Illumina Sequencing Methods

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

Illumina sequencing libraries were prepared using the tagmentation-based and PCR-based Illumina DNA Prep kit and custom IDT 10bp unique dual indices (UDI) with a target insert size of 280 bp. No additional DNA fragmentation or size selection steps were performed. Illumina sequencing was performed on an Illumina NovaSeq X Plus sequencer in one or more multiplexed shared-flow-cell runs, producing 2x151bp paired-end reads. Demultiplexing, quality control and adapter trimming was performed with bcl-convert[1] (v4.2.4). Sequencing statistics are included in the '*DNA Sequencing Stats.xlsx*' file.

What is an md5sum?

The md5sum functions as a file's compact digital fingerprint. md5sums are used to verify the integrity of files between two servers. If you calculate the md5sum of the file on your server, it should match that of the file listed in the '*DNA Sequencing Stats.xlsx*' file. If it does not match, the file was corrupted either being uploaded or downloaded to box, please let SeqCenter know if this is the case.

Calculating the md5sum:

- Windows users, please see this tutorial.
- MacOS users, please see this tutorial.
- Linux users, please see this tutorial.

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[1] bcl-convert: A proprietary Illumina software for the conversion of bcl files to basecalls.

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 3 minutes of rest for 2 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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