

## GO Enrichment

Run the `render()` function below and everything will be run with report at end.

```
library(rmarkdown)
render("skeleton_GO.Rmd", "pdf_document", output_file = paste(sample1,"_",sample2,"_", "GO.pdf",sep=""))
```

## Read in YAML guide

```
library(yaml)
ymls <- yaml.load_file("de.yml")
sample1 <- ymls$sample1
sample2 <- ymls$sample2

sample1
```

```
## [1] "tf2bmbr"
```

```
sample2
```

```
## [1] "tf2bother"
```

```
library(goseq)
library(GO.db)
```

## Setting up the DE table for GO analysis

### File Input

Input the output from DE analysis. This is made for a list that includes only the significant genes.

```
sigOnly <- read.table(paste(sample1,"_",sample2,"_DE_sig.txt", sep=""), header = TRUE, fill = TRUE)
sigOnly$logFC <- as.numeric(as.character(sigOnly$logFC))
colnames(sigOnly)[1] <- "itag"
```

### Subset

First I need to subset the list to up or down regulated, then add a new column that specifies 1. This column is needed for merging.

```
upITAG <- subset(sigOnly, logFC > 0, select = c(itag))
upITAG$up <- 1

downITAG <- subset(sigOnly, logFC < 0, select = c(itag))
downITAG$down <- 1

allITAG <- subset(sigOnly, select = c(itag))
allITAG$all <- 1
```

## Merge I - with normalized ITAG length gene list

read in guide.

```
geneLength <- read.csv("../normalized_genes_length.csv")
head(geneLength)
```

```
##           itag length
## 1 Solyc00g005040.2.1   357
## 2 Solyc00g005050.2.1   588
## 3 Solyc00g005060.1.1   273
## 4 Solyc00g005070.1.1    81
## 5 Solyc00g005080.1.1   297
## 6 Solyc00g005150.1.1  1143
```

```
#isolate just the gene list
genes <- subset(geneLength, select = c(itag))
```

First merge each table to geneLength

```
upITAGmerge <- merge(genes, upITAG, by = "itag", all= TRUE)
downITAGmerge <- merge(genes, downITAG, by = "itag", all= TRUE)
allITAGmerge <- merge(genes, allITAG, by = "itag", all= TRUE)
```

## Merge II - Merge them all together.

```
matrixGOupdown <- merge(upITAGmerge, downITAGmerge, by = "itag", all = TRUE)
matrixGOupdownall <- merge(matrixGOupdown, allITAG, by = "itag", all = TRUE)
matrixGO <- merge(matrixGOupdownall, geneLength, by = "itag", all = TRUE)
```

## Clean Up

```
matrixGO[is.na(matrixGO)] <- 0
head(matrixGO)
```

```
##           itag up down all length
## 1 Solyc00g005040.2.1 0   0   0   357
## 2 Solyc00g005050.2.1 0   0   0   588
## 3 Solyc00g005060.1.1 0   0   0   273
## 4 Solyc00g005070.1.1 0   1   1    81
## 5 Solyc00g005080.1.1 0   0   0   297
## 6 Solyc00g005150.1.1 0   0   0  1143
```

This is if you want to write out the table of the GO matrix. `#write.table(matrixGO, "mydata.txt", sep=" ", quote= FALSE)`

## GO enrichment

This is the input of the GOSlim categories. There are only two columns 1. itag and 2. go

```
pat <- matrixGO
head(pat)
```

```
##               itag up down all length
## 1 Solyc00g005040.2.1 0    0  0    357
## 2 Solyc00g005050.2.1 0    0  0    588
## 3 Solyc00g005060.1.1 0    0  0    273
## 4 Solyc00g005070.1.1 0    1  1     81
## 5 Solyc00g005080.1.1 0    0  0    297
## 6 Solyc00g005150.1.1 0    0  0   1143
```

```
cate <- read.table("../melted.GOTable.txt",header=TRUE)
head(cate)
```

```
##               itag               go
## 1 Solyc00g005000.2.1 GO:0006508
## 2 Solyc00g005040.2.1 GO:0005774
## 3 Solyc00g005050.2.1 GO:0005829
## 4 Solyc00g005080.1.1 GO:0005524
## 5 Solyc00g005130.1.1 GO:0006508
## 6 Solyc00g005150.1.1 GO:0003676
```

### Subsetting for GO analysis

Specify the column you are interested in `pat$all` refers to all the DE gene regardless if they are up or down regulated. If you want to specify down regulated, specify `pat$down`. I am going to put this into a loop, where each time the loop goes through it will perform GO enrichment on all three types of lists of significant genes and then write them to a table.

```
sigType <- c("up", "down", "all")

for(type in sigType) {

  genes = as.integer(pat[,type])
  names(genes) = pat$itag
  table(genes)
  length(genes)

  pwf = nullp(genes,bias.data=pat$length)

  GO.wall = goseq(pwf, gene2cat = cate)
  head(GO.wall)

#This is going to correct for multiple testing. You can specify the p-value cut-off of GO categories y

  enriched.GO = GO.wall$category[p.adjust(GO.wall$over_represented_pvalue, method = "BH") < 0.05]

  enriched.GO
```

```

my.GO <- as.character(enriched.GO)
my.GO.table <- Term(my.GO)
my.GO.table
t <- as.matrix(my.GO.table)

print(type) #this is for the knitr document
print(t) #this is for the knitr document

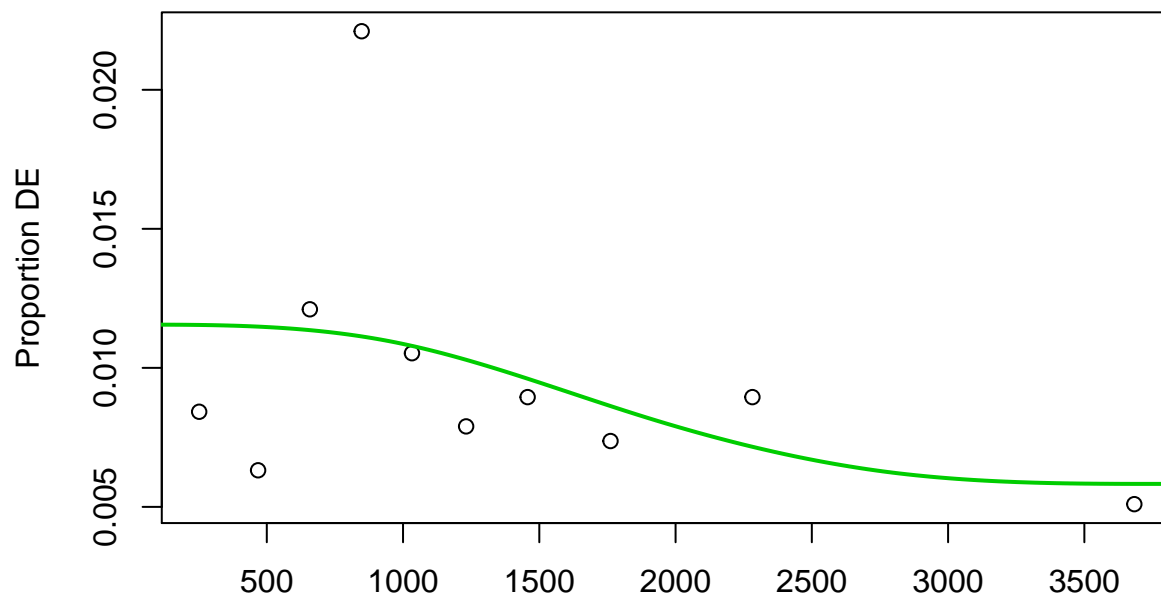
write.table(t, file=paste(sample1,"_",sample2,"DE1_sigonly_",type,"_GO.txt", sep=""))
}

```

```

## Using manually entered categories.
## For 2936 genes, we could not find any categories. These genes will be excluded.
## To force their use, please run with use_genes_without_cat=T (see documentation).
## This was the default behavior for version 1.15.1 and earlier.
## Calculating the p-values...

```



Biased Data in 1900 gene bins.

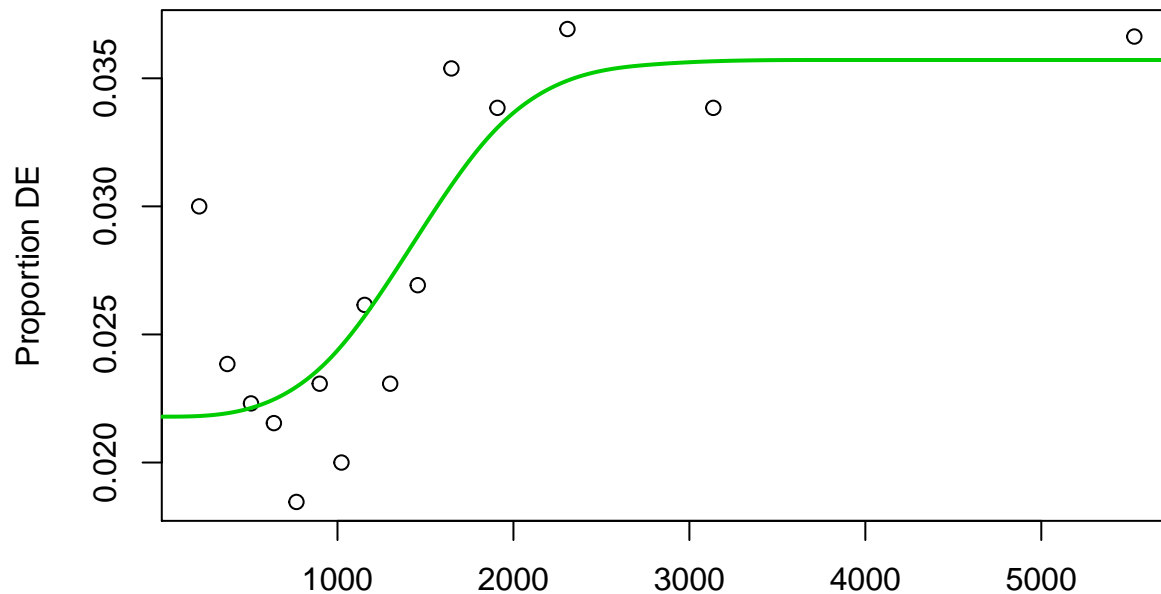
```

## [1] "up"
##           [,1]
## G0:0009765 "photosynthesis, light harvesting"
## G0:0009523 "photosystem II"
## G0:0018298 "protein-chromophore linkage"
## G0:0009522 "photosystem I"
## G0:0016168 "chlorophyll binding"
## G0:0009535 "chloroplast thylakoid membrane"
## G0:0006833 "water transport"
## G0:0015250 "water channel activity"
## G0:0030076 "light-harvesting complex"
## G0:0046872 "metal ion binding"
## G0:0016021 "integral component of membrane"

```

```
## G0:0010287 "plastoglobule"
## G0:0016020 "membrane"
## G0:0042807 "central vacuole"
## G0:0009768 "photosynthesis, light harvesting in photosystem I"
## G0:0048046 "apoplast"
## G0:0010487 "thermospermine synthase activity"
## G0:0048759 "xylem vessel member cell differentiation"
## G0:0015200 "methylammonium transmembrane transporter activity"
## <NA>      NA
## G0:0072489 "methylammonium transmembrane transport"
## G0:0009638 "phototropism"
```

```
## Using manually entered categories.
## For 2936 genes, we could not find any categories. These genes will be excluded.
## To force their use, please run with use_genes_without_cat=T (see documentation).
## This was the default behavior for version 1.15.1 and earlier.
## Calculating the p-values...
```

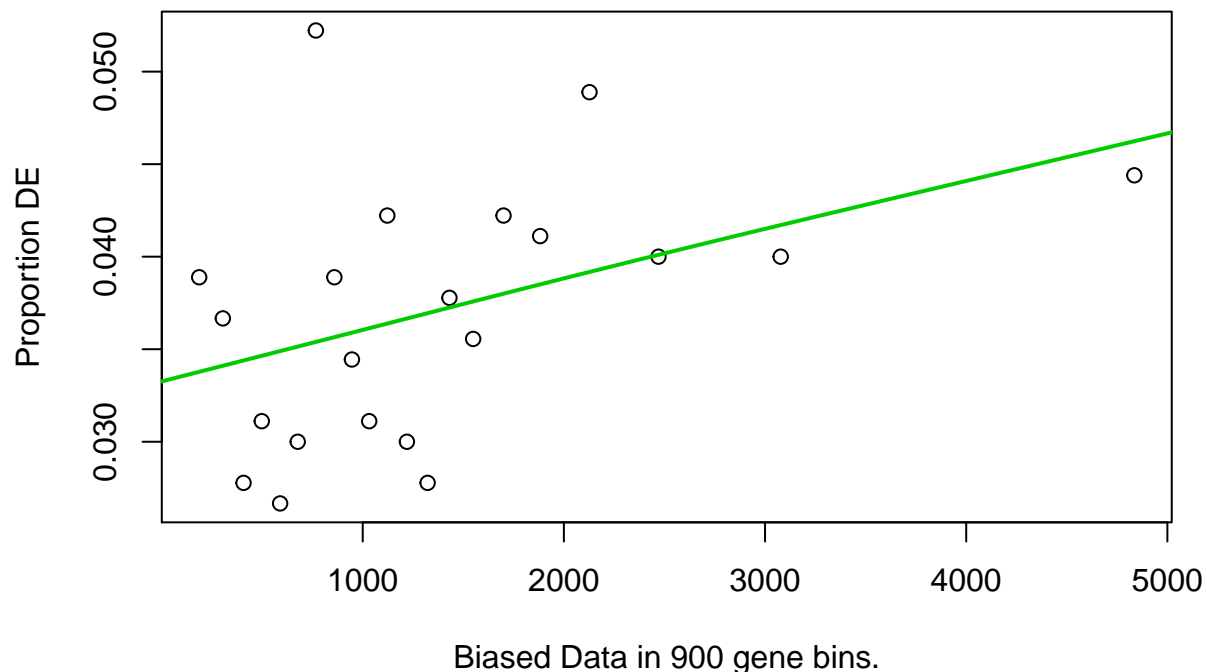


Biased Data in 1300 gene bins.

```
## [1] "down"
##      [,1]
## G0:0015074 "DNA integration"
## G0:0003964 "RNA-directed DNA polymerase activity"
## G0:0006278 "RNA-dependent DNA replication"
## G0:0006333 "chromatin assembly or disassembly"
## G0:0003682 "chromatin binding"
## G0:0000785 "chromatin"
## G0:0031969 "chloroplast membrane"
## G0:0003723 "RNA binding"
## G0:0043229 "intracellular organelle"
## G0:0016651 "oxidoreductase activity, acting on NAD(P)H"
## G0:0004190 "aspartic-type endopeptidase activity"
```

```
## GO:0006310 "DNA recombination"
## GO:0003676 "nucleic acid binding"
## GO:0005030 "neurotrophin receptor activity"
## GO:0003677 "DNA binding"
## GO:0008270 "zinc ion binding"
## GO:0006259 "DNA metabolic process"
## GO:0043170 "macromolecule metabolic process"
```

```
## Using manually entered categories.
## For 2936 genes, we could not find any categories. These genes will be excluded.
## To force their use, please run with use_genes_without_cat=T (see documentation).
## This was the default behavior for version 1.15.1 and earlier.
## Calculating the p-values...
```



```
## [1] "all"
##      [,1]
## GO:0015074 "DNA integration"
## GO:0003964 "RNA-directed DNA polymerase activity"
## GO:0006278 "RNA-dependent DNA replication"
## GO:0009523 "photosystem II"
## GO:0018298 "protein-chromophore linkage"
## GO:0009522 "photosystem I"
## GO:0009765 "photosynthesis, light harvesting"
## GO:0016168 "chlorophyll binding"
## GO:0006333 "chromatin assembly or disassembly"
## GO:0003682 "chromatin binding"
## GO:0000785 "chromatin"
## GO:0031969 "chloroplast membrane"
## GO:0009535 "chloroplast thylakoid membrane"
## GO:0030076 "light-harvesting complex"
## GO:0006833 "water transport"
```

## G0:0016651 "oxidoreductase activity, acting on NAD(P)H"  
## G0:0043229 "intracellular organelle"  
## G0:0006310 "DNA recombination"  
## G0:0016021 "integral component of membrane"  
## G0:0015250 "water channel activity"  
## G0:0004190 "aspartic-type endopeptidase activity"  
## G0:0042807 "central vacuole"  
## G0:0005030 "neurotrophin receptor activity"  
## G0:0046872 "metal ion binding"  
## G0:0009768 "photosynthesis, light harvesting in photosystem I"