mini RNASeq Project

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Background

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

Loading libraries

```r
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: GenomeInfoDb

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
```

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

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

```
library(gage)
```

```
library(gageData)
library(ggplot2)
```

Data Import

```
metaFile <- "GSE37704_metadata.csv"
countFile <- "GSE37704_featurecounts.csv"

metadata = read.csv(metaFile)
counts = read.csv(countFile, row.names=1)
```

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

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

Also lets remove low count genes

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

Let's remove low count genes

```
threshold = 0
del.inds <- tot.counts > threshold
counts <- counts[del.inds,]
```

Check correspondance of `metadata` and `counts` (columns in counts matches rows of metadata)

```
test_cols<-!all(colnames(counts)==metadata$id)
```

```
if(test_cols){
  message("Your metadata and counts do not match")
  break
}
```

Setup for DESeq

```
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
```

Run DESeq

```
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)
```

Add annotation

```
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 0.3248215   0.551863 5.81042e-01
ENSG00000187634  183.229650      0.4264571 0.1402658   3.040350 2.36304e-03
ENSG00000188976 1651.188076     -0.6927205 0.0548465 -12.630156 1.43993e-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
```

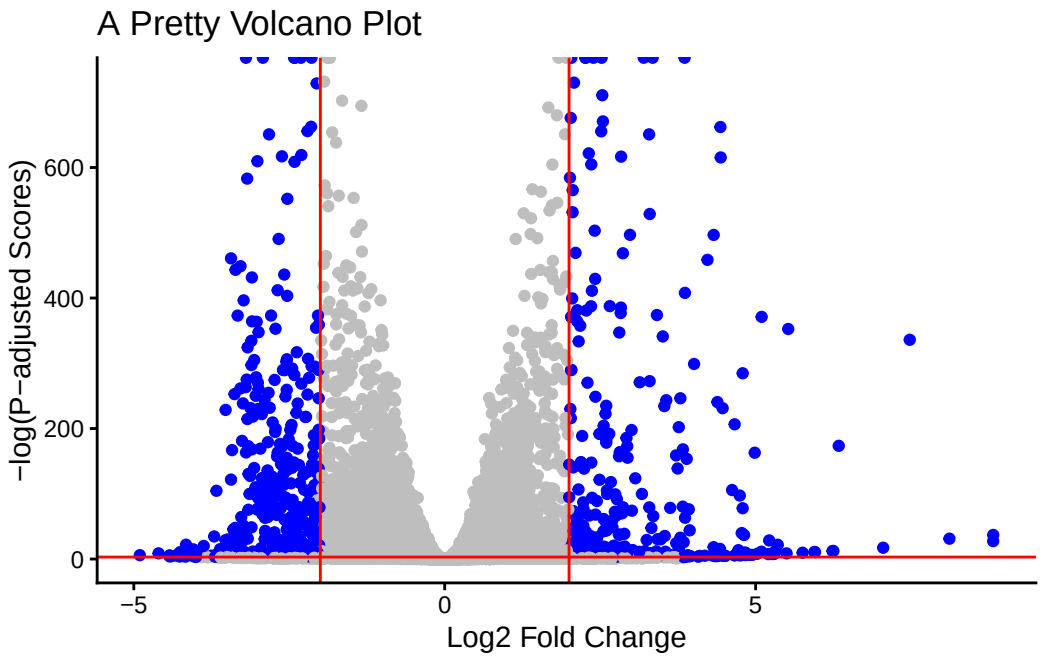
```
ENSG00000187642   11.979750      0.5428105 0.5215598   1.040744 2.97994e-01
ENSG00000188290  108.922128      2.0570638 0.1969053  10.446970 1.51281e-25
ENSG00000187608  350.716868      0.2573837 0.1027266   2.505522 1.22271e-02
ENSG00000188157 9128.439422      0.3899088 0.0467164   8.346302 7.04333e-17
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 1.76553e-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 4.21970e-16        AGRN      375790
ENSG00000237330          NA      RNF223      401934
```

Visualize results

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

ggplot(res) +
  aes(log2FoldChange, -log(padj)) +
  geom_point(col = my_cols) +
  geom_vline(xintercept = c(-2,2), col="red") +
  geom_hline(yintercept = -log(0.05), col="red") +
  labs(x = "Log2 Fold Change",
y = "-log(P-adjusted Scores)",
title = "A Pretty Volcano Plot") +
  theme_classic()
```

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



Pathway analysis

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

For **gage** we want a named vector of importance

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

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

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

```
Info: Working in directory /Users/brad/UCSD/Bioinformatics (BGGN 213)/mini RNASeq project
```

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

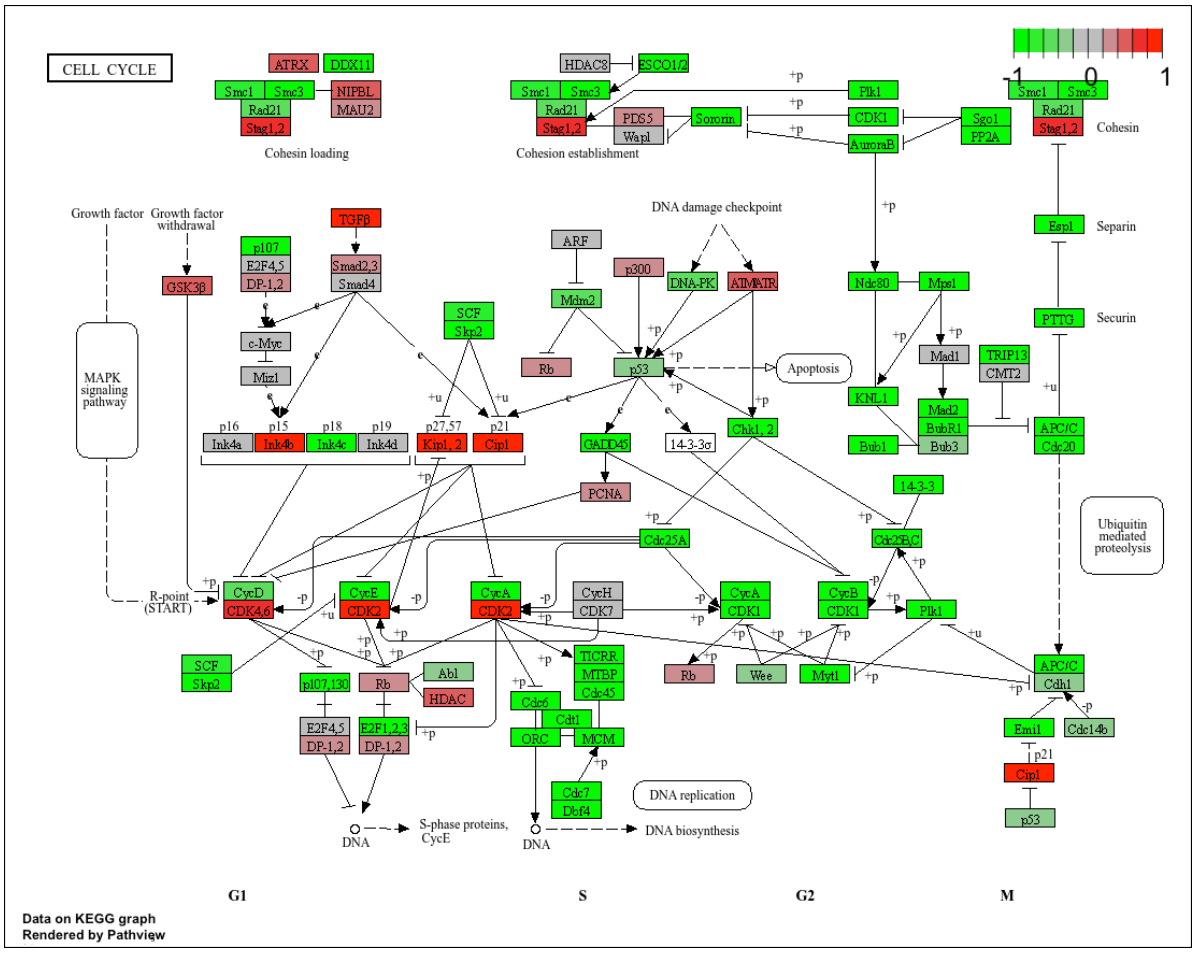


Figure 1: "Cool pathway picture"

GO Analysis

Let's try GO analysis and compare to KEGG 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 people really like Reactome online (webpage) rather than the R package of the same name.

To use the website viewer we want to upload our set of gene symbols for the genes we want to focus on.

```
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=
```

Save results

```
write.csv(res, file="myresults.csv")
save(res, file="my_results.RData")
```