

Identification of Informative and Uninformative Features

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Objective

Identification of informative and uninformative features. Informative features defined as features with observed counts in all pre-treatment samples and all titration samples and all or none of the post-treatment replicates.

Feature Categories

- Null - features not present in more than one PCR replicate for any sample of a biological replicate, and pipeline.

Informative

- Full - features present all PCR replicates for all samples (unmixed and titrations) for a biological replicate.
- Pre - present in all PCR replicates for unmixed pre-treatment samples, not observed in any PCR replicates of the unmixed post treatment samples, and present in all titration PCR replicates.
- Post - present in all PCR replicates for the unmixed post-treatment samples, not observed in any PCR replicates of the unmixed pre-treatment samples, and present all titration PCR replicates.

Characterization of low abundance features * Mix - features present in mixed samples but not observed in any of the unmixed sample PCR replicates.

```
## Extracting a tidy dataframe with count values from MRexpiment objects
get_count_df <- function(mrojb, agg_genus = FALSE){
  if(agg_genus){
    mrojb <- aggregateByTaxonomy(mrojb, lvl = "Rank6",
                                norm = FALSE, log = FALSE, sl = 1)
  }

  mrojb <- cumNorm(mrojb, p = 0.75)
  mrojb %>%
    # 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)
}

get_rep_info <- function(count_df){
  count_replicate_df <- count_df %>%
    mutate(detect = if_else(count > 1, 1, 0)) %>%
    group_by(pipe, biosample_id, titration, t_fctr, feature_id) %>%
    summarise(total_detect = sum(detect),
              n_replicates = n(),
              avg_non0_count = sum(count)/total_detect) %>%
    mutate(detect_prop = total_detect/n_replicates) %>%
}
```

```

        select(-total_detect)

count_replicate_df %>% ungroup() %>%
mutate(t_fctr = paste0("T",t_fctr)) %>%
select(pipe, biosample_id, feature_id, t_fctr, detect_prop)
}

assign_cat <- function(rep_info){
  prop_summary <- rep_info %>%
    group_by(pipe, biosample_id, feature_id) %>%
    summarise(prop_max = max(detect_prop),
              prop_min = min(detect_prop),
              prop_sum = sum(detect_prop))

  unmix_prop <- rep_info %>%
    filter(t_fctr %in% c("T0", "T20")) %>%
    spread(t_fctr, detect_prop)

  left_join(prop_summary, unmix_prop) %>%
    mutate(cat_null = if_else(prop_max == 0, 1, 0),
           cat_full = if_else(prop_min == 1, 1, 0),
           cat_near_full = if_else(prop_min == 0.75, 1, 0),
           cat_mix = if_else(prop_max == 1 & T0 == 0 & T20 == 0, 1, 0),
           ## Post prop 5 - expected at least three replicates for titrations 4, 5, 10, and 15
           ## Pre prop 3 - expected at least three replicates for titrations 1, 2, 3, and 4
           ## titration 4, is ~94% post
           ## titration 4, is ~94% post
           cat_pre = if_else(T20 == 1 & T0 == 0 & prop_sum == 8, 1, 0),
           cat_post = if_else(T0 == 1 & T20 == 0 & prop_sum == 8, 1, 0),
           cat_none = if_else(cat_null + cat_full + cat_near_full + cat_mix + cat_pre + cat_pos
    }

```

Feature Level Category Assignments

```

count_df <- mrex %>% map_df(get_count_df, .id = "pipe") %>%
  left_join(pData(mrex$dada2)) %>%
  filter(biosample_id != "NTC")

#count_df
rep_info <- get_rep_info(count_df)

#rep_info
feature_info <- assign_cat(rep_info)

feature_cat <- feature_info %>%
  select(-prop_max, -prop_min, -prop_sum, -T0, -T20) %>%
  gather(cat, value, -pipe, -biosample_id, -feature_id) %>%
  filter(value == 1) %>% select(-value)

feature_cat %>% saveRDS("../data/feature_categories_df.rds")

```

Category Sanity Check

```
cat_check <- feature_cat %>%
  group_by(pipe, biosample_id, feature_id) %>%
  summarise(n_cat = n())
cat_check %>% filter(n_cat != 1)

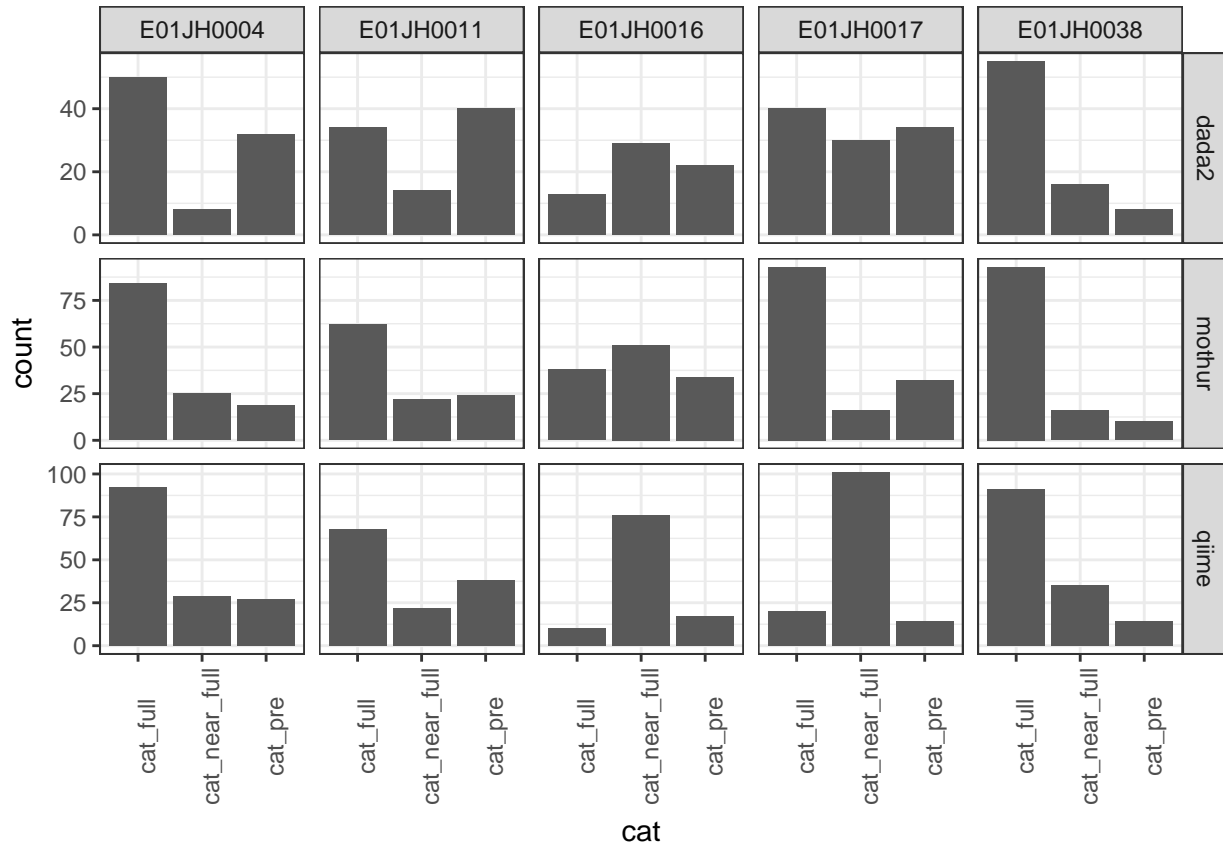
## Source: local data frame [0 x 4]
## Groups: pipe, biosample_id [0]
##
## # ... with 4 variables: pipe <chr>, biosample_id <chr>, feature_id <chr>,
## #   n_cat <int>

# cat_check <- feature_info %>%
#   select(-prop_max, -prop_min, -prop_sum, -T0, -T20) %>%
#   gather(cat, value, -pipe, -biosample_id, -feature_id) %>%
#   group_by(pipe, biosample_id, feature_id) %>%
#   mutate(n_cat = sum(value)) %>%
#   filter(n_cat != 1, value != 0)
# cat_check %>% arrange(feature_id)

prop_summary <- rep_info %>%
  group_by(pipe, biosample_id, feature_id) %>%
  summarise(prop_max = max(detect_prop),
            prop_min = min(detect_prop),
            prop_sum = sum(detect_prop))
```

Informative Features

```
feature_cat %>% filter(cat != "cat_null", cat != "cat_none", cat != "cat_mix") %>%
  ggplot() + geom_bar(aes(x = cat)) +
  facet_grid(pipe ~ biosample_id, scales = "free_y") +
  theme_bw() + theme(axis.text.x = element_text(angle = 90))
```



Recovering Semi-Informative Features

```
cat_none_df <- feature_cat %>% left_join(rep_info) %>% filter(cat == "cat_none")
total_prop_cat_none <- cat_none_df %>% group_by(pipe, biosample_id, feature_id) %>%
  summarise(total_prop = sum(detect_prop))
```

Most of the uncategorized features were observed in less than 4 PCR replicates

```
total_prop_cat_none %>% mutate(total_prop = floor(total_prop)) %>%
  group_by(pipe, total_prop) %>% summarise(count = n()) %>%
  spread(pipe, count) %>% knitr::kable()
```

total_prop	dada2	mothur	qiime
0	877	28995	14341
1	405	1090	2473
2	93	337	877
3	75	201	447
4	82	148	298
5	87	140	270
6	98	156	189
7	186	168	310
8	64	103	113

Assuming 4 PCR replicates for all samples. Using proportions if samples are excluded from analysis, for example samples with few reads compared to the rest of the samples.

```
cat_none_df %>% filter(!(detect_prop %in% c(0,0.25,0.5,0.75,1)))
```

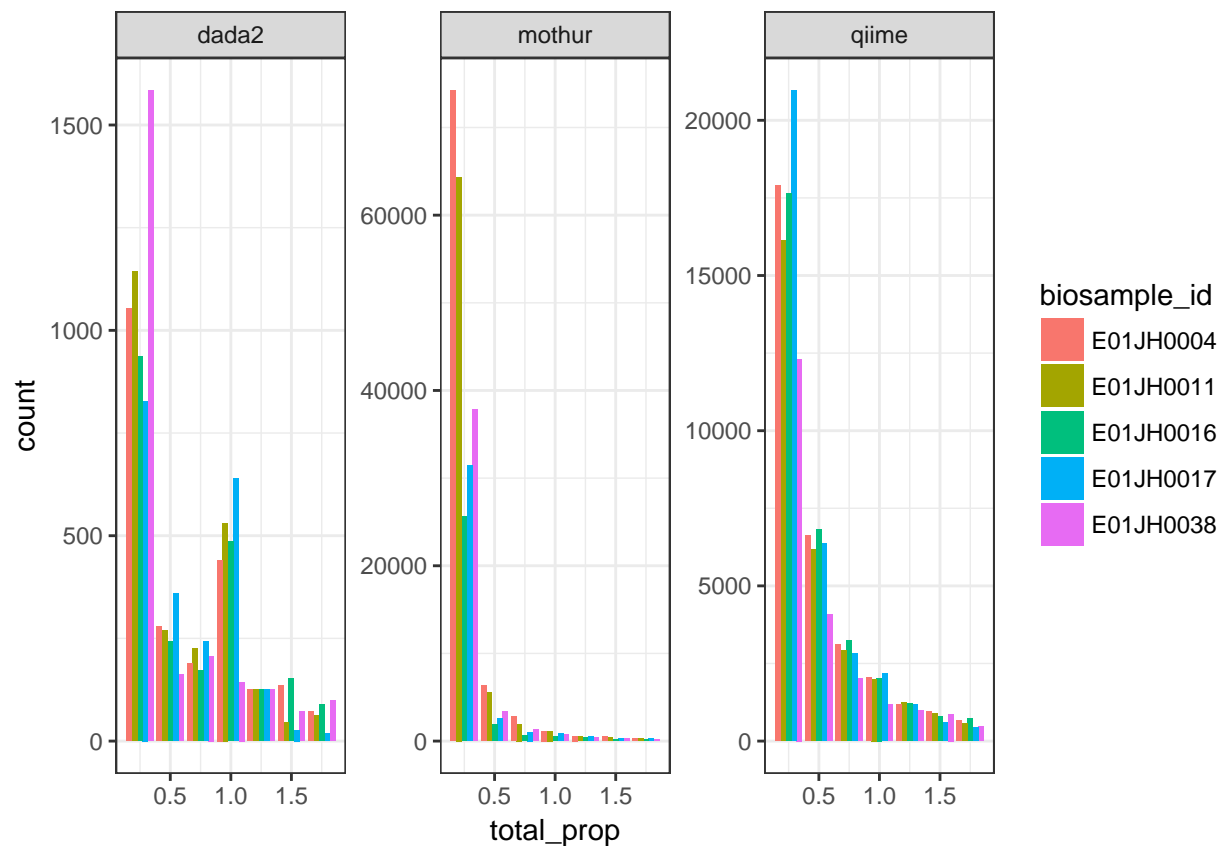
```
## Source: local data frame [0 x 6]
## Groups: pipe, biosample_id [0]
##
## # ... with 6 variables: pipe <chr>, biosample_id <chr>, feature_id <chr>,
## #   cat <chr>, t_fctr <chr>, detect_prop <dbl>
```

What is the detect_proportions for samples with total prop < 2.

```
cat_none_low <- cat_none_df %>% group_by(pipe, biosample_id, feature_id) %>%
  mutate(total_prop = sum(detect_prop)) %>% filter(total_prop > 0, total_prop < 2)
```

Most of the low total detect proportion features were only observed in one PCR replicate.

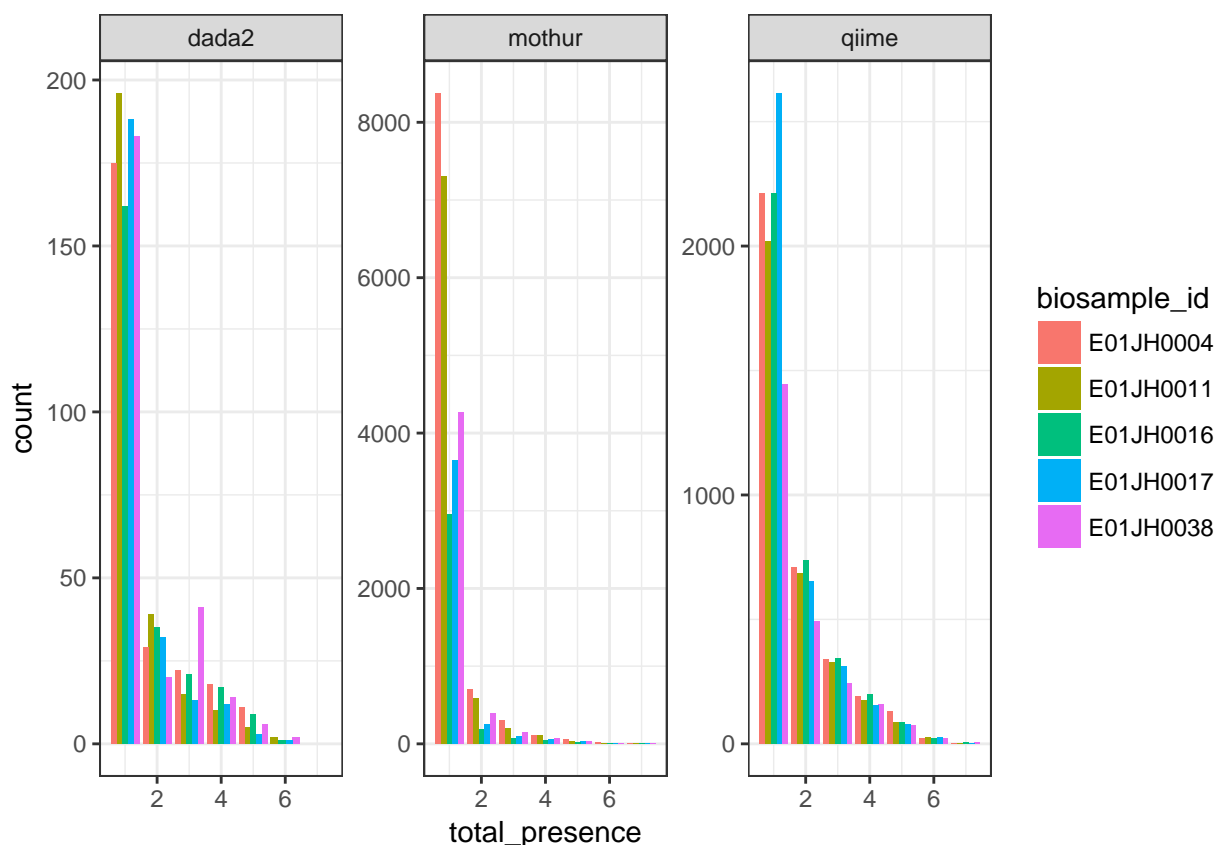
```
cat_none_low %>%
  ggplot() + geom_bar(aes(x = total_prop, fill = biosample_id),
    position = "dodge") +
  facet_wrap(~pipe, scales = "free_y") + theme_bw()
```



```
# cat_none_low %>% filter(total_prop > 0.5) %>% spread(t_fctr, detect_prop) %>% arrange(total_prop)
```

Most of the low detect features are only present in 1 sample

```
cat_none_low %>% mutate(pa = if_else(detect_prop == 0, 0, 1)) %>% summarise(total_presence = sum(pa)) %>%
  ggplot() + geom_bar(aes(x = total_presence, fill = biosample_id),
    position = "dodge") +
  facet_wrap(~pipe, scales = "free_y") + theme_bw()
```

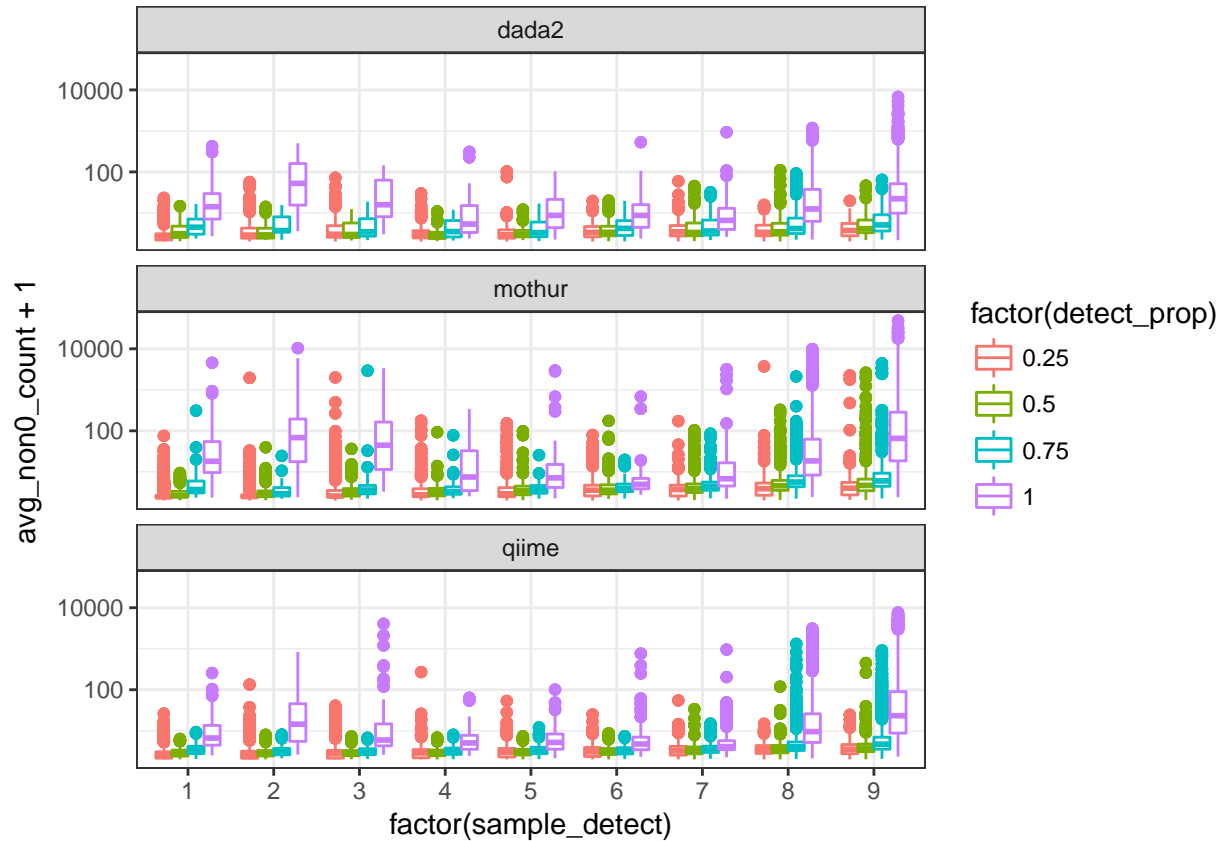


Open Question Are low detect features due to random sampling or bioinformatic/ experimental artifact. *
 Approach - average counts by number of PCR replicates and number of samples with observed counts.

```
count_replicate_df <- count_df %>%
  mutate(detect = if_else(count > 1, 1, 0)) %>%
  group_by(pipe, biosample_id, titration, t_fctr, feature_id) %>%
  mutate(total_detect = sum(detect),
         n_replicates = n(),
         avg_non0_count = sum(count)/total_detect) %>%
  mutate(detect_prop = total_detect/n_replicates) %>%
  select(-total_detect)
count_rep_pa <- count_replicate_df %>%
  group_by(pipe, biosample_id, feature_id, t_fctr) %>%
  summarise(pa = if_else(sum(detect) != 0, 1, 0)) %>%
  group_by(pipe, biosample_id, feature_id) %>%
  mutate(sample_detect = sum(pa))
count_replicate_pa_df <- count_rep_pa %>% filter(sample_detect != 0) %>% left_join(count_replicate_df)
```

X-axis: Number of samples out of the 2 unmixed and 7 titrations with at least one of the four PCR replicates with observed counts Y-axis: Mean counts for PCR replicates with non-zero count values Color: Of the sample (either unmixed or titration) proportion of PCR replicates with observed counts

```
count_replicate_pa_df %>% ggplot() +
  geom_boxplot(aes(y = avg_non0_count + 1, x = factor(sample_detect), color = factor(detect_prop))) +
  scale_y_log10() + facet_wrap(~pipe, ncol = 1) + theme_bw()
```



Extracting additional informative features

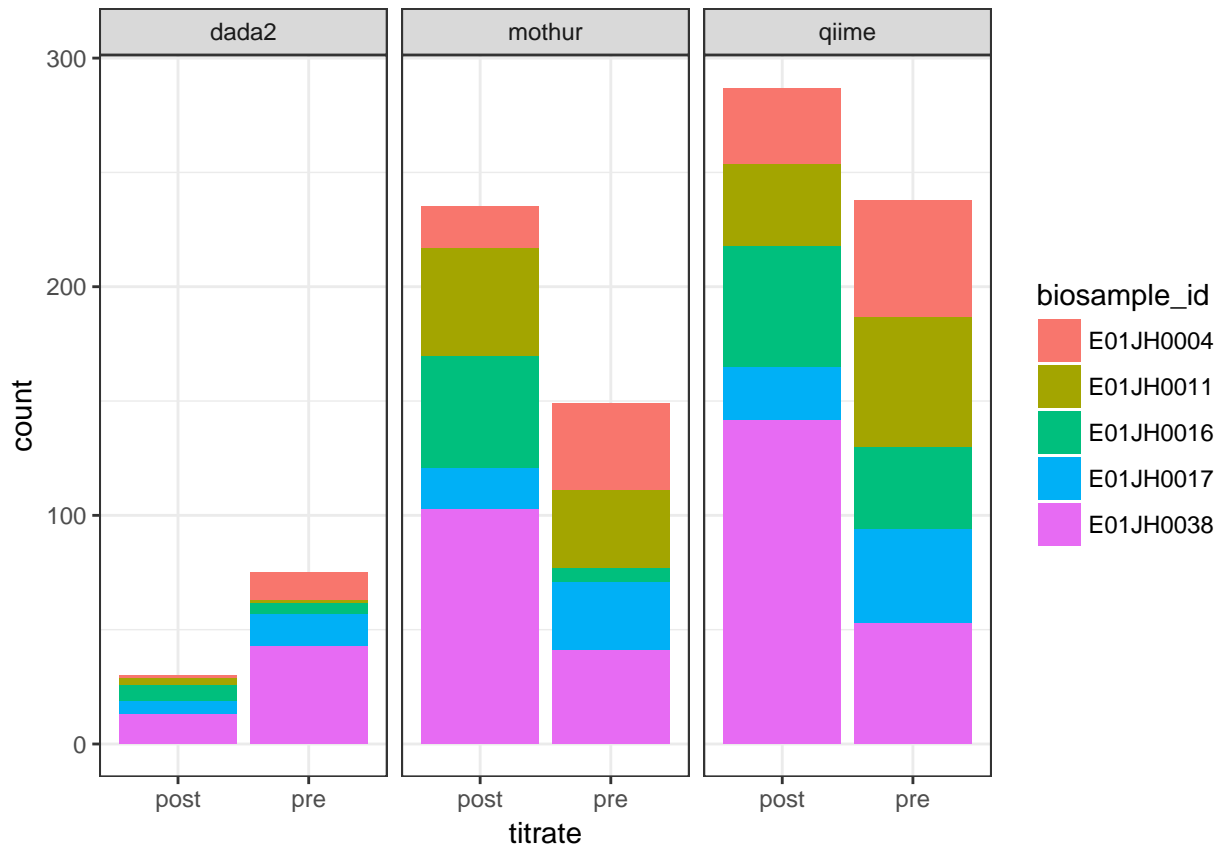
```
pre_post_titrate <- cat_none_df %>% spread(t_fctr, detect_prop) %>%
  mutate(pre_titration = if_else(T20 != 0 & T15 != 0 &
    T20 >= T15 & T15 >= T10 & T10 >= T5 &
    T5 >= T4 & T4 >= T3 & T3 >= T2 &
    T2 >= T1 & T1 >= T0, 1, 0),
  post_titration = if_else(T0 != 0 & T1 != 0 &
    T20 <= T15 & T15 <= T10 & T10 <= T5 &
    T5 <= T4 & T4 <= T3 & T3 <= T2 &
    T2 <= T1 & T1 <= T0, 1, 0)) %>%
  filter(pre_titration == 1 | post_titration == 1)
```

Feature assigned to both groups if present in the same number of PCR replicates for all samples

```
pre_post_titrate %>% mutate(t_sum = pre_titration + post_titration) %>% filter(t_sum > 1)
```

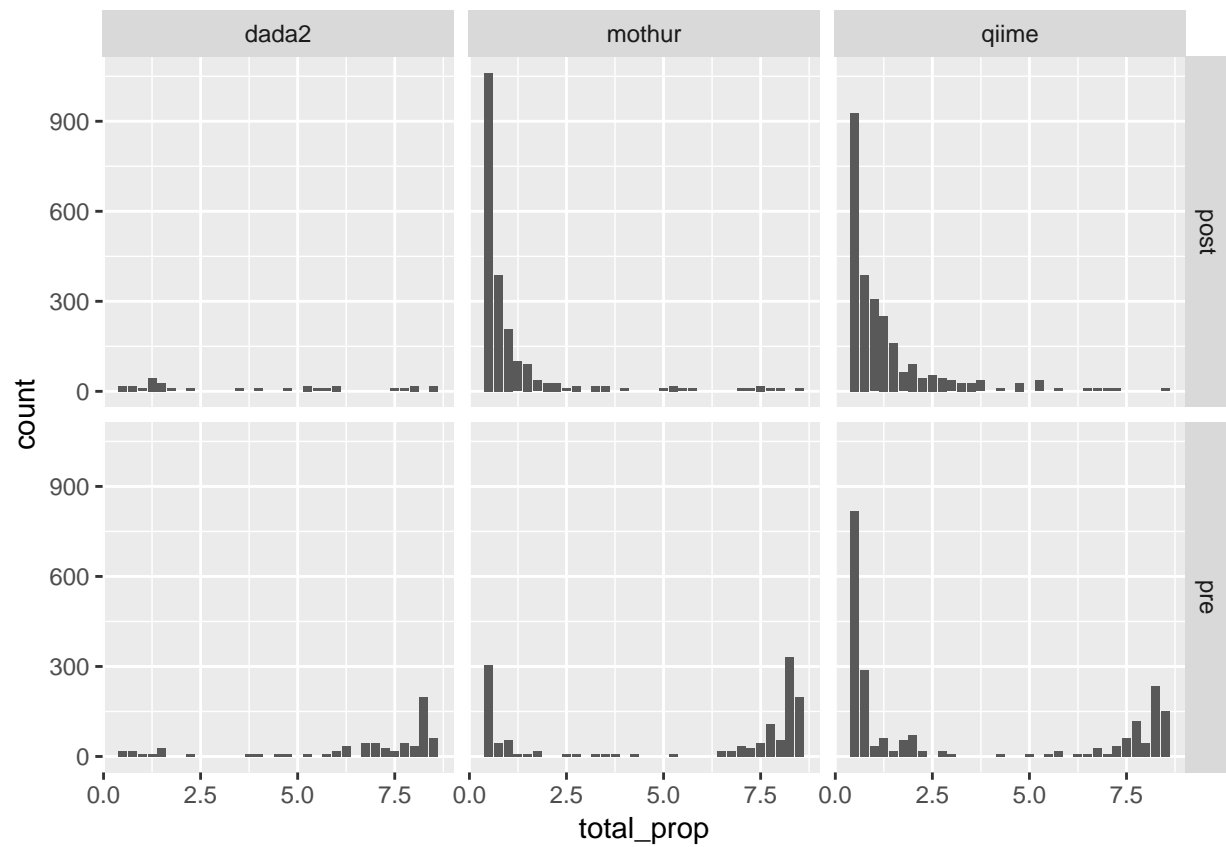
```
## Source: local data frame [0 x 16]
## Groups: pipe, biosample_id [0]
##
## # ... with 16 variables: pipe <chr>, biosample_id <chr>, feature_id <chr>,
## #   cat <chr>, T0 <dbl>, T1 <dbl>, T10 <dbl>, T15 <dbl>, T2 <dbl>,
## #   T20 <dbl>, T3 <dbl>, T4 <dbl>, T5 <dbl>, pre_titration <dbl>,
## #   post_titration <dbl>, t_sum <dbl>
```

```
pre_post_titrate %>% mutate(t_sum = pre_titration + post_titration) %>% filter(t_sum == 1) %>%
  mutate(titrate = if_else(pre_titration == 1, "pre", "post")) %>%
  ggplot() + geom_bar(aes(x = titrate, fill = biosample_id)) + facet_wrap(~pipe) + theme_bw()
```



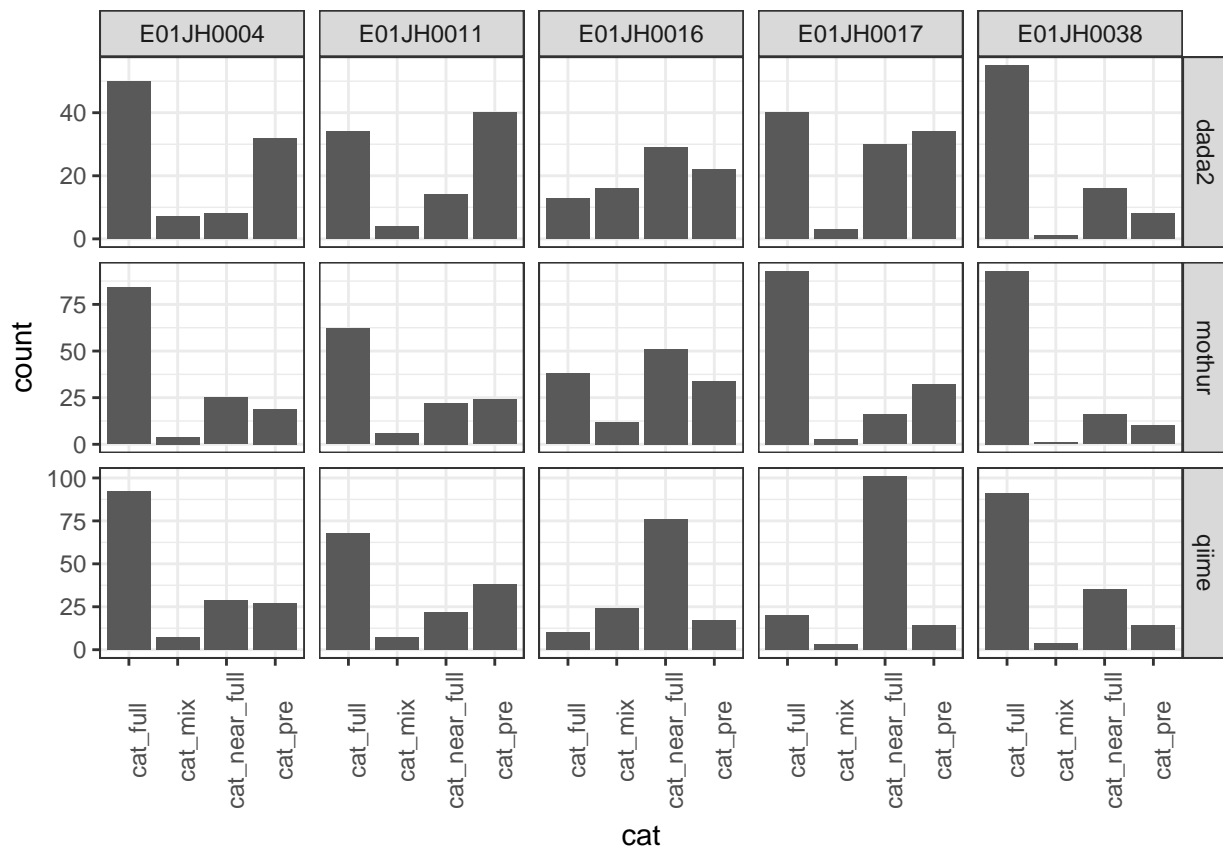
```
total_prop_titrate <- pre_post_titrate %>% mutate(t_sum = pre_titration + post_titration) %>% filter(t_sum == 1) %>%
  mutate(titrate = if_else(pre_titration == 1, "pre", "post")) %>%
  select(pipe, biosample_id, feature_id, titrate) %>% left_join(cat_none_df) %>%
  group_by(pipe, biosample_id, feature_id, titrate) %>% mutate(total_prop = sum(detect_prop))

total_prop_titrate %>% ggplot() + geom_bar(aes(x = total_prop)) + facet_grid(titrate~pipe)
```

Summary Figures

```
feature_cat %>% filter(cat != "cat_null", cat != "cat_none") %>%
  ggplot() + geom_bar(aes(x = cat)) +
  facet_grid(pipe ~ biosample_id, scales = "free_y") +
  theme_bw() + theme(axis.text.x = element_text(angle = 90))
```



Genus Level Category Assignments

```
count_df <- mrex %>% map_df(get_count_df, agg_genus = TRUE, .id = "pipe") %>%
  left_join(pData(mrex$dada2)) %>%
  filter(biosample_id != "NTC")

rep_info <- get_rep_info(count_df)
rep_info %>% saveRDS("../data/genus_rep_info_df.rds")

feature_info <- assign_cat(rep_info)
feature_info %>% saveRDS("../data/genus_info_df.rds")

feature_cat <- feature_info %>%
  select(pipe, biosample_id, feature_id,
         cat_null, cat_full, cat_mix, cat_pre, cat_post, cat_none) %>%
  gather(cat, value, -pipe, -biosample_id, -feature_id) %>%
  filter(value == 1) %>% select(-value)

feature_cat %>% saveRDS("../data/genus_categories_df.rds")
```

Category Sanity Check

```
cat_check <- feature_cat %>%
  group_by(pipe, biosample_id, feature_id) %>%
```

```

summarise(n_cat = n())
cat_check %>% filter(n_cat != 1)

## Source: local data frame [0 x 4]
## Groups: pipe, biosample_id [0]
##
## # ... with 4 variables: pipe <chr>, biosample_id <chr>, feature_id <chr>,
## #   n_cat <int>
# cat_check <- feature_categories %>%
#   select(pipe, biosample_id, feature_id,
#     cat_null, cat_full, cat_mix, cat_pre, cat_post, cat_none) %>%
#   gather(cat, value, -pipe, -biosample_id, -feature_id) %>%
#   group_by(pipe, biosample_id, feature_id) %>%
#   mutate(n_cat = sum(value)) %>%
#   filter(n_cat != 1, value != 0)
# cat_check %>% arrange(feature_id)

```

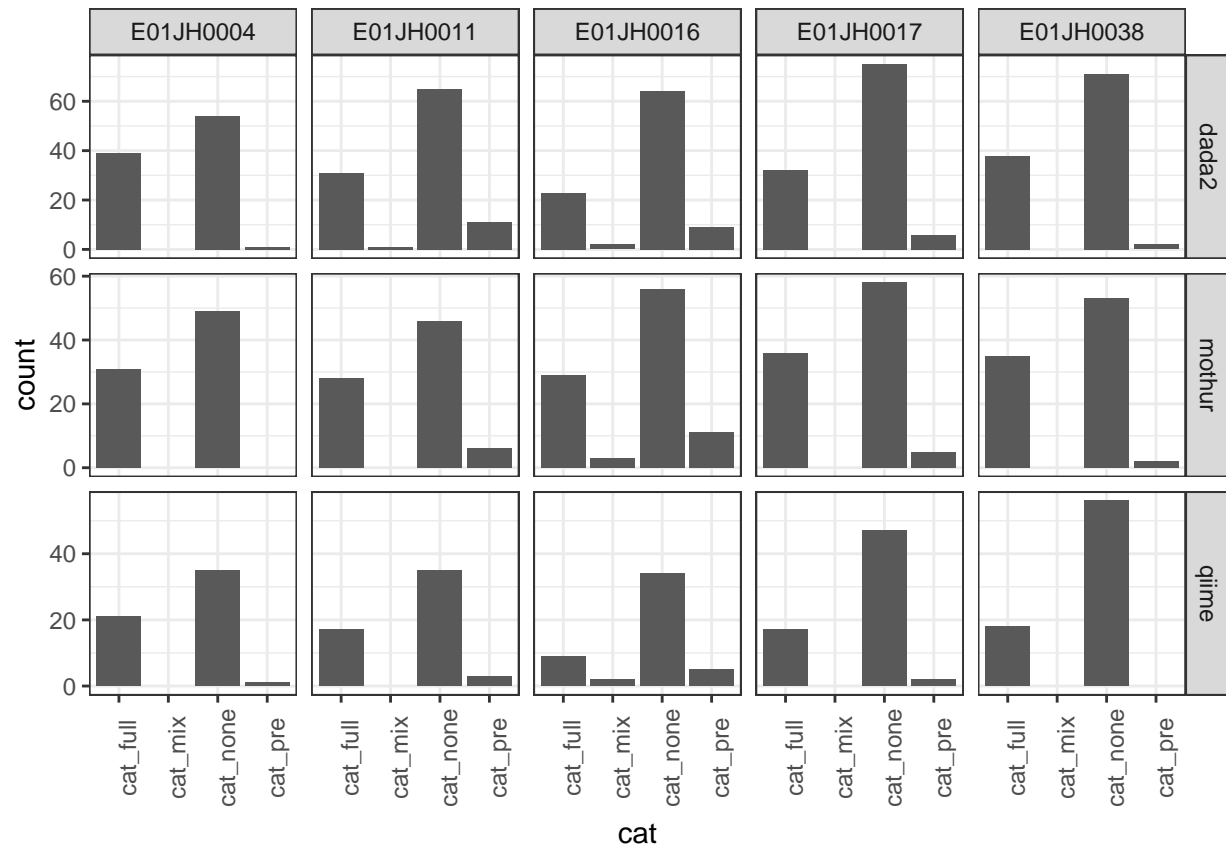
Summary Figures

Larger proportion of full category features and fewer mix specific features when aggregating to the genus level compared to unaggregated features.

```

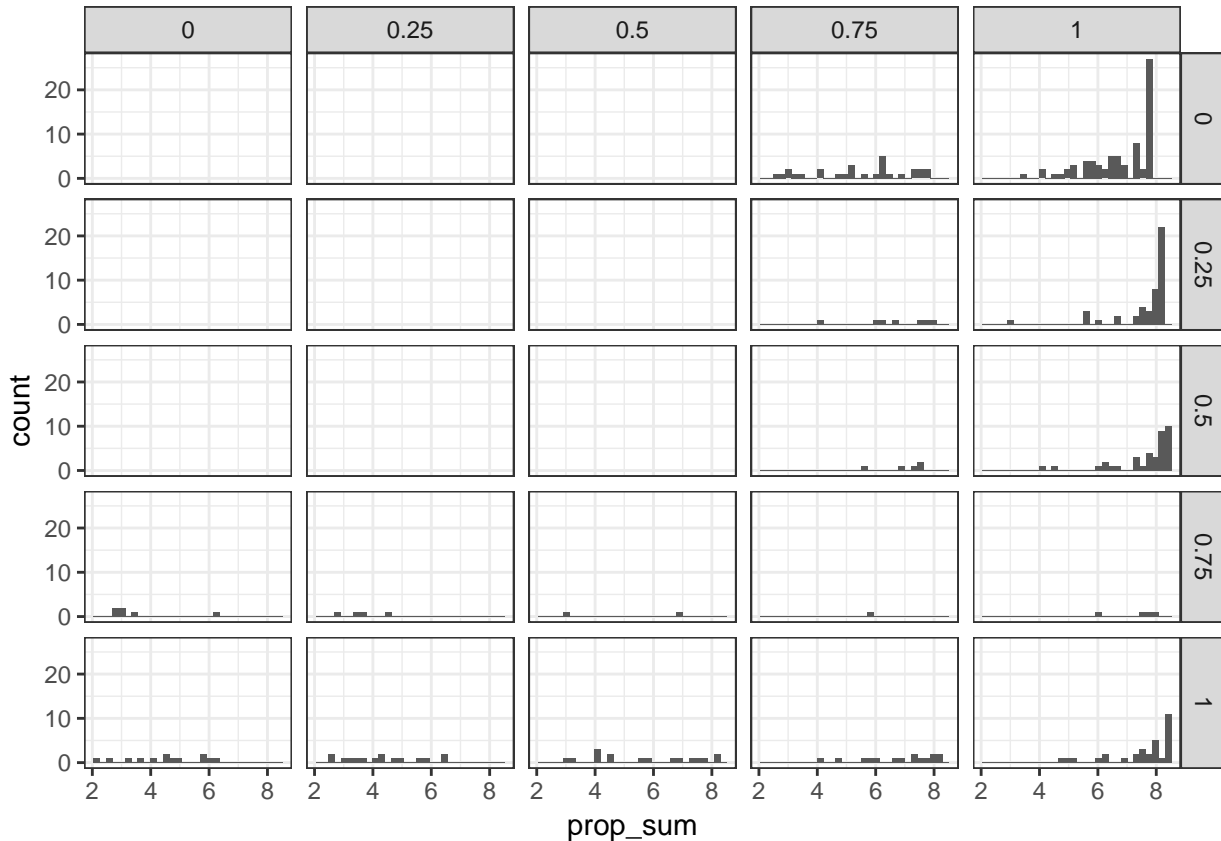
feature_cat %>% filter(cat != "cat_null") %>%
  ggplot() + geom_bar(aes(x = cat)) +
  facet_grid(pipe ~ biosample_id, scales = "free_y") +
  theme_bw() + theme(axis.text.x = element_text(angle = 90))

```



While there are a large number of unclassified features, few are potentially informative. Ones that stand out are features detected in 4 T0 (pre-treatment features) with prop sum value close to 8.

```
feature_info %>% filter(cat_none == 1, prop_sum > 2, T0 > 0.5 | T20 > 0.5) %>%
  ggplot() + geom_histogram(aes( x= prop_sum)) + facet_grid(T0 ~ T20) + theme_bw()
```



Alternative Characterization

```
detect_idx <- rep_info %>% mutate(detect = detect_prop * 4,
  t_fctr = fct_relevel(t_fctr, paste0("T",c(0:5,10,15,20))),
  idx_buffer = 10^(as.numeric(t_fctr)-1),
  idx = detect * idx_buffer) %>%
  group_by(pipe,biosample_id,feature_id) %>% mutate(idx = sum(idx)) %>%
  filter(idx != 0)

detect_idx %>%
  group_by(idx, pipe, biosample_id, feature_id) %>%
  mutate(total_detect = sum(detect)) %>%
  filter(total_detect > 9) %>%
  group_by(idx, pipe) %>%
  summarise(count = n()) %>%
  arrange(desc(count))
```

```
## Source: local data frame [348 x 3]
## Groups: idx [313]
##
```

```
##           idx   pipe count
##           <dbl> <chr> <int>
## 1 4444444444 dada2 1467
## 2 4444444444 mothur 1431
## 3 4444444444 qiime 738
## 4 4444444440 dada2 261
## 5 4444444440 mothur 216
## 6 4443444444 dada2 135
## 7 4444344444 dada2 117
## 8 4444444440 qiime 99
## 9 4444444441 dada2 99
## 10 4443444440 dada2 90
## # ... with 338 more rows
```

Appendix

Table with the total proportion of PCR replicates by biosample and pipeline.

```
total_prop_cat_none %>% mutate(total_prop = floor(total_prop)) %>%
  group_by(pipe, biosample_id, total_prop) %>% summarise(count = n()) %>%
  spread(biosample_id, count) %>% knitr::kable()
```

pipe	total_prop	E01JH0004	E01JH0011	E01JH0016	E01JH0017	E01JH0038
dada2	0	169	182	150	159	217
dada2	1	86	85	95	90	49
dada2	2	14	18	18	20	23
dada2	3	17	9	17	15	17
dada2	4	15	12	17	20	18
dada2	5	14	13	20	19	21
dada2	6	9	12	26	19	32
dada2	7	24	21	71	46	24
dada2	8	13	7	9	14	21
mothur	0	9266	7982	3125	3893	4729
mothur	1	284	270	136	218	182
mothur	2	99	62	44	53	79
mothur	3	51	41	25	33	51
mothur	4	25	27	25	36	35
mothur	5	27	24	26	22	41
mothur	6	29	20	30	36	41
mothur	7	24	21	57	35	31
mothur	8	20	22	12	23	26
qiime	0	3070	2801	3075	3351	2044
qiime	1	539	522	531	489	392
qiime	2	174	205	181	163	154
qiime	3	87	107	88	78	87
qiime	4	61	70	53	59	55
qiime	5	51	49	60	50	60
qiime	6	42	49	36	33	29
qiime	7	56	57	81	69	47
qiime	8	21	29	15	22	26

Session 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

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)
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

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