RNA data processing

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

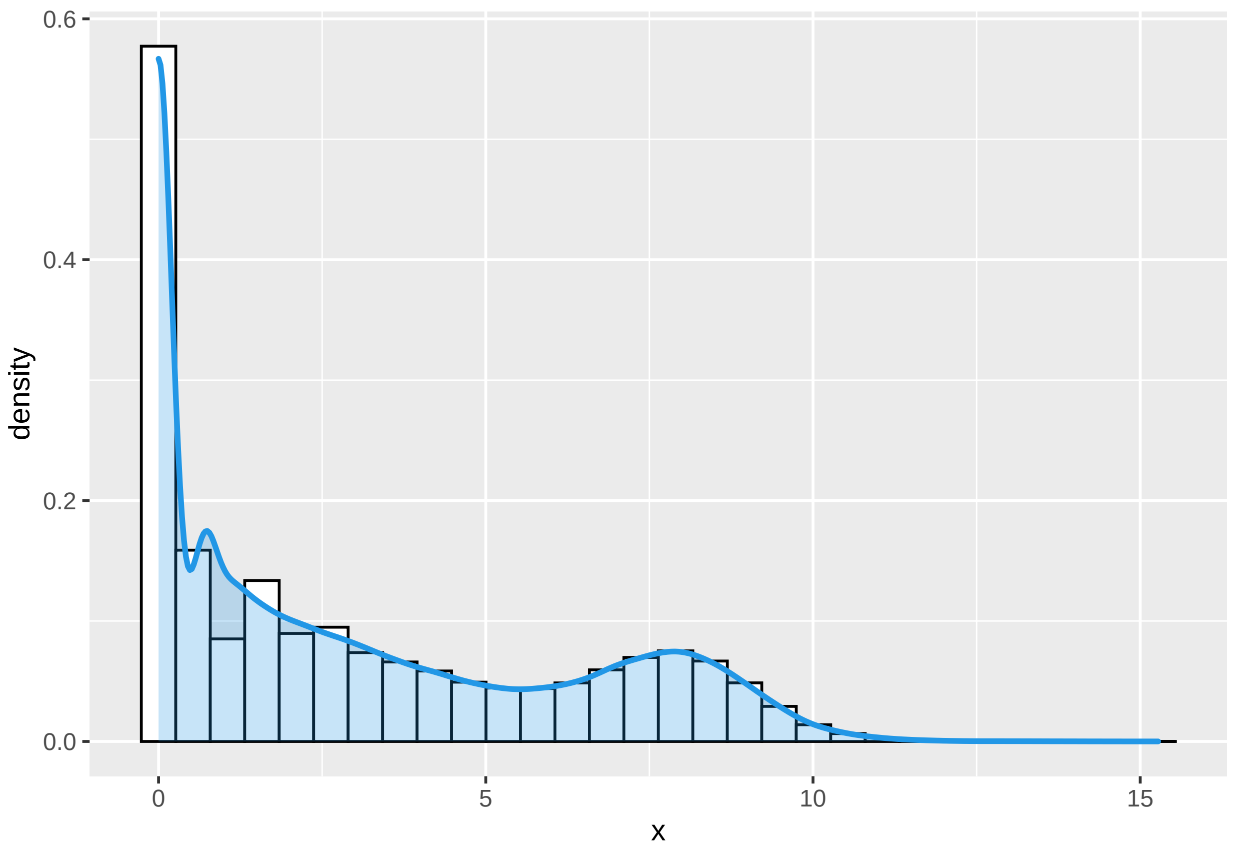
```
## read-in newest missing protein list
missing_protein_df <- readxl::read_xlsx('support-data/PE2-5.xlsx')
missing_protein_df <- missing_protein_df[-c(1:12), ]
# head(missing_protein_df)
accession_to_symbol <- missing_protein_df %>% dplyr::select(Accession, `gene name(s)`)
accession_to_symbol <- base::data.frame(gene_name = accession_to_symbol$`gene name(s)`, row.names = acc
```

Section 0: Exploration

overall distribution

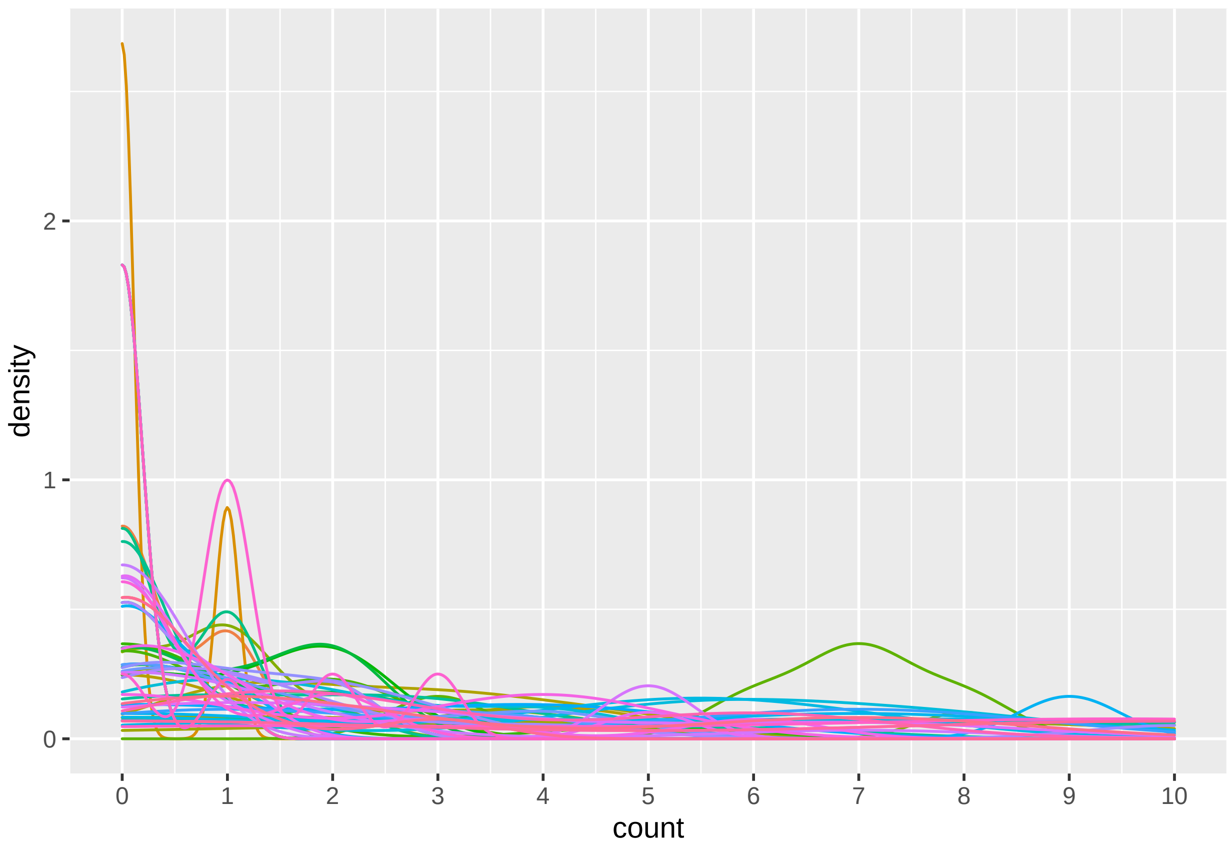
```
count_df <- as.data.frame(count[-1])
pseudo_counts <- log(count_df + 1)
ggplot(data = data.frame(x=c(as.matrix(pseudo_counts))), aes(x=x)) +
  geom_histogram(aes(y = ..density..),
               colour = 1, fill = "white") +
  geom_density(lwd = 1, colour = 4,
              fill = 4, alpha = 0.25)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

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



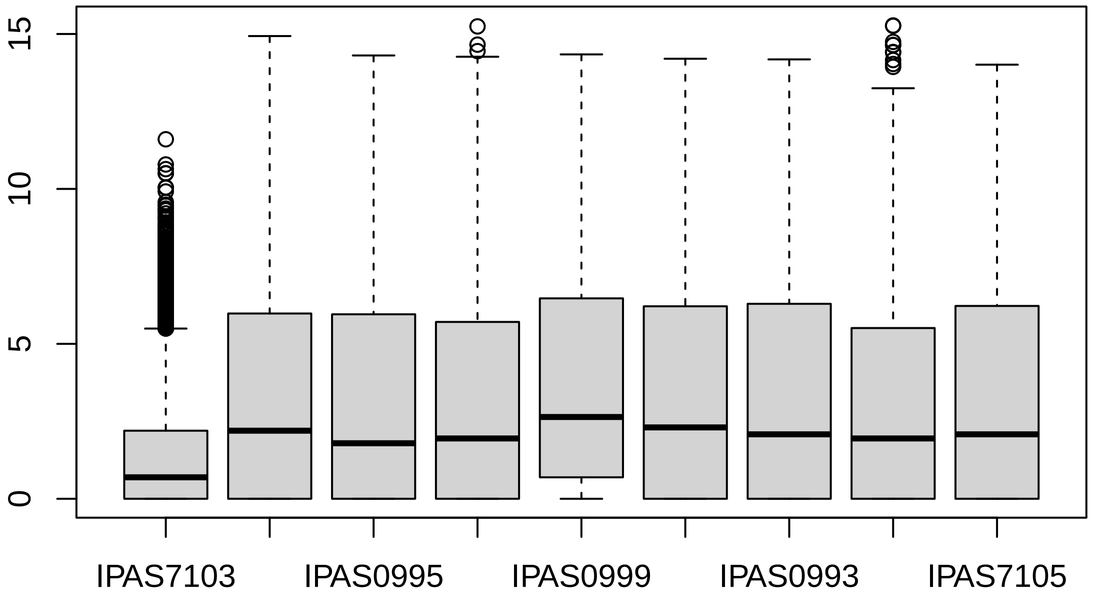
tag-wise distribution

```
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)

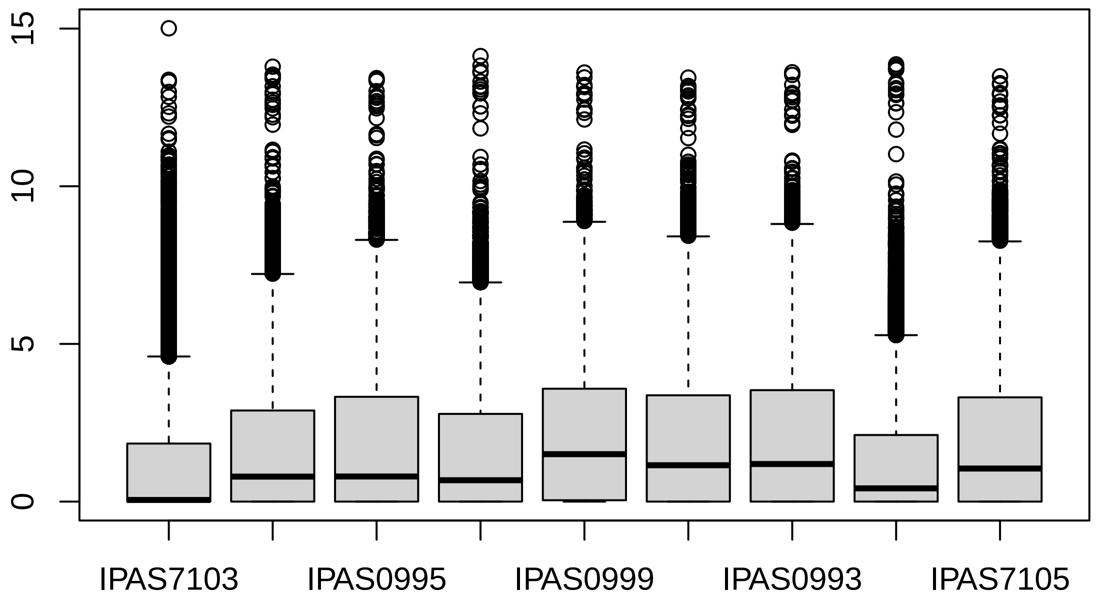
```
gene_length_df <- dict_ensembl_to_symbol %>% dplyr::select(ensembl_gene_id, gene_length) %>% unique()
gene_length_df <- data.frame(length=gene_length_df$gene_length, row.names=gene_length_df$ensembl_gene_i
count_TPM_mat <- TPM(count_mat, gene_length_df)
head(count_TPM_mat)
```

```
##                    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')
```

recheck sample-level expression distribution after TPM normalization

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


IPAS7103
IPAS0995
IPAS0999
IPAS0993
IPAS7105