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Ultra-deep, long-read nanopore sequencing of mock microbial community standards

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1 Works for me dx.doi.org/10.17504/protocols.io.x9tfr6n

Long Read Club
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ABSTRACT

Background: Long sequencing reads are information-rich: aiding de novo assembly and reference mapping, and consequently have great potential for the study of microbial communities. However, the best approaches for analysis of long-read metagenomic data are unknown. Additionally, rigorous evaluation of bioinformatics tools is hindered by a lack of long-read data from validated samples with known composition.

Methods: We sequenced two commercially-available mock communities containing ten microbial species (ZymoBIOMICS Microbial Community Standards) with Oxford Nanopore GridION and PromethION. Both communities and the ten individual species isolates were also sequenced with Illumina technology.

Data: We generated 14 and 16 Gbp from GridION owcells and 150 and 153 Gbp from PromethION owcells for the evenly-distributed and log-distributed communities respectively. Read length N50 was 5.3 Kbp and 5.2 Kbp for the even and log community, respectively. Basecalls and corresponding signal data are made available (4.2 TB in total).

Results: Alignment to Illumina-sequenced isolates demonstrated the expected microbial species at anticipated abundances, with the limit of detection for the lowest abundance species below 50 cells (GridION). De novo assembly of metagenomes recovered long contiguous sequences without the need for pre-processing techniques such as binning.

Conclusions: We present ultra-deep, long-read nanopore datasets from a well-dened mock community. These datasets will be useful for those developing bioinformatics methods for long-read metagenomics and for the validation and comparison of current laboratory and software pipelines.

EXTERNAL LINK

https://www.biorxiv.org/content/biorxiv/early/2018/12/04/487033.full.pdf

GUIDELINES

This protocol has been writen for those wishing to reproduce the DNA extractions used to generate the Nanopore sequencing data presented in the publication.

MATERIALS

NAME	CATALOG #	VENDOR
ZymoBIOMICS Microbial Community Standard	D6300	Zymo Research
ZymoBIOMICS Microbial Community Standard II (Log Distribution)	D6310	Zymo Research
ZymoBIOMICS DNA Miniprep Kit	D4300	Zymo Research

STEPS MATERIALS

NAME	CATALOG #	VENDOR
ZymoBIOMICS Microbial Community Standard II (Log Distribution)	D6310	Zymo Research
ZymoBIOMICS Microbial Community Standard	D6300	Zymo Research
ZymoBIOMICS DNA Miniprep Kit	D4300	Zymo Research

SAFETY WARNINGS

Read the specific MSDS documents accociated with the materials used in the protocol.

BEFORE STARTING

Thaw all frozen reagents on ice, mix well and spin down.

Prepare standard

1 Transfer **75 µl Microbial Community Standard** or **375 µl Microbial Community Standard II (Log distribution)** to a **1.5 ml** Eppendorf tube.

Each 75 ul of the even community is expected to yield 2000 ng and the log community 220 ng therefore a larger starting volume of the later is required.

ZymoBIOMICS Microbial Community Standard II (Log Distribution)
by Zymo Research
Catalog #: D6310

ZymoBIOMICS Microbial Community Standard
by Zymo Research
Catalog #: D6300

Retain supernatant

2 Centrifuge the standard at 6,000 xg for **00:05:00** before transferring the supernatant to a new **1.5 ml** Eppendorf

The Microbial Community Standard is shipped in DNA/RNA Shield for safety reasons but this causes lysis of Gram-negative species. Retaining the supernatant prevents unecessary shearing during the subsequent bead-beating step.

Resuspend pellet

3 Resuspend the cell pellet in **750 µl** Lysis Solution from the ZymoBIOMICS DNA Miniprep Kit and transfer the resuspended cells to a ZR BashingBead Lysis Tube.

ZymoBIOMICS DNA Miniprep Kit
by Zymo Research
Catalog #: D4300

Bead-beat

4 Load the lysis tube into a FastPrep-24 5G instrument and bead-beat at 6.0 m/s for 2 cycles of **00:00:40** removing the tube and chilling on ice for **00:05:00** between cycles.

FastPrep-24 5G
Bead beater
MP Biomedicals 116005500

5 Centrifuge the lysis tube at 10,000 xg for **00:01:00** and transfer **400 µl** supernatant to a Zymo-Spin III-F Filter in a collection tube.

6 Centrifuge the filter column at 8,000 xg for **00:01:00** and transfer **400 µl** filtrate to a new **15 ml** Falcon tube.

7 For the Microbial Community Standard add **45 µl** of the supernatant from Step 2 and **1.485 ml** Binding buffer to the tube and mix well. For the Microbial Community Standard II (Log distribution) add **225 µl** of the supernatant from Step 2 and **2.025 ml** instead.

8 Load **800 µl** onto a Zymo-Spin IIC-Z Column, centrifuge at 8,000 xg for **00:01:00** and discard flow through. Repeat as many times as needed to process all the mixture.

9 Wash and elute the sample as per the ZymoBIOMICS DNA Miniprep Kit instruction manual.

10 Prepare sequencing libraries using the Ligation Sequencing Kit SQK-LSK109 (Oxford Nanopore Technologies) using **1400 ng** input DNA and loading **50 ng (MinION)** or **400 ng (PromethION)** completed library onto the flowcell.