Class 14: RNA-Seq analysis mini project

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Table of contents

Background

The data for this 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". For our session we have used their Sailfish gene-level estimated counts and hence are restricted to protein-coding genes only

1

Data import

```
library(DESeq2)
```

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

Inspect and tidy data

Does the `counts` columns match the `colData` rows?

```
head(colData)
```

```
          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
```

```
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
```

Counts has a lot of data going on. The first column, "length" does not match the colData values.

```
colData$id
```

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

```
colnames(counts)
```

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

We need to remove the first column from counts to make it match up. Only run this once or it will keep taking away columns.

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

```
colnames(countData) == colData$id
```

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

> Q1. How many genes in total?

```
nrow(countData)
```

```
[1] 19808
```

> Q2. Filter to remove zero count genes (rows where the gene activity is zero in all columns)

```
#library(dplyr)
#countData %>% filter (rowSums(countData) == 0)

to.keep.inds <- rowSums(countData) >0
```

```
new.counts<- countData[to.keep.inds,]
nrow(new.counts)
```

```
[1] 15975
```

Setup for DESeq

Already called the function in by library above

```r
dds <- DESeqDataSetFromMatrix(countData = new.counts,
                              colData = colData,
                              design= ~condition)
```

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

Run DESeq

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

estimating size factors

estimating dispersions

gene-wise dispersion estimates

mean-dispersion relationship

final dispersion estimates

fitting model and testing

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

```r
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
```
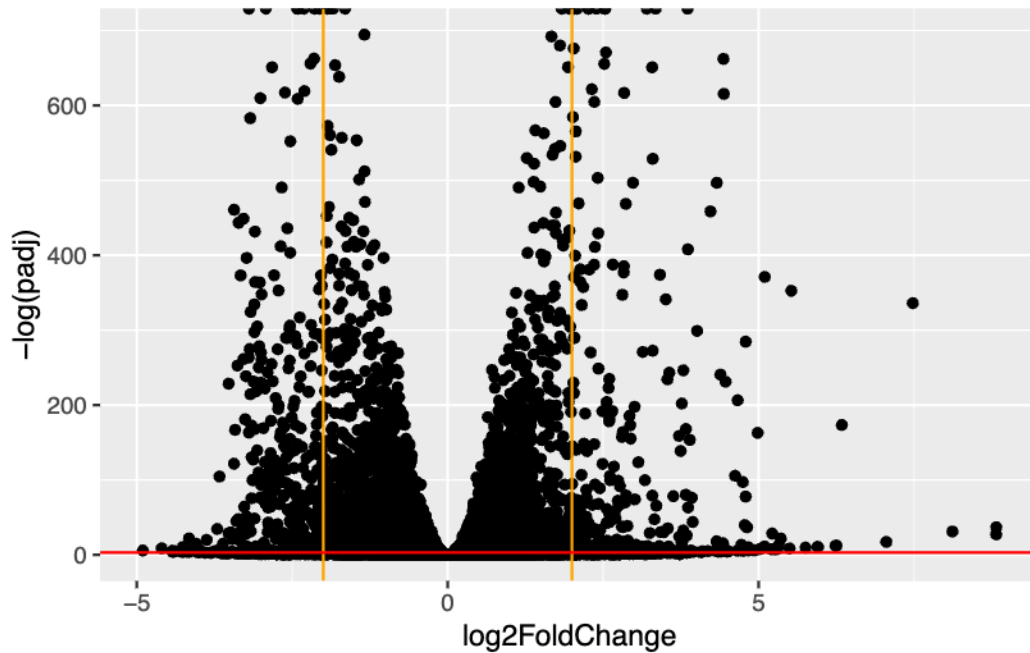
Volcano plot of results

```
library(ggplot2)
```

```
ggplot(res) +
      aes(log2FoldChange, -log(padj)) +
      geom_point() +
      geom_vline (xintercept= c(-2,2), col="orange")+
      geom_hline (yintercept = -log(0.05), col="red")
```

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

Gene annotation

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

```
res$symbol = mapIds(org.Hs.eg.db,
                    keys=rownames(res),
                    keytype="ENSEMBL",
                    column="SYMBOL")
```

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

```
res$entrez = mapIds(org.Hs.eg.db,
                    keys=rownames(res),
                    keytype="ENSEMBL",
                    column="ENTREZID")
```

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

```
res$name =    mapIds(org.Hs.eg.db,
                     keys=row.names(res),
                     keytype= "ENSEMBL",
                     column="GENENAME")
```

'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 ..
```

**Pathway analysis**

```r
library(gage)
```

```r
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).
############################################################################
```

Input vector for gage()

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

```r
data("kegg.sets.hs")
keggres = gage (foldchanges, gsets=kegg.sets.hs)
```

Cell cycle figure

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

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

```
Info: Working in directory /Users/dylanmullaney/Desktop/BIMM143/Class 14
```

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

MAPK Signaling Pathway

Let's look at a second pathway for Caffeine Metabolism

```
kegg.sets.hs$`hsa00232 Caffeine metabolism`
```

```
[1] "10"   "1544" "1548" "1549" "1553" "7498" "9"
```

```
pathview(foldchanges, pathway.id= "hsa00232")
```

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

```
Info: Working in directory /Users/dylanmullaney/Desktop/BIMM143/Class 14
```

```
Info: Writing image file hsa00232.pathview.png
```
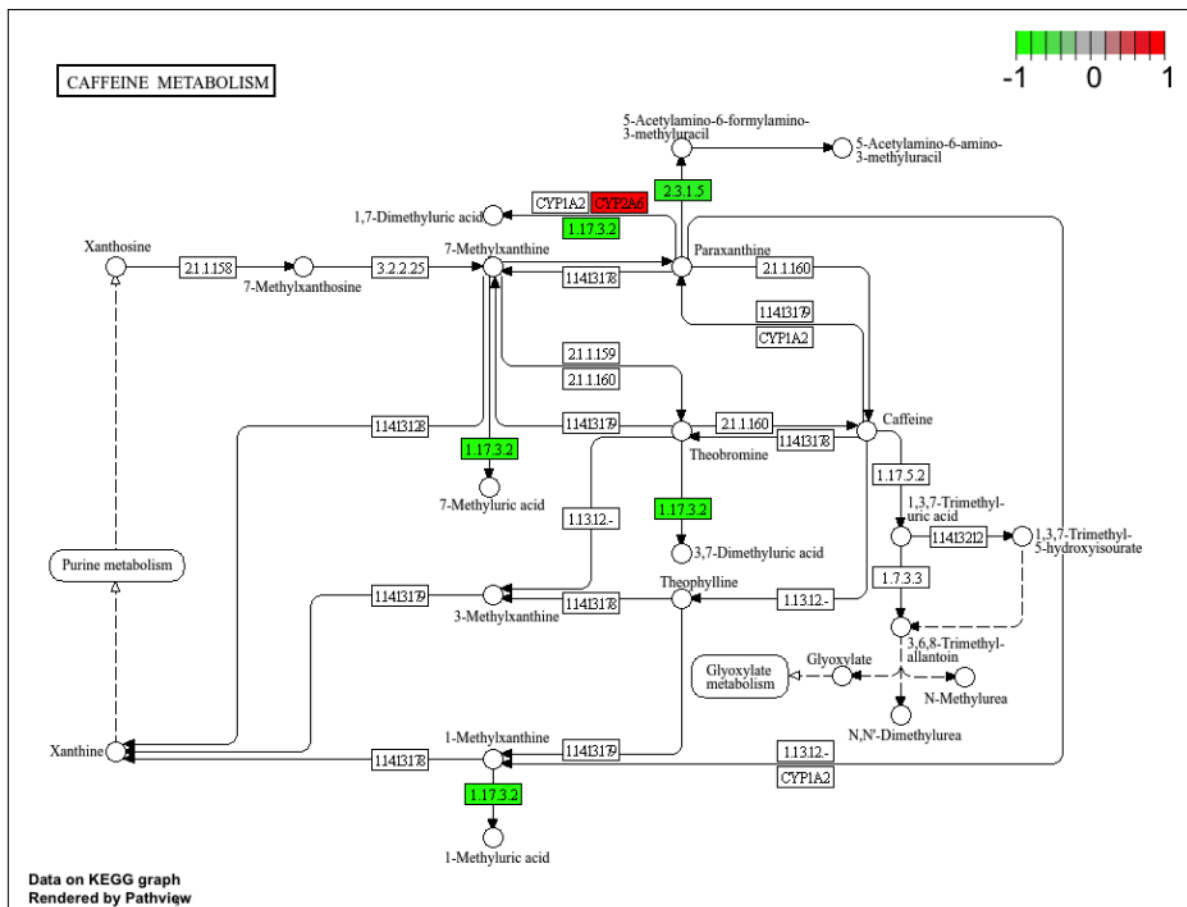
Okay one last pathway for primary immunodeficiency

```
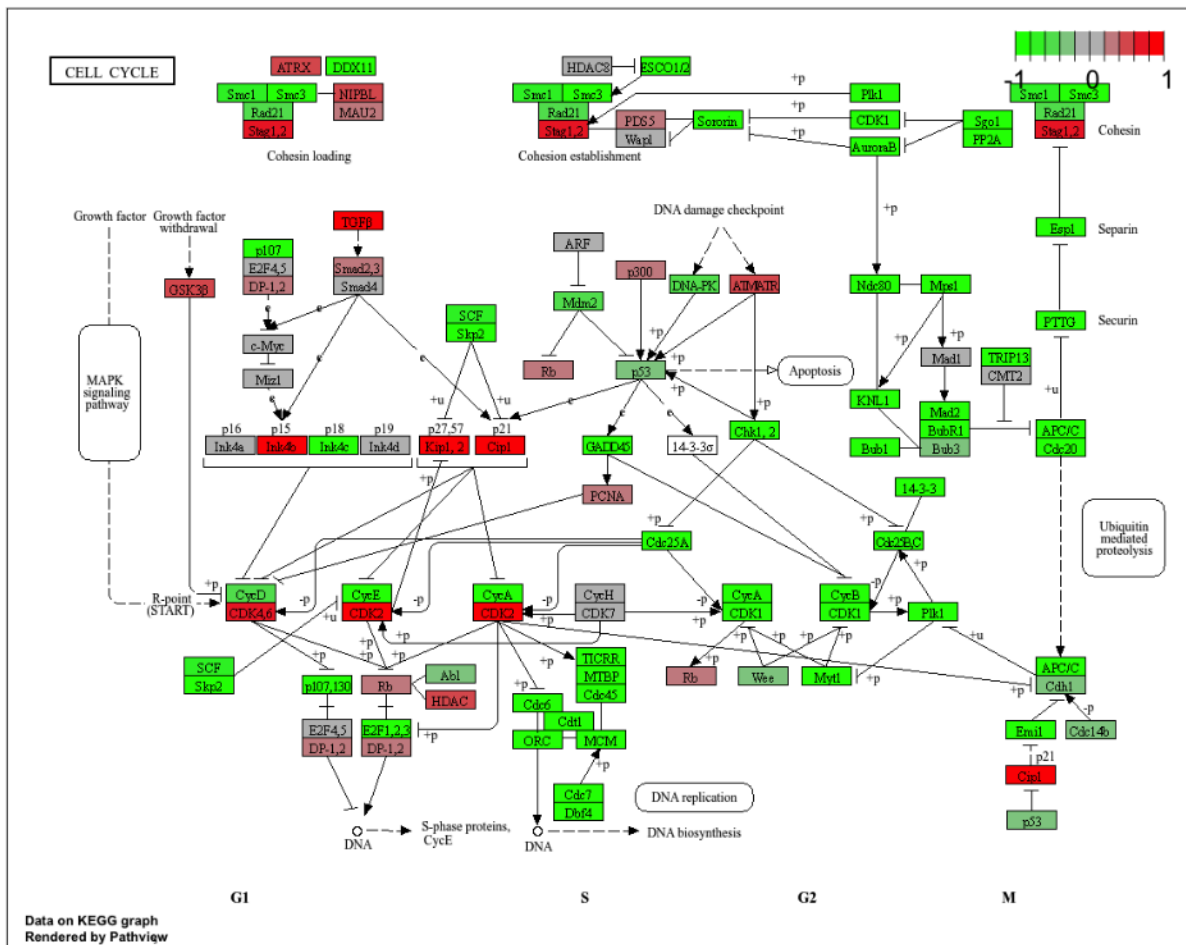pathview(foldchanges, pathway.id= "hsa05340")
```

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

Info: Working in directory /Users/dylanmullaney/Desktop/BIMM143/Class 14

Info: Writing image file hsa05340.pathview.png
```

10

Gene Ontology (GO)

Let's look at biological processes within gene ontology

```
data(go.sets.hs)
data(go.subs.hs)

#biological processes subset
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
```

11

```
GO:0007156 homophilic cell adhesion            0.1951953       113 8.519724e-05
GO:0002009 morphogenesis of an epithelium 0.1951953           339 1.396681e-04
GO:0048729 tissue morphogenesis              0.1951953         424 1.432451e-04
GO:0007610 behavior                          0.1967577         426 1.925222e-04
GO:0060562 epithelial tube morphogenesis     0.3565320         257 5.932837e-04
GO:0035295 tube development                   0.3565320        391 5.953254e-04

$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

$stats
                                         stat.mean     exp1
GO:0007156 homophilic cell adhesion       3.824205 3.824205
GO:0002009 morphogenesis of an epithelium 3.653886 3.653886
GO:0048729 tissue morphogenesis           3.643242 3.643242
GO:0007610 behavior                       3.565432 3.565432
GO:0060562 epithelial tube morphogenesis  3.261376 3.261376
GO:0035295 tube development               3.253665 3.253665
```

This divides into values greater than and values less than. I'm not sure exactly what I did here.

Reactome Analysis

Reactome is database consisting of biological molecules and their relation to pathways and processes (https://reactome.org/).

```
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?

The "Cel Cycle" has the most significant 'entities P-value'. Most of these pathways seem to align with the previous predictions. The cell growth and programmed cell death section feel like they could counteract each other.