Bio-Rad Take Home Project

Todd Warczak

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```
library(tidyverse)
library(data.table)
library(here)
library(gridExtra)
library(gt)
library(ggtext)
library(ggdark)
```

```
count_tbl_filt <- data.table::fread(here("data", "count_tbl_filt.csv"))</pre>
```

Data set dimensions (rows, columns)

```
dim(count_tbl_filt)
```

[1] 128804 5580

First 4 rows and 2 columns of count_tbl_filt.

```
count_tbl_filt[1:4, 1:2]
```

```
## 1: hg19_chr1:713971-714221 0
## 2: hg19_chr1:714480-714730 0
## 3: hg19_chr1:762720-762970 0
## 4: hg19_chr1:825991-826241 0
```

Create useful variables from the gene ID (V1) column.

Now we can group_by, filter, ... count_tbl_filt_2 by reference genome, chromosome, or locus. The drops_with_gene variable was created earlier when filtering out genes present in < 1% of droplets.

First 4 rows and 5 columns of count_tbl_filt_2.

count_tbl_filt_2[1:4, 1:5]

```
##
      ref_genome chromosome from_loc to_loc drops_with_gene
## 1:
                           1
                                713971 714221
            hg19
## 2:
                                714480 714730
            hg19
                           1
                                                            88
## 3:
            hg19
                           1
                                762720 762970
                                                            189
## 4:
            hg19
                           1
                                825991 826241
                                                            56
```

Find total gene read counts for both reference genomes in each droplet.

```
count_tbl_sum <- count_tbl_filt_2 %>%
  group_by(ref_genome) %>%
  summarise(across(5:last_col(), .fns = sum))
```

View total read counts for both reference genomes in first 2 droplets.

count_tbl_sum[,1:3]

Reshape $count_tbl_sum$ so droplets are observations (rows). Filter to retain droplets that have > 800 read counts in at least 1 reference genome.

```
count_tbl_sum_long <- count_tbl_sum %>%
  pivot_longer(cols = -ref_genome) %>%
  pivot_wider(names_from = ref_genome, values_from = value) %>%
  rename(total_hg19_counts = hg19, total_mm10_counts = mm10) %>%
  filter(total_hg19_counts > 800 | total_mm10_counts > 800)
```

View first 4 rows in count_tbl_sum_long.

count_tbl_sum_long[1:4,]

```
## # A tibble: 4 x 3
##
     name
                                              total_hg19_counts total_mm10_counts
##
     <chr>
                                                           <int>
                                                                              <int>
## 1 alignments.possorted.tagged_BC00001_N01
                                                              51
                                                                                962
## 2 alignments.possorted.tagged_BC00002_N02
                                                             146
                                                                               3724
## 3 alignments.possorted.tagged_BC00003_N03
                                                              78
                                                                               2067
## 4 alignments.possorted.tagged_BC00004_N03
                                                           12796
                                                                                149
```

Create a summary table to tally unique genes expressed in each droplet. This is different than total counts. If a gene in the count_tbl is >= 1, count_tbl_tally will count it as 1.

```
count_tbl_tally <- count_tbl_filt_2 %>%
  group_by(ref_genome) %>%
  summarise(across(5:last_col(), ~ length(which(.x != 0))))
```

Create drops_filter to get names of droplets with > 800 read counts in at least 1 reference genome. Then use drops_filter to filter count_tbl_tally.

```
drops_filter <- count_tbl_sum_long %>% pull(name)

count_tbl_tally_long <- count_tbl_tally %>%
  pivot_longer(cols = -ref_genome) %>%
  pivot_wider(names_from = ref_genome, values_from = value) %>%
  rename(unique_hg19_genes = hg19, unique_mm10_genes = mm10) %>%
  filter(name %in% drops_filter)
```

View first 4 rows in count_tbl_tally_long.

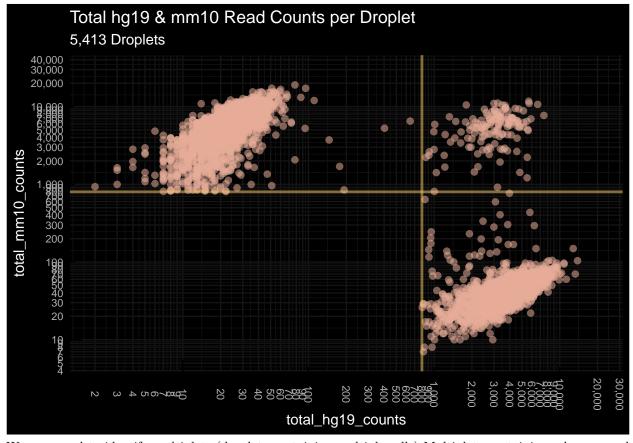
```
count_tbl_tally_long[1:4,]
```

```
## # A tibble: 4 x 3
##
    name
                                              unique_hg19_genes unique_mm10_genes
##
     <chr>>
                                                           <int>
                                                                              <int>
## 1 alignments.possorted.tagged_BC00001_N01
                                                              51
                                                                                935
## 2 alignments.possorted.tagged_BC00002_N02
                                                                               3554
                                                             144
## 3 alignments.possorted.tagged_BC00003_N03
                                                              78
                                                                               1935
## 4 alignments.possorted.tagged BC00004 NO3
                                                           10853
                                                                                141
```

Join count_tbl_sum_long and count_tbl_tally_long summary tables. Create 2 variables for the ratios of unique genes/total counts, one for each reference genome.

Add a plot here of log10(hg19 Counts) vs log10(mm10 Counts), no grouping. Just to show its easy to identify Mouse + Human doublets. Count those and use that number to find the number of expected Human + Human and Mouse + Doublets.

```
= 1) +
geom_vline(xintercept = c(800),
                      = "#FFCF6A",
           color
           alpha
                      = 0.5,
                      = 1) +
ggdark::dark_theme_minimal() +
                    = element_text(angle = -90, vjust = 0.5, size = rel(0.9)),
theme(axis.text.x
                      = element text(size = rel(0.9)),
      axis.text.y
                      = element_text(size = rel(1.0)),
      title
      legend.position = c(0.4, 0.3),
      legend.text
                      = element_text(size = rel(0.8))) +
annotation_logticks(scaled = FALSE) +
             = "Total hg19 & mm10 Read Counts per Droplet",
labs(title
     subtitle = "5,413 Droplets") +
guides(shape = "none")
```



We now need to identify multiplets (droplets containing multiple cells) Multiplets containing a human and mouse cell are easiest to identify: Droplets with total read counts for both reference genomes > 800 or all droplets in quadrant I.

Count droplets in each quadrant and use those counts to find expected number of multiplets in each quadrant.

```
quad_1_count <- count_tbl_join %>%
  filter(total_hg19_counts > 800 & total_mm10_counts > 800) %>%
   nrow()

quad_4_count <- count_tbl_join %>%
```

```
filter(total_hg19_counts > 800 & total_mm10_counts < 800) %>%
    nrow()

quad_2_count <- count_tbl_join %>%
    filter(total_hg19_counts < 800 & total_mm10_counts > 800) %>%
    nrow()

ratio_quad_2_4 <- quad_2_count/quad_4_count
ratio_quad_4_2 <- quad_4_count/quad_2_count

expect_quad_2_multi_count <- round(quad_1_count*ratio_quad_2_4, dig = 0)
expect_quad_4_multi_count <- round(quad_1_count*ratio_quad_4_2, dig = 0)</pre>
```

expect_quad_2_multi_count is the expected n doublets in quadrant II. Droplets containing 2+ human OR 2+ mouse cells can be identified by having lower ratio of unique genes to total read counts in the hg19 or mm10 reference genome, respectively. Droplets containing 1+ human AND 1+ mouse cells will have typical ratios of unique genes to total read counts. Find the value for nth lowest unique_count_ratio. Droplets with values <= to the nth lowest unique_count_ratio will be classified as multiplets. Remember to filter counts for quadrant II and IV.

Create new variables to label multiplets in the different quadrants:

- Logical variables to label Human + Human in quadrant IV (hg19_multiplet), Mouse + Mouse in quadrant II (mm10_multiplet), and Human + Mouse in quadrant I (hg19_mm10_multiplet).
- Logical variable that labels any multiplet droplet (Multiplet).
- Character variable that labels the type of multiplet (Multiplet Type).
- Character variable that labels multiplet type and singlet type (Droplet_Contents).

View new variables in count_tbl_summary.

glimpse(count_tbl_summary)

```
## Rows: 5,413
## Columns: 13
                            <chr> "alignments.possorted.tagged_BC00001_N01", "al~
## $ name
## $ total_hg19_counts
                            <int> 51, 146, 78, 12796, 8706, 49, 63, 39, 5323, 38~
                            <int> 962, 3724, 2067, 149, 105, 11183, 5610, 10488,~
## $ total_mm10_counts
                            <int> 51, 144, 78, 10853, 7761, 48, 59, 35, 4829, 34~
## $ unique_hg19_genes
## $ unique mm10 genes
                            <int> 935, 3554, 1935, 141, 104, 9559, 5208, 9176, 9~
## $ hg19_unique_count_ratio <dbl> 1.0000000, 0.9972323, 1.0000000, 0.9825851, 0.~
## $ mm10_unique_count_ratio <dbl> 0.9958556, 0.9943175, 0.9913555, 0.9889714, 0.~
## $ hg19_multiplet
                            <lgl> FALSE, FALSE, FALSE, TRUE, TRUE, FALSE, FALSE,~
                            <lgl> FALSE, FALSE, FALSE, FALSE, FALSE, FALSE, FALSE,
## $ mm10_multiplet
## $ hg19_mm10_multiplet
                            <lgl> FALSE, FALSE, FALSE, FALSE, FALSE, FALSE, FALSE, FALSE
                            <lgl> FALSE, FALSE, FALSE, TRUE, TRUE, FALSE, FALSE,~
## $ Multiplet
                            <chr> "Singlet", "Singlet", "Human + Huma~
## $ Multiplet_Type
## $ Droplet_Contents
                            <chr> "Mouse", "Mouse", "Human + Human", "H~
```

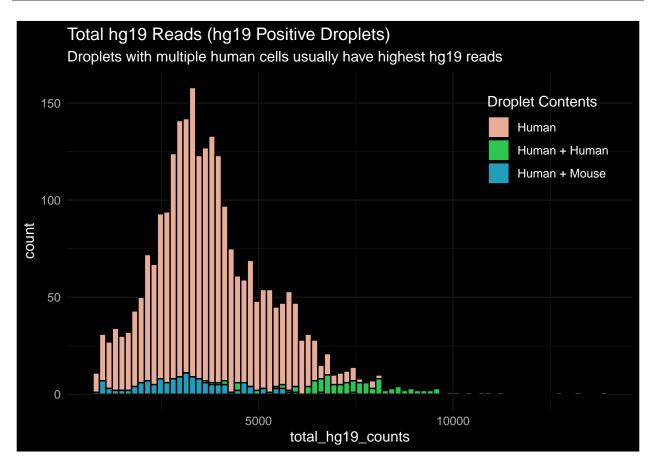
Make sure the number of expected multiplets we calculated in expect_quad_4_multi_count and expect_quad_2_multi_count match the number of TRUE in count_tbl_summary\$hg19_multiplet and count_tbl_summary\$mm10_multiplet.

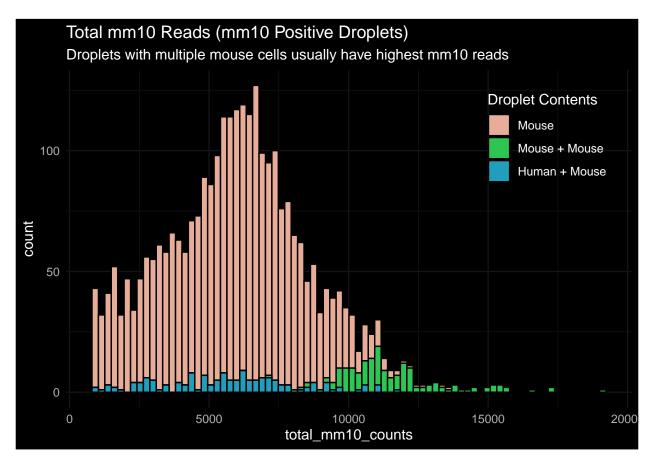
[1] TRUE

[1] TRUE

Droplets with 2+ Humand OR 2+ Mouse cells typically have higher read counts per droplet for that reference genome. Droplets with 1+ Humand AND 1+ Mouse cells have typical read counts for each reference genome. View histogram of hg19 and mm10 reads on separate plots.

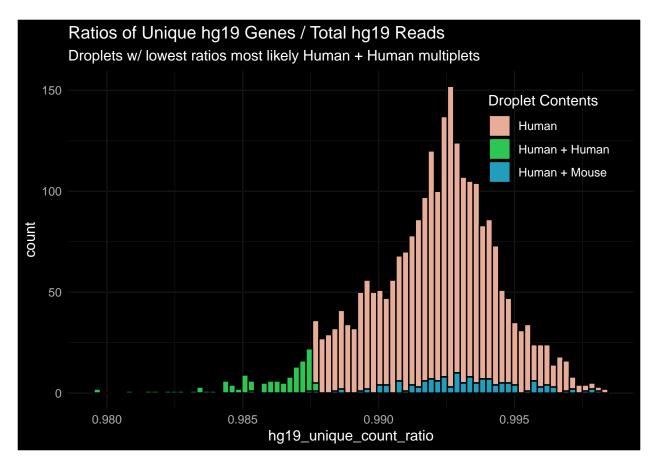
```
count_tbl_summary %>%
  filter(total_hg19_counts > 800) %>%
  ggplot(aes(x = total_hg19_counts, fill = Droplet_Contents)) +
  geom_histogram(bins = 80, color = "black") +
  scale_fill_manual(values = c("#e7ad99", "#2dc653", "#219ebc")) +
  ggdark::dark_theme_minimal() +
  labs(title = "Total hg19 Reads (hg19 Positive Droplets)",
      subtitle = "Droplets with multiple human cells usually have highest hg19 reads",
      fill = "Droplet Contents") +
  theme(legend.position = c(0.85, 0.8),
      legend.text = element_text(size = rel(0.8)))
```

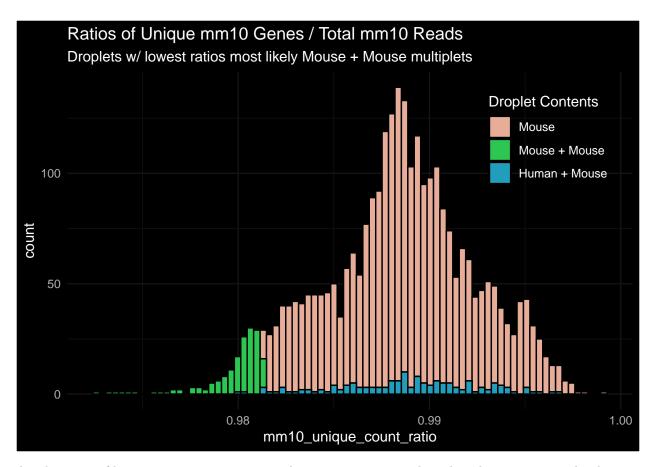




Droplets with 2+ Humand OR 2+ Mouse cells typically have the lowest unique gene count to total read count ratios for that reference genome. Droplets with 1+ Humand AND 1+ Mouse cells have typical ratios for each reference genome.

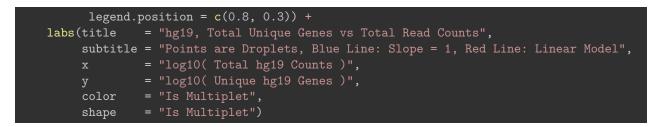
View histogram of hg19 and mm10 ratios on separate plots.

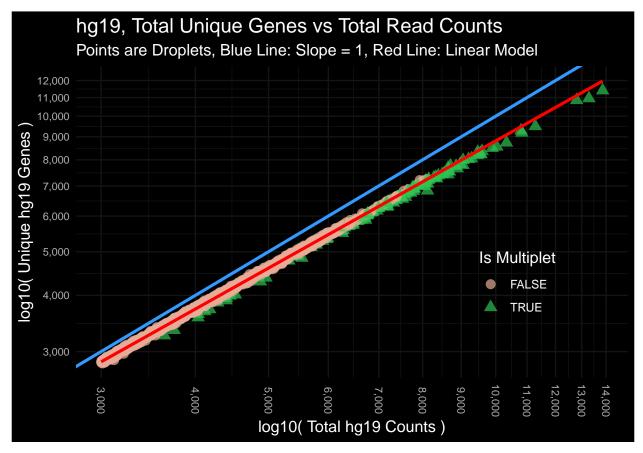




Another view of how unique gene count to total gene count ratios and total read counts are good indicators of multiplets. Fit a linear model on total_hg19_counts for low count droplets, and see how high count droplets deviate from that model.

```
count_tbl_summary %>%
  filter(total_hg19_counts > 3000) %>%
  ggplot(aes(x = total_hg19_counts, y = unique_hg19_genes, color = hg19_multiplet)) +
    geom_point(aes(shape = hg19_multiplet), alpha = 0.7, size = 3.2) +
                          = count_tbl_summary %>% filter(total_hg19_counts < 6000
    geom_smooth(data
                                                         & total_hg19_counts > 3000),
                          = "lm".
                method
                fullrange = TRUE,
                color
                          = "red",
                size
                          = 1.1) +
    scale_color_manual(values = c("#e7ad99", "#2dc653")) +
    scale_y_log10(breaks = scales::breaks_log(n = 12),
                  labels = scales::number_format(accuracy = 1, big.mark = ",")) +
    scale_x_log10(breaks = scales::breaks_log(n = 12),
                  labels = scales::number_format(accuracy = 1, big.mark = ",")) +
    geom_abline(slope = 1,
                color = "#3399FF",
                size = 1.1) +
    ggdark::dark_theme_minimal() +
    theme(axis.text.x = element_text(angle = -90, vjust = 0.5, size = rel(0.9)),
                          = element_text(size = rel(0.9)),
          title
                          = element_text(size = rel(1.1)),
```





Same for $total_mm10_counts$.

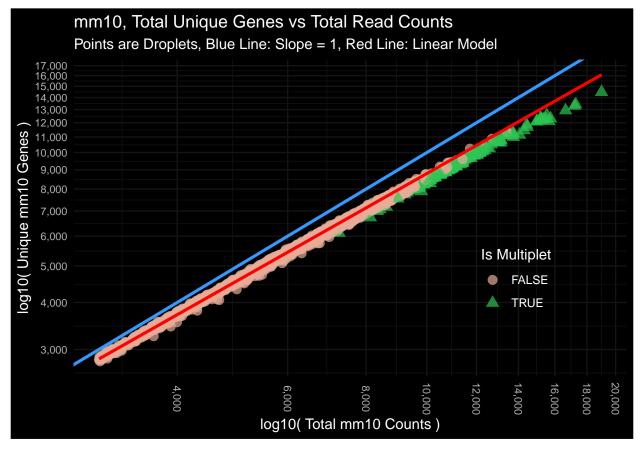
```
count_tbl_summary %>%
  filter(total_mm10_counts > 3000) %>%
  ggplot(aes(x = total_mm10_counts, y = unique_mm10_genes, color = mm10_multiplet)) +
    geom_point(aes(shape = mm10_multiplet), alpha = 0.7, size = 3.2) +
    geom smooth(data
                          = count_tbl_summary %>% filter(total_mm10_counts < 6000
                                                         & total_mm10_counts > 3000),
                method
                          = "lm",
                fullrange = TRUE,
                          = "red",
                color
                          = 1.1) +
                size
    scale_color_manual(values = c("#e7ad99", "#2dc653")) +
    scale_y_log10(breaks = scales::breaks_log(n = 12),
                  labels = scales::number_format(accuracy = 1, big.mark = ",")) +
    scale_x_log10(breaks = scales::breaks_log(n = 12),
                  labels = scales::number_format(accuracy = 1, big.mark = ",")) +
    geom_abline(slope = 1,
```

```
color = "#3399FF",
    size = 1.1) +

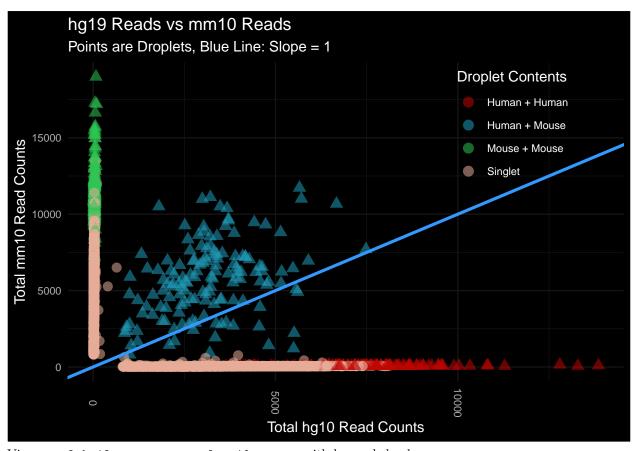
ggdark::dark_theme_minimal() +

theme(axis.text.x = element_text(angle = -90, vjust = 0.5, size = rel(0.9)),
    axis.text.y = element_text(size = rel(0.9)),
    title = element_text(size = rel(1.0)),
    legend.position = c(0.8, 0.3)) +

labs(title = "mm10, Total Unique Genes vs Total Read Counts",
    subtitle = "Points are Droplets, Blue Line: Slope = 1, Red Line: Linear Model",
    x = "log10( Total mm10 Counts )",
    y = "log10( Unique mm10 Genes )",
    color = "Is Multiplet",
    shape = "Is Multiplet")
```

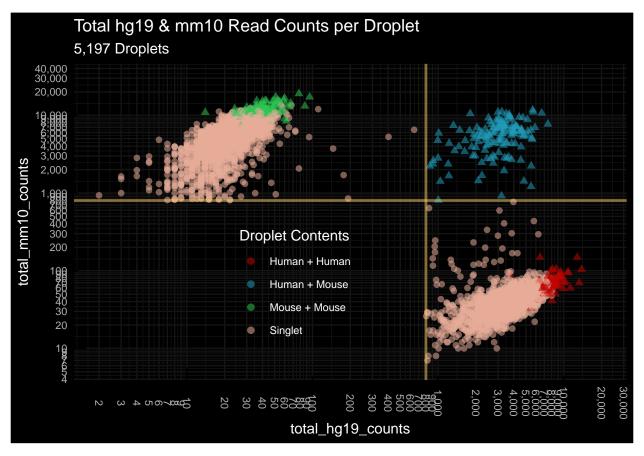


View total_hg19_counts vs total_mm10_counts without log scaled values.



View total_hg19_counts vs total_mm10_counts with \log scaled values.

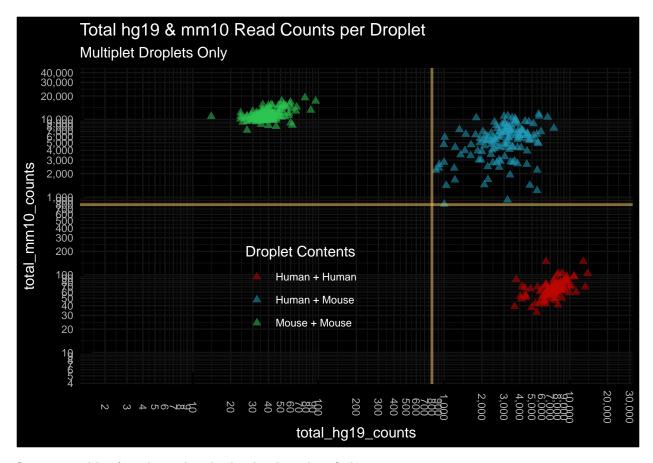
```
= "#FFCF6A",
           color
           alpha
           size
                      = 1) +
geom_vline(xintercept = c(800),
           color
                     = "#FFCF6A",
          alpha
          size
                     = 1) +
ggdark::dark_theme_minimal() +
                   = element_text(angle = -90, vjust = 0.5, size = rel(0.9)),
theme(axis.text.x
     axis.text.y
                     = element_text(size = rel(0.9)),
                     = element_text(size = rel(1.0)),
     title
     legend.position = c(0.4, 0.3),
     legend.text
                     = element_text(size = rel(0.75))) +
annotation_logticks(scaled = FALSE) +
labs(title
            = "Total hg19 & mm10 Read Counts per Droplet",
     subtitle = "5,197 Droplets",
     color
            = "Droplet Contents") +
guides(shape = "none")
```



View total_hg19_counts vs total_mm10_counts with log scaled values, but just the multiplets.

```
count_tbl_summary %>% filter(Multiplet == TRUE) %>%
  ggplot(aes(x = total_hg19_counts, y = total_mm10_counts)) +
    geom_point(aes(color = Multiplet_Type),
        alpha = 0.55,
        size = 2,
```

```
shape = 17) +
scale_y_log10(breaks = scales::breaks_log(n = 38),
             labels = scales::number_format(accuracy = 1, big.mark = ","),
             limit = c(6, 30000)) +
scale_x_log10(breaks = scales::breaks_log(n = 40),
             labels = scales::number_format(accuracy = 1, big.mark = ","),
             limit = c(2, 20000)) +
scale_color_manual(values = c("#bf0603", "#219ebc", "#2dc653")) +
geom_hline(yintercept = c(800),
          color = "#FFCF6A",
          alpha
          size
                   = 1) +
geom_vline(xintercept = c(800),
          alpha
                    = 0.5,
          size
                    = 1) +
ggdark::dark_theme_minimal() +
theme(axis.text.x = element_text(angle = -90, vjust = 0.5, size = rel(0.9)),
                   = element_text(size = rel(0.9)),
     axis.text.y
                  = element_text(size = rel(1.0)),
     title
     legend.position = c(0.4, 0.3),
     legend.text = element_text(size = rel(0.75))) +
annotation_logticks(scaled = FALSE) +
labs(title = "Total hg19 & mm10 Read Counts per Droplet",
     subtitle = "Multiplet Droplets Only",
guides(shape = "none")
```



Summary table of singlet and multiplet droplets identified.

Droplet Contents 5413 Total Droplets

	n
Human	2328
Human + Human	124
Human + Mouse	145
Mouse	2652
Mouse + Mouse	164

Save count_tbl_summary for further exploratory data analysis.

data.table::fwrite(count_tbl_summary, here("data", "count_tbl_summary.csv"))