Recruitment Data Challenge

The Bioinformatics & Biostatistics Group @ The Francis Crick Institute

Introduction

Here you will find the data from an RNA-Seq and ATAC-Seq experiment. Both experiments have the same design. There is a treatment and control group each containing three replicates making a total of six samples per experiment. The data files are defined as follows (all files are tab delimited text files):

RNA-Seq Data

- **rnaseq_design.txt**: Sample ids and corresponding condition labels.
- **rnaseq_gene_counts.txt**: Raw (not normalised) gene-level read counts for each sample.
- **rnaseq_annotation.txt**: Gene level annotation.

ATAC-Seq Data

- **atacseq_design.txt**: Sample ids and corresponding condition labels.
- **atacseq_peak_counts.txt**: Raw (not normalised) ATAC-Seq peak level counts for each sample.
- **atacseq_peaks.bed**: A bed file defining the peak loci

All sequence data were aligned to the human genome reference hg38.

The Challenge

The treatment here is thought to activate a transcriptional program via remodelling of the chromatin architecture. The aim here is to:
1. Identify genes that may be regulated in this fashion.
2. Identify the possible transcriptional programs involved.
3. Present candidate transcription factors that may be responsible for the underlying regulation.

Please produce a 20 minute presentation detailing your exploration of the data, your analysis approach and findings?

Analysis

Strategy

1. Identify genes with significant changes in expression.
2. Identify zones with significant changes in accessibility.
3. Detect hotspots in accessibility changes over gene regulatory areas of differentially expressed genes.
4. Detect enriched TF motifs in zones presenting accessibility changes.
5. Detect enriched TF motifs in hotspots.
6. Perform GO Analysis to put genes in context.

GO Analysis with clusterProfiler

[1]
```
library(clusterProfiler)
library("org.Hs.eg.db")
```

```
Registered S3 method overwritten by 'enrichplot':
  method               from
  fortify.enrichResult DOSE

clusterProfiler v3.12.0  For help:
https://guangchuangyu.github.io/software/clusterProfiler

If you use clusterProfiler in published research, please cite:
Guangchuang Yu, Li-Gen Wang, Yanyan Han, Qing-Yu He. clusterProfiler: an
R package for comparing biological themes among gene clusters. OMICS: A
Journal of Integrative Biology. 2012, 16(5):284-287.

Loading required package: AnnotationDbi

Loading required package: stats4

Loading required package: BiocGenerics

Loading required package: parallel


Attaching package: 'BiocGenerics'


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

    clusterApply, clusterApplyLB, clusterCall, clusterEvalQ,
    clusterExport, clusterMap, parApply, parCapply, parLapply,
    parLapplyLB, parRapply, parSapply, parSapplyLB
```

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

    IQR, mad, sd, var, xtabs


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

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


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")'.


Loading required package: IRanges

Loading required package: S4Vectors


Attaching package: 'S4Vectors'


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

    expand.grid
```

Re-load files if necessary:

[2]
```
rna_diffx <- read.table(file="output/rna_diffx.bed", sep =
"\t",col.names =
c("chr","fstart","fend","name","score","strand"))
rna_cts <-
as.matrix(read.csv("data_challenge/rnaseq_gene_counts.txt",sep="\
t",row.names="featureid"))
```

```
rna_up <- read.table(file="output/rna_up.bed", sep = "\t",
col.names = c("chr","fstart","fend","name","score","strand"))
rna_dw <- read.table(file="output/rna_dw.bed", sep = "\t",
col.names = c("chr","fstart","fend","name","score","strand"))
head(rna_diffx,2)
head(rna_cts,2)
```

A data.frame: 2 × 6

chr	fstart	fend	name	score	strand
<fct>	<int>	<int>	<fct>	<dbl>	<fct>
1	169853073	169888888	ENSG00000000457	0.4596662	-
1	27612668	27626569	ENSG00000000938	1.9510985	-

A matrix: 2 × 6 of type int

	s69	s70	s71	s75	s76	s77
ENSG00000000003	1	1	0	8	2	1
ENSG00000000005	0	0	0	0	0	0

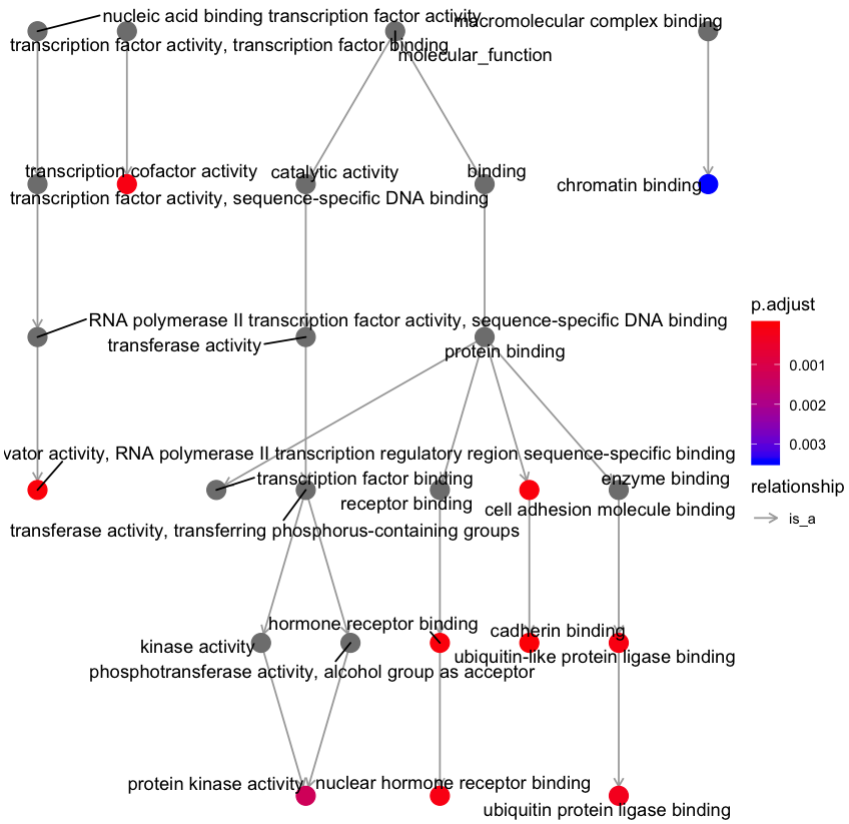
GO Analysis for Up/Down Regulated Gene Sets

[3]
```
rna_bgd <- row.names(rna_cts[rowSums(rna_cts) > 0,])
rna_up_list <- as.vector(rna_up$name)
rna_dw_list <- as.vector(rna_dw$name)
rna_up_ego <- enrichGO(gene = rna_up_list, universe = rna_bgd,
OrgDb = org.Hs.eg.db, keyType = "ENSEMBL")
rna_dw_ego <- enrichGO(gene = rna_dw_list, universe = rna_bgd,
OrgDb = org.Hs.eg.db, keyType = "ENSEMBL")
```

[4]
```
rna_up_ego_gn <- setReadable(rna_up_ego, 'org.Hs.eg.db',
'ENSEMBL')
rna_dw_ego_gn <- setReadable(rna_dw_ego, 'org.Hs.eg.db',
'ENSEMBL')
```

[5]
```
goplot(rna_up_ego_gn)
goplot(rna_dw_ego_gn)
png("output/plot/rna_up_goplot.png", width = 1600, height = 1600)
goplot(rna_up_ego_gn)
dev.off()
png("output/plot/rna_dw_goplot.png", width = 1600, height = 1600)
goplot(rna_dw_ego_gn)
```

```
dev.off()
```



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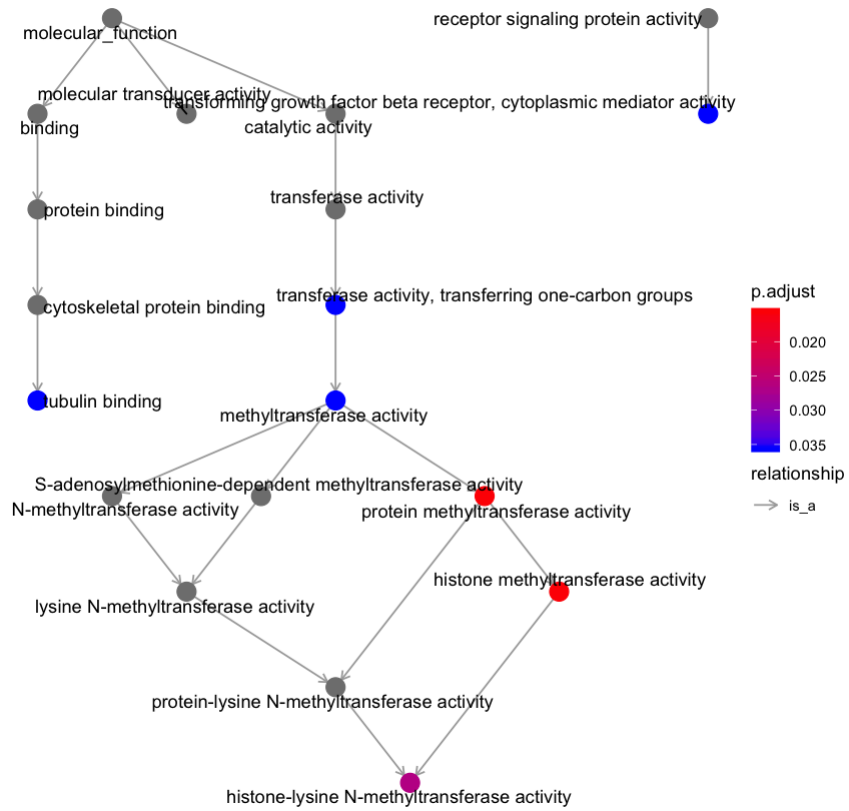
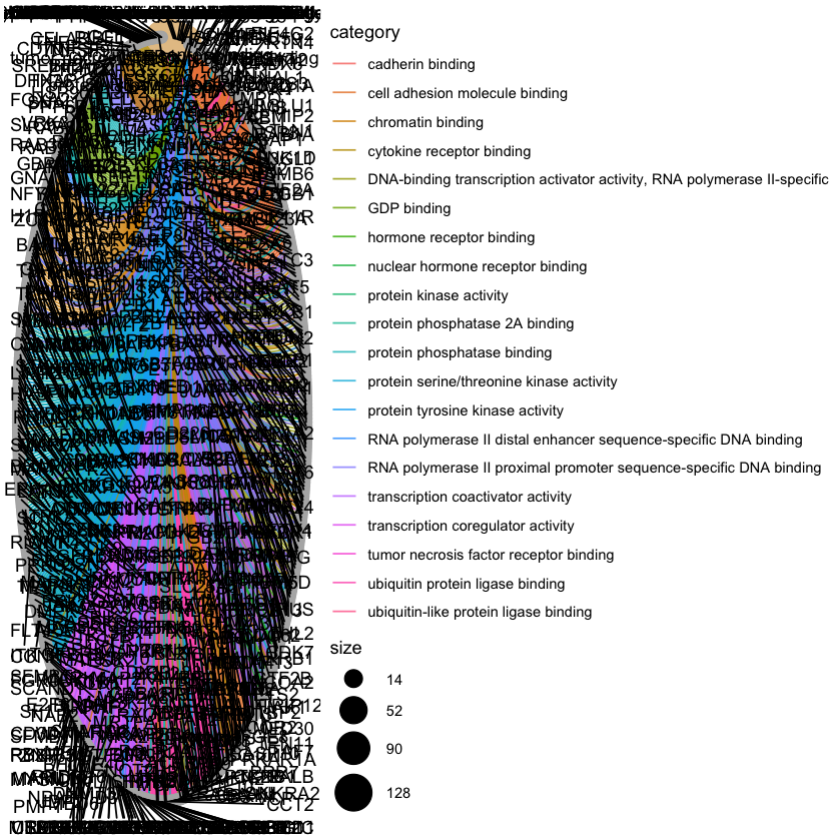


[6]
```
cnetplot(rna_up_ego_gn, circular=TRUE, colorEdge=TRUE,
showCategory = 20)
cnetplot(rna_dw_ego_gn, circular=TRUE, colorEdge=TRUE,
showCategory = 20)
```

```
png("output/plot/rna_up_cnet.png", width = 1600, height = 1600)
cnetplot(rna_up_ego_gn, circular=TRUE, colorEdge=TRUE,
showCategory = 20)
dev.off()
png("output/plot/rna_dw_cnet.png", width = 1600, height = 1600)
cnetplot(rna_dw_ego_gn, circular=TRUE, colorEdge=TRUE,
showCategory = 20)
dev.off()
```



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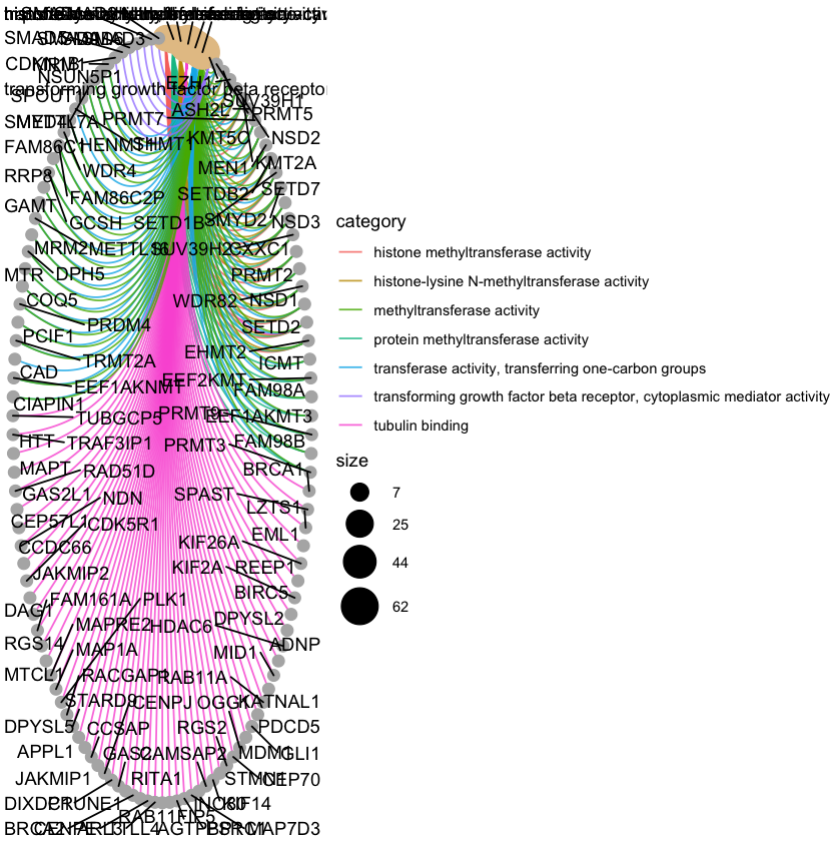
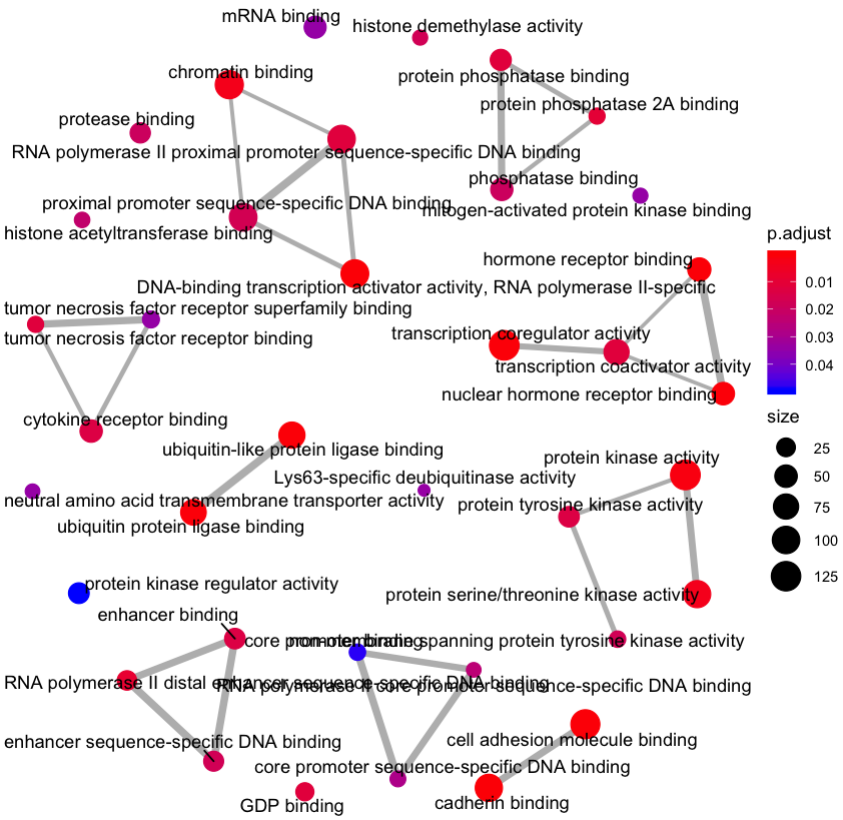
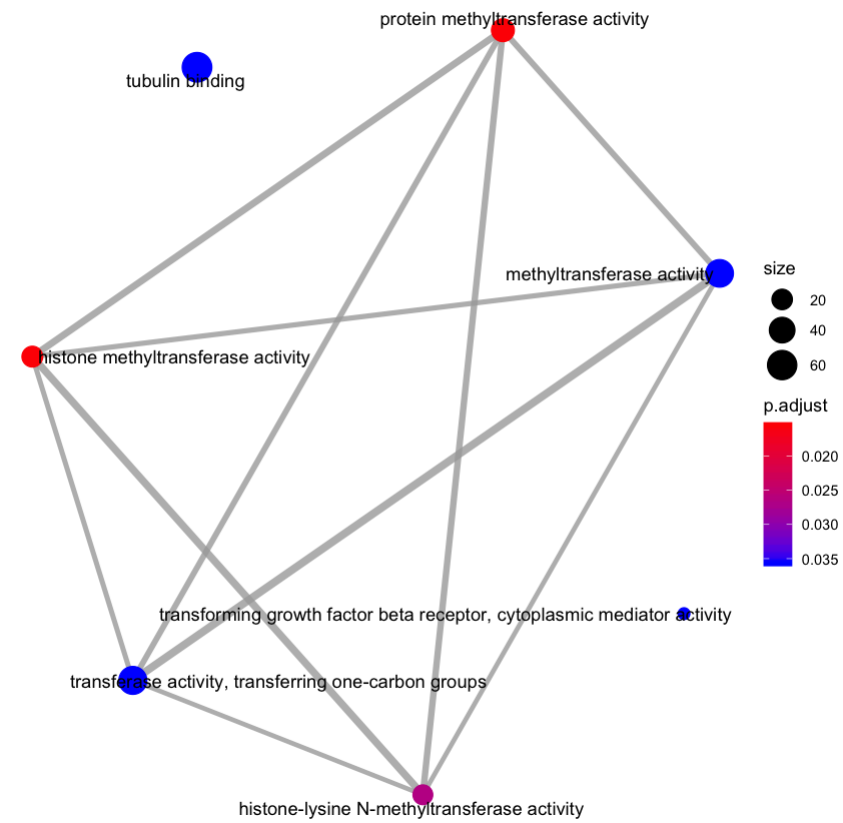
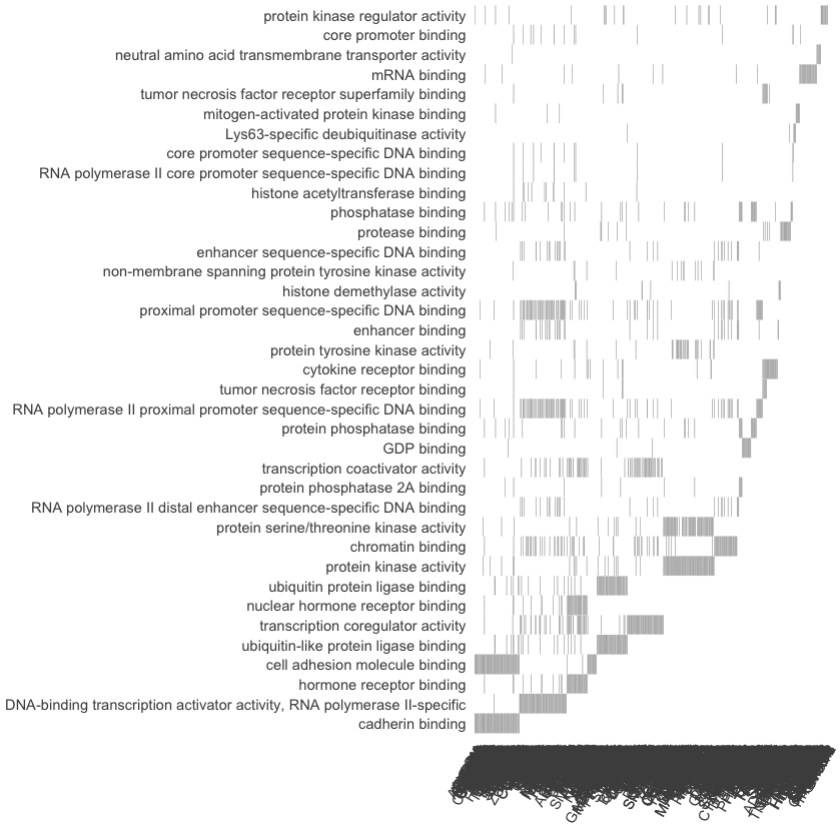


[7]

```
emapplot(rna_up_ego_gn, showCategory = 100)
emapplot(rna_dw_ego_gn, showCategory = 100)
heatplot(rna_up_ego_gn, showCategory = 50)
heatplot(rna_dw_ego_gn, showCategory = 50)
png("output/plot/rna_up_emap.png", width = 1000, height = 1000)
emapplot(rna_up_ego_gn, showCategory = 100)
dev.off()
png("output/plot/rna_dw_emap.png", width = 1000, height = 1000)
emapplot(rna_dw_ego_gn, showCategory = 100)
dev.off()
png("output/plot/rna_up_heat.png", width = 1600, height = 800)
heatplot(rna_up_ego_gn, showCategory = 50)
dev.off()
png("output/plot/rna_dw_heat.png", width = 1600, height = 800)
heatplot(rna_dw_ego_gn, showCategory = 50)
dev.off()
```


mRNA binding
histone demethylase activity
chromatin binding
protein phosphatase binding
protein phosphatase 2A binding
protease binding
RNA polymerase II proximal promoter sequence-specific DNA binding
phosphatase binding
proximal promoter sequence-specific DNA binding
mitogen-activated protein kinase binding
histone acetyltransferase binding
hormone receptor binding
DNA-binding transcription activator activity, RNA polymerase II-specific
tumor necrosis factor receptor superfamily binding
tumor necrosis factor receptor binding
transcription coregulator activity
transcription coactivator activity
nuclear hormone receptor binding
cytokine receptor binding
ubiquitin-like protein ligase binding
protein kinase activity
Lys63-specific deubiquitinase activity
neutral amino acid transmembrane transporter activity
protein tyrosine kinase activity
ubiquitin protein ligase binding
protein kinase regulator activity
protein serine/threonine kinase activity
enhancer binding
core promoter binding
spanning protein tyrosine kinase activity
RNA polymerase II distal enhancer sequence-specific DNA binding
RNA polymerase II core promoter sequence-specific DNA binding
enhancer sequence-specific DNA binding
cell adhesion molecule binding
core promoter sequence-specific DNA binding
GDP binding
cadherin binding
p.adjust
0.01
0.02
0.03
0.04
size
25
50
75
100
125


protein methyltransferase activity
tubulin binding
methyltransferase activity
histone methyltransferase activity
transforming growth factor beta receptor, cytoplasmic mediator activity
transferase activity, transferring one-carbon groups
histone-lysine N-methyltransferase activity
size
20
40
60
p.adjust
0.020
0.025
0.030
0.035



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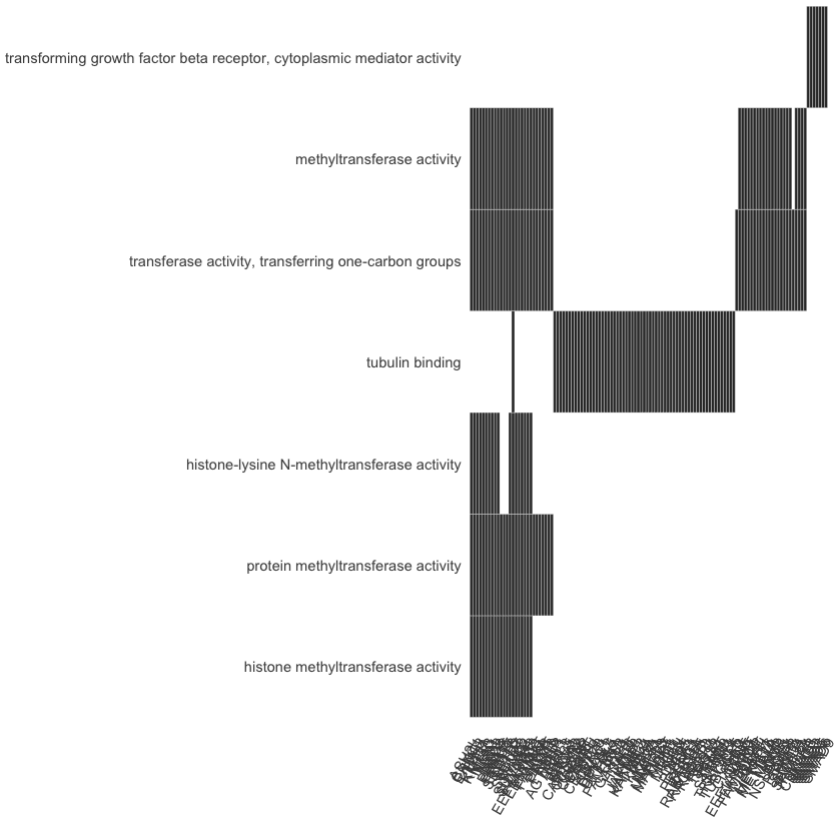

[8]
```
rna_dx_list <- rna_diffx[,5]
names(rna_dx_list) = as.character(rna_diffx[,4])
rna_dx_list = sort(rna_dx_list, decreasing = TRUE)
head(rna_dx_list)
```

ENSG00000198788 10.7500622971149

ENSG00000134460

9.95780645848594 **ENSG00000054179**

8.95868722141504 **ENSG00000225968**

7.97441284526463 **ENSG00000120738**

7.59632267158719 **ENSG00000122877**

7.28922841148145

[9]
```
rna_gsea <- gseGO(geneList = rna_dx_list, OrgDb = org.Hs.eg.db,
keyType = 'ENSEMBL')
```

```
preparing geneSet collections...

GSEA analysis...

leading edge analysis...

done...
```

[10]
```
rna_gsea_short <- setReadable(rna_gsea, 'org.Hs.eg.db',
'ENSEMBL')
head(rna_gsea_short,2)
```

	ID	Description	setSize	enrichmentScore
	<chr>	<chr>	<int>	<dbl>
GO:0001932	GO:0001932	regulation of protein phosphorylation	470	0.4108816
GO:0009968	GO:0009968	negative regulation of signal transduction	457	0.4069802

[28]
```
png("output/plot/rna_diffx_gsea.png", width = 9000, height = 800)
heatplot(rna_gsea_short, foldChange = rna_dx_list)
dev.off()
```

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GO Analysis for Up/Down Gene Sets in combination with Hi/Lo Accessibility in their regulatory regions (-10000 to +1000)

[12]
```
up_hi <- read.table(file="output/upregby_hiprom.bed", sep =
"\t",col.names =
c("chr","fstart","fend","name","score","strand"))
dw_hi <- read.table(file="output/dwregby_hiprom.bed", sep =
"\t",col.names =
c("chr","fstart","fend","name","score","strand"))
up_lo <- read.table(file="output/upregby_loprom.bed", sep =
"\t",col.names =
c("chr","fstart","fend","name","score","strand"))
dw_lo <- read.table(file="output/dwregby_loprom.bed", sep =
"\t",col.names =
c("chr","fstart","fend","name","score","strand"))
head(dw_hi,2)
```

A data.frame: 2 × 6

chr	fstart	fend	name	score	strand
<fct>	<int>	<int>	<fct>	<dbl>	<fct>
6	82169982	82247754	ENSG00000005700	-0.2436118	-
13	77044657	77327044	ENSG00000005810	-0.4716991	-

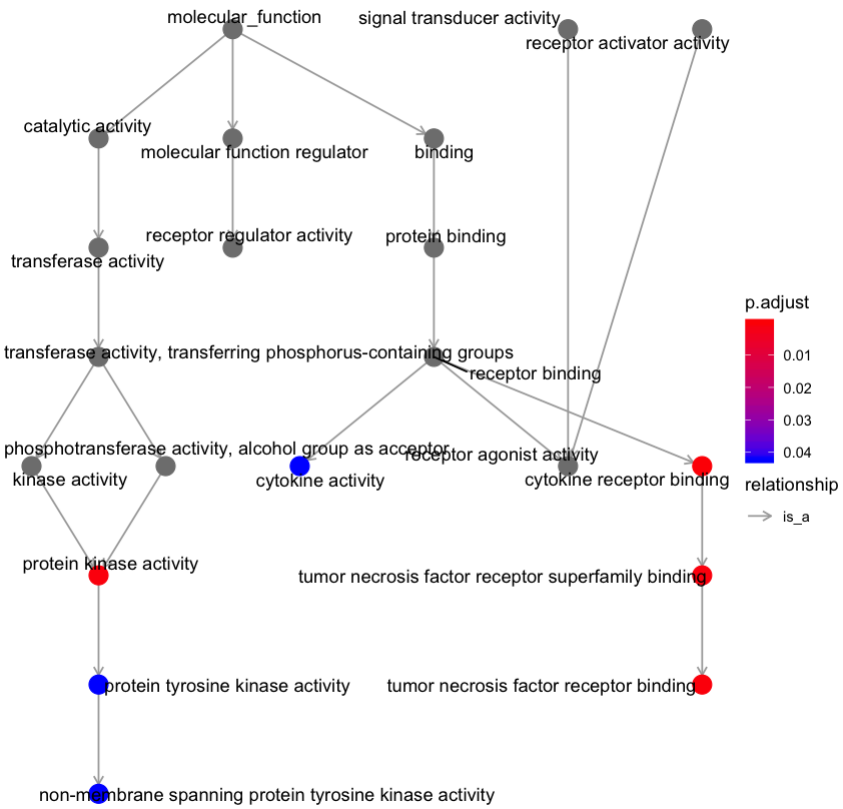
[13]
```
up_hi_list <- as.vector(up_hi$name)
dw_hi_list <- as.vector(dw_hi$name)
up_lo_list <- as.vector(up_lo$name)
dw_lo_list <- as.vector(dw_lo$name)
up_hi_ego <- enrichGO(gene = up_hi_list, universe = rna_bgd,
OrgDb = org.Hs.eg.db, keyType = "ENSEMBL")
dw_hi_ego <- enrichGO(gene = dw_hi_list, universe = rna_bgd,
OrgDb = org.Hs.eg.db, keyType = "ENSEMBL")
up_lo_ego <- enrichGO(gene = up_lo_list, universe = rna_bgd,
OrgDb = org.Hs.eg.db, keyType = "ENSEMBL")
dw_lo_ego <- enrichGO(gene = dw_lo_list, universe = rna_bgd,
OrgDb = org.Hs.eg.db, keyType = "ENSEMBL")
```

[14]
```
up_hi_ego_gn <- setReadable(up_hi_ego, 'org.Hs.eg.db', 'ENSEMBL')
dw_hi_ego_gn <- setReadable(dw_hi_ego, 'org.Hs.eg.db', 'ENSEMBL')
```

```
up_lo_ego_gn <- setReadable(up_lo_ego, 'org.Hs.eg.db', 'ENSEMBL')
dw_lo_ego_gn <- setReadable(dw_lo_ego, 'org.Hs.eg.db', 'ENSEMBL')
```

[15]

```
goplot(up_hi_ego_gn)
goplot(up_lo_ego_gn)
#no data?
#goplot(dw_hi_ego_gn)
#goplot(dw_lo_ego_gn)
png("output/plot/up_hi_goplot.png", width = 900, height = 900)
goplot(up_hi_ego_gn)
dev.off()
png("output/plot/up_lo_goplot.png", width = 900, height = 900)
goplot(up_lo_ego_gn)
dev.off()
```



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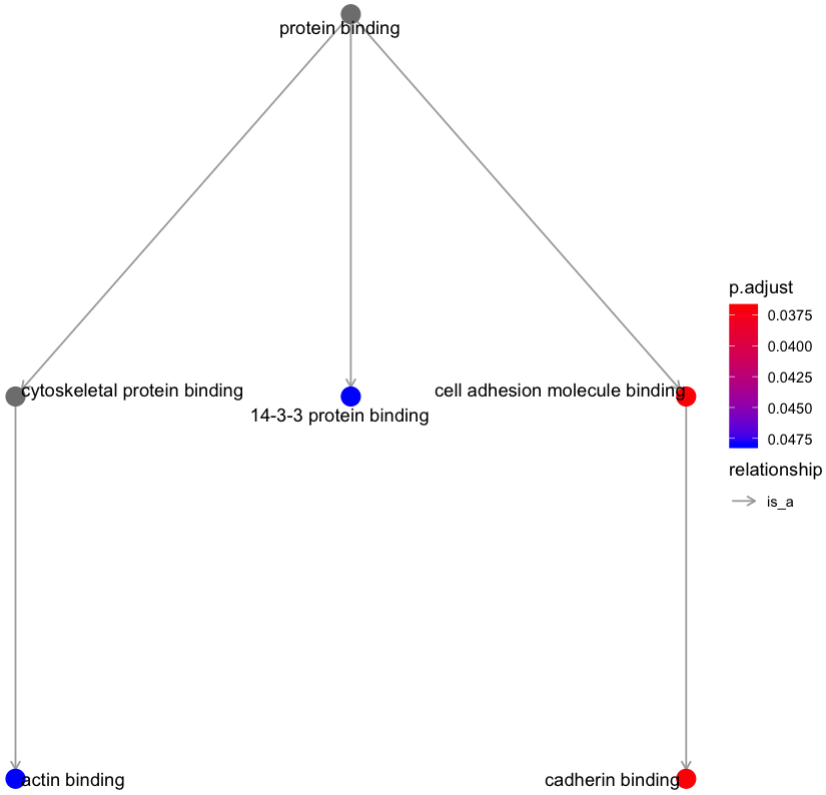
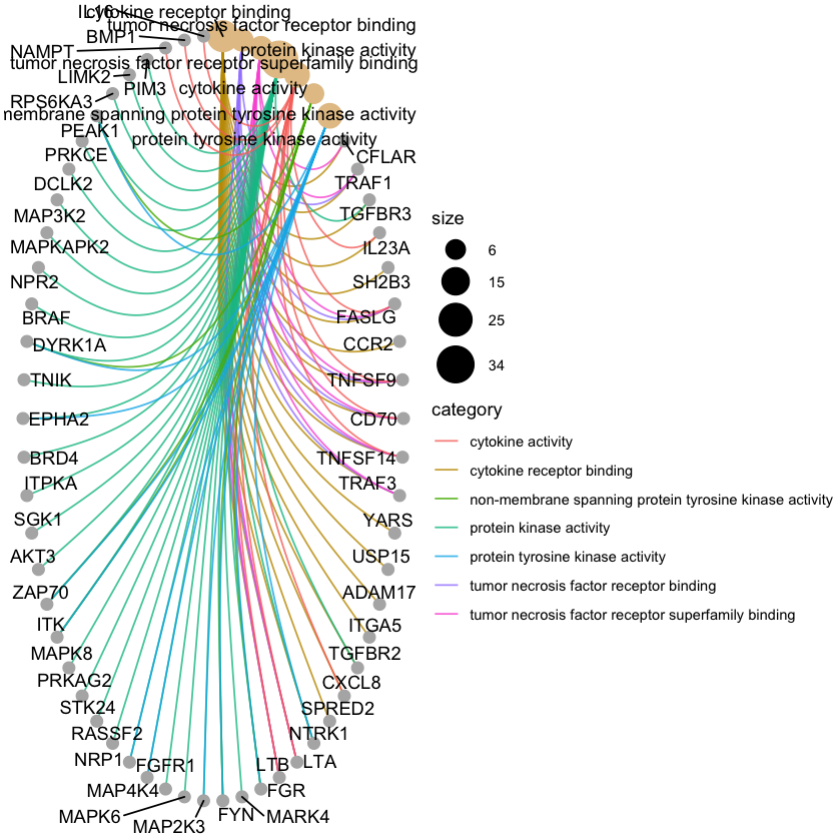


[16]
```
cnetplot(up_hi_ego_gn, circular=TRUE, colorEdge=TRUE,
showCategory = 20)
cnetplot(up_lo_ego_gn, circular=TRUE, colorEdge=TRUE,
showCategory = 20)
png("output/plot/up_hi_cnet.png", width = 1600, height = 1600)
cnetplot(up_hi_ego_gn, circular=TRUE, colorEdge=TRUE,
showCategory = 20)
dev.off()
png("output/plot/up_lo_cnet.png", width = 1600, height = 1600)
cnetplot(up_lo_ego_gn, circular=TRUE, colorEdge=TRUE,
showCategory = 20)
dev.off()
```



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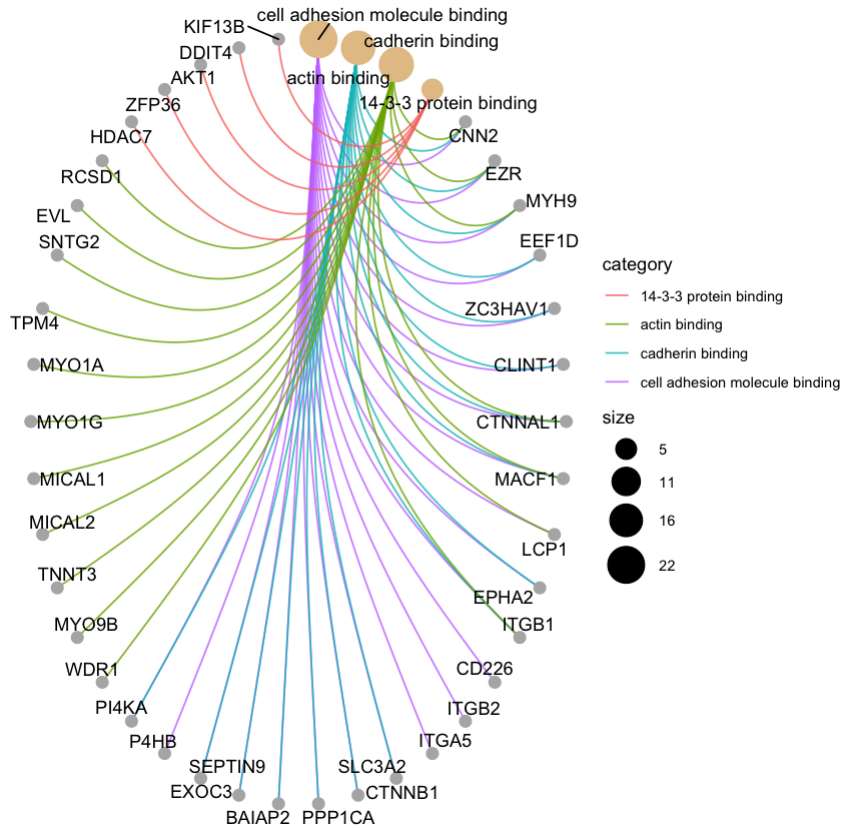
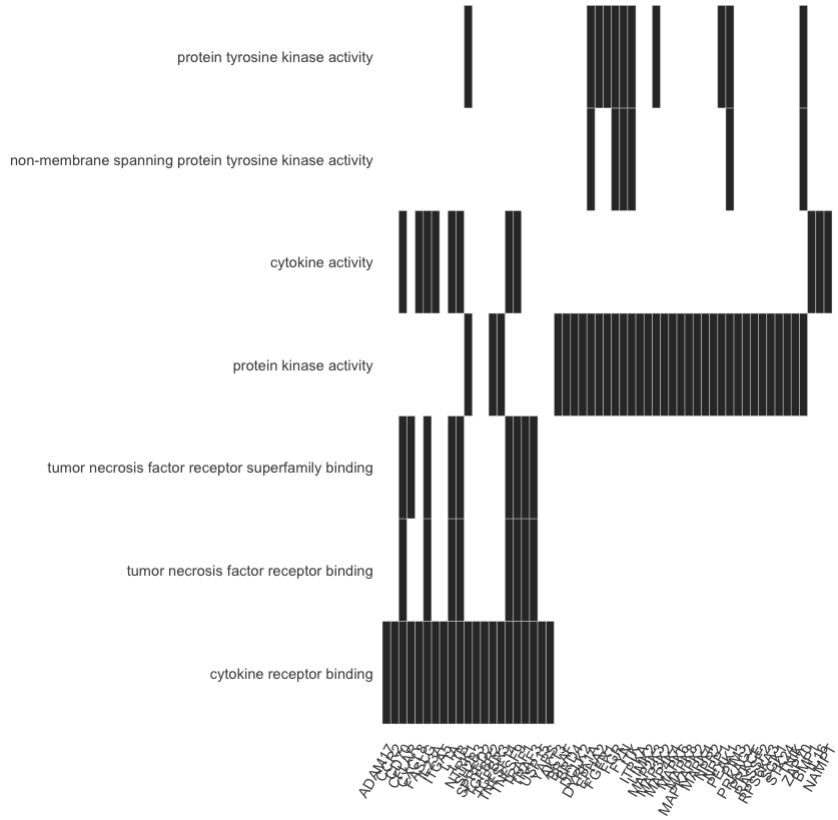



```
[17]  heatplot(up_hi_ego_gn, showCategory = 50)
      heatplot(up_lo_ego_gn, showCategory = 50)
      png("output/plot/up_hi_heat.png", width = 1600, height = 800)
      heatplot(up_hi_ego_gn, showCategory = 50)
      dev.off()
```

```
png("output/plot/up_lo_heat.png", width = 1600, height = 800)
heatplot(up_lo_ego_gn, showCategory = 50)
dev.off()
```



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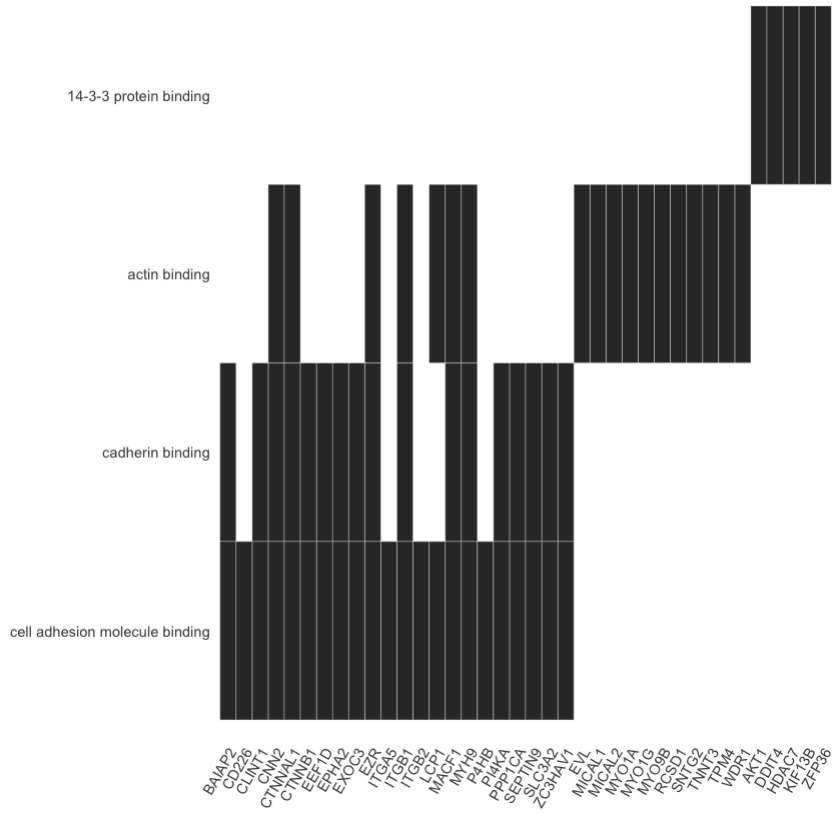


GO Analysis on Shortlists

[18]
```
stock_anno <-
read.csv("data_challenge/rnaseq_annotation.txt",sep="\t",header =
TRUE)
colnames(stock_anno)
```

'featureid' 'chr' 'start' 'end' 'width' 'strand' 'source' 'type' 'score' 'phase' 'gene_id' 'gene_version' 'gene_name' 'gene_source' 'gene_biotype' 'havana_gene' 'havana_gene_version' 'transcript_id' 'transcript_version' 'transcript_name' 'transcript_source' 'transcript_biotype' 'havana_transcript' 'havana_transcript_version' 'tag' 'transcript_support_level' 'exon_number' 'exon_id' 'exon_version' 'ccds_id' 'protein_id' 'protein_version'

[19]
```
id2name <- stock_anno[c("gene_id","gene_name")]
head(id2name)
```

A data.frame: 6 × 2

gene_id	gene_name
<fct>	<fct>
ENSG00000000003	TSPAN6
ENSG00000000005	TNMD
ENSG00000000419	DPM1
ENSG00000000457	SCYL3
ENSG00000000460	C1orf112
ENSG00000000938	FGR

[20]
```
rna_diffx_anno <- merge(id2name, rna_diffx, by.x = "gene_id",
by.y = "name", all.y = TRUE)
```

[21]
```
uphiprox_TFs <-
read.csv("output/uphiprox_TFMs.tsv",sep="\t",header = TRUE)
uphiprox_TFs_diffx <- merge(rna_diffx_anno, uphiprox_TFs, by.x =
"gene_name", by.y = "motif_alt_ID", all.y = TRUE)
uphiprox_TFs_diffx
```

A data.frame: 6 × 11

gene_name	gene_id	chr	fstart	fend	score
<fct>	<fct>	<fct>	<int>	<int>	<dbl>
CTCFL	NA	NA	NA	NA	NA
KLF15	ENSG00000163884	3	126342634	126357442	-4.3128
TFAP2A	NA	NA	NA	NA	NA
TFAP2B	NA	NA	NA	NA	NA
TFAP2C	NA	NA	NA	NA	NA
ZNF148	NA	NA	NA	NA	NA

[22]
```
hi_TFs <- read.csv("output/hi_TFMs.tsv",sep="\t",header = TRUE)
hi_TFs_diffx <- merge(rna_diffx_anno, hi_TFs, by.x = "gene_name",
by.y = "motif_alt_ID", all.y = TRUE)
hi_TFs_diffx <- hi_TFs_diffx[!duplicated(hi_TFs_diffx$gene_id),]
hi_TFs_diffx <- na.omit(hi_TFs_diffx)
head(hi_TFs_diffx)
```

A data.frame: 6 ×

	gene_name	gene_id	chr	fstart	fend
	<fct>	<fct>	<fct>	<int>	<int>
3	ATF4	ENSG00000128272	22	39520563	39522683
4	BACH1	ENSG00000156273	21	29298870	29346148
5	BACH2	ENSG00000112182	6	89926528	90296742
14	CREB1	ENSG00000118260	2	207529911	207603431
29	FOS	ENSG00000170345	14	75278773	75282230
34	FOSB	ENSG00000125740	19	45467994	45475178

[23]
```
lo_TFs <- read.csv("output/lo_TFMs.tsv",sep="\t",header = TRUE)
lo_TFs_diffx <- merge(rna_diffx_anno, lo_TFs, by.x = "gene_name",
by.y = "motif_alt_ID", all.y = TRUE)
lo_TFs_diffx <- hi_TFs_diffx[!duplicated(lo_TFs_diffx$gene_id),]
lo_TFs_diffx <- na.omit(lo_TFs_diffx)
lo_TFs_vshi <- merge(lo_TFs_diffx, hi_TFs_diffx, by.x =
"gene_id", by.y = "gene_id", all.x = TRUE)
lo_TFs_vshi <- lo_TFs_diffx[is.na(lo_TFs_diffx$gene_name.y),]
lo_TFs_vshi
```

A data.frame: 0 × 11

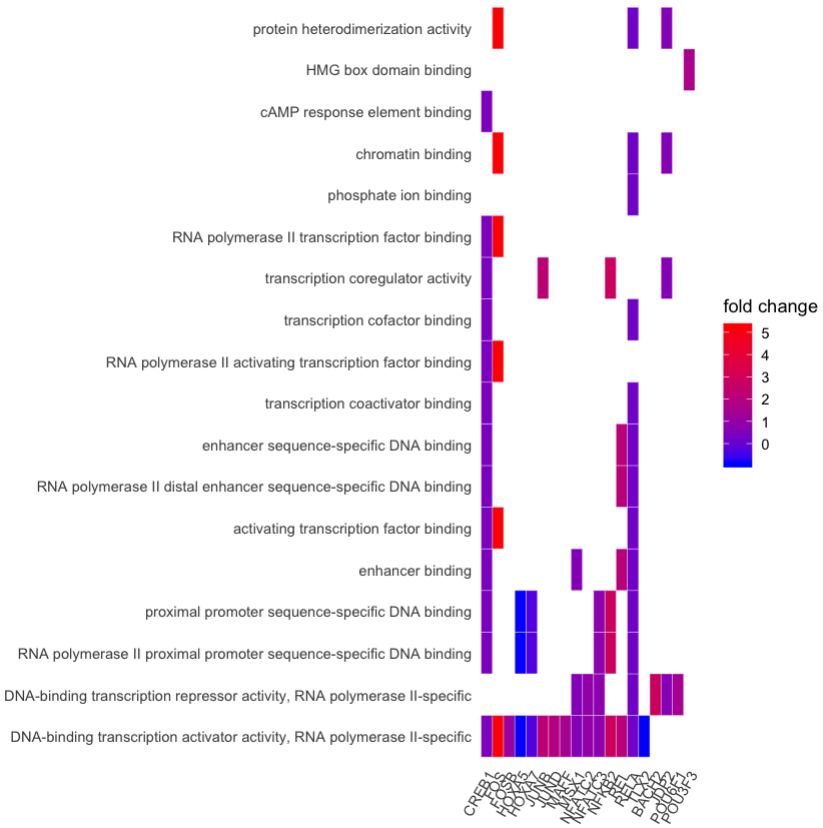
gene_name	gene_id	chr	fstart	fend	score	strand	rank
<fct>	<fct>	<fct>	<int>	<int>	<dbl>	<fct>	<int>

None of the lo_TF sites are unique versus hi_TF sites

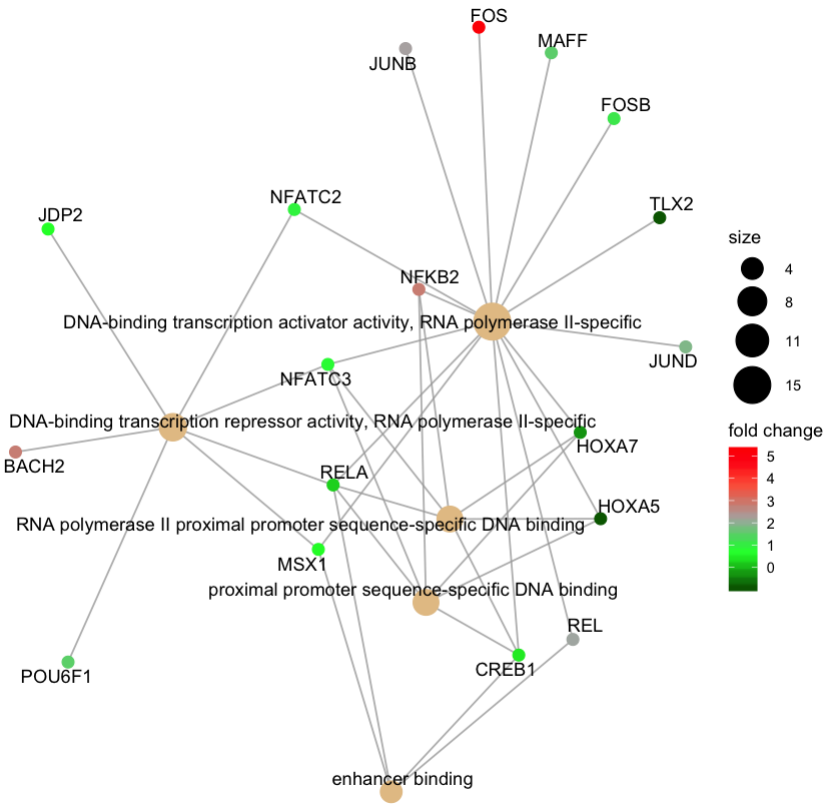
[24]
```
hi_TFs_vslo <- merge(hi_TFs_diffx, lo_TFs_diffx, by.x =
"gene_id", by.y = "gene_id", all.x = TRUE)
hi_TFs_vslo <- hi_TFs_vslo[is.na(hi_TFs_vslo$gene_name.y),]
```

[25]
```
hi_TFs_vslo_list <- hi_TFs_vslo[,6]
names(hi_TFs_vslo_list) = as.character(hi_TFs_vslo[,1])
hi_TFs_vslo_list = sort(hi_TFs_vslo_list, decreasing = TRUE)
hi_TFs_vslo_vec <- names(hi_TFs_vslo_list)
hi_TFs_vslo_ego <- enrichGO(gene = hi_TFs_vslo_vec, universe =
rna_bgd, OrgDb = org.Hs.eg.db, keyType = "ENSEMBL")
hi_TFs_vslo_ego <- setReadable(hi_TFs_vslo_ego, 'org.Hs.eg.db',
'ENSEMBL')
```

[26]
```
heatplot(hi_TFs_vslo_ego, foldChange=hi_TFs_vslo_list)
cnetplot(hi_TFs_vslo_ego, foldChange=hi_TFs_vslo_list)
png("output/plot/hiTFs_cnet.png", width = 900, height = 900)
cnetplot(hi_TFs_vslo_ego, foldChange=hi_TFs_vslo_list)
dev.off()
png("output/plot/hiTFs_heat.png", width = 900, height = 900)
heatplot(hi_TFs_vslo_ego, foldChange=hi_TFs_vslo_list)
dev.off()
```



pdf: 2
pdf: 2



Interpretation

The GO Analysis on the RNA-Seq Data clearly showed many genes involved in histone methyltransferase activity are downregulated, which may be a clue to the mechanism of

chromatin accessibility changes induced. The upregulated genes indicated cytokine/TNF receptors were activated leading to a cascade of changes triggering changes in phosphorylation activity, chromatin and transcription factor activation. These points are especially clear in the emap plots and heatmaps.

There is also some indication of hormone receptor binding activity although the cytokine/TNF pathway looks most strongly activated via the chromatin remodelling as evidenced by the GO terms associated with genes upregulated with increased accessibility in their regulatory regions (up_hi_goplot). Perhaps the hormone receptor binding is more upstream of the chromatin remodelling program and potentially the treatment applied?

The Gene Set Enrichment Analysis Heatmap (rna_diffx_gsea) also indicated a number of leukocyte activation genes are regulated. Most notably IL2RA is upregulated indicating activation of T or B cells. TNF cytokine expression is also highly upregulated so this may be an immune cell line responding to some pro-inflammatory treatment? EGR1 is also highly expressed and upstream of DNA demethylation pathways.

It appears that decrease in chromatin accessibility liberated some genes involved in cell adhesion from repressors (up_lo_goplot).

The motif search showed some specific TF motifs are enriched in the more accessible regions. Those TFs found to be differentially expressed by cross referencing with the RNASeq form a fairly small list: CREB1, FOS, FOSB, HOXA5, HOXA7, JUNB, JUND, MAFF, MSX1, NFATC2, NFATC3, NFKB2, REL, RELA, TLX2, BACH2, JDP2, POU6F1, POU3F3.

FOS or c-Fos is the most highly expressed of these TFs, it's also connected to chromatin binding activity. Together with EGR1 it's a top candidate stimulated by growth factors (hormones) or cytokine stimuli.