ITS Preparation and Sequencing Methods

Sequences were imported into Qiime2[1] for analysis. Primer sequences were removed using Qiime2's cutadapt[2] plugin using the following sequences.

Region	Forward Trim Sequence	Reverse Trim Sequence
ITS2	GCATCGATGAAGAACGCAG	TCCTCCGCTTATTGATATGC

Sequences were then denoised using Qiime2's dada2 plugin[3]. Denoised sequences were placed into a feature table detailing which amplicon sequence variants (ASVs) were observed in which samples, and how many times each ASV was observed in each sample. Identified ASVs were taxonomically assessed using the Unite 8 99% full-length sequence database and the VSEARCH[4] utility within Qiime2's feature-classifier plugin. ASVs were then collapsed to their lowest taxonomic units (strain, species, genus, etc.), and their counts were converted to reflect their relative frequency within a sample. A table of ASV relative frequencies is available in the '*Microbial Composition.tsv*' file. Representative sequences for each ASV can be found in the *'Representative ASV Metadata.qzv'* which can be viewed in Qiime2View. Likewise, a visualization of ASV relative frequencies is available in the '*Microbial Compostion.html*' file. You can explore, reformat and export a visualization of your data by uploading the '*Microbial Composition.qzv*' file to Qiime2's website. Provided there are at least two samples in the analysis, the following alpha diversity metrics can be found in '*Alpha Diversity Metrics.tsv*':

- Shannon's diversity index (a quantitative measure of community richness)
- Observed Features (a qualitative measure of community richness)
- Faith's Phylogenetic Diversity (a qualitative measure of community richness that incorporates phylogenetic relationships between the features)
- Evenness (or Pielou's Evenness; a measure of community evenness)

Provided there are at least two samples in the analysis, the following beta diversity metrics can be found as Principal Component Analysis (PCA) visualizations. You may open these files using Qiime2's website.

- Jaccard Distance Visualization.qzv (a qualitative measure of community dissimilarity)

[1] Bolyen, E., Rideout, J.R., Dillon, M.R. et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37, 852–857 (2019). https://doi.org/10.1038/s41587-019-0209-9

[2] Marcel Martin. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet. journal, 17(1):pp–10, 2011. doi:10.14806/ej.17.1.200.

[3] Benjamin J Callahan, Paul J McMurdie, Michael J Rosen, Andrew W Han, Amy Jo A Johnson, and Susan P Holmes. Dada2: high-resolution sample inference from illumina amplicon data. Nature methods, 13(7):581, 2016. doi:10.1038/nmeth.3869.

[4] Torbjørn Rognes, Tomáš Flouri, Ben Nichols, Christopher Quince, and Frédéric Mahé. Vsearch: a versatile open source tool for metagenomics. PeerJ, 4:e2584, 2016. doi:10.7717/peerj.2584.

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- Bray Curtis Visualization.qzv (a quantitative measure of community dissimilarity)
- Unweighted UniFrac Visualization.qzv (a qualitative measure of community dissimilarity that incorporates phylogenetic relationships between the features)
- Weighted UniFrac Visualization.qzv (a quantitative measure of community dissimilarity that incorporates phylogenetic relationships between the features)

Tool	Version
Qiime2	2023.9.1 (q2cli) from quay.io/qiime2 Docker Image

Qiime2 Commands	Qiime2 Options
tools import	--type 'SampleData[PairedEndSequencesWithQuality]' --input-format PairedEndFastqManifestPhred33V2
cutadapt trim-paired	primer sequence from table above, default parameters
dada2 denoise-paired	--p-trim-left-f 0 --p-trim-left-r 0 --p-trunc-len-f 0 --p-trunc-len-r 0 --p-max-ee-f 5 --p-max-ee-r 5 --p-min-overlap 9
phylogeny align-to-tree-mafft-fasttree	default parameters
feature-classifier classify-consensus-vsearch	db listed above, default parameters
taxa barplot	default parameters
taxa collapse	default parameters
feature-table relative-frequency	default parameters
diversity core-metrics-phylogenetic	--p-sampling-depth 1000, default parameters
feature-table transpose	default parameters
metadata tabulate	default parameters

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DNA Extraction Methods

All standard DNA extractions at SeqCenter follow the ZymoBIOMICS™ DNA Miniprep Kit[5]. Samples submitted on agar plates had a loopfµl of cells (~50-100mg) aseptically scraped from the agar and resuspended in 750 µl of lysis solution. Samples submitted as liquid aliquots had 200 µl of media transferred into 550 µl of lysis solution. Samples submitted as cell pellets were resuspended in 750 µl of lysis solution. Samples submitted as solid masses including but not limited to soil, fecal material, food products, plant, and/or tissue materials were sampled following the guidelines in Appendix B of the ZymoBIOMICS™ DNA Miniprep Kit.

Cells suspended in lysis solution were transferred into the ZR BashingBead™ Lysis Tubes and mechanically lysed using the MP FastPrep-24™ lysis system with 1 minute of lysis at maximum speed and 5 minutes of rest for 5 cycles. Samples were then centrifuged at 10,000rcf for 1 minute. 400µl of supernatant was transferred from the ZR BashingBead™ Lysis Tube to a Zymo-Spin™ III-F Filter and centrifuged at 8,000rcf for 1 minute. 1,200 µl of ZymoBIOMICS™ DNA Binding Buffer was added to the effluent and mixed via pipetting. 800µl of this solution was transferred to a Zymo-Spin™ IICR Column and centrifuged at 10,000rcf for 1 minute. This step was repeated until all material was loaded onto the Zymo-Spin™ IICR Column.

DNA bound to the Zymo-Spin™ IICR Column was washed 3 times with 400µl and 700µl of ZymoBIOMICS™ DNA Wash Buffer 1 and then 200 µl of ZymoBIOMICS™ DNA Wash Buffer 2 with a 1-minute spin down at 10,000rcf for each, respectively. Washed DNA was eluted using 75µl of ZymoBIOMICS™ DNase/RNase Free Water following a 5-minute incubation at room temperature and a 1-minute spin down at 10,000rcf. The Zymo-Spin™ III-HRC Filter was prepared using 600 µl of the ZymoBIOMICS™ HRC Prep Solution and a centrifugation at 8,000rcf for 3 minutes. Eluted DNA was then purified by running the effluent through the prepared Zymo-Spin™ III-HRC Filter.

Final DNA concentrations were determined via Qubit[6].

[5] ZymoBIOMICS DNA Miniprep Kit. https://www.zymoresearch.com/products/zymobiomics-dna-miniprep-kit
[6] Qubit 1X dsDNA assays: simplified workflow and improved performance. http://assets.thermofisher.com/TFS-Assets/BID/Technical-Notes/qubit-1x-dsdna-assays-simplified-workflow-tech-note.pdf

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