No Template Control Characterization

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Objective

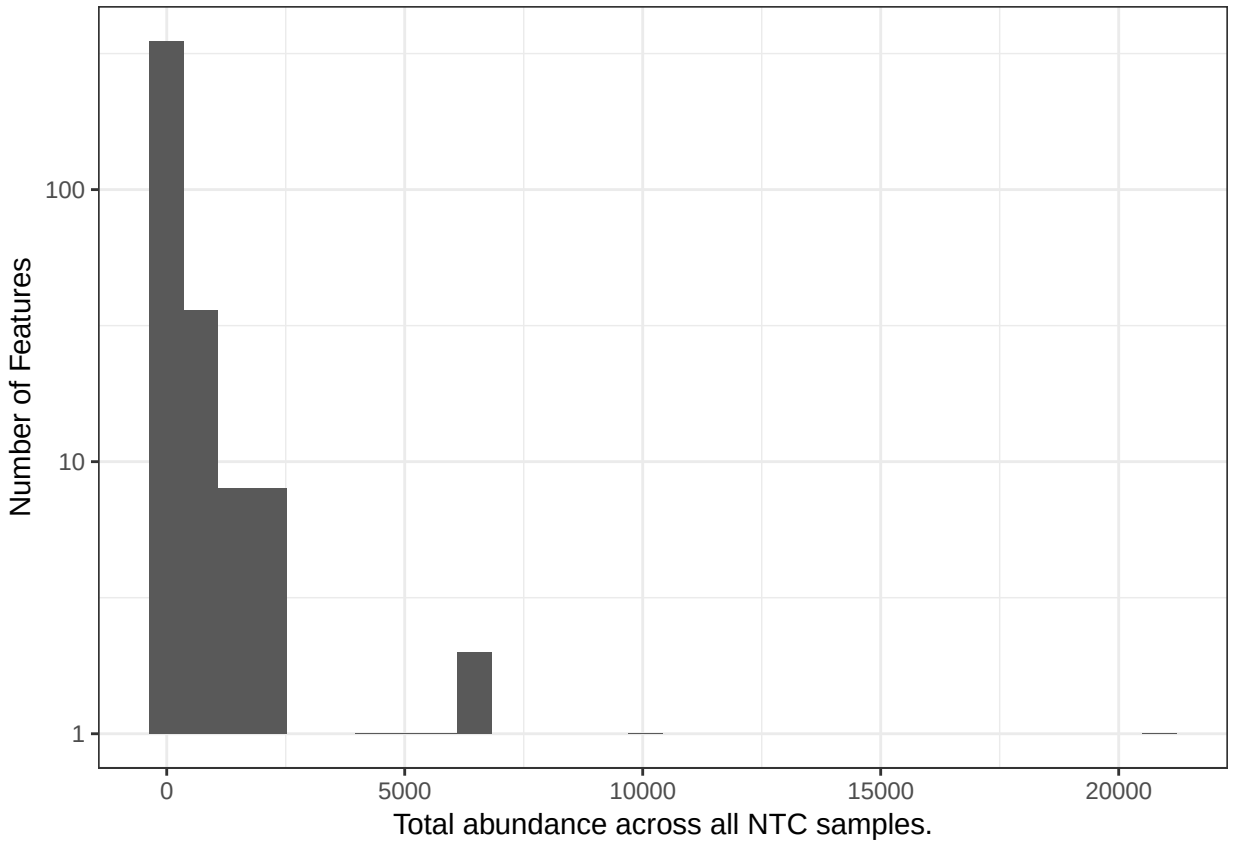
Identify and characterize features present in No Template Controls.

```
get_count_df <- function(mrobj, agg_genus = FALSE){
      if(agg_genus){
            mrobj <- aggregateByTaxonomy(mrobj, lvl = "Rank6",
                                         norm = FALSE, log = FALSE, sl = 1)
      }

      mrobj <- cumNorm(mrobj, p = 0.75)
      mrobj %>%
            # not sure whether or not to normalize counts prior to analysis
            MRcounts(norm = TRUE, log = FALSE, sl = 1000) %>%
            as.data.frame() %>%
            rownames_to_column(var = "feature_id") %>%
            gather("id","count", -feature_id)
}

pipeline_dir <- "../../mgtst_pipelines"
mrexp <- get_mrexp(pipeline_dir)

count_df <- mrexp %>% map_df(get_count_df, .id = "pipe") %>%
      left_join(pData(mrexp$dada2))
```

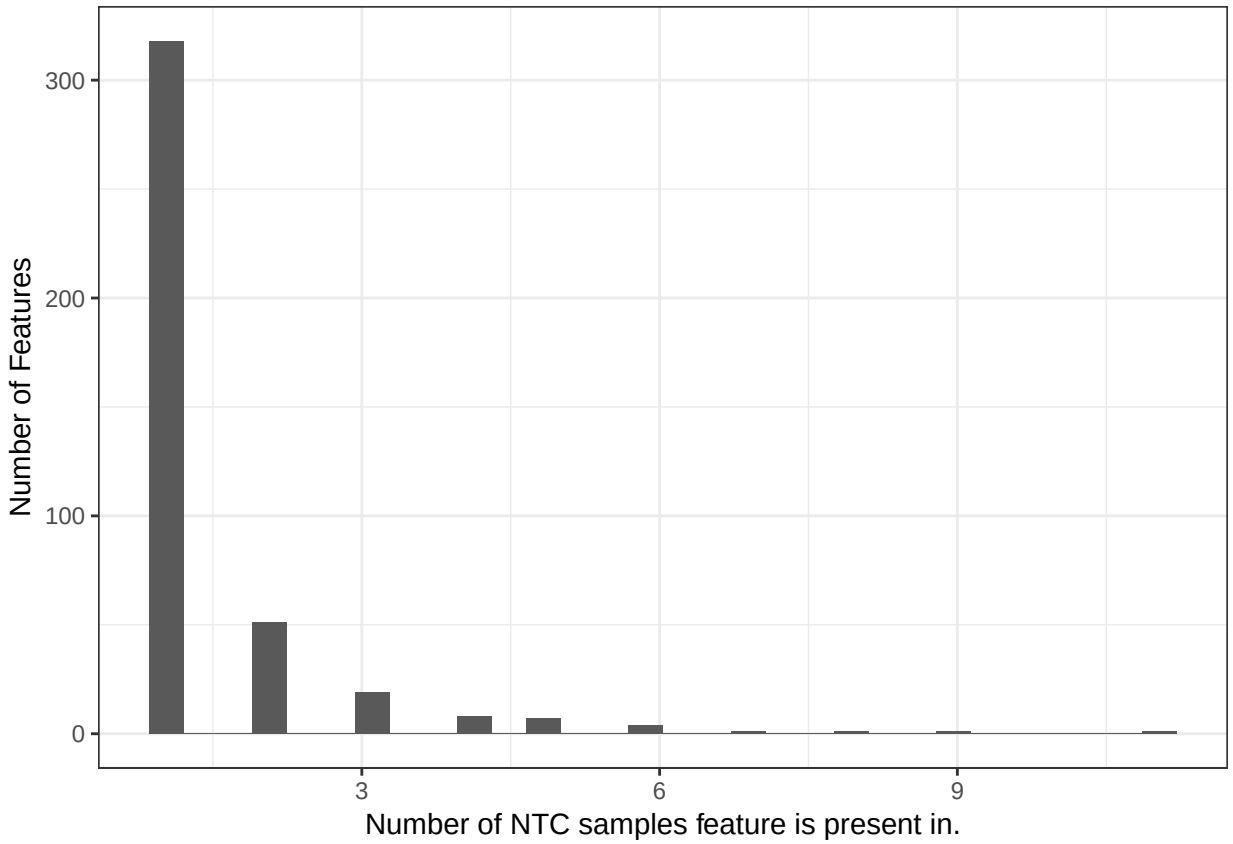
```
ntc_summary <- count_df %>% filter(biosample_id == "NTC") %>%
      filter(count != 0) %>%
      group_by(pipe, feature_id) %>%
      summarise(total_count = sum(count),
                med_count = median(count),
                n_present = n())
```

Note count values are CSS normalized and scaled.

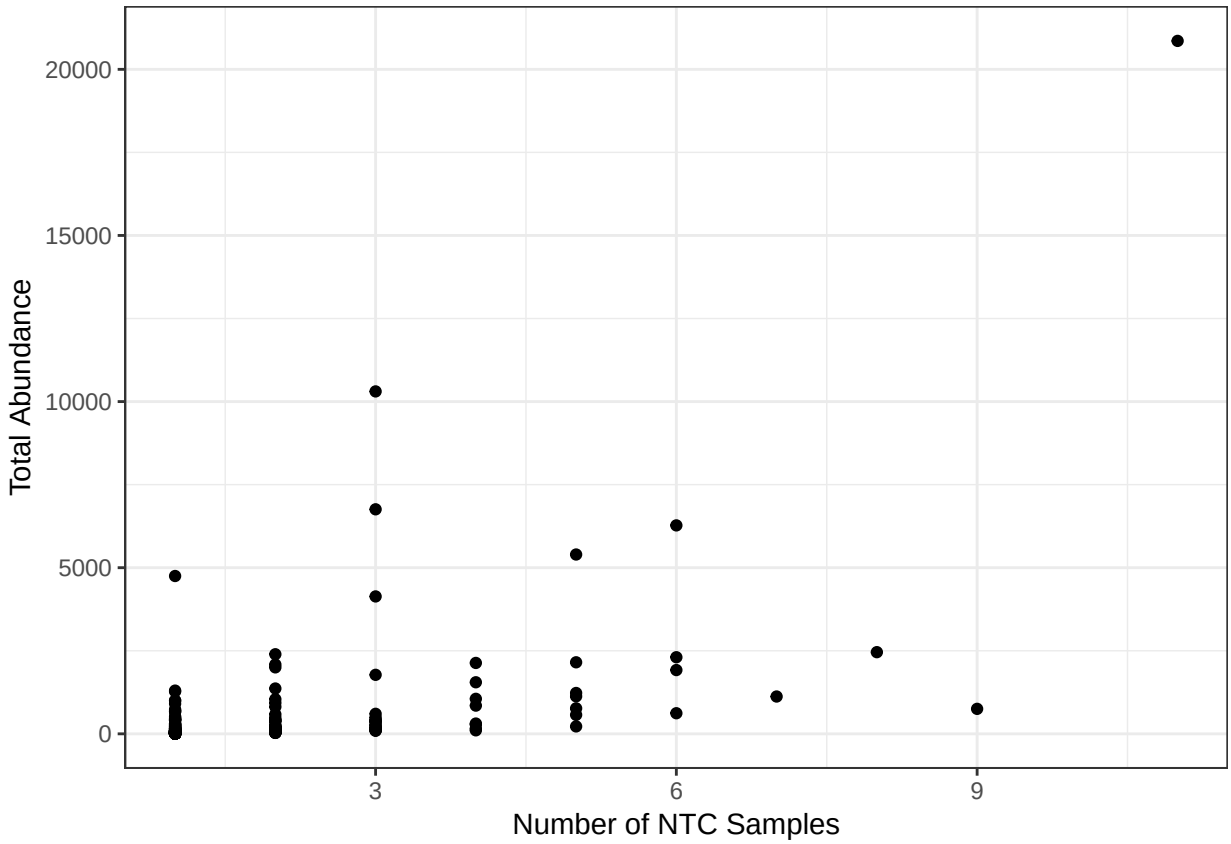
```
ntc_summary %>% ggplot() +
      geom_histogram(aes(x = total_count)) + scale_y_log10() + theme_bw() +
      labs(x = "Total abundance across all NTC samples.", y = "Number of Features")
```



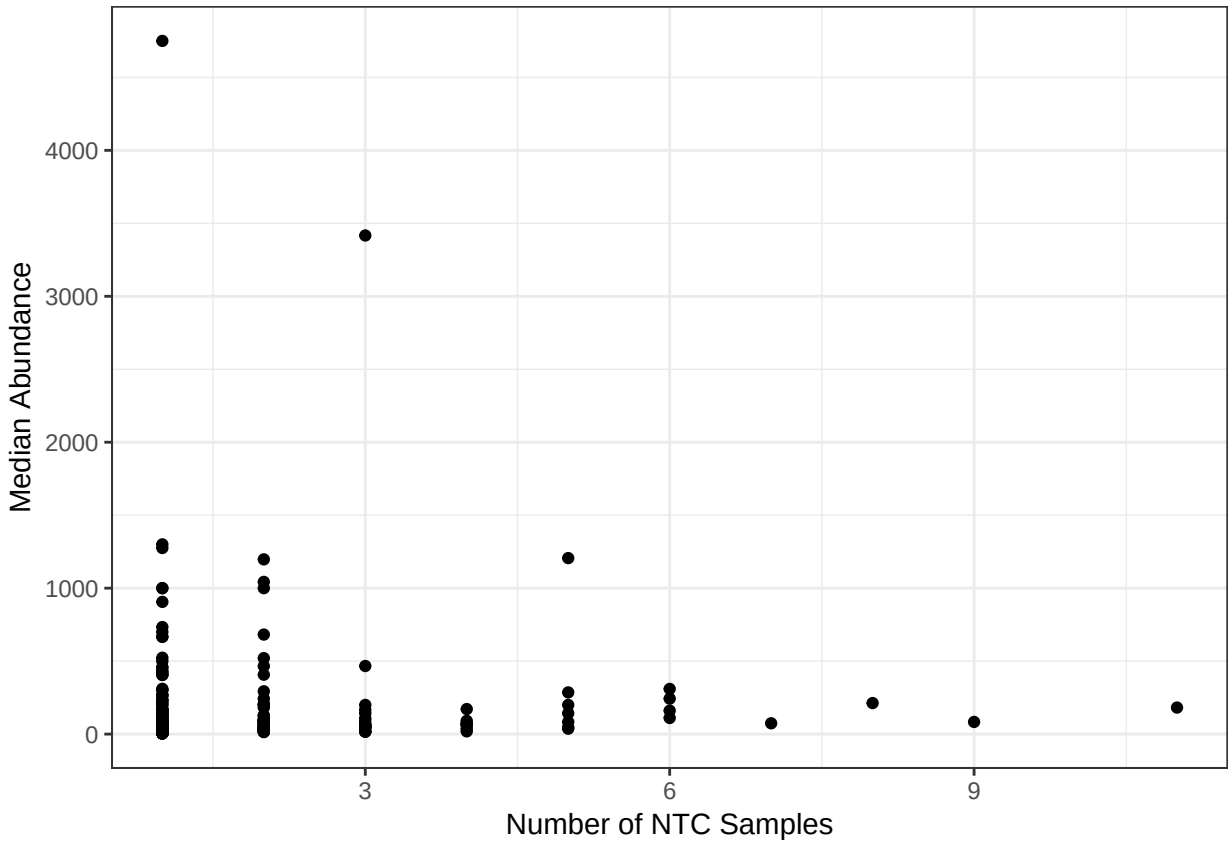

```
ntc_summary %>% ggplot() +
      geom_histogram(aes(x = n_present)) + theme_bw() +
      labs(x = "Number of NTC samples feature is present in.",
           y = "Number of Features")
```




```
ntc_summary %>% ggplot() +
      geom_point(aes(x = n_present, y = total_count)) + theme_bw() +
      labs(x = "Number of NTC Samples", y = "Total Abundance")
```




```
ntc_summary %>% ggplot() +
      geom_point(aes(x = n_present, y = med_count)) + theme_bw() +
      labs(x = "Number of NTC Samples", y = "Median Abundance")
```



High Abundance Features * Features with a high total abundance or count and low median count are features with a high abundance count in few samples and low abundance in other samples.

```
ntc_summary %>% filter(total_count > 4000) %>%
      arrange(desc(total_count)) %>% knitr::kable()
```

pipe	feature_id	total_count	med_count	n_present
mothur	Otu00002	20855.382	181.81818	11
dada2	SV727	10305.556	3416.66667	3
mothur	Otu00010	6757.330	45.45455	3
qiime	513445	6273.634	242.85714	6
dada2	SV1	5397.178	1205.88235	5
dada2	SV90	4750.000	4750.00000	1
qiime	348304	4134.931	466.66667	3

High Frequency Features

```
ntc_summary %>% filter(n_present > 6) %>%
      arrange(desc(n_present)) %>% knitr::kable()
```

pipe	feature_id	total_count	med_count	n_present
mothur	Otu00002	20855.3824	181.81818	11
mothur	Otu00003	751.5403	83.33333	9
mothur	Otu00001	2457.8397	212.53071	8
mothur	Otu00004	1122.8116	74.07407	7

Taxonomy of high abundance and fequency features

- Taxonomy of high abundance no template control features is not consistent between pipelines.

```
feature_taxa <- mrexp %>% map_df(fData, .id = "pipe") %>%
      dplyr::rename(feature_id = OTUname)
ntc_summary %>% filter(n_present > 6 | total_count > 4000) %>%
      left_join(feature_taxa) %>% select(pipe, feature_id, n_present, total_count, Rank5, Rank6) %>%
      arrange(Rank5) %>% knitr::kable()
```

pipe	feature_id	n_present	total_count	Rank5	Rank6
mothur	Otu00002	11	20855.3824	Bacteroidaceae	Bacteroides
mothur	Otu00004	7	1122.8116	Bacteroidaceae	Bacteroides
mothur	Otu00010	3	6757.3301	Bacteroidaceae	Bacteroides
dada2	SV1	5	5397.1779	Enterobacteriaceae	Escherichia/Shigella
dada2	SV727	3	10305.5556	Enterobacteriaceae	Serratia
mothur	Otu00001	8	2457.8397	Enterobacteriaceae	Escherichia/Shigella
qiime	348304	3	4134.9313	f__Bacteroidaceae	g__Bacteroides
qiime	513445	6	6273.6337	f__Bacteroidaceae	g__Bacteroides
dada2	SV90	1	4750.0000	Prevotellaceae	Prevotella_9
mothur	Otu00003	9	751.5403	Prevotellaceae	Prevotella

Saving data

```
ntc_summary %>% saveRDS("../data/ntc_features.rds")
```

Session information

Git repo commit information

```
repo <- repository(path = "../")
last_commit <- commits(repo)[[1]]
```

The current git commit of this file is 8cfea3db5826b66b88213e1000afd6647989f832, which is on the master branch and was made by nate-d-olson on 2017-04-06 18:38:12. The current commit message is bio replicate and pipe analysis with mixed-effects model. The repository is online at https://github.com/nate-d-olson/mgtst-pub

Platform Information

```
s_info <- devtools::session_info()
print(s_info$platform)
```

```
##  setting  value
##  version  R version 3.3.3 (2017-03-06)
##  system   x86_64, darwin15.6.0
##  ui       unknown
##  language (EN)
##  collate  en_US.UTF-8
```

```
##  tz       America/New_York
##  date     2017-04-11
```

Package Versions

```
s_info$packages %>% filter(`*` == "*") %>% select(-`*`) %>%
      knitr::kable()
```

package	version	date	source
bbmle	1.0.18	2016-02-11	CRAN (R 3.3.2)
Biobase	2.34.0	2016-11-07	Bioconductor
BiocGenerics	0.20.0	2016-11-07	Bioconductor
BiocParallel	1.8.1	2016-11-07	Bioconductor
Biostrings	2.42.1	2016-12-19	Bioconductor
DESeq	1.26.0	2016-11-28	Bioconductor
DESeq2	1.15.28	2017-02-02	bioc (readonly/DESeq2@125913)
dplyr	0.5.0	2016-06-24	CRAN (R 3.3.2)
edgeR	3.16.5	2017-02-02	Bioconductor
forcats	0.2.0	2017-01-23	CRAN (R 3.3.2)
foreach	1.4.3	2015-10-13	CRAN (R 3.3.1)
GenomeInfoDb	1.10.3	2017-03-28	Bioconductor
GenomicAlignments	1.10.1	2017-03-28	Bioconductor
GenomicRanges	1.26.4	2017-03-28	Bioconductor
ggplot2	2.2.1	2016-12-30	CRAN (R 3.3.2)
git2r	0.18.0	2017-01-01	CRAN (R 3.3.2)
glmnet	2.0-5	2016-03-17	CRAN (R 3.3.1)
IRanges	2.8.2	2017-03-28	Bioconductor
knitr	1.15.1	2016-11-22	CRAN (R 3.3.2)
lattice	0.20-34	2016-09-06	CRAN (R 3.3.3)
limma	3.30.13	2017-03-28	Bioconductor
locfit	1.5-9.1	2013-04-20	CRAN (R 3.3.1)
Matrix	1.2-8	2017-01-20	CRAN (R 3.3.3)
metagenomeSeq	1.16.0	2016-11-07	Bioconductor
modelr	0.1.0	2016-08-31	cran (@0.1.0)
permute	0.9-4	2016-09-09	CRAN (R 3.3.1)
phyloseq	1.19.1	2017-01-04	Bioconductor
ProjectTemplate	0.7	2016-08-11	CRAN (R 3.3.1)
purrr	0.2.2	2016-06-18	CRAN (R 3.3.1)
RColorBrewer	1.1-2	2014-12-07	CRAN (R 3.3.1)
readr	1.1.0	2017-03-22	CRAN (R 3.3.2)
readxl	0.1.1	2016-03-28	cran (@0.1.1)
Rqc	1.8.0	2016-11-07	Bioconductor
Rsamtools	1.26.1	2016-11-07	Bioconductor
S4Vectors	0.12.2	2017-03-28	Bioconductor
sads	0.3.1	2016-05-13	CRAN (R 3.3.2)
savR	1.12.0	2016-11-07	Bioconductor
ShortRead	1.32.1	2017-03-28	Bioconductor
stringr	1.2.0	2017-02-18	CRAN (R 3.3.2)
SummarizedExperiment	1.4.0	2016-11-07	Bioconductor
tibble	1.3.0	2017-04-01	CRAN (R 3.3.3)
tidyr	0.6.1	2017-01-10	CRAN (R 3.3.2)
tidyverse	1.1.1	2017-01-27	CRAN (R 3.3.2)

package	version	date	source
vegan	2.4-3	2017-04-07	CRAN (R 3.3.3)
XVector	0.14.1	2017-03-28	Bioconductor