Single Peptide Results Analysis

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Introduction

The single peptide analysis methods and results are contained in this document.

Five peptide analysis

The five peptides are as follows:

```
fivepep <- c('AFLPGSLVDTRPVR',
             'DIAMQIAAVNPTYLNREEVPTEVIEHEK',
             'DLFKNPIHPYTK',
             'DVTIEAVNSLYEK',
             'EVPDWAAQLNENTNVKGLRIAVPK')
```

Unipept

Process

- Paste the tabular list of peptides into the Unipept 'Metaproteomics Analysis' web application (https://morty.ugent.be/mpa)

- Parameters:
  - Equate I and L: FALSE
  - Filter duplicate peptides: FALSE
  - Advanced missed cleavage handling: TRUE
- Download results
- Annotate each peptide with only the GO terms that are present in 5% or more of the proteins (percentages are returned in GO term column)

```
library(dplyr)
library(stringr)
cov_pat <- "\ \\(.{3,4}\\)"
uni <- read.csv('unipept_20_peptides_result.csv',
                stringsAsFactors = FALSE) %>%
    select(peptide,
           uni_go_bp = GO..biological.process.,
           uni_go_mf = GO..molecular.function.,
           uni_go_cc = GO..cellular.component.) %>%
    filter(peptide %in% fivepep) %>%
    mutate(uni_go_bp = str_replace_all(string = uni_go_bp, pattern = cov_pat, replacement = ""),
           uni_go_mf = str_replace_all(string = uni_go_mf, pattern = cov_pat, replacement = ""),
           uni_go_cc = str_replace_all(string = uni_go_cc, pattern = cov_pat, replacement = ""))
```

eggNOG mapper

- Use the Galaxy version of eggNOG mapper, on Galaxy-P
- Parameters:
  - Annotation type: DIAMOND
  - Scoring matrix and gap costs: PAM30, 9 and 1
  - Taxonomic Scope: Bacteria
  - Orthologs: use all orthologs
  - Gene Ontology evidence: use non-electronic terms
  - Seed Orthology Search Options
    - Min e-value: 200000
    - Min bit score: 20
- Download and compare GO terms

```
em <- read.delim("eggnog_mapper_20_sequences_results.tabular",
                 stringsAsFactors = FALSE,
                 header=FALSE) %>%
    select(peptide = V1, em_prot = V2, em_go = V6, em_gene = V5, em_descript = V13) %>%
    filter(peptide %in% fivepep) %>%
    mutate(em_go = str_replace_all(em_go, pattern = ",", replacement = "; "))
```

BLASTP against UniProt

- Use the UniProtKB BLAST web search on each peptide, one-by-one
- Parameters
  - Target database: UniProtKB
  - E-Threshold: 10
  - Matrix: Auto
  - Filtering: None
  - Gapped: Yes
  - Hits: 50
- For each peptide, download the result list and get all GO terms and TaxID associated with that peptide
- To match Unipept, annotate each peptide with only the GO terms that are present in 5% or more of the proteins
- Get the most frequent protein name
- For taxonomy, we can also calculate the lowest common ancestor of each peptide (TODO)

```
peptide <- rep(0, 5)
blast_go <- rep(0, 5)
files <- list.files('uniprot_blastp_outputs')
for (i in 1:5){
    peptide[i] <- fivepep[i]
    result <- read.delim(paste('uniprot_blastp_outputs', paste(fivepep[i], '.tab', sep=""), sep="/"),
                    stringsAsFactors = FALSE,
                    na.strings = c("", "NA", "NaN"))
    gos <- table(unlist(str_split(result$Gene.ontology.IDs, "; ")))/50
    blast_go[i] <- paste(names(gos)[which(gos > 0.05)], collapse = "; ")
}
blast <- data.frame(peptide, blast_go, stringsAsFactors = FALSE)
```

MetaGOmics

- Upload HOMD to metaGOmics

- Parameters:
    - Uniprot database: Uniprot sprot
    - Blast e-value cutoff: 1e-10
    - Use only top hit?: TRUE
- Result URL: https://www.yeastrc.org/metagomics/viewUploadedFasta.do?uid=42jgJAcLHHZBoRQk
- One-by-one, upload peptides and run
- Download results individually, combine into table

```
peptide <- rep(0, 5)
mg_go <- rep(0, 5)
dir <- 'metaGOmics_single_peptides_outputs/'
files <- list.files(dir)
for (i in 1:5){
    peptide[i] <- fivepep[i]
    result <- read.delim(paste(dir, paste(fivepep[i], '.txt', sep=""), sep=""),
                  stringsAsFactors = FALSE,
                  na.strings = c("", "NA", "NaN"),
                  comment.char = "#")
    gos <- result$GO.acc
    mg_go[i] <- paste(gos, collapse = "; ")
}
mg <- data.frame(peptide, mg_go, stringsAsFactors = FALSE)
```

Combine all of the results:

```
all_results <- plyr::join_all(list(em, blast, mg, uni), by = "peptide")
```

All of the results are below:

```
library(pander)
# knitr::kable(all_results)
pander::pander(all_results, split.cell = 80, split.table = Inf)
```

peptide	em_prot	em_go	em_gene	em_descript	blast_go	mg_go	uni_go_bp	uni_go_mf	uni_go_cc
AFLPG[illegible]	[illegible]	GO:0003674; GO:0003676; GO:0003723; GO:0003729; GO:0003735; GO:0005198; GO:0005488; GO:0005575; GO:0005622; GO:0005623; GO:0005737; GO:0005829; GO:0005840; GO:0006412; GO:0006417; GO:0008150; GO:0008152; GO:0009058; GO:0009059; GO:0009889; GO:0009890; GO:0009892; GO:0009987; GO:0010467; GO:0010468; GO:0010556; GO:0010558; GO:0010605; GO:0010608; GO:0015935; GO:0016020; GO:0017148; GO:0019222; GO:0019538; GO:0022626; GO:0022627; GO:0030529; GO:0031323; GO:0031324; GO:0031326; GO:0031327; GO:0032268; GO:0032269; GO:0032991; GO:0034645; GO:0043170; GO:0043226; GO:0043228; GO:0043229; GO:0043232; GO:0044237; GO:0044238; GO:0044249; GO:0044260; GO:0044267; GO:0044391; GO:0044422; GO:0044424; GO:0044444;	RPSA	thus facilitating recognition of the initiation point. It is needed to translate mRNA with a short Shine-Dalgarno (SD) purine-rich sequence (By similarity)	GO:0003723; GO:0003735; GO:0005840; GO:0006412	GO:0005840; GO:0005198; GO:1901363; GO:0006412; GO:0006417; GO:0017148; GO:0008150; GO:0010556; GO:0015935; GO:0010558; GO:0034248; GO:0034249; GO:0005575; GO:0032991; GO:0008152; GO:0044391; GO:0000028; GO:0043603; GO:0043604; GO:0003674; GO:0005737; GO:0003676; GO:0010605; GO:0043043; GO:0010608; GO:0050789; GO:0050794; GO:0044422; GO:0044424; GO:2000112; GO:2000113; GO:0065003; GO:0065007; GO:0010629; GO:0071826; GO:0044444; GO:0044445; GO:0044446; GO:0009058; GO:0009059; GO:0006518; GO:0071840; GO:0016043; GO:0044464; GO:0003723; GO:0043933; GO:0003729; GO:0006807; GO:0030529; GO:0003735; GO:0034622; GO:0032268; GO:0032269; GO:0060255; GO:0022607; GO:0009889; GO:0009890; GO:0009892; GO:0051171;	GO: 0006412	GO: 0003723; GO: 0003735	GO: 0005840

peptide	em_prot	em_go	em_gene	em_descript	blast_go	mg_go	uni_go_bp	uni_go_mf	uni_go_cc
DIAMQ[illegible]VPTISVIEHEK	479436.Vpar_0867	GO:0003674; GO:0003676; GO:0003723; GO:0003746; GO:0005488; GO:0005575; GO:0005618; GO:0005622; GO:0005623; GO:0005737; GO:0005829; GO:0005886; GO:0006412; GO:0006414; GO:0008135; GO:0008150; GO:0008152; GO:0008270; GO:0009058; GO:0009059; GO:0009987; GO:0010467; GO:0016020; GO:0019538; GO:0030312; GO:0034645; GO:0040007; GO:0043167; GO:0043169; GO:0043170; GO:0044237; GO:0044238; GO:0044249; GO:0044260; GO:0044267; GO:0044424; GO:0044444; GO:0044464; GO:0046872; GO:0046914; GO:0071704; GO:0071944; GO:0097159; GO:1901363; GO:1901576	tsf	Associates with the EF-Tu.GDP complex and induces the exchange of GDP to GTP. It remains bound to the aminoacyl-tRNA.EF-Tu.GTP complex up to the GTP hydrolysis stage on the ribosome (By similarity)	GO:0003746; GO:0005737	GO:0008152; GO:0043170; GO:0044464; GO:0003723; GO:0044267; GO:0043603; GO:0043604; GO:0006807; GO:0044271; GO:0003674; GO:0008135; GO:0005737; GO:0003676; GO:1901363; GO:0019538; GO:0003746; GO:0043043; GO:0006412; GO:1901564; GO:1901566; GO:0006414; GO:0044424; GO:0008150; GO:1901576; GO:0034641; GO:0034645; GO:0044237; GO:0044238; GO:0005622; GO:0009987; GO:0097159; GO:0044249; GO:0009058; GO:0005575; GO:0009059; GO:0006518; GO:0071704; GO:0005488; GO:0044260		GO: 0003746	GO: 0005737

peptide	em_prot	em_go	em_gene	em_descript	blast_go	mg_go	uni_go_bp	uni_go_mf	uni_go_cc
DLFKNPTPYTK	457405.FSDG_00110			ATP-binding protein	GO:0005524; GO:0015833; GO:0016887	GO:0016817; GO:0016818; GO:0044464; GO:0048869; GO:0051716; GO:0043934; GO:0009605; GO:1901363; GO:0006810; GO:0008104; GO:0048646; GO:0032549; GO:0032550; GO:0008150; GO:0032553; GO:0050896; GO:0032555; GO:0051179; GO:0032559; GO:0044763; GO:0071496; GO:0044765; GO:1901265; GO:0044767; GO:0036094; GO:1902578; GO:0005575; GO:0030554; GO:0005488; GO:0043167; GO:0045184; GO:0043168; GO:0007154; GO:0015833; GO:0042886; GO:0000166; GO:0097367; GO:0016020; GO:0017111; GO:0016462; GO:0051234; GO:0003674; GO:0005886; GO:0031668; GO:0030420; GO:0030154; GO:0005524; GO:0017076; GO:0016887; GO:0030435; GO:0016787; GO:0033036; GO:0044699; GO:0032502; GO:0015031; GO:0001882; GO:0003824; GO:0009987; GO:0035639;	GO: 0015833	GO: 0005524; GO: 0016887	

peptide	em_prot	em_go	em_gene	em_descript	blast_go	mg_go	uni_go_bp	uni_go_mf	uni_go_cc
DVTIEAVNSKYEK	479436.Vpar_0475		MOAB	molybdenum cofactor	GO:0001732; GO:0003743; GO:0005829; GO:0006777; GO:0016282; GO:0032436; GO:0033290; GO:0034613; GO:0070196; GO:0071540; GO:1900182	GO:0008152; GO:0043170; unknownfun; GO:0018130; unknowncmp; GO:0006732; GO:0051189; GO:1901360; GO:0006807; GO:0003674; GO:1901362; GO:0019538; GO:0043545; GO:1901564; GO:0090407; GO:1901566; GO:0046483; GO:0008150; GO:1901576; GO:0051186; GO:0051188; GO:0009108; GO:0044237; GO:0044238; GO:0006777; GO:0019637; GO:0009987; GO:0044249; GO:0009058; GO:0005575; GO:0071704; GO:0006793; GO:0019720; GO:0006796; GO:0032324	GO: 0006777		

peptide	em_prot	em_go	em_gene	em_descript	blast_go	mg_go	uni_go_bp	uni_go_mf	uni_go_cc
EVPDW[illegible]	[illegible]	GO:0006412; GO:0040007	[illegible]	Allows the formation of correctly charged Gln-tRNA(Gln) through the transamidation of misacylated Glu-tRNA(Gln) in organisms which lack glutaminyl-tRNA synthetase. The reaction takes place in the presence of glutamine and ATP through an activated gamma-phospho-Glu-tRNA(Gln) (By similarity)	GO:0004040; GO:0005524; GO:0006412; GO:0016740; GO:0030956; GO:0050567	GO:0044464; GO:0006807; GO:1901363; GO:0006412; GO:0032549; GO:0032550; GO:0008150; GO:0032553; GO:0032555; GO:0034641; GO:0032559; GO:0034645; GO:0044237; GO:0044238; GO:1901265; GO:0036094; GO:0030956; GO:0044249; GO:0005575; GO:0030554; GO:0016874; GO:0004040; GO:0032991; GO:0005488; GO:0043167; GO:0016879; GO:0044260; GO:0043168; GO:0000166; GO:0008152; GO:0043170; GO:0097367; GO:0016884; GO:0044267; GO:0043603; GO:0043604; GO:0044271; GO:0003674; GO:0019538; GO:0043043; GO:1901564; GO:1901566; GO:0005524; GO:0017076; GO:0044424; GO:1901576; GO:0016787; GO:0009987; GO:0001882; GO:0003824; GO:0035639; GO:0001883; GO:0097159; GO:0009058; GO:0009059; GO:0006518; GO:0071704; GO:0050567; GO:0016810;	GO: 0006412	GO: 0004040; GO: 0005524; GO: 0050567	GO: 0030956

peptide	em_prot	em_go	em_gene	em_descript	blast_go	mg_go	uni_go_bp	uni_go_mf	uni_go_cc

Let’s go through the peptides one-by-one

AFLPGSLVDTRPVR

Here, BLAST and Unipept give the same four GO terms:

```r
buni <- all_results[1, 'blast_go']
buni
```

```
## [1] "GO:0003723; GO:0003735; GO:0005840; GO:0006412"
```

Let’s go to the QuickGO API to get the names of these 4.

```r
library(httr)
library(jsonlite)
buni_split <- str_split(buni, "; ", simplify = TRUE)
get_go_names <- function(id_vector){
    base_url <- 'https://www.ebi.ac.uk/QuickGO/services/ontology/go/terms/'
    terms <- str_replace(id_vector, ":", "%3A")
    joined_terms <- paste(terms, collapse="%2C")
    term_url <- paste(base_url, joined_terms, sep="")
    term_info <- GET(term_url, accept("application/json"))
    json <- toJSON(content(term_info))
    names <- unlist(fromJSON(json)$results$name)
    names
}
get_go_names(buni_split)
```

```
## [1] "translation"
## [2] "ribosome"
## [3] "RNA binding"
## [4] "structural constituent of ribosome"
```

On the other hand, both eggNOG mapper and metaGOmics give huge lists of GO terms. Are the 4 BLAST+Unipept terms contained in these lists?

Does eggnog mapper contain all 4?

```r
all(str_detect(all_results[1, ]$em_go, pattern = buni_split))
```

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

Does metagomics contain all 4?

```r
all(str_detect(all_results[1, ]$mg_gos, pattern = buni_split))
```

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

The question is, then, what the other terms are.

We can look at this in 3 ways:

1) Are the extra terms more general (ancestors) of the 4 we found?
2) Are there any terms which are more specific (children) of the 4 we found?
3) Are there terms that are not ancestors or children? These may be false hits.

Let’s look at question 1):

```r
# get all ancestors of the 4 terms
get_go_ancestors <- function(id_vector){
    base_url <- 'https://www.ebi.ac.uk/QuickGO/services/ontology/go/terms/'
    terms <- str_replace(id_vector, ":", "%3A")
    joined_terms <- paste(terms, collapse="%2C")
    term_url <- paste(base_url, joined_terms, '/ancestors?relations=is_a', sep="")
    term_info <- GET(term_url, accept("application/json"))
    json <- toJSON(content(term_info))
    names <- unlist(fromJSON(json)$results$ancestors)
    names
}
ancestors <- get_go_ancestors(buni_split)
get_go_names(ancestors)
```

```
##  [1] "molecular_function"
##  [2] "amide biosynthetic process"
##  [3] "cellular amide metabolic process"
##  [4] "biological_process"
##  [5] "metabolic process"
##  [6] "organonitrogen compound metabolic process"
##  [7] "organonitrogen compound biosynthetic process"
##  [8] "organic substance biosynthetic process"
##  [9] "heterocyclic compound binding"
## [10] "translation"
## [11] "nitrogen compound metabolic process"
## [12] "peptide metabolic process"
## [13] "ribosome"
## [14] "cellular_component"
## [15] "macromolecule metabolic process"
## [16] "intracellular organelle"
## [17] "organelle"
## [18] "non-membrane-bounded organelle"
## [19] "intracellular non-membrane-bounded organelle"
## [20] "nucleic acid binding"
## [21] "structural molecule activity"
## [22] "peptide biosynthetic process"
## [23] "macromolecule biosynthetic process"
## [24] "biosynthetic process"
## [25] "cellular nitrogen compound metabolic process"
## [26] "cellular macromolecule biosynthetic process"
## [27] "RNA binding"
## [28] "structural constituent of ribosome"
## [29] "organic cyclic compound binding"
## [30] "ribonucleoprotein complex"
## [31] "protein-containing complex"
## [32] "binding"
## [33] "organic substance metabolic process"
## [34] "protein metabolic process"
## [35] "cytoplasmic part"
## [36] "cell part"
## [37] "intracellular part"
## [38] "cellular process"
## [39] "primary metabolic process"
## [40] "cellular metabolic process"
## [41] "cellular biosynthetic process"
## [42] "cellular macromolecule metabolic process"
## [43] "cellular protein metabolic process"
## [44] "cellular nitrogen compound biosynthetic process"
```

Now, let's look at the overlap between the ancestors (including the original 4 terms) and eggnog mapper, and between the ancestors and metaGOmics:

```
four_and_ancestors <- c(ancestors, buni_split)
```

```
metaGOmics_list <- c(str_split(all_results[1, 'mg_go'], "; ", simplify=TRUE))
overlap <- length(intersect(four_and_ancestors, metaGOmics_list))
overlap
```

```
## [1] 44
```

```
eggnog_list <- c(str_split(all_results[1, 'em_go'], "; ", simplify =TRUE))
overlap <- length(intersect(four_and_ancestors, eggnog_list))
overlap
```

```
## [1] 34
```

Let's do the same for the children:

```
# get all childen of the 4 terms
get_go_descendants <- function(id_vector){
    base_url <- 'https://www.ebi.ac.uk/QuickGO/services/ontology/go/terms/'
    terms <- str_replace(id_vector, ":", "%3A")
    joined_terms <- paste(terms, collapse="%2C")
    term_url <- paste(base_url, joined_terms, '/descendants?relations=is_a', sep="")
    term_info <- GET(term_url, accept("application/json"))
    json <- toJSON(content(term_info))
    names <- unlist(fromJSON(json)$results$descendants)
    names
}
descendants <- get_go_descendants(buni_split)
get_go_names(descendants)
```

```
##   [1] "7S RNA binding"
##   [2] "poly(U) RNA binding"
##   [3] "ribonuclease P RNA binding"
##   [4] "CUA codon-amino acid adaptor activity"
##   [5] "CUC codon-amino acid adaptor activity"
##   [6] "CUU codon-amino acid adaptor activity"
##   [7] "UAG codon-amino acid adaptor activity"
##   [8] "UAA codon-amino acid adaptor activity"
##   [9] "UAC codon-amino acid adaptor activity"
##  [10] "UGG codon-amino acid adaptor activity"
##  [11] "UGA codon-amino acid adaptor activity"
##  [12] "UGC codon-amino acid adaptor activity"
##  [13] "UGU codon-amino acid adaptor activity"
##  [14] "UAU codon-amino acid adaptor activity"
##  [15] "UCG codon-amino acid adaptor activity"
##  [16] "UCA codon-amino acid adaptor activity"
##  [17] "UCC codon-amino acid adaptor activity"
##  [18] "UUU codon-amino acid adaptor activity"
##  [19] "UCU codon-amino acid adaptor activity"
## [20] "UUG codon-amino acid adaptor activity"
## [21] "UUA codon-amino acid adaptor activity"
## [22] "UUC codon-amino acid adaptor activity"
## [23] "GGA codon-amino acid adaptor activity"
## [24] "GGC codon-amino acid adaptor activity"
## [25] "GGU codon-amino acid adaptor activity"
## [26] "GAG codon-amino acid adaptor activity"
## [27] "GCG codon-amino acid adaptor activity"
## [28] "GCA codon-amino acid adaptor activity"
## [29] "GCC codon-amino acid adaptor activity"
## [30] "GCU codon-amino acid adaptor activity"
## [31] "GAA codon-amino acid adaptor activity"
## [32] "GAC codon-amino acid adaptor activity"
## [33] "GAU codon-amino acid adaptor activity"
## [34] "GUG codon-amino acid adaptor activity"
## [35] "GUA codon-amino acid adaptor activity"
## [36] "GUC codon-amino acid adaptor activity"
## [37] "AGU codon-amino acid adaptor activity"
## [38] "AAG codon-amino acid adaptor activity"
## [39] "AAA codon-amino acid adaptor activity"
## [40] "AAC codon-amino acid adaptor activity"
## [41] "GUU codon-amino acid adaptor activity"
## [42] "AGG codon-amino acid adaptor activity"
## [43] "AGA codon-amino acid adaptor activity"
## [44] "AGC codon-amino acid adaptor activity"
## [45] "AAU codon-amino acid adaptor activity"
## [46] "ACG codon-amino acid adaptor activity"
## [47] "ACA codon-amino acid adaptor activity"
## [48] "AUC codon-amino acid adaptor activity"
## [49] "AUU codon-amino acid adaptor activity"
## [50] "CGG codon-amino acid adaptor activity"
## [51] "CGA codon-amino acid adaptor activity"
## [52] "ACC codon-amino acid adaptor activity"
## [53] "ACU codon-amino acid adaptor activity"
## [54] "AUG codon-amino acid adaptor activity"
## [55] "AUA codon-amino acid adaptor activity"
## [56] "CGC codon-amino acid adaptor activity"
## [57] "CGU codon-amino acid adaptor activity"
## [58] "CAG codon-amino acid adaptor activity"
## [59] "CCA codon-amino acid adaptor activity"
## [60] "CCC codon-amino acid adaptor activity"
## [61] "CCU codon-amino acid adaptor activity"
## [62] "CUG codon-amino acid adaptor activity"
## [63] "CAA codon-amino acid adaptor activity"
## [64] "CAC codon-amino acid adaptor activity"
## [65] "CAU codon-amino acid adaptor activity"
## [66] "CCG codon-amino acid adaptor activity"
## [67] "GGG codon-amino acid adaptor activity"
## [68] "large ribosomal subunit rRNA binding"
## [69] "small ribosomal subunit rRNA binding"
## [70] "poly(A) binding"
## [71] "translation factor activity, RNA binding"
## [72] "poly-pyrimidine tract binding"
## [73] "pre-mRNA branch point binding"
##  [74] "G-quadruplex RNA binding"
##  [75] "steroid receptor RNA activator RNA binding"
##  [76] "cytoplasmic translation"
##  [77] "poly-purine tract binding"
##  [78] "mRNA 3'-UTR AU-rich region binding"
##  [79] "telomerase RNA binding"
##  [80] "RNA stem-loop binding"
##  [81] "snRNA stem-loop binding"
##  [82] "translation termination factor activity"
##  [83] "5S rRNA primary transcript binding"
##  [84] "5S rRNA binding"
##  [85] "selenocysteine insertion sequence binding"
##  [86] "translation release factor activity, codon nonspecific"
##  [87] "translation release factor activity, codon specific"
##  [88] "translation"
##  [89] "mRNA binding involved in posttranscriptional gene silencing"
##  [90] "pre-mRNA binding"
##  [91] "mRNA 5'-UTR binding"
##  [92] "tRNA binding"
##  [93] "miRNA binding"
##  [94] "siRNA binding"
##  [95] "double-stranded miRNA binding"
##  [96] "mRNA cap binding"
##  [97] "mitochondrial ribosome"
##  [98] "polysomal ribosome"
##  [99] "BRE binding"
## [100] "ribosome"
## [101] "triplet codon-amino acid adaptor activity"
## [102] "snoRNA binding"
## [103] "RNA 2'-O-ribose methylation guide activity"
## [104] "tRNA pseudouridylation guide activity"
## [105] "snRNA 2'-O-ribose methylation guide activity"
## [106] "rRNA 2'-O-ribose methylation guide activity"
## [107] "snRNA pseudouridylation guide activity"
## [108] "tRNA 2'-O-ribose methylation guide activity"
## [109] "snRNA modification guide activity"
## [110] "RNA pseudouridylation guide activity"
## [111] "tRNA modification guide activity"
## [112] "rRNA pseudouridylation guide activity"
## [113] "rRNA modification guide activity"
## [114] "RNA modification guide activity"
## [115] "U6atac snRNA binding"
## [116] "U5 snRNA binding"
## [117] "U12 snRNA binding"
## [118] "U11 snRNA binding"
## [119] "pre-mRNA 3'-splice site binding"
## [120] "pre-mRNA 5'-splice site binding"
## [121] "U6 snRNA 3'-end binding"
## [122] "U2 snRNA binding"
## [123] "U4atac snRNA binding"
## [124] "U4 snRNA binding"
## [125] "U1 snRNA binding"
## [126] "chloroplast ribosome"
## [127] "N6-methyladenosine-containing RNA binding"
## [128] "regulatory region RNA binding"
## [129] "transcription regulatory region RNA binding"
## [130] "pre-miRNA binding"
## [131] "primary miRNA binding"
## [132] "RNA strand annealing activity"
## [133] "GU repeat RNA binding"
## [134] "alpha-aminoacyl-tRNA binding"
## [135] "snRNA binding"
## [136] "AU-rich element binding"
## [137] "U6 snRNA binding"
## [138] "rRNA binding"
## [139] "rRNA primary transcript binding"
## [140] "base pairing with mRNA"
## [141] "base pairing with RNA"
## [142] "cytosolic ribosome"
## [143] "7SK snRNA binding"
## [144] "RNA cap binding"
## [145] "RNA cap 4 binding"
## [146] "RNA trimethylguanosine cap binding"
## [147] "RNA 7-methylguanosine cap binding"
## [148] "organellar ribosome"
## [149] "mRNA CDS binding"
## [150] "histone pre-mRNA DCP binding"
## [151] "U7 snRNA binding"
## [152] "histone pre-mRNA stem-loop binding"
## [153] "misfolded RNA binding"
## [154] "regulatory RNA binding"
## [155] "RNA strand-exchange activity"
## [156] "poly(G) binding"
## [157] "mitochondrial ribosomal large subunit rRNA binding"
## [158] "mRNA 3'-UTR binding"
## [159] "RNA binding"
## [160] "double-stranded RNA binding"
## [161] "single-stranded RNA binding"
## [162] "mRNA binding"
## [163] "structural constituent of ribosome"
## [164] "translation initiation factor activity"
## [165] "translation elongation factor activity"
## [166] "translation release factor activity"
## [167] "pre-mRNA intronic binding"
## [168] "pre-mRNA intronic pyrimidine-rich binding"
## [169] "sequence-specific mRNA binding"
## [170] "methionyl-initiator methionine tRNA binding"
## [171] "5.8S rRNA binding"
## [172] "trans-activation response element binding"
## [173] "iron-responsive element binding"
## [174] "poly(C) RNA binding"
## [175] "uridine-rich cytoplasmic polyadenylylation element binding"
## [176] "base pairing with tRNA"
## [177] "base pairing with snRNA"
## [178] "base pairing with rRNA"
## [179] "telomeric repeat-containing RNA binding"
## [180] "plastid ribosome"
## [181] "box C/D snoRNA binding"
## [182] "U3 snoRNA binding"
## [183] "box H/ACA snoRNA binding"
## [184] "piRNA binding"
## [185] "21U-RNA binding"
## [186] "translation at presynapse"
## [187] "translation at presynapse, modulating chemical synaptic transmission"
## [188] "translation at synapse"
## [189] "translation at postsynapse"
## [190] "mitochondrial translation"
## [191] "plastid translation"
```

```
four_and_descendants <- c(descendants, buni_split)
```

MetaGOmics overlap:

```
length(intersect(four_and_descendants, metaGOmics_list))
```

```
## [1] 5
```

eggNOG overlap with descendants:

```
eggnog_list <- c(str_split(all_results[1, 'em_go'], "; ", simplify =TRUE))
length(intersect(four_and_descendants, eggnog_list))
```

```
## [1] 6
```

Ok, so still not a lot of overlap. Let's examine the terms that are neither the 4 terms or their descendants or ancestors:

```
full_tree <- c(buni_split, ancestors, descendants)
```

metaGOmics

```
diff <- setdiff(metaGOmics_list, full_tree)
get_go_names(diff)
```

```
##  [1] "cellular component organization"
##  [2] "membrane"
##  [3] "biological regulation"
##  [4] "protein-containing complex assembly"
##  [5] "regulation of translation"
##  [6] "negative regulation of cellular biosynthetic process"
##  [7] "regulation of cellular biosynthetic process"
##  [8] "regulation of cellular metabolic process"
##  [9] "negative regulation of cellular metabolic process"
## [10] "regulation of cellular process"
## [11] "negative regulation of cellular macromolecule biosynthetic process"
## [12] "regulation of cellular macromolecule biosynthetic process"
## [13] "protein-containing complex subunit organization"
## [14] "regulation of biological process"
## [15] "ribosomal small subunit assembly"
## [16] "regulation of protein metabolic process"
## [17] "negative regulation of protein metabolic process"
## [18] "negative regulation of nitrogen compound metabolic process"
## [19] "regulation of nitrogen compound metabolic process"
## [20] "regulation of macromolecule metabolic process"
## [21] "cytoplasm"
## [22] "regulation of primary metabolic process"
## [23] "small ribosomal subunit"
## [24] "regulation of cytoplasmic translation"
## [25] "negative regulation of cytoplasmic translation"
## [26] "cellular protein-containing complex assembly"
## [27] "cytosolic small ribosomal subunit"
## [28] "ribonucleoprotein complex assembly"
## [29] "RNA binding"
## [30] "negative regulation of biological process"
## [31] "negative regulation of cellular process"
## [32] "negative regulation of translation"
## [33] "regulation of cellular amide metabolic process"
## [34] "negative regulation of cellular amide metabolic process"
## [35] "regulation of biosynthetic process"
## [36] "regulation of gene expression"
## [37] "cellular component assembly"
## [38] "negative regulation of gene expression"
## [39] "posttranscriptional regulation of gene expression"
## [40] "negative regulation of macromolecule metabolic process"
## [41] "regulation of macromolecule biosynthetic process"
## [42] "negative regulation of macromolecule biosynthetic process"
## [43] "cytosolic part"
## [44] "intracellular organelle part"
## [45] "organelle part"
## [46] "negative regulation of metabolic process"
## [47] "negative regulation of biosynthetic process"
## [48] "regulation of metabolic process"
## [49] "ribonucleoprotein complex subunit organization"
## [50] "regulation of cellular protein metabolic process"
## [51] "negative regulation of cellular protein metabolic process"
## [52] "cellular component organization or biogenesis"
## [53] "ribosomal subunit"
## [54] "ribonucleoprotein complex"
```

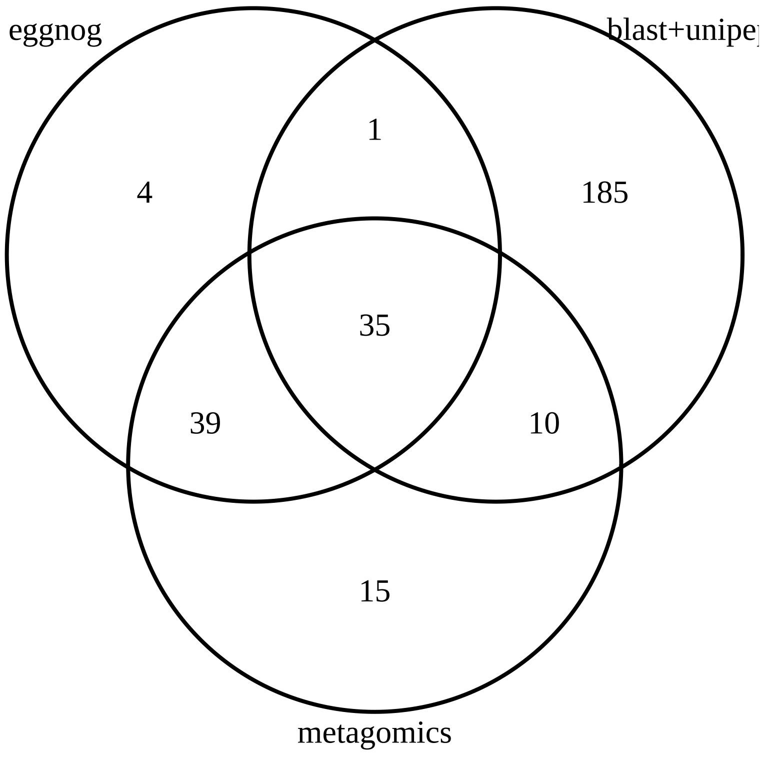
eggNOG mapper

```r
em_diff <- setdiff(eggnog_list, full_tree)
get_go_names(em_diff)
```

```
##  [1] "membrane"
##  [2] "biological regulation"
##  [3] "regulation of translation"
##  [4] "negative regulation of cellular biosynthetic process"
##  [5] "regulation of cellular biosynthetic process"
##  [6] "regulation of cellular metabolic process"
##  [7] "negative regulation of cellular metabolic process"
##  [8] "regulation of cellular process"
##  [9] "negative regulation of cellular macromolecule biosynthetic process"
## [10] "regulation of cellular macromolecule biosynthetic process"
## [11] "regulation of biological process"
## [12] "regulation of protein metabolic process"
## [13] "negative regulation of protein metabolic process"
## [14] "regulation of macromolecule metabolic process"
## [15] "cytoplasm"
## [16] "cytosol"
## [17] "cell"
## [18] "intracellular"
## [19] "regulation of primary metabolic process"
## [20] "small ribosomal subunit"
## [21] "regulation of cytoplasmic translation"
## [22] "negative regulation of cytoplasmic translation"
## [23] "cytosolic small ribosomal subunit"
## [24] "negative regulation of biological process"
## [25] "negative regulation of cellular process"
## [26] "negative regulation of translation"
## [27] "regulation of biosynthetic process"
## [28] "regulation of gene expression"
## [29] "gene expression"
## [30] "posttranscriptional regulation of gene expression"
## [31] "negative regulation of macromolecule metabolic process"
## [32] "regulation of macromolecule biosynthetic process"
## [33] "negative regulation of macromolecule biosynthetic process"
## [34] "cytosolic part"
## [35] "intracellular organelle part"
## [36] "organelle part"
## [37] "negative regulation of metabolic process"
## [38] "negative regulation of biosynthetic process"
## [39] "regulation of metabolic process"
## [40] "regulation of cellular protein metabolic process"
## [41] "negative regulation of cellular protein metabolic process"
## [42] "ribosomal subunit"
## [43] "ribonucleoprotein complex"
```

Visualize the overlap between the full Blast+Unipept tree (descendants, ancestors) and the eggNOG and metaGOmics term lists.

```r
library(VennDiagram)
grid.newpage()
grid.draw(venn.diagram(
    list("eggnog" = eggnog_list, "blast+unipept" = full_tree, "metagomics" = metaGOmics_list),
    NULL))
```



```r
file.remove(list.files(pattern = "VennDiagram.*log")) # venn diagram log files
```

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

Future directions

1) repeat this for the other 4 peptides
2) how do we handle terms that are not descendants or ancestors? We could define some distance cutoff, and say that everything beyond that is a false hit. For example, we could say that if the shortest path between a metaGOmics or eggNOG term and any term in the full B+U tree has length greater than or equal to 2 than it is a false hit.

Distance

Idea: if a term is not a child of any term in the full expanded graph of the Uniprot-assigned terms, it is a false hit.

```r
get_children <- function(goids){
    base_url <- 'https://www.ebi.ac.uk/QuickGO/services/ontology/go/terms/'
    terms <- str_replace(goids, ":", "%3A")
    joined_terms <- paste(terms, collapse="%2C")
    term_url <- paste(base_url, joined_terms, sep="")
    term_info <- GET(term_url, accept("application/json"))
    json <- toJSON(content(term_info))
    children <- fromJSON(json)$results$children
    children_is_a <- lapply(children, function(x) unlist(x[x$relation == "is_a", ]$id))
    return(children_is_a)
}
```

Let's do this for the full tree above.

```
full_tree_with_children <- get_children(full_tree)

# combine term-specific sets of children
all_children <- c(full_tree, unlist(full_tree_with_children))

# how many terms are not in the full tree with children?
em_diff_with_kids <- setdiff(eggnog_list, all_children)
mg_diff_with_kids <- setdiff(metaGOmics_list, all_children)
```

Calculate proportions to answer three questions: 1) How many of Uniprot's terms does the tool pick up? 2) What is the proportion of total terms from the tool that are extraneous?

Eggnog

```
# answer to 1
length(intersect(eggnog_list, buni_split)) / length(buni_split)
```

```
## [1] 1
```

```
# answer to 2
length(em_diff_with_kids)/length(eggnog_list)
```

```
## [1] 0.4050633
```

MetaGOmics

```
# answer to 1
length(intersect(metaGOmics_list, buni_split)) / length(buni_split)
```

```
## [1] 1
```

```
# answer to 2
length(mg_diff_with_kids)/length(metaGOmics_list)
```

```
## [1] 0.4545455
```

GO glossary

Here, I get the names of all the above GO terms.

```
library(httr)
library(jsonlite)
base_url <- 'https://www.ebi.ac.uk/QuickGO/services/ontology/go/terms/'
term_url <- paste(base_url, 'GO%3A0008150%2CGO%3A0008152', sep="")
term_info <- GET(term_url, verbose(), accept("application/json"))
json <- toJSON(content(term_info))
df <- fromJSON(json)$results
```