Supplementary Information for "Emu: Species-Level Microbial Community Profiling for Full-Length Nanopore 16S Reads"

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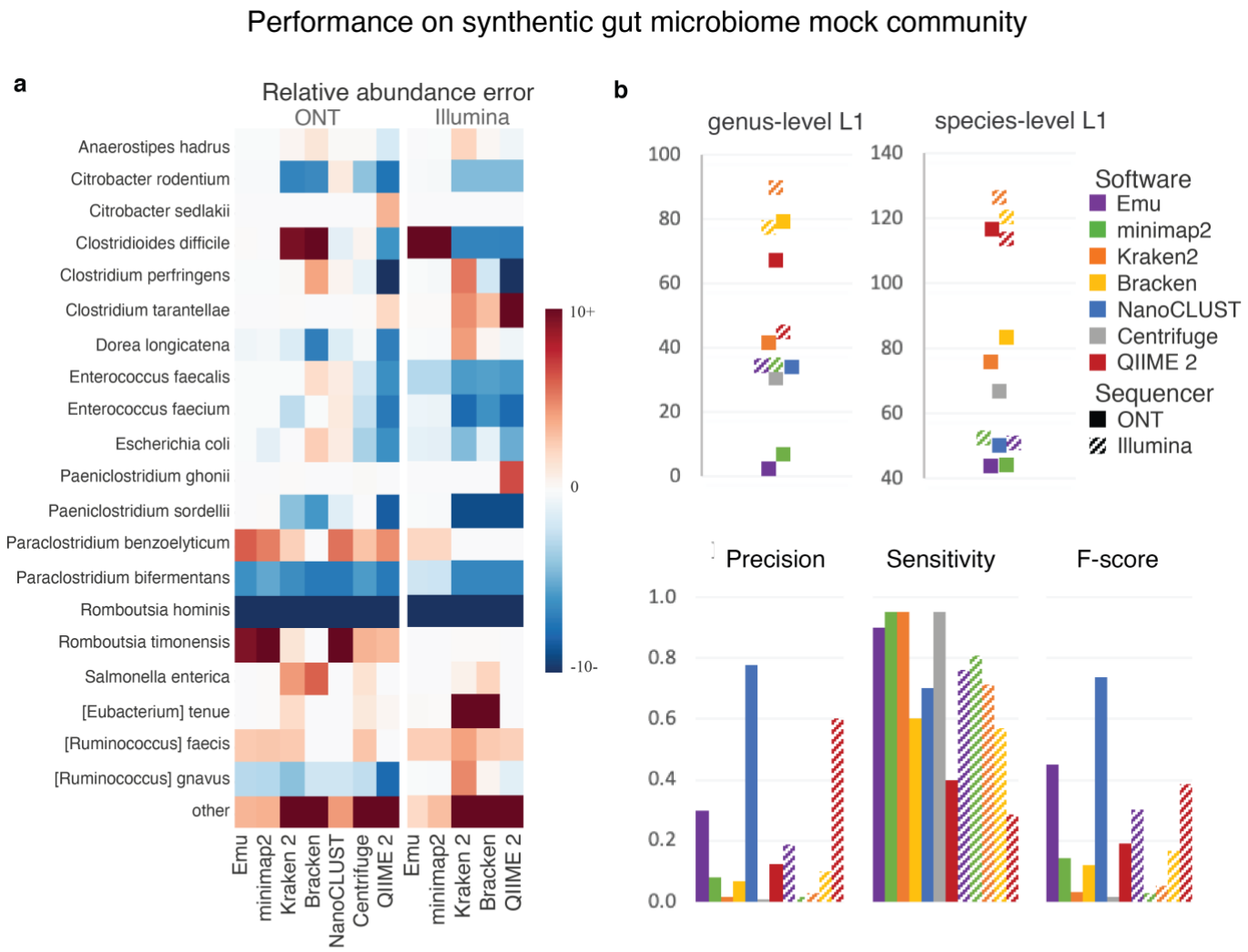
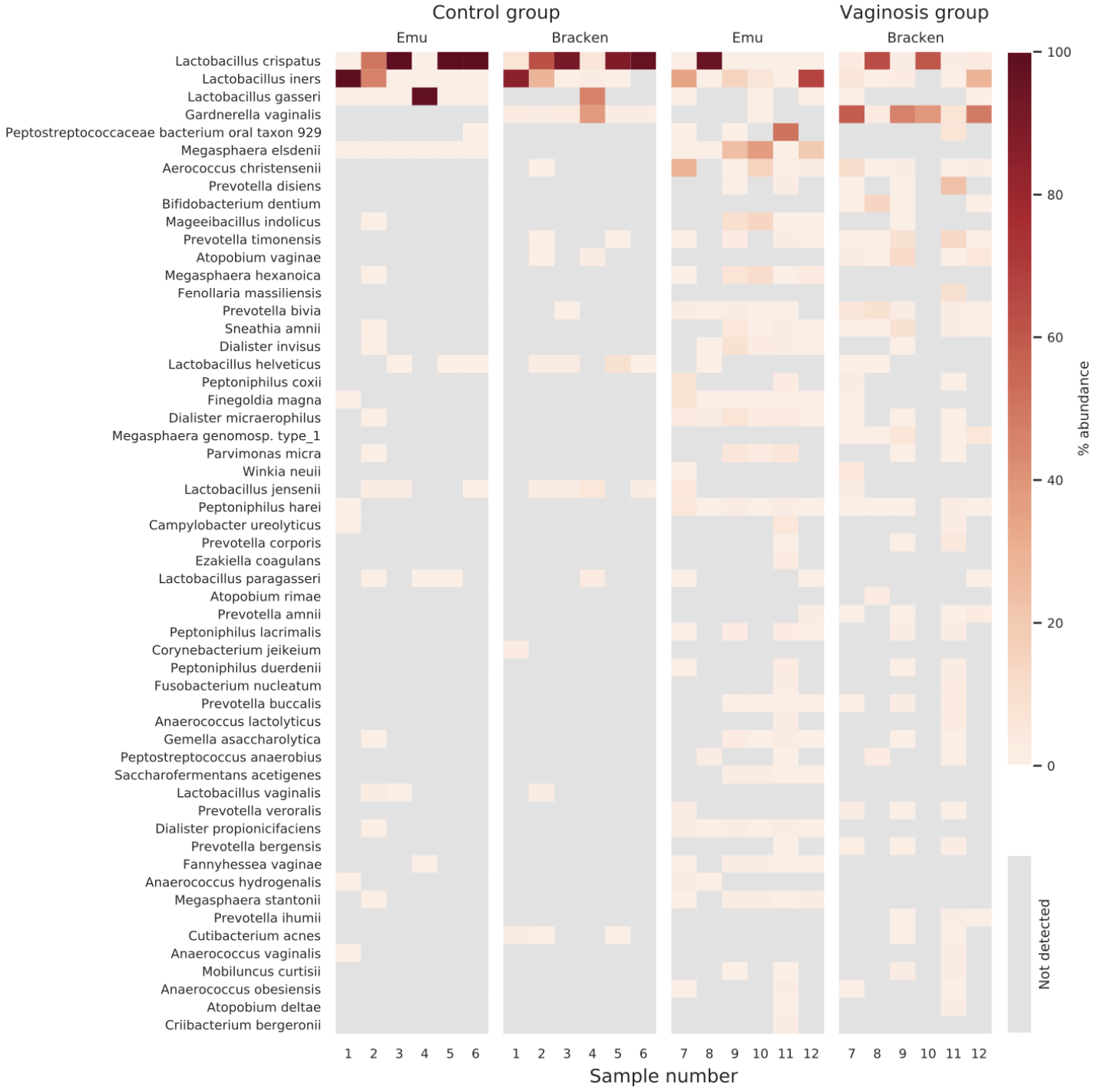
Supplementary Figure 1: Performance on Synthetic Gut Microbiome Mock Community



Supplementary Figure 1. Synthetic gut microbiome mock community. Composition accuracy for our synthetic gut microbiome mock community sequenced by Oxford Nanopore Technologies (ONT) and Illumina devices. **a** Species-level error between established ground truth abundance and estimated relative abundance. All ONT results are compared to the established truth for the ONT dataset, while all Illumina results are compared to established ground truth for the Illumina dataset. Color scheme is capped at ±10, resulting in error greater than ±10% observing the maximum error colors. Displayed are the 20 species claiming the largest abundance in any of the software results. "Other" represents the sum of all species not shown in figure for the respective column. **b** Genus-level L1-norm, as well as species-level L1-norm, precision, sensitivity, and F-score for all results expressed in panel **a**.

Supplementary Figure 2: Full Abundance Profile Comparison on Vaginal Samples



Supplementary Figure 2. Bacterial community of 12 vaginal samples. Species with estimated abundance of over 1% in at least one sample with either Emu or Bracken are shown. Data is grouped by condition: healthy control or vaginosis.

Supplementary Table 1: Ground Truth Abundance Profiles for Three Communities

a) MBARC-26 Simulated

Nanopore (%)	strain	NCBI Accession
0.14	Clostridium perfringens ATCC 13124 (F)	NR_121697_2
1.79	Coraliomargarita akajimensis DSM 45221 (V)	NR_041496_1, NR_074901_1
0.13	Corynebacterium glutamic Corynebacterium glutamicum	NR_074663_1, NR_041817_1
7.64	Desulfotomaculum gibsoniae DSM 7213 (F)	NR_114759_1, NR_114761_1, NR_037081_1, NR_114760_1, NR_103939_1
6.99	Desulfosporosinus acidiphilus SJ4 DSM 22704 (F)	NR_074663_1, NR_041817_1
2.58	Desulfosporosinus meridiei DSM 13257 (F)	NR_074129_1
0.33	Echinicola vietnamensis DSM 17526 (B)	NR_102438_1
0.10	Escherichia coli K-12, MG1655 (P)	NR_114042_1, NR_112558_1, NR_024570_1
12.15	Fervidobacterium pennivorans DSM 9078 (T)	NR_074097_1, NR_117583_1, NR_117584_1, NR_117582_1
7.41	Frateuria aurantia DSM 6220 (P)	NR_074107_1, NR_040947_1
6.10	Halovivax ruber XH-70 (E)	NR_112853_1, NR_042522_1, NR_113517_1, NR_102445_1
4.64	Hirschia baltica ATCC 49814 (P)	NR_118878_1, NR_042123_1, NR_074121_1
0.11	Clostridium thermocellum ATCC 27405 (F)	NR_074629_1
11.42	Meiothermus Silvanus DSM 9946 (D)	NR_074273_1, NR_027600_1
7.85	Natronobacterium gregoryi SP2 (E)	NR_102442_1, NR_113531_1, NR_028223_1
5.61	Natronococcus occultus DSM 3396 (E)	NR_113534_1, NR_102453_1, NR_112871_1, NR_028255_1
0.01	Nocardiopsis dassonvillei DSM 43111 (AT)	NR_074635_1
8.87	Olsenella uli DSM 7084 (AT)	NR_074414_1, NR_036820_1, NR_115110_1, NR_116937_1
3.79	Pseudomonas stutzeri RCH2 (P)	NR_113652_1, NR_116489_1, NR_114751_1, NR_118798_1, NR_103934_2
0.04	Salmonella bongori NCTC 12419 (P)	NR_074888_1
0.17	Salmonella enterica subsp. arizonae serovar RSK2980 (	NR_041696_1, NR_116125_1
7.25	Spirochaeta smaragdinae DSM 11293 (S)	NR_074741_1, NR_027585_1
3.18	Segniliparus rotundus DSM 44985 (AT)	NR_116690_1, NR_043018_1, NR_074426_1
0.40	Streptococcus pyogenes M1 GAS SF370 (F)	NR_112088_1, NR_028598_1
1.25	Terriglobus roseus DSM 18391 (AD)	NR_043918_1
0.06	Thermobacillus composti KWC4, DSM 18247 (F)	NR_041449_1, NR_102440_1

b) ZymoBIOMICS Community Standard

theoretical (%)	Nanopore (%)	Illumina (%)	species
17.4	21.7	19.3	Bacillus subtilis
9.9	12.2	9.0	Enterococcus faecalis
10.1	8.9	9.4	Escherichia coli
18.4	9.3	14.3	Limosilactobacillus fermetum
14.1	15.2	12.7	Listeria monocytogenes
4.2	1.9	4.8	Psedudomonas aeruginosa
10.4	13.0	13.3	Salmonella enterica
15.5	17.7	17.2	Staphylococcus aureus

c) Gut Microbiome Mock Synthetic

Nanopore (%)	Illumina (%)	species
0.060	0.830	[Clostridium] innocuum
0.002	0.001	[Clostridium] leptum
0.740	0.481	[Clostridium] scindens
11.011	7.518	[Ruminococcus] gnavus
2.108	3.385	Anaerostipes hadrus
0.009	0.756	Bacteroides thetaiotaomicro
0	2.047	Bifidobacterium dentium
7.286	4.441	Citrobacter rodentium
5.949	6.784	Clostridioides difficile
17.890	15.415	Clostridium perfringens
6.910	6.722	Dorea longicatena
6.192	5.706	Enterococcus faecalis
7.123	7.832	Enterococcus faecium
6.120	4.998	Escherichia coli
0.004	0.001	Gemmiger formicilis
0.594	0.766	Intestinibacter bartlettii
8.301	8.939	Paeniclostridium sordellii
7.045	6.596	Paraclostridium bifermentan
0.019	0.942	Phocaeicola vulgatus
12.176	12.701	Romboutsia hominis
0.462	3.139	Roseburia faecis

Supplementary Table 2: Computational Performance Metrics

		Nanopore						Illumina			
		Emu	minimap2	Kraken 2	Bracken	NanoCLUST	Centrifuge	Emu	minimap2	Kraken 2	Bracken
MBARC-26	time (m)	249	234	2	2	249	20				
	RAM (GB)*	319	318	0	0	16	0				
Zymo	time (m)	1048	886	3	3	204	38	62	53	0	0
	RAM (GB)	420	408	0	0	34	0	2	1	0	0
Gut Mock	time (m)	221	200	1	1	270	12	10	6	0	0
	RAM (GB)	373	368	0	0	34	0	1	1	0	1

*RAM usage represented maximum resident set size

Supplementary Table 3: Comparison of Database Performance using Kraken2/Bracken

The following table shows an evaluation of performance Kraken 2/Bracken on the ZymoBIOMICS ONT data with different databases. The middle three are those made available with Kraken 2. The rightmost column includes results of Kraken/Bracken (that is, Kraken 1) on the default database. The leftmost column is the performance on the combined rrnDB/NCBI database that was built for Emu.

Data set: ZymoBIOMICS (ONT) **read count**: 1,096,959

	database	**Emu**	**Greengenes**	**Silva**	**RDP**	**Kraken**
	classifier	Kraken 2	Kraken 2	Kraken 2	Kraken 2	Kraken
genus	TP [8]	8	5	7	8	7
	FP	60	35	24	72	60
	L1-norm	23	102	58	63	40
	precision	0.12	0.13	0.25	0.09	0.10
	sensitivity	1.00	0.63	1.00	0.88	0.88
	F-score	0.21	0.21	0.40	0.16	0.19
	reads kept*	1,040,877	747,277	1,092,151	1,011,567	1,077,148
	% reads kept	94.9	68.1	99.6	92.2	98.2
species	TP [8]	8	2	-	-	7
	FP	191	44	-	-	171
	L1-norm	66	179	-	-	78
	precision	0.04	0.04	-	-	0.04
	sensitivity	1.00	0.25	-	-	0.88
	F-score	0.08	0.07	-	-	0.08
	reads kept	1,035,110	557,256	0	0	1,075,754
	% reads kept	94.4	50.8	0.0	0.0	98.1

*"new_est_reads" column sum in Bracken output

Supplementary File 2: Complete Abundance Results for Quantitative Study

File: supp_file2_complete_abundance_results.xlsx (included with submission)

This Excel file contains the complete abundance profile outputs for each of the methods and data sets contained in Table 1. The file has 5 sheets, each one with a self-descriptive name and corresponding to a segment of Table 1:

A) MBARC-26
B) Zymo - Nanopore
C) Zymo - Illumina
D) Gut - Nanopore
E) Gut - Illumina

In each table, the first column is labeled with the ground truth abundances. In tab (A), it is labeled “truth” since the data is simulated and ground truths are known. In tabs (B) – (E) it is labeled “theoretical” since an algorithm was used to compute a putative ground truth value for these communities.

Supplementary Note 1: Commands Used to Run Quantitative Study

Below are commands used to generate comparison results on our 3 Oxford Nanopore Technologies (ONT) datasets and 2 Illumina datasets. The following variables are used:

```
<sample path>: path to sample(s), ONT or Illumina
<sample>: ONT reads [.fastq]
<sample f>: Illumina PE forward reads [.fastq]
<sample r>: Illumina PE reverse reads [.fastq]
<db fasta>: custom database sequences [.fasta]
<db nodes>: custom database nodes [.dmp]
<db names>: custom database names [.dmp]
<db seq2tax map>: custom database sequence id to tax id map [.txt]
```

Trim barcodes

ONT

Guppy Basecalling Software v4.4.2:

```
$ guppy_barcoder --input_path <sample_path> --save_path <output_path>
--barcode_kits <kits> --trim_barcodes
```

- Our ZymoBIOMICS sample used kits SQK-RAB201, while our gut mock sample used SQK-16S024.

Illumina

Trimmomatic v0.39

```
$ java -jar trimmomatic-0.39.jar PE <sample_f> <sample_r> <out_sample_f>
   <out_sample_r> ILLUMINACLIP:adapters/TruSeq3-PE.fa:2:30:10:2:keepBothReads
```

Emu v1.0.1

ONT

```
$ emu abundance <sample>
```

Illumina

```
$ emu abundance --type sr <sample_f> <sample_r>
```

Minimap2 v.2.17

The following commands were used to collect primary alignments. Further processing was completed to calculate relative abundance from results.

ONT

```
$ minimap2 -ax map-ont -N 1 <db_fasta> <sample>
```

Illumina

```
$ minimap2 -ax sr -N 1 <db_fasta> <sample_f> <sample_r>
```

Kraken 2 v2.1.1

Build database

```
$ kraken2-build --download-taxonomy --db <db_name>
#replace name.dmp & nodes.dmp files
$ kraken2-build --add-to-library <db_fasta> --db <db_name>
$ kraken2-build --build --db <db_name>
```

ONT

```
$ kraken2 --db <db_name> <sample> --output <out_classification> --report
    <out_kreport>
```

Illumina

```
$ kraken2 --db <db_name> <sample-f> <sample_r> --output <out_classification>
    --report <out_kreport>
```

Bracken v2.5.0

Build databases

```
$ bracken-build -d <KRAKEN2_DB_PATH>/<db_name> -l <read_len> -k 35 -x
    <kraken_installation>
```

- read len 1500 was used for our ONT reads, while 300 and 250 were used for our Illumina reads.

ONT

```
$ bracken -d <KRAKEN2_DB_PATH>/<db_name> -i <kraken2_kreport> -r 1500 -l [G|S] -o
    <out_file>
```

Illumina

```
$ bracken -d <KRAKEN2_DB_PATH>/<db_name> -i <kraken2_kreport> -r [250|300] -l [G|S]
    -o <out_file>
```

- read len 250 was used for our ZymoBIOMICS community, and 300 for our gut mock community.

NanoCLUST v1.9

Build databases

```
$ makeblastdb -in <db_fasta> -parse_seqids -blastdb_version 5 -taxid_map
    <db_seq2tax_map> -title <db_name> -dbtype prot
```

ONT

```
$ nextflow run main.nf -profile docker --reads <sample> --db <db_fasta> --tax
    <db_taxonomy_path> --outdir <out_dir>
```

Centrifuge v1.0.4

Build databases

```
$ centrifuge-build --conversion-table <db_seq2tax_map> --taxonomy-tree <db_nodes> -
    -name-table <db_names> <db_fasta> <db_name>
```

ONT

```
$ centrifuge -q -x <db_name> <sample> -S <out_classification> --report-file
    <out_report>
$ centrifuge-kreport -x <db_name> <out_report> > <out_kreport>
```

QIIME 2 v2020.11

Import database

```
$ qiime tools import --input-path <db_fasta> --type FeatureData[Sequence] --output-
    path <reference_sequences>
```

```
$ qiime tools import --input-path <db_taxonomy_path> --type FeatureData[Taxonomy] -
    -output-path <reference_taxonomy>
```

Extract reference sequences with primers

```
$ qiime feature-classifier extract-reads --i-sequences <reference_sequences> --p-f-
    primer <forward_primer> --p-r-primer <reverse_primer> --o-reads
    <reference_extracted_reads>
```

- All ONT reads used full-length 16S primers: AGAGTTTGATCMTGGCTCAG, CGGTTACCTTGTTACGACTT.
- ZymoBIOMICS Illumina reads were targeted for hypervariable regions V4-6 using primers: GTGCCAGCMGCCGCGGTAA, ACAACACGAGCTGACGAC.
- The synthetic gut mock community used the v4 region with primers: GTGCCAGCMGCCGCGGTAA, GGACTACHVGGGTWTCTAAT.

Fit classier

```
$ qiime feature-classifier fit-classifier-naive-bayes --i-reference-reads
    <reference_extracted_reads> --i-reference-taxonomy <reference_taxonomy> --o-
    classifier <classifier>
```

ONT

```
$ qiime tools import --type SampleData[SequencesWithQuality] --input-path
    <path_to_sample> --input-format CasavaOneEightSingleLanePerSampleDirFmt --
    output-path <demultiplexed_sample>
$ qiime vsearch dereplicate-sequences --i-demultiplexed-seqs <demultiplexed-sample>
    --o-dereplicated-table <sample_derep_table> --o-dereplicated-sequences
    <sample_derep_seqs>
$ qiime feature-classifier classify-sklearn --i-classifier <classifier> --i-reads
    <sample_derep_seqs> --p-n-jobs <num_threads> --o-classification
    <sample_sklearn_classification>
$ qiime taxa collapse --i-table <sample_derep_table> --i-taxonomy
    <sample_sklearn_classification> --p-level [6|7] --o-collapsed-table
    <sample_collapsed_table>
$ qiime feature-table relative-frequency --i-table <sample_collapsed_table> --o-
    relative-frequency-table <sample_collapsed_rel_frequency>
```

Illumina

```
$ qiime tools import --type SampleData[PairedEndSequencesWithQuality] --input-path
    <path_to_sample> --input-format CasavaOneEightSingleLanePerSampleDirFmt --
    output-path <demultiplexed_sample>
$ qiime dada2 denoise-paired --i-demultiplexed-seqs <demultiplexed_sample> --p-n-
    threads <num_threads> --p-trunc-len-f 0 --p-trunc-len-r 0 --o-table
    <sample_denoised_table> --o-representative-sequences <sample_denoised_sequences>
    --o-denoising-stats <sample_denoising_stats>
$ qiime feature-classifier classify-sklearn --i-classifier <classifier> --i-reads
    <sample_denoised_seqs> --p-n-jobs <num_threads> --o-classification
    <sample_sklearn_classification>
$ qiime taxa collapse --i-table <sample_denoised_table> --i-taxonomy
    <sample_sklearn_classification> --p-level [6|7] --o-collapsed-table
    <sample_collapsed_table>
$ qiime feature-table relative-frequency --i-table <sample_collapsed_table> --o-
    relative-frequency-table <sample_collapsed_rel_frequency>
```

- Taxa level 6 is used for collapsing to genus level.
- Taxa level 7 is used for collapsing to species level.

Establish ground truth

The following commands were used to collect primary alignments. Further processing was completed to calculate relative abundance from results. For our ZymoBIOMICS community, restricted database sequences contains all 8 assembled sequences from ZymoBIOMICS. For our gut mock community, all NCBI RefSeq sequences from the 21 expected species are included. Detailed descriptions of both processes are provided in the manuscript.

ONT

```
$ minimap2 -ax map-ont <restricted_db_sequences> <sample>
```

Illumina

```
$ bwa index <restricted_db_sequences>
$ bwa mem restricted_db_sequences> <sample_f> <sample_r>
```