Causality in Coexpression

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```
files_in_zip <- unzip(zipfile = "data/zipped_data.zip", list = T)
for (datafile in files_in_zip$Name){
  if (!file.exists(paste0("data/", "datafile"))){
    unzip(zipfile = "data/zipped_data.zip", exdir = "data/")
  }
}
```

Data from **Albert FW, Bloom JS, Siegel J, Day L, Kruglyak L. 2018. Genetics of trans-regulatory variation in gene expression. eLife 7: 1–39.** Source data or source data

Files to download:

* SI_Data_01_expressionValues.txt (in the repo) * SI_Data_03_genotypes.txt (too big for the repo) * SI_Data_04_eQTL.csv

I started with :

* **phenotype matrix** - contains the gene expression data (expression levels in units of log2(TPM) for all genes and segregants)

* **genotype matrix** - contains the genotype information (genotypes at 42,052 markers for all segregants. BY (i.e. reference) alleles are denoted by '−1'. RM alleles are denoted by '1'.)

* **eqtl_results** - Genes with a local eQTL and significant Allele-specific expression (ASE), and discordant direction of effect. (1) Positive values indicate higher expression in RM compared to BY. (2) Shown is the less sig- nificant p-value from the two ASE datasets. (3) The table shows only genes where both ASE datasets agreed in the direction of effect. Shown is the average effect.

1 for the actual analysis jump to "Do analysis with new parameters"

Original parameters used

```
var.exp.lim <- 0.1


nSNPs <- length(colnames(genotype)) - 1
nGenes <- length(colnames(phenotype)) - 1
# nSNPs <- 42052
# nGenes <- 5720


snp.pval <- 0.05 / (as.numeric(nGenes) * as.numeric(nSNPs))
snp.pval.nsign <- as.numeric(1e-5)


corr.pval <-  0.05 / (nGenes * nGenes)
```

2 Get effects table

Probably best to run in uppmax

What's happening here:
- create a table with all the possible combinations of geneA-eqtlA with geneB-eqtlB
- ANOVA to test the effect of an eQTL on a gene
- combines the anova results with the table with the geneA-eqtlA and geneB-eqtlB combinations
- get correlation between genes
- adds the correlation between genes to the previous table

Output is a table with all combinations of gene-eqtl pairs (all geneA must have a cis-eqtl but not all of geneB have to have an eqtl (in cis or not)), if their eqtl is in cis or not, the variance explained by each gene-eqtl pair, the anova p-value and r2 of the effect of an eqtl on a gene and the correlation value and p-value between genes.

```
library(data.table)
if (!file.exists("results/effects_table.Rdata")){
```

```r
eqtl_results.sub <- eqtl_results[,.(gene, pmarker, cis, var.exp)]

genesB <- colnames(phenotype[,2:ncol(phenotype)])
effectsA_B.sepA_B <- create_ini_table(eqtl_results.sub, genesB, var.exp.lim)

### Find effect of eqtls from geneA in expression of geneB ####
eqtls.A <- unique(effectsA_B.sepA_B$eqtl.A)
genes.B <- unique(effectsA_B.sepA_B$geneB)
res.tot.eqtlA_geneB <- data.table(expand.grid(gene=genes.B, eqtl=eqtls.A))#, anv.res=NA))
res.eqtlA_geneB <- res.tot.eqtlA_geneB

# run anova in parallel -- effect of eqtlA on geneB
message("running anova for the effect of eqtlA on geneB")
cl = makeCluster(detectCores() - 1, type="FORK")
res.eqtlA_geneB$anv.res <- parApply(cl=cl,res.eqtlA_geneB,1,effect_eqtl_gene, phenotype, genotype)
stopCluster(cl)


### Find effect of eqtls from geneB in expression of geneA
message("running anova for the effect of eqtlB on geneA")
eqtls.B <- na.omit(unique(effectsA_B.sepA_B$eqtl.B))
genes.A <- unique(effectsA_B.sepA_B$geneA)
res.tot.eqtlB_geneA <- data.table(expand.grid(gene=genes.A, eqtl=eqtls.B))#, anv.res=NA))
res.eqtlB_geneA <- res.tot.eqtlB_geneA

# run the anova function in parallel -- effect of eqtlB on geneA
cl = makeCluster(detectCores() - 1, type="FORK")
res.eqtlB_geneA$anv.res <- parApply(cl=cl,res.eqtlB_geneA,1,effect_eqtl_gene, phenotype, genotype)
stopCluster(cl)

## merge anova results with the information table to create an effects table
```

```r
  # results from effect of eqtlA on geneB
  effects_table.eqtlA_geneB <- merge_after_anova(res.eqtlA_geneB, "B", "A", effectsA_B.sepA_B)

  # results from effect of eqtlB on geneA
  effects_table.anova <- merge_after_anova(res.eqtlB_geneA, gene.AB="A", eqtl.AB="B", effects_table.eqtl
  setcolorder(effects_table.anova, c("geneA", "geneB", "eqtl.A", "eqtl.B", "cis.A", "cis.B"))

  ### correlation ####
  message("getting correlation between genes")
  cor_traits <- rcorr(as.matrix(phenotype[,2:ncol(phenotype)])) # to remove the sample names

  cor_traits.cor <- cor_traits$r
  cor_traits.p <- cor_traits$P

  my_cor_matr_flat <- flat_cor_mat(cor_traits.cor,cor_traits.p)
  my_cor_matr_flat <- data.table(my_cor_matr_flat)

  cor_matr <- my_cor_matr_flat[!duplicated(t(apply(my_cor_matr_flat, 1, sort))), ]

  # merge the correlation values with the table that has the anova results
  setnames(cor_matr, old=c("row","column", "pval"), new=c("geneA","geneB", "cor.pval"))

  message("combining anova results with correlation")
  effects_table.cor <- merge(effects_table.anova, cor_matr, by=c("geneA","geneB"), all.x=T)

  message("saving effects table with correlation between genes")
  save(effects_table, file="results/effects_table.Rdata")

}
```

2.1 Test different parameters and create summary table with results from testing the different parameters

Test different parameter values combinations to see if I there is a certain combination that gives optimal results in the causality inference.
Takes a while, probably better to use uppmax

Assumptions:
* geneA is in cis with eqtlA
* geneB is in cis with eqtlB
* var.explained for geneA must be > var.exp.lim
* correlation pval is < corr.pval
Inferred if gene A is affecting geneB or if geneB is affecting geneA.
* geneA != geneB

There are two categories and several end results:
Categories:
* A affects B: A->B
* B affects A: B->A

End results:
* **A->B = T and B->A = F** or **A->B = F and B->A = T** –> this is the case we are mostly interested in. It means we can say that a gene affects the other, but it's not affected by it.
* **A->B = T and B->A = NA** or **A->B = NA and B->A = T** –> we can say that a gene affects the other, but we can't say if the second gene affects the first
* **A->B = NA and B->A = NA** –> we can't say anything about causality
* **A->B = F and B->A = T** or **A->B = F and B->A = T** –> neither gene affects the other
* **A->B = T and B->A = T** or **A->B = T and B->A = T**

How it works:
* **A->B = T** if anova p-value for the effect of eqtlA on geneB is < snp.pval
* **A->B = F** if the anova p-value of the effect of eqtlA on geneB is > snp.pval.nsign and geneA and geneB have different eqtls
* **B->A = T** if anova p-value for the effect of eqtlB on geneA is < snp.pval
* **B->A = F** if the anova p-value of the effect of eqtlB on geneA is > snp.pval.nsign and geneA and geneB have different eqtls

What's happening here:

- set different values for an effect or a correlation to be significant or non-significant

- find causality (A->B and B->A) using the diferent parameters - the object "res" is a list of tables where each table corresponds to the causality inference done with different parameters (specified in each table)

geneA <- causal genes

geneB <- affected genes

- create table with a summary of the results:
  - number of different geneA and geneB found
  - number of different eqtlA and eqtlB found
  - number of unique gene pairs
  - number of different gene-eqtl pairs for geneA-eqtlA and geneB-eqtlB
  - the parameter cutoffs

```r
if (!file.exists("results/resparams.Rdata")){

  message("loading effects_table")
  load("results/effects_table.Rdata")

  sign_p <- c(1e-17, 1e-16, 1e-15, 1e-14, 1e-13, 1e-12, 1e-11, 1e-10, 1e-9, 1e-8, 1e-7, 1e-6, 1e-5, 1e-
  non_sign_p <- c(1e-7, 1e-6, 1e-5, 1e-4, 1e-3, 1e-2)
  cor_p <- c(1e-9, 1e-8, 1e-7, 1e-6, 1e-5, 1e-4, 1e-3, 1e-2)
  params0 <- data.table(expand.grid(sign_p=sign_p, non_sign_p=non_sign_p, cor_p=cor_p))

  message("testing parameters")

  cl = makeCluster(detectCores() - 1, type="FORK")
  res <- parApply(cl=cl,params0,1, testparams, effects_table, var.exp.lim)
  stopCluster(cl)
  save(res, file="results/resparams.Rdata")

}

if (!file.exists("results/res_table_params.Rdata")){
  message("loading resparams")
  load(file = "results/resparams.Rdata")

  # create summary table with results from testing the different parameters
  message("creating summary table")
  cl = makeCluster(detectCores()-1, type="FORK")
  res_table.temp <- parLapply(cl=cl, res, create_res_table)
  stopCluster(cl)

  res_table <- rbindlist(res_table.temp)
  res_table <- res_table[order(sign.p, -nonsign.p, cor.p)]

  save(res_table, file="results/res_table_params.Rdata")

} else if (file.exists("results/res_table_params.Rdata")){
  message("loading res_table with the results of testing the several parameters")
  load("results/res_table_params.Rdata")
}
```

```
## loading res_table with the results of testing the several parameters
```

2.1.1 Plot number of times geneA -> geneB with the different parameters tested

Only the cases where A->B=T and B->A=F

```
par(mfrow=c(2,4))

cor.pvals <- unique(res_table$cor.p)
linetype <- c(1:length(unique(res_table$nonsign.p)))
```

```
for (p in cor.pvals){
  xrange <- range(-log(res_table$sign.p)) # set x-axis range
  yrange <- range(res_table$unique.genepairs) # set y-axis range
  plot(xrange, yrange, type = "n", main=paste("corr pval = ", p), xlab = "-log(sign.p)",
       ylab = " #unique gene pairs") # empty plot
  colors <- rainbow(length(unique(res_table$nonsign.p)))
  for (i in 1:length(unique(res_table$nonsign.p))){
    x <- -log(res_table[nonsign.p==nonsign.p[i] & cor.p==p]$sign.p)
    y <- res_table[nonsign.p==nonsign.p[i] & cor.p==p]$unique.genepairs
    lines(x,y,  type="l", lwd=1.5,lty=linetype[i], col=colors[i])
  }
  #legend(x=20, yrange[2], unique(res_table$nonsign.p), col=colors, lty=linetype, cex=0.8)
}
```

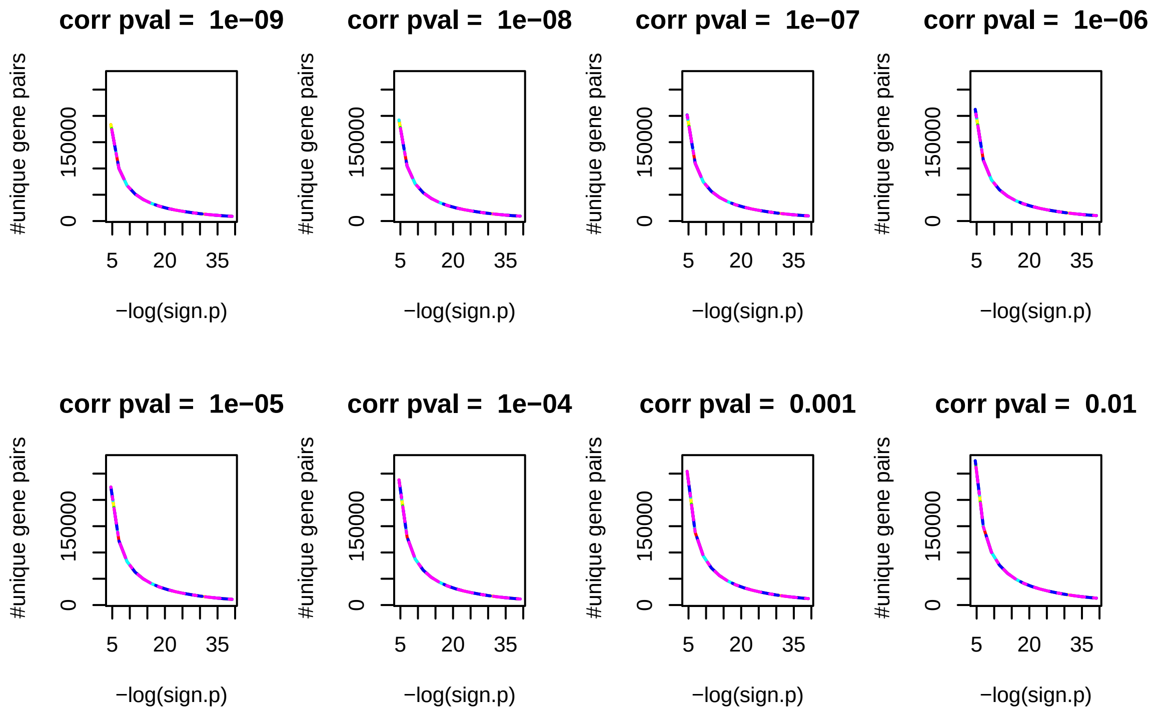


Figure 1: Number of unique gene pairs found when using different cutoffs. Each pannel corresponds to a different correlation p-value cutff. X-axis is the -log(pvalue) (for the effect to be significant) and differenc colors represent different values for the non-significant p-value (for the effect to be considered non-significant)

```
par(mfrow=c(1,1))


xrange <- range(-log(res_table$sign.p)) # set x-axis range
yrange <- range(res_table$unique.genepairs) # set y-axis range
plot(xrange, yrange, type = "n", xlab = "-log(p)", ylab = "#unique gene pairs",
     main="#gene pairs where A->B") # empty plot
colors <- rainbow(length(unique(res_table$nonsign.p)))
linetype <- c(1:length(unique(res_table$nonsign.p)))
for (i in 1:length(unique(res_table$nonsign.p))){
  x <- -log(res_table$sign.p[res_table$nonsign.p==unique(res_table$nonsign.p)[i]])
  y <- res_table$unique.genepairs[res_table$nonsign.p==unique(res_table$nonsign.p)[i]]
  lines(x,y,  type="l", lwd=1.5,lty=linetype[i], col=colors[i])
}
legend(x=20, yrange[2], unique(res_table$nonsign.p), col=colors, lty=linetype, cex=0.8)
```

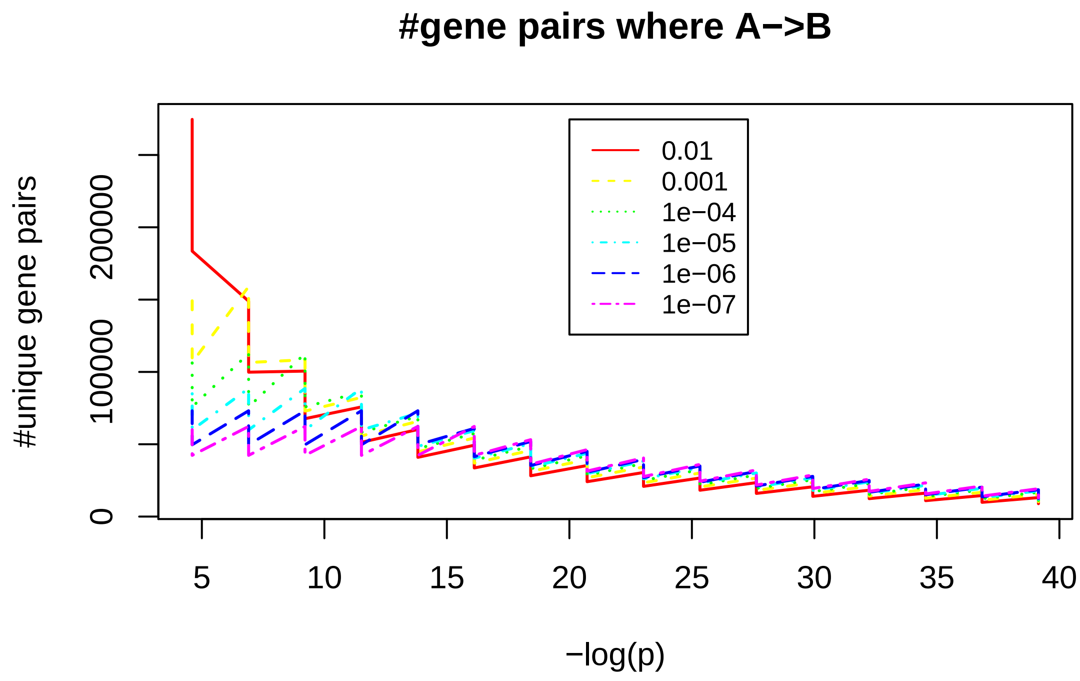


Figure 2: Number of unique gene pairs found when using different cutoffs. X-axis is the -log(pvalue) (for the effect to be significant) and differenc colors represent different values for the non-significant p-value (for the effect to be considered non-significant)

3 Inferring causality with new parameters

```
var.exp.lim <- 0.1

# nSNPs <- length(colnames(genotype))-1
# nGenes <- length(colnames(phenotype))-1

nSNPs <- 42052
nGenes <- 5720

snp.pval <- 0.01
snp.pval.nsign <- as.numeric(1e-5)

corr.pval <- 0.05/choose(nGenes,2)
```

Assumptions (same as before):

* geneA is in cis with eqtlA

* geneB is in cis with eqtlB

* var.explained for geneA must be > var.exp.lim

* correlation pval is < corr.pval

Inferred if gene A is affecting geneB or if geneB is affecting geneA.

* geneA != geneB

There are two categories and several end results:

Categories:

* A affects B: A->B

* B affects A: B->A

End results:

* **A->B = T and B->A = F** or **A->B = F and B->A = T** –> this is the case we are mostly interested in. It means we can say that a gene affects the other, but it's not affected by it.

* **A->B = T and B->A = NA** or **A->B = NA and B->A = T** –> we can say that a gene affects the other, but we can't say if the second gene affects the first

* **A->B = NA and B->A = NA** –> we can't say anything about causality

* **A->B = F and B->A = T** or **A->B = F and B->A = T** –> neither gene affects the other

* **A->B = T and B->A = T** or **A->B = T and B->A = T**

How it works:

* **A->B = T** if anova p-value for the effect of eqtlA on geneB is < snp.pval

* **A->B = F** if the anova p-value of the effect of eqtlA on geneB is > snp.pval.nsign and geneA and geneB have different eqtls

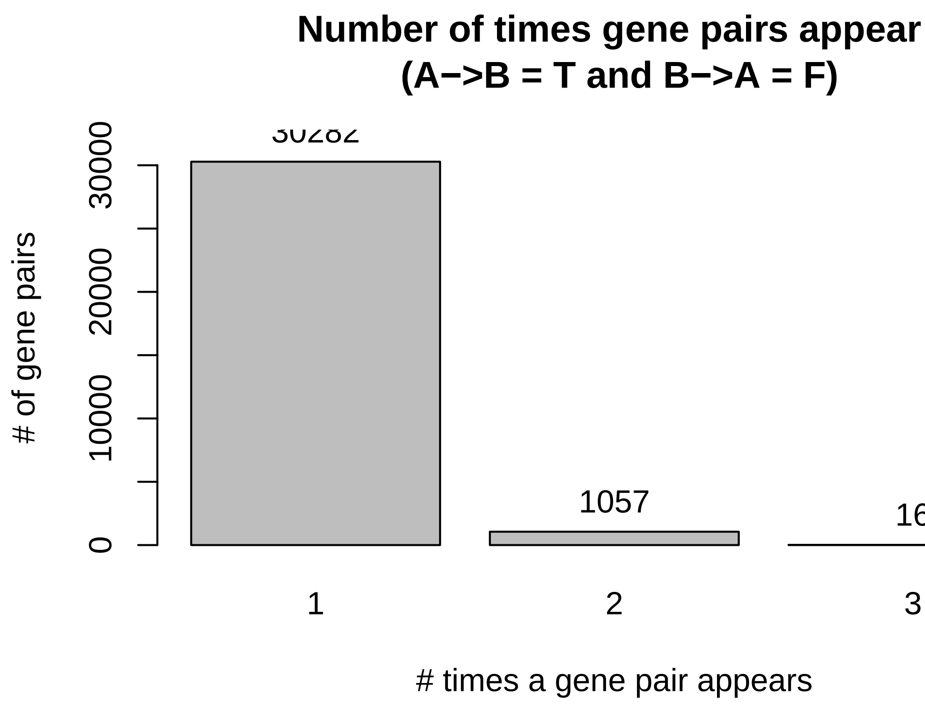
* **B->A = T** if anova p-value for the effect of eqtlB on geneA is < snp.pval

* **B->A = F** if the anova p-value of the effect of eqtlB on geneA is > snp.pval.nsign and geneA and geneB have different eqtls

3.1 Get the effect of each gene on the other and the cases where A->B = T and B->A = F

3.1.1 Plot number of times a gene pair appears

```r
find.effects_TF.numpairs <- find.effects_TF[, .N, by=.(geneA, geneB)]


numpairs.table <- data.table(N = unique(find.effects_TF.numpairs$N), numpairs =NA)
for (i in 1:nrow(numpairs.table)){
  numpairs.table$numpairs[i] <- nrow(find.effects_TF.numpairs[N==numpairs.table$N[i]])
}


# plot the number of times a gene pair appears
bp <- barplot(numpairs.table[order(-numpairs)]$numpairs,
              numpairs.table[order(-numpairs)]$N, names.arg=unique(find.effects_TF.numpairs[order(N)]$N
              width = 0.5, space=0.2, legend.text = F, ylim = c(0,max(numpairs.table$numpairs)+2500),
              main = "Number of times gene pairs appear \n (A->B = T and B->A = F)", xlab = "# times a 
              ylab = "# of gene pairs")
text(bp,numpairs.table[order(-numpairs)]$numpairs,
     labels=numpairs.table[order(-numpairs)]$numpairs, cex=1, pos=3)
```

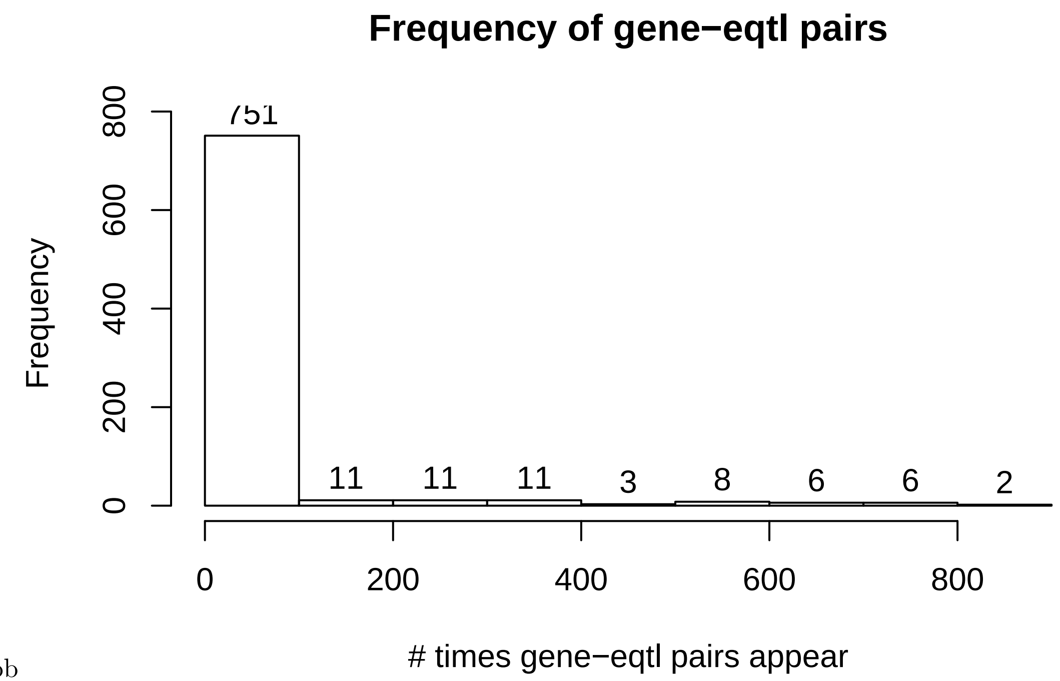


the number of times a gene pair appears-1.bb

3.1.2 Plot the number of times a gene-eqtl pair appears

3.1.3 Frequency of gene-eqtl pairs

```
find.effects_TF.geneeqtl.A <- find.effects_TF[, .N, by=.(geneA, eqtl.A)]
find.effects_TF.geneeqtl.A.plot <- find.effects_TF.geneeqtl.A %>% unite(gene_eqtlA, geneA, eqtl.A, sep =
numpairs.table2 <- data.table(N = unique(find.effects_TF.geneeqtl.A.plot$N), numpairs =NA)
for (i in 1:nrow(numpairs.table2)){
  numpairs.table2$numpairs[i] <- nrow(find.effects_TF.geneeqtl.A[N==numpairs.table2$N[i]])
}
numpairs.table2 <- numpairs.table2[order(N)]


hist(find.effects_TF.geneeqtl.A.plot$N, main = "Frequency of gene-eqtl pairs", xlab = "# times gene-eqtl
```



gene-eqtl pairs-1.bb

```
# the majority of gene-eqtl pairs appear between 0-100 times
```

3.1.4 Plot the network

Takes a very long time and you can't really see anything so it's not very worth it.

There are no subclusters, it's just one bit network

```
plot_TF <- graph_from_edgelist(as.matrix(find.effects_TF[,.(geneA, geneB)]),directed=TRUE)
# components(plot_TF)

memb <- components(plot_TF)$membership
nodes <- data.table(id = names(memb),
                    group_id = memb)
nodes <- nodes[order(nodes$id), ]

if(length(unique(nodes$group_id))> 1){
  message(paste0("There are ", length(unique(nodes$group_id)), " subclusters!"))
} else {
  message("There are no subclusters")
}
```

```
## There are no subclusters
```

```
#plot(plot_TF, layout=layout_with_gem,edge.arrow.size=.5, vertex.label=NA)
```

4 GO analysis

In order to find the GO terms associated with my genes I used YeastMine (Balakrishnan et al., 2012) (https://yeastmine.yeastgenome.org/yeastmine/begin.do). Since I was not being able to do it in R, using the YeastMine API, I used python to run my queries. To be able to run the queries with python, first I needed to create an account and request an API key. Since you can generate python code from the website, I used it as a guide and added/ removed parameters to get what I needed.

To get an API key, go to https://yeastmine.yeastgenome.org/yeastmine/begin.do and create an account. Go to account details and create a new key. Save the key in a file called yeastmineAPI.txt

“genelist” is a list of the genes involved in the causality - Uploaded into yeastmine and saved in my area

1. create gene list

```
if (!file.exists("data/genelist.txt")){
  genelist <- data.table(unique(c(find.effects_TF$geneA, find.effects_TF$geneB)))
  fwrite(genelist, "data/genelist.txt", col.names = F)
}


genelist <- fread("data/genelist.txt", header = F)
genes_in_find.effects <- data.table(unique(c(find.effects_TF$geneA, find.effects_TF$geneB)))
if (nrow(genelist) != nrow(genes_in_find.effects)){
  update_genelist <- T
  stop("YOU'LL NEED TO REUPLOAD THE GENELIST INTO YEASTMINE AND RERUN THE PYTHON CODE BELOW")
} else {
  update_genelist <- F
}
```

2. upload list into yeastmine giving it the name genelist
3. Continue once you have uploaded the genelist into yeastmine

Get table with geneID, gene symbol, gene name, GO identifier, GO term name, GO namespace and evidence code

```
# Sometimes breaks Rstudio, might be better to run it in a script/python console
import os.path
from os import path
```

```
os.chdir("/Users/Carolina/Documents/GitHub/DegreeProject/summarizing/")
if not path.isfile("results/genelistwithGOterm.txt" or r.update_genelist):
  # The following lines will be needed in every python script:
  from intermine.webservice import Service
  yeastmineAPItoken_file = open('data/yeastmineAPI.txt', 'r')
  yeastmineAPItoken = yeastmineAPItoken_file.readline().rstrip()
  service = Service("https://yeastmine.yeastgenome.org/yeastmine/service", token = yeastmineAPItoken)

  # Get a new query on the class (table) you will be querying:
  query = service.new_query("Gene")

  # The view specifies the output columns
  query.add_view(
      "secondaryIdentifier", "symbol", "name",
      "ontologyAnnotations.ontologyTerm.identifier",
      "ontologyAnnotations.ontologyTerm.name",
      "ontologyAnnotations.ontologyTerm.namespace",
      "ontologyAnnotations.evidence.code.code"
  )

  # You can edit the constraint values below
  query.add_constraint("Gene", "IN", "genelist", code = "A")

  terms = "gene", "symbol", "gene.name", "GO.identifier", "GO.term", "GO.namespace", "evidence"

  terms_query = ["secondaryIdentifier", "symbol", "name", "ontologyAnnotations.ontologyTerm.identifier"
  print("saving file")
  with open("results/genelistwithGOterm.txt", "w") as file:
    # write headers
    for term in terms[:-1]:
      file.write(term)
      file.write("\t")
    else:
      file.write(terms[-1])
    file.write("\n")
    #write content
    for row in query.rows():
      for t in terms_query[:-1]:
        if row[t] != None:
          file.write(str(row[t]))
          file.write("\t")
        else:
          file.write("NA")
          file.write("\t")
      if row[terms_query[-1]] != None:
        file.write(row[terms_query[-1]])
      else:
        file.write("NA")
      file.write("\n")
else:
  print("file exists")
```

```
## file exists
```

Load file generated with python - contains gene, gene symbol, gene name, GO code, GO term, GO namespace and evidence code

```
genes_GO.table <- fread("results/genelistwithGOterm.txt")
genes_GO.table <- unique(genes_GO.table)
```

Subset to have only the things we'll need for the next analysis - GO terms related to biological processes and remove unecessary columns

```
genes_GO.bio <- unique(genes_GO.table[GO.namespace=="biological_process"])
genes_GO.bio.noevidence <- data.table(genes_GO.bio)
genes_GO.bio.noevidence[,evidence :=NULL]
genes_GO.bio.noevidence[,GO.namespace :=NULL]
genes_GO.bio.noevidence <- unique(genes_GO.bio.noevidence)
```

Run to get different measures

```
#count number of times each ontology term appears (number of diferent genes that have this GO term)
GOterms.count <- genes_GO.bio.noevidence[, .(description=unique(GO.term), count = .N), by = GO.identifi


# find genes that have "transcription factor" in the GO term
GO_trans_factor <- unique(genes_GO.bio.noevidence[grepl("transcription factor",GO.term, fixed = F)])


# gene symbol + GO term + gene name
GO_transc_reg <- unique(genes_GO.bio.noevidence[(grepl(" transcription ",GO.term, fixed = F) & grepl(" 
```

4.0.1 number of times each geneA and geneB point to another gene

```
numlinks_from_gene.A <- find.effects_TF[, .(geneB, count.A=.N), by=geneA]
numlinks_from_gene.B <- find.effects_TF[, .(count.B=.N), by=geneB]
numlinks_from_gene <- merge(numlinks_from_gene.A, numlinks_from_gene.B, by="geneB", all=T)
setcolorder(numlinks_from_gene, c("geneA", "geneB", "count.A", "count.B"))
head(numlinks_from_gene[order(-count.A, count.B)])
```

geneA	geneB	count.A	count.B
YLR270W	YDR236C	897	2
YLR270W	YJL008C	897	2
YLR270W	YJL193W	897	3
YLR270W	YLR343W	897	3
YLR270W	YGL193C	897	4
YLR270W	YLR455W	897	4

4.0.2 number of times each geneA points to another gene + GO term

```
links_perGO_pergene <- merge(unique(genes_GO.bio.noevidence),
                             unique(numlinks_from_gene[,.(geneA, count.A)]),
                             by.x="gene", by.y="geneA", all=T)
```

```r
head(links_perGO_pergene[order(-count.A), .(gene, GO.identifier, GO.term, count.A)])
```

gene	GO.identifier	GO.term	count.A
YLR270W	GO:0000290	deadenylation-dependent decapping of nuclear-transcribed mRNA	897
YLR270W	GO:0009267	cellular response to starvation	897
YLR270W	GO:0031086	nuclear-transcribed mRNA catabolic process, deadenylation-independent decay	897
YLR270W	GO:1901919	positive regulation of exoribonuclease activity	897
YLR264W	GO:0000028	ribosomal small subunit assembly	834
YLR264W	GO:0002181	cytoplasmic translation	834

```r
links_perGO <- links_perGO_pergene[,.(gocount=sum(count.A, na.rm = T)), by=c("GO.identifier", "GO.term"
```

4.0.3 find the GOs that have transcription factor or transcription regulation in the name

```r
links_perGO_transfactor <- links_perGO[(grepl(" transcription ",GO.term, fixed = F) &
                                        grepl(" regulation ",GO.term, fixed = F)) |
                                        grepl("transcription factor",GO.term, fixed = F)]
head(links_perGO_transfactor[order(-gocount)][,.(GO.term, gocount)])
```

GO.term	gocount
positive regulation of transcription by RNA polymerase II	568
negative regulation of transcription by RNA polymerase II	479
positive regulation of transcription elongation from RNA polymerase II promoter	127
negative regulation of transcription by transcription factor localization	119
positive regulation of transcription initiation from RNA polymerase II promoter	100
positive regulation of transcription by RNA polymerase I	43

There are 65 different terms with “transcription” and “regulation” or “transcription factor” in their name

4.0.3.1 causal links GO is regulating transcription or a transcription factor

```r
bp <-
  barplot(
    links_perGO_transfactor[order(-gocount)][gocount>0]$gocount,
    names.arg = links_perGO_transfactor[gocount>0]$GO.identifier,
    ylab = "# links",
    xlab = "" ,
    ylim = c(0, 650),
    main = "Number of links going out",
    las=2,
    cex.names = 0.7
  )
text(
  bp,
  links_perGO_transfactor[order(-gocount)][gocount>0]$gocount,
  links_perGO_transfactor[order(-gocount)][gocount>0]$gocount,
  cex = 0.8,
  pos = 3
)
```

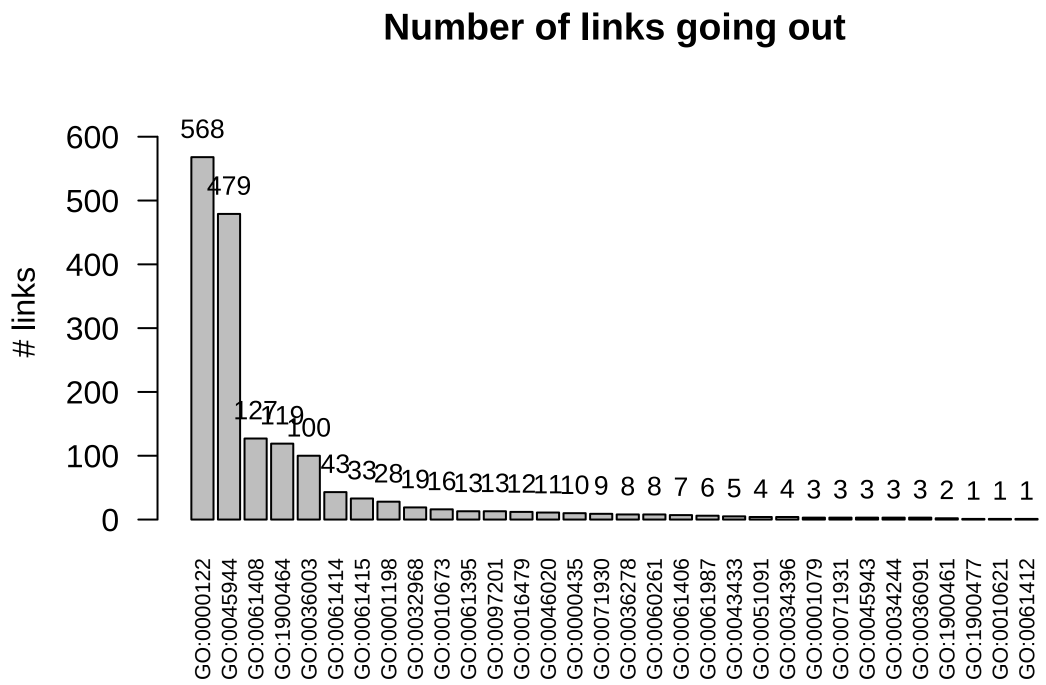


Figure 3: Number of links going out of causal genes that have terms with 'transcription factor' or 'transcription' and 'regulation'

```
hist(links_perGO_transfactor[order(-gocount)]$gocount,
     main = "# causal links \n GO is regulating transcription or a transcription factor")
```

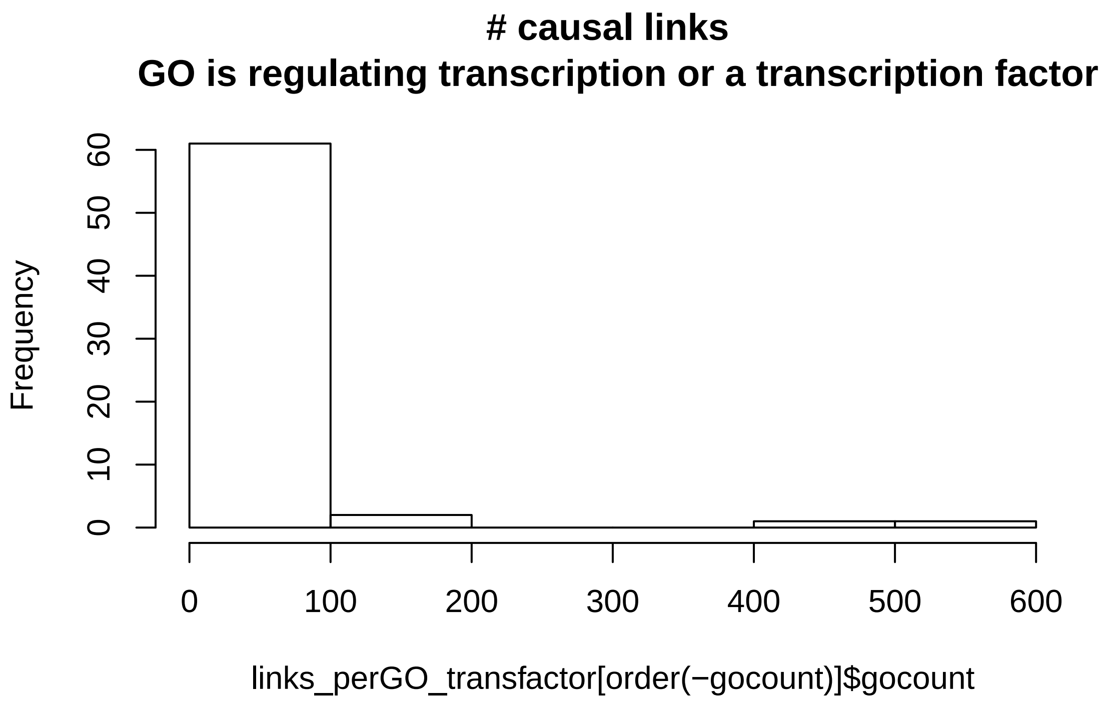


Figure 4: Number of links going out of causal genes that have terms with 'transcription factor' or 'transcription' and 'regulation'

4.0.4 Num of links going out of genes with "transcription" "regulation" or "transcription factor" as its GO term and number of links going out of genes with other descriptions

```
data.table(links_perGO[(grepl(" transcription ",GO.term, fixed = F) & grepl(" regulation ",GO.term, fix
```

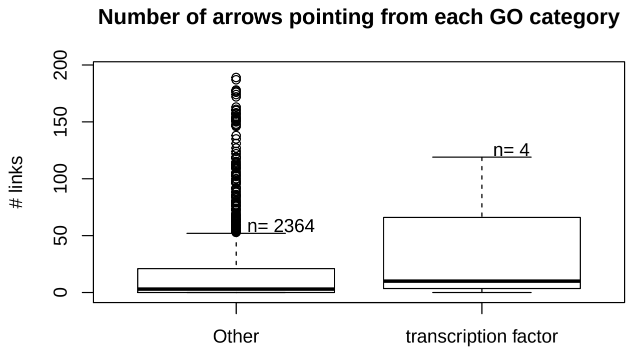
sum_trans	sum_others
1662	164609

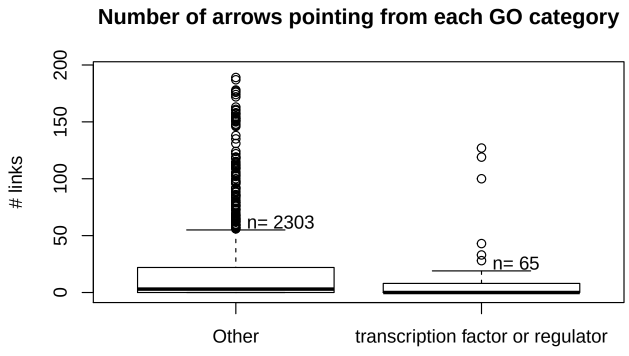
4.0.5 Num of links having "transcription" and "regulation" or "transcription factor" in the description

(with outliers)

```
bp_tfactor <-
  boxplot(
```

```r
    gocount ~ grepl("transcription factor", GO.term, fixed = F),
    data = links_perGO,
    outline = T,
    names = c("Other", "transcription factor"),
    xlab = "",
    ylab = "# links",
    main = "Number of arrows pointing from each GO category",
    ylim = c(-1, 195))

text(1:length(bp_tfactor$n),
     bp_tfactor$stats[5, ] + 5,
     paste("n=", bp_tfactor$n),
     pos = 4)

bp_tfactor_reg <-boxplot(
    gocount ~ ((grepl(" transcription ", GO.term, fixed = F) &
                grepl(" regulation ", GO.term, fixed = F)) |
                grepl("transcription factor", GO.term, fixed = F)),
    data = links_perGO,
    outline = T,
    names = c("Other", "transcription factor or regulator"),
    xlab = "",
    ylab = "# links",
    main = "Number of arrows pointing from each GO category",
    ylim = c(-1, 195))

text(1:length(bp_tfactor_reg$n),
  bp_tfactor_reg$stats[5, ] + 5,
  paste("n=", bp_tfactor_reg$n),
  pos = 4)
```



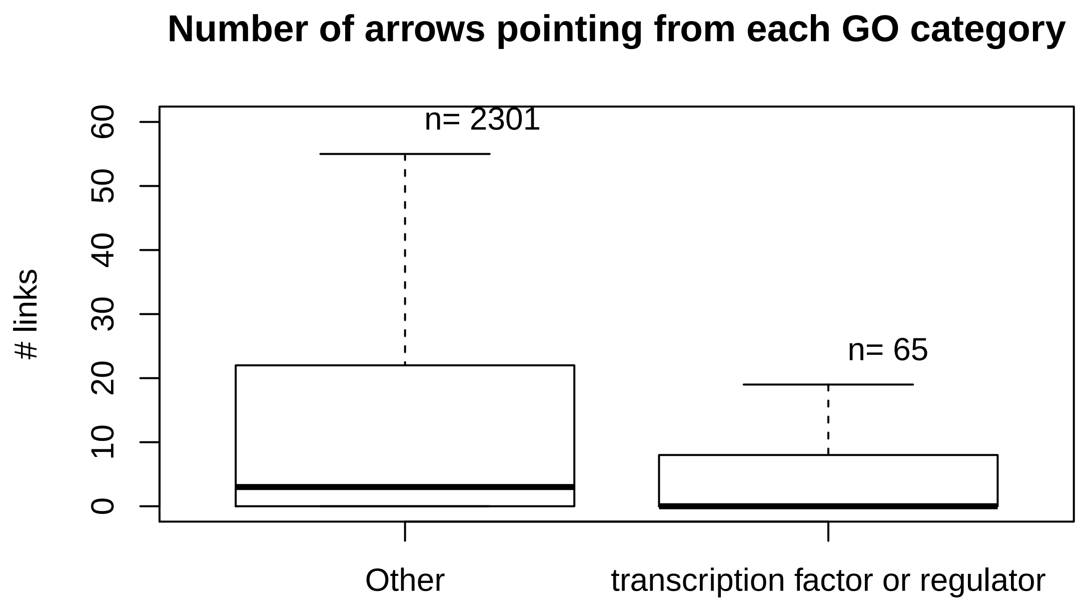


would make sense for there to be an enrichment of transcription regulators/factors in the genes A

4.0.6 Num of links having "transcription" and "regulation" or "transcription factor" in the description

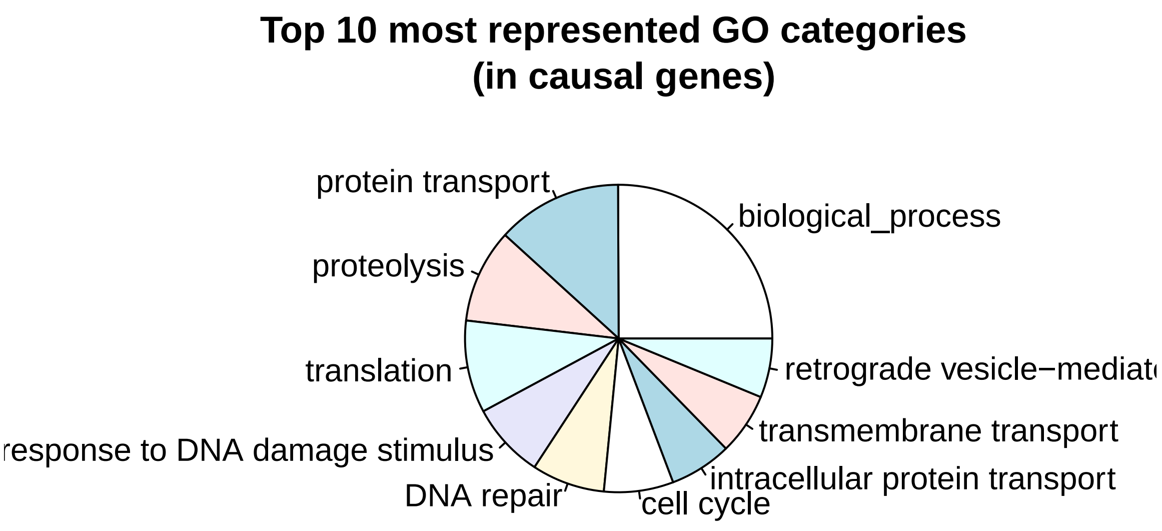
(without outliers)

```
links_perGO.nobioprocess <-
  links_perGO[!GO.term == "biological_process"]


bp_tfactor_reg <-boxplot(
    gocount ~ ((grepl(" transcription ", GO.term, fixed = F) &
                  grepl(" regulation ", GO.term, fixed = F)) |
                  grepl("transcription factor", GO.term, fixed = F)),
    data = links_perGO.nobioprocess,
    outline = F,
    names = c("Other", "transcription factor or regulator"),
    xlab = "",
    ylab = "# links",
    main = "Number of arrows pointing from each GO category",
    ylim = c(0,60)
  )


text(
  1:length(bp_tfactor_reg$n),
  bp_tfactor_reg$stats[5, ] + 5,
  paste("n=", bp_tfactor_reg$n),
  pos = 4
)
```



4.0.7 10 most represented GO categories

```
nms <- links_perGO[order(-gocount)]$GO.term[1:10]
mytable <- links_perGO[order(-gocount)]$gocount[1:10]
# labels with count
lbls <- paste( links_perGO[order(-gocount)]$GO.term[1:10], " -- ", mytable, sep="")
pie(mytable, labels = nms,
    main = "Top 10 most represented GO categories \n (in causal genes)")
```



According to EBI, the GO term “biological process” is “any process that modulates the frequency, rate or extent of a biological process” it’s usually used when the actual function of the gene is not known.

High values might be because there are many genes associated with a certain term or because the genes that are associated with that term have many “arrows” going out (they affect many other genes)

5 GO Enrichment

I used GOstats, an R package (bioconductor), to test GO terms for over representation. I used both a classical hypergeometric test and a conditional hypergeometric test, which uses the relationships among GO terms to decorrelate the results

First I needed to define a few parameters:
* **universe** - all the genes in the dataset (can be involved in the causality or not) (num genes = 5720)
* **interesting genes** - causal genes (n=2658) or affected genes (n=2478)

Falcon & Gentleman (2007) the universe can be reduced by not using the genes that are not being expressed (in this case I would say not involved in the causality). Taking this into account, it would be interesting to perform the hypergeometric test using only the genes involved in the causality (genes that affect the expression of other genes and genes that are affected) as universe. Falcon & Gentleman (2007) also suggest removing genes that do not map to any GO term
I'm performing the hypergeometric test twice, once for the causal genes and once for the affected genes to see if there's a different enrichment in both groups. It would be expected that the causal group would be enriched for genes involved in regulation.

From Falcon & Gentleman (2007)
"In the hypergeometric model, each term is treated as an independent classification. Each gene is classified according to whether or not it has been selected and whether or not it is annotated at a particular term. A hypergeometric probability is computed to assess whether the number of selected genes associated with the term is larger than expected."

Performed new hypergeometric test with
* **universe** - all the genes involved in the causality (num genes = 2861)
* **interesting genes** - causal genes or affected genes

I checked if the resulting enrichment table was the same for both universes tested and it was.
I will continue by using the results from the second test, where the universe was comprised of genes involved in the causality.

5.1 Get enrichment for the "causal" genes and for the affected genes

5.1.1 Hypergeometric test

Causal - genesA

Affected - genesB

Test universe as all the genes in the dataset and universe as all the genes involved in the causality

```
if (!exists("genes_GO.bio")){
  genes_GO.table <- fread("results/genelistwithGOterm.txt")
  genes_GO.table <- unique(genes_GO.table)
  genes_GO.bio   <- unique(genes_GO.table[GO.namespace=="biological_process"])
}

# create geneset
goframeData <- unique(genes_GO.bio[,.(GO.identifier, evidence, gene)])
gs <- getgeneset(goframeData)



genesA <- unlist(unique(find.effects_TF[,geneA]))
genesB <- unlist(unique(find.effects_TF[,geneB]))

# universe is genes involved in causality
universe <- unique(c(genesA, genesB))



# get enrichment for genesA
res.geneA <- getenrichment(gs, universe = universe, interestinggenes = genesA)
res.geneA.dt <- data.table(summary(res.geneA))

# get enrichment for genesB
res.geneB <- getenrichment(gs, universe = universe, interestinggenes = genesB)
res.geneB.dt <- data.table(summary(res.geneB))
```

```
# universe is all the genes
universe.all <- names(phenotype[,2:ncol(phenotype)])


res.geneA.uniall <- getenrichment(gs, universe = universe.all, interestinggenes = genesA)
res.geneA.uniall.dt <- data.table(summary(res.geneA.uniall))


# get enrichment for genesB
res.geneB.uniall <- getenrichment(gs, universe = universe.all, interestinggenes = genesB)
res.geneB.uniall.dt <- data.table(summary(res.geneB.uniall))



# check if I got the same results using the universe = all genes and universe = genes involved in causa
if (!isTRUE(all.equal(res.geneA, res.geneA.uniall)) | isTRUE(all.equal(res.geneB, res.geneB.uniall))) {
  message("the hypergeometric test with the different universes didn't give the same results")
} else {
  message("The hypergeometric test for the different universes gave the same results")
}
```

```
## the hypergeometric test with the different universes didn't give the same results
```

5.1.1.1 To get the GO term graph for the geneA or geneB enrichment

(where universe is all the genes involved in the causality)

```
#save all the graphs to a pdf
if (!file.exists("results/figures/termgraph_A.pdf") | update_genelist){


  termgrA <- termGraphs(res.geneA, use.terms = T, pvalue = 0.05)
  pdf(file = "results/figures/termgraph_A.pdf", onefile = T)
  for (i in 1:length(termgrA)){
    plotGOTermGraph(termgrA[[i]], r = res.geneA, add.counts = T,
                    node.colors=c(sig="green", not="white"), max.nchar=30)
  }
  dev.off()
```

```r
  message("saved the pdf with GO term graphs for the causal genes")

} else {
  message("pdf with graphs for the causal genes already exists")
}
```

```
## pdf with graphs for the causal genes already exists
```

```r
if (!file.exists("results/figures/termgraph_B.pdf") | update_genelist){

  termgrB <- termGraphs(res.geneB, use.terms = T, pvalue = 0.05)

  pdf(file = "results/figures/termgraph_B.pdf", onefile = T)
  for (i in 1:length(termgrB)){
    plotGOTermGraph(termgrB[[i]], r = res.geneB, add.counts = T,
                    node.colors=c(sig="green", not="white"), max.nchar=30)
  }
  dev.off()
  message("saved the pdf with GO term graphs for the affected genes")

} else {
  message("pdf with graphs for the affected genes already exists")
}
```

```
## pdf with graphs for the affected genes already exists
```

5.1.2 Conditional Hypergeometric test

```r
if (!exists("genes_GO.bio")){
  genes_GO.table <- fread("results/genelistwithGOterm.txt")
  genes_GO.table <- unique(genes_GO.table)
  genes_GO.bio   <- unique(genes_GO.table[GO.namespace=="biological_process"])
}

# create geneset
```

```r
goframeData <- unique(genes_GO.bio[,.(GO.identifier, evidence, gene)])
gs <- getgeneset(goframeData)



genesA <- unlist(unique(find.effects_TF[,geneA]))
genesB <- unlist(unique(find.effects_TF[,geneB]))

# universe is genes involved in causality
universe <- unique(c(genesA, genesB))

# get enrichment
hgCondA <- getenrichment(gs, universe = universe, interestinggenes = genesA, cond = T)
hgCondA.dt <- data.table(summary(hgCondA))

hgCondB <- getenrichment(gs, universe = universe, interestinggenes = genesB, cond = T)
hgCondB.dt <- data.table(summary(hgCondB))

# causal genes
hgCondA
```

```
## Gene to GO BP Conditional test for over-representation
## 3138 GO BP ids tested (137 have p < 0.05)
## Selected gene set size: 808
##     Gene universe size: 2480
##     Annotation package: Based on a GeneSetCollection Object
```

```r
# affected genes
hgCondB
```

```
## Gene to GO BP Conditional test for over-representation
## 4337 GO BP ids tested (16 have p < 0.05)
## Selected gene set size: 2377
##     Gene universe size: 2480
##     Annotation package: Based on a GeneSetCollection Object
```

```
hgCondA.dt
```

GOBPID	Pvalue	OddsRatio	ExpCount	Count	Size	Term
GO:0015074	0.0000017	7.409149	8.7967742	21	27	DNA integration
GO:0044260	0.0000493	1.407163	329.7161290	375	1012	cellular macromolecule metabolic process
GO:0032774	0.0001334	1.594596	100.0225806	129	307	RNA biosynthetic process
GO:2000112	0.0001536	1.741600	65.0561509	89	204	regulation of cellular macromolecule biosynthetic pr
GO:0006310	0.0001578	2.588475	20.7334963	35	64	DNA recombination
GO:0006351	0.0001738	1.585169	98.7193548	127	303	transcription, DNA-templated
GO:0050789	0.0002188	1.402571	219.7579204	257	678	regulation of biological process
GO:0031328	0.0002471	1.707768	65.8129032	89	202	positive regulation of cellular biosynthetic process
GO:0009889	0.0002639	1.685235	68.4890228	92	215	regulation of biosynthetic process
GO:0006508	0.0003048	1.691701	66.1387097	89	203	proteolysis
GO:0080090	0.0003075	1.572287	93.5558430	120	292	regulation of primary metabolic process
GO:0010557	0.0003752	1.691413	63.8580645	86	196	positive regulation of macromolecule biosynthetic pr
GO:0018130	0.0005465	1.421242	157.0387097	188	482	heterocycle biosynthetic process
GO:0032197	0.0005672	3.032484	12.7064516	23	39	transposition, RNA-mediated
GO:0006278	0.0005672	3.032484	12.7064516	23	39	RNA-dependent DNA biosynthetic process
GO:0010628	0.0007401	1.647514	63.2064516	84	194	positive regulation of gene expression
GO:0019438	0.0009689	1.399374	154.7580645	184	475	aromatic compound biosynthetic process
GO:0006449	0.0011812	Inf	1.9548387	6	6	regulation of translational termination
GO:1901362	0.0014664	1.368941	167.1387097	196	513	organic cyclic compound biosynthetic process
GO:0031323	0.0017321	1.492755	87.0693717	109	273	regulation of cellular metabolic process
GO:0051254	0.0017756	1.614944	57.6677419	76	177	positive regulation of RNA metabolic process
GO:0090502	0.0021940	2.383743	15.9645161	26	49	RNA phosphodiester bond hydrolysis, endonucleoly
GO:0009890	0.0023687	1.634437	51.1516129	68	157	negative regulation of biosynthetic process
GO:0090305	0.0026634	1.837383	31.6032258	45	97	nucleic acid phosphodiester bond hydrolysis
GO:2000113	0.0027213	1.646845	47.8935484	64	147	negative regulation of cellular macromolecule biosyn
GO:0051173	0.0030367	1.527000	66.5951896	85	206	positive regulation of nitrogen compound metabolic
GO:0044271	0.0033930	1.300038	218.2903226	247	670	cellular nitrogen compound biosynthetic process
GO:1903508	0.0036026	1.571207	55.3870968	72	170	positive regulation of nucleic acid-templated transcr
GO:0010603	0.0036405	Inf	1.6290323	5	5	regulation of cytoplasmic mRNA processing body as
GO:0071704	0.0043227	1.271438	514.1225806	544	1578	organic substance metabolic process

GOBPID	Pvalue	OddsRatio	ExpCount	Count	Size	Term
GO:1903008	0.0046312	2.698373	10.4258065	18	32	organelle disassembly
GO:1903507	0.0046587	1.663822	40.0741935	54	123	negative regulation of nucleic acid-templated transc
GO:0098813	0.0047088	1.986659	21.5032258	32	66	nuclear chromosome segregation
GO:0043632	0.0051799	1.718655	34.2096774	47	105	modification-dependent macromolecule catabolic pro
GO:0048522	0.0053085	1.434294	82.2409488	101	255	positive regulation of cellular process
GO:0006974	0.0056948	1.616138	43.0064516	57	132	cellular response to DNA damage stimulus
GO:0009057	0.0058392	1.480644	66.1387097	83	203	macromolecule catabolic process
GO:0007165	0.0058473	1.567173	49.1967742	64	151	signal transduction
GO:1902532	0.0058693	3.585786	6.1903226	12	19	negative regulation of intracellular signal transducti
GO:0042908	0.0059707	12.501247	2.2806452	6	7	xenobiotic transport
GO:0048585	0.0061125	2.794613	9.1225806	16	28	negative regulation of response to stimulus
GO:0006357	0.0063853	2.269841	13.7361111	22	43	regulation of transcription by RNA polymerase II
GO:0031929	0.0064349	3.832288	5.5387097	11	17	TOR signaling
GO:0006139	0.0065210	1.257750	262.2741935	290	805	nucleobase-containing compound metabolic process
GO:0031146	0.0070948	7.297129	2.9322581	7	9	SCF-dependent proteasomal ubiquitin-dependent pr
GO:0031324	0.0074633	1.481335	61.2516129	77	188	negative regulation of cellular metabolic process
GO:0006511	0.0091638	1.705703	29.8990715	41	92	ubiquitin-dependent protein catabolic process
GO:0016042	0.0096995	2.204066	13.3580645	21	41	lipid catabolic process
GO:0140013	0.0099863	2.100637	14.9870968	23	46	meiotic nuclear division
GO:0051253	0.0102045	1.568729	41.3774194	54	127	negative regulation of RNA metabolic process
GO:0051172	0.0106382	1.459986	59.2967742	74	182	negative regulation of nitrogen compound metabolic
GO:0034250	0.0111024	2.616646	8.7967742	15	27	positive regulation of cellular amide metabolic proce
GO:0043007	0.0112114	Inf	1.3032258	4	4	maintenance of rDNA
GO:0031145	0.0112114	Inf	1.3032258	4	4	anaphase-promoting complex-dependent catabolic p
GO:0034063	0.0112114	Inf	1.3032258	4	4	stress granule assembly
GO:0016567	0.0114159	1.900248	19.2225806	28	59	protein ubiquitination
GO:0010648	0.0128494	2.662469	8.1451613	14	25	negative regulation of cell communication
GO:0023057	0.0128494	2.662469	8.1451613	14	25	negative regulation of signaling
GO:0044257	0.0131998	1.551010	40.0741935	52	123	cellular protein catabolic process
GO:0016070	0.0140420	1.376252	78.1974050	94	250	RNA metabolic process
GO:0045143	0.0146346	3.755444	4.5612903	9	14	homologous chromosome segregation

GOBPID	Pvalue	OddsRatio	ExpCount	Count	Size	Term
GO:0043624	0.0148611	2.717736	7.4935484	13	23	cellular protein complex disassembly
GO:0046686	0.0159375	10.404732	1.9548387	5	6	response to cadmium ion
GO:0006817	0.0170293	4.861839	3.2580645	7	10	phosphate ion transport
GO:0006928	0.0170293	4.861839	3.2580645	7	10	movement of cell or subcellular component
GO:0010608	0.0178532	1.675291	25.7387097	35	79	posttranscriptional regulation of gene expression
GO:0051246	0.0192575	1.436182	52.4548387	65	161	regulation of protein metabolic process
GO:0033043	0.0204253	1.483516	42.6806452	54	131	regulation of organelle organization
GO:0032270	0.0212220	1.741170	20.8516129	29	64	positive regulation of cellular protein metabolic proc
GO:0006325	0.0222035	1.517417	36.5939788	47	113	chromatin organization
GO:0000086	0.0228064	2.980666	5.5387097	10	17	G2/M transition of mitotic cell cycle
GO:0051171	0.0234895	1.848214	15.8704589	23	51	regulation of nitrogen compound metabolic process
GO:0000122	0.0236022	1.652535	24.4354839	33	75	negative regulation of transcription by RNA polyme
GO:0010629	0.0236386	1.389130	59.2967742	72	182	negative regulation of gene expression
GO:1903506	0.0236932	1.917149	14.2400000	21	45	regulation of nucleic acid-templated transcription
GO:0045944	0.0242023	1.467288	42.1178862	53	130	positive regulation of transcription by RNA polyme
GO:0007049	0.0244254	1.311048	89.9225806	105	276	cell cycle
GO:1903047	0.0253338	1.538433	32.4115494	42	100	mitotic cell cycle process
GO:0043543	0.0253897	1.989100	12.7064516	19	39	protein acylation
GO:0010468	0.0255923	1.827773	15.9833102	23	51	regulation of gene expression
GO:0001302	0.0268090	2.505528	7.1677419	12	22	replicative cell aging
GO:0003333	0.0268090	2.505528	7.1677419	12	22	amino acid transmembrane transport
GO:0006333	0.0268090	2.505528	7.1677419	12	22	chromatin assembly or disassembly
GO:0006950	0.0279536	1.274141	109.1451613	125	335	response to stress
GO:0043620	0.0288900	2.250145	8.7967742	14	27	regulation of DNA-templated transcription in respor
GO:1903825	0.0297046	2.090909	10.4258065	16	32	organic acid transmembrane transport
GO:0036003	0.0313313	2.550258	6.5161290	11	20	positive regulation of transcription from RNA polyr
GO:0000422	0.0313313	2.550258	6.5161290	11	20	autophagy of mitochondrion
GO:1903432	0.0338113	3.644195	3.5838710	7	11	regulation of TORC1 signaling
GO:0006448	0.0338113	3.644195	3.5838710	7	11	regulation of translational elongation
GO:0036211	0.0342190	1.247119	122.1774194	138	375	protein modification process
GO:2000059	0.0344977	Inf	0.9774194	3	3	negative regulation of ubiquitin-dependent protein c

GOBPID	Pvalue	OddsRatio	ExpCount	Count	Size	Term
GO:1903710	0.0344977	Inf	0.9774194	3	3	spermine transmembrane transport
GO:1903711	0.0344977	Inf	0.9774194	3	3	spermidine transmembrane transport
GO:0045842	0.0344977	Inf	0.9774194	3	3	positive regulation of mitotic metaphase/anaphase t
GO:0002949	0.0344977	Inf	0.9774194	3	3	tRNA threonylcarbamoyladenosine modification
GO:0120174	0.0344977	Inf	0.9774194	3	3	stress-induced homeostatically regulated protein deg
GO:0005980	0.0344977	Inf	0.9774194	3	3	glycogen catabolic process
GO:0071168	0.0344977	Inf	0.9774194	3	3	protein localization to chromatin
GO:0051446	0.0344977	Inf	0.9774194	3	3	positive regulation of meiotic cell cycle
GO:1905820	0.0344977	Inf	0.9774194	3	3	positive regulation of chromosome separation
GO:0062033	0.0344977	Inf	0.9774194	3	3	positive regulation of mitotic sister chromatid segreg
GO:0051228	0.0344977	Inf	0.9774194	3	3	mitotic spindle disassembly
GO:0006384	0.0344977	Inf	0.9774194	3	3	transcription initiation from RNA polymerase III pr
GO:0051130	0.0354937	1.575484	25.0870968	33	77	positive regulation of cellular component organizatio
GO:0010498	0.0355812	1.665512	19.8741935	27	61	proteasomal protein catabolic process
GO:0030148	0.0366405	2.606516	5.8645161	10	18	sphingolipid biosynthetic process
GO:0040008	0.0366405	2.606516	5.8645161	10	18	regulation of growth
GO:0016570	0.0378869	1.690149	18.2451613	25	56	histone modification
GO:0033045	0.0398432	2.276382	7.4935484	12	23	regulation of sister chromatid segregation
GO:0098656	0.0402081	1.720291	16.6161290	23	51	anion transmembrane transport
GO:0030705	0.0408544	5.199253	2.2806452	5	7	cytoskeleton-dependent intracellular transport
GO:0060237	0.0408544	5.199253	2.2806452	5	7	regulation of fungal-type cell wall organization
GO:0046015	0.0408544	5.199253	2.2806452	5	7	regulation of transcription by glucose
GO:0007346	0.0410701	1.600770	21.8290323	29	67	regulation of mitotic cell cycle
GO:0071824	0.0410701	1.600770	21.8290323	29	67	protein-DNA complex subunit organization
GO:0048017	0.0414949	8.313433	1.6290323	4	5	inositol lipid-mediated signaling
GO:0030149	0.0414949	8.313433	1.6290323	4	5	sphingolipid catabolic process
GO:2000104	0.0414949	8.313433	1.6290323	4	5	negative regulation of DNA-dependent DNA replica
GO:0000076	0.0414949	8.313433	1.6290323	4	5	DNA replication checkpoint
GO:0045903	0.0414949	8.313433	1.6290323	4	5	positive regulation of translational fidelity
GO:0051785	0.0414949	8.313433	1.6290323	4	5	positive regulation of nuclear division
GO:0015740	0.0414949	8.313433	1.6290323	4	5	C4-dicarboxylate transport

GOBPID	Pvalue	OddsRatio	ExpCount	Count	Size	Term
GO:0015828	0.0414949	8.313433	1.6290323	4	5	tyrosine transport
GO:0015813	0.0414949	8.313433	1.6290323	4	5	L-glutamate transmembrane transport
GO:0046173	0.0414949	8.313433	1.6290323	4	5	polyol biosynthetic process
GO:0051252	0.0426191	1.721470	15.8106297	22	50	regulation of RNA metabolic process
GO:0048285	0.0438284	1.495061	28.9967742	37	89	organelle fission
GO:0044089	0.0465924	1.705306	15.9645161	22	49	positive regulation of cellular component biogenesis
GO:0007093	0.0469081	2.293852	6.8419355	11	21	mitotic cell cycle checkpoint
GO:0015867	0.0469081	2.293852	6.8419355	11	21	ATP transport
GO:0051716	0.0477139	1.249970	97.7518248	111	306	cellular response to stimulus
GO:1901264	0.0477616	1.957755	10.1000000	15	31	carbohydrate derivative transport
GO:0000226	0.0481370	2.086793	8.4709677	13	26	microtubule cytoskeleton organization
GO:0000725	0.0481370	2.086793	8.4709677	13	26	recombinational repair
GO:0006413	0.0481370	2.086793	8.4709677	13	26	translational initiation
GO:0007568	0.0481370	2.086793	8.4709677	13	26	aging

```
hgCondB.dt
```

GOBPID	Pvalue	OddsRatio	ExpCount	Count	Size	Term
GO:0044281	0.0002792	3.965604	388.17944	400	405	small molecule metabolic process
GO:0006753	0.0031634	Inf	126.51774	132	132	nucleoside phosphate metabolic process
GO:0016053	0.0070694	Inf	109.26532	114	114	organic acid biosynthetic process
GO:1901135	0.0133808	4.212170	177.31653	183	185	carbohydrate derivative metabolic process
GO:0055114	0.0151275	3.314832	208.94597	215	218	oxidation-reduction process
GO:0019752	0.0156343	3.297889	207.98750	214	217	carboxylic acid metabolic process
GO:1901605	0.0157001	Inf	92.01290	96	96	alpha-amino acid metabolic process
GO:0006732	0.0171490	Inf	90.09597	94	94	coenzyme metabolic process
GO:0090407	0.0259591	5.757333	122.68387	127	128	organophosphate biosynthetic process
GO:0009165	0.0290788	Inf	78.59435	82	82	nucleotide biosynthetic process
GO:0072521	0.0331686	Inf	75.71895	79	79	purine-containing compound metabolic process
GO:0009259	0.0362065	Inf	73.80202	77	77	ribonucleotide metabolic process
GO:0006790	0.0431330	Inf	69.96815	73	73	sulfur compound metabolic process

GOBPID	Pvalue	OddsRatio	ExpCount	Count	Size	Term
GO:0051188	0.0450604	Inf	69.00968	72	72	cofactor biosynthetic process
GO:0005975	0.0453705	5.043709	108.30685	112	113	carbohydrate metabolic process
GO:0008652	0.0491747	Inf	67.09274	70	70	cellular amino acid biosynthetic process

to filter the results, the following parameters can be added to the "summary()" call:

Optional arguments pvalue and categorySize allow specification of maximum p-value and minimum categorySize

5.1.2.1 Other analysis

```r
if (exists("res.geneA") & exists("hgCondA") & exists("res.geneB") & exists("hgCondB")){
  # GO terms that are marked significant by the standard hypergeo test, but not by the conditional test
  stdIdsA = sigCategories(res.geneA)
  condIdsA = sigCategories(hgCondA)
  # num of GO terms that were not significant with the conditional hypergeo test
  # print(length(setdiff(stdIdsA, condIdsA)))

  stdIdsB = sigCategories(res.geneB)
  condIdsB = sigCategories(hgCondB)
  # num of GO terms that were not significant with the conditional hypergeo test
  # print(length(setdiff(stdIdsB, condIdsB)))

  # terms that are enriched in the hypergeo test but not on the conditional
  goterms_notin_condA <- res.geneA.dt[GOBPID %in% setdiff(stdIdsA, condIdsA)]
  goterms_notin_condB <- res.geneB.dt[GOBPID %in% setdiff(stdIdsB, condIdsB)]

  # create HTML reports (tables with enrichment) where the GO terms have links that
  # take you to a page where you can learn more about them
  htmlReport(hgCondA, file="results/hgCondA_htmlreport.html")
  htmlReport(hgCondB, file="results/hgCondB_htmlreport.html")
} else {
  message("Please perform the conditional and the 'normal' hypergeometric test")
}
```

Number of GO terms that were not significant with the conditional hypergeo test:

* for the causal genes - 122

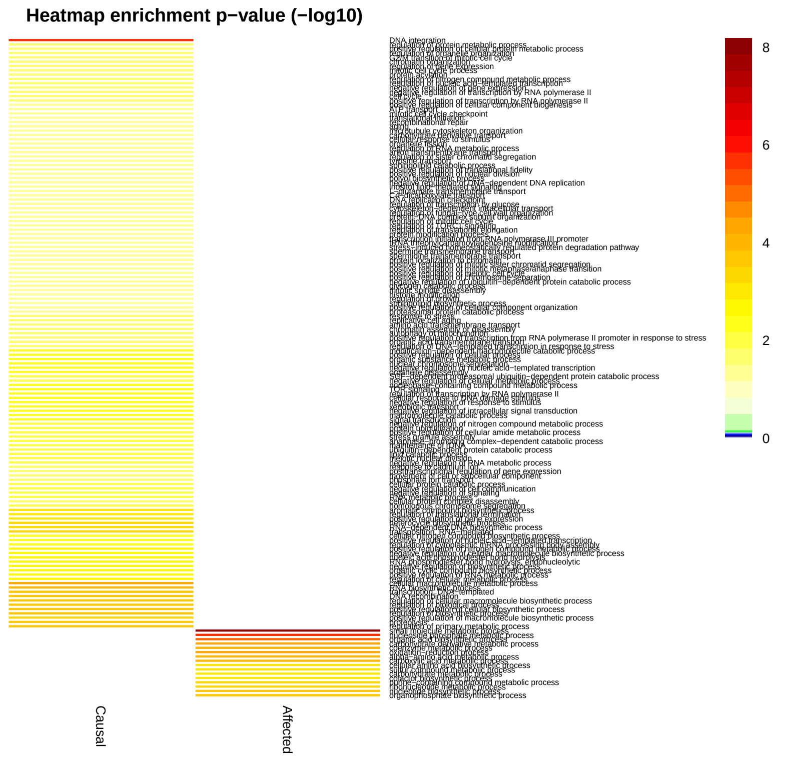
* for the affected genes - 12

set of genes	Hypergeo	Conditional hypergeo
causal		
affected		

5.1.2.2 GO Enrichment Heatmap (-log10(pval))

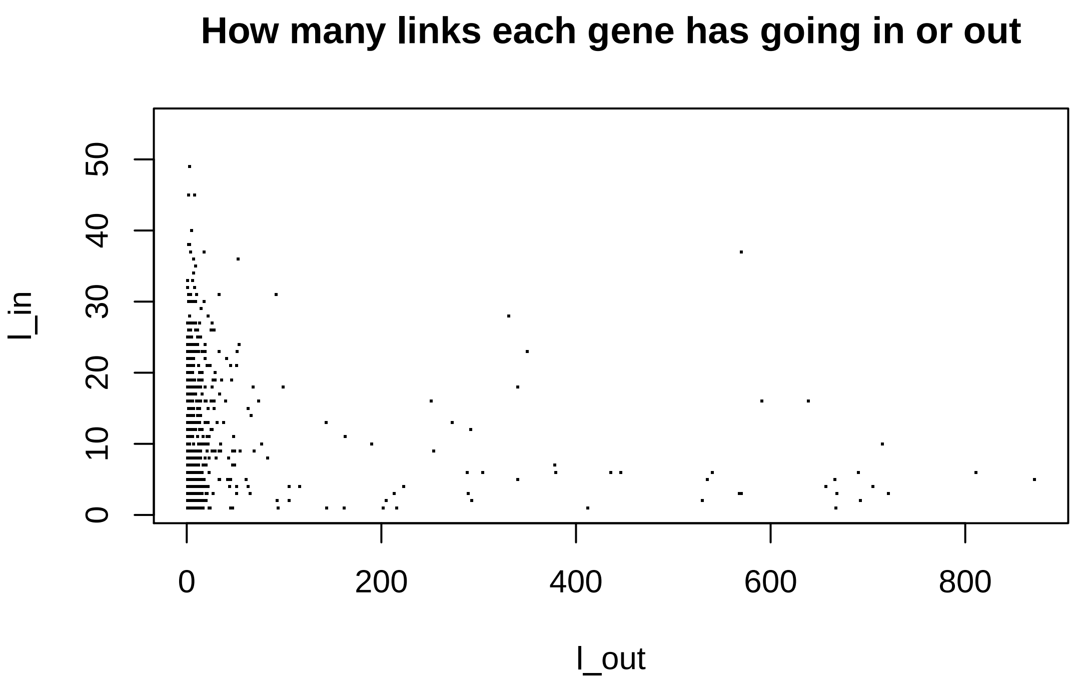
```
if (exists("hgCondA.dt") & exists("hgCondB.dt")){
  tocombine.A <- data.table(-log10(hgCondA.dt$Pvalue), hgCondA.dt$Term)
  tocombine.B <- data.table(-log(hgCondB.dt$Pvalue), hgCondB.dt$Term)
  combined <- merge(tocombine.A, tocombine.B, by="V2", all=T)
  colnames(combined) <- c("term", "Causal", "Affected")

  # Causal <- combined$Causal
  # Affected <- combined$Affected

  if (!file.exists("results/figures/heatmap_enrichmentpvals.pdf") | update_genelist){

    pdf("results/figures/heatmap_enrichmentpvals.pdf")
    combined[is.na(Causal)]$Causal <- 0
    combined[is.na(Affected)]$Affected <- 0
    plot_enrichment_heatmap(combined)
    dev.off()
    plot_enrichment_heatmap(combined)
  } else {
    combined[is.na(Causal)]$Causal <- 0
    combined[is.na(Affected)]$Affected <- 0
    plot_enrichment_heatmap(combined)
```

```r
  }
} else {
  stop("Please perform the conditional hypergeometric test")
}
```

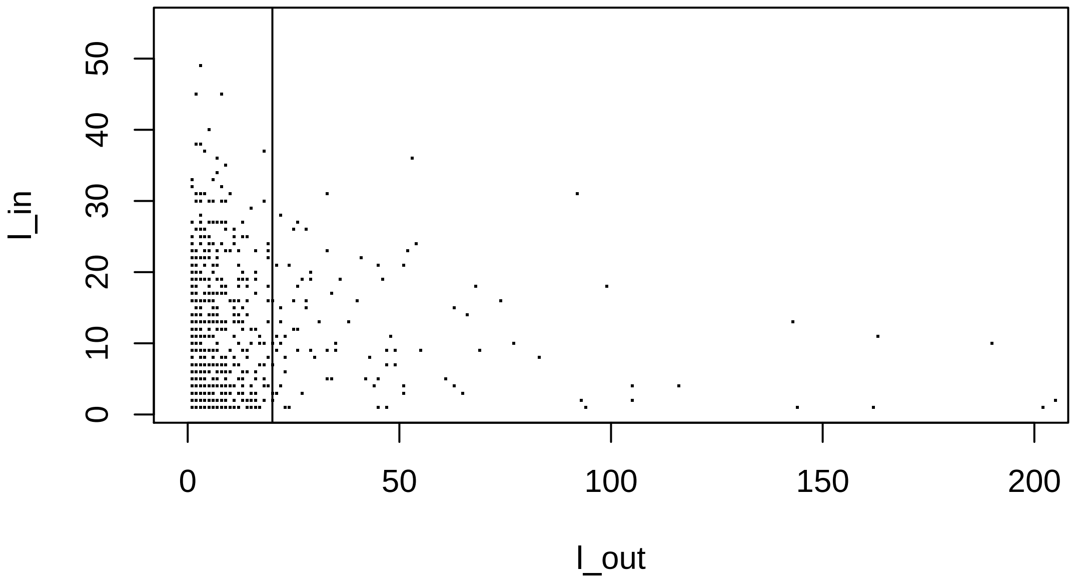


5.2 Using a subset of the causal and the affected genes

```r
if (!exists("find.effects_TF")){
  find.effects_TF <- fread("results/findeffects_TF_newparams.gz")
}
numlinks_from_geneA <- unique(find.effects_TF[, .(geneA, geneB)])[, .(l_out = .N), by = geneA]
numlinks_from_geneB <- unique(find.effects_TF[, .(geneA, geneB)])[, .(l_in = .N), by = geneB]

# table with how many links go in and out of a gene
genes <-data.table(genes = unique(c(find.effects_TF$geneA, find.effects_TF$geneB)))
merge_lin <-merge( genes, numlinks_from_geneA, by. = "genes", by.y = "geneA", all.x = T)
genes_nlinks <-merge( merge_lin, numlinks_from_geneB, by.x = "genes", by.y = "geneB", all.x = T)
# genes_nlinks[order(-l_out, l_in)]
# scatterplot of link distribution
plot(genes_nlinks[, .(l_out, l_in)], main = "How many links each gene has going in or out", pch =".")
```



```r
plot(genes_nlinks[, .(l_out, l_in)], xlim = c(0, 200), pch = ".")
abline(v = 20)
```

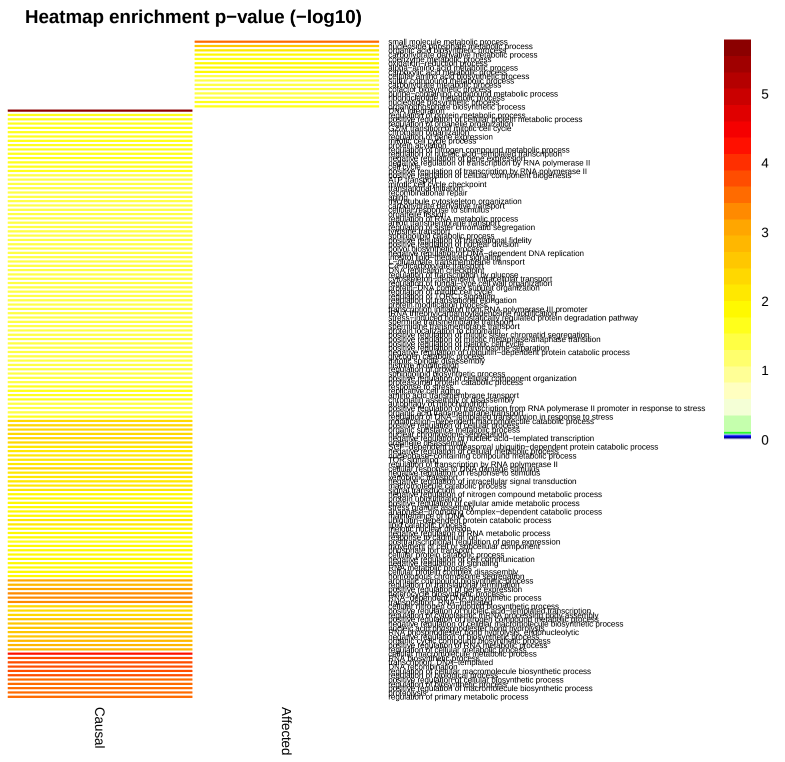


There are 3 genes that have more links going in than out in the causal genes.

5.2.1 Conditional hypergeometric test with new subset of causal and affected genes

```
# creating the sets of genes to use
# defined causalgenes as the genes that have more than 20 links going out
causalgenes <- genes_nlinks[l_out > 20]
# defined affectedgenes as the genes that have no links going out
affectedgenes <- genes_nlinks[is.na(l_out) & !is.na(l_in)]

# the "interesting genes" - either genesA (the causal ones) or genesB (on the receiving end)
# the "universe" - in this case, only the genes that are involved in the causality
genesA <- causalgenes$genes
genesB <- affectedgenes$genes

universe <- unlist(unique(c(find.effects_TF[, geneA], find.effects_TF[, geneB])))

# create geneset
gs <- getgeneset(goframeData)

# get enrichment for genesA
hgCondA.sub <- getenrichment(gs, universe = universe, interestinggenes = genesA, cond = T)
hgCondA.sub.dt <- data.table(summary(hgCondA.sub))

hgCondB.sub <- getenrichment(gs, universe = universe, interestinggenes = genesB, cond = T)
hgCondB.sub.dt <- data.table(summary(hgCondB.sub))


# table with all enriched GO terms found for both the causal and the affected genes with the correspond
tocombine.A <- data.table(-log10(hgCondA.dt$Pvalue), hgCondA.dt$Term)
tocombine.B <- data.table(-log10(hgCondB.dt$Pvalue), hgCondB.dt$Term)
combined <- merge(tocombine.A, tocombine.B, by = "V2", all = T)
colnames(combined) <- c("term", "Causal", "Affected")
combined[is.na(Causal)]$Causal <- 0
combined[is.na(Affected)]$Affected <- 0
```

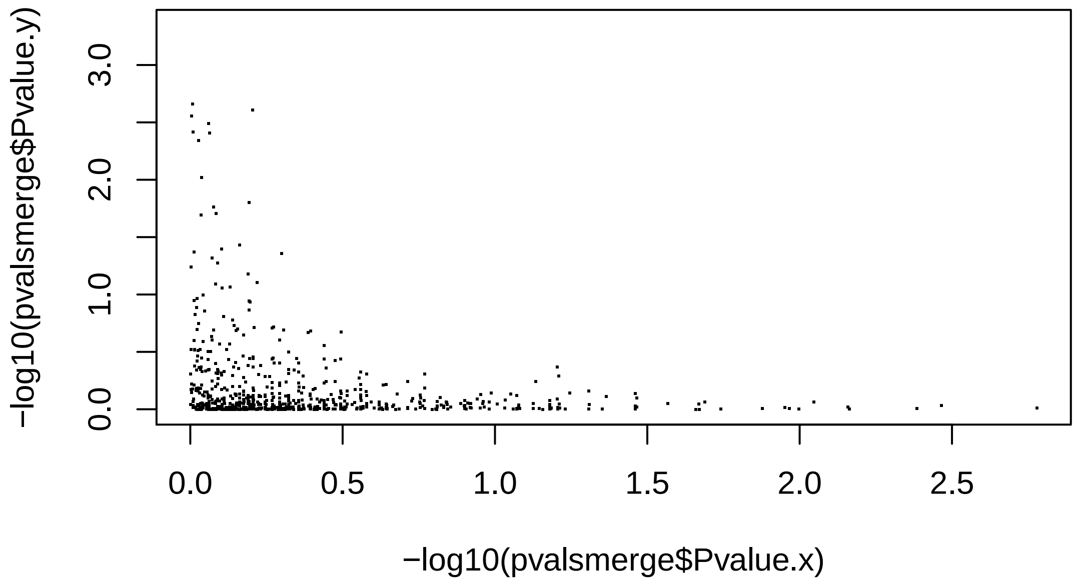
```
if (!file.exists("results/figures/heatmap_enrichmentpvals_geneslinks_c20.pdf") | update_genelist){
  # plot GO enrichment heatmap
  pdf("results/figures/heatmap_enrichmentpvals_geneslinks_c20.pdf")
  plot_enrichment_heatmap(combined)
  dev.off()

  plot_enrichment_heatmap(combined)
} else {
  plot_enrichment_heatmap(combined)
}
```



5.2.1.1 Plot of the p-values of enrichment of all terms for all genes

(subset of causal and affected genes)

```
hgCondA.nopvallim <- getenrichment(gs, universe = universe, interestinggenes = genesA, cond = T, pval =
hgCondA.nopvallim.dt <- data.table(summary(hgCondA.nopvallim))


hgCondB.nopvallim <- getenrichment(gs, universe = universe, interestinggenes = genesB, cond = T, pval =
hgCondB.nopvallim.dt <- data.table(summary(hgCondB.nopvallim))


pvalsmerge <- merge(hgCondA.nopvallim.dt[,.(GOBPID, Term, Pvalue)], hgCondB.nopvallim.dt[,.(GOBPID, Terr
plot(-log10(pvalsmerge$Pvalue.x), -log10(pvalsmerge$Pvalue.y), pch=".")
```



6 Plot similar to the one from the paper

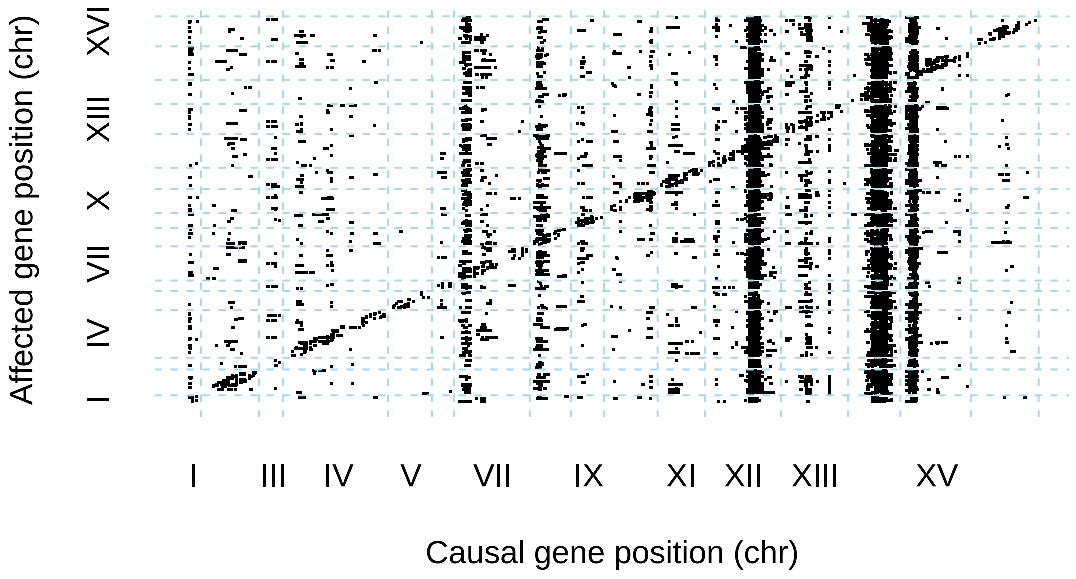
Albert FW, Bloom JS, Siegel J, Day L, Kruglyak L. 2018. Genetics of trans-regulatory variation in gene expression. eLife 7: 1–39.

6.1 get gene position

6.1.1 Plot affected genes x causal genes

```
genepos <- fread("results/gene_pos.gz")
if (!exists("find.effects_TF")){
  find.effects_TF <- fread("results/findeffects_TF_newparams.gz")
}


# Add chomosome and start position to each gene
causal.pos.A <- merge(find.effects_TF, genepos, by.x="geneA", by.y="gene", all.x=T)
colnames(causal.pos.A) <- c("geneA", "geneB", "eqtl.A", "eqtl.B", "A->B", "B->A", "strand.A", "start.A"
```

```r
causal.pos.B <- merge(causal.pos.A, genepos, by.x="geneB", by.y="gene", all.x=T)



# Keep olnly columns with genes, start positions and chromosomes
causal.pos.B.2 <- unique(causal.pos.B[,.(geneA, geneB, start.A, chr.A, chr.start, chr.id)])
colnames(causal.pos.B.2) <- c("geneA", "geneB", "start.A", "chr.A", "start.B", "chr.B")

## transform chromosome ids into numbers
# remove "chr" part of the chromosome name
causal.pos.B.2$chr.A <-gsub('chr', '', causal.pos.B.2$chr.A)
causal.pos.B.2$chr.B <-gsub('chr', '', causal.pos.B.2$chr.B)
colnames(causal.pos.B.2) <- c("geneA", "geneB", "start.A", "chr.A", "start.B", "chr.B")

# convert roman chromosome numbers to numbers
causal.pos.B.2$chr.A <- as.numeric(as.roman(causal.pos.B.2$chr.A))
causal.pos.B.2$chr.B <- as.numeric(as.roman(causal.pos.B.2$chr.B))

# order values
causal.pos.B.2.order <- causal.pos.B.2[order(chr.A, start.A, chr.B, start.B)]



# organize coordinates so that they are ordered by chromosome
# vector of chromosomes
vchr <- 1:16
# how much space will be separating chromosomes
separator <- 1e5

coordinates_plot <- sort_by_chr(vchr = vchr, causal.pos.B.2.order, separator = separator)

plot_sorted_coordinates(coordinates_plot, separator = separator)
```



6.1.2 Which genes in the paper are also in my results

Table with the number of times a gene form the paper is the causal or the affected gene

```
# genes in hotspots referred to in the text
genes_in_text <- c("GPA1", "ERC1", "STB5", "HAP1", "KRE33", "MKT1", "IRA2")


# genes from the list above that are in my dataset
my_genes_in_text <- unique(genes_GO.bio[symbol %in% genes_in_text, .(gene, symbol)])


# keep causality results where either geneA or geneB are in the list above
my_genes_in_text.effects <- find.effects_TF[geneA %in% my_genes_in_text$gene |
                                            geneB %in% my_genes_in_text$gene]
# my_genes_in_text.effects <- find.effects_TF[geneA %in% my_genes_in_text$gene]


# replace my geneIds with the gene symbol (like what they have in the paper)
for (rown in 1:nrow(my_genes_in_text)){
  my_genes_in_text.effects[geneA == my_genes_in_text[rown]$gene]$geneA <- my_genes_in_text[rown]$symbol
  my_genes_in_text.effects[geneB == my_genes_in_text[rown]$gene]$geneB <- my_genes_in_text[rown]$symbol
}
# my_genes_in_text.effects[geneA %in% my_genes_in_text$symbol,.N, by=geneA]
# my_genes_in_text.effects[geneB %in% my_genes_in_text$symbol,.N, by=geneB]
```

```r
# number of times a gene is the causal one or the affected one
mygenes_paper.count <- merge(my_genes_in_text.effects[geneA %in% my_genes_in_text$symbol,.N, by=geneA],
      my_genes_in_text.effects[geneB %in% my_genes_in_text$symbol,.N, by=geneB],
      by.x="geneA", by.y="geneB", all=T)
setnames(mygenes_paper.count, c("gene", "causal", "affected"))
mygenes_paper.count[order(-causal)]
```

gene	causal	affected
GPA1	77	16
ERC1	36	9
STB5	8	1
KRE33	NA	8