Class 13: RNAseq mini project

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Background

Today we will run through a complete RNAseq analysis pipeline.

The data for 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.

Data import

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

Check correspondance of `metadata` and `counts`.

```
colnames(counts)
```

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

```
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
```

Right now the metadata id's and the counts columns are not a 1:1 correspondance.

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

```
Warning in colnames(counts) == metadata$id: longer object length is not a
multiple of shorter object length
```

```
[1] FALSE FALSE FALSE FALSE FALSE FALSE FALSE
```

```
colnames(counts)[-1] == metadata$id
```

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

Fix to remove that first "length" column of `counts`

```
counts <- counts[,-1]
head(counts, 3)
```

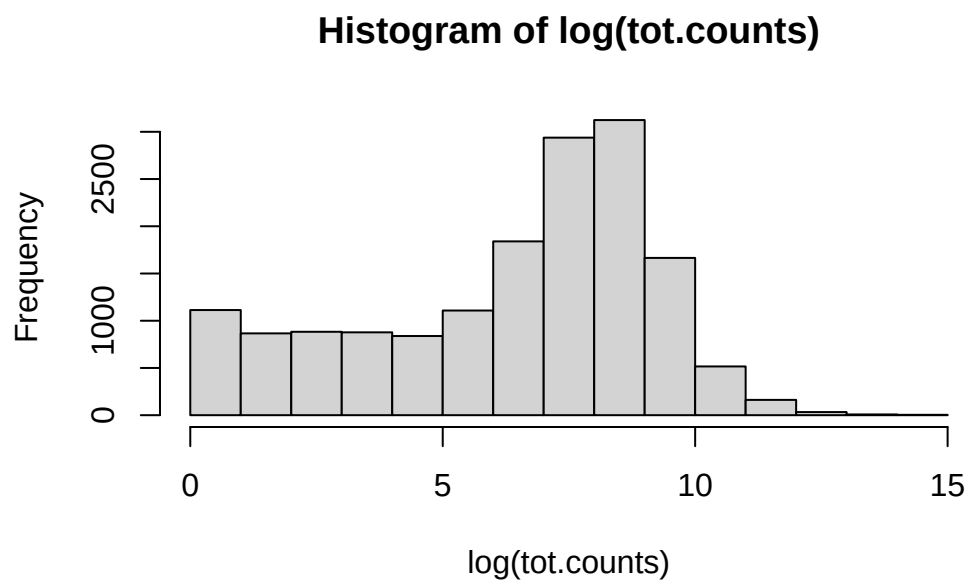
```
                SRR493366 SRR493367 SRR493368 SRR493369 SRR493370 SRR493371
ENSG00000186092         0         0         0         0         0         0
ENSG00000279928         0         0         0         0         0         0
ENSG00000279457        23        28        29        29        28        46
```

Also let's remove low count genes

```
tot.counts <- rowSums(counts)
head(tot.counts)
```

```
ENSG00000186092 ENSG00000279928 ENSG00000279457 ENSG00000278566 ENSG00000273547
              0               0             183               0               0
ENSG00000187634
           1129
```

```
hist(log(tot.counts),)
```



```
nonzero.inds <- tot.counts != 0
counts <- counts[nonzero.inds,]
```

```
# we can reuse this logical in the future
test_cols <- !all(colnames(counts) == metadata$id)
if (test_cols){
  message("there's an error")
} else {
  message("no error")
}
```

```
no error
```

Setup for DESeq

```
library(DESeq2)
```

```
Loading required package: S4Vectors
```

```
Loading required package: stats4
```

```
Loading required package: BiocGenerics
```

```
Loading required package: generics
```

```

Attaching package: 'generics'
```

```
The following objects are masked from 'package:base':

    as.difftime, as.factor, as.ordered, intersect, is.element, setdiff,
    setequal, union
```

```

Attaching package: 'BiocGenerics'
```

```
The following objects are masked from 'package:stats':

    IQR, mad, sd, var, xtabs

The following objects are masked from 'package:base':

    anyDuplicated, aperm, append, as.data.frame, basename, cbind,
    colnames, dirname, do.call, duplicated, eval, evalq, Filter, Find,
    get, grep, grepl, is.unsorted, lapply, Map, mapply, match, mget,
    order, paste, pmax, pmax.int, pmin, pmin.int, Position, rank,
    rbind, Reduce, rownames, sapply, saveRDS, table, tapply, unique,
    unsplit, which.max, which.min


Attaching package: 'S4Vectors'

The following object is masked from 'package:utils':

    findMatches

The following objects are masked from 'package:base':

    expand.grid, I, unname

Loading required package: IRanges

Loading required package: GenomicRanges

Loading required package: Seqinfo

Loading required package: SummarizedExperiment

Loading required package: MatrixGenerics

Loading required package: matrixStats


Attaching package: 'MatrixGenerics'
```

```
The following objects are masked from 'package:matrixStats':

    colAlls, colAnyNAs, colAnys, colAvgsPerRowSet, colCollapse,
    colCounts, colCummaxs, colCummins, colCumprods, colCumsums,
    colDiffs, colIQRDiffs, colIQRs, colLogSumExps, colMadDiffs,
    colMads, colMaxs, colMeans2, colMedians, colMins, colOrderStats,
    colProds, colQuantiles, colRanges, colRanks, colSdDiffs, colSds,
    colSums2, colTabulates, colVarDiffs, colVars, colWeightedMads,
    colWeightedMeans, colWeightedMedians, colWeightedSds,
    colWeightedVars, rowAlls, rowAnyNAs, rowAnys, rowAvgsPerColSet,
    rowCollapse, rowCounts, rowCummaxs, rowCummins, rowCumprods,
    rowCumsums, rowDiffs, rowIQRDiffs, rowIQRs, rowLogSumExps,
    rowMadDiffs, rowMads, rowMaxs, rowMeans2, rowMedians, rowMins,
    rowOrderStats, rowProds, rowQuantiles, rowRanges, rowRanks,
    rowSdDiffs, rowSds, rowSums2, rowTabulates, rowVarDiffs, rowVars,
    rowWeightedMads, rowWeightedMeans, rowWeightedMedians,
    rowWeightedSds, rowWeightedVars

Loading required package: Biobase

Welcome to Bioconductor

    Vignettes contain introductory material; view with
    'browseVignettes()'. To cite Bioconductor, see
    'citation("Biobase")', and for packages 'citation("pkgname")'.


Attaching package: 'Biobase'

The following object is masked from 'package:MatrixGenerics':

    rowMedians

The following objects are masked from 'package:matrixStats':

    anyMissing, rowMedians
```

```
dds <- DESeqDataSetFromMatrix(countData=counts,
                              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
```

Get results

```
res <- results(dds)
head(res,3)
```

```
log2 fold change (MLE): condition hoxa1 kd vs control sirna
Wald test p-value: condition hoxa1 kd vs control sirna
DataFrame with 3 rows and 6 columns
                 baseMean log2FoldChange     lfcSE       stat      pvalue
                <numeric>      <numeric> <numeric>  <numeric>   <numeric>
ENSG00000279457   29.9136       0.179257 0.3248215   0.551863 5.81042e-01
ENSG00000187634  183.2296       0.426457 0.1402658   3.040350 2.36304e-03
ENSG00000188976 1651.1881      -0.692720 0.0548465 -12.630156 1.43993e-36
                       padj
                  <numeric>
ENSG00000279457 6.86555e-01
ENSG00000187634 5.15718e-03
ENSG00000188976 1.76553e-35
```

Add annotation

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

```
res$entrezid <- 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$entrezid)
```

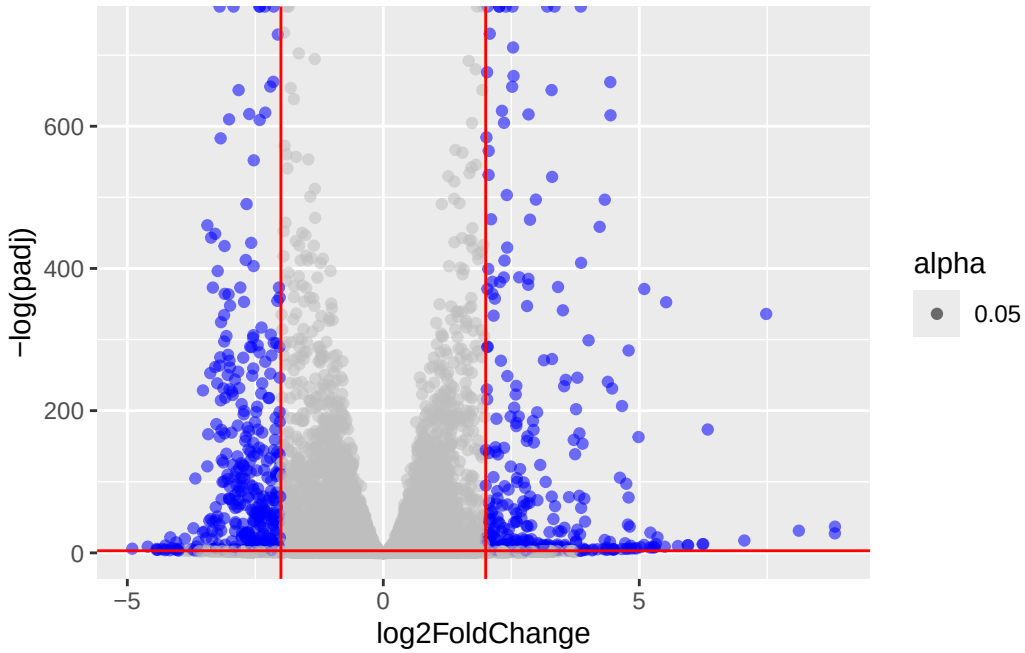
```
ENSG00000279457 ENSG00000187634 ENSG00000188976 ENSG00000187961 ENSG00000187583
             NA        "148398"         "26155"        "339451"         "84069"
ENSG00000187642
        "84808"
```

Visualize results

```
library(ggplot2)
mycols <- rep("gray", nrow(res))
mycols[abs(res$log2FoldChange) >= 2 & res$padj<0.05] <- "blue"

ggplot(res) + aes(x=log2FoldChange, y=-log(padj), alpha = 0.05) + geom_point(col=mycols) + g
```

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



##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)

# Examine the first 2 pathways in this kegg set for humans
head(kegg.sets.hs, 2)
```

```
$`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"
```

```
foldchanges <- res$log2FoldChange
names(foldchanges) <- res$entrezid
head(foldchanges)
```

```
       <NA>      148398       26155      339451       84069       84808
 0.17925708  0.42645712 -0.69272046  0.72975561  0.04057653  0.54281049
```

```
keggres = gage(foldchanges, 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 0.065461280
hsa03440 Homologous recombination           3.066756e-03 0.128803765
```

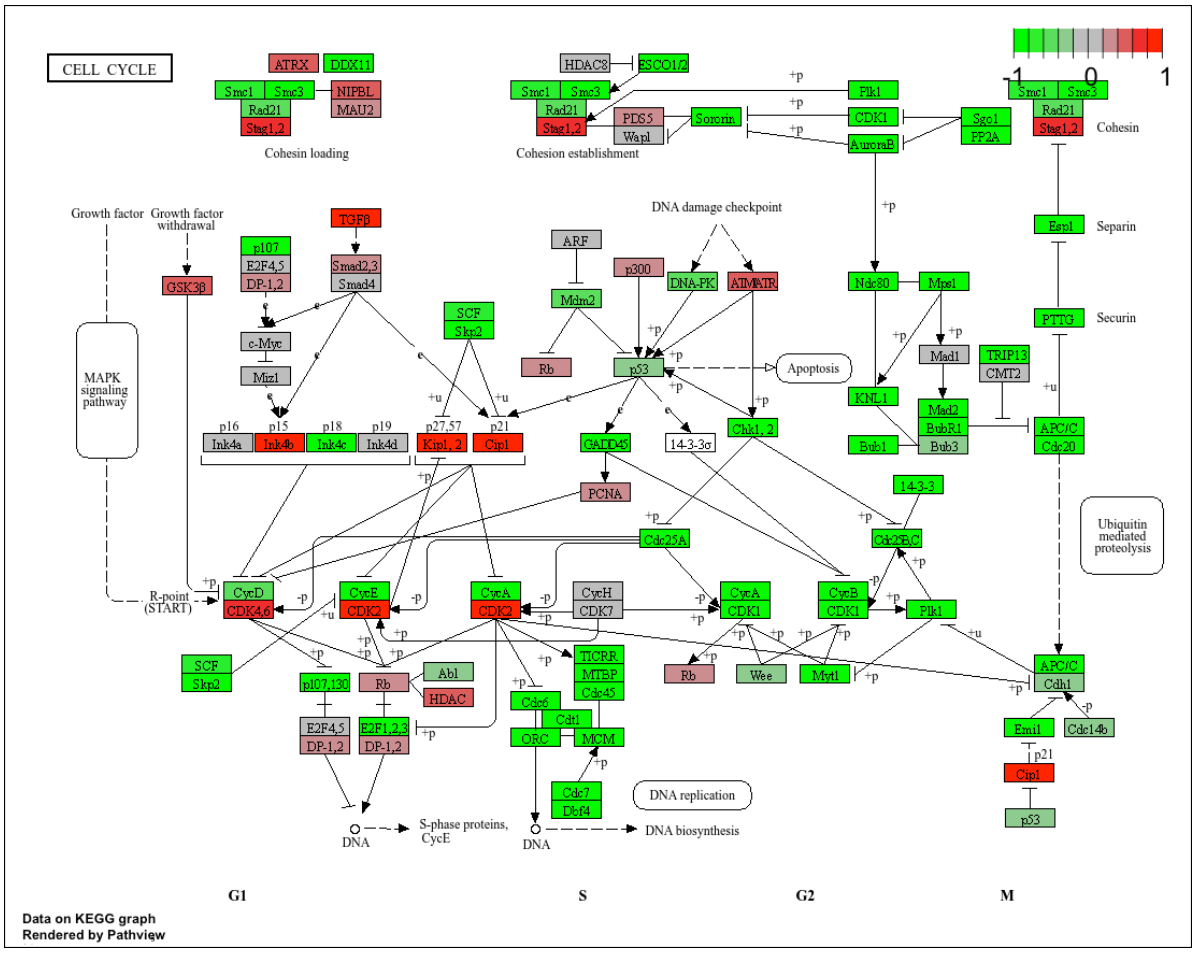
```
hsa04114 Oocyte meiosis                          3.784520e-03 0.132458190
                                                 set.size         exp1
hsa04110 Cell cycle                                   121 8.995727e-06
hsa03030 DNA replication                               36 9.424076e-05
hsa05130 Pathogenic 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
```

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

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

```
Info: Working in directory /Users/emilychase/Desktop/phd/bggn213/class13_RNAseqminiproj
```

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



GO analysis

```
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

Some folks really like Reactome online (ie their webpage viewer) rather than the R package of the same name (can get from bioconductor).

To use the website viewer we want to upload our set of gene symbols for the genes we want to focus on (here those with a P-value<0.05)

```
res$symbol <- mapIds(org.Hs.eg.db,
                     keys=row.names(res),
                     keytype="ENSEMBL",
                     column="SYMBOL",
                     multiVals="first")
```

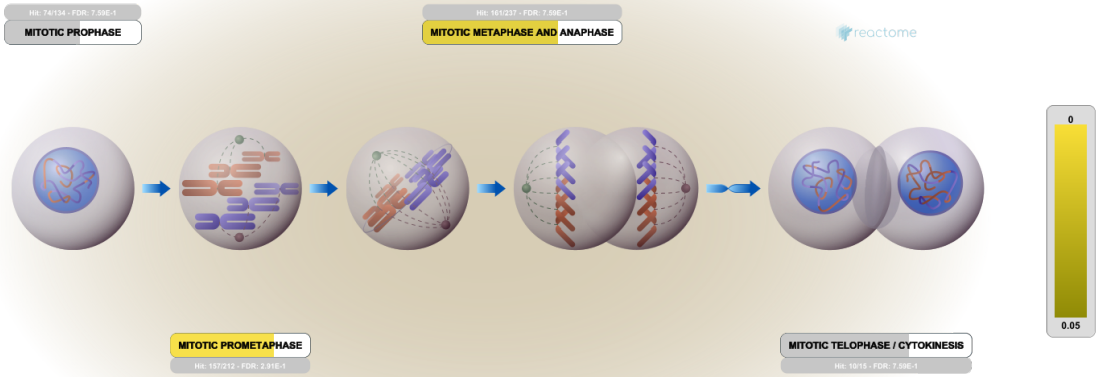
```
'select()' returned 1:many mapping between keys and columns
```

```
head(res$symbol)
```

```
ENSG00000279457 ENSG00000187634 ENSG00000188976 ENSG00000187961 ENSG00000187583
             NA        "SAMD11"         "NOC2L"        "KLHL17"       "PLEKHN1"
ENSG00000187642
        "PERM1"
```

```
sig_genes <- res[res$padj <= 0.05 & !is.na(res$padj), "symbol"]
write.table(sig_genes, file="significant_genes.txt", row.names=FALSE, col.names=FALSE, quote=
```

Then, to perform pathway analysis online go to the Reactome website (https://reactome.org/PathwayBrowser/# Select "choose file" to upload your significant gene list. Then, select the parameters "Project to Humans", then click "Analyze".



Save results

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

#another option
# save(res, file="my_results.RData") #save as an R object to be loaded back into R
```