Mock community example

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For installtion instructions please see https://github.com/cdiener/microbiome. We recommend using the docker image in the cloud or locally as this ensures that all requirements are fulfilled in the correct version.

0.1 Loading dependencies

In order to facilitate use of the pipeline we provide the `mbtools` R package in this repository which serves two major purposes:

1. It implements additional helper functions for the analysis and benchmarking of microbial community data.
2. It depends on all additional packages required for analysis and load them upon import.

So loading `mbtools` should be the first step when running the analysis

```
library(mbtools)
```

0.2 Getting the mock community data

`mbtools` includes helper functions to obtain benchmark mock data sets from the mockrobiota database. For instance to download the mock-3 data set (relatively small) we can use

```
if (!file.exists("mock3.rds")) {
    mock <- mockrobiota("mock-3", "mock3")
    saveRDS(mock, "mock3.rds")
} else mock <- readRDS("mock3.rds")
```

Here `mock` now includes annotations for the data set as a list.

```
names(mock)
```

```
##  [1] "description" "forward"     "reverse"     "index"       "citation"
##  [6] "fragment"    "equipment"   "samples"     "tax_gg"      "tax_silva"
```

```
mock$samples
```

```
##                 SampleID BarcodeSequence LinkerPrimerSequence
## 1      HMPMockV1.1.Even1    TGTACGGATAAC  GTGCCAGCMGCCGCGGTAA
## 2      HMPMockV1.1.Even2    CAAATGGTCGTC  GTGCCAGCMGCCGCGGTAA
## 3 HMPMockV1.2.Staggered1    AATCAACTAGGC  GTGCCAGCMGCCGCGGTAA
## 4 HMPMockV1.2.Staggered2    ACACATAAGTCG  GTGCCAGCMGCCGCGGTAA
##          ReversePrimer PrimerName Description
## 1 GGACTACHVGGGTWTCTAAT  515f-806r    Nenehozi
## 2 GGACTACHVGGGTWTCTAAT  515f-806r    Tofekoca
## 3 GGACTACHVGGGTWTCTAAT  515f-806r    Kalofiyo
## 4 GGACTACHVGGGTWTCTAAT  515f-806r    Pewizifo
```

0.3 Preparing the reads

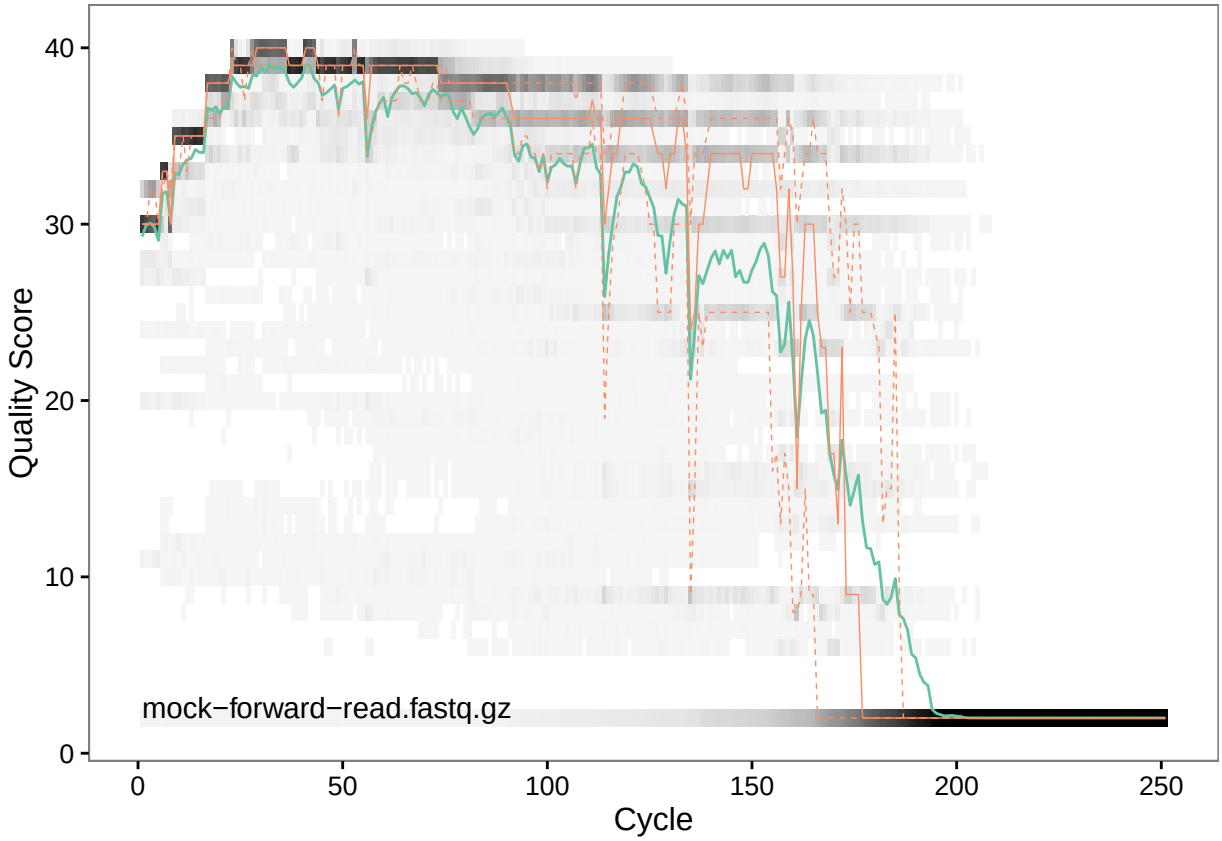
As we can see we have 3 read files, the forward and backward reads and one index file mapping the sample barcodes to the sequences. However, we have 4 samples: a uniform and staggered community in duplicates each. In order to map sequence variants to samples `dada2` expects read files to be separated by sample. `mbtools` includes helper functions to obtain this splitting.

```
reads <- c(mock$forward, mock$reverse)
barcodes <- mock$samples$BarcodeSequence
names(barcodes) <- mock$samples[,1]
bcs <- split_barcodes(reads, mock$index, "split", barcodes)
fwd <- list.files("split", pattern="forward", full.names=T)
bwd <- list.files("split", pattern="reverse", full.names=T)

fwd
```

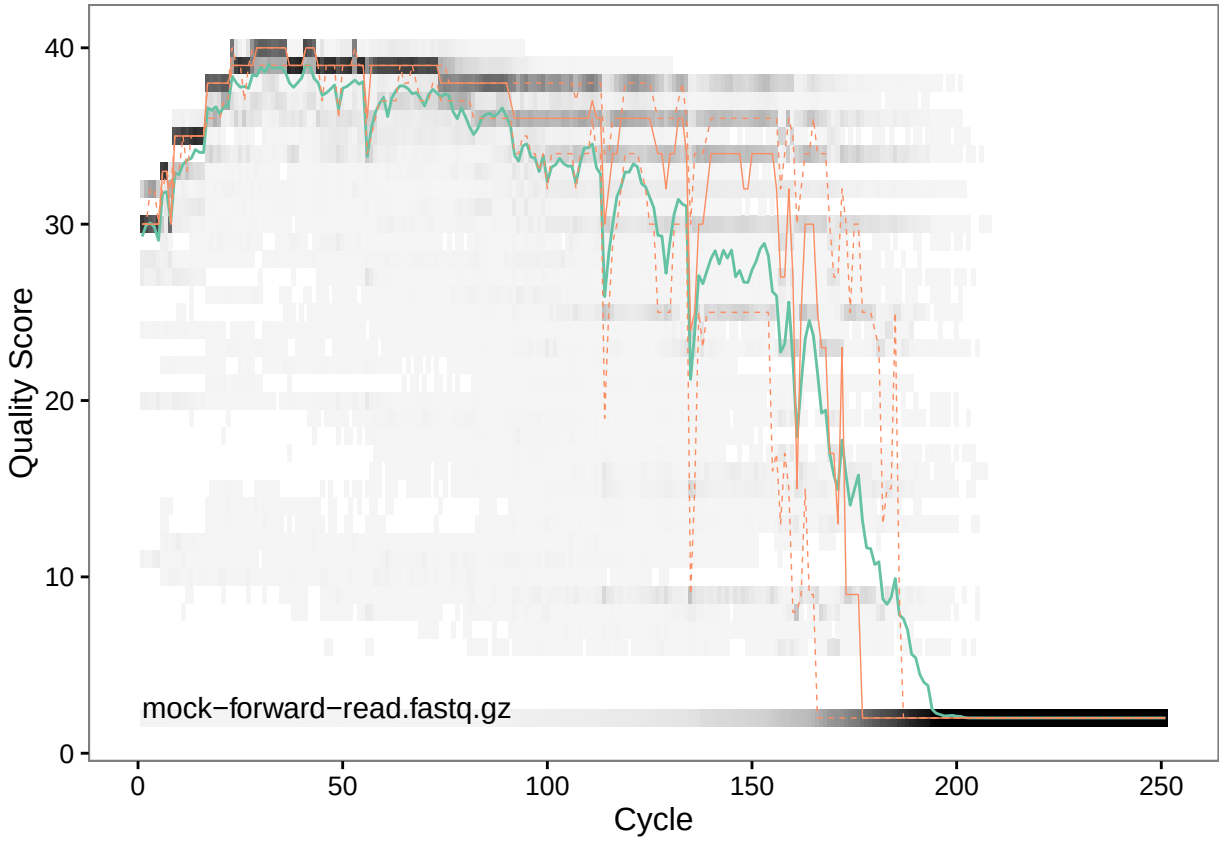
```
## [1] "split/HMPMockV1.1.Even1_mock-forward-read.fastq.gz"
## [2] "split/HMPMockV1.1.Even2_mock-forward-read.fastq.gz"
## [3] "split/HMPMockV1.2.Staggered1_mock-forward-read.fastq.gz"
## [4] "split/HMPMockV1.2.Staggered2_mock-forward-read.fastq.gz"
```

As we see that now gives us the reads separated by sample. The orginal read still include some valid information, particularly they include the read qualities across all samples.

```
plotQualityProfile(reads[1])
```




```
plotQualityProfile(reads[1])
```



As we can see both qualities detoriate extremely with read lengths. Thus, we will now pass trim the reads. From the plots we can see that the forward reads have decent quality up to a length of 150 bp whereas the reverse reads are acceptable up to 100 bp.

```
dir.create("filtered")
```

```
## Warning in dir.create("filtered"): 'filtered' already exists
```

```
fwd_filt <- file.path("filtered", basename(fwd))
bwd_filt <- file.path("filtered", basename(bwd))
for (i in seq_along(fwd)) {
    fastqPairedFilter(c(fwd[i], bwd[i]), c(fwd_filt[i], bwd_filt[i]),
        truncLen=c(150, 100), compress=T, verbose=T)
}
```

```
## Overwriting file:filtered/HMPMockV1.1.Even1_mock-forward-read.fastq.gz

## Overwriting file:filtered/HMPMockV1.1.Even1_mock-reverse-read.fastq.gz

## Read in 10536 paired-sequences, output 8792 filtered paired-sequences.

## Overwriting file:filtered/HMPMockV1.1.Even2_mock-forward-read.fastq.gz

## Overwriting file:filtered/HMPMockV1.1.Even2_mock-reverse-read.fastq.gz
```

```
## Read in 13000 paired-sequences, output 10147 filtered paired-sequences.
## Overwriting file:filtered/HMPMockV1.2.Staggered1_mock-forward-read.fastq.gz
## Overwriting file:filtered/HMPMockV1.2.Staggered1_mock-reverse-read.fastq.gz
## Read in 23463 paired-sequences, output 19012 filtered paired-sequences.
## Overwriting file:filtered/HMPMockV1.2.Staggered2_mock-forward-read.fastq.gz
## Overwriting file:filtered/HMPMockV1.2.Staggered2_mock-reverse-read.fastq.gz
## Read in 8980 paired-sequences, output 7263 filtered paired-sequences.
```

We will follow by dereplicating the reads which will yield the unique sequences in the samples.

```
derepFs <- derepFastq(fwd_filt, verbose=TRUE)
```

```
## Dereplicating sequence entries in Fastq file: filtered/HMPMockV1.1.Even1_mock-forward-read.fastq.gz
## Encountered 1302 unique sequences from 8792 total sequences read.
## Dereplicating sequence entries in Fastq file: filtered/HMPMockV1.1.Even2_mock-forward-read.fastq.gz
## Encountered 1644 unique sequences from 10147 total sequences read.
## Dereplicating sequence entries in Fastq file: filtered/HMPMockV1.2.Staggered1_mock-forward-read.fast
## Encountered 2597 unique sequences from 19012 total sequences read.
## Dereplicating sequence entries in Fastq file: filtered/HMPMockV1.2.Staggered2_mock-forward-read.fast
## Encountered 1126 unique sequences from 7263 total sequences read.
```

```
derepRs <- derepFastq(bwd_filt, verbose=TRUE)
```

```
## Dereplicating sequence entries in Fastq file: filtered/HMPMockV1.1.Even1_mock-reverse-read.fastq.gz
## Encountered 1681 unique sequences from 8792 total sequences read.
## Dereplicating sequence entries in Fastq file: filtered/HMPMockV1.1.Even2_mock-reverse-read.fastq.gz
## Encountered 2268 unique sequences from 10147 total sequences read.
## Dereplicating sequence entries in Fastq file: filtered/HMPMockV1.2.Staggered1_mock-reverse-read.fast
## Encountered 3176 unique sequences from 19012 total sequences read.
## Dereplicating sequence entries in Fastq file: filtered/HMPMockV1.2.Staggered2_mock-reverse-read.fast
## Encountered 1517 unique sequences from 7263 total sequences read.
```

```
# Name the derep-class objects by the sample names
names(derepFs) <- names(derepRs) <- names(barcodes)
```

0.4 Obtaining the sequence variants (sequence OTUs)

With the trimmed and dereplicated reads we can now advance to running the dada2 algorithm to discover the unqiue sequence variants in our reads. We will do this separately for the forward and backward reads.

```
dadaFs <- dada(derepFs, err=NULL, selfConsist = TRUE)
```

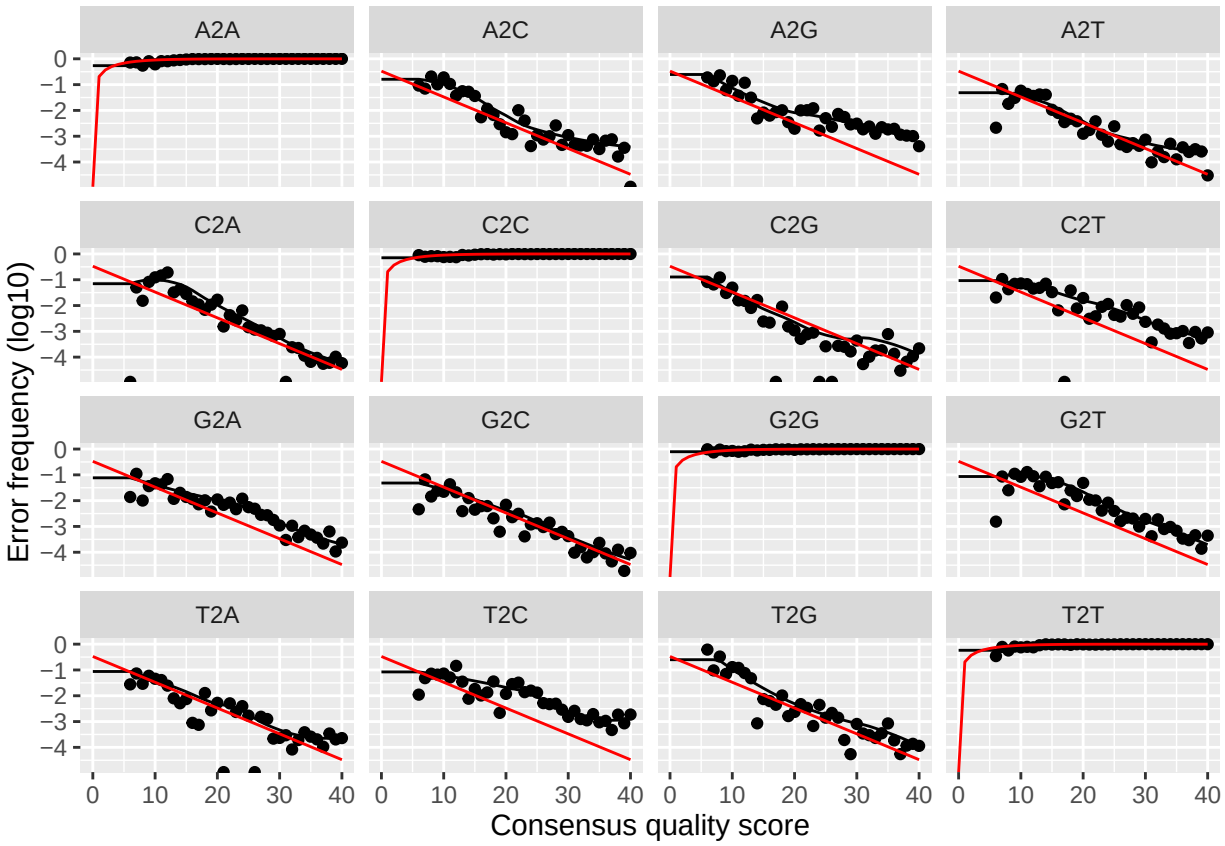
```
## Initial error matrix unspecified. Error rates will be initialized to the maximum possible estimate f

## Initializing error rates to maximum possible estimate.
## Sample 1 - 8792 reads in 1302 unique sequences.
## Sample 2 - 10147 reads in 1644 unique sequences.
## Sample 3 - 19012 reads in 2597 unique sequences.
## Sample 4 - 7263 reads in 1126 unique sequences.
##    selfConsist step 2
##    selfConsist step 3
##    selfConsist step 4
##
##
## Convergence after  4  rounds.
```

```
dadaRs <- dada(derepRs, err=NULL, selfConsist = TRUE)
```

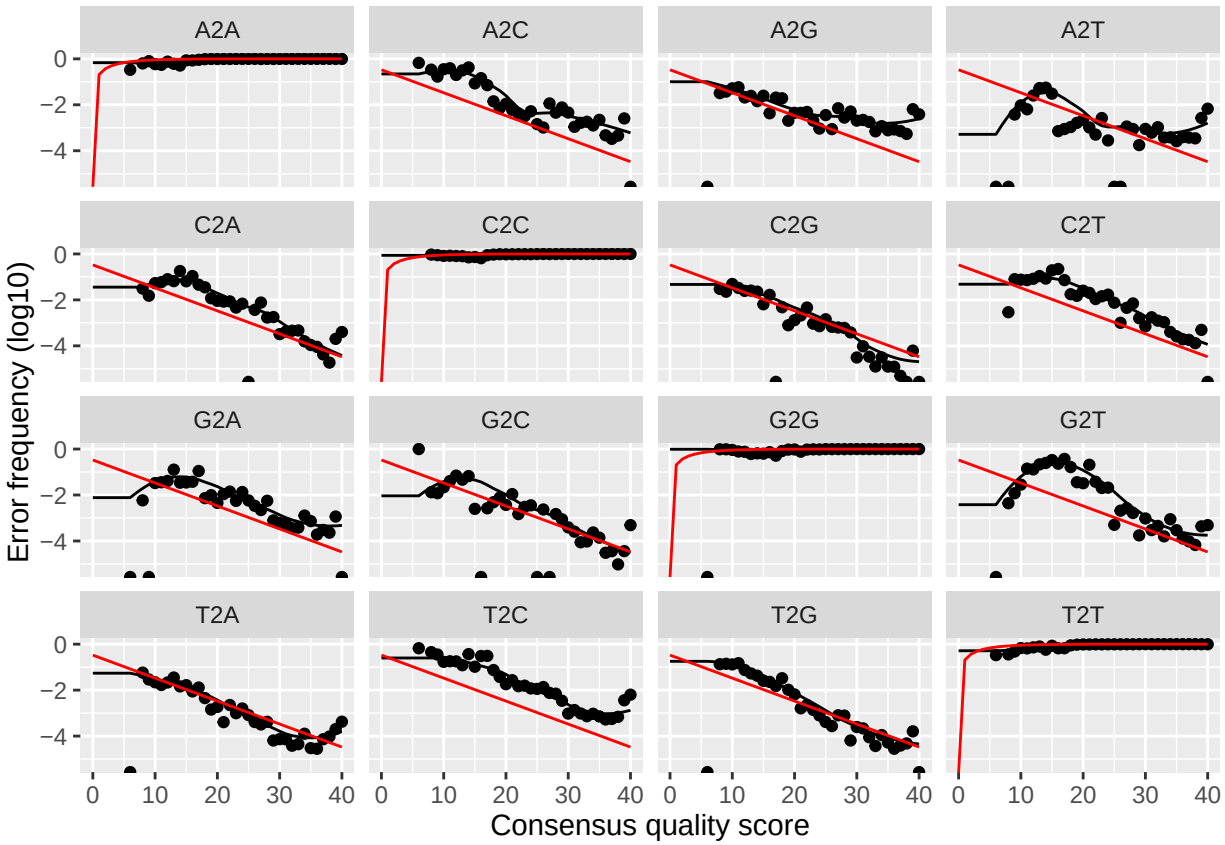
```
## Initial error matrix unspecified. Error rates will be initialized to the maximum possible estimate f

## Initializing error rates to maximum possible estimate.
## Sample 1 - 8792 reads in 1681 unique sequences.
## Sample 2 - 10147 reads in 2268 unique sequences.
## Sample 3 - 19012 reads in 3176 unique sequences.
## Sample 4 - 7263 reads in 1517 unique sequences.
##    selfConsist step 2
##    selfConsist step 3
##    selfConsist step 4
##
##
## Convergence after  4  rounds.
```

This will fit an error model that deconvolutes the original sequence variants in the sample. We can investigate how well the error model reproduces our data as well.

```
plotErrors(dadaFs, nominalQ=TRUE)
```




```
plotErrors(dadaRs, nominalQ=TRUE)
```



We will now quantify the sequence variants for both samples and combine them. Normally we would try to actually combine the forward and backwards reads into larger reads and quantify those, however our read qualities were so bad in this data set that there is no sufficient overlap. Thus, we will treat the forward and backward reads independently.

```
seqtab <- cbind(
    makeSequenceTable(dadaFs),
    makeSequenceTable(dadaRs))
```

Finally, we will also remove bimeras from the data set.

```
seqtab_nochim <- removeBimeraDenovo(seqtab, verbose=TRUE)
```

```
## Identified 6 bimeras out of 48 input sequences.
```

0.5 Taxonomy assignment and validation

In order to classify taxonomies for the individual sequence variants we will use the RDP algorithm with the green genes reference sequences. If you do not use the docker image this data set has to be downloaded first from here.

```
file.exists("gg_13_8_train_set_97.fa.gz")
```

```
## [1] TRUE
```

Axonomies can now be assigned by

```
taxa <- assignTaxonomy(seqtab_nochim, "gg_13_8_train_set_97.fa.gz")
colnames(taxa) <- c("Kingdom", "Phylum", "Class", "Order", "Family", "Genus", "Species")
head(unname(taxa))
```

```
##      [,1]          [,2]               [,3]
## [1,] "k__Bacteria" "p__Firmicutes"    "c__Bacilli"
## [2,] "k__Bacteria" "p__Firmicutes"    "c__Bacilli"
## [3,] "k__Bacteria" "p__Proteobacteria" "c__Gammaproteobacteria"
## [4,] "k__Bacteria" "p__[Thermi]"      "c__Deinococci"
## [5,] "k__Bacteria" "p__Proteobacteria" "c__Gammaproteobacteria"
## [6,] "k__Bacteria" "p__Proteobacteria" "c__Alphaproteobacteria"
##      [,4]                [,5]                   [,6]
## [1,] "o__Bacillales"     "f__Staphylococcaceae" "g__Staphylococcus"
## [2,] "o__Lactobacillales" "f__Streptococcaceae"  "g__Streptococcus"
## [3,] "o__Pseudomonadales" "f__Moraxellaceae"     "g__Acinetobacter"
## [4,] "o__Deinococcales"   "f__Deinococcaceae"    "g__Deinococcus"
## [5,] "o__Pseudomonadales" "f__Pseudomonadaceae"  "g__Pseudomonas"
## [6,] "o__Rhodobacterales" "f__Rhodobacteraceae"  "g__Rhodobacter"
##      [,7]
## [1,] "s__"
## [2,] "s__"
## [3,] "s__"
## [4,] "s__"
## [5,] "s__"
## [6,] "s__sphaeroides"
```

This can be combined with quantifications using the phyloseq package.

```
ps <- phyloseq(otu_table(seqtab_nochim, taxa_are_rows=FALSE),
               tax_table(taxa))
```

We can create a table quantifying the taxa across samples with

```
taxa <- cbind(as.data.frame(tax_table(ps)), t(otu_table(ps)))
rownames(taxa) <- NULL

head(taxa)
```

```
##       Kingdom            Phylum                 Class              Order
## 1 k__Bacteria     p__Firmicutes             c__Bacilli      o__Bacillales
## 2 k__Bacteria     p__Firmicutes             c__Bacilli o__Lactobacillales
## 3 k__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Pseudomonadales
## 4 k__Bacteria       p__[Thermi]          c__Deinococci   o__Deinococcales
## 5 k__Bacteria p__Proteobacteria c__Gammaproteobacteria o__Pseudomonadales
## 6 k__Bacteria p__Proteobacteria c__Alphaproteobacteria o__Rhodobacterales
##                Family             Genus        Species HMPMockV1.1.Even1
## 1 f__Staphylococcaceae g__Staphylococcus            s__              5934
## 2  f__Streptococcaceae  g__Streptococcus            s__               937
## 3     f__Moraxellaceae  g__Acinetobacter            s__                51
## 4    f__Deinococcaceae    g__Deinococcus            s__                 0
```

```
## 5  f__Pseudomonadaceae    g__Pseudomonas            s__               187
## 6  f__Rhodobacteraceae    g__Rhodobacter s__sphaeroides               600
##   HMPMockV1.1.Even2 HMPMockV1.2.Staggered1 HMPMockV1.2.Staggered2
## 1              5512                 11062                  4745
## 2               234                   267                   933
## 3               790                  1379                    60
## 4               905                  1059                     0
## 5               332                   657                   182
## 6                88                   149                   455
```

We can now compare this table to our reference table from mockrobiota. We will start by quantifying how many of the real taxa were found in our taxonomy assignment.

```
taxa_metrics(taxa[, 1:7], mock$tax_gg[, 1:7])
```

```
##     level     found  n
## 1 Kingdom 1.0000000  2
## 2  Phylum 1.0000000  6
## 3   Class 1.0000000 10
## 4   Order 1.0000000 12
## 5  Family 1.0000000 18
## 6   Genus 1.0000000 18
## 7 Species 0.7368421 19
```

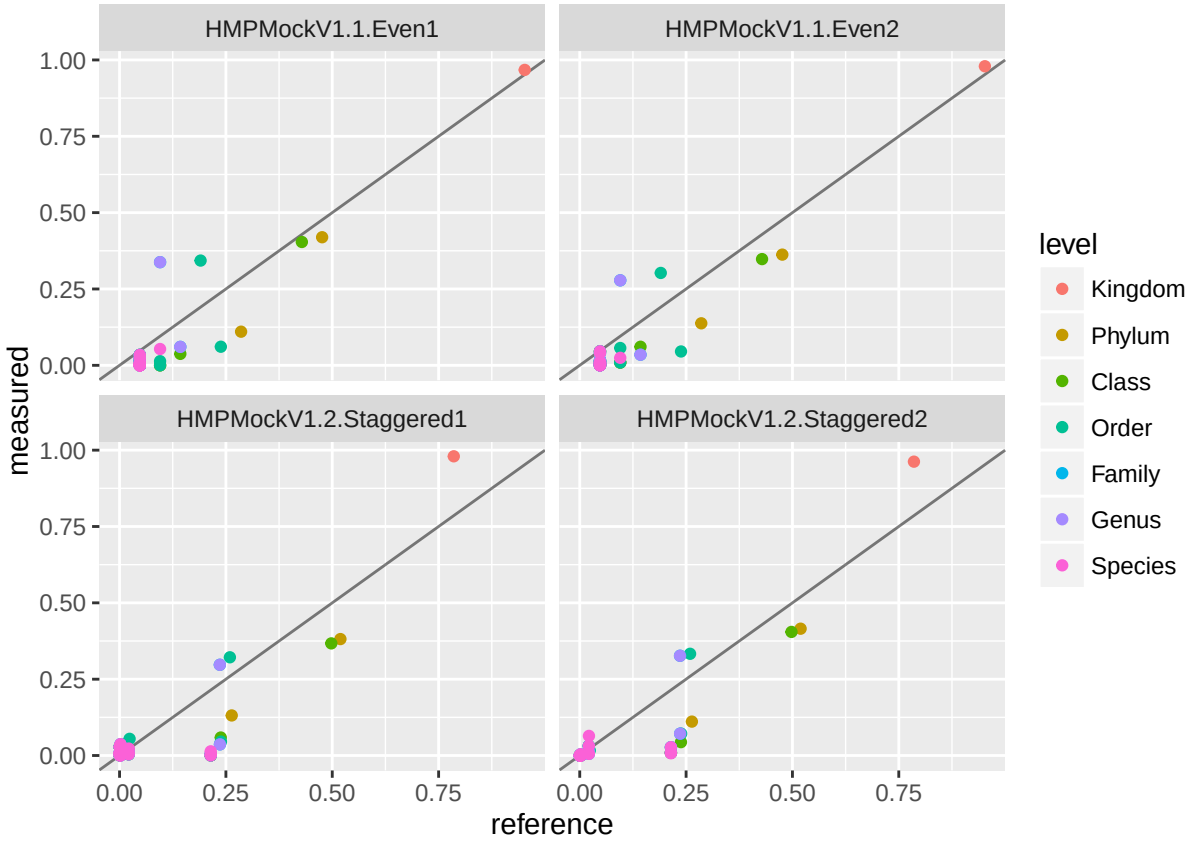
As we can see we are pretty good in identifying the taxa in our samples. However, how do we perform in terms of taxa quantification? We will start by stratifying across the samples:

```
res <- NULL

# ierate over samples
for (i in 8:11) {
    tq <- taxa_quants(taxa[, c(1:7, i)], mock$tax_gg[, c(1:7, i)], normalize=T)
    res <- rbind(res, cbind(tq, sample=colnames(taxa)[i]))
}

head(res)
```

```
##                                level                       name
## k__Bacteria                  Kingdom                  k__Bacteria
## k__Archaea                   Kingdom                   k__Archaea
## k__Bacteria;p__Firmicutes     Phylum     k__Bacteria;p__Firmicutes
## k__Bacteria;p__Proteobacteria  Phylum k__Bacteria;p__Proteobacteria
## k__Bacteria;p__[Thermi]       Phylum       k__Bacteria;p__[Thermi]
## k__Bacteria;p__Actinobacteria  Phylum k__Bacteria;p__Actinobacteria
##                                measured  reference            sample
## k__Bacteria                  0.96738574 0.95238095 HMPMockV1.1.Even1
## k__Archaea                   0.00762707 0.04761905 HMPMockV1.1.Even1
## k__Bacteria;p__Firmicutes    0.41943195 0.47619048 HMPMockV1.1.Even1
## k__Bacteria;p__Proteobacteria 0.11013717 0.28571429 HMPMockV1.1.Even1
## k__Bacteria;p__[Thermi]      0.00000000 0.04761905 HMPMockV1.1.Even1
## k__Bacteria;p__Actinobacteria 0.00000000 0.09523810 HMPMockV1.1.Even1
```

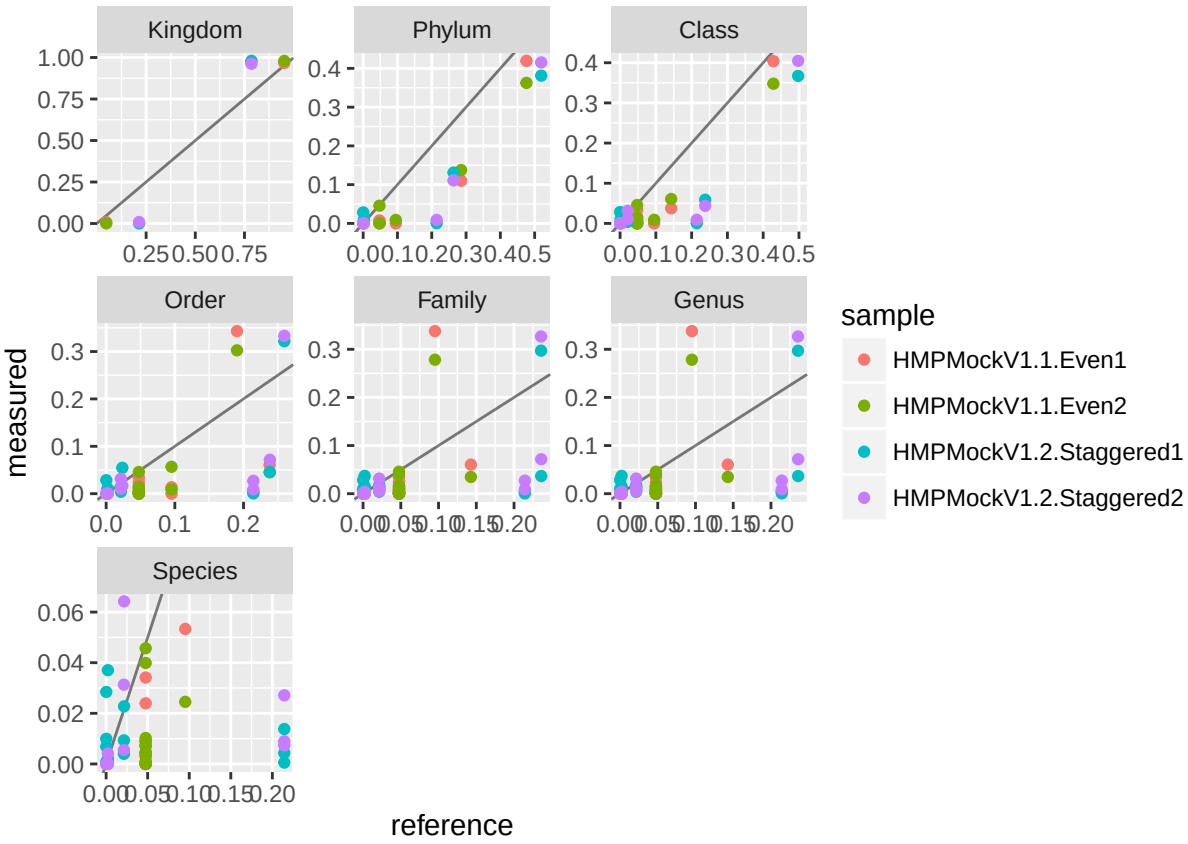
`taxa_quants` already qunatifies across a possible levels of taxonomy. So we can plot the performance stratified by sample with

```
ggplot(res, aes(x=reference, y=measured, col=level)) +
    geom_abline(alpha=0.5) +
    geom_point() +
    facet_wrap(~ sample)
```



Additionally, we can also combine all samples and rather stratify by taxonomy level.

```
ggplot(res, aes(x=reference, y=measured, col=sample)) +
    geom_abline(alpha=0.5) +
    geom_point() +
    facet_wrap(~ level, scale = "free")
```



This looks pretty ok with larger variations on the species level, which is to be expected. We can quantify the performance by a correlation test.

```
cor.test(res$measured, res$reference)
```

```
##
##  Pearson's product-moment correlation
##
## data:  res$measured and res$reference
## t = 28.408, df = 318, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.8127945 0.8753158
## sample estimates:
##      cor
## 0.846958
```

So we get a correlation of about 0.85 which is okay for the low number of reads we used here.