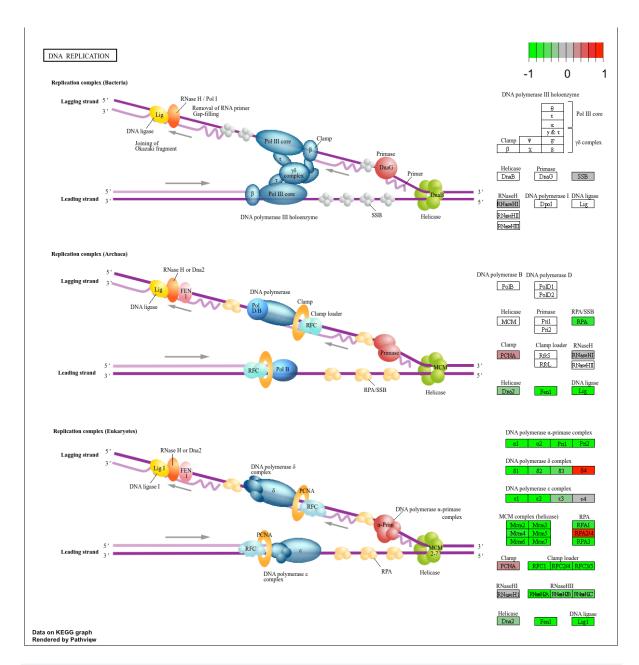
pathview(gene.data=foldchanges,
pathway.id = "hsa03030")
```

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

Info: Working in directory /Users/noellim/Desktop/BIMM143/class14

Info: Writing image file hsa03030.pathview.png





nrow(countData)

[1] 19808

nrow(res)

[1] 19808

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

```
head(gobpres$less)
```

```
p.geomean stat.mean
GO:0048285 organelle fission
                                        6.386337e-16 -8.175381 6.386337e-16
GO:0000280 nuclear division
                                        1.726380e-15 -8.056666 1.726380e-15
GO:0007067 mitosis
                                        1.726380e-15 -8.056666 1.726380e-15
GO:0000087 M phase of mitotic cell cycle 4.593581e-15 -7.919909 4.593581e-15
GO:0007059 chromosome segregation
                                        9.576332e-12 -6.994852 9.576332e-12
GO:0051301 cell division
                                        8.718528e-11 -6.455491 8.718528e-11
                                               q.val set.size
GO:0048285 organelle fission
                                        2.515911e-12
                                                          386 6.386337e-16
GO:0000280 nuclear division
                                                          362 1.726380e-15
                                        2.515911e-12
GO:0007067 mitosis
                                                          362 1.726380e-15
                                        2.515911e-12
GO:0000087 M phase of mitotic cell cycle 5.020784e-12
                                                          373 4.593581e-15
GO:0007059 chromosome segregation
                                        8.373545e-09
                                                          146 9.576332e-12
GO:0051301 cell division
                                        6.352901e-08
                                                          479 8.718528e-11
```

Reactome Analysis

```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
print(paste("Total number of significant genes:", length(sig_genes)))</pre>
```

[1] "Total number of significant genes: 8146"

```
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?

Most significant pathway is cell cycle (mitotic), they are slightly different from KEGG results (more specific)

Save Results

write.csv(res, file="myresults.csv")