Workflow analysis of Prussin et al. (2019)

Air filters from a daycare in Virginia.

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2024-04-12

Taking a break from working on P2RA datasets, we're also working on a review of air sampling for viral pathogen detection. For that study, we're collecting and analyzing air MGS data that could give us a high-level idea of the likely viral composition of such samples.

The first dataset I'm looking at for this work is Prussin et al. (2019), a study of HVAC filter samples in a Virginia daycare center between 2014 and 2015. Samples were eluted from MERV-14 air filters collected every two weeks, with pairs of successive samples combined into four-week sampling periods. Like Brumfield et al, this study conducted both RNA and DNA sequencing; all samples were sequenced on an Illumina NextSeq500 with 2x150bp reads.

The raw data

The Prussin dataset comprised sequencing data from 14 timepoints spread across the year, from 20th January 2014 to 2nd February 2015. Each sample represents a four-week sampling period. In addition to the 14 on-site samples, there were also two control samples, a negative control (NC) and an "unexposed filter" control (UFC), which were collected on December 23rd 2014.

```r
# Data input paths
data_dir <- "./"
libraries_path <- file.path(data_dir, "harmon-sample-metadata.csv")
basic_stats_path <- file.path(data_dir, "qc_basic_stats.tsv")
adapter_stats_path <- file.path(data_dir, "qc_adapter_stats.tsv")
quality_base_stats_path <- file.path(data_dir, "qc_quality_base_stats.tsv")
quality_seq_stats_path <- file.path(data_dir, "qc_quality_sequence_stats.tsv")

# Import libraries and extract metadata from sample names
libraries_raw <- read_csv(libraries_path, show_col_types = FALSE)
libraries <- libraries_raw %>%
  arrange(desc(na_type)) %>% mutate(na_type = fct_inorder(na_type)) %>%
  arrange(date) %>% rename(start_date = date) %>%
  mutate(end_date = start_date + 28) %>%
  mutate(date = fct_inorder(as.character(end_date)),
         ctrl = ifelse(grepl("Negative_Control", sample_alias), "NC",
                       ifelse(grepl("Unexposed_Filter", sample_alias),
                              "UFC", "Non-Control")),
         ctrl = factor(ctrl, levels = c("Non-Control",
                                        "NC",
                                        "UFC")),
       open = (season != "Closed") & (ctrl == "On-Site"))

# Import QC data
```

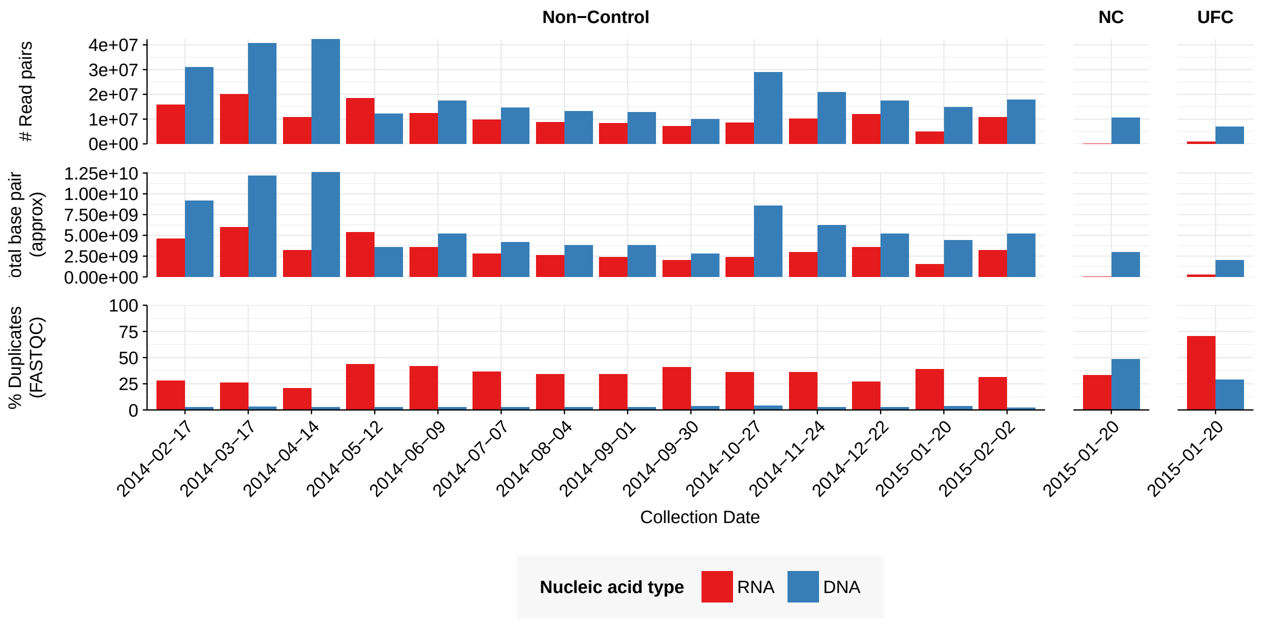
```r
stages <- c("raw_concat", "cleaned", "dedup", "ribo_initial", "ribo_secondary")
basic_stats <- read_tsv(basic_stats_path, show_col_types = FALSE) %>%
  inner_join(libraries, by="sample") %>%
  mutate(stage = factor(stage, levels = stages),
         sample = fct_inorder(sample))
adapter_stats <- read_tsv(adapter_stats_path, show_col_types = FALSE) %>%
    mutate(sample = sub("_2$", "", sample)) %>%
  inner_join(libraries, by="sample") %>%
  mutate(stage = factor(stage, levels = stages),
         read_pair = fct_inorder(as.character(read_pair)))
quality_base_stats <- read_tsv(quality_base_stats_path, show_col_types = FALSE) %>%
  inner_join(libraries, by="sample") %>%
  mutate(stage = factor(stage, levels = stages),
         read_pair = fct_inorder(as.character(read_pair)))
quality_seq_stats <- read_tsv(quality_seq_stats_path, show_col_types = FALSE) %>%
  inner_join(libraries, by="sample") %>%
  mutate(stage = factor(stage, levels = stages),
         read_pair = fct_inorder(as.character(read_pair)))

# Filter to raw data
basic_stats_raw <- basic_stats %>% filter(stage == "raw_concat")
adapter_stats_raw <- adapter_stats %>% filter(stage == "raw_concat")
quality_base_stats_raw <- quality_base_stats %>% filter(stage == "raw_concat")
quality_seq_stats_raw <- quality_seq_stats %>% filter(stage == "raw_concat")

# Get key values for readout
raw_read_counts <- basic_stats_raw %>% group_by(na_type, ctrl) %>%
  summarize(rmin = min(n_read_pairs), rmax=max(n_read_pairs),
            rmean=mean(n_read_pairs), .groups = "drop")
raw_read_totals <- basic_stats_raw %>% group_by(na_type, ctrl) %>%
  summarize(n_read_pairs = sum(n_read_pairs),
            n_bases_approx = sum(n_bases_approx), .groups = "drop")
raw_dup <- basic_stats_raw %>% group_by(na_type, ctrl) %>%
  summarize(dmin = min(percent_duplicates), dmax=max(percent_duplicates),
            dmean=mean(percent_duplicates), .groups = "drop")
```

The 14 positive samples from the dataset yielded 5M-20M (mean 11.3M) RNA-sequencing reads and 10M-42M (mean 21.0M) DNA-sequencing reads per sample, for a total of 159M RNA read pairs and 294M DNA read pairs (46.3 and 87.0 gigabases of sequence, respectively). Controls contributed an additional 1M RNA read pairs and 17.5M DNA read pairs.

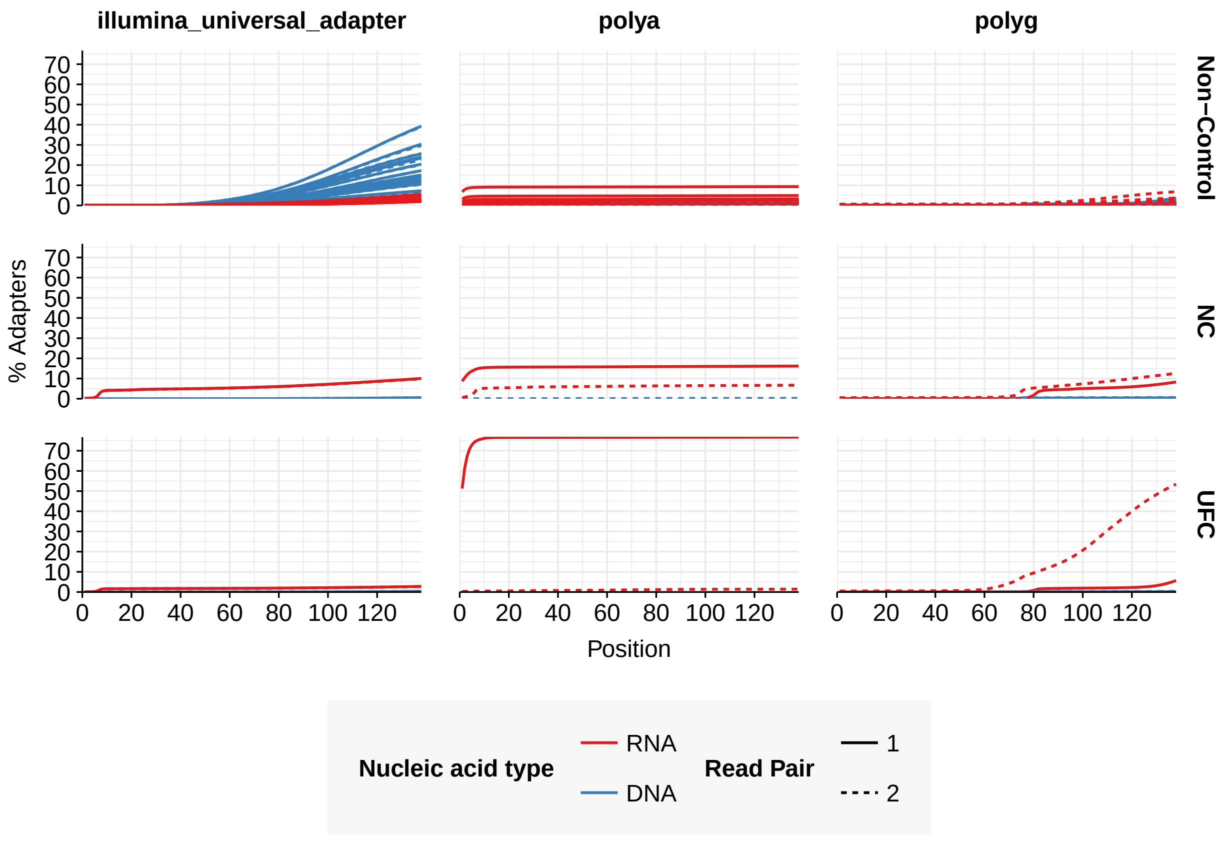
In positive samples, read qualities were mostly high but tailed off slightly at the 3' end in some samples, suggesting the need for trimming. Adapter levels were high. Inferred duplication levels were low (2-4%) in DNA reads and moderate (21-44%) in RNA reads. Control sample reads were more problematic, with higher duplication and adapter levels and lower quality.

```r
# Prepare data
basic_stats_raw_metrics <- basic_stats_raw %>%
  select(date, na_type, ctrl,
         `# Read pairs` = n_read_pairs,
         `Total base pairs\n(approx)` = n_bases_approx,
         `% Duplicates\n(FASTQC)` = percent_duplicates) %>%
  pivot_longer(-(date:ctrl), names_to = "metric", values_to = "value") %>%
  mutate(metric = fct_inorder(metric))
```

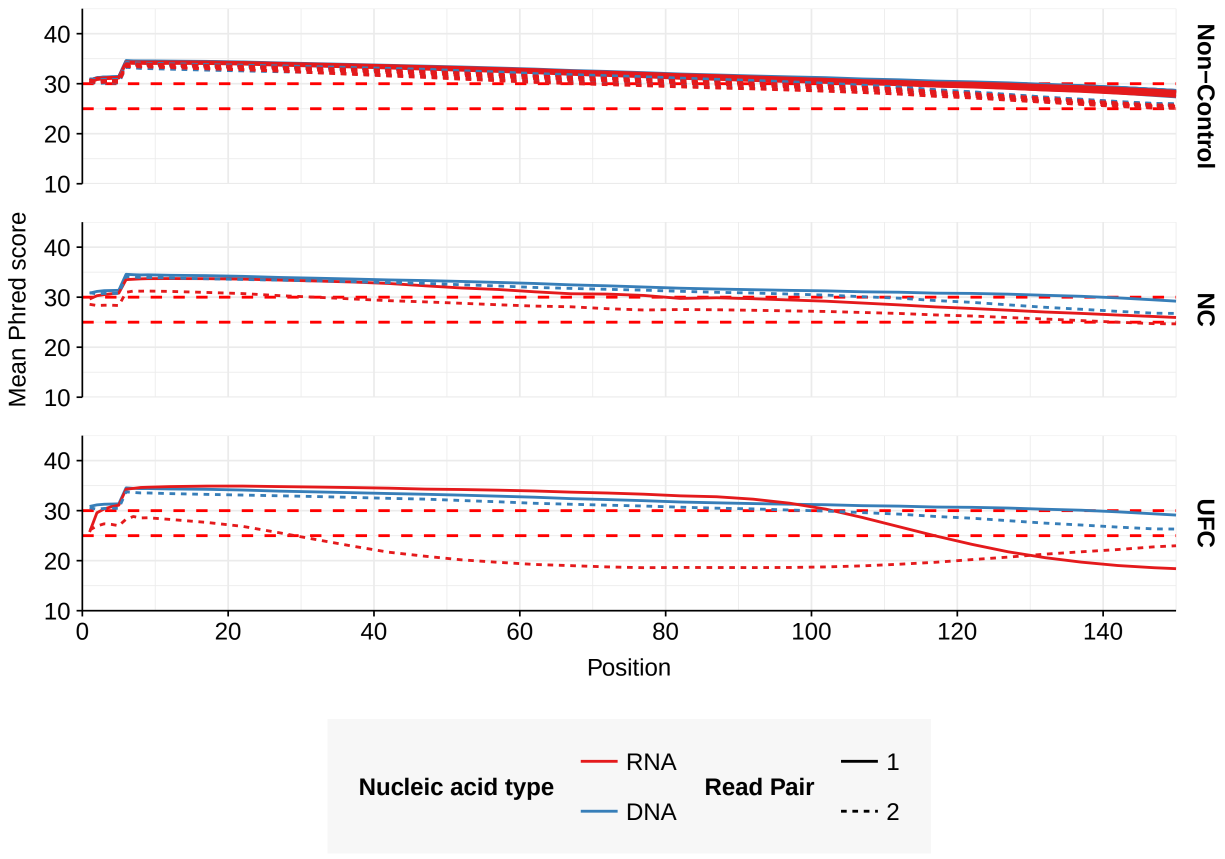
```r
# Set up plot templates
scale_fill_na <- purrr::partial(scale_fill_brewer, palette="Set1",
                                name="Nucleic acid type")

g_basic <- ggplot(basic_stats_raw_metrics,
                  aes(x=date, y=value, fill=na_type)) +
  geom_col(position = "dodge") +
  scale_x_discrete(name="Collection Date") +
  scale_y_continuous(expand=c(0,0)) +
  expand_limits(y=c(0,100)) +
  scale_fill_na() +
  facet_grid(metric~ctrl, scales = "free", space="free_x", switch="y") +
  #facet_grid(metric~ctrl, scales = "free", space="free_x", switch="y", ylim = list(
  #  "# Read pairs" = c(0, 4e+07),
  #  "% Duplicates\n(FASTQC)" = c(0, 1.25e+10)
  #)) +
  theme_rotate + theme(
    axis.title.y = element_blank(),
    strip.text.y = element_text(face="plain")
  )
g_basic
```



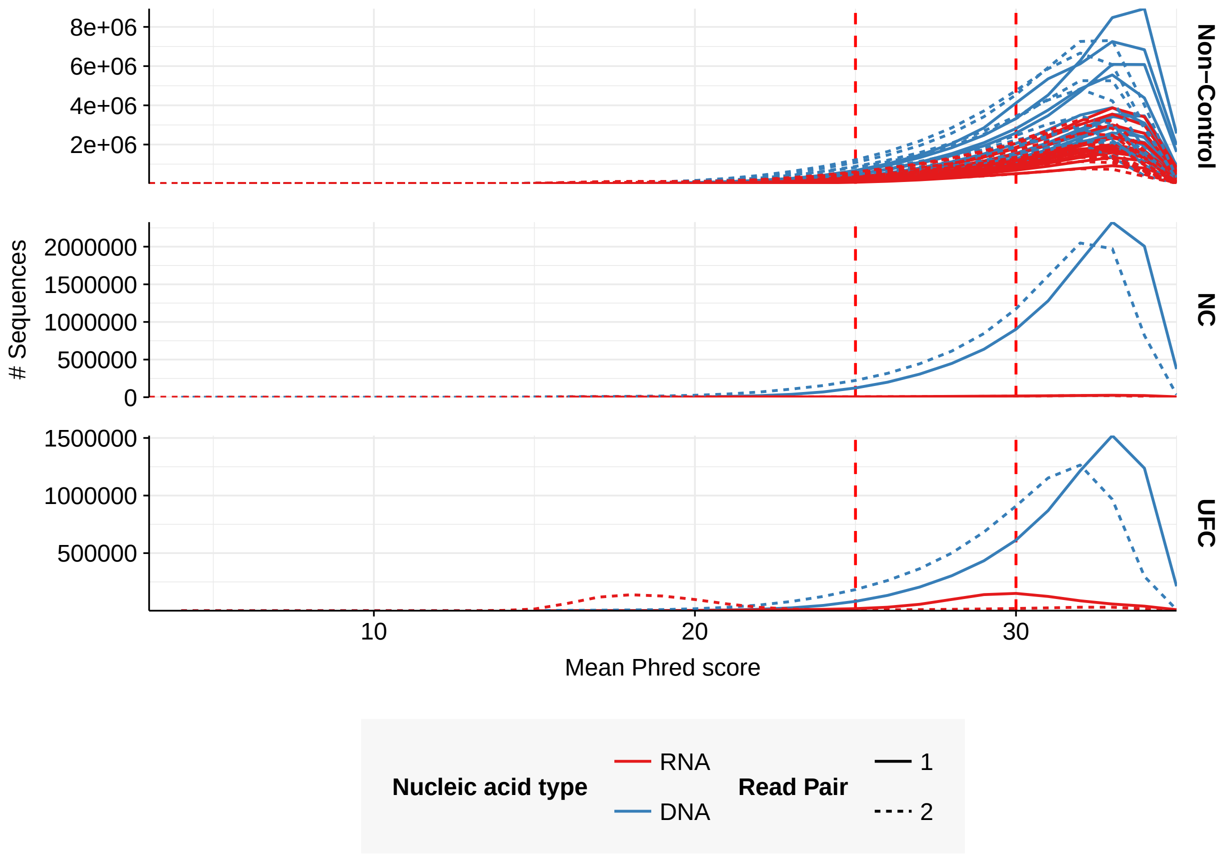
```r
# Set up plotting templates
scale_color_na <- purrr::partial(scale_color_brewer, palette="Set1",
                                 name="Nucleic acid type")
g_qual_raw <- ggplot(mapping=aes(color=na_type, linetype=read_pair,
                         group=interaction(sample,read_pair))) +
  scale_color_na() + scale_linetype_discrete(name = "Read Pair") +
  guides(color=guide_legend(nrow=2,byrow=TRUE),
         linetype = guide_legend(nrow=2,byrow=TRUE)) +
  theme_base

# Visualize adapters
g_adapters_raw <- g_qual_raw +
  geom_line(aes(x=position, y=pc_adapters), data=adapter_stats_raw) +
  scale_y_continuous(name="% Adapters", limits=c(0,NA),
                     breaks = seq(0,100,10), expand=c(0,0)) +
  scale_x_continuous(name="Position", limits=c(0,NA),
                     breaks=seq(0,140,20), expand=c(0,0)) +
  facet_grid(ctrl~adapter)
g_adapters_raw
```



```
# Visualize quality
g_quality_base_raw <- g_qual_raw +
  geom_hline(yintercept=25, linetype="dashed", color="red") +
  geom_hline(yintercept=30, linetype="dashed", color="red") +
  geom_line(aes(x=position, y=mean_phred_score), data=quality_base_stats_raw) +
  scale_y_continuous(name="Mean Phred score", expand=c(0,0), limits=c(10,45)) +
  scale_x_continuous(name="Position", limits=c(0,NA),
                     breaks=seq(0,140,20), expand=c(0,0)) +
  facet_grid(ctrl~.)
g_quality_base_raw
```



```r
g_quality_seq_raw <- g_qual_raw +
  geom_vline(xintercept=25, linetype="dashed", color="red") +
  geom_vline(xintercept=30, linetype="dashed", color="red") +
  geom_line(aes(x=mean_phred_score, y=n_sequences), data=quality_seq_stats_raw) +
  scale_x_continuous(name="Mean Phred score", expand=c(0,0)) +
  scale_y_continuous(name="# Sequences", expand=c(0,0)) +
  facet_grid(ctrl~., scales = "free_y")
g_quality_seq_raw
```



Preprocessing

The average fraction of reads lost at each stage in the preprocessing pipeline is shown in the following table. For positive samples, on average, cleaning and deduplication removed about 19% of total read pairs from RNA libraries and about 7% from DNA libraries. Subsequent ribodepletion removed a further ~32% of total read pairs on average from RNA libraries but <0.5% of total read pairs from DNA libraries.

Control samples, meanwhile, lost an average of 63% of RNA read pairs and 21% of DNA read pairs during cleaning and deduplication, consistent with the lower qualities observed above. Subsequent ribodepletion removed an additional 9% of total RNA read pairs and 0.3% of total DNA read pairs.

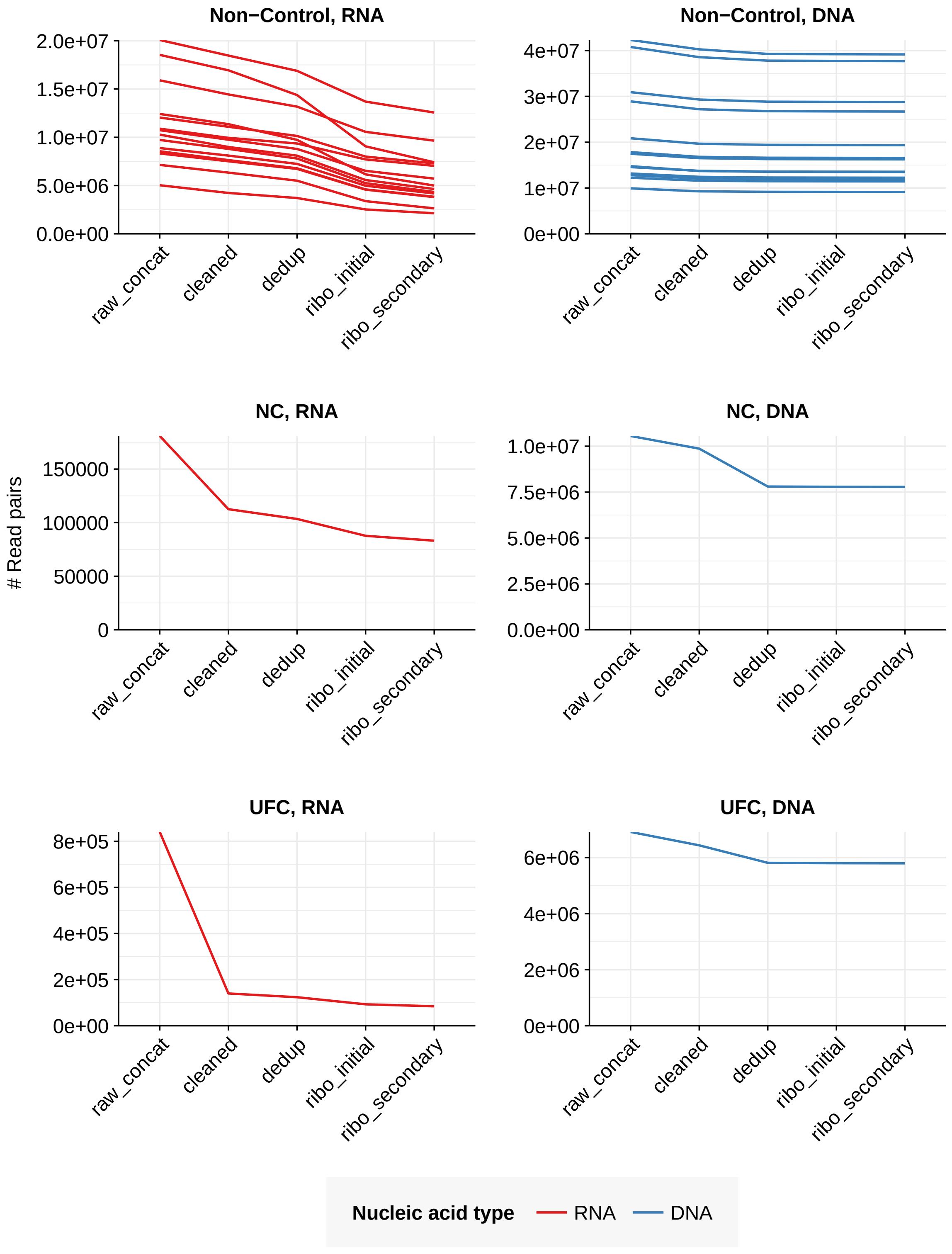
Harmon note: My numbers are slightly higher than Will's when trimming & filtering

```
n_reads_rel <- basic_stats %>%
  select(sample, date, na_type, ctrl, stage,
         percent_duplicates, n_read_pairs) %>%
  group_by(sample, na_type) %>% arrange(sample, na_type, stage) %>%
  mutate(p_reads_retained = replace_na(n_read_pairs / lag(n_read_pairs), 0),
         p_reads_lost = 1 - p_reads_retained,
         p_reads_retained_abs = n_read_pairs / n_read_pairs[1],
         p_reads_lost_abs = 1-p_reads_retained_abs,
         p_reads_lost_abs_marginal = replace_na(p_reads_lost_abs - lag(p_reads_lost_abs), 0))
n_reads_rel_display <- n_reads_rel %>%
  rename(Stage=stage, `NA Type`=na_type, `Control?`=ctrl) %>%
  group_by(`Control?`, `NA Type`, Stage) %>%
  summarize(`% Total Reads Lost (Cumulative)` = paste0(round(min(p_reads_lost_abs*100),1), "-", round(m
            `% Total Reads Lost (Marginal)` = paste0(round(min(p_reads_lost_abs_marginal*100),1), "-", 
```

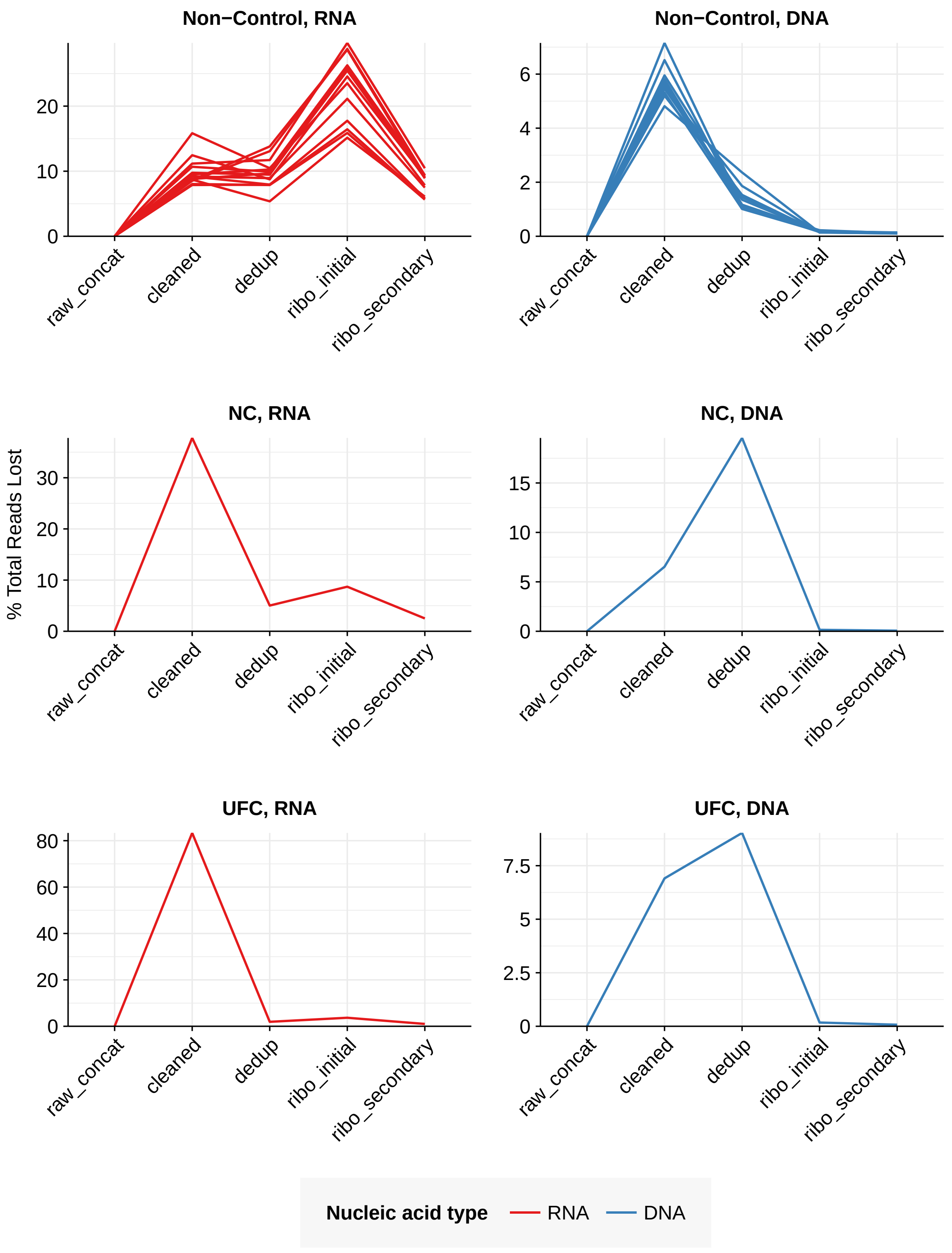
```r
  filter(Stage != "raw_concat") %>%
  mutate(Stage = Stage %>% as.numeric %>% factor(labels=c("Trimming & filtering", "Deduplication", "Ini
n_reads_rel_display
```

```
## # A tibble: 24 x 5
##    `Control?`  `NA Type` Stage                  % Total Reads Lost (Cumu~1 % Total Reads Lost (~2
##    <fct>       <fct>     <fct>                  <chr>                      <chr>
##  1 Non-Control RNA       Trimming & filtering   7.9-15.8 (mean 9.9)        7.9-15.8 (mean 9.9)
##  2 Non-Control RNA       Deduplication          14.1-26.3 (mean 19.5)      5.4-13.8 (mean 9.7)
##  3 Non-Control RNA       Initial ribodepletion  29.2-52.6 (mean 42.7)      15.1-29.7 (mean 23.2)
##  4 Non-Control RNA       Secondary ribodepletion 35.3-63 (mean 50.8)       5.6-10.5 (mean 8.1)
##  5 Non-Control DNA       Trimming & filtering   4.8-7.2 (mean 5.7)         4.8-7.2 (mean 5.7)
##  6 Non-Control DNA       Deduplication          6.3-8.3 (mean 7)           1-2.4 (mean 1.3)
##  7 Non-Control DNA       Initial ribodepletion  6.5-8.5 (mean 7.2)         0.1-0.2 (mean 0.2)
##  8 Non-Control DNA       Secondary ribodepletion 6.6-8.6 (mean 7.3)        0.1-0.1 (mean 0.1)
##  9 NC          RNA       Trimming & filtering   37.8-37.8 (mean 37.8)      37.8-37.8 (mean 37.8)
## 10 NC          RNA       Deduplication          42.8-42.8 (mean 42.8)      5-5 (mean 5)
## # i 14 more rows
## # i abbreviated names: 1: `% Total Reads Lost (Cumulative)`, 2: `% Total Reads Lost (Marginal)`
```

```r
g_stage_trace <- ggplot(basic_stats, aes(x=stage, color=na_type, group=sample)) +
  scale_color_na() +
  facet_wrap(ctrl~na_type, scales="free", ncol=2,
             labeller = label_wrap_gen(multi_line=FALSE)) +
  theme_kit

# Plot reads over preprocessing
g_reads_stages <- g_stage_trace +
  geom_line(aes(y=n_read_pairs)) +
  scale_y_continuous("# Read pairs", expand=c(0,0), limits=c(0,NA))
g_reads_stages
```

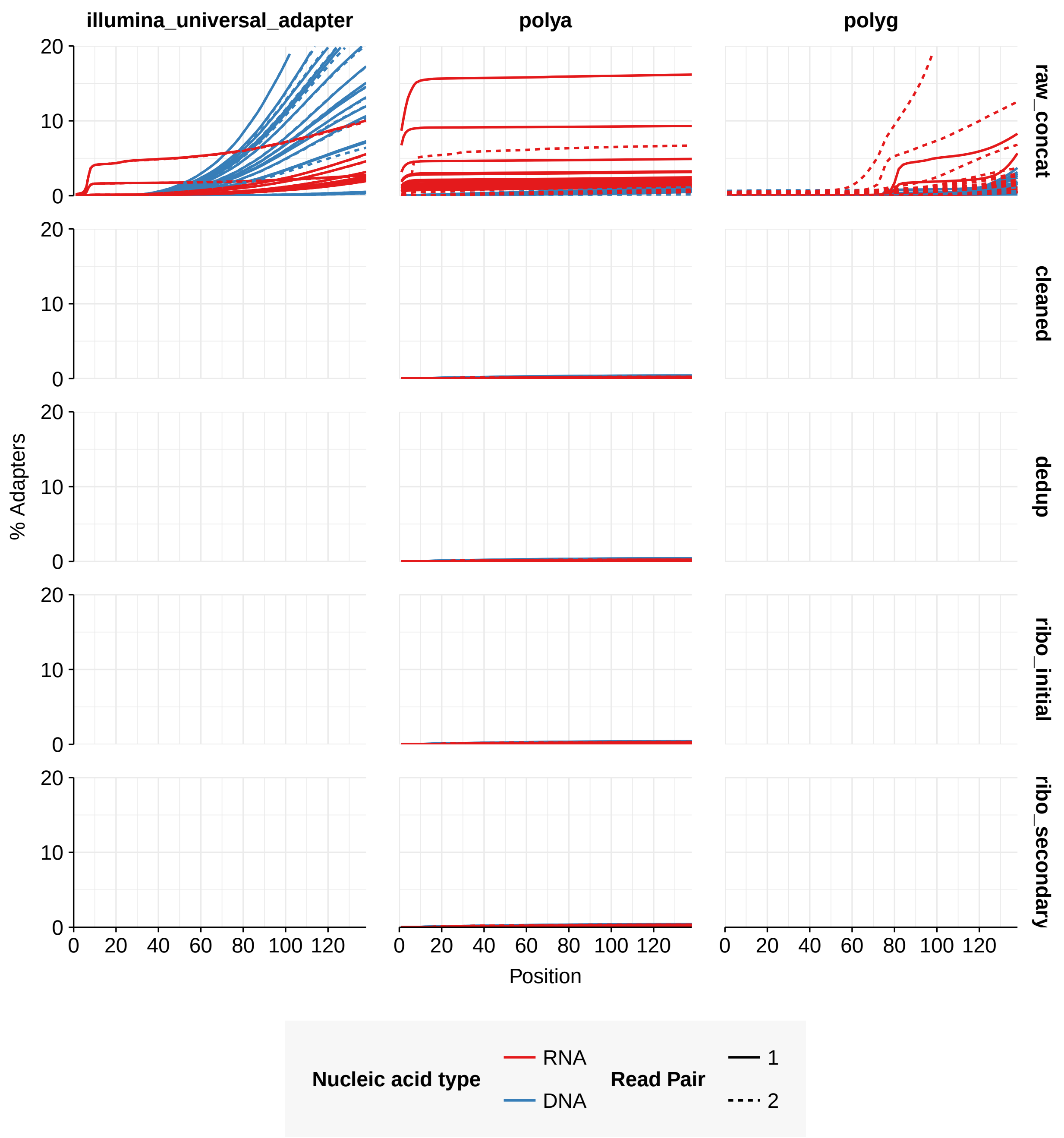


```r
# Plot relative read losses during preprocessing
g_reads_rel <- ggplot(n_reads_rel, aes(x=stage, color=na_type, group=sample)) +
  geom_line(aes(y=p_reads_lost_abs_marginal)) +
  scale_y_continuous("% Total Reads Lost", expand=c(0,0),
                     labels = function(x) x*100) +
  scale_color_na() +
  facet_wrap(ctrl~na_type, scales="free", ncol=2,
             labeller = label_wrap_gen(multi_line=FALSE)) +
  theme_kit
g_reads_rel
```

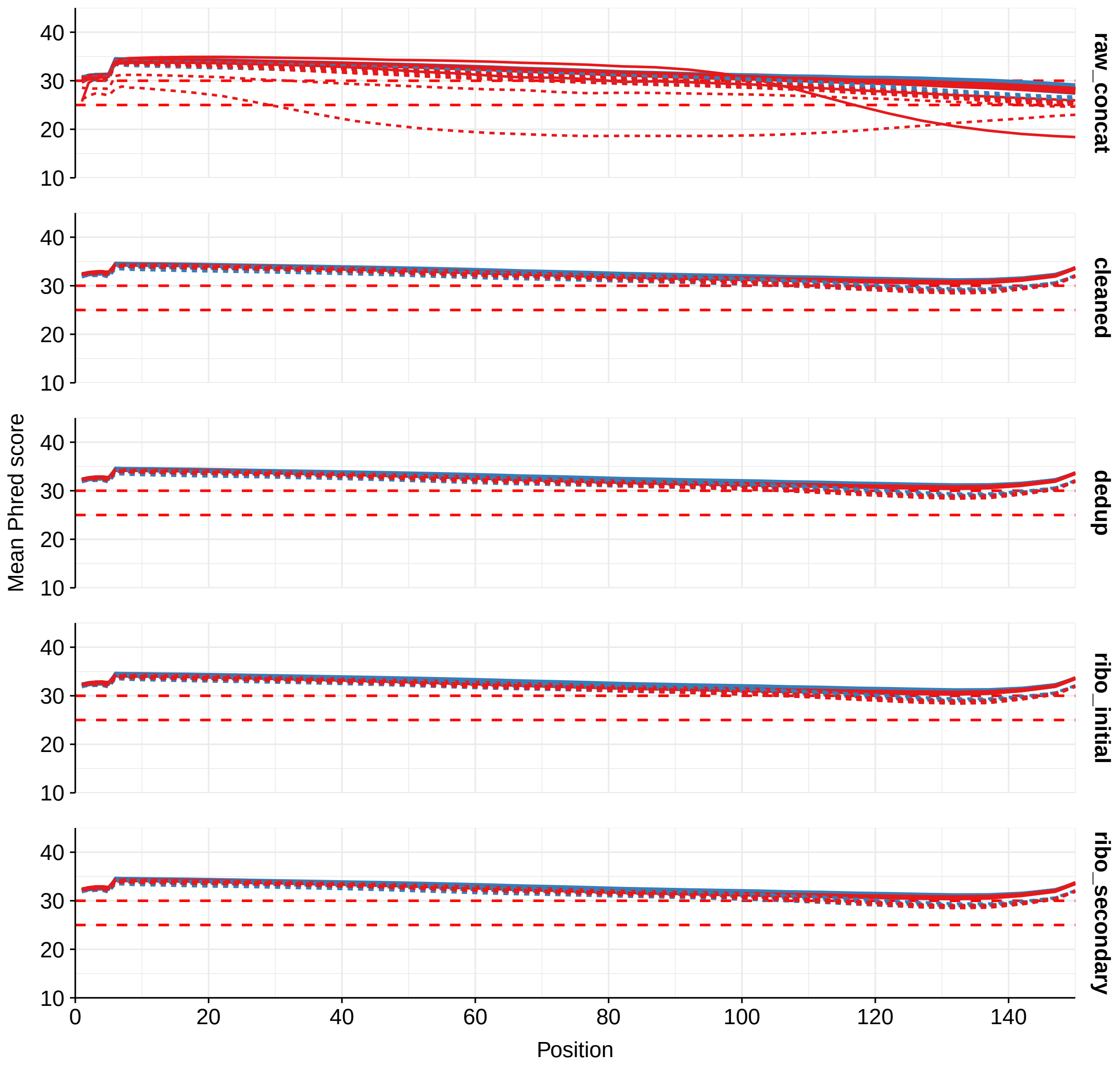


In both positive and control samples, data cleaning with FASTP was very successful at removing adapters, with very few adapter sequences found by FASTQC at any stage after the raw data. FASTP was also successful at improving read quality.

```r
g_qual <- ggplot(mapping=aes(color=na_type, linetype=read_pair,
                         group=interaction(sample,read_pair))) +
  scale_color_na() + scale_linetype_discrete(name = "Read Pair") +
  guides(color=guide_legend(nrow=2,byrow=TRUE),
         linetype = guide_legend(nrow=2,byrow=TRUE)) +
  theme_base

# Visualize adapters
g_adapters <- g_qual +
  geom_line(aes(x=position, y=pc_adapters), data=adapter_stats) +
  scale_y_continuous(name="% Adapters", limits=c(0,20),
                     breaks = seq(0,50,10), expand=c(0,0)) +
  scale_x_continuous(name="Position", limits=c(0,NA),
                     breaks=seq(0,140,20), expand=c(0,0)) +
  facet_grid(stage~adapter)
g_adapters
```

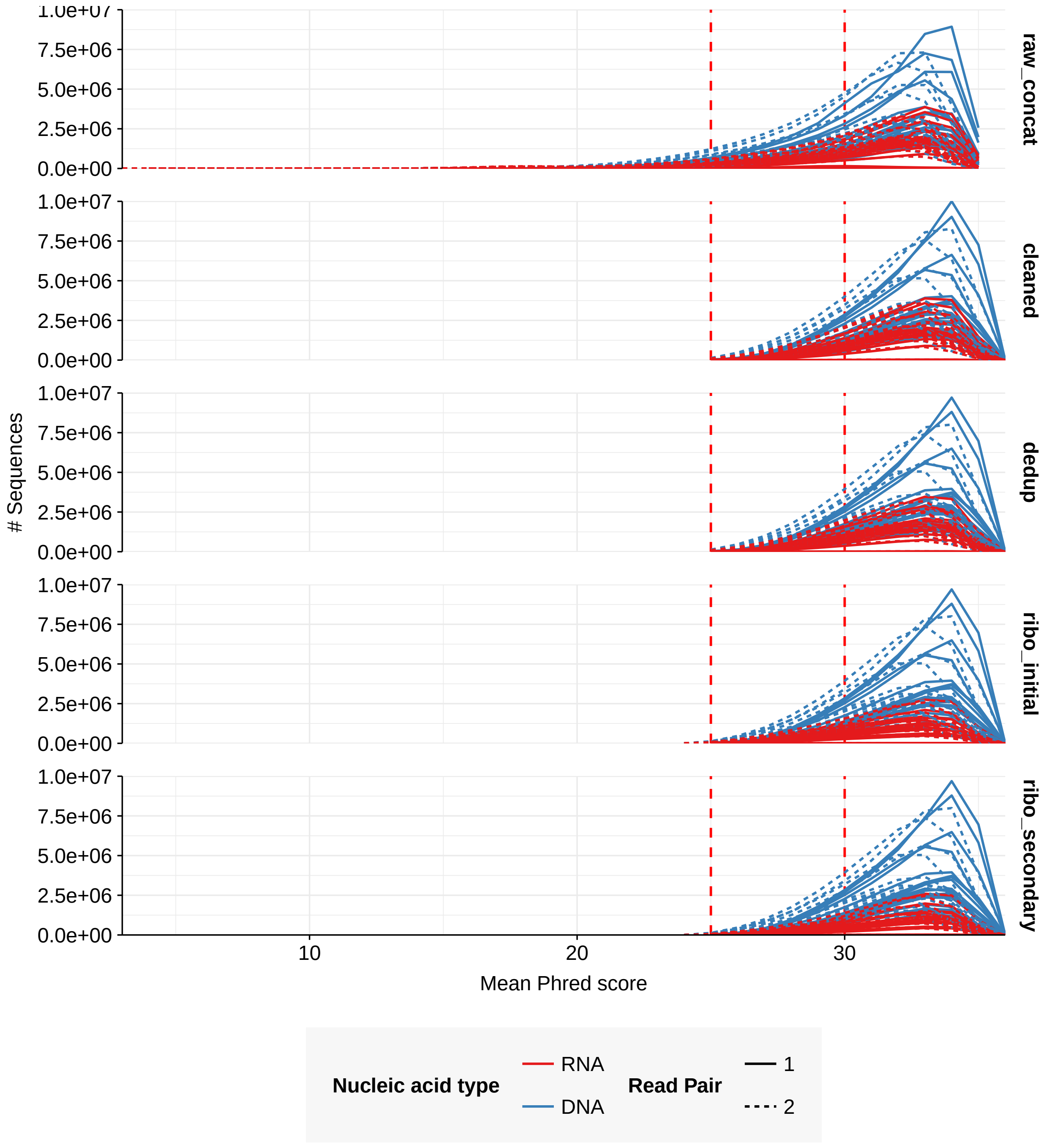


```r
# Visualize quality
g_quality_base <- g_qual +
  geom_hline(yintercept=25, linetype="dashed", color="red") +
  geom_hline(yintercept=30, linetype="dashed", color="red") +
  geom_line(aes(x=position, y=mean_phred_score), data=quality_base_stats) +
  scale_y_continuous(name="Mean Phred score", expand=c(0,0), limits=c(10,45)) +
  scale_x_continuous(name="Position", limits=c(0,NA),
                     breaks=seq(0,140,20), expand=c(0,0)) +
  facet_grid(stage~.)
g_quality_base
```



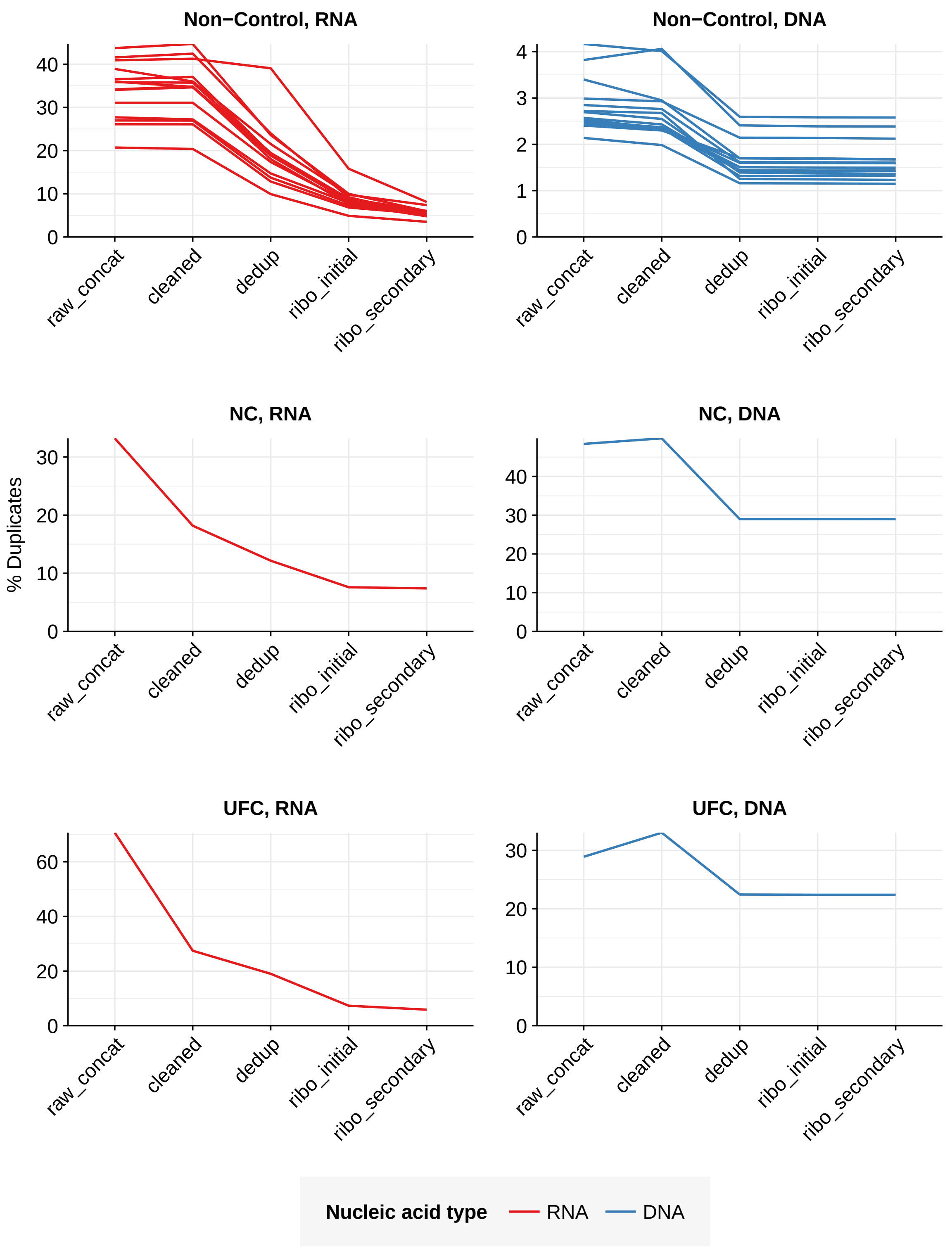
Nucleic acid type
RNA
DNA
Read Pair
1
2

```
g_quality_seq <- g_qual +
  geom_vline(xintercept=25, linetype="dashed", color="red") +
  geom_vline(xintercept=30, linetype="dashed", color="red") +
  geom_line(aes(x=mean_phred_score, y=n_sequences), data=quality_seq_stats) +
  scale_x_continuous(name="Mean Phred score", expand=c(0,0)) +
  scale_y_continuous(name="# Sequences", expand=c(0,0)) +
  facet_grid(stage~.)
g_quality_seq
```

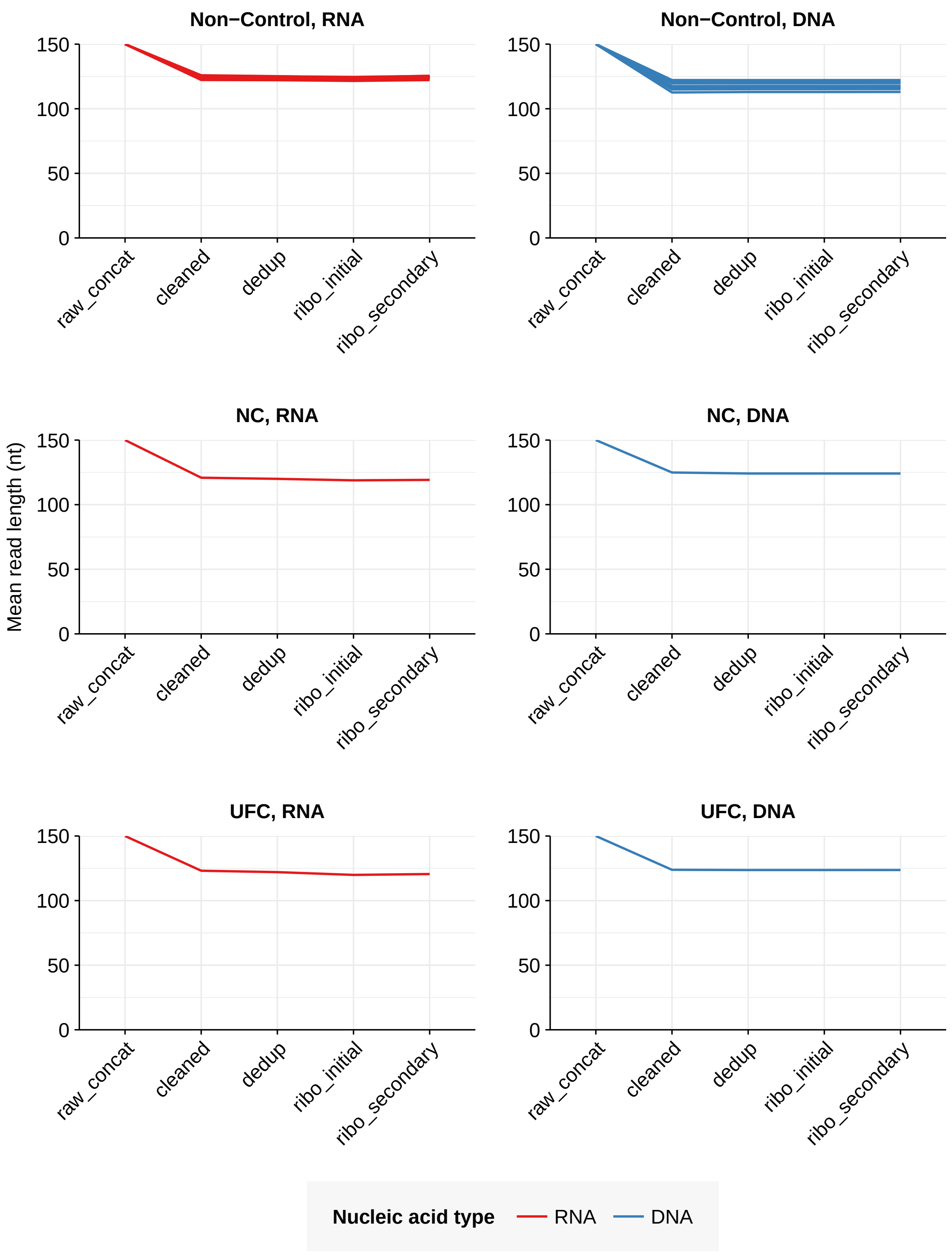


According to FASTQC, deduplication was moderately effective at reducing measured duplicate levels in on-site samples, with FASTQC-measured levels falling from an average of 34% to 23% for RNA reads and from 2.7% to 2.2% for DNA reads.

```
stage_dup <- basic_stats %>% group_by(na_type, ctrl, stage) %>%
  summarize(dmin = min(percent_duplicates), dmax=max(percent_duplicates),
            dmean=mean(percent_duplicates), .groups = "drop")

g_dup_stages <- g_stage_trace +
  geom_line(aes(y=percent_duplicates)) +
  scale_y_continuous("% Duplicates", limits=c(0,NA), expand=c(0,0))
g_dup_stages
```



```
g_readlen_stages <- g_stage_trace + geom_line(aes(y=mean_seq_len)) +
  scale_y_continuous("Mean read length (nt)", expand=c(0,0), limits=c(0,NA))
g_readlen_stages
```

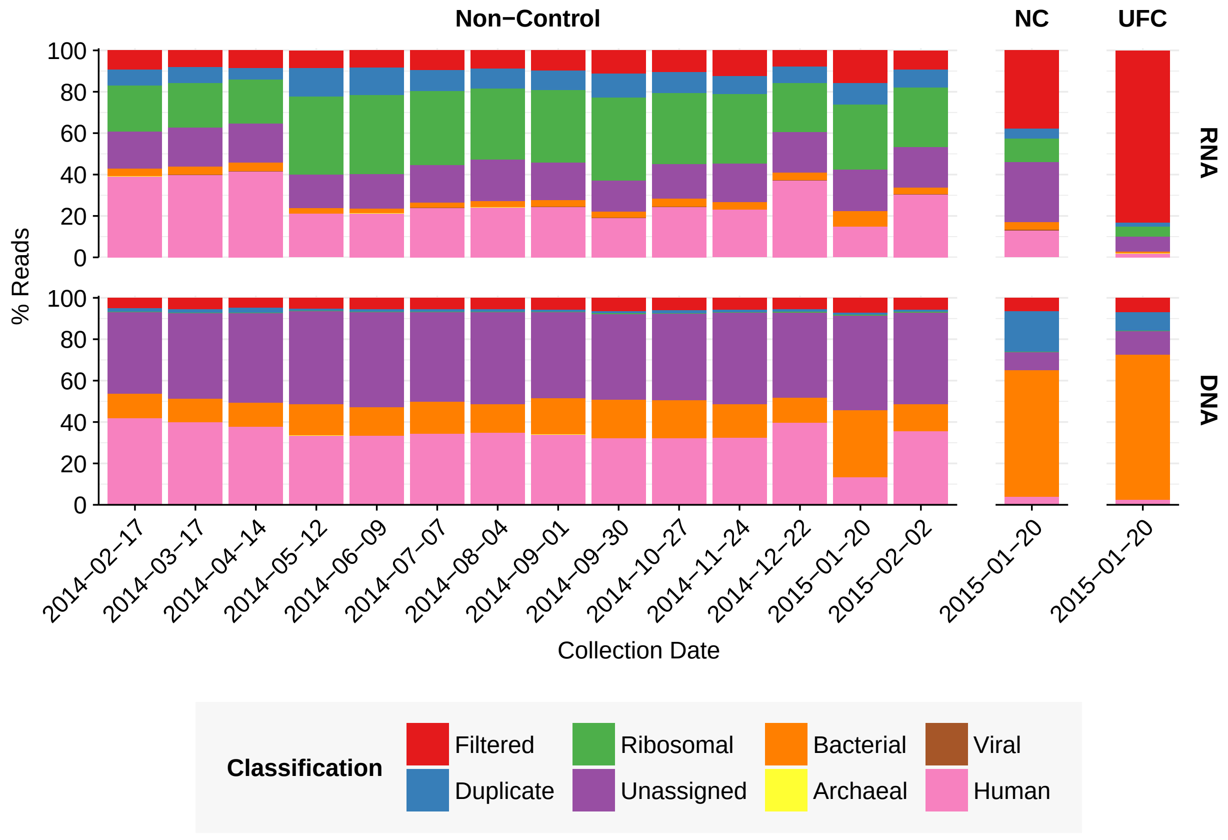


High-level composition

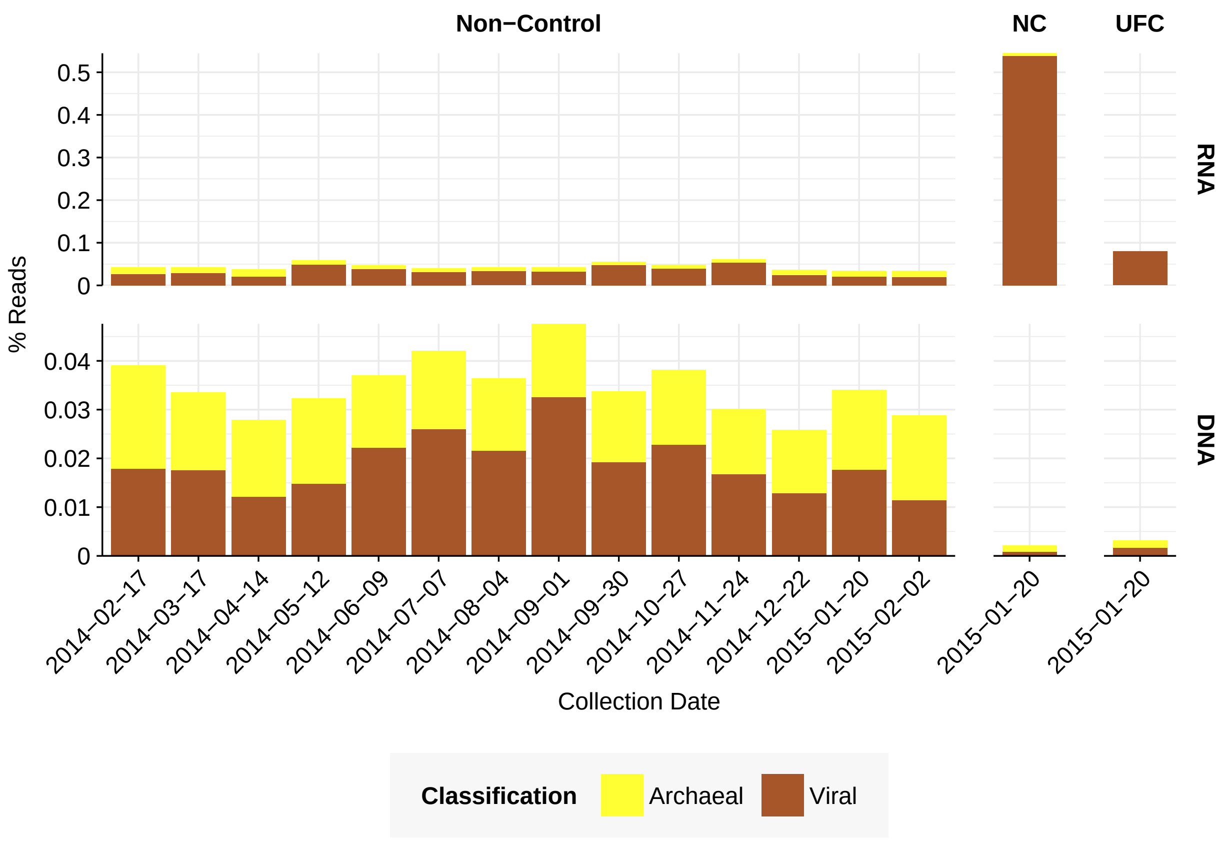
As before, to assess the high-level composition of the reads, I ran the ribodepleted files through Kraken (using the Standard 16 database) and summarized the results with Bracken. Combining these results with the read counts above gives us a breakdown of the inferred composition of the samples:

```r
# Import Bracken data
bracken_path <- file.path(data_dir, "bracken_counts.tsv")
bracken <- read_tsv(bracken_path, show_col_types = FALSE)
total_assigned <- bracken %>% group_by(sample) %>% summarize(
  name = "Total",
  kraken_assigned_reads = sum(kraken_assigned_reads),
  added_reads = sum(added_reads),
  new_est_reads = sum(new_est_reads),
  fraction_total_reads = sum(fraction_total_reads)
)
bracken_spread <- bracken %>% select(name, sample, new_est_reads) %>%
  mutate(name = tolower(name)) %>%
  pivot_wider(id_cols = "sample", names_from = "name",
              values_from = "new_est_reads")

# Count reads
read_counts_preproc <- basic_stats %>%
  select(sample, na_type, ctrl, date, stage, n_read_pairs) %>%
  pivot_wider(id_cols = c("sample", "na_type", "ctrl", "date"),
              names_from="stage", values_from="n_read_pairs")
read_counts <- read_counts_preproc %>%
  inner_join(total_assigned %>% select(sample, new_est_reads), by = "sample") %>%
  rename(assigned = new_est_reads) %>%
  inner_join(bracken_spread, by="sample")

# Assess composition
read_comp <- transmute(read_counts, sample=sample, na_type=na_type,
                       ctrl = ctrl, date = date,
                       n_filtered = raw_concat-cleaned,
                       n_duplicate = cleaned-dedup,
                       n_ribosomal = (dedup-ribo_initial) + (ribo_initial-ribo_secondary),
                       n_unassigned = ribo_secondary-assigned,
                       n_bacterial = bacteria,
                       n_archaeal = archaea,
                       n_viral = viruses,
                       n_human = eukaryota)
read_comp_long <- pivot_longer(read_comp, -(sample:date),
                               names_to = "classification",
                               names_prefix = "n_", values_to = "n_reads") %>%
  mutate(classification = fct_inorder(str_to_sentence(classification))) %>%
  group_by(sample) %>% mutate(p_reads = n_reads/sum(n_reads))

# Summarize composition
read_comp_summ <- read_comp_long %>%
  group_by(na_type, ctrl, classification) %>%
  summarize(n_reads = sum(n_reads), .groups = "drop_last") %>%
  mutate(n_reads = replace_na(n_reads,0),
    p_reads = n_reads/sum(n_reads),
    pc_reads = p_reads*100)
```

```r
# Prepare plotting templates
g_comp_base <- ggplot(mapping=aes(x=date, y=p_reads, fill=classification)) +
  scale_x_discrete(name="Collection Date") +
  facet_grid(na_type~ctrl, scales = "free", space = "free_x") +
  theme_rotate
scale_y_pc_reads <- purrr::partial(scale_y_continuous, name = "% Reads",
                                   expand = c(0,0), labels = function(y) y*100)

# Plot overall composition
g_comp <- g_comp_base + geom_col(data = read_comp_long, position = "stack") +
  scale_y_pc_reads(limits = c(0,1.01), breaks = seq(0,1,0.2)) +
  scale_fill_brewer(palette = "Set1", name = "Classification")
g_comp
```



```
# Plot composition of minor components
read_comp_minor <- read_comp_long %>%
  filter(classification %in% c("Archaeal", "Viral", "Other"))
palette_minor <- brewer.pal(9, "Set1")[c(6,7,9)]
g_comp_minor <- g_comp_base + geom_col(data=read_comp_minor, position = "stack") +
  scale_y_pc_reads() +
  scale_fill_manual(values=palette_minor, name = "Classification")
g_comp_minor
```



```r
p_reads_summ_group <- read_comp_long %>%
  mutate(classification = ifelse(classification %in% c("Filtered", "Duplicate", "Unassigned"), "Exclude
         classification = fct_inorder(classification)) %>%
  group_by(classification, sample, na_type, ctrl) %>%
  summarize(p_reads = sum(p_reads), .groups = "drop") %>%
  group_by(classification, na_type, ctrl) %>%
  summarize(pc_min = min(p_reads)*100, pc_max = max(p_reads)*100,
            pc_mean = mean(p_reads)*100, .groups = "drop")
p_reads_summ_prep <- p_reads_summ_group %>%
  mutate(classification = fct_inorder(classification),
         pc_min = pc_min %>% signif(digits=2) %>% sapply(format, scientific=FALSE, trim=TRUE, digits=2)
         pc_max = pc_max %>% signif(digits=2) %>% sapply(format, scientific=FALSE, trim=TRUE, digits=2)
         pc_mean = pc_mean %>% signif(digits=2) %>% sapply(format, scientific=FALSE, trim=TRUE, digits=
         display = paste0(pc_min, "-", pc_max, "% (mean ", pc_mean, "%)"))
p_reads_summ <- p_reads_summ_prep %>%
  select(ctrl, classification, na_type, display) %>%
  pivot_wider(names_from=na_type, values_from = display) %>%
  arrange(ctrl, classification)
p_reads_summ
```

```
## # A tibble: 18 x 4
##    ctrl        classification RNA                              DNA
##    <fct>       <fct>          <chr>                            <chr>
##  1 Non-Control Excluded       33-46% (mean 38%)                46-54% (mean 50%)
##  2 Non-Control Ribosomal      21-40% (mean 31%)                0.24-0.34% (mean 0.29%)
##  3 Non-Control Bacterial      2.3-7.6% (mean 3.6%)             11-33% (mean 16%)
##  4 Non-Control Archaeal       0.0077-0.017% (mean 0.012%)      0.013-0.021% (mean 0.016%)
##  5 Non-Control Viral          0.02-0.053% (mean 0.033%)        0.011-0.033% (mean 0.019%)
##  6 Non-Control Human          15-42% (mean 27%)                 13-42% (mean 34%)
##  7 NC          Excluded       72-72% (mean 72%)                 35-35% (mean 35%)
##  8 NC          Ribosomal      11-11% (mean 11%)                 0.21-0.21% (mean 0.21%)
##  9 NC          Bacterial      3.5-3.5% (mean 3.5%)              61-61% (mean 61%)
## 10 NC          Archaeal       0.0061-0.0061% (mean 0.0061%)     0.0013-0.0013% (mean 0.0013%)
## 11 NC          Viral          0.54-0.54% (mean 0.54%)           0.0008-0.0008% (mean 0.0008%)
## 12 NC          Human          13-13% (mean 13%)                 3.8-3.8% (mean 3.8%)
## 13 UFC         Excluded       93-93% (mean 93%)                 27-27% (mean 27%)
## 14 UFC         Ribosomal      4.7-4.7% (mean 4.7%)              0.25-0.25% (mean 0.25%)
## 15 UFC         Bacterial      0.76-0.76% (mean 0.76%)           70-70% (mean 70%)
## 16 UFC         Archaeal       0.00059-0.00059% (mean 0.00059%) 0.0016-0.0016% (mean 0.0016%)
## 17 UFC         Viral          0.08-0.08% (mean 0.08%)           0.0016-0.0016% (mean 0.0016%)
## 18 UFC         Human          1.7-1.7% (mean 1.7%)              2.3-2.3% (mean 2.3%)
```

What we see is a very different picture from the wastewater samples I've been analyzing so far. Most notably, the fraction of (non-ribosomal) human reads is *much* higher. Even the 2015-01-20 samples, which were taken when the daycare center was closed for the winter holidays, showed human read fractions (for both nucleic-acid types) of >12%; non-control samples as a whole averaged 27% for RNA reads and 34% for DNA reads. Compare Brumfield (average 0.08% for RNA and 0.02% for DNA), Yang (mean 0.05% for RNA) or even Rothman (mean 1.8% for non-panel-enriched RNA samples).

Conversely, total viral reads are very low: mean 0.033% for RNA reads and 0.019% for DNA reads. Wastewater RNA datasets have typically had much higher total viruses: mean 0.5% for Brumfield, about the same for Crits-Christoph, 5.5% for Yang, 4.5% for Rothman. Brumfield's DNA data contained substantially fewer viruses than their RNA data, but still more than Prussin: about 0.08% on average.

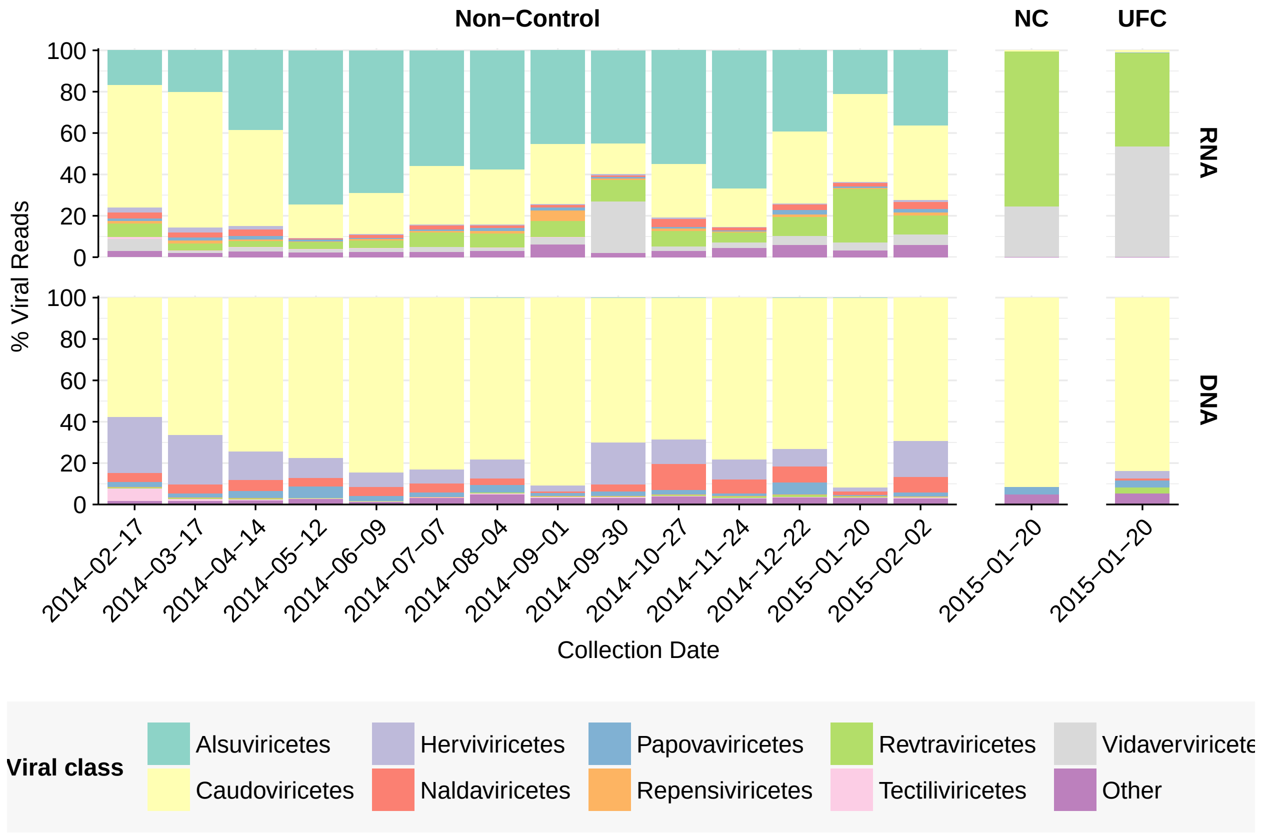
Looking at viral families…was less informative than usual, especially for DNA reads. It turns out that these samples contain a lot of viral reads that Kraken2 was only able to classify to the class level. In DNA reads, samples were dominated by *Caudoviricetes* phages, though *Herviviricetes* (which includes herpesviruses) and *Naldaviricetes* (a class of arthropod-infecting viruses) also put in a respectable showing. In RNA reads, *Caudoviricetes* was again a major presence, but *Alsuviricetes* (a family of primarily plant pathogens) was often as or more prevalent, and *Revtraviricetes* (a class that includes Hepatitis B virus and retroviruses) was also significant.

```r
# Get viral taxonomy
viral_taxa_path <- file.path(data_dir, "viral-taxids.tsv.gz")
viral_taxa <- read_tsv(viral_taxa_path, show_col_types = FALSE)

# Import Kraken reports & extract viral taxa
samples <- as.character(basic_stats_raw$sample)
#col_names <- c("pc_reads_total", "n_reads_clade", "n_reads_direct",
#                "rank", "taxid", "name")
#report_paths <- paste0(data_dir, "kraken/", samples, ".report.gz")
#kraken_reports <- lapply(1:length(samples), function(n)
#  read_tsv(report_paths[n], col_names = col_names, show_col_types = FALSE) %>%
#    mutate(sample = samples[n])) %>% bind_rows

kraken_reports <- read_tsv(sprintf('%s/kraken_reports.tsv', data_dir))
```

```
## Rows: 377006 Columns: 9
## -- Column specification --------------------------------------------------------------------------------
## Delimiter: "\t"
## chr (3): rank, name, sample
## dbl (6): pc_reads_total, n_reads_clade, n_reads_direct, n_minimizers_total, n_minimizers_disti...
##
## 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.
```

```r
kraken_reports_viral <- filter(kraken_reports, taxid %in% viral_taxa$taxid) %>%
  group_by(sample) %>%
  mutate(p_reads_viral = n_reads_clade/n_reads_clade[1])
kraken_reports_viral_cleaned <- kraken_reports_viral %>%
  inner_join(libraries, by="sample") %>%
  select(-pc_reads_total, -n_reads_direct) %>%
  select(name, taxid, p_reads_viral, n_reads_clade, everything())

viral_classes <- kraken_reports_viral_cleaned %>% filter(rank == "C")
viral_families <- kraken_reports_viral_cleaned %>% filter(rank == "F")
```

```r
# Identify major viral classes
viral_classes_major_tab <- viral_classes %>%
  group_by(name, taxid) %>%
  summarize(p_reads_viral_max = max(p_reads_viral), .groups="drop") %>%
  filter(p_reads_viral_max >= 0.04)
viral_classes_major_list <- viral_classes_major_tab %>% pull(name)
viral_classes_major <- viral_classes %>%
  filter(name %in% viral_classes_major_list) %>%
  select(name, taxid, sample, na_type, ctrl, date, p_reads_viral)
viral_classes_minor <- viral_classes_major %>%
  group_by(sample, na_type, ctrl, date) %>%
  summarize(p_reads_viral_major = sum(p_reads_viral), .groups = "drop") %>%
  mutate(name = "Other", taxid=NA, p_reads_viral = 1-p_reads_viral_major) %>%
  select(name, taxid, sample, na_type, ctrl, date, p_reads_viral)
viral_classes_display <- bind_rows(viral_classes_major, viral_classes_minor) %>%
  arrange(desc(p_reads_viral)) %>%
  mutate(name = factor(name, levels=c(viral_classes_major_list, "Other"))) %>%
  rename(p_reads = p_reads_viral, classification=name)

palette_viral <- c(brewer.pal(12, "Set3"), brewer.pal(8, "Set2"))
g_classes <- g_comp_base +
  geom_col(data=viral_classes_display, position = "stack") +
  scale_y_continuous(name="% Viral Reads", limits=c(0,1.01), breaks = seq(0,1,0.2),
                     expand=c(0,0), labels = function(y) y*100) +
  scale_fill_manual(values=palette_viral, name = "Viral class")
g_classes
```

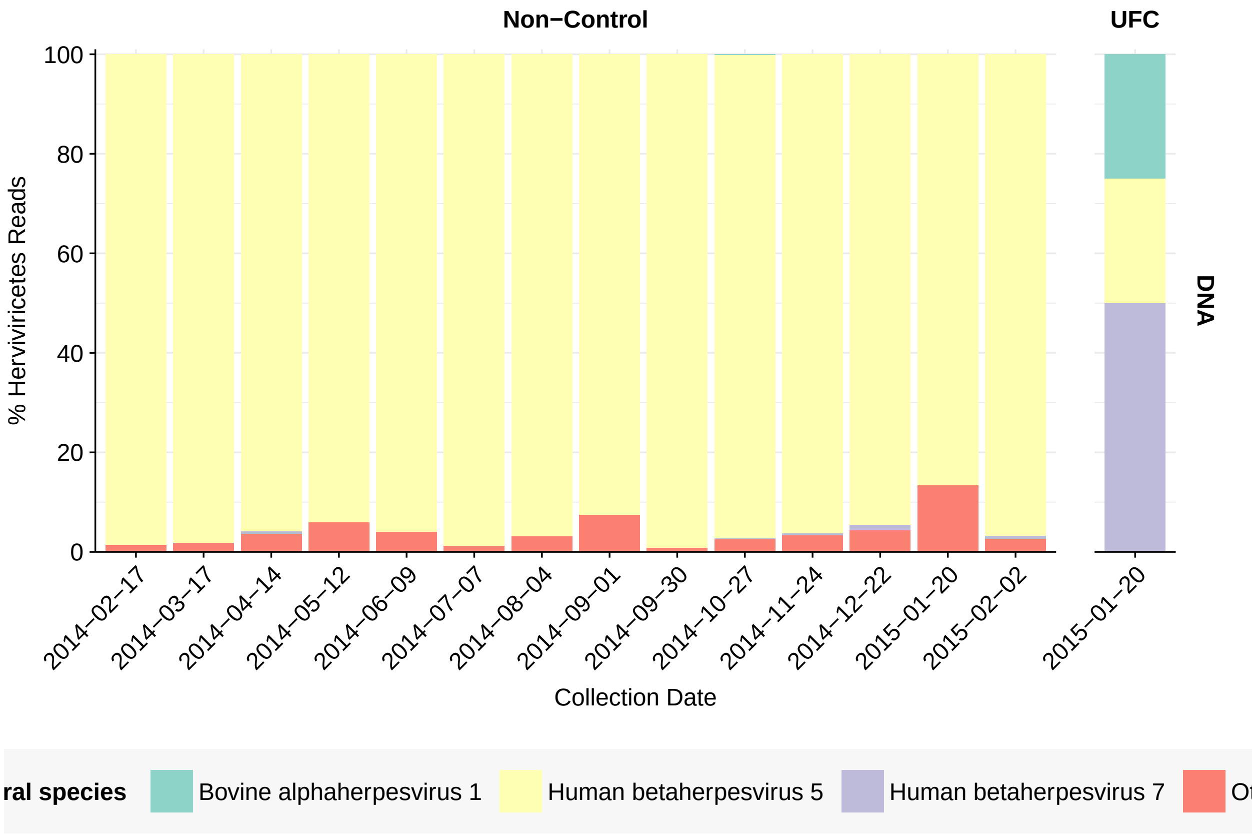


Of these, the most interesting are the strong presence of *Herviviricetes* in the DNA reads and *Revtraviricetes* in the RNA reads, as both of these are families that contain important human pathogens.

Digging into the former, it turns out these reads are composed almost exclusively of *Herpesviridae* at the family level. Within non-control samples, these arise overwhelmingly from *Cytomegalovirus*. Digging in at the species level, these in turn are primarily attributed to a single CMV, Human betaherpesvirus 5, a.k.a. human cytomegalovirus (HCMV). I was excited to see this: this is the first time a single human pathogen, or even all human pathogens combined, have constituted a significant fraction of all viral reads in a sample I've analyzed with this pipeline.

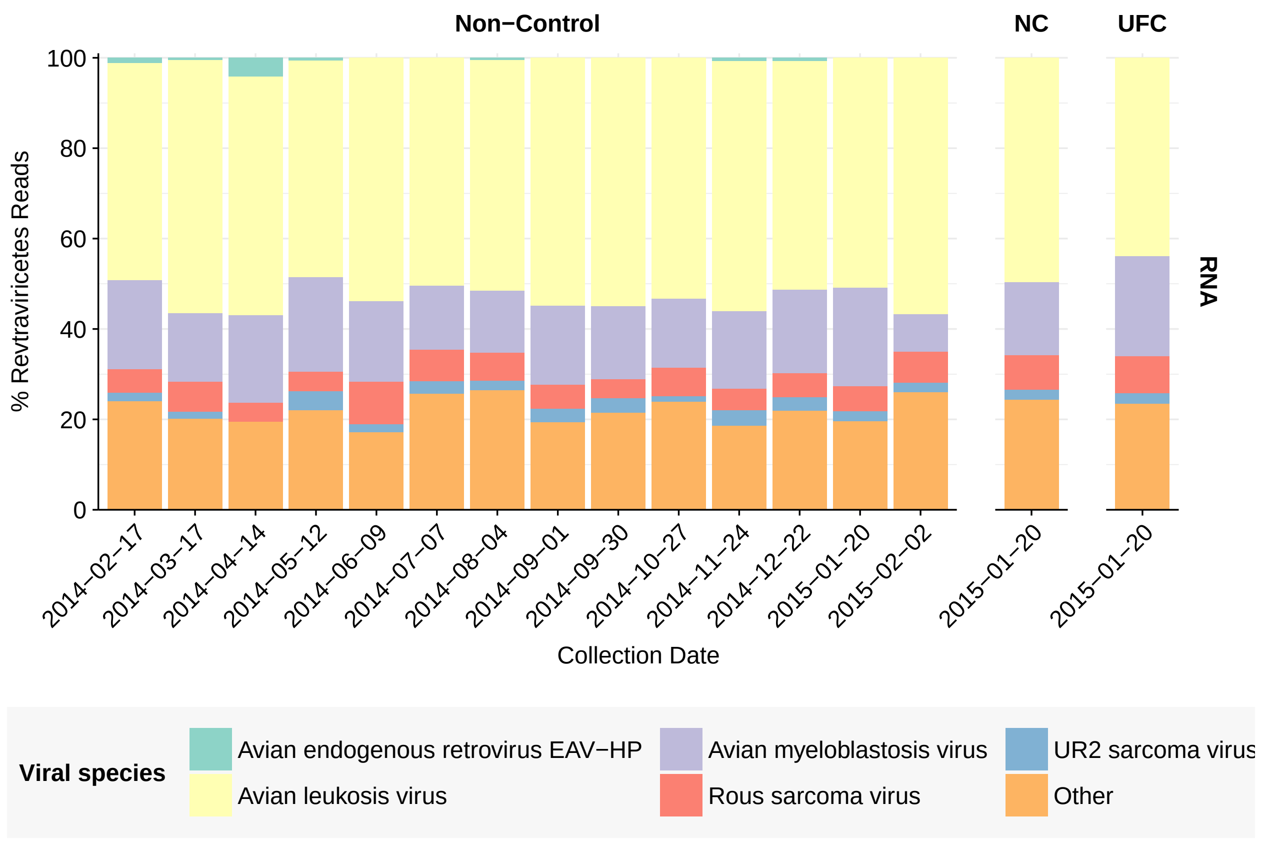
```
# Get all read counts in class
hervi_taxid <- 2731363
hervi_desc_taxids_old <- hervi_taxid
hervi_desc_taxids_new <- unique(c(hervi_desc_taxids_old, viral_taxa %>% filter(parent_taxid %in% hervi_d
while (length(hervi_desc_taxids_new) > length(hervi_desc_taxids_old)){
  hervi_desc_taxids_old <- hervi_desc_taxids_new
  hervi_desc_taxids_new <- unique(c(hervi_desc_taxids_old, viral_taxa %>% filter(parent_taxid %in% hervi
}
hervi_counts <- kraken_reports_viral_cleaned %>%
  filter(taxid %in% hervi_desc_taxids_new) %>%
  mutate(p_reads_hervi = n_reads_clade/n_reads_clade[1])

# Get genus composition
hervi_genera <- hervi_counts %>% filter(rank == "S", na_type == "DNA")
hervi_genera_major_tab <- hervi_genera %>%
  group_by(name, taxid) %>%
  summarize(p_reads_hervi_max = max(p_reads_hervi), .groups="drop") %>%
  filter(p_reads_hervi_max >= 0.04)
```

```r
hervi_genera_major_list <- hervi_genera_major_tab %>% pull(name)
hervi_genera_major <- hervi_genera %>%
  filter(name %in% hervi_genera_major_list) %>%
  select(name, taxid, sample, na_type, ctrl, date, p_reads_hervi)
hervi_genera_minor <- hervi_genera_major %>%
  group_by(sample, na_type, ctrl, date) %>%
  summarize(p_reads_hervi_major = sum(p_reads_hervi), .groups = "drop") %>%
  mutate(name = "Other", taxid=NA, p_reads_hervi = 1-p_reads_hervi_major) %>%
  select(name, taxid, sample, na_type, ctrl, date, p_reads_hervi)
hervi_genera_display <- bind_rows(hervi_genera_major, hervi_genera_minor) %>%
  arrange(desc(p_reads_hervi)) %>%
  mutate(name = factor(name, levels=c(hervi_genera_major_list, "Other"))) %>%
  rename(p_reads = p_reads_hervi, classification=name)

# Plot
g_hervi_genera <- g_comp_base +
  geom_col(data=hervi_genera_display, position = "stack") +
  scale_y_continuous(name="% Herviviricetes Reads", limits=c(0,1.01),
                     breaks = seq(0,1,0.2),
                     expand=c(0,0), labels = function(y) y*100) +
  scale_fill_manual(values=palette_viral, name = "Viral species")
g_hervi_genera
```



Revtraviricetes reads are similarly dominated by a single viral genus, *Alpharetrovirus*. Digging in at the species level, we see contributions from a variety of avian oncoviruses. To my (admittedly non-expert) knowledge, none of these infect humans, and I think they are probably primarily arising from local birds (or possibly rats).

```
# Get all read counts in class
revtra_taxid <- 2732514
revtra_desc_taxids_old <- revtra_taxid
revtra_desc_taxids_new <- unique(c(revtra_desc_taxids_old, viral_taxa %>% filter(parent_taxid %in% revt
while (length(revtra_desc_taxids_new) > length(revtra_desc_taxids_old)){
  revtra_desc_taxids_old <- revtra_desc_taxids_new
  revtra_desc_taxids_new <- unique(c(revtra_desc_taxids_old, viral_taxa %>% filter(parent_taxid %in% re
}
revtra_counts <- kraken_reports_viral_cleaned %>%
  filter(taxid %in% revtra_desc_taxids_new) %>%
  mutate(p_reads_revtra = n_reads_clade/n_reads_clade[1])

# Get genus composition
revtra_species <- revtra_counts %>% filter(rank == "S", na_type == "RNA")
revtra_species_major_tab <- revtra_species %>%
  group_by(name, taxid) %>%
  summarize(p_reads_revtra_max = max(p_reads_revtra), .groups="drop") %>%
  filter(p_reads_revtra_max >= 0.04)
revtra_species_major_list <- revtra_species_major_tab %>% pull(name)
revtra_species_major <- revtra_species %>%
  filter(name %in% revtra_species_major_list) %>%
  select(name, taxid, sample, na_type, ctrl, date, p_reads_revtra)
revtra_species_minor <- revtra_species_major %>%
  group_by(sample, na_type, ctrl, date) %>%
  summarize(p_reads_revtra_major = sum(p_reads_revtra), .groups = "drop") %>%
  mutate(name = "Other", taxid=NA, p_reads_revtra = 1-p_reads_revtra_major) %>%
  select(name, taxid, sample, na_type, ctrl, date, p_reads_revtra)
revtra_species_display <- bind_rows(revtra_species_major, revtra_species_minor) %>%
  arrange(desc(p_reads_revtra)) %>%
  mutate(name = factor(name, levels=c(revtra_species_major_list, "Other"))) %>%
  rename(p_reads = p_reads_revtra, classification=name)

# Plot
g_revtra_species <- g_comp_base +
  geom_col(data=revtra_species_display, position = "stack") +
  scale_y_continuous(name="% Revtraviricetes Reads", limits=c(0,1.01),
                     breaks = seq(0,1,0.2),
                     expand=c(0,0), labels = function(y) y*100) +
  scale_fill_manual(values=palette_viral, name = "Viral species")
g_revtra_species
```



Human-infecting virus reads: validation

Next, I investigated the human-infecting virus read content of these unenriched samples. Using the same workflow I used for Brumfield et al, I identified 2811 RNA read pairs and 12792 DNA read pairs as putatively human viral: 0.003% and 0.005% of surviving reads, respectively.

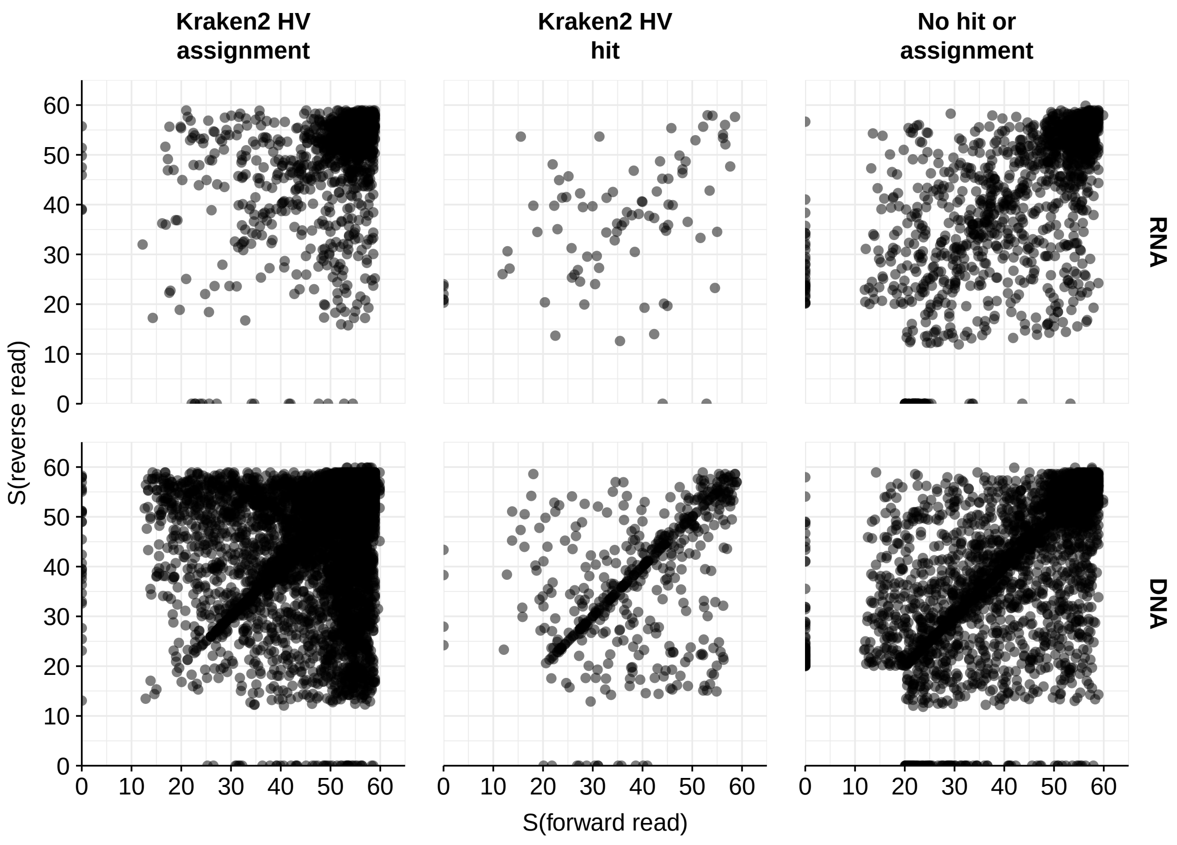
```
#hv_reads_filtered_path <- file.path(data_dir, "hv_hits_putative_filtered.tsv.gz")
#hv_reads_filtered <- read_tsv(hv_reads_filtered_path, show_col_types = FALSE) %>%
#  inner_join(libraries, by="sample") %>%
#  arrange(date, na_type)
hv_reads_filtered <- read_tsv(sprintf('%s/hv_hits_putative_collapsed.tsv', data_dir))
```

```
## Rows: 14530 Columns: 21
## -- Column specification --------------------------------------------------------------------------
## Delimiter: "\t"
## chr  (8): seq_id, sample, genome_id, taxid, query_seq_fwd, query_seq_rev, assigned_name, encod...
## dbl (13): taxid_best, best_alignment_score_fwd, best_alignment_score_rev, query_len_fwd, query...
##
## 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.
```

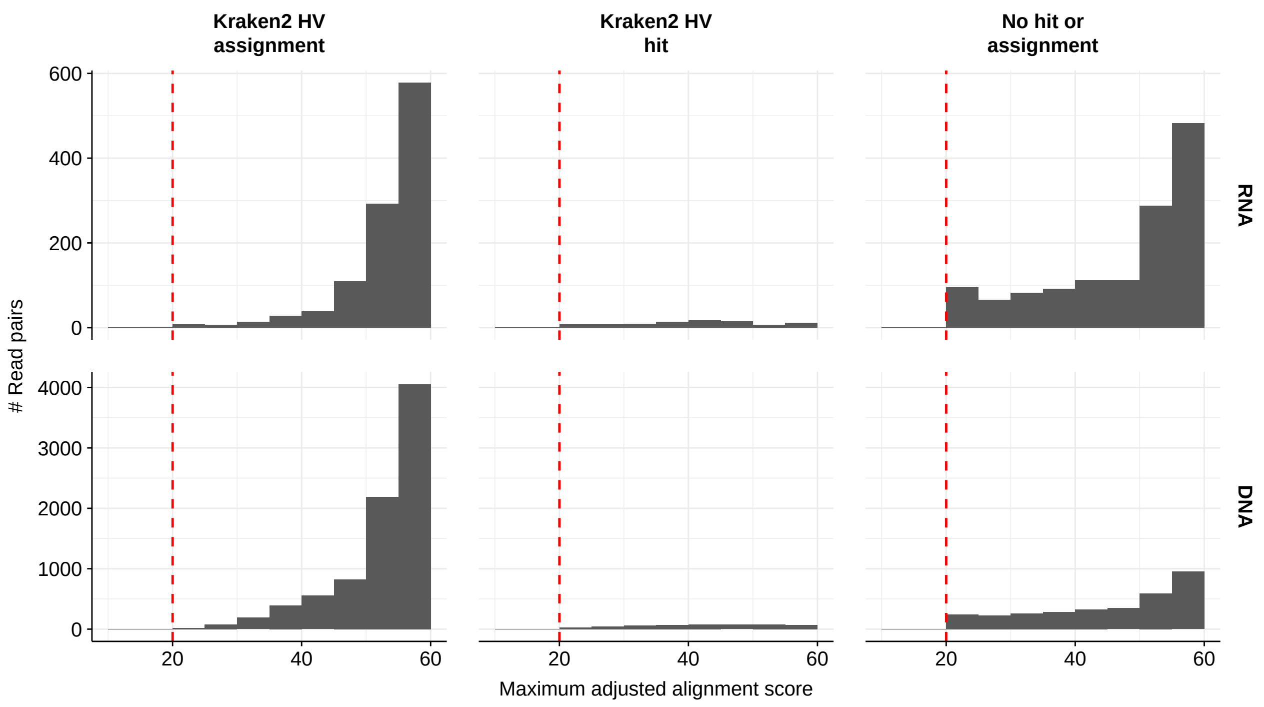
```
hv_reads_filtered <- left_join(hv_reads_filtered, libraries)
```

```
## Joining with `by = join_by(sample)`
```

```
n_hv_filtered <- hv_reads_filtered %>% group_by(sample, date, na_type, ctrl) %>% count %>% inner_join(ba
n_hv_filtered_summ <- n_hv_filtered %>% group_by(na_type, ctrl) %>% summarize(n_putative = sum(n_putati
mrg <- hv_reads_filtered %>%
  mutate(kraken_label = ifelse(assigned_hv, "Kraken2 HV\nassignment",
                               ifelse(hit_hv, "Kraken2 HV\nhit",
                                      "No hit or\nassignment"))) %>%
  group_by(sample, na_type) %>% arrange(desc(adj_score_fwd), desc(adj_score_rev)) %>%
  mutate(seq_num = row_number(),
         adj_score_max = pmax(adj_score_fwd, adj_score_rev))
# Import Bowtie2/Kraken data and perform filtering steps
g_mrg <- ggplot(mrg, aes(x=adj_score_fwd, y=adj_score_rev)) +
  geom_point(alpha=0.5, shape=16) +
  scale_x_continuous(name="S(forward read)", limits=c(0,65), breaks=seq(0,100,10), expand = c(0,0)) +
  scale_y_continuous(name="S(reverse read)", limits=c(0,65), breaks=seq(0,100,10), expand = c(0,0)) +
  facet_grid(na_type~kraken_label, labeller = labeller(kit = label_wrap_gen(20))) +
  theme_base + theme(aspect.ratio=1)
g_mrg
```



```
g_hist_0 <- ggplot(mrg, aes(x=adj_score_max)) + geom_histogram(binwidth=5,boundary=0,position="dodge")
g_hist_0
```



As previously described, I ran BLASTN on these reads via a dedicated EC2 instance, using the same parameters I've used for previous datasets.

Harmon note: I did not run BLAST

```
mrg_fasta <-  mrg %>%
  mutate(seq_head = paste0(">", seq_id)) %>%
  ungroup %>%
  select(header1=seq_head, seq1=query_seq_fwd,
         header2=seq_head, seq2=query_seq_rev) %>%
  mutate(header1=paste0(header1, "_1"), header2=paste0(header2, "_2"))
mrg_fasta_out <- do.call(paste, c(mrg_fasta, sep="\n")) %>% paste(collapse="\n")
write(mrg_fasta_out, file.path(data_dir, "blast/putative-viral.fasta"))
```

```
## Warning in file(file, ifelse(append, "a", "w")): cannot open file
## './/blast/putative-viral.fasta': No such file or directory

## Error in file(file, ifelse(append, "a", "w")): cannot open the connection
```

```
# Import BLAST results
# Pre-filtering BLAST results to save space
# blast_results_path <- file.path(data_dir, "blast/putative-viral.blast.gz")
# blast_cols <- c("qseqid", "sseqid", "sgi", "staxid", "qlen", "evalue", "bitscore", "qcovs", "length",
# blast_results <- read_tsv(blast_results_path, show_col_types = FALSE,
#                           col_names = blast_cols, col_types = cols(.default="c"))
blast_results_path <- file.path(data_dir, "blast/putative-viral-best.blast.gz")
blast_results <- read_tsv(blast_results_path, show_col_types = FALSE)
```

```
## Error: './/blast/putative-viral-best.blast.gz' does not exist in current working directory ('/Users/
```

```
# Filter for best hit for each query/subject combination
blast_results_best <- blast_results %>% group_by(qseqid, staxid) %>%
  filter(bitscore == max(bitscore)) %>%
  filter(length == max(length)) %>% filter(row_number() == 1)
```

```
## Error in group_by(., qseqid, staxid): object 'blast_results' not found
```

```
# Rank hits for each query and filter for high-ranking hits
blast_results_ranked <- blast_results_best %>%
  group_by(qseqid) %>% mutate(rank = dense_rank(desc(bitscore)))
```

```
## Error in group_by(., qseqid): object 'blast_results_best' not found
```

```
blast_results_highrank <- blast_results_ranked %>% filter(rank <= 5) %>%
    mutate(read_pair = str_split(qseqid, "_") %>% sapply(nth, n=-1),
         seq_id = str_split(qseqid, "_") %>% sapply(nth, n=1)) %>%
    mutate(bitscore = as.numeric(bitscore))
```

```
## Error in filter(., rank <= 5): object 'blast_results_ranked' not found
```

```
# Summarize by read pair and taxid
blast_results_paired <- blast_results_highrank %>%
  group_by(seq_id, staxid) %>%
  summarize(bitscore_max = max(bitscore), bitscore_min = min(bitscore),
            n_reads = n(), .groups = "drop")
```

```
## Error in group_by(., seq_id, staxid): object 'blast_results_highrank' not found
```

```
# Add viral status
blast_results_viral <- mutate(blast_results_paired, viral = staxid %in% viral_taxa$taxid) %>%
  mutate(viral_full = viral & n_reads == 2)
```

```
## Error in mutate(blast_results_paired, viral = staxid %in% viral_taxa$taxid): object 'blast_results_pa
```

```
# Compare to Kraken & Bowtie assignments
mrg_assign <- mrg %>% select(sample, seq_id, taxid, assigned_taxid, adj_score_max, na_type)
blast_results_assign <- left_join(blast_results_viral, mrg_assign, by="seq_id") %>%
    mutate(taxid_match_bowtie = (staxid == taxid),
           taxid_match_kraken = (staxid == assigned_taxid),
           taxid_match_any = taxid_match_bowtie | taxid_match_kraken)
```

```
## Error in left_join(blast_results_viral, mrg_assign, by = "seq_id"): object 'blast_results_viral' not
```

```
blast_results_out <- blast_results_assign %>%
  group_by(seq_id) %>%
  summarize(viral_status = ifelse(any(viral_full), 2,
                                  ifelse(any(taxid_match_any), 2,
                                         ifelse(any(viral), 1, 0))),
            .groups = "drop")
```

```
## Error in group_by(., seq_id): object 'blast_results_assign' not found
```

```
# Merge BLAST results with unenriched read data
mrg_blast <- full_join(mrg, blast_results_out, by="seq_id") %>%
  mutate(viral_status = replace_na(viral_status, 0),
         viral_status_out = ifelse(viral_status == 0, FALSE, TRUE))
```

```
## Error in is.data.frame(y): object 'blast_results_out' not found
```

```
mrg_blast_rna <- mrg_blast %>% filter(na_type == "RNA")
```

```
## Error in filter(., na_type == "RNA"): object 'mrg_blast' not found
```

```
mrg_blast_dna <- mrg_blast %>% filter(na_type == "DNA")
```

```
## Error in filter(., na_type == "DNA"): object 'mrg_blast' not found
```

```
# Plot RNA
g_mrg_blast_rna <- mrg_blast_rna %>%
  ggplot(aes(x=adj_score_fwd, y=adj_score_rev, color=viral_status_out)) +
  geom_point(alpha=0.5, shape=16) +
  scale_x_continuous(name="S(forward read)", limits=c(0,65), breaks=seq(0,100,10), expand = c(0,0)) +
  scale_y_continuous(name="S(reverse read)", limits=c(0,65), breaks=seq(0,100,10), expand = c(0,0)) +
  scale_color_brewer(palette = "Set1", name = "Viral status") +
  facet_grid(viral_status_out~kraken_label, labeller = labeller(kit = label_wrap_gen(20))) +
  theme_base + labs(title="RNA") +
  theme(aspect.ratio=1, plot.title = element_text(size=rel(2), hjust=0))
```

```
## Error in ggplot(., aes(x = adj_score_fwd, y = adj_score_rev, color = viral_status_out)): object 'mrg_
```

```
g_mrg_blast_rna
```

```
## Error in eval(expr, envir, enclos): object 'g_mrg_blast_rna' not found
```

```
# Plot DNA
g_mrg_blast_dna <- mrg_blast_dna %>%
  ggplot(aes(x=adj_score_fwd, y=adj_score_rev, color=viral_status_out)) +
  geom_point(alpha=0.5, shape=16) +
  scale_x_continuous(name="S(forward read)", limits=c(0,65), breaks=seq(0,100,10), expand = c(0,0)) +
  scale_y_continuous(name="S(reverse read)", limits=c(0,65), breaks=seq(0,100,10), expand = c(0,0)) +
  scale_color_brewer(palette = "Set1", name = "Viral status") +
  facet_grid(viral_status_out~kraken_label, labeller = labeller(kit = label_wrap_gen(20))) +
  theme_base + labs(title="DNA") +
  theme(aspect.ratio=1, plot.title = element_text(size=rel(2), hjust=0))
```

```
## Error in ggplot(., aes(x = adj_score_fwd, y = adj_score_rev, color = viral_status_out)): object 'mrg_
```

```
g_mrg_blast_dna
```

```
## Error in eval(expr, envir, enclos): object 'g_mrg_blast_dna' not found
```

```
g_hist_1 <- ggplot(mrg_blast, aes(x=adj_score_max)) + geom_histogram(binwidth=5,boundary=0,position="do
```

```
## Error in ggplot(mrg_blast, aes(x = adj_score_max)): object 'mrg_blast' not found
```

```
g_hist_1
```

```
## Error in eval(expr, envir, enclos): object 'g_hist_1' not found
```

These results look good on visual inspection, and indeed precision and sensitivity are both very high. For a disjunctive score threshold of 20, my updated workflow achieves an F1 score of 96.7% for RNA sequences and 98.2% for DNA sequences.

```
test_sens_spec <- function(tab, score_threshold, conjunctive, include_special){
  if (!include_special) tab <- filter(tab, viral_status_out %in% c("TRUE", "FALSE"))
  tab_retained <- tab %>% mutate(
    conjunctive = conjunctive,
    retain_score_conjunctive = (adj_score_fwd > score_threshold & adj_score_rev > score_threshold),
    retain_score_disjunctive = (adj_score_fwd > score_threshold | adj_score_rev > score_threshold),
    retain_score = ifelse(conjunctive, retain_score_conjunctive, retain_score_disjunctive),
    retain = assigned_hv | hit_hv | retain_score) %>%
    group_by(viral_status_out, retain) %>% count
  pos_tru <- tab_retained %>% filter(viral_status_out == "TRUE", retain) %>% pull(n) %>% sum
  pos_fls <- tab_retained %>% filter(viral_status_out != "TRUE", retain) %>% pull(n) %>% sum
  neg_tru <- tab_retained %>% filter(viral_status_out != "TRUE", !retain) %>% pull(n) %>% sum
  neg_fls <- tab_retained %>% filter(viral_status_out == "TRUE", !retain) %>% pull(n) %>% sum
  sensitivity <- pos_tru / (pos_tru + neg_fls)
  specificity <- neg_tru / (neg_tru + pos_fls)
  precision   <- pos_tru / (pos_tru + pos_fls)
  f1 <- 2 * precision * sensitivity / (precision + sensitivity)
  out <- tibble(threshold=score_threshold, include_special = include_special,
                conjunctive = conjunctive, sensitivity=sensitivity,
                specificity=specificity, precision=precision, f1=f1)
  return(out)
}
range_f1 <- function(intab, inc_special, inrange=15:45){
  tss <- purrr::partial(test_sens_spec, tab=intab, include_special=inc_special)
  stats_conj <- lapply(inrange, tss, conjunctive=TRUE) %>% bind_rows
  stats_disj <- lapply(inrange, tss, conjunctive=FALSE) %>% bind_rows
  stats_all <- bind_rows(stats_conj, stats_disj) %>%
    pivot_longer(!(threshold:conjunctive), names_to="metric", values_to="value") %>%
    mutate(conj_label = ifelse(conjunctive, "Conjunctive", "Disjunctive"))
  return(stats_all)
}
inc_special <- FALSE
stats_rna <- range_f1(mrg_blast_rna, inc_special) %>% mutate(na_type = "RNA")
```

```
## Error in filter(tab, viral_status_out %in% c("TRUE", "FALSE")): object 'mrg_blast_rna' not found
```

```
stats_dna <- range_f1(mrg_blast_dna, inc_special) %>% mutate(na_type = "DNA")
```

```
## Error in filter(tab, viral_status_out %in% c("TRUE", "FALSE")): object 'mrg_blast_dna' not found
```

```
stats_0 <- bind_rows(stats_rna, stats_dna) %>% mutate(attempt=0)
```

```
## Error in list2(...): object 'stats_rna' not found
```

```
threshold_opt_0 <- stats_0 %>% group_by(conj_label,attempt,na_type) %>%
  filter(metric == "f1") %>%
  filter(value == max(value)) %>% filter(threshold == min(threshold))
```

```
## Error in group_by(., conj_label, attempt, na_type): object 'stats_0' not found
```

```
g_stats_0 <- ggplot(stats_0, aes(x=threshold, y=value, color=metric)) +
  geom_vline(data = threshold_opt_0, mapping = aes(xintercept=threshold),
             color = "red", linetype = "dashed") +
  geom_line() +
  scale_y_continuous(name = "Value", limits=c(0,1), breaks = seq(0,1,0.2), expand = c(0,0)) +
  scale_x_continuous(name = "Threshold", expand = c(0,0)) +
  scale_color_brewer(palette="Set3") +
  facet_grid(na_type~conj_label) +
  theme_base
```

```
## Error in ggplot(stats_0, aes(x = threshold, y = value, color = metric)): object 'stats_0' not found
```

```
g_stats_0
```

```
## Error in eval(expr, envir, enclos): object 'g_stats_0' not found
```

Looking into the composition of different read groups, nothing stands out except the predominance of HCMV (human betaherpesvirus 5), which is consistent with the Kraken results above and borne out by the BLASTN alignments:

```r
viral_taxa$name[viral_taxa$taxid == 211787] <- "Human papillomavirus type 92"
viral_taxa$name[viral_taxa$taxid == 509154] <- "Porcine endogenous retrovirus C"

major_threshold <- 0.05
fp <- mrg_blast %>%
  group_by(na_type, viral_status_out,
           highscore = adj_score_max >= 20, taxid) %>% count %>%
  group_by(na_type, viral_status_out, highscore) %>% mutate(p=n/sum(n)) %>%
  left_join(viral_taxa, by="taxid") %>%
  arrange(desc(p)) %>%
  mutate(name = ifelse(taxid == 194958, "Porcine endogenous retrovirus A", name))
```

```
## Error in group_by(., na_type, viral_status_out, highscore = adj_score_max >= : object 'mrg_blast' no
```

```r
fp_major_tab <- fp %>% filter(p > major_threshold) %>% arrange(desc(p))
```

```
## Error in filter(., p > major_threshold): object 'fp' not found
```

```r
fp_major_list <- fp_major_tab %>% pull(name) %>% sort %>% unique %>% c(., "Other")
```

```
## Error in pull(., name): object 'fp_major_tab' not found
```

```r
fp_major <- fp %>% mutate(major = p > major_threshold) %>%
  mutate(name_display = ifelse(major, name, "Other")) %>%
  group_by(na_type, viral_status_out, highscore, name_display) %>%
  summarize(n=sum(n), p=sum(p), .groups = "drop")  %>%
  mutate(name_display = factor(name_display, levels = fp_major_list),
         score_display = ifelse(highscore, "S >= 20", "S < 20"),
         status_display = ifelse(viral_status_out, "True positive", "False positive"))
```

```
## Error in mutate(., major = p > major_threshold): object 'fp' not found
```

```r
g_fp <- ggplot(fp_major, aes(x=score_display, y=p, fill=name_display)) +
  geom_col(position="stack") +
  scale_x_discrete(name = "True positive?") +
  scale_y_continuous(name = "% reads", limits = c(0,1.01),
                     breaks = seq(0,1,0.2), expand = c(0,0)) +
  scale_fill_manual(values = palette_viral, name = "Viral\ntaxon") +
  facet_grid(na_type~status_display) +
  guides(fill=guide_legend(ncol=3)) +
  theme_kit
```

```
## Error in ggplot(fp_major, aes(x = score_display, y = p, fill = name_display)): object 'fp_major' not
```

```r
g_fp
```

```
## Error in eval(expr, envir, enclos): object 'g_fp' not found
```

```r
# Configure
ref_taxid <- 10359

# Get taxon names
tax_names_path <- file.path(data_dir, "taxid-names.tsv.gz")
tax_names <- read_tsv(tax_names_path, show_col_types = FALSE)
```

```
## Error: './/taxid-names.tsv.gz' does not exist in current working directory ('/Users/harmonbhasin/wor
```

```r
# Add missing names
tax_names_new <- tribble(~staxid, ~name,
                         3050295, "Cytomegalovirus humanbeta5",
                         459231, "FLAG-tagging vector pFLAG97-TSR")
tax_names <- bind_rows(tax_names, tax_names_new)
```

```
## Error in list2(...): object 'tax_names' not found
```

```
ref_name <- tax_names %>% filter(staxid == ref_taxid) %>% pull(name)
```

```
## Error in filter(., staxid == ref_taxid): object 'tax_names' not found
```

```
# Get major matches
fp_staxid <- mrg_blast %>% filter(taxid == ref_taxid) %>%
  group_by(na_type, highscore = adj_score_max >= 20) %>% mutate(n_seq = n()) %>%
  left_join(blast_results_paired, by="seq_id") %>%
  mutate(staxid = as.integer(staxid)) %>%
  left_join(tax_names, by="staxid") %>% rename(sname=name) %>%
  left_join(tax_names %>% rename(taxid=staxid), by="taxid")
```

```
## Error in filter(., taxid == ref_taxid): object 'mrg_blast' not found
```

```
fp_staxid_count <- fp_staxid %>%
  group_by(viral_status_out, highscore, na_type,
           taxid, name, staxid, sname, n_seq) %>%
  count %>%
  group_by(viral_status_out, highscore, na_type, taxid, name) %>%
  mutate(p=n/n_seq)
```

```
## Error in group_by(., viral_status_out, highscore, na_type, taxid, name, : object 'fp_staxid' not fou
```

```
fp_staxid_count_major <- fp_staxid_count %>%
  filter(n>1, p>0.1, !is.na(staxid)) %>%
  mutate(score_display = ifelse(highscore, "S >= 20", "S < 20"),
         status_display = ifelse(viral_status_out,
                                 "True positive", "False positive"))
```

```
## Error in filter(., n > 1, p > 0.1, !is.na(staxid)): object 'fp_staxid_count' not found
```

```
# Plot
g <- ggplot(fp_staxid_count_major, aes(x=p, y=sname)) +
  geom_col() +
  facet_grid(na_type~status_display+score_display, scales="free",
             labeller = label_wrap_gen(multi_line = FALSE)) +
  scale_x_continuous(name="% mapped reads", limits=c(0,1), breaks=seq(0,1,0.2),
                     expand=c(0,0)) +
  labs(title=paste0(ref_name, " (taxid ", ref_taxid, ")")) +
  theme_base + theme(
    axis.title.y = element_blank(),
    plot.title = element_text(size=rel(1.5), hjust=0, face="plain"))
```

```
## Error in ggplot(fp_staxid_count_major, aes(x = p, y = sname)): object 'fp_staxid_count_major' not fo
```

```
g
```

```
## Error in eval(expr, envir, enclos): object 'g' not found
```

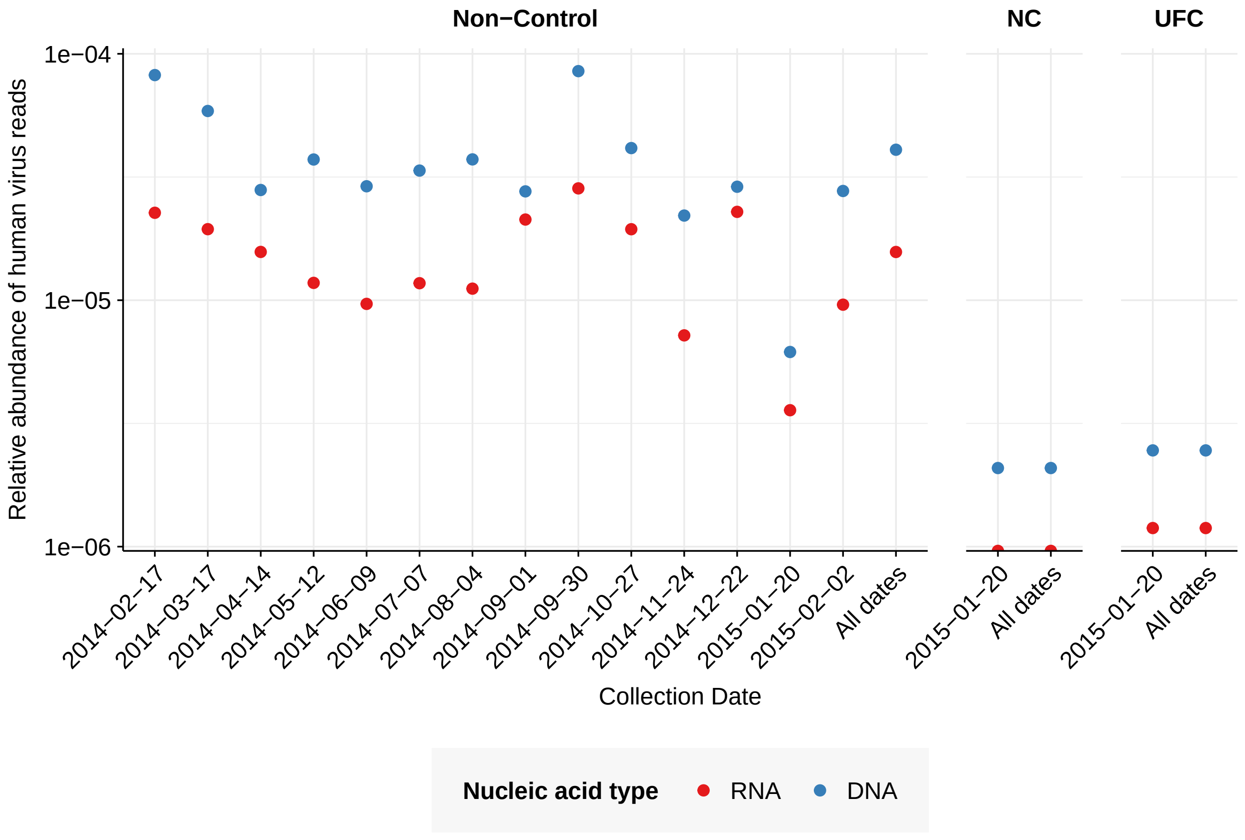
Human-infecting viruses: overall relative abundance

Harmon note: picking up back here.

```
# Get raw read counts
read_counts_raw <- basic_stats_raw %>%
  select(sample, date, na_type, ctrl, n_reads_raw = n_read_pairs)

# Get HV read counts
mrg_hv <- mrg %>% mutate(hv_status = assigned_hv | hit_hv | adj_score_max >= 20)
read_counts_hv <- mrg_hv %>% filter(hv_status) %>% group_by(sample) %>%
  count(name="n_reads_hv")
read_counts <- read_counts_raw %>% left_join(read_counts_hv, by="sample") %>%
  mutate(n_reads_hv = replace_na(n_reads_hv, 0))

# Aggregate
read_counts_total <- read_counts %>% group_by(na_type, ctrl) %>%
  summarize(n_reads_raw = sum(n_reads_raw),
            n_reads_hv = sum(n_reads_hv), .groups="drop") %>%
  mutate(sample= "All samples", date = "All dates")
read_counts_agg <- read_counts %>% arrange(date) %>%
  arrange(sample) %>%
  bind_rows(read_counts_total) %>%
  mutate(sample = fct_inorder(sample),
         p_reads_hv = n_reads_hv/n_reads_raw)
```

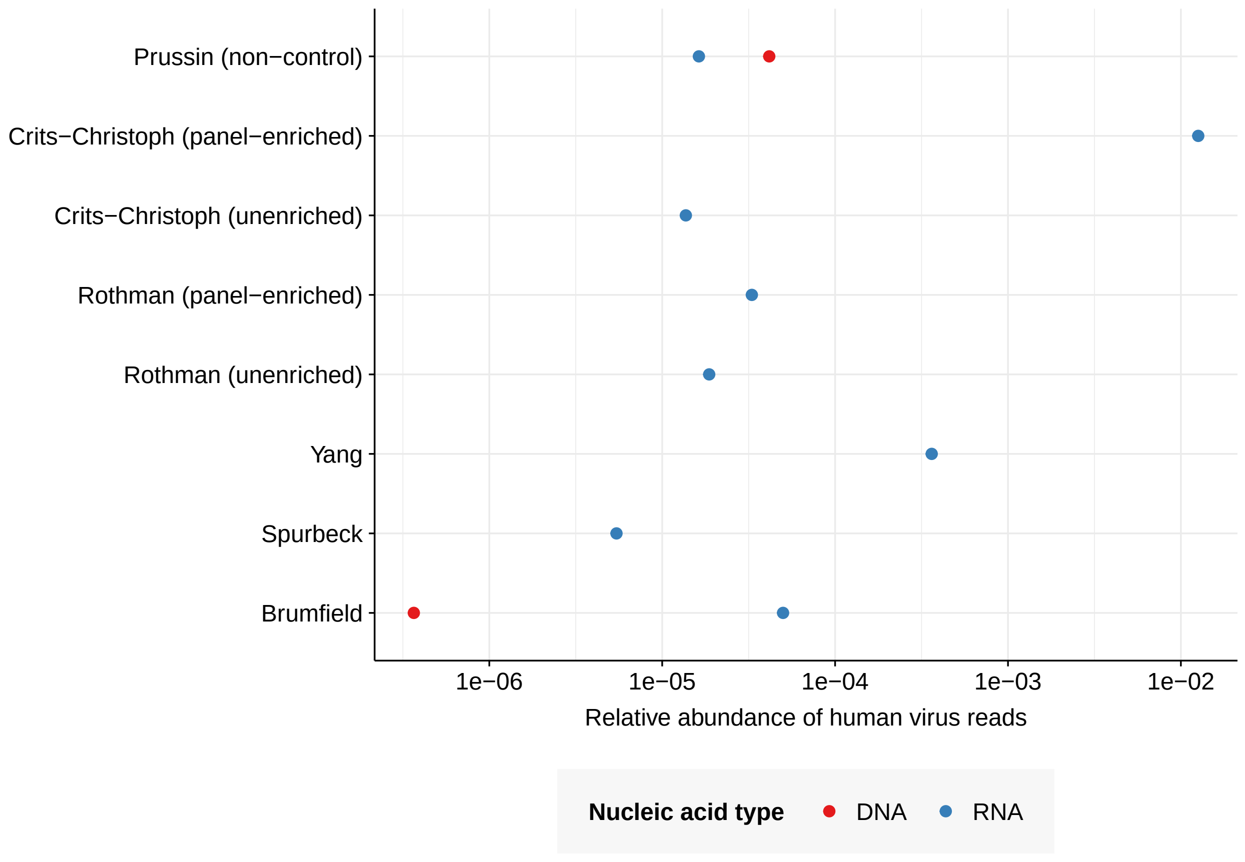
In non-control samples, applying a disjunctive cutoff at S=20 identifies 2591 RNA reads and 12236 DNA reads as human-viral. This gives an overall relative HV abundance of 1.63×10^{-5} for RNA reads and 4.16×10^{-5} for DNA reads. Reassuringly, relative abundances in control samples are at least 10x lower. We also see a sharp drop in relative abundance for both nucleic-acid types for the period when the daycare was closed (2015-01-20):

```
# Visualize
g_phv_agg <- ggplot(read_counts_agg, aes(x=date, color=na_type)) +
  geom_point(aes(y=p_reads_hv)) +
  scale_y_log10("Relative abundance of human virus reads") +
  scale_x_discrete(name="Collection Date") +
  facet_grid(.~ctrl, scales = "free", space = "free_x") +
  scale_color_na() + theme_rotate
g_phv_agg
```



These overall RA values are similar to those we've seen previously for non-panel-enriched wastewater RNA data. That said, it's notable that the DNA read RA seen here is much higher than that seen in the only DNA wastewater dataset I've analyzed so far (Brumfield):

```
# Collate past RA values
ra_past <- tribble(~dataset, ~ra, ~na_type, ~panel_enriched,
                   "Brumfield", 5e-5, "RNA", FALSE,
                   "Brumfield", 3.66e-7, "DNA", FALSE,
                   "Spurbeck", 5.44e-6, "RNA", FALSE,
                   "Yang", 3.62e-4, "RNA", FALSE,
                   "Rothman (unenriched)", 1.87e-5, "RNA", FALSE,
                   "Rothman (panel-enriched)", 3.3e-5, "RNA", TRUE,
                   "Crits-Christoph (unenriched)", 1.37e-5, "RNA", FALSE,
                   "Crits-Christoph (panel-enriched)", 1.26e-2, "RNA", TRUE)

# Collate new RA values
ra_new <- tribble(~dataset, ~ra, ~na_type, ~panel_enriched,
                   "Prussin (non-control)", 1.63e-5, "RNA", FALSE,
                   "Prussin (non-control)", 4.16e-5, "DNA", FALSE)

# Plot
ra_comp <- bind_rows(ra_past, ra_new) %>% mutate(dataset = fct_inorder(dataset))
g_ra_comp <- ggplot(ra_comp, aes(y=dataset, x=ra, color=na_type)) +
  geom_point() +
  scale_color_na() +
  scale_x_log10(name="Relative abundance of human virus reads") +
  theme_base + theme(axis.title.y = element_blank())
g_ra_comp
```



Human-infecting viruses: taxonomy and composition

Harmon note: Looks different, but maybe taxid's are changing? who knows, I checked that some of the taxid's show up as NA but none of them are the NC or UFC which seem to be the most different.

At the family level, we see that *Papillomaviridae*, *Herpesviridae*, and *Polyomaviridae* are the most abundant families in both DNA and RNA reads, with *Adenoviridae* and (in RNA reads) *Picornaviridae* also making a respectable showing. The *Herpesviridae* reads are, predictably, overwhelmingly from HCMV, while the *Papillomaviridae* and *Polyomaviridae* reads are split up among a larger number of related viruses:

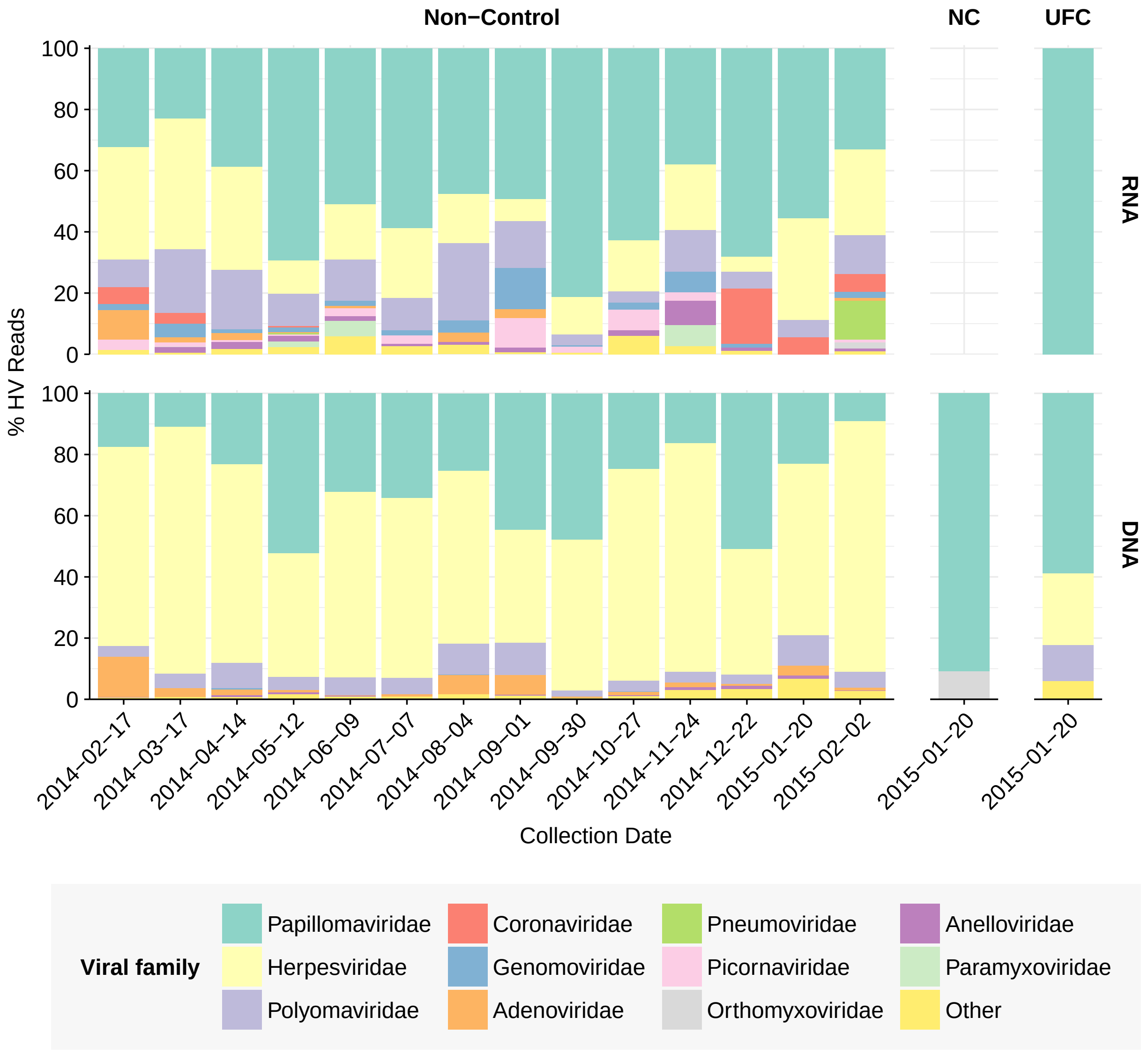
```
# Get viral taxon names for putative HV reads
viral_taxa$name[viral_taxa$taxid == 249588] <- "Mamastrovirus"
viral_taxa$name[viral_taxa$taxid == 194960] <- "Kobuvirus"
viral_taxa$name[viral_taxa$taxid == 688449] <- "Salivirus"
viral_taxa$name[viral_taxa$taxid == 585893] <- "Picobirnaviridae"
viral_taxa$name[viral_taxa$taxid == 333922] <- "Betapapillomavirus"
viral_taxa$name[viral_taxa$taxid == 334207] <- "Betapapillomavirus 3"
viral_taxa$name[viral_taxa$taxid == 369960] <- "Porcine type-C oncovirus"
viral_taxa$name[viral_taxa$taxid == 333924] <- "Betapapillomavirus 2"
viral_taxa$name[viral_taxa$taxid == 687329] <- "Anelloviridae"
viral_taxa$name[viral_taxa$taxid == 325455] <- "Gammapapillomavirus"
viral_taxa$name[viral_taxa$taxid == 333750] <- "Alphapapillomavirus"
viral_taxa$name[viral_taxa$taxid == 694002] <- "Betacoronavirus"
viral_taxa$name[viral_taxa$taxid == 334202] <- "Mupapillomavirus"
viral_taxa$name[viral_taxa$taxid == 197911] <- "Alphainfluenzavirus"
viral_taxa$name[viral_taxa$taxid == 186938] <- "Respirovirus"
viral_taxa$name[viral_taxa$taxid == 333926] <- "Gammapapillomavirus 1"
```

```
viral_taxa$name[viral_taxa$taxid == 337051] <- "Betapapillomavirus 1"
viral_taxa$name[viral_taxa$taxid == 337043] <- "Alphapapillomavirus 4"
viral_taxa$name[viral_taxa$taxid == 694003] <- "Betacoronavirus 1"
viral_taxa$name[viral_taxa$taxid == 334204] <- "Mupapillomavirus 2"
viral_taxa$name[viral_taxa$taxid == 334208] <- "Betapapillomavirus 4"

viral_taxa$name[viral_taxa$taxid == 334208] <- "Betapapillomavirus 4"
viral_taxa$name[viral_taxa$taxid == 334208] <- "Betapapillomavirus 4"
viral_taxa$name[viral_taxa$taxid == 334208] <- "Betapapillomavirus 4"


mrg_hv_named <- mrg_hv %>% mutate(taxid = as.integer(taxid)) %>% left_join(viral_taxa, by="taxid")
```

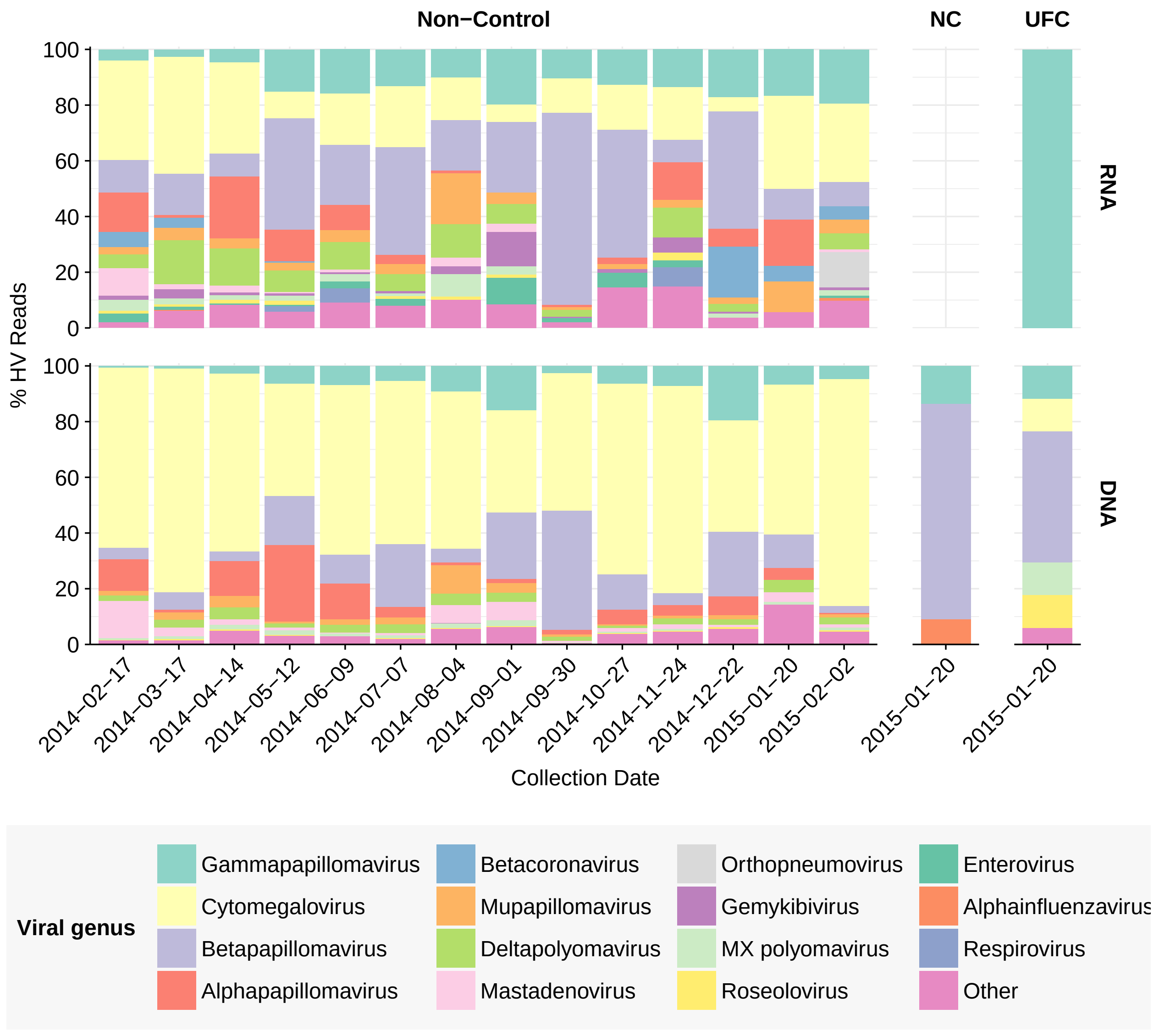
```
## Warning: There were 4 warnings in `mutate()`.
## The first warning was:
## i In argument: `taxid = as.integer(taxid)`.
## i In group 1: `sample = "AP-DNA-1"`, `na_type = DNA`.
## Caused by warning:
## ! NAs introduced by coercion
## i Run `dplyr::last_dplyr_warnings()` to see the 3 remaining warnings.
```

```
# Discover viral species & genera for HV reads
raise_rank <- function(read_db, taxid_db, out_rank = "species", verbose = FALSE){
  # Get higher ranks than search rank
  ranks <- c("subspecies", "species", "subgenus", "genus", "subfamily", "family", "suborder", "order",
  rank_match <- which.max(ranks == out_rank)
  high_ranks <- ranks[rank_match:length(ranks)]
  # Merge read DB and taxid DB
  reads <- read_db %>% select(-parent_taxid, -rank, -name) %>%
    left_join(taxid_db, by="taxid")
  # Extract sequences that are already at appropriate rank
  reads_rank <- filter(reads, rank == out_rank)
  # Drop sequences at a higher rank and return unclassified sequences
  reads_norank <- reads %>% filter(rank != out_rank, !rank %in% high_ranks, !is.na(taxid))
  while(nrow(reads_norank) > 0){ # As long as there are unclassified sequences...
    # Promote read taxids and re-merge with taxid DB, then re-classify and filter
    reads_remaining <- reads_norank %>% mutate(taxid = parent_taxid) %>%
      select(-parent_taxid, -rank, -name) %>%
      left_join(taxid_db, by="taxid")
    reads_rank <- reads_remaining %>% filter(rank == out_rank) %>%
      bind_rows(reads_rank)
    reads_norank <- reads_remaining %>%
      filter(rank != out_rank, !rank %in% high_ranks, !is.na(taxid))
  }
  # Finally, extract and append reads that were excluded during the process
  reads_dropped <- reads %>% filter(!seq_id %in% reads_rank$seq_id)
  reads_out <- reads_rank %>% bind_rows(reads_dropped) %>%
    select(-parent_taxid, -rank, -name) %>%
    left_join(taxid_db, by="taxid")
  return(reads_out)
}
hv_reads_species <- raise_rank(mrg_hv_named, viral_taxa, "species")
hv_reads_genus <- raise_rank(mrg_hv_named, viral_taxa, "genus")
hv_reads_family <- raise_rank(mrg_hv_named, viral_taxa, "family")
```

```r
threshold_major_family <- 0.06

# Count reads for each human-viral family
hv_family_counts <- hv_reads_family %>%
  group_by(sample, date, ctrl, na_type, name, taxid) %>%
  count(name = "n_reads_hv") %>%
  group_by(sample, date, ctrl, na_type) %>%
  mutate(p_reads_hv = n_reads_hv/sum(n_reads_hv))

# Identify high-ranking families and group others
hv_family_major_tab <- hv_family_counts %>% group_by(name) %>%
  filter(p_reads_hv == max(p_reads_hv)) %>% filter(row_number() == 1) %>%
  arrange(desc(p_reads_hv)) %>% filter(p_reads_hv > threshold_major_family)
hv_family_counts_major <- hv_family_counts %>%
  mutate(name_display = ifelse(name %in% hv_family_major_tab$name, name, "Other")) %>%
  group_by(sample, date, ctrl, na_type, name_display) %>%
  summarize(n_reads_hv = sum(n_reads_hv), p_reads_hv = sum(p_reads_hv),
            .groups="drop") %>%
  mutate(name_display = factor(name_display,
                               levels = c(hv_family_major_tab$name, "Other")))
hv_family_counts_display <- hv_family_counts_major %>%
  rename(p_reads = p_reads_hv, classification = name_display)

# Plot
g_hv_family <- g_comp_base +
  geom_col(data=hv_family_counts_display, position = "stack") +
  scale_y_continuous(name="% HV Reads", limits=c(0,1.01),
                     breaks = seq(0,1,0.2),
                     expand=c(0,0), labels = function(y) y*100) +
  scale_fill_manual(values=palette_viral, name = "Viral family")
g_hv_family
```

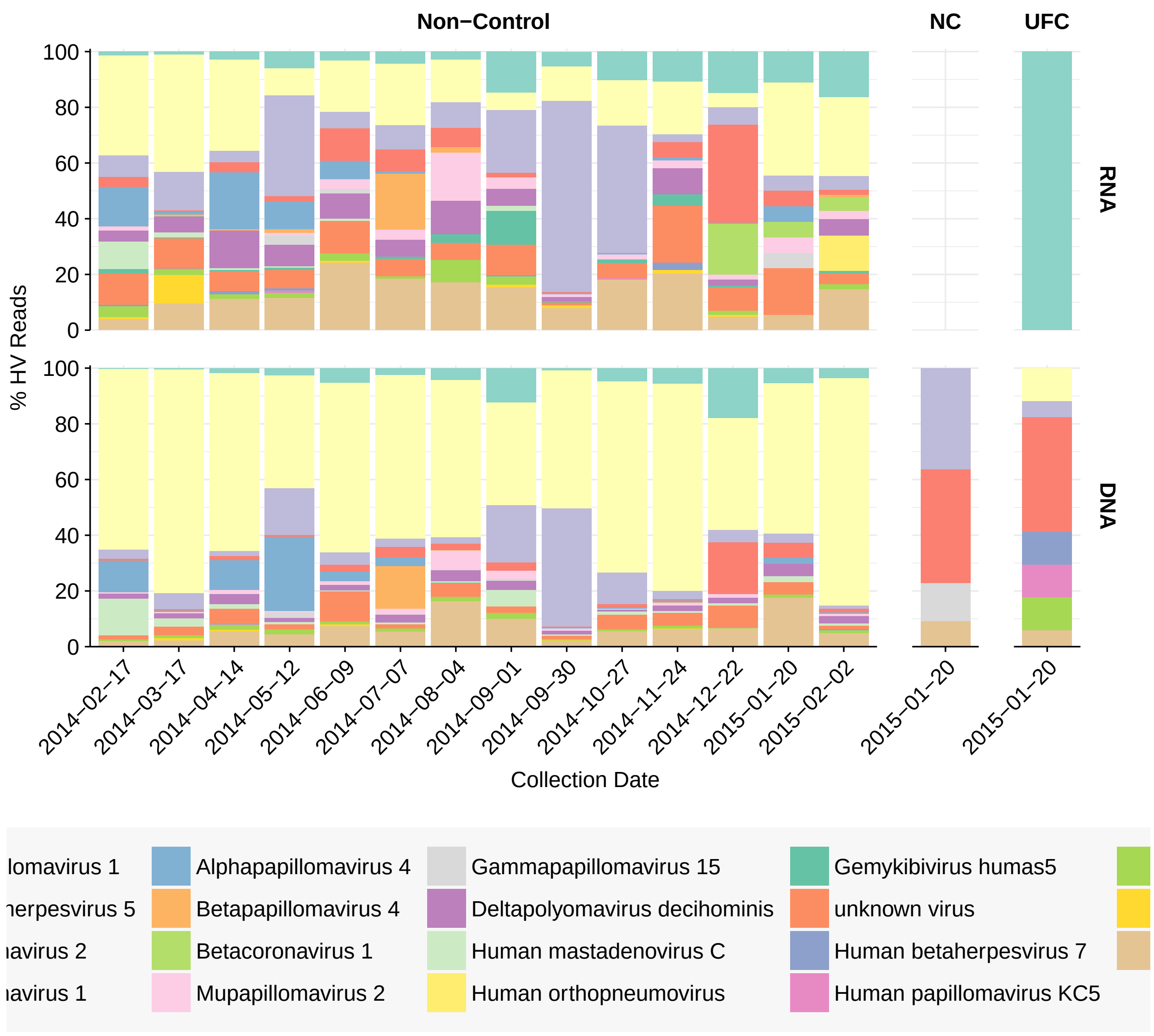


```r
threshold_major_genus <- 0.06

# Count reads for each human-viral genus
hv_genus_counts <- hv_reads_genus %>%
  group_by(sample, date, ctrl, na_type, name, taxid) %>%
  count(name = "n_reads_hv") %>%
  group_by(sample, date, ctrl, na_type) %>%
  mutate(p_reads_hv = n_reads_hv/sum(n_reads_hv))

# Identify high-ranking families and group others
hv_genus_major_tab <- hv_genus_counts %>% group_by(name) %>%
  filter(p_reads_hv == max(p_reads_hv)) %>% filter(row_number() == 1) %>%
  arrange(desc(p_reads_hv)) %>% filter(p_reads_hv > threshold_major_genus)
hv_genus_counts_major <- hv_genus_counts %>%
  mutate(name_display = ifelse(name %in% hv_genus_major_tab$name, name, "Other")) %>%
  group_by(sample, date, ctrl, na_type, name_display) %>%
  summarize(n_reads_hv = sum(n_reads_hv), p_reads_hv = sum(p_reads_hv),
            .groups="drop") %>%
  mutate(name_display = factor(name_display,
                               levels = c(hv_genus_major_tab$name, "Other")))
hv_genus_counts_display <- hv_genus_counts_major %>%
  rename(p_reads = p_reads_hv, classification = name_display)

# Plot
g_hv_genus <- g_comp_base +
  geom_col(data=hv_genus_counts_display, position = "stack") +
  scale_y_continuous(name="% HV Reads", limits=c(0,1.01),
                     breaks = seq(0,1,0.2),
                     expand=c(0,0), labels = function(y) y*100) +
  scale_fill_manual(values=palette_viral, name = "Viral genus")
g_hv_genus
```



```
threshold_major_species <- 0.10

# Count reads for each human-viral species
hv_species_counts <- hv_reads_species %>%
```

```r
  group_by(sample, date, ctrl, na_type, name, taxid) %>%
  count(name = "n_reads_hv") %>%
  group_by(sample, date, ctrl, na_type) %>%
  mutate(p_reads_hv = n_reads_hv/sum(n_reads_hv))

# Identify high-ranking families and group others
hv_species_major_tab <- hv_species_counts %>% group_by(name) %>%
  filter(p_reads_hv == max(p_reads_hv)) %>% filter(row_number() == 1) %>%
  arrange(desc(p_reads_hv)) %>% filter(p_reads_hv > threshold_major_species)
hv_species_counts_major <- hv_species_counts %>%
  mutate(name_display = ifelse(name %in% hv_species_major_tab$name, name, "Other")) %>%
  group_by(sample, date, ctrl, na_type, name_display) %>%
  summarize(n_reads_hv = sum(n_reads_hv), p_reads_hv = sum(p_reads_hv),
            .groups="drop") %>%
  mutate(name_display = factor(name_display,
                               levels = c(hv_species_major_tab$name, "Other")))
hv_species_counts_display <- hv_species_counts_major %>%
  rename(p_reads = p_reads_hv, classification = name_display)

# Plot
g_hv_species <- g_comp_base +
  geom_col(data=hv_species_counts_display, position = "stack") +
  scale_y_continuous(name="% HV Reads", limits=c(0,1.01),
                     breaks = seq(0,1,0.2),
                     expand=c(0,0), labels = function(y) y*100) +
  scale_fill_manual(values=palette_viral, name = "Viral species")
g_hv_species
```



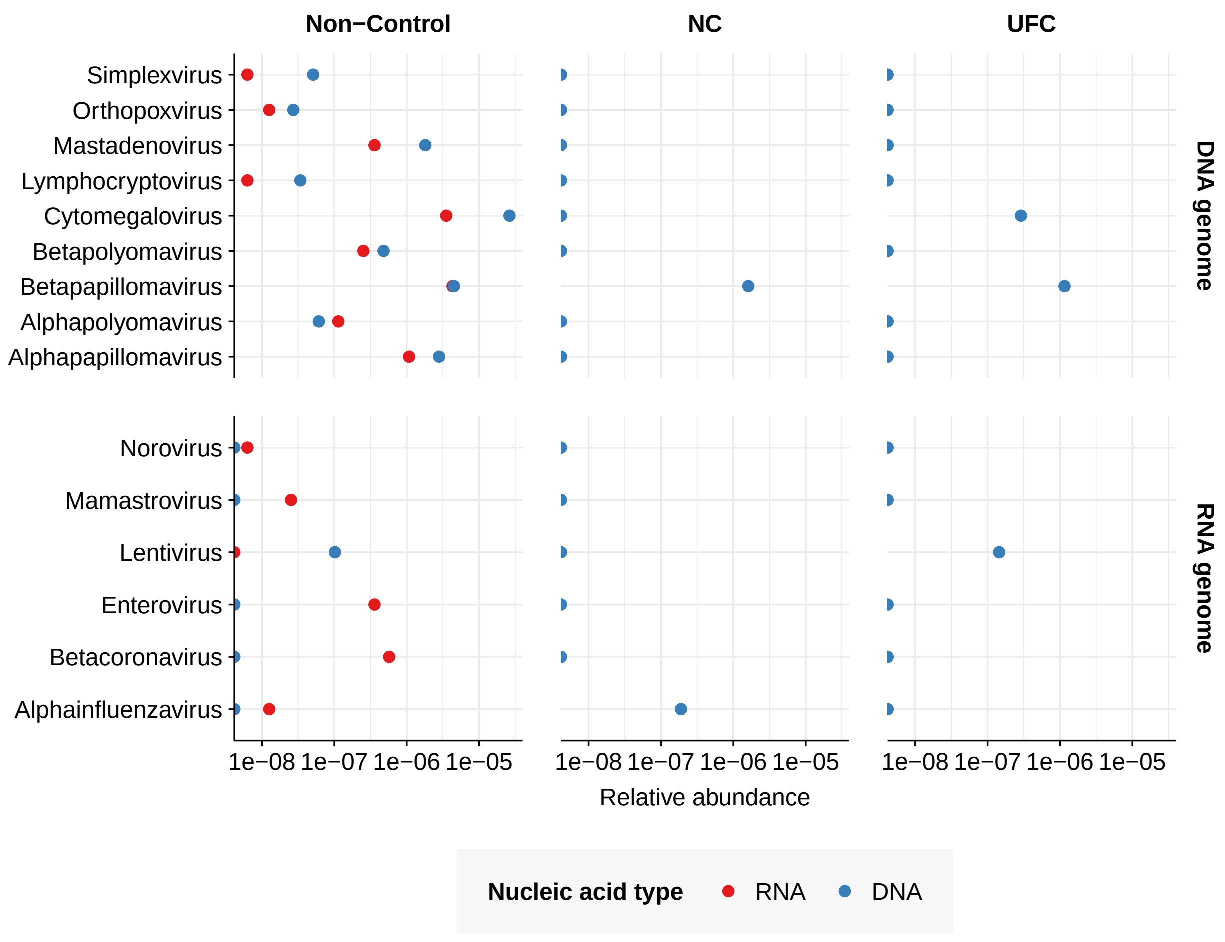
Compared to the previous datasets I've analyzed, the most notable difference is the absence of enteric viruses: *Norovirus*, *Rotavirus*, and *Enterovirus* are all absent from the list of abundant human-viral taxa, as are ~all other gastrointestinal pathogens.

Finally, here are the overall relative abundances of a set of specific viral genera picked out manually on the basis of scientific or medical interest. In the future, I'll quantify the RA of these genera across all datasets analyzed with this pipeline to date, which should give us a better sense of how these data compare to others' under this pipeline.

```r
# Define reference genera
path_genera_rna <- c("Mamastrovirus", "Enterovirus", "Salivirus", "Kobuvirus", "Norovirus", "Sapovirus"
                     "Alphainfluenzavirus", "Betainfluenzavirus", "Lentivirus")
path_genera_dna <- c("Mastadenovirus", "Alphapolyomavirus", "Betapolyomavirus", "Alphapapillomavirus",
                     "Lymphocryptovirus", "Cytomegalovirus", "Dependoparvovirus")
path_genera <- bind_rows(tibble(name=path_genera_rna, genome_type="RNA genome"),
                         tibble(name=path_genera_dna, genome_type="DNA genome")) %>%
  left_join(viral_taxa, by="name")

# Count in each sample
```

```
n_path_genera <- hv_reads_genus %>%
  group_by(sample, date, na_type, ctrl, name, taxid) %>%
  count(name="n_reads_viral") %>%
  inner_join(path_genera, by=c("name", "taxid")) %>%
  left_join(read_counts_raw, by=c("sample", "date", "na_type", "ctrl")) %>%
  mutate(p_reads_viral = n_reads_viral/n_reads_raw)

# Pivot out and back to add zero lines
n_path_genera_out <- n_path_genera %>% ungroup %>% select(sample, name, n_reads_viral) %>%
  pivot_wider(names_from="name", values_from="n_reads_viral", values_fill=0) %>%
  pivot_longer(-sample, names_to="name", values_to="n_reads_viral") %>%
  left_join(read_counts_raw, by="sample") %>%
  left_join(path_genera, by="name") %>%
  mutate(p_reads_viral = n_reads_viral/n_reads_raw)

## Aggregate across dates
n_path_genera_stype <- n_path_genera_out %>%
  group_by(na_type, ctrl,
           name, taxid, genome_type) %>%
  summarize(n_reads_raw = sum(n_reads_raw),
            n_reads_viral = sum(n_reads_viral), .groups = "drop") %>%
  mutate(sample="All samples", date="All dates",
         p_reads_viral = n_reads_viral/n_reads_raw)

# Plot
g_path_genera <- ggplot(n_path_genera_stype,
                        aes(y=name, x=p_reads_viral, color=na_type)) +
  geom_point() +
  scale_x_log10(name="Relative abundance") +
  scale_color_na() +
  facet_grid(genome_type~ctrl, scales="free_y") +
  theme_base + theme(axis.title.y = element_blank())
g_path_genera
```

```
## Warning: Transformation introduced infinite values in continuous x-axis
```



Conclusion

This is the first air-sampling dataset I've analyzed using this pipeline, and it was interesting to see the differences from the wastewater datasets I've been analyzing so far. Among the more striking differences were:

- A much higher total fraction of human reads;
- A lower total fraction of viral reads;
- Near-total absence of enteric viruses and *Tobamovirus*;
- Much higher relative abundance of herpesviruses, particularly HCMV, and papillomaviruses among human-infecting virus reads.

Conversely, one thing that was not notably different, at least in RNA viruses, was the total relative abundance of human-infecting viruses as a whole. Given the lower fraction of reads made up of all viruses, this means that the fraction of total viruses arising from human-infecting viruses is much higher here than we've historically seen with wastewater data. In particular, HCMV represents a nontrivial fraction of total viruses for many DNA libraries, the first time I've seen a human pathogen show up significantly in the total virus composition data.

Going forward, I have two more air sampling datasets to analyze, Rosario et al. 2018 and Leung et al. 2021. It will be interesting to see whether the findings from this dataset generalize to other air sampling contexts.

Addendum: Checking HIV

Jeff from my team asks:

> Do you think the HIV reads are real? The v1 pipeline shows 39 HIV reads in that dataset

My pipeline finds 62 *Lentivirus* reads for this dataset, 34 from HIV-1 and 28 from HIV-2. Oddly, and perhaps suspiciously, these nearly all come from DNA libraries:

```
lenti_read_ids <- hv_reads_genus %>% filter(name == "Lentivirus") %>% pull(seq_id)
lenti_reads <- hv_reads_species %>% filter(seq_id %in% lenti_read_ids)
lenti_read_count <- lenti_reads %>% group_by(name, na_type) %>% count(name="n_reads")
lenti_read_count
```

```
## # A tibble: 1 x 3
## # Groups:   name, na_type [1]
##   name                           na_type n_reads
##   <chr>                          <fct>     <int>
## 1 Human immunodeficiency virus 1 DNA          31
```

Here’s what I get when I look at the subject taxa that BLASTN maps these reads to:

```
# Configure
ref_taxid <- 11646

# Get taxon names
tax_names_path <- file.path(data_dir, "taxid-names.tsv.gz")
tax_names <- read_tsv(tax_names_path, show_col_types = FALSE)
```

```
## Error: './/taxid-names.tsv.gz' does not exist in current working directory ('/Users/harmonbhasin/wor
```

```
# Add missing names
tax_names_new <- tribble(~staxid, ~name,
                         3050295, "Cytomegalovirus humanbeta5",
                         459231, "FLAG-tagging vector pFLAG97-TSR",
                         257877, "Macaca thibetana thibetana",
                         256321, "Lentiviral transfer vector pHsCXW",
                         419242, "Shuttle vector pLvCmvMYOCDHA",
                         419243, "Shuttle vector pLvCmvLacZ",
                         421868, "Cloning vector pLvCmvLacZ.Gfp",
                         421869, "Cloning vector pLvCmvMyocardin.Gfp",
                         426303, "Lentiviral vector pNL-GFP-RRE(SA)",
                         436015, "Lentiviral transfer vector pFTMGW",
                         454257, "Shuttle vector pLvCmvMYOCD2aHA",
                         476184, "Shuttle vector pLV.mMyoD::ERT2.eGFP",
                         476185, "Shuttle vector pLV.hMyoD.eGFP",
                         591936, "Piliocolobus tephrosceles",
                         627481, "Lentiviral transfer vector pFTM3GW",
                         680261, "Self-inactivating lentivirus vector pLV.C-EF1a.cyt-bGal.dCpG",
                         2952778, "Expression vector pLV[Exp]-EGFP:T2A:Puro-EF1A",
                         3022699, "Vector PAS_122122",
                         3025913, "Vector pSIN-WP-mPGK-GDNF",
                         3105863, "Vector pLKO.1-ZsGreen1",
                         3105864, "Vector pLKO.1-ZsGreen1 mouse Wfs1 shRNA",
                         3108001, "Cloning vector pLVSIN-CMV_Neo_v4.0",
                         3109234, "Vector pTwist+Kan+High",
                         3117662, "Cloning vector pLV[Exp]-CBA>P301L",
                         3117663, "Cloning vector pLV[Exp]-CBA>P301L:T2A:mRuby3",
                         3117664, "Cloning vector pLV[Exp]-CBA>hMAPT[NM_005910.6](ns):T2A:mRuby3",
                         3117665, "Cloning vector pLV[Exp]-CBA>mRuby3",
                         3117666, "Cloning vector pLV[Exp]-CBA>mRuby3/NFAT3 fusion protein",
                         3117667, "Cloning vector pLV[Exp]-Neo-mPGK>{EGFP-hSEPT6}"

)
tax_names <- bind_rows(tax_names, tax_names_new)
```

```
## Error in list2(...): object 'tax_names' not found
```

```
ref_name <- tax_names %>% filter(staxid == ref_taxid) %>% pull(name)
```

```
## Error in filter(., staxid == ref_taxid): object 'tax_names' not found
```

```
# Get major matches
fp_staxid <- hv_reads_genus %>% filter(taxid == ref_taxid) %>%
  group_by(na_type, highscore = adj_score_max >= 20) %>% mutate(n_seq = n()) %>%
  select(-name) %>%
  left_join(blast_results_paired, by="seq_id") %>%
  mutate(staxid = as.integer(staxid)) %>%
  left_join(tax_names, by="staxid") %>% rename(sname=name) %>%
  left_join(tax_names %>% rename(taxid=staxid), by="taxid")
```

```
## Error in is.data.frame(y): object 'blast_results_paired' not found
```

```
fp_staxid_count <- fp_staxid %>%
  group_by(na_type, taxid, name, staxid, sname, n_seq) %>%
  count %>%
  group_by(na_type, taxid, name) %>%
  mutate(p=n/n_seq)
```

```
## Error in group_by(., na_type, taxid, name, staxid, sname, n_seq): object 'fp_staxid' not found
```

```
fp_staxid_count_major <- fp_staxid_count %>%
  filter(n>1, p>0.1, !is.na(staxid)) %>%
  arrange(desc(p)) %>%
  mutate(sname = fct_inorder(sname)) %>%
  group_by(na_type) %>% filter(row_number() <= 20)
```

```
## Error in filter(., n > 1, p > 0.1, !is.na(staxid)): object 'fp_staxid_count' not found
```

```
# Plot
g <- ggplot(fp_staxid_count_major, aes(x=p, y=sname)) +
  geom_col() +
  facet_grid(na_type~., scales="free",
             labeller = label_wrap_gen(multi_line = FALSE)) +
  scale_x_continuous(name="% mapped reads", limits=c(0,1), breaks=seq(0,1,0.2),
                     expand=c(0,0)) +
  labs(title=paste0(ref_name, " (taxid ", ref_taxid, ")")) +
  theme_base + theme(
    axis.title.y = element_blank(),
    plot.title = element_text(size=rel(1.5), hjust=0, face="plain"))
```

```
## Error in ggplot(fp_staxid_count_major, aes(x = p, y = sname)): object 'fp_staxid_count_major' not fo
```

```
g
```

```
## Error in eval(expr, envir, enclos): object 'g' not found
```

The four RNA reads seem very likely not to be true HIV reads, since they map primarily to humans and other primates. The 58 DNA reads are more ambiguous; they do map to HIV, but also to a range of synthetic cloning vectors. On the other hand, I just now took a random slice from the HIV-1 genome and BLASTed it online, and it also mapped to cloning vectors; it's just the case that a lot of people use lentiviruses as the backbones for their cloning vectors.

This makes disambiguating whether or not these reads are "real" a bit tricky. The fact that they're DNA rather than RNA makes me suspicious, as does the fact that so few of the reads apparently come from regions of the HIV genome not included in cloning vectors. On balance, I think these are probably not real HIV reads! But it's hard to be highly confident one way or the other.

If we decide to treat these reads as artifacts arising from contamination with cloning vectors, what do we do about it? Just removing any read that matches a lentiviral cloning vector seems like a risky strategy when these vectors share so much sequence with real viruses. For now, I'll leave my contamination database as it is and continue checking HIV reads manually. But I'll need to develop a better solution to this sooner or later.