16S Preparation and Sequencing Methods

DNA extractions, if applicable, follow the methods reported on Page 2.

Samples were prepared using Zymo Research's Quick-16S kit with phased primers targeting the V3/V4 regions of the 16S gene. The specific primer sequences are found below:

Region	Forward Sequence	Reverse Sequence
	341f	806r
V3/V4	CCTACGGGDGGCWGCAG CCTAYGGGGYGCWGCAG	GACTACNVGGGTMTCTAATCC

Following clean up and normalization, samples were sequenced on a P1 600cyc NextSeq2000 Flowcell to generate 2x301bp paired end (PE) reads. Quality control and adapter trimming was performed with bcl-convert[1] (v4.2.4)

[1] bcl-convert: A proprietary Illumina software for the conversion of bcl files to basecalls. https://support-docs.illumina.com/SW/BCL_Convert/Content/SW/BCLConvert/Introduction_swBCL.htm

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DNA Extraction Methods

All standard DNA extractions at SeqCenter follow the ZymoBIOMICS™ DNA Miniprep Kit[2]. 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[3].

[2] ZymoBIOMICS DNA Miniprep Kit. https://www.zymoresearch.com/products/zymobiomics-dna-miniprep-kit
[3] 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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