missing-protein-unique-peptides

2023-04-06

```
library(tidyverse)
```

```
## -- Attaching packages --------------------------------------- tidyverse 1.3.2 --
## v ggplot2 3.3.6     v purrr   0.3.5
## v tibble  3.2.1     v dplyr   1.1.2
## v tidyr   1.2.1     v stringr 1.4.1
## v readr   2.1.3     v forcats 0.5.2
## -- Conflicts ------------------------------------------ tidyverse_conflicts() --
## x dplyr::filter() masks stats::filter()
## x dplyr::lag()    masks stats::lag()
```

```
if (!require("protools", quietly = TRUE))
   devtools::install_github("https://github.com/FDUguchunhui/protools")
library(protools)
```

Import peptides of identified missing proteins

```
MP_final_peptide <- readxl::read_xlsx('Supplementary file 2 identified missing protein details.xlsx', sl
head(MP_final_peptide)
```

```
## # A tibble: 6 x 93
##   Gene     protein.key protein.Entry    protein.Accession protein.Description
##   <chr>          <dbl> <chr>            <chr>             <chr>
## 1 C1orf141        3363 A0A0A0MTM1_HUMAN A0A0A0MTM1        Isoform of Q5JVX7_ Un~
## 2 C1orf141        3363 A0A0A0MTM1_HUMAN A0A0A0MTM1        Isoform of Q5JVX7_ Un~
## 3 C1orf141        3363 A0A0A0MTM1_HUMAN A0A0A0MTM1        Isoform of Q5JVX7_ Un~
## 4 C1orf141        3363 A0A0A0MTM1_HUMAN A0A0A0MTM1        Isoform of Q5JVX7_ Un~
## 5 C1orf141        3363 A0A0A0MTM1_HUMAN A0A0A0MTM1        Isoform of Q5JVX7_ Un~
## 6 C1orf141        3363 A0A0A0MTM1_HUMAN A0A0A0MTM1        Isoform of Q5JVX7_ Un~
## # i 88 more variables: protein.dataBaseType <chr>, protein.score <dbl>,
## #   protein.falsePositiveRate <dbl>, protein.avgMass <dbl>,
## #   protein.MatchedProducts <dbl>, protein.matchedPeptides <dbl>,
## #   protein.digestPeps <dbl>, `protein.seqCover(%)` <dbl>,
## #   protein.MatchedPeptideIntenSum <dbl>,
## #   protein.top3MatchedPeptideIntenSum <dbl>,
## #   protein.MatchedProductIntenSum <dbl>, protein.fmolOnColumn <lgl>, ...
```

Import uniqueness checking

```
uniqueness_checking <- readxl::read_xlsx('Supplementary file 2 identified missing protein details.xlsx'
head(uniqueness_checking)
```

```
## # A tibble: 6 x 7
##   peptide   UniquenessWithoutVar~1 countIsoMatchedWitho~2 listIsoMatchedWithou~3
##   <chr>     <chr>                                   <dbl> <chr>
## 1 HTGSGILS~ N                                           0 <NA>
## 2 RPAFPVIH~ Y                                           1 NX_Q8N687-1
## 3 VYGPAESQ~ Y                                           2 NX_Q9UIL4-1 NX_Q9UIL4~
## 4 GHVGIFFI~ Y                                           2 NX_Q8N5U1-2 NX_Q8N5U1~
## 5 PLLPSTVG~ Y                                           1 NX_Q9H3Y0-1
## 6 ILQKEEEA~ Y                                           1 NX_A6NFK2-1
## # i abbreviated names: 1: UniquenessWithoutVariant,
## #   2: countIsoMatchedWithoutVariant, 3: listIsoMatchedWithoutVariant
## # i 3 more variables: UniquenessWithVariant <chr>,
## #   countAdditionalIsoMatchedWithVariant <dbl>,
## #   listAdditionalIsoMatchedWithVariant <chr>
```

Processing data before computing the number of unique peptides for each identified missing proteins

```
MP_final_peptide$Source <- str_extract(MP_final_peptide$Source, '^IPAS[0-9]+')
MP_final_peptide$unique <- ifelse((MP_final_peptide$unique =='.' | MP_final_peptide$unique == 'N'), 0 ,
```

Import peptides of 177 identified missing proteins

```
# MP_204_products <- readxl::read_xlsx('Supplementary file 2 identified missing protein details.xlsx',
MP_177_products <- read_csv('detected_177_MP_products_with_RNA.csv')
```

```
## Rows: 177 Columns: 6
## -- Column specification --------------------------------------------------------
## Delimiter: ","
## chr (4): accession, IPAS, type, gene_symbol
## dbl (2): NSAF, TPM
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```
MP_177_products$IPAS <- paste0('IPAS', str_extract(MP_177_products$IPAS, '(?<=IP).+(?=_)'))
```

Calculate the number of unique peptides for each detected MPs (and identified MPs)

```
unique_peptide_summary <- MP_final_peptide %>% filter(unique == 1) %>% group_by(protein.Accession, Sour
```

```
## `summarise()` has grouped output by 'protein.Accession'. You can override using
## the `.groups` argument.
```

Get Summary of number of unique peptides for each of the 177 MPs

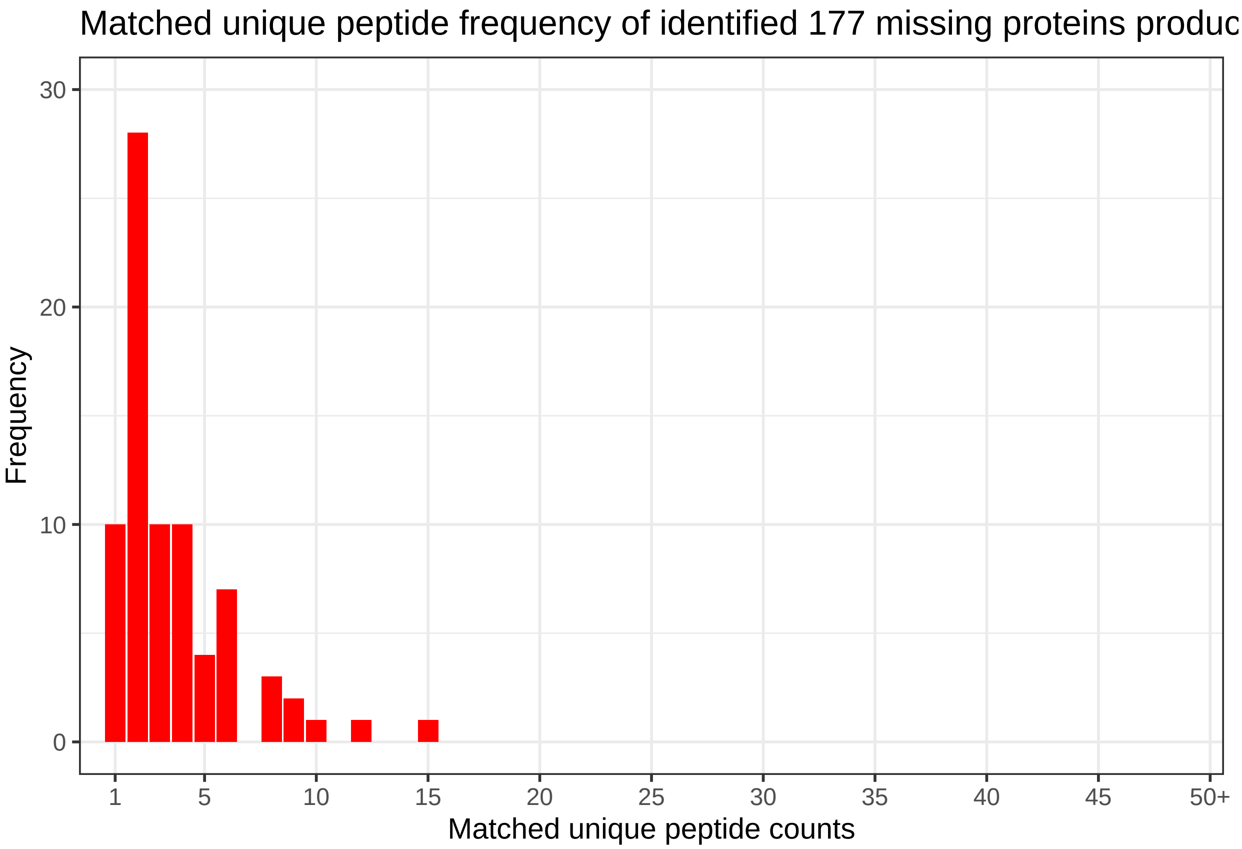
```
unique_peptide_summary <- MP_177_products %>% left_join(unique_peptide_summary, by=c('accession' = 'pro
# write_csv(unique_peptide_summary, 'missing_protein_unique_peptides.csv')
```

plot unique peptide frequency for MP with/without RNA expression

```
unique_peptide_summary <-  unique_peptide_summary %>% mutate(count_discrete=ifelse(n >= 50, '50+', n))
unique_peptide_summary$count_discrete <- factor(unique_peptide_summary$count_discrete, levels=c(as.chara

unique_peptide_summary %>%
  ggplot(aes(x=count_discrete)) +
  geom_bar(position = 'identity', fill='red') +
theme_bw() +
  xlab('Matched unique peptide counts') +
  ylab('Frequency') +
  ggtitle('Matched unique peptide frequency of identified 177 missing proteins products') +
  scale_x_discrete(breaks=c('1', seq(5, 49, 5), '50+'), drop=FALSE) +
  scale_y_continuous(limits=c(0, 30))
```

```
## Warning: Removed 1 rows containing missing values (geom_bar).
```



```
unique_peptide_summary %>% summary()
```

```
##   accession             IPAS                NSAF             type          
##  Length:177         Length:177         Min.   :  9.264   Length:177        
##  Class :character   Class :character   1st Qu.: 48.719   Class :character  
##  Mode  :character   Mode  :character   Median : 78.349   Mode  :character  
##                                        Mean   :138.911                     
##                                        3rd Qu.:159.265                     
##                                        Max.   :950.963                     
##                                                                            
##  gene_symbol             TPM                 n           unique_peptide    
##  Length:177         Min.   :  0.0000   Min.   : 0.000   Length:177        
##  Class :character   1st Qu.:  0.0000   1st Qu.: 0.000   Class :character  
##  Mode  :character   Median :  0.0000   Median : 0.000   Mode  :character  
##                     Mean   :  7.3663   Mean   : 1.565                     
##                     3rd Qu.:  0.7085   3rd Qu.: 2.000                     
##                     Max.   :321.2116   Max.   :15.000                     
##                                                                           
##  count_discrete
##  0      :100   
##  2      : 28   
##  1      : 10   
##  3      : 10   
##  4      : 10   
##  6      :  7   
##  (Other): 12   
```

```
unique_peptide_summary %>% filter(n >= 2)
```

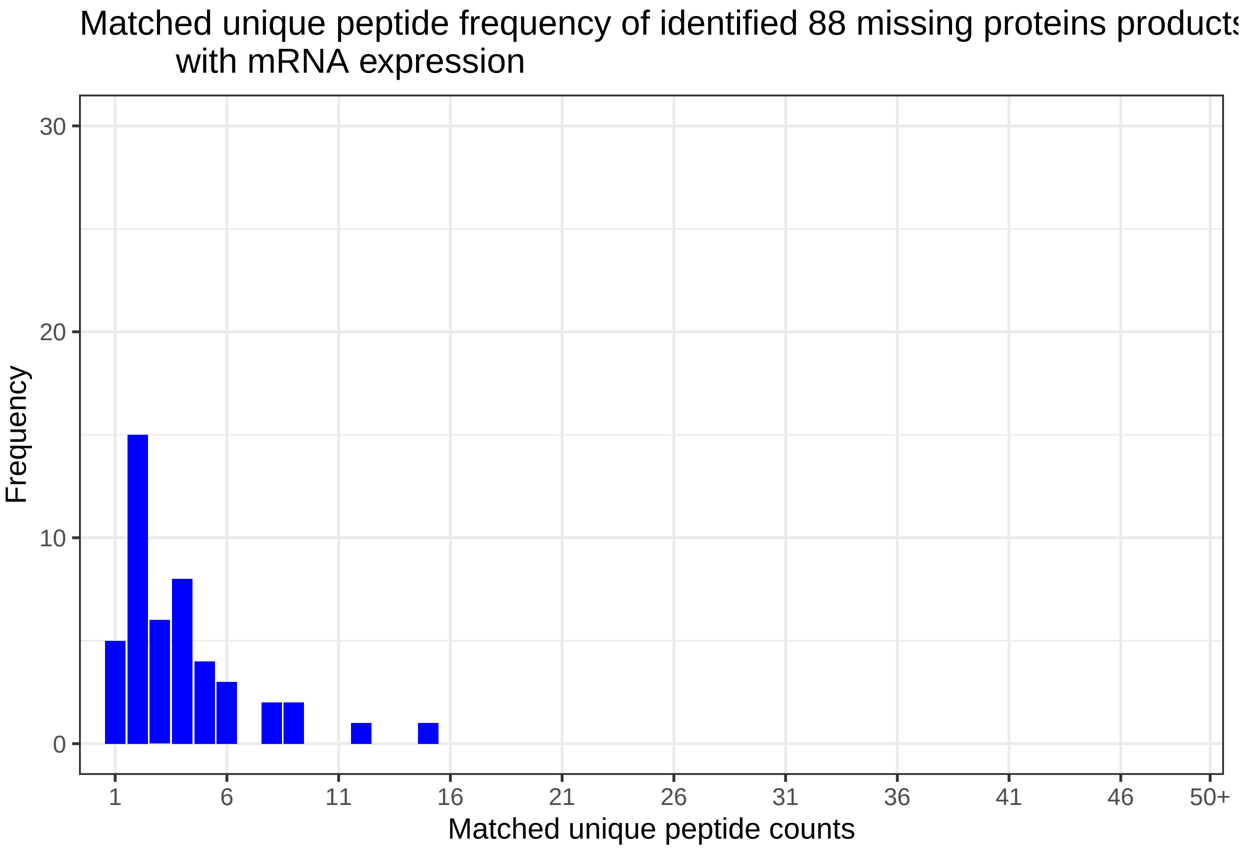
```
## # A tibble: 67 x 9
##    accession  IPAS      NSAF type  gene_symbol       TPM     n unique_peptide
##    <chr>      <chr>    <dbl> <chr> <chr>           <dbl> <int> <chr>
##  1 A0A075B6T7 IPAS7105 193.  MP    TRAV6         11.6        2 QSLFHITASQPADSAT~
##  2 A0A087WT02 IPAS7100 375.  MP    TRAV9-2       84.9        5 GSVQVSDSAVYFCALS~
##  3 A0A096LP55 IPAS7105 560.  MP    UQCRHL       321.         5 SHTEEDCTEELFDFLH~
##  4 A0A0A6YYG3 IPAS0995 146.  MP    TRBV6-8        0          6 QDPGMGLRLIYYSAAA~
##  5 A0A0B4J237 IPAS0995 218.  MP    TRAV8-2        0          2 SETSFHLTKPSAHMSD~
##  6 A0A0J9YX75 IPAS0982  78.3 MP    TRBV6-9        0          4 MSIGLLCCVAFSLLWA~
##  7 A4D1E1     IPAS7105  56.7 MP    ZNF804B        0         10 ISECLDEFSSLEPSEQ~
##  8 A6NFK2     IPAS0982  72.0 MP    GRXCR2         0.0128     8 ILQKEEEAEEESLMNK~
##  9 A6NFK2     IPAS7100 203.  MP    GRXCR2         0          8 QVFEDGQELESPKEEY~
## 10 A6NFN9     IPAS0999  67.6 MP    ANKUB1         0.00357    2 VALYIAAFCGYIELTE~
## # i 57 more rows
## # i 1 more variable: count_discrete <fct>
```

plot unique peptide frequency for MP with RNA expression

```
unique_peptide_summary_88 <- unique_peptide_summary %>% filter(TPM > 0)
unique_peptide_summary_88 <-  unique_peptide_summary_88 %>% mutate(count_discrete=ifelse(n >= 50, '50+'
unique_peptide_summary_88$count_discrete <- factor(unique_peptide_summary_88$count_discrete, levels=c(a

unique_peptide_summary_88 %>%
  ggplot(aes(x=count_discrete)) +
  geom_bar(position = 'identity', fill='blue') +
theme_bw() +
  xlab('Matched unique peptide counts') +
  ylab('Frequency') +
  ggtitle('Matched unique peptide frequency of identified 88 missing proteins products
          with mRNA expression') +
  scale_x_discrete(breaks=c('1', seq(1, 49, 5), '50+'), drop=FALSE) +
  scale_y_continuous(limits=c(0, 30))
```

```
## Warning: Removed 1 rows containing missing values (geom_bar).
```



```
unique_peptide_summary_88 %>% summary()
```

```
##   accession             IPAS                NSAF             type          
##  Length:88          Length:88          Min.   :  9.264   Length:88         
##  Class :character   Class :character   1st Qu.: 43.362   Class :character  
##  Mode  :character   Mode  :character   Median : 77.131   Mode  :character  
##                                        Mean   :139.252                     
##                                        3rd Qu.:155.888                     
##                                        Max.   :950.963                     
##                                                                            
##  gene_symbol             TPM                  n          unique_peptide    
##  Length:88          Min.   :  0.0036   Min.   : 0.000   Length:88         
##  Class :character   1st Qu.:  0.1063   1st Qu.: 0.000   Class :character  
##  Mode  :character   Median :  0.7315   Median : 1.000   Mode  :character  
##                     Mean   : 14.8163   Mean   : 2.091                     
##                     3rd Qu.:  7.5493   3rd Qu.: 3.000                     
##                     Max.   :321.2116   Max.   :15.000                     
##                                                                           
##  count_discrete
##  0      :41    
##  2      :15    
##  4      : 8    
##  3      : 6    
##  1      : 5    
##  5      : 4    
##  (Other): 9    
```

```r
unique_peptide_summary_88 %>% filter(n >= 2)
```

```
## # A tibble: 42 x 9
##    accession  IPAS      NSAF type  gene_symbol       TPM     n unique_peptide
##    <chr>      <chr>    <dbl> <chr> <chr>           <dbl> <int> <chr>
##  1 A0A075B6T7 IPAS7105 193.  MP    TRAV6        11.6         2 QSLFHITASQPADSAT~
##  2 A0A087WT02 IPAS7100 375.  MP    TRAV9-2      84.9         5 GSVQVSDSAVYFCALS~
##  3 A0A096LP55 IPAS7105 560.  MP    UQCRHL      321.          5 SHTEEDCTEELFDFLH~
##  4 A6NFK2     IPAS0982  72.0 MP    GRXCR2        0.0128      8 ILQKEEEAEEESLMNK~
##  5 A6NFN9     IPAS0999  67.6 MP    ANKUB1        0.00357     2 VALYIAAFCGYIELTE~
##  6 A6NH13     IPAS7100 227.  MP    DNAJC9-AS1    0.269       2 PGGDTTPEEAAAPSCA~
##  7 A6NHG4     IPAS0982 400.  MP    DDTL          0.0891      5 FPTVLSTSPAAHGGPR~
##  8 A6NJJ6     IPAS0995  68.9 MP    C19orf67      0.906       2 MATEQWFEGSLPLDPG~
##  9 A6NK53     IPAS0982  26.7 MP    ZNF233        1.01        4 FQEMVTFKDVAVVFTR~
## 10 A6NK53     IPAS7105  76.1 MP    ZNF233       10.3         4 ESSQHSIIQSGEQTSD~
## # i 32 more rows
## # i 1 more variable: count_discrete <fct>
```