Results Section: Public Metadata

```
library(staphopia)
library(ggplot2)
library(reshape2)
```

Aggregating Data For Public Samples

First we'll get all publicly available *S. aureus* samples.

```
ps <- get_public_samples()
```

Variation From *S. aureus* N315

In Staphopia all samples had variants (SNPs and InDels) called using *S. aureus* N315 as the reference genome. In this section we'll visualize the total number of variants each sample has. This will give us an idea of the sequenced genitic diversity with respect to N315.

Gather Variant Counts

We will use `get_variant_counts()` to get the variant counts for each sample. We will also order the counts by the total.

```
variant_counts <- get_variant_counts(ps$sample_id)
variant_counts <- variant_counts[order(total),]
```

Summary of Variant Counts

Total Variants (SNPs and InDels)

```
summary(variant_counts$total)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##      10   19457   23891   26505   37343  146962
```

SNPs

```
summary(variant_counts$snp_count)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##       6   18712   23162   25560   36062  141893
```

InDels

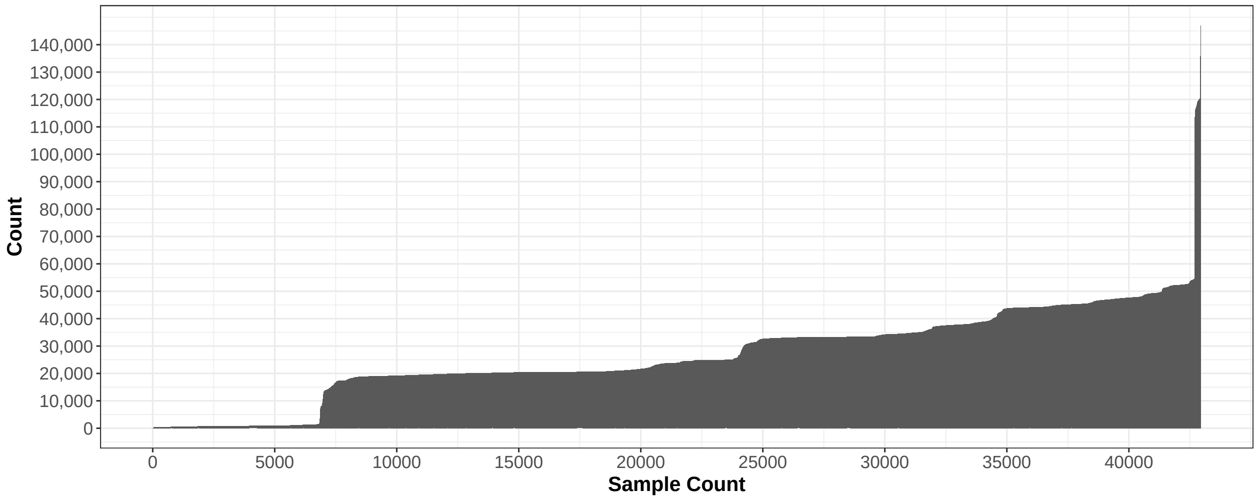
```
summary(variant_counts$indel_count)
```

```
##    Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
##     1.0   709.0   901.0   944.4  1293.0  5125.0
```

Visualizing Variant Counts

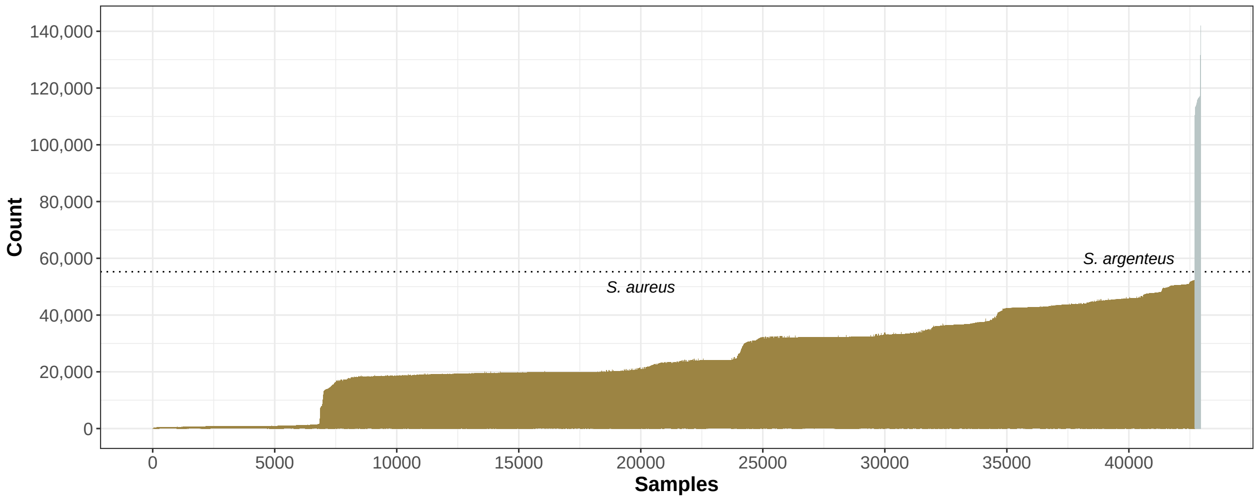
Total Variants (SNPs and InDels)

```r
p <- ggplot(data=variant_counts, aes(x=seq(1,nrow(variant_counts)), y=total)) +
    xlab("Sample Count") +
    ylab("Count") +
    geom_bar(stat='identity') +
    scale_x_continuous(breaks = seq(0, nrow(variant_counts), by = 5000)) +
    scale_y_continuous(breaks = seq(0, max(variant_counts$total), by=10000),
                       labels = scales::comma) +
    theme_bw() +
    theme(axis.text=element_text(size=12),
          axis.title=element_text(size=14,face="bold"))
p
```



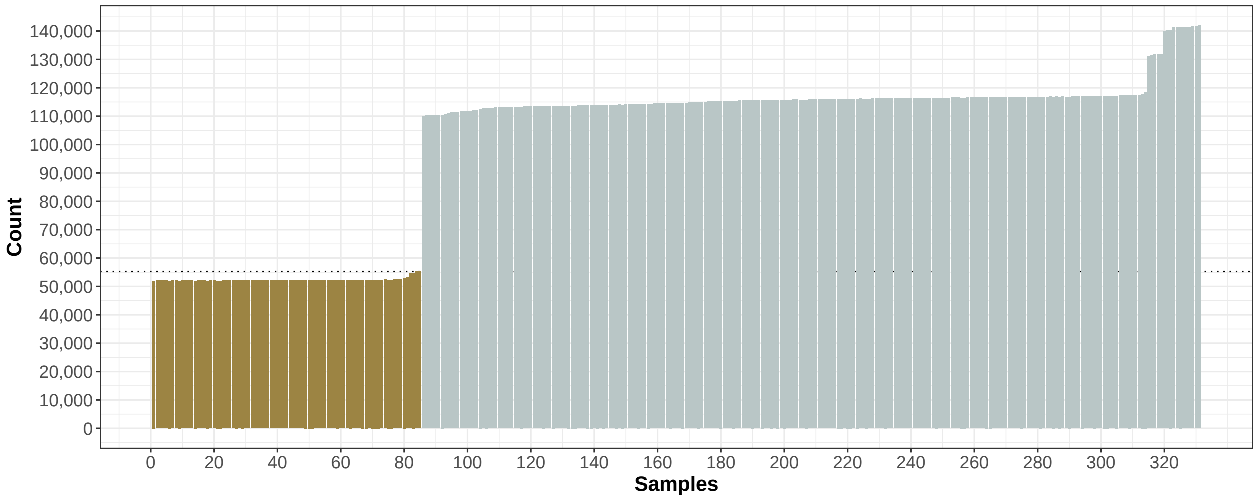
SNPs Only

```r
cutoff <- max(variant_counts[variant_counts$snp_count < 60000,]$snp_count)
variant_counts$fill <- ifelse(variant_counts$snp_count > cutoff, TRUE, FALSE)
p <- ggplot(data=variant_counts, aes(x=seq(1,nrow(variant_counts)), y=snp_count,
                                     fill=fill)) +
    xlab("Samples") +
    ylab("Count") +
    geom_hline(yintercept = cutoff, linetype="dotted") +
    geom_bar(stat='identity') +
    annotate("text", x = 40000, y = 60000, label = "S. argenteus", fontface=3) +
    annotate("text", x = 20000, y = 50000, label = "S. aureus", fontface=3) +
    scale_x_continuous(breaks = seq(0, nrow(variant_counts), by = 5000)) +
    scale_y_continuous(breaks = seq(0, max(variant_counts$snp_count), by=20000),
                       labels = scales::comma) +
    scale_fill_manual(values=c("#9C8443", "#B9C6C6")) +
    theme_bw() +
    theme(axis.text=element_text(size=12),
          axis.title=element_text(size=14,face="bold"),
          legend.position="none")
p
```



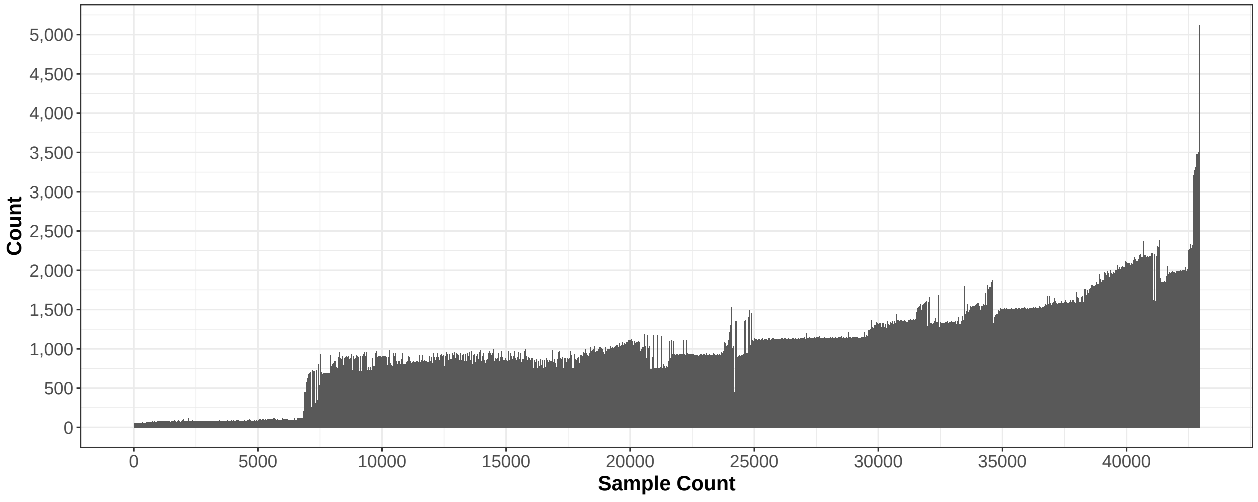
```r
# Output plot to PDF and PNG
staphopia::write_plot(p, paste0(getwd(), '/../figures/figure-09-snp-accumulation'))
```

```r
cutoff <- max(variant_counts[variant_counts$snp_count < 60000,]$snp_count)
variant_counts$fill <- ifelse(variant_counts$snp_count > cutoff, TRUE, FALSE)
p <- ggplot(data=variant_counts[variant_counts$snp_count > 52000,], aes(
        x=seq(1,nrow(variant_counts[variant_counts$snp_count > 52000,])),
        y=snp_count,
        fill=fill)
    ) +
    xlab("Samples") +
    ylab("Count") +
    geom_hline(yintercept = cutoff, linetype="dotted") +
    geom_bar(stat='identity') +
    scale_x_continuous(breaks = seq(
        0, nrow(variant_counts[variant_counts$snp_count > 52000,]), by = 20)
    ) +
    scale_y_continuous(breaks = seq(0, max(variant_counts$snp_count), by=10000),
                       labels = scales::comma) +
    scale_fill_manual(values=c("#9C8443", "#B9C6C6")) +
    theme_bw() +
    theme(axis.text=element_text(size=12),
          axis.title=element_text(size=14,face="bold"),
          legend.position="none")
p
```



InDels Only

```
p <- ggplot(data=variant_counts, aes(x=seq(1,nrow(variant_counts)), y=indel_count)) +
    xlab("Sample Count") +
    ylab("Count") +
    geom_bar(stat='identity') +
    scale_x_continuous(breaks = seq(0, nrow(variant_counts), by = 5000)) +
    scale_y_continuous(breaks = seq(0, max(variant_counts$indel_count), by=500),
                       labels = scales::comma) +
    theme_bw() +
    theme(axis.text=element_text(size=12),
          axis.title=element_text(size=14,face="bold"))
p
```



Non-Redundant Diversity Dataset

Below are the set of commands used to generate the NRD dataset. These were executed on a local server using Staphopia functions not available through the API due to execution times.

```
# Create NRD dataset (non-redundant-set.txt)
~/staphopia-web/manage.py create_nonredundant_set nrd-dataset --published --rank 3 \
                                                              --singletons

# Extract all genes without InDels and validate SNPs via 31-mers
NRD="nrd-annotation-summary.txt"
STAPHOPIA="/home/rpetit/cgc-staphopia/staphopia"
mkdir extract-genome
awk '{print $8}' non-redundant-set.txt | \
    grep -v sample_id | \
    xargs -I {} ~/staphopia-web/manage.py extract_genome {} $NRD $STAPHOPIA

# Identify genes with less than 3 samples with missing 31-mers
cat extract-genome/*-annotation-details.txt | \
    awk '{if ($3=="False"){print $2}}' | \
    sort | \
    uniq -c | \
    awk '{if ($1 >= 377){print $2}}' | \
    sort -n > validated-genes.txt

# Extract validated genes (extract-core.fasta, extract-core.phylip)
~/staphopia-web/manage.py extract_core validated-genes.txt extract-genome/

# Build Phylogeny
# Start Tree
iqtree -s extract-core.phylip -m GTR -nt AUTO -fast -pre start-tree | \
    tee iqtree-start.stdout.txt

# Identify Recombination
ClonalFrameML start-tree.treefile extract-core.fasta clonalframe -emsim 100 | \
    tee clonalframe.stdout.txt

# Remove Recombination
maskrc-svg.py clonalframe --aln extract-core.fasta \
                          --out extract-core-cfmasked.fasta --symbol '-'| \
    tee maskrc-svg.stdout.txt

# Final Tree
iqtree -s extract-core-cfmasked.fasta -m GTR -nt 11 -pre final-tree \
       -bb 1000 -alrt 1000 -wbt -wbtl -alninfo | \
    tee iqtree-final.stdout.txt
```

Session Info

```
sessionInfo()
```

```
## R version 3.4.3 (2017-11-30)
## Platform: x86_64-pc-linux-gnu (64-bit)
## Running under: Ubuntu 16.04.2 LTS
##
## Matrix products: default
## BLAS: /usr/lib/libblas/libblas.so.3.6.0
## LAPACK: /usr/lib/lapack/liblapack.so.3.6.0
##
## locale:
##  [1] LC_CTYPE=en_US.UTF-8       LC_NUMERIC=C
##  [3] LC_TIME=en_US.UTF-8        LC_COLLATE=en_US.UTF-8
##  [5] LC_MONETARY=en_US.UTF-8    LC_MESSAGES=en_US.UTF-8
##  [7] LC_PAPER=en_US.UTF-8       LC_NAME=C
##  [9] LC_ADDRESS=C               LC_TELEPHONE=C
## [11] LC_MEASUREMENT=en_US.UTF-8 LC_IDENTIFICATION=C
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
## [1] reshape2_1.4.3  ggplot2_2.2.1   staphopia_0.1.9
##
## loaded via a namespace (and not attached):
##  [1] Rcpp_0.12.15       knitr_1.20         magrittr_1.5
##  [4] munsell_0.4.3      colorspace_1.3-2   R6_2.2.2
##  [7] rlang_0.1.6        stringr_1.2.0      httr_1.3.1
## [10] plyr_1.8.4         tools_3.4.3        grid_3.4.3
## [13] data.table_1.10.4-3 gtable_0.2.0       htmltools_0.3.6
## [16] yaml_2.1.18        lazyeval_0.2.1     rprojroot_1.3-2
## [19] digest_0.6.15      tibble_1.4.2       curl_3.1
## [22] evaluate_0.10.1    rmarkdown_1.9      stringi_1.1.6
## [25] compiler_3.4.3     pillar_1.1.0       scales_0.5.0
## [28] backports_1.1.2    jsonlite_1.5
```