NHNW_report

Raphael Eisenhofer

Northern Hairy-nosed Wombat faecal metagenomics

This report contains data from only the NHNW samples.

Preprocessing report

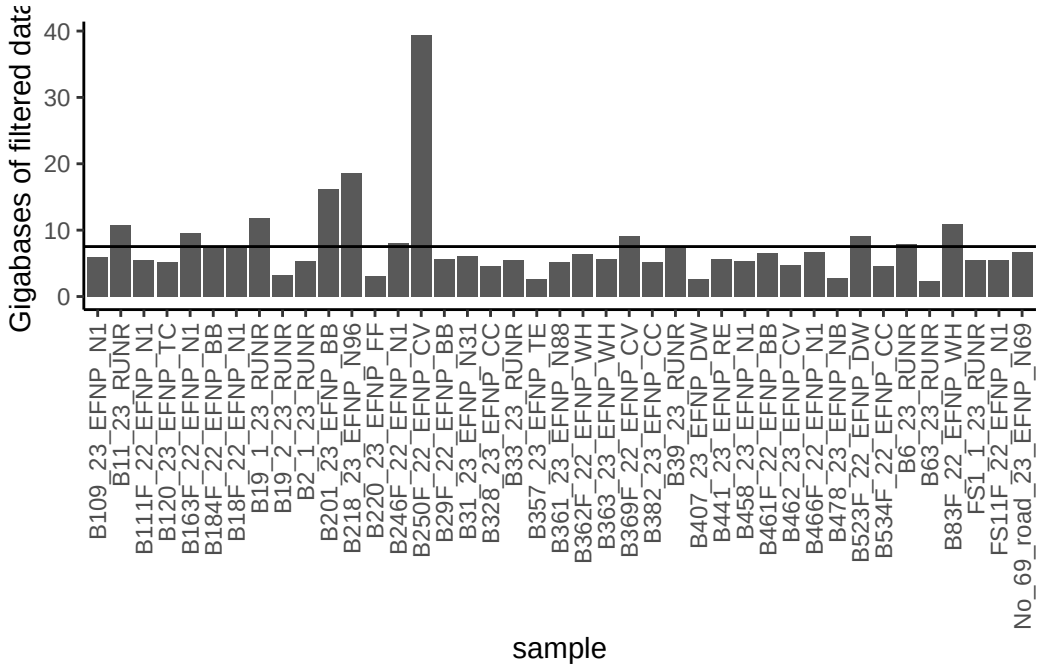
Here are the results for the preprocessing of the data, namely, fastp to trim/quality filter the paired reads and the mapping of filtered reads to the bare-nosed wombat genomes.

```
library(tidyverse)
library(scales)
library(ggtext)
library(ggforce)
library(ggdist)
library(patchwork)

ppr <- read_delim("../data/NHNW/preprocessing_report.tsv")

mean_gbp <- mean(ppr$bases_post_filt) / 1e9
mean_host <- mean(ppr$host_percentage)
max_host <- max(ppr$host_percentage)

ppr %>%
  ggplot(aes(y = bases_post_filt / 1000000000, x = sample)) +
  geom_histogram(stat = "identity") +
  geom_hline(yintercept = mean(ppr$bases_post_filt) / 1e9) +
  theme_classic() +
  theme(
    axis.text.x = element_text(angle = 90, hjust = 1, vjust = 0.5)
  ) +
  ylab("Gigabases of filtered data")
```



Horizontal line indicates the mean (7.5) Gbp of data for the samples. There was minimal mapping of reads to the host genome: max = 2% mean = 0.21%.

Coassembly report

Here's a summary of the coassembly and binning of all samples together.

```
coasb <- read_delim("../data/NHNW/nhnw_coassembly_summary.tsv")

nbin <- max(coasb$num_bins)
ncontig <- max(coasb$num_contigs)
total_length <- max(coasb$total_length / 1e9)
longest_contig <- max(coasb$largest_contig)
n50 <- max(coasb$N50)
coasb_mapping_mean <- percent(mean(coasb$assembly_mapping_percent/100), accuracy = 0.1)
coasb_mapping_max <- percent(max(coasb$assembly_mapping_percent/100), accuracy = 0.1)
coasb_mapping_min <- percent(min(coasb$assembly_mapping_percent/100), accuracy = 0.1)
```

Coassembly yielded 2.19412×10^5 contigs with a total length of 1.8294628 Gbp. The longest contig was 8.16345×10^5 bp, with an average N50 of 2.2118×10^4 bp. Overall the coassembly captured most of the metagenomic reads, with a mean mapping rate of 94.7% (max = 97.6%; min = 90.8%). Binning of these contigs yielded 600 metagenome assembled genomes (MAGs).

Dereplication report

Here is the report on the dereplication of the MAGs at an average nucleotide identity (ANI) of 98%. This is to help mitigate cross-mapping between highly similar MAGs in the final table. **No MAGs were dereplicated at this level.**

Final MAG quality report

Here are some stats for the quality of the final MAGs.

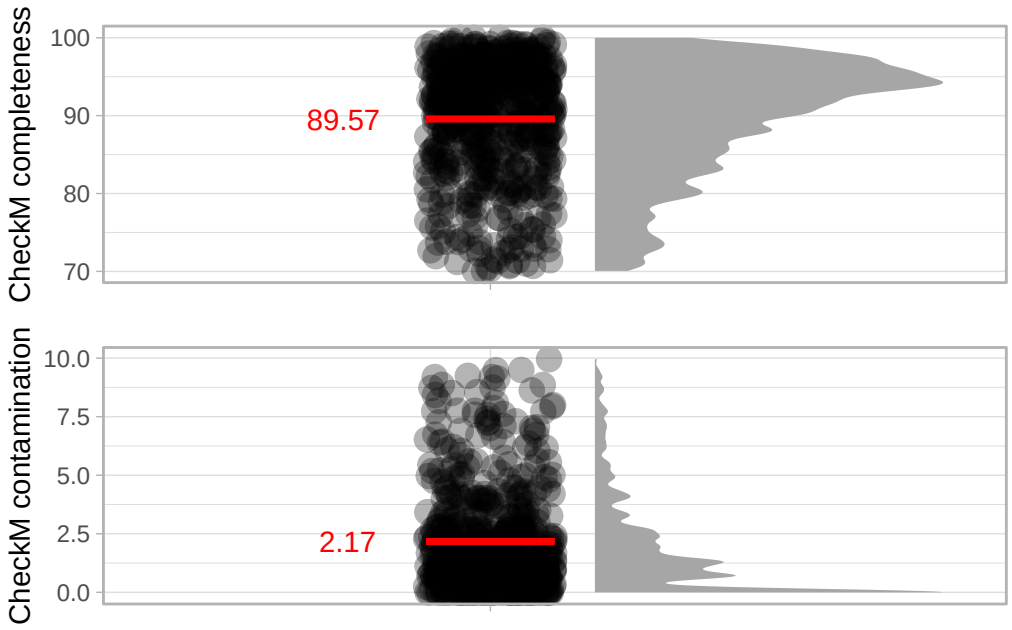
```
mags <- read_delim("../data/NHNW/nhnw_metawrap_70_10_bins.stats")

comp <- mags %>%
  ggplot(aes(y = completeness, x = "")) +
  stat_halfeye(
    adjust = .5,
    width = .6,
    .width = 0,
    justification = -.3,
    point_colour = NA
  ) +
  geom_point(
    size = 4,
    alpha = .3,
    position = position_jitter(seed = 1, width = .1)
  ) +
  stat_summary(
    fun = "mean",
    geom = "crossbar",
    colour = "red",
    width = 0.2
  ) +
  stat_summary(
    fun = "mean",
    geom = "text",
    aes(label = round(..y.., 2)),
    hjust = 2.5,
    colour = "red"
  ) +
  theme_light() +
  theme(
    legend.box.background = element_rect(size = 0.5),
```

```
    legend.margin = margin(-5, 5, 0, 0),
    axis.title.x = element_blank(),
    legend.position = "none") +
  coord_cartesian(xlim = c(1.2, NA), clip = "off") +
  labs(y = "CheckM completeness")

cont <- mags %>%
  ggplot(aes(y = contamination, x = "")) +
  stat_halfeye(
    adjust = .5,
    width = .6,
    .width = 0,
    justification = -.3,
    point_colour = NA
  ) +
  geom_point(
    size = 4,
    alpha = .3,
    position = position_jitter(seed = 1, width = .1)
  ) +
  stat_summary(
    fun = "mean",
    geom = "crossbar",
    colour = "red",
    width = 0.2,
  ) +
  stat_summary(
    fun = "mean",
    geom = "text",
    aes(label = round(..y.., 2)),
    hjust = 3,
    colour = "red"
  ) +
  theme_light() +
  theme(
    legend.box.background = element_rect(size = 0.5),
    legend.margin = margin(-5, 5, 0, 0),
    axis.title.x = element_blank(),
    legend.position = "none") +
  coord_cartesian(xlim = c(1.2, NA), clip = "off") +
  labs(y = "CheckM contamination")
```

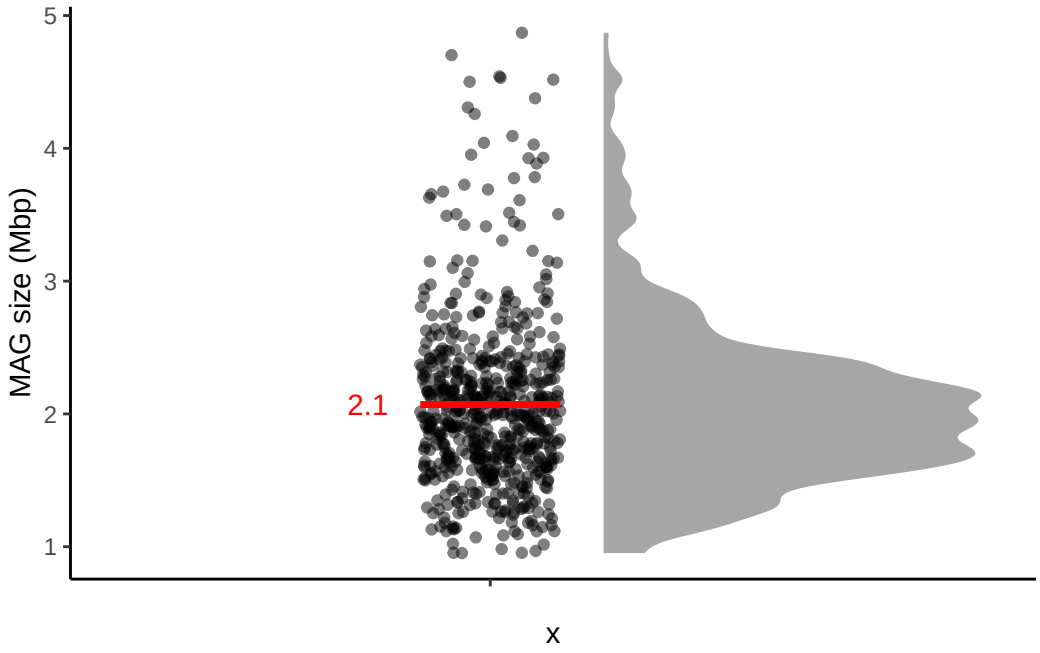
```
comp / cont
```



Most MAGs are of decent quality.

```
mags %>%
  ggplot(aes(x = "", y = size / 1000000)) +
  geom_jitter(width = 0.1, height = 0, alpha = 0.5) +
  stat_halfeye(
    adjust = .5,
    width = .6,
    .width = 0,
    justification = -.3,
    point_colour = NA
  ) +
  stat_summary(
    fun = "mean",
    geom = "crossbar",
    colour = "red",
    width = 0.2,
  ) +
  stat_summary(
    fun = "mean",
    geom = "text",
```

```
    aes(label = number(mean(mags$size / 1000000), accuracy = 0.1)),
    hjust = 3.5,
    colour = "red"
  ) +
  theme_classic() +
  ylab("MAG size (Mbp)")
```



The mean MAG size is 2.1 Mbp. This is pretty low, but cool, as it suggests that most of these bacteria probably can't live outside the NHNW gut!

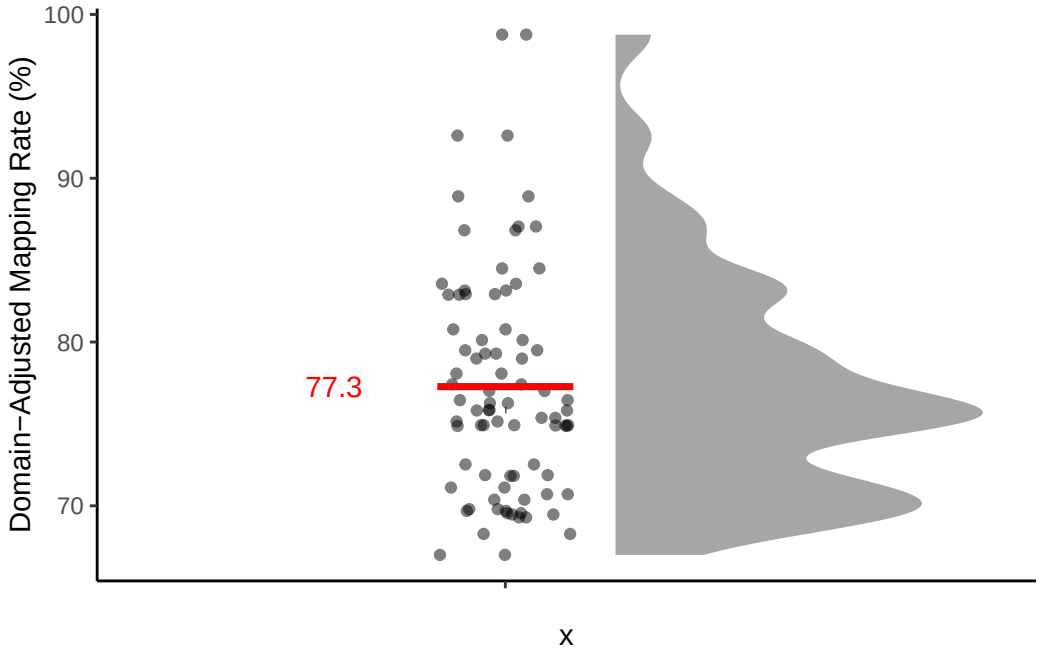
How well did we capture the metagenomic samples?

Using 'SingleM microbial_fraction', we can estimate how much prokaryotic DNA is in our metagenomes. We can then compare this to the mapping rate to calculate a **D**omain-**A**djusted **M**apping **R**ate (**DAMR**). This lets us know how well we've captured the prokaryotic community using our assembly/binning. For more info, see our paper describing the method: https://www.biorxiv.org/content/10.1101/2024.05.16.594470v1

```
smf <- read_delim("../data/NHNW/smf_nhnw.tsv") %>%
  mutate(sample = str_replace_all(sample, "_M_1", ""))
mag_mapping <- read_delim("../data/NHNW/mag_mapping_rate.txt") %>%
  pivot_longer(., !Genome) %>%
```

```
  filter(Genome == "unmapped") %>%
    mutate(name = str_replace_all(name, " Relative Abundance \\(%\\)", ""),
           mapped = 100 - value) %>%
  inner_join(., smf, by = join_by(name == sample)) %>%
  mutate(DAMR = mapped / as.numeric(read_fraction))

mag_mapping %>%
  ggplot(aes(x = "", y = DAMR * 100)) +
  geom_jitter(width = 0.1, height = 0, alpha = 0.5) +
  stat_halfeye(
    adjust = .5,
    width = .6,
    .width = 0,
    justification = -.3,
    point_colour = NA
  ) +
  stat_summary(
    fun = "mean",
    geom = "crossbar",
    colour = "red",
    width = 0.2,
  ) +
  stat_summary(
    fun = "mean",
    geom = "text",
    aes(label = number(mean(mag_mapping$DAMR * 100), accuracy = 0.1)),
    hjust = 3.5,
    colour = "red"
  ) +
  theme_classic() +
  ylab("Domain-Adjusted Mapping Rate (%)")
```



Overall, we've captured most of the prokaryote DNA in the samples (mean 77.3%). This is pretty decent considering the complexity of these microbial communities!