Promega

Product Application

Purification of Microbiome DNA from Human Feces in OMNIgene®•GUT

Purify microbiome DNA from human feces collected in OMNIgene®•GUT using bead beating and 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: Human Feces collected in OMNIgene®•GUT

Input: Up to 250µl

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**

Materials Required:

- OMNIgene®•GUT (e.g., DNA Genotek Cat.# OM-200)
- 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 set to 95°C and 56°C

Protocol:

1. Collect human feces in a DNA Genotek OMNIgene®•GUT tube, as instructed by the manufacturer.
2. Transfer up to 250µl of feces-containing solution into a ZR BashingBead™ Lysis Tube.
3. Add 1ml of Lysis Buffer and 40µl of Proteinase K to each sample, and cap the tubes tightly.
4. Place tubes in a horizontal tube adapter assembled on a vortex.
5. Vortex tubes at maximum speed (~3,000rpm) for 30 minutes.
6. Continue with Step 3 in Section 4.B. of the Maxwell® RSC Fecal Microbiome DNA Kit Technical Manual (TM640).

Results:

DNA was purified from human feces collected in OMNIgene®•GUT devices with the Maxwell® RSC Fecal Microbiome DNA Kit, as described above. Samples were purified within 1-2 hours of collection; long-term storage of feces in the collection device prior to purification was not tested. Purified DNA was used in 16S V3/V4 metagenomic sequencing, and the resulting microbial profiles are shown in Figure 1. Purified DNA was also used for Oxford Nanopore Technologies long-read sequencing, and the taxomic tree of the detected microbes is shown in Figure 2.

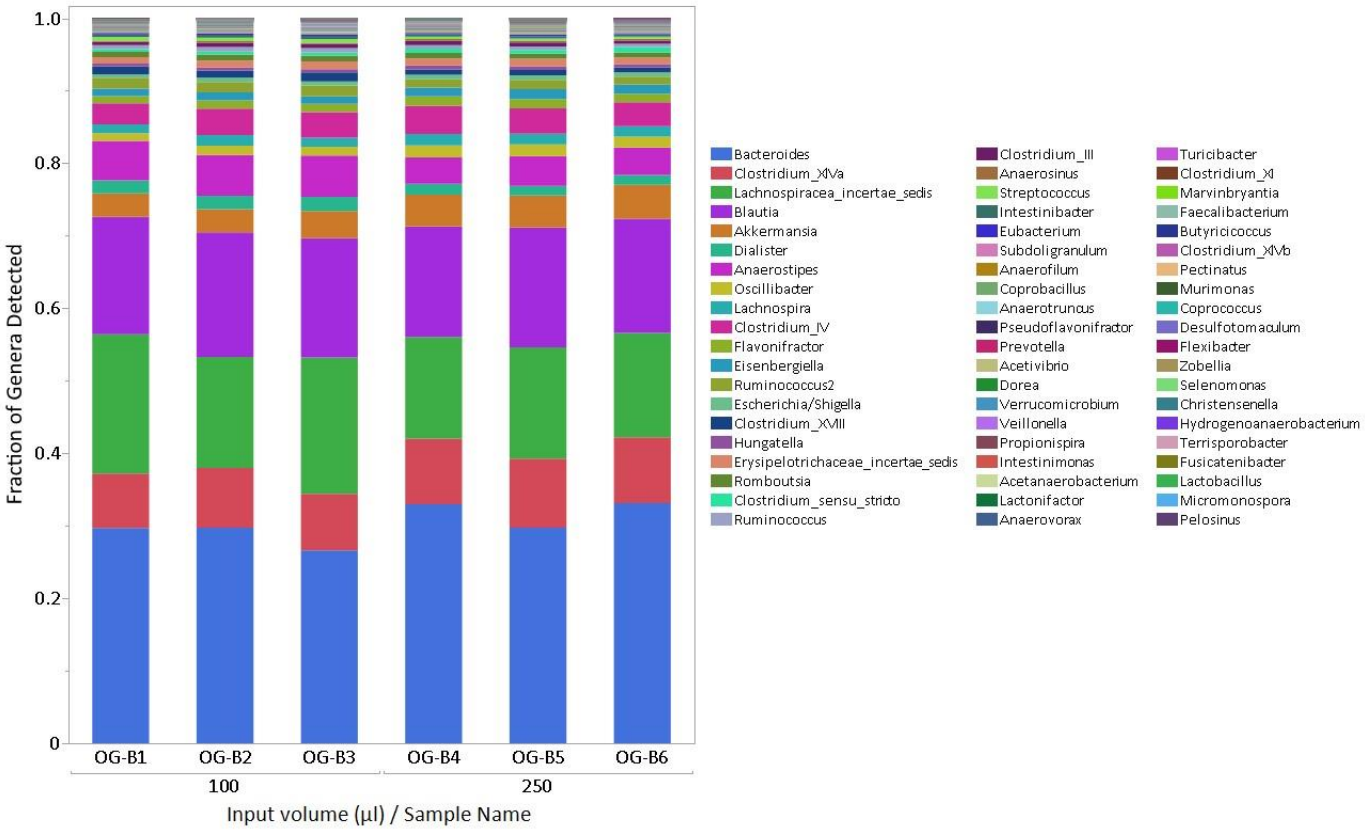

Figure 1. Example 16S V3/V4 metagenomic sequencing analysis for DNA purified from human feces collected in OMNIgene®•GUT. A feces sample was collected from one individual in OMNIgene®•GUT, according to manufacturer's instructions. DNA was purified from 100µl or 250µl of feces-containing solution, as described above. The microbial DNA was sequenced over the V3 and V4 variable regions of the 16S rRNA gene following the Illumina 16S Metagenomic Sequencing Library Preparation Guide[1] to prepare libraries, with the following modifications: GoTaq® Long PCR Master Mix (Cat.# M4021) was used for Amplicon PCR and Index PCR, clean up steps were performed with ProNex® Size Selective Purification System (Cat.# NG2001) using a 1.25X ProNex® Chemistry to sample ratio, and libraries were normalized and pooled based on quantification with the ProNex® NGS Library Quant Kit (Cat.# NG1201). Libraries were sequenced on an Illumina MiSeq Instrument with the v3 600-cycle reagent kit. Sequencing results are shown for the genus-level bacterial profiles of DNA purified in triplicate for each input volume from a single source sample collected in two OMNIgene®•GUT devices. Sequencing data were analyzed using the Illumina 16S Metagenomics Basespace application.

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

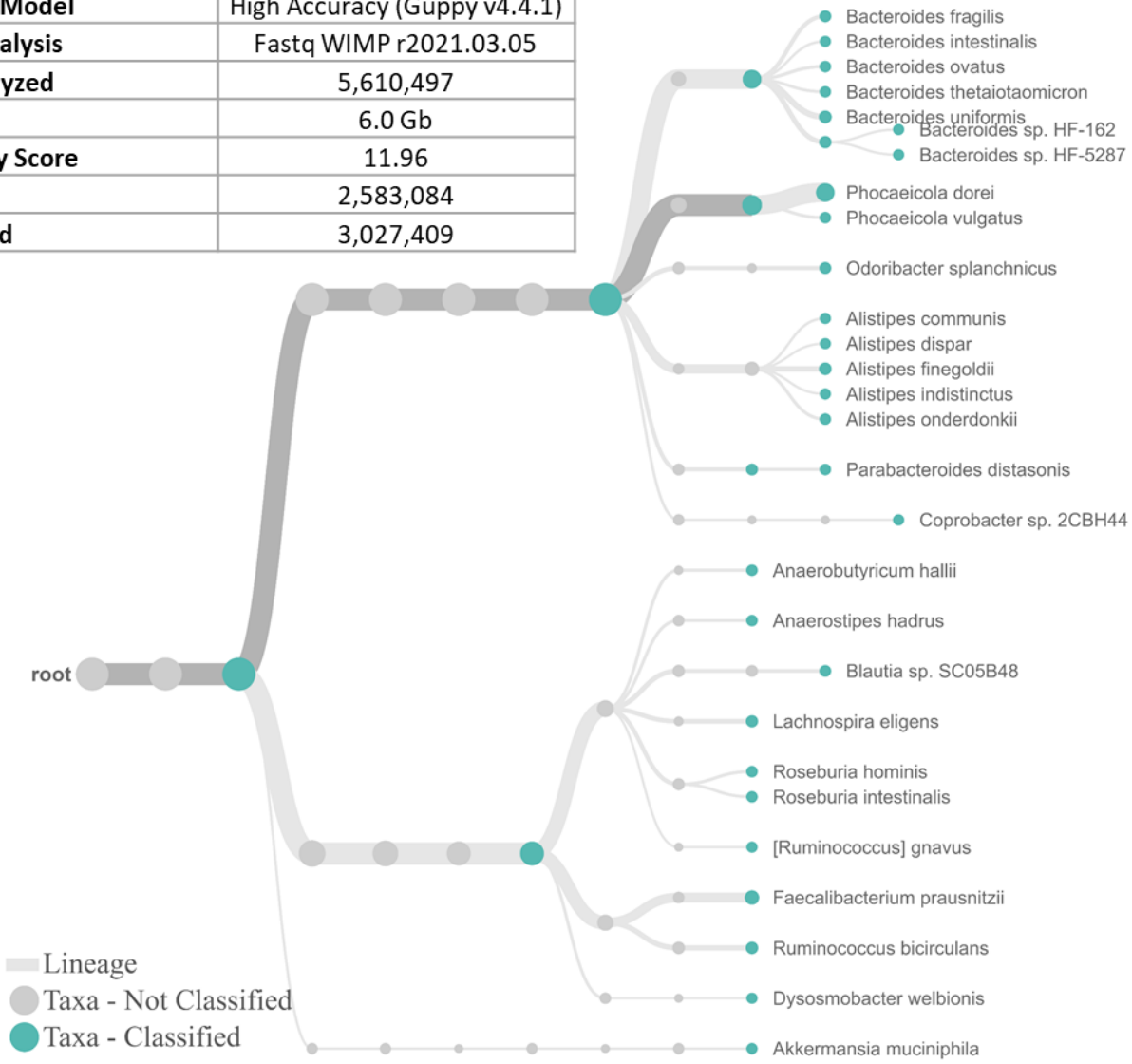

Figure 2. Oxford Nanopore Genomic DNA by Ligation Sequencing results for an individual fecal sample purified with the Maxwell® RSC Fecal Microbiome DNA Kit with optional bead beating. DNA was purified from 250µl of feces-containing solution from an OMNIgene®•GUT collection device according to the protocol described above. 500ng of purified DNA was sequenced according to manufacturer's protocol using the Ligation Sequencing Kit (SQK-LSK109)[2,3] using the Oxford Nanopore MinION with a Flongle adapter. 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. Part # 15044223 Rev. B
2. Oxford Nanopore Genomic DNA by Ligation (SQK-LSK110) Protocol. Version GDE_9108_v110_revD_10Nov2020 Last update 02/02/2021.
3. ProNex® Chemistry-Based Clean-up in the Oxford Nanopore Ligation Sequencing Kit. PA411. 10/19.