Promega

Product Application

Automated Purification of DNA from Cow Feces

Purify DNA suitable for next-generation sequencing (NGS) of microbial populations from cow feces using bead beating and the Maxwell® RSC Fecal Microbiome DNA Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Fecal Microbiome DNA Kit

Analyses: NGS sequencing of the 16S V3/V4 region

Sample Type: bovine feces

Input: 200-250mg

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:

- Maxwell® RSC Fecal Microbiome DNA Kit (Cat.# AS1700)
- Maxwell® RSC Instrument (Cat.# AS4500) or Maxwell® RSC 48 Instrument (Cat.# AS8500)
- ZR BashingBead™ Lysis Tubes ,(0.1 & 0.5mm) (Zymo Research, Cat.# S6012-50)
- Vortex Genie 2 Digital (Scientific Industrics, Cat.# SI-A236) or similar
- Horizontal Vortex Adaptor for 24 Tubes (Qiagen, Cat.# 13000-V1-24) or similar
- Heat block suitable for 2.0ml microcentrifuge tubes
- Microcentrifuge

Protocol:

1. Weigh feces into ZR BashingBead™ Lysis Tubes (0.1 & 0.5mm).
2. Add 1ml of Lysis Buffer and 40µl of Proteinase K to the tube.
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 was purified from the ZymoBIOMICS® Gut Microbiome Standard or fresh cow feces using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating according to the protocol described above. DNA was used for 16S V3/V4 metagenomic sequencing and the taxonomic distributions are shown at the genus level (Figs.1-2). For the ZymoBIOMICS® Gut Microbiome Standard, the 15 expected genera were present in all replicates and accounted for >99.9% of mapped reads (Fig.1). The expected profile for the cow fecal samples is unknown (Fig.2). However, the average Bray-Curtis dissimilarity for taxonomic distribution between purification replicates averaged 0.030 and 0.108 for the ZymoBIOMICS® Gut Microbiome Standard and cow feces, respectively, indicating a high level of reproducibility in microbial purification and library preparation using this method.

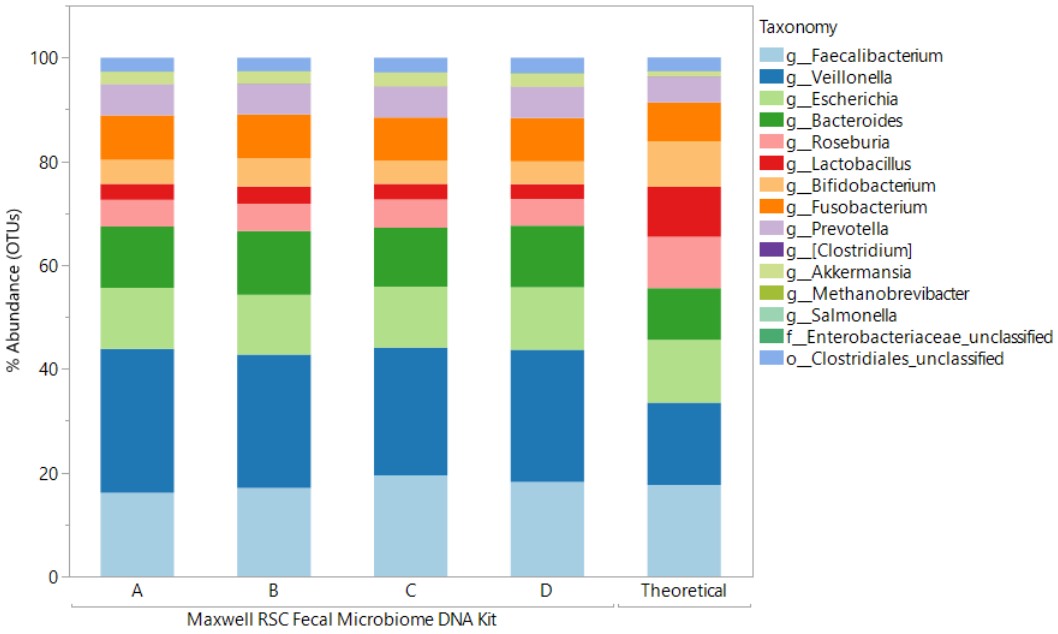

Figure 1. Taxonomic distributions from 16S V3/V4 metagenomic sequencing of the ZymoBIOMICS® Gut Microbiome Standard purified using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating. DNA was purified in quadruplicate from 50µl of the ZymoBIOMICS® Gut Microbiome Standard (Zymo Research, Cat.# D6331) using the above protocol. 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] with the following differences: DNA input for amplicon PCR was reduced to 1ng with 2 additional PCR cycles; GoTaq® Long PCR Master Mix (Cat.# M4021) was used for all amplification steps; and the ProNex® Size-Selective Purification System (Cat.# NG2001) was used for all purification steps. 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 a v3 600-cycle reagent kit. Sequencing data was analyzed at the genus level using a pipeline based on the *mothur* open source software package (v1.43.0)[2]. Percent abundance of the top 15 OTUs are shown compared to the theoretical distribution of the standard based on 16S copy number.

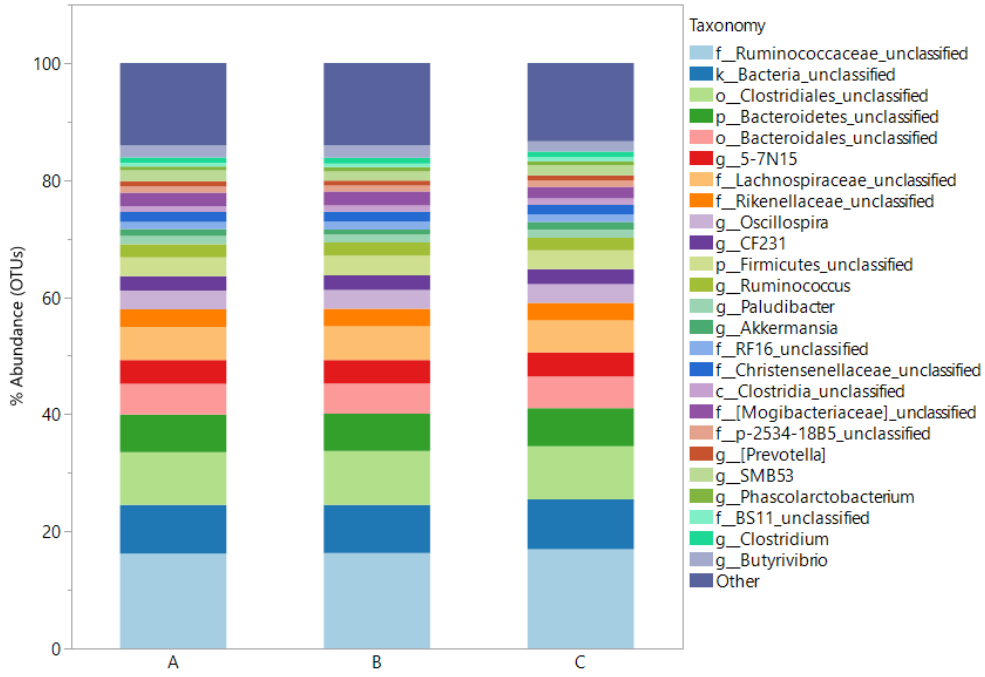

Figure 2. Bovine microbiome taxonomic distributions from 16S V3/V4 metagenomic sequencing of cow fecal DNA purified using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating. DNA was purified in triplicate from 200-250mg of fresh cow feces using the above protocol. Microbial DNA was sequenced over the V3 and V4 variable regions of the 16S gene following the Illumina 16S Metagenomic Sequencing Library Preparation Guide[1] with the following differences: DNA input for amplicon PCR was reduced to 1ng with 2 additional PCR cycles; GoTaq® Long PCR Master Mix (Cat.# M4021) was used for all amplification steps; and the ProNex® Size-Selective Purification System (Cat.# NG2001) was used for all purification steps. 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 a v3 600-cycle reagent kit. Sequencing data was analyzed at the genus level using a pipeline based on the *mothur* open source software package (v1.43.0)[2]. Percent abundance of the top 25 OTUs are shown.

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/illuminasupport/documents/documentation/chemistry_documentation/16s/16s-metagenomic-libraryprep-guide-15044223-b.pdf. Accessed 04/2021.
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.