Promega

Product Application

Automated Purification of Fecal DNA with a Bead Beating Step

An optional bead beating step is used to enhance lysis of bacteria in fecal samples before purification of fecal microbiome DNA with the Maxwell® RSC Fecal Microbiome DNA Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Fecal Microbiome DNA Kit (Cat.# AS1700)

Analyses: Next-generation sequencing (NGS)

Sample Type(s): Feces

Input: ≤300mg

Materials Required:

- Maxwell® RSC Fecal Microbiome DNA Kit (Cat.# AS1700)
- Maxwell® RSC Instrument (Cat.# AS4500)
- ZR BashingBead™ Lysis Tubes (Zymo, Cat.# S6012-50)
- Vortex
- Horizontal Vortex Adapter for 1.5/2.0ml Tubes (e.g. Qiagen, Cat.#13000-V1-24)
- Heat Blocks

This protocol was developed by Promega Applications Scientists and is intended for research use only.

Users are responsible for determining suitability of the protocol for their application.

For further information, see Technical Manual TM640, available at: **www.promega.com/protocols**

or contact Technical Services at: **techserv@promega.com**

Protocol:

1. Weigh ≤300mg of feces into ZR BashingBead™ Lysis Tubes.
2. Add 1ml of Lysis Buffer and 40µl of Proteinase K to each sample, and cap the tubes tightly.
3. Place tubes in a horizontal tube adapter assembled on a vortex. Vortex tubes at maximum speed (~3000rpm) for 30 minutes.
4. Continue with Step 3 in Section 4.B. of the Maxwell® RSC Fecal Microbiome DNA Kit Technical Manual (TM640).

Results:

DNA purified from the ZymoBIOMICS Microbial Community Standard with the Maxwell® RSC Fecal Microbiome DNA Kit with optional bead beating was used in 16S V3/V4 metagenomic sequencing. The microbial profile in the purified DNA is concordant with the theoretical species representation reported by the manufacturer (Figure 1, top panel). Also shown are example sequencing results for DNA purified from fecal samples of two individuals (Figure 1, bottom panel). Microbiome DNA purified with the Maxwell® RSC Fecal Microbiome DNA Kit with optional bead beating was also compatible with Oxford Nanopore sequencing to detect the bacterial species present (Figure 2).

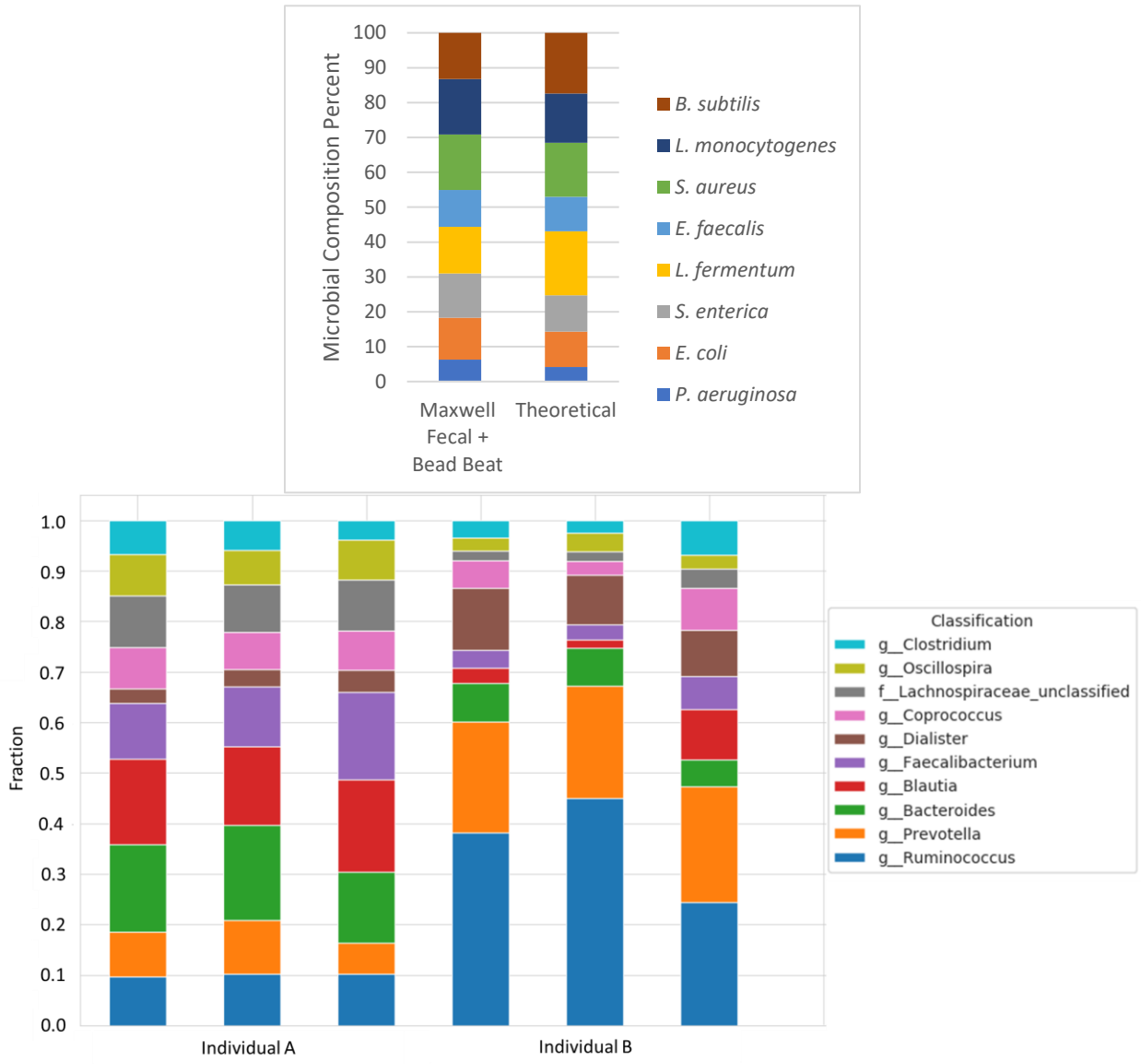

Figure 1. Example 16S V3/V4 metagenomic sequencing analysis for a microbial community standard and fecal samples purified with the Maxwell® RSC Fecal Microbiome DNA Kit with optional bead beating. DNA was purified from the ZymoBIOMICS Microbial Community Standard or 250-300mg of feces from two individuals according to the protocol described above. Following purification, the microbial DNA was sequenced over the V3

and V4 variable regions of the 16S gene following the Illumina 16S Metagenomic Sequencing Library Preparation Guide[1] to prepare libraries. Clean up steps were performed with ProNex® Size Selective Purification System (Cat.# NG2001) rather than AMPure XP Beads. Libraries were normalized and pooled based on quantification with the ProNex® Library Quant Kit (Cat.# NG1201) and were sequenced on an Illumina MiSeq Instrument with the v3 600-cycle reagent kit. Top Panel shows the sequencing results for the species-level bacterial profile of DNA purified from the microbial community standard as compared to the theoretical composition accounting for 16S copy number. Bottom Panel shows the sequencing results for the genus-level bacterial profile of DNA purified from triplicate stool samples from two individuals. Sequencing data was analyzed using an internal analysis pipeline running the Mothur[2] open source software package.

Sample ID	PB1-d
Library Prep Kit	SQK-LSK110
Flow Cell Type (# Pores)	MinION (1303)
BaseCaller Model	High Accuracy (Guppy v4.4.1)
EPI2ME Analysis	Fastq WIMP r2021.03.05
Reads Analyzed	5,610,497
Total Yield	6.0 Gb
Avg Quality Score	11.96
Classified	2,583,084
Unclassified	3,027,409

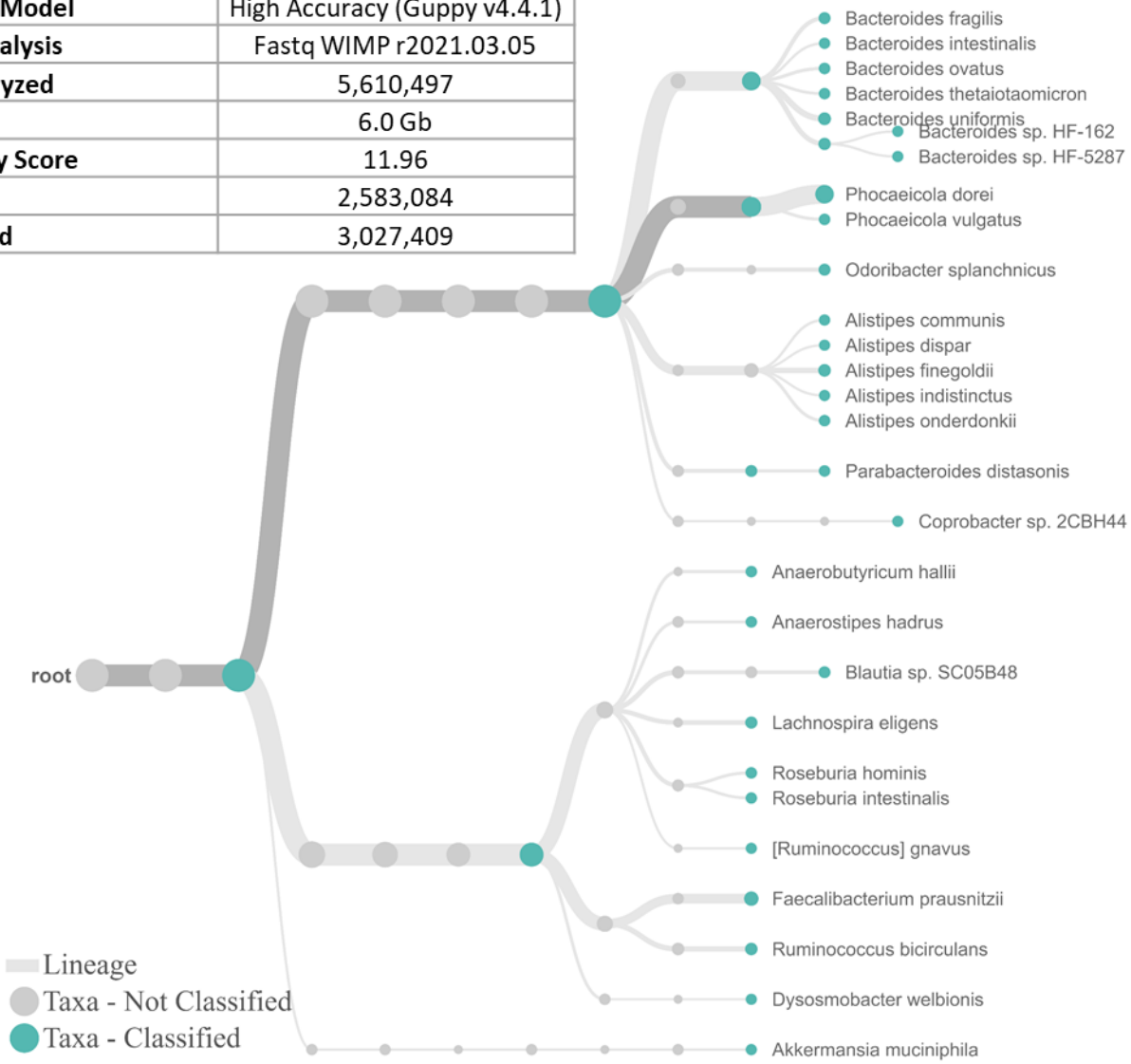

Figure 2. Oxford Nanopore Genomic DNA by Ligation Sequencing Results for an individual fecal sample purified with the Maxwell® RSC Fecal Microbiome DNA Kit with optional bead beating. DNA was purified from 250-300mg of feces from an individual according to the protocol described above. Following purification, 1µg of purified fecal microbiome DNA was sequenced according to manufacturer's protocol using the Ligation Sequencing Kit (SQK-LSK110)[3,4] using the Oxford Nanopore MinION. Fast5 sequences were base called using Guppy and analyzed using EPI2ME v3.3.0 FASTQ WIMP workflow for taxonomic classification. Left Table: Sequencing run details. Right: Species level subtree representing the NCBI taxonomy associated to the most common assignments (minimum 0.5% abundance cutoff).

References:

1. Illumina. 16S Metagenomic Sequencing Library Preparation – Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System. https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223-b.pdf. Accessed 03/2020
2. Schloss P.D., Westcott S.L., Ryabin T., Hall J.R., Hartmann M., Hollister E.B., Lesniewski R.A., Oakley B.B., Parks D.H., Robinson C.J., Sahl J.W., Stres B., Thallinger G.G., Van Horn D.J., Weber C.F. (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microbiol. 75: 7537-41.
3. Oxford Nanopore Genomic DNA by Ligation (SQK-LSK110) Protocol. Version GDE_9108_v110_revD_10Nov2020 Last update 02/02/2021.
4. ProNex® Chemistry-Based Clean-up in the Oxford Nanopore Ligation Sequencing Kit. PA411. 10/19.