Class 14: RNAseq mini project

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Background

The data for for hands-on session comes from GEO entry: GSE37704, which is associated with the following publication:

Trapnell C, Hendrickson DG, Sauvageau M, Goff L et al. "Differential analysis of gene regulation at transcript resolution with RNA-seq". Nat Biotechnol 2013 Jan;31(1):46-53. PMID: 23222703

The authors report on differential analysis of lung fibroblasts in response to loss of the developmental transcription factor HOXA1. Their results and others indicate that HOXA1 is required for lung fibroblast and HeLa cell cycle progression. In particular their analysis show that "loss of HOXA1 results in significant expression level changes in thousands of individual transcripts, along with isoform switching events in key regulators of the cell cycle".

Data Import

Reading in the counts and the metadata

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

Tidy and verify data

Q. How many genesa re in this dataset?

```
nrow(counts)
```

```
[1] 19808
```

Q. How many control and knockdown experiments are there?

```
table(metadata$condition)
```

```
control_sirna      hoxa1_kd
            3             3
```

Q. Does the `metadata` match the `countdata`

```
colnames(counts)
```

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

```
metadata$id
```

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

Does not match, counts has extra length column

Fix countdata to match metadata

```
newcounts <- counts[,-1]
```

```
colnames(newcounts) == metadata$id
```

```
[1] TRUE TRUE TRUE TRUE TRUE TRUE
```

Remove zero count genes

```
to.keep <- rowSums(newcounts) !=0
countData = newcounts[to.keep,]
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

PCA quality control

We can use `prcomp()` function

```
pc <- prcomp(t(countData), scale = T)
summary(pc)
```

```
Importance of components:
                          PC1     PC2      PC3      PC4      PC5       PC6
Standard deviation     87.7211 73.3196 32.89604 31.15094 29.18417 7.373e-13
Proportion of Variance  0.4817  0.3365  0.06774  0.06074  0.05332 0.000e+00
Cumulative Proportion   0.4817  0.8182  0.88594  0.94668  1.00000 1.000e+00
```

Make plot of PCA data

Color by "control" - blue and "knockdown" - red

```
metadata$condition
```

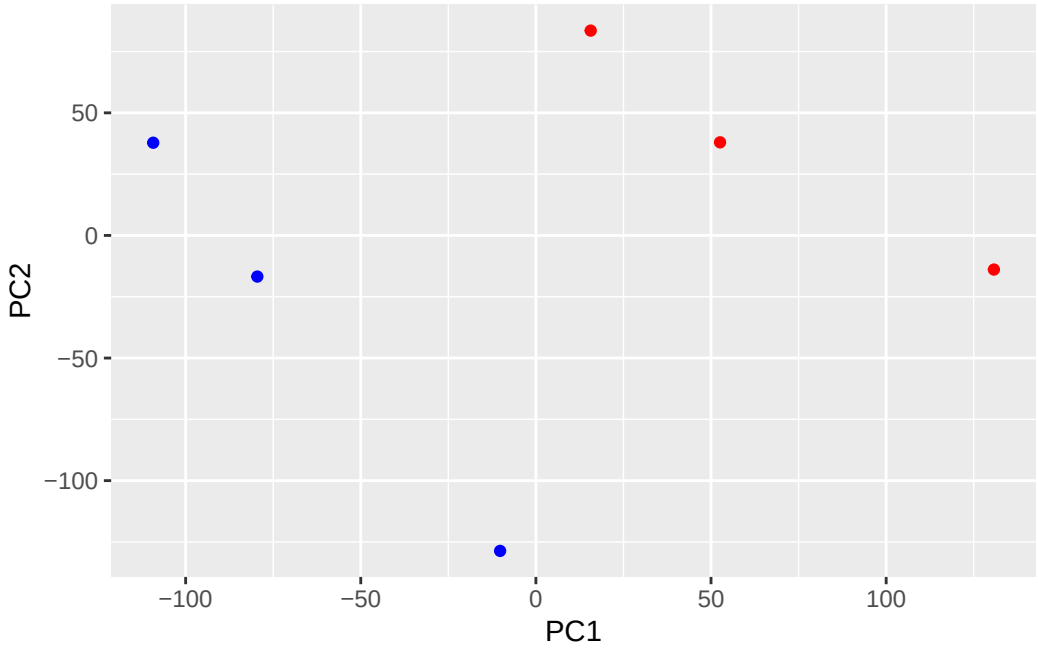
```
[1] "control_sirna" "control_sirna" "control_sirna" "hoxa1_kd"
[5] "hoxa1_kd"      "hoxa1_kd"
```

```
mycols <- c(rep("blue", 3), rep("red",3))
mycols
```

```
[1] "blue" "blue" "blue" "red"  "red"  "red"
```

```
library(ggplot2)

ggplot(pc$x) +
  aes(PC1, PC2) +
  geom_point(col=mycols)
```



Q. How many genes do we have left after filtering?

```
nrow(countData)
```

```
[1] 15975
```

DESeq analysis

```
library(DESeq2)
```

Set up the DESeq input object

```
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
```

Run DESeq

```
dds <- DESeq(dds)
```

```
estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing
```

Extract results

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

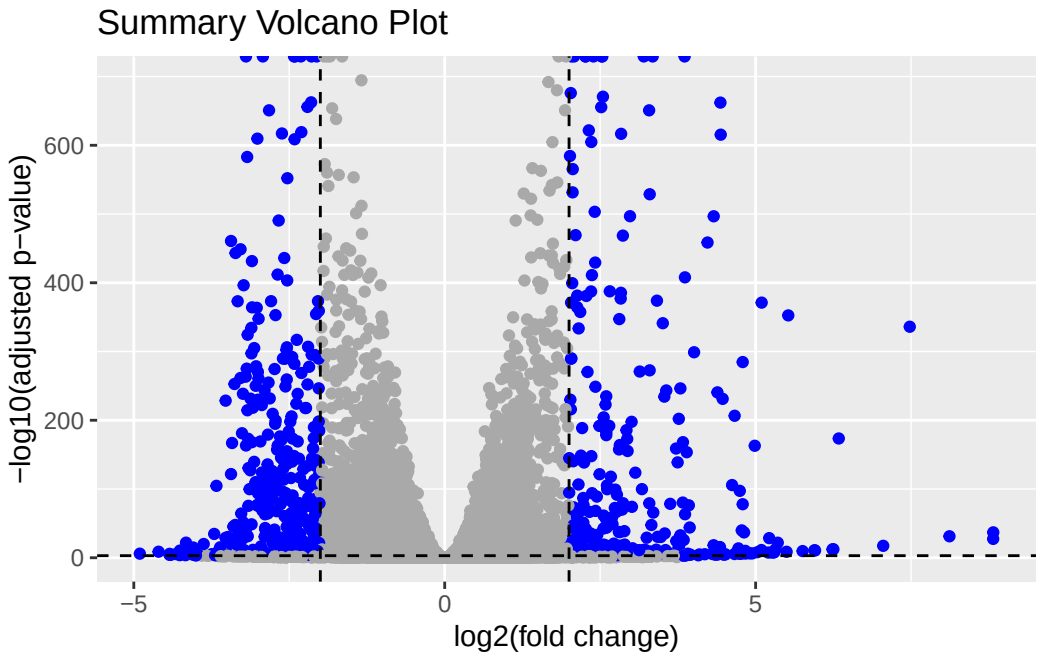
```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 6 rows and 6 columns
                 baseMean log2FoldChange     lfcSE       stat      pvalue
                <numeric>      <numeric> <numeric>  <numeric>   <numeric>
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 1.43990e-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 0.5215598   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

```
mycols <- rep("darkgray", nrow(res))
mycols[res$log2FoldChange >= 2] <- "blue"
mycols[res$log2FoldChange <= -2] <- "blue"
mycols[ res$padj >= 0.05] <- "darkgray"
```

```
ggplot(res) +
aes(log2FoldChange, -log(padj)) +
geom_point(col=mycols) +
labs(title = "Summary Volcano Plot") +
xlab( "log2(fold change)") +
ylab ("-log10(adjusted p-value)") +
geom_vline(xintercept = c(-2,2), col = "black", lty = 2) +
geom_hline (yintercept = -log(0.05), col = "black", lty =2)
```

```
Warning: Removed 1237 rows containing missing values or values outside the scale range
(`geom_point()`).
```



Add 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(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
```

```
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 8 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
	<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
ENSG00000188290	1.30538e-24	HES4	57801
ENSG00000187608	2.37452e-02	ISG15	9636
ENSG00000188157	4.21963e-16	AGRN	375790
ENSG00000237330	NA	RNF223	401934

Save results

```
write.csv(res, file = "class14results.csv")
```

Pathway analysis

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

KEGG

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

```
head(kegg.sets.hs, 1)
```

```
$`hsa00232 Caffeine metabolism`
[1] "10"   "1544" "1548" "1549" "1553" "7498" "9"
```

Make an input vector for `gage()` called `foldchanges` that has `names()` attribute set to ENTREZIDs

```
foldchanges <- res$log2FoldChange
names(foldchanges) <- res$entrez
```

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

```
attributes(keggres)
```

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

```
head(keggres$less, 2)
```

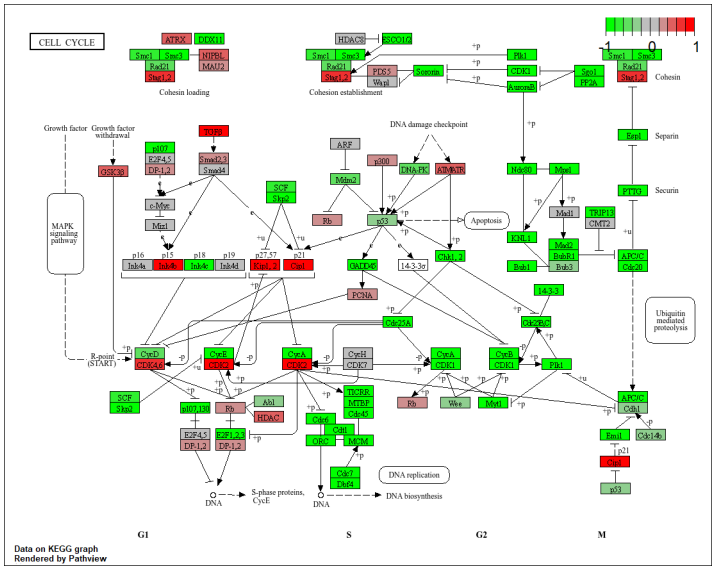
```
                          p.geomean stat.mean        p.val       q.val
hsa04110 Cell cycle      8.995727e-06 -4.378644 8.995727e-06 0.001889103
hsa03030 DNA replication 9.424076e-05 -3.951803 9.424076e-05 0.009841047
                         set.size         exp1
hsa04110 Cell cycle           121 8.995727e-06
hsa03030 DNA replication       36 9.424076e-05
```

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

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

```
Info: Working in directory C:/Users/yiyuw/Desktop/BGGN 213/class14
```

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

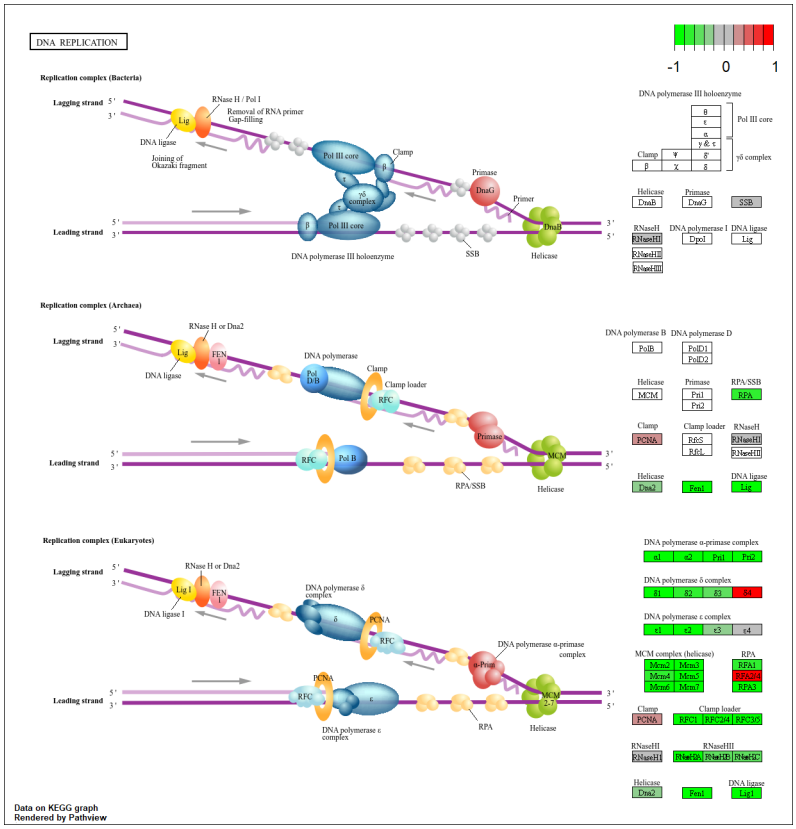



```
pathview(foldchanges, pathway.id = "hsa03030")
```

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

```
Info: Working in directory C:/Users/yiyuw/Desktop/BGGN 213/class14
```

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




```
head(keggres$greater, 2)
```

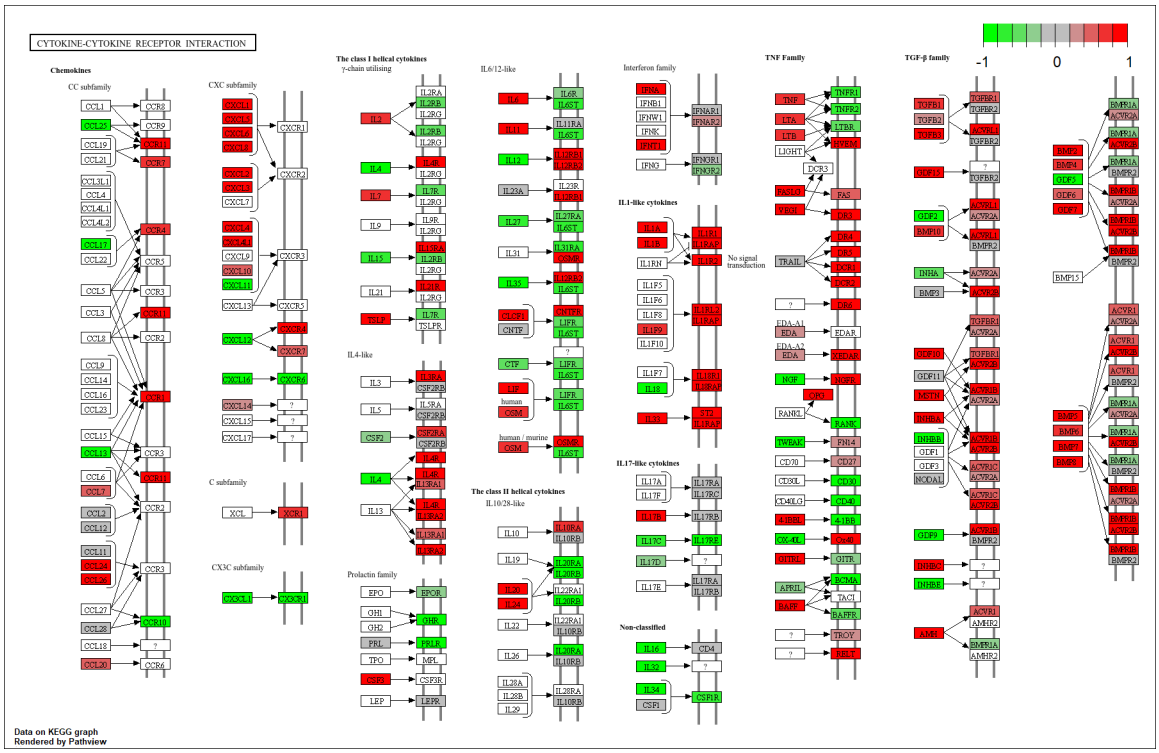
```
                                                    p.geomean stat.mean
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06  4.358967
hsa05323 Rheumatoid arthritis                   1.809824e-04  3.666793
                                                          p.val       q.val
hsa04060 Cytokine-cytokine receptor interaction 9.131044e-06 0.001917519
hsa05323 Rheumatoid arthritis                   1.809824e-04 0.019003147
                                                set.size         exp1
hsa04060 Cytokine-cytokine receptor interaction      177 9.131044e-06
hsa05323 Rheumatoid arthritis                         72 1.809824e-04
```

```
pathview(foldchanges, pathway.id = "hsa04060")
```

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

```
Info: Working in directory C:/Users/yiyuw/Desktop/BGGN 213/class14
```

```
Info: Writing image file hsa04060.pathview.png
```



GO 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)
```

```
head(gobpres$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
```

Reactome

We can use reactome via R or via their fancy new website interface. The web interface wants a set of ENTREZ ID values for your genes of interes.t Let's generate that.

```
inds <- abs(res$log2FoldChange)>=2 & res$padj <= 0.05
top.genes <- res$entrez[inds]
```

```
write.table(top.genes, file="top_genes.txt", row.names=FALSE, col.names=FALSE, quote=FALSE)
```