Promega

Product Application

Purification of Microbiome DNA from Feces in Stool Transport and Recovery (S.T.A.R.) Buffer

Purify microbiome DNA from human feces collected in 50% S.T.A.R. Buffer 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 50% S.T.A.R. Buffer

Input: Up to 300µl

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:

- S.T.A.R. Buffer (Stool Transport and Recovery Buffer) (Roche Diagnostics Cat.# 03335208001)
- 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. Dilute S.T.A.R. Buffer to 50% in Nuclease-free water.
2. Add 50mg of human feces to a 5ml screw cap tube
3. Add 2ml of 50% S.T.A.R. Buffer.
4. Briefly vortex the sample to homogenize, and allow particulates to settle.
5. Transfer 300µl of feces-containing solution into a ZR BashingBead™ Lysis Tube.
6. Add 1ml of Lysis Buffer and 40µl of Proteinase K to each sample, and cap the tubes tightly.
7. Place tubes in a horizontal tube adapter assembled on a vortex.
8. Vortex tubes at maximum speed (~3,000rpm) for 30 minutes.
9. Continue with Step 3 in Section 4.B. of the Maxwell® RSC Fecal Microbiome DNA Kit Technical Manual (TM640).

Results:

DNA was purified from fresh human feces collected in 50% S.T.A.R. Buffer with the Maxwell® RSC Fecal Microbiome DNA Kit, as described above. Samples were purified within 1-2 hours of collection; storage of feces in the collection solution prior to purification was not tested. Microbiome DNA was used in 16S V3/V4 metagenomic sequencing, and the resulting microbial profile is shown in Figure 1.

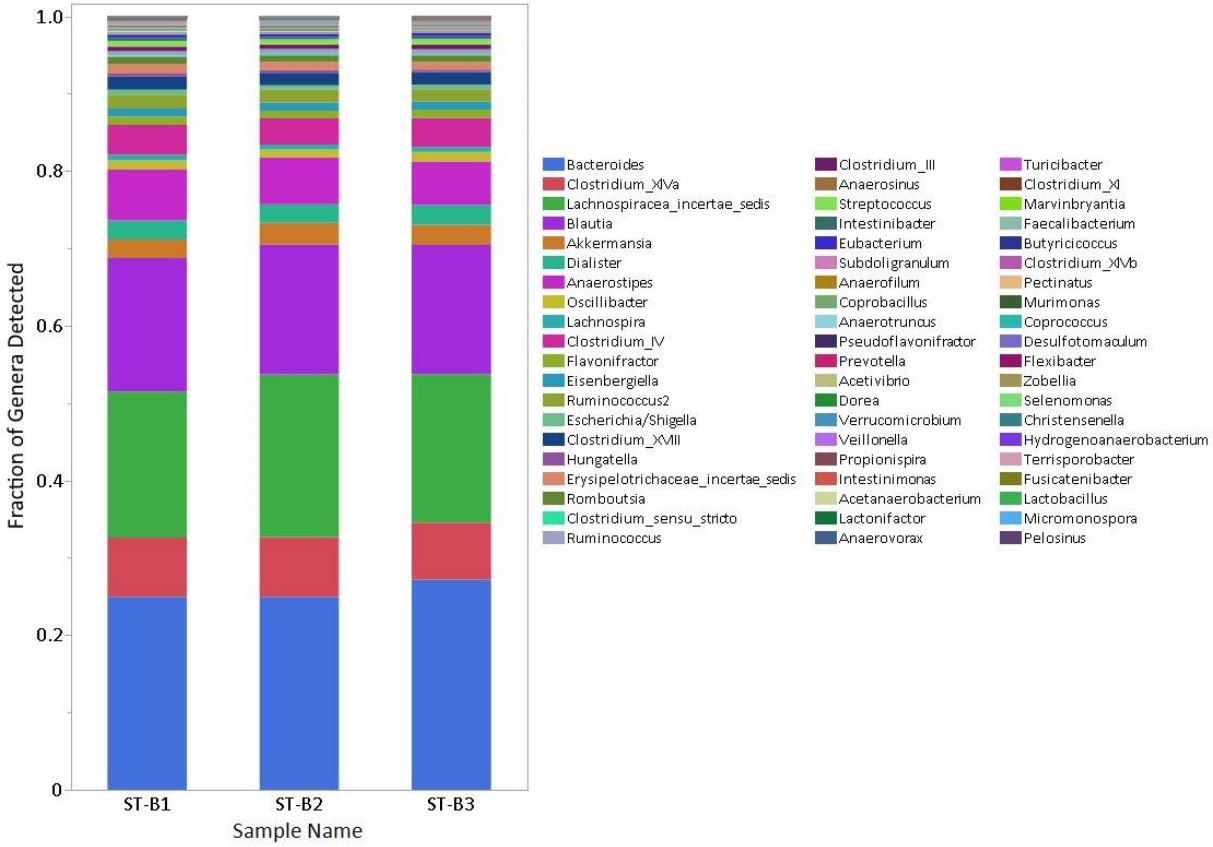

Figure 1. Example 16S V3/V4 metagenomic sequencing analysis for microbiome DNA from human feces collected in 50% S.T.A.R. Buffer. Human feces was collected from one individual in 50% S.T.A.R. Buffer, and DNA was purified from 300µl of feces-containing solution, as described above. The microbial DNA was 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. Libraries were normalized and pooled based on quantification with the ProNex® NGS Library Quant Kit (Cat.# NG1201) and were sequenced on an Illumina MiSeq Instrument with the v3 600-cycle reagent kit. Sequencing results are shown for the genus-level bacterial profile of DNA purified from triplicate stool samples for each input volume. Sequencing data were analyzed using the Illumina 16S Metagenomics Basespace application.

Reference:

1. Illumina. 16S Metagenomic Sequencing Library Preparation – Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq System. Part # 15044223 Rev. B