Promega

Product Application

Automated Purification of Animal Fecal Microbiome DNA from PERFORMAbiome™•GUT Collection Devices

Purify DNA suitable for next-generation sequencing (NGS) of microbial populations from animal feces collected in PERFORMAbiome™•GUT devices using bead beating and the Maxwell® RSC Fecal Microbiome DNA Kit on the Maxwell® RSC Instrument.

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**

Kit: Maxwell® RSC Fecal Microbiome DNA Kit

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

Sample Type: Feces from cow, cat, and dog collected in PERFORMAbiome™•GUT devices

Input: 250µl fecal sample in PERFORMAbiome™•GUT devices

Materials Required:

- PERFORMAbiome™•GUT collection device (DNA genotek®, Cat.# PB-200)
- 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. Follow manufacturer's directions for collecting feces in a PERFORMAbiome™•GUT collection device.

   Note: Promega has tested compatibility of the PERFORMAbiome™•GUT collection device with the Maxwell® RSC Fecal Microbiome DNA Kit, but has not tested the manufacturer's claims of microbiome stability and reduced bias.

2. Immediately before purification, shake the PERFORMAbiome™•GUT collection device for 10 seconds. Transfer 250µl of lysate to a ZR BashingBead™ Lysis Tube (0.1 & 0.5mm) using a wide bore pipet tip.
3. Add 1ml of Lysis Buffer and 40µl of Proteinase K to the ZR BashingBead™ Lysis Tube (0.1 & 0.5mm).
4. Place tubes in a horizontal tube adapter assembled on a vortex. Vortex tubes at maximum speed (~3000rpm) for 30 minutes.
5. Continue with Step 3 in Section 4.B of the Maxwell® RSC Fecal Microbiome DNA Kit Technical Manual (TM640).

Results:

DNA was purified using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating (as described above) from fresh cow feces or from the same feces sampled with a PERFORMAbiome™•GUT collection device. DNA was used for 16S V3/V4 metagenomic sequencing and the taxonomic distributions are shown at the genus level (Fig.1). Recovered microbial genera and distributions were similar between fresh cow feces and feces collected in the PERFORMAbiome™•GUT collection device. DNA was also purified from cat and dog feces collected in a PERFORMAbiome™•GUT device and microbial populations identified using 16S V3/V4 metagenomic sequencing (Figs.2,3).

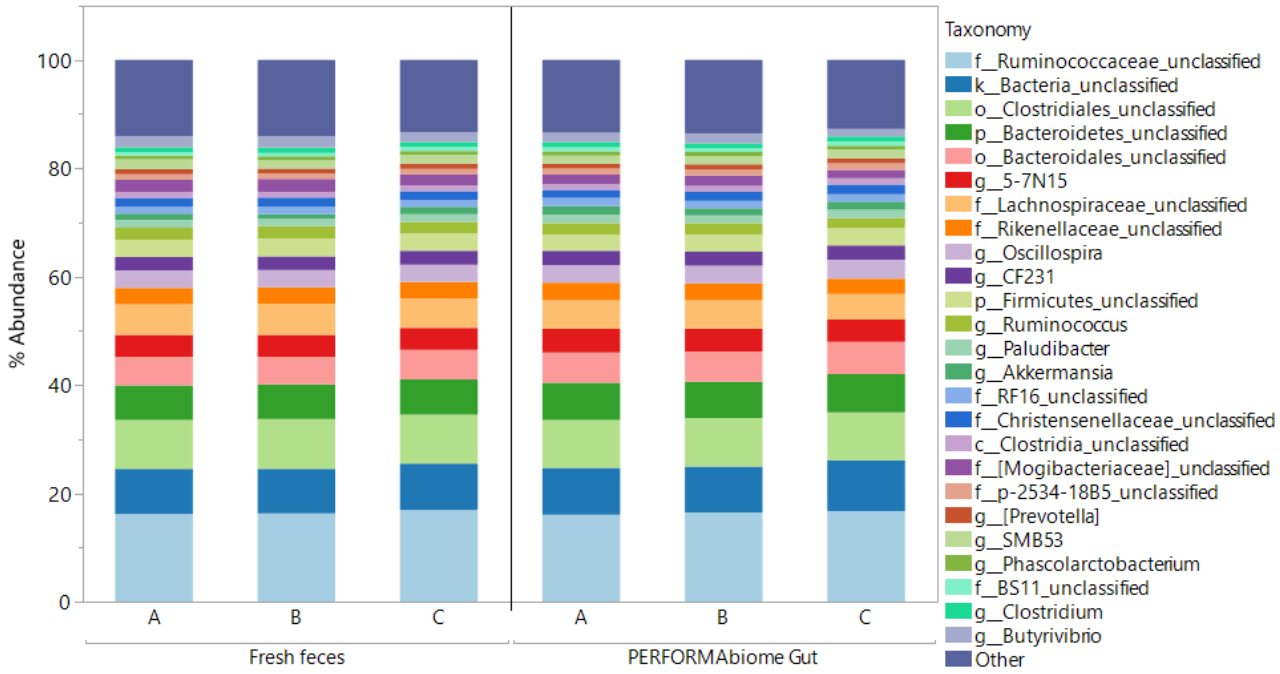

Figure 1. Bovine microbiome taxonomic distributions from 16S V3/V4 metagenomic sequencing of cow fecal DNA purified using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating. DNA was purified in triplicate from 200-250mg of fresh cow feces or from 250µl of the same feces collected in a PERFORMAbiome™•GUT device (purified within 2 hours of collection in the device). 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.

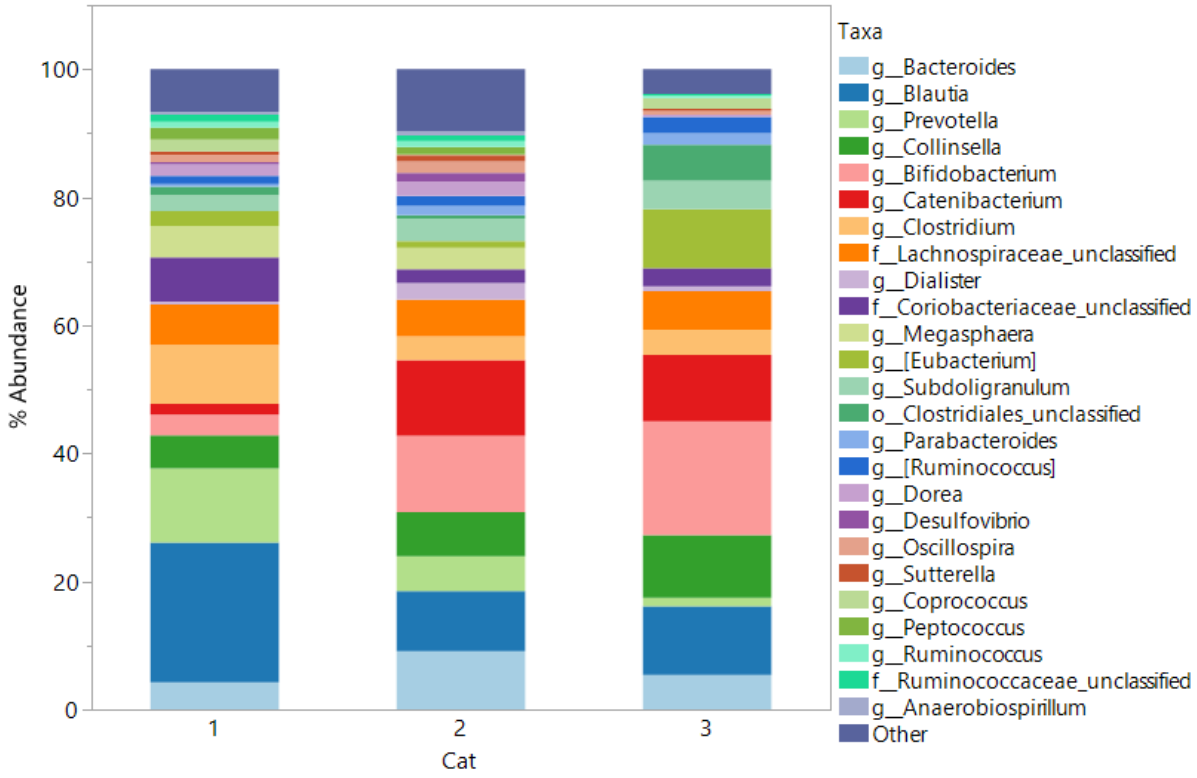

Figure 2. Feline microbiome taxonomic distributions from 16S V3/V4 metagenomic sequencing of cat fecal DNA purified from PERFORMAbiome™•GUT devices using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating. DNA was purified from 200-250mg frozen feces of three cats collected into PERFORMAbiome™•GUT devices and stored at room temperature for 2-3 days before purification. 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.

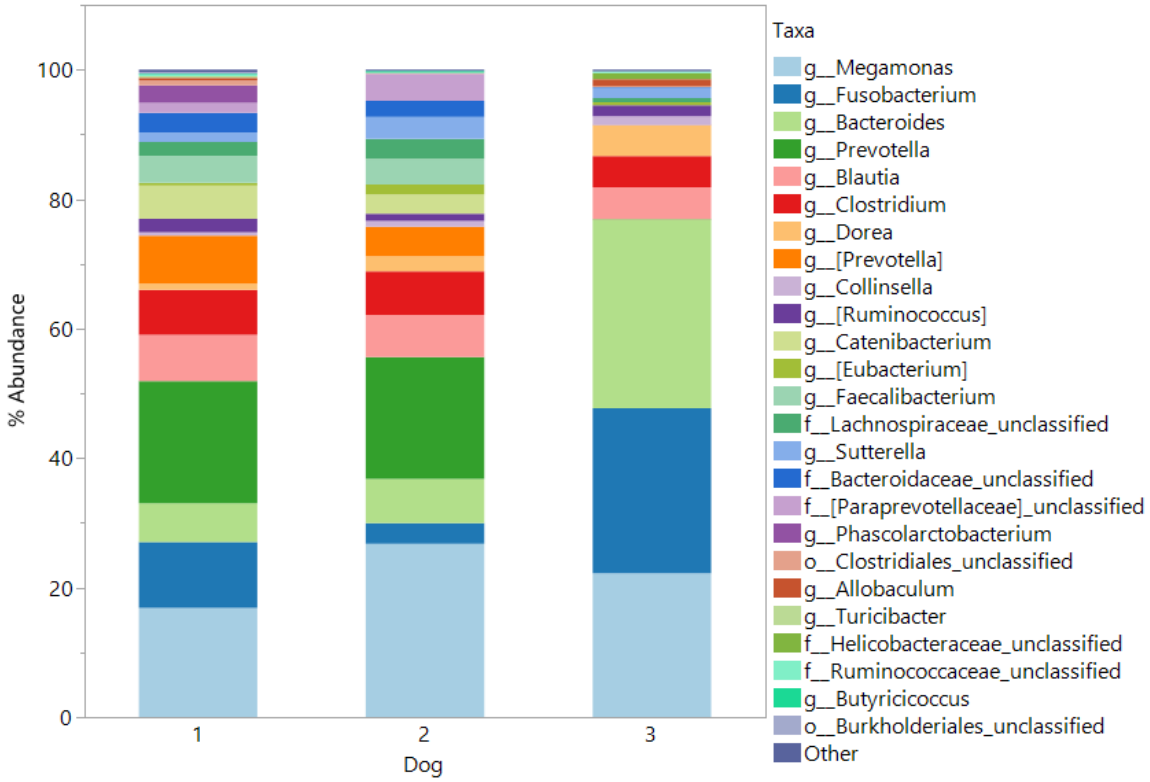

Figure 3. Canine microbiome taxonomic distributions from 16S V3/V4 metagenomic sequencing of dog fecal DNA purified from PERFORMAbiome™•GUT devices using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating. DNA was purified from 200-250mg fresh feces of three dogs collected into PERFORMAbiome™•GUT devices and stored at room temperature for 2-6 days before purification. 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.