RNA data processing and exploration

2022-09-27

```
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 the RNA data

```
count <- read_csv('RNA-data/merged_IPAS_RNAseq_counts_gastric.csv')
```

```
## New names:
## Rows: 63930 Columns: 10
## -- Column specification
## -------------------------------------------------------- Delimiter: "," chr
## (1): ...1 dbl (9): IPAS7103, IPAS0972, IPAS0995, IPAS0982, IPAS0999, IPAS7100,
## IPAS099...
## 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.
## * `` -> `...1`
```

```
count <- count[-c(1:5), ]
colnames(count) <- c('Ensemble_ID', colnames(count)[-1])
count$Ensemble_ID <-str_extract(count$Ensemble_ID, pattern = '.+(?=\\.)')
head(count)
```

```
## # A tibble: 6 x 10
##   Ensemble_ID     IPAS7103 IPAS0972 IPAS0995 IPAS0982 IPAS0999 IPAS7100 IPAS0993
##   <chr>              <dbl>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl>    <dbl>
## 1 ENSG00000000003        0     3891     2139     1397     1755      365     1907
## 2 ENSG00000000005        0        0        0        0        0        0        0
```

```
## 3 ENSG00000000419       25     2173     5350     1388     1574     2032     2414
## 4 ENSG00000000457      175     2392     3390     1130     2927     1572     1546
## 5 ENSG00000000460      269     1602     2865      788     1368      839     1359
## 6 ENSG00000000938        3     1746      173      361     3089    27631     3783
## # i 2 more variables: IPAS0981 <dbl>, IPAS7105 <dbl>
```

data wrangling

combine duplicate ensemble_id 63,920 -> 63,875

```
count <- count %>% group_by(Ensemble_ID) %>% summarise_all(sum)
```

63,875 -> 43,939 remove rows that all cells are 0

```
count <- count[-which(rowSums(count[-1]) == 0),]
```

import missing protein information

```
## read-in newest missing protein list
missing_protein_df <- readxl::read_xlsx('support-data/PE2-5.xlsx')
missing_protein_df <- missing_protein_df[-c(1:12), ]
head(missing_protein_df)
```

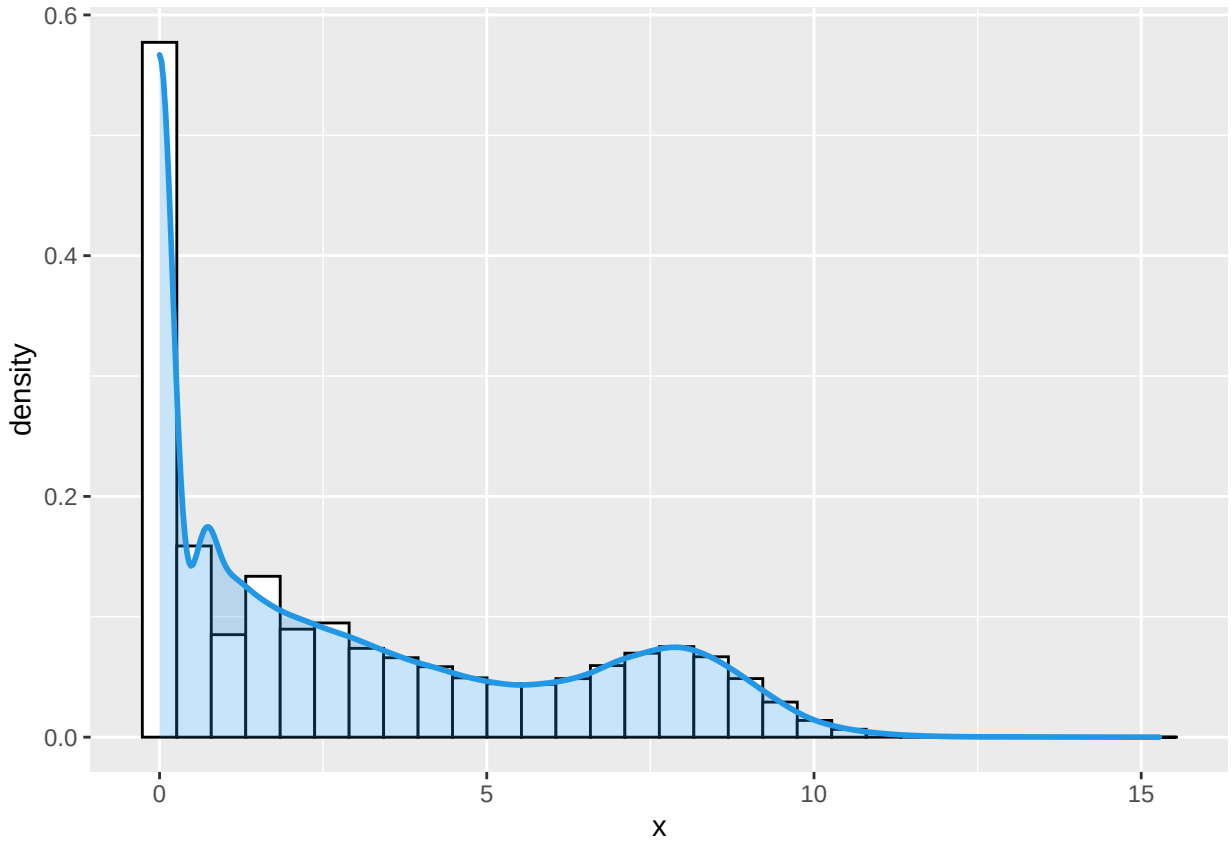
```
## # A tibble: 6 x 14
##   `acc. code`      Accession `protein name` `gene name(s)` chromosome proteomics
##   <chr>            <chr>     <chr>          <chr>          <chr>      <chr>
## 1 "{\"NX_A0A087WT~ A0A087WT~ Putative pota~ KCNE1B         21p11.2    no
## 2 "{\"NX_A0A087WU~ A0A087WU~ Neuroblastoma~ NBPF19         1q21.2     yes
## 3 "{\"NX_A0A087WX~ A0A087WX~ Putative elon~ ELOA3CP        18q21.1    no
## 4 "{\"NX_A0A087X1~ A0A087X1~ Putative cyto~ CYP2D7         22q13.2    yes
## 5 "{\"NX_A0A0B4J2~ A0A0B4J2~ Putative glut~ GATD3B         21p12      yes
## 6 "{\"NX_A0A0B4J2~ A0A0B4J2~ Putative seri~ SIK1B          21p12      yes
## # i 8 more variables: disease <chr>, structure <chr>, `#isof.` <dbl>,
## #   `#variants` <dbl>, `#PTMS` <dbl>, mutagenesis <chr>, `tissue expr.` <chr>,
## #   PE <chr>
```

Section 0: Exploration

overall distribution

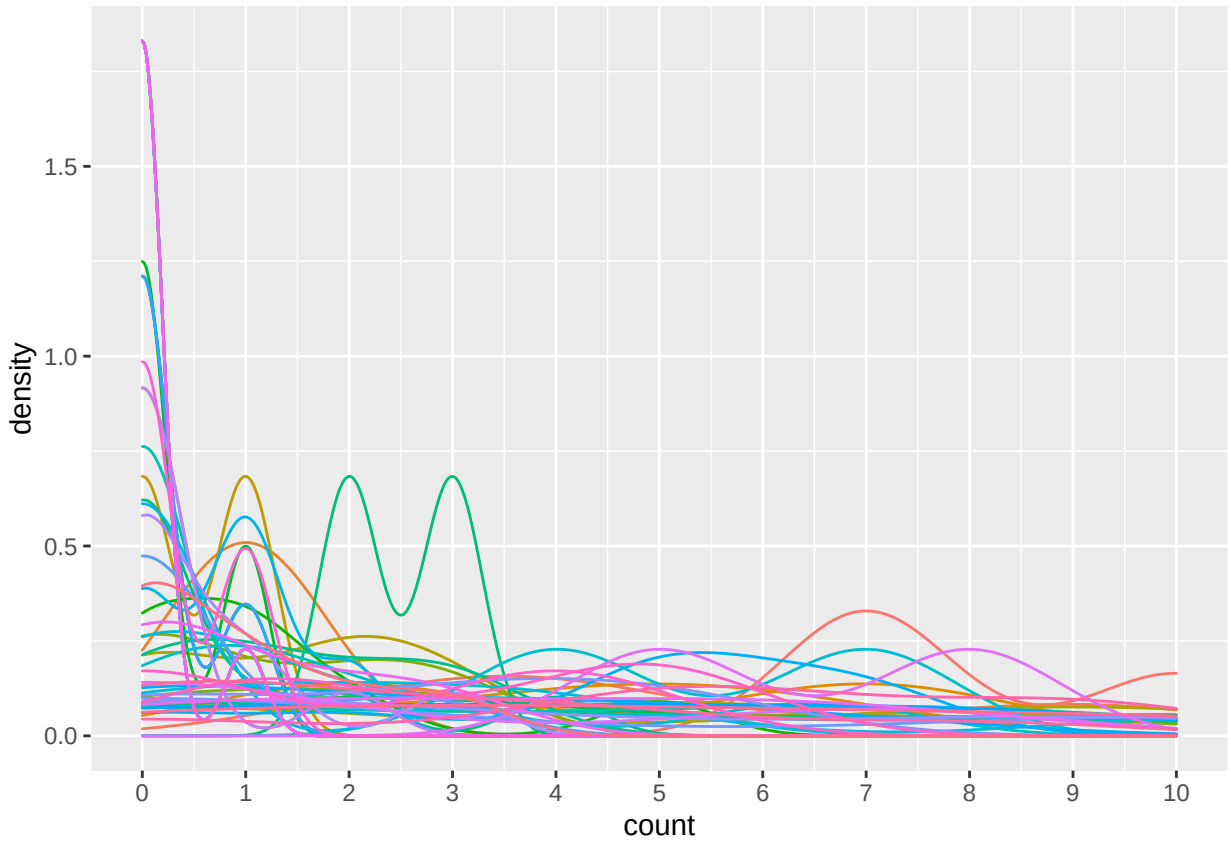
```
count_df <- as.data.frame(count[-1])
pseudo_counts <- log(count_df + 1)
ggplot(data = data.frame(x=c(as.matrix(pseudo_counts))), aes(x=x)) +
  geom_histogram(aes(y = ..density..),
                 colour = 1, fill = "white") +
  geom_density(lwd = 1, colour = 4,
               fill = 4, alpha = 0.25)
```

```
## `stat_bin()` using `bins = 30`. Pick better value with `binwidth`.
```



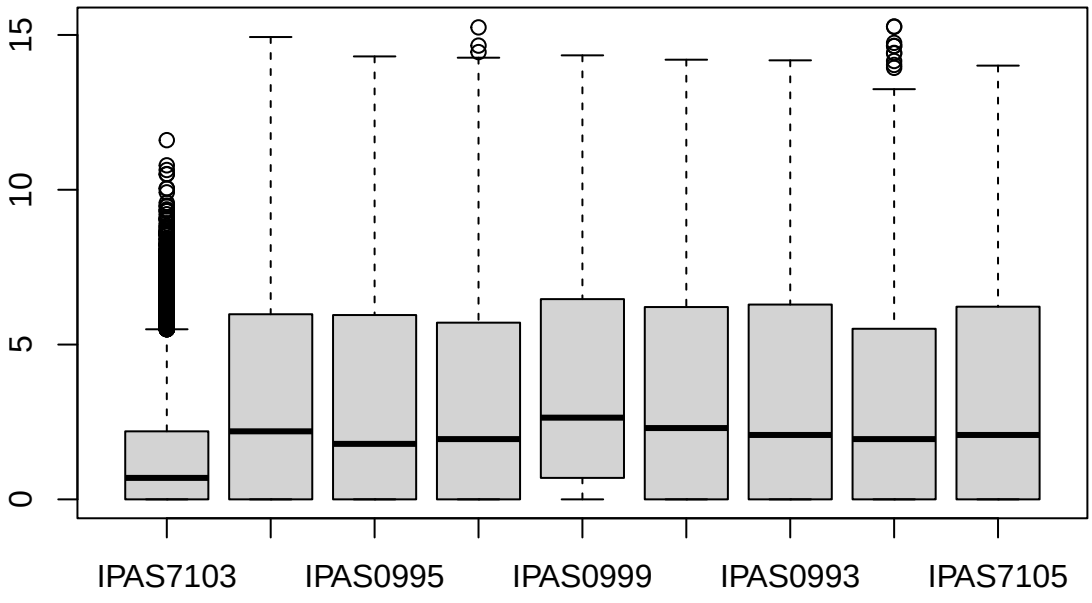
tag-wise distribution

```
sample_filter <- sample(1:nrow(count_df), 100)
density_plot_dat <- t(count_df[sample_filter,]) %>% as_tibble() %>% pivot_longer(cols = everything(), na
plot <- ggplot(data = density_plot_dat, aes(x=count, color=ensemble_id)) +
  geom_density(alpha = 0.2) +
  scale_x_continuous(breaks=0:10, limits=c(0, 10)) +
  theme(legend.position="none")
suppressWarnings(print(plot))
```



sample-wise distribution

```
boxplot(log(count_df+1))
```



Section 1 data processing

do the conversion using biomaRt

the result is better than using org.Hs.eg.db no missing gene symbol

! there are some Ensembl gene id cannot be found by biomart https://support.bioconductor.org/p/111608/

```
# Uncomment the following code if you want to get the dictionary for mapping ensemble ID to gene symbol
# mart <- biomaRt::useDataset("hsapiens_gene_ensembl", useMart("ensembl"))
# dict_ensembl_to_symbol <- biomaRt::getBM(filters= "ensembl_gene_id", attributes= c("ensembl_gene_id",
# dict_ensembl_to_symbol$gene_length=dict_ensembl_to_symbol$end_position - dict_ensembl_to_symbol$start_
# dict_ensembl_to_symbol[dict_ensembl_to_symbol == ''] <- NA
# save(dict_ensembl_to_symbol, file='cache/dict_ensemble_to_symbol.rda')
load('cache/dict_ensemble_to_symbol.rda')
head(dict_ensembl_to_symbol)
```

```
##   ensembl_gene_id hgnc_symbol start_position end_position gene_length
## 1 ENSG00000000003      TSPAN6      100627108    100639991       12883
## 2 ENSG00000000419        DPM1       50934867     50959140       24273
## 3 ENSG00000000457       SCYL3      169849631    169894267       44636
## 4 ENSG00000000460    C1orf112      169662007    169854080      192073
## 5 ENSG00000000938         FGR       27612064     27635185       23121
## 6 ENSG00000000971         CFH      196651754    196752476      100722
```

```
write_csv(dict_ensembl_to_symbol, 'support-data/dict_ensembl_to_symbol.csv')

# code below can be used to check those unmapped ensembl gene id
# count$Ensemble_ID[!count$Ensemble_ID %in% dict_ensembl_to_symbol$ensembl_gene_id]
```

filtering unmapped ensemble gene id

```
count <- count %>% filter(Ensemble_ID %in% dict_ensembl_to_symbol$ensembl_gene_id)
```

recreate data.frame and matrix

after several necessary pre-processing create data.frame and matrix version of the data

```
count_df <- as.data.frame(count[-1])
rownames(count_df) <- count$Ensemble_ID
count_mat <- as.matrix(count_df)
```

normalization: calculate TPM (transcripts per million)

```
gene_length_df <- dict_ensembl_to_symbol %>% dplyr::select(ensembl_gene_id, gene_length) %>% unique()
gene_length_df <- data.frame(length=gene_length_df$gene_length, row.names=gene_length_df$ensembl_gene_i
count_TPM_mat <- TPM(count_mat, gene_length_df)
head(count_TPM_mat)
```

```
##                   IPAS7103   IPAS0972    IPAS0995  IPAS0982   IPAS0999
## ENSG00000000003  0.0000000 148.723868 127.9468305 54.739058 100.699035
## ENSG00000000419  7.1717515  44.083160 169.8502069 28.865822  47.934309
## ENSG00000000457 27.2999253  26.388357  58.5261806 12.779419  48.473307
## ENSG00000000460  9.7520213   4.107075  11.4946052  2.070989   5.264838
## ENSG00000000938  0.9034899  37.185538   5.7660080  7.881675  98.758956
## ENSG00000000971  0.0000000   4.072463   0.1147632 17.731742   4.851123
##                   IPAS7100   IPAS0993  IPAS0981   IPAS7105
## ENSG00000000003  20.516786 129.648564 37.334118 130.934586
## ENSG00000000419  60.622482  87.105952 29.002314  92.134711
## ENSG00000000457  25.503556  30.335995  6.555727  28.402952
## ENSG00000000460   3.163218   6.197081  1.173263   6.298571
## ENSG00000000938 865.413082 143.305795 17.820355 138.584587
## ENSG00000000971  31.742405   8.643628 13.801552  10.639516
```

```
# save(count_TPM_mat, file='cache/count_TPM.rda')
```

Transform RNA expression data into gene symbol level

The original RNA data is indexed by ensembl_gene_id (ENSG), to used it with proteomics data at gene symbol level, we need to mapping ensembl_gene_id to gene symbol.

create a dictionary used for mapping ensembl_gene_id to gene symbol

When a same ensembl_gene_id can be map to multiple gene symbol (not common) use only the first one (ordered by alphabeta)

```
# import information for mapping between accession, gene symbol, and ensembl ID
omics_metadata <- read_csv('support-data/omics_metadata.csv', show_col_types = FALSE)

dict_ensembl_to_gen_symbol <- omics_metadata %>% select(ensembl_gene_id, gene_symbol) %>% unique()
dict_ensembl_to_gen_symbol <- dict_ensembl_to_gen_symbol %>% dplyr::group_by(ensembl_gene_id) %>%
      dplyr::arrange(gene_symbol) %>%
      dplyr::summarise(map_to=first(gene_symbol))

rm(omics_metadata)
```

mapping ensembl_gene_id to gene symbol and pre-process data

1. Remove row with 0 in all cells
2. Only keep the 8 gastric primary cell samples
3. aggregate row with the same index by summing across row axis

```
count_TPM_gene_symbol <- count_TPM_mat
# for rowname, mapping ensembl ID to gene symbol
rownames(count_TPM_gene_symbol) <- mapping(rownames(count_TPM_mat),
                                           dictionary = dict_ensembl_to_gen_symbol)
# remove all 0 rows
count_TPM_gene_symbol <- count_TPM_gene_symbol[rowSums(count_TPM_gene_symbol > 0) > 0, ]
```

```
# aggregate row with same gene symbol index
temp <- as_tibble(count_TPM_gene_symbol, rownames='symbol') %>% filter(!is.na(symbol)) %>% group_by(syml
count_TPM_gene_symbol <- as.matrix(temp[-1])
rownames(count_TPM_gene_symbol) <- temp$symbol

# change the column name the same as in spectral count matrix
colnames(count_TPM_gene_symbol) <- c('IP7103_1701', 'IP0972_1701', 'IP0995_1701', 'IP0982_1701', 'IP099
count_TPM_gene_symbol <- count_TPM_gene_symbol[, c("IP0981_1701", "IP0982_1701",
                                                   "IP0993_1701", "IP0995_1701",
                                                   "IP0999_1701", "IP7100_1701",
                                                   "IP7103_1701", "IP7105_1701")]

# remove row with NA index
count_TPM_gene_symbol <- count_TPM_gene_symbol[!is.na(rownames(count_TPM_gene_symbol)), ]

head(count_TPM_gene_symbol)
```

```
##          IP0981_1701 IP0982_1701 IP0993_1701  IP0995_1701 IP0999_1701
## A1BG      0.12136630   0.9714666   1.8962540   8.06389727   0.8891093
## A1BG-AS1  0.69555945   1.3701390   8.9431089 137.05080510   5.6369555
## A1CF      3.60652496   5.0558246   7.3000066   0.00000000   6.4181129
## A2M       6.77333151   3.2430162 605.7338518   6.07730643  34.4754551
## A2M-AS1   0.85851339   3.4359501  10.6812031   2.84116724  18.0294027
## A2ML1     0.02089757   0.0000000   0.2448813   0.04787897   0.4248308
##           IP7100_1701 IP7103_1701  IP7105_1701
## A1BG       2.00332441   2.5125801   3.39680657
## A1BG-AS1  44.92645048   7.1998934   7.00430202
## A1CF       1.54458435   0.0000000   6.29969386
## A2M      676.87508306   0.2867578 524.78950976
## A2M-AS1   24.64520330  27.6474070   2.16469545
## A2ML1      0.01124819   0.1081578   0.02371145
```

```
nrow(count_TPM_gene_symbol)
```

```
## [1] 31742
```

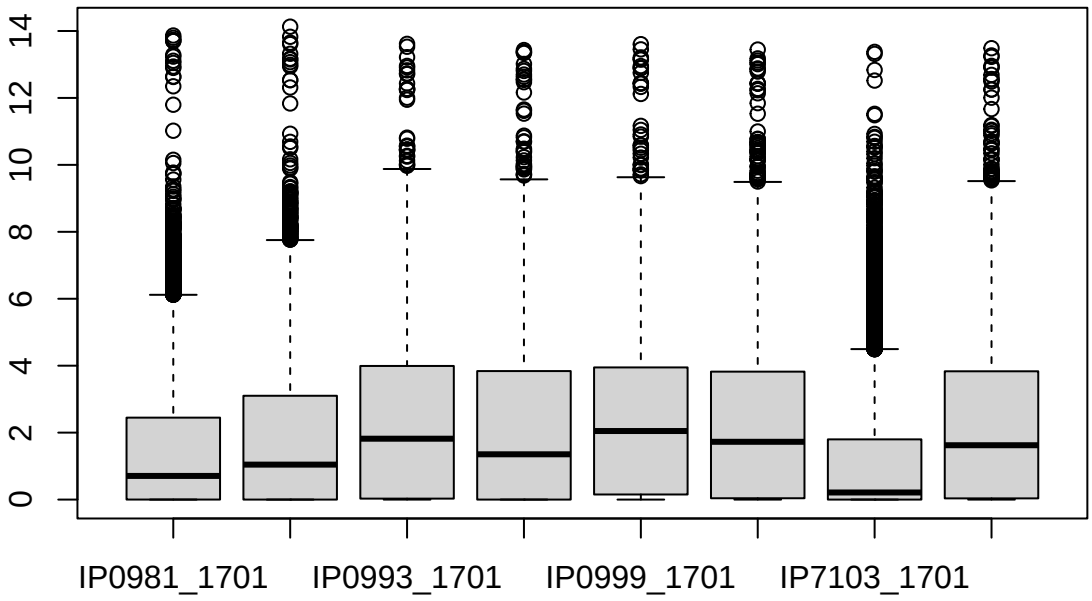
```
# write.csv(as.data.frame(count_TPM_gene_symbol), file='TPM_gastric_cancer_primary_cell.csv')
```

export the processed RNA data

```
write.csv(as.data.frame(count_TPM_gene_symbol), 'RNA-data/processed_RNA_data.csv')
```

recheck sample-level expression distribution after TPM normalization

```
boxplot(as.data.frame(log(count_TPM_gene_symbol+1)))
```


0
2
4
6
8
10
12
14
IP0981_1701
IP0993_1701
IP0999_1701
IP7103_1701