

Automated Purification of Companion Animal Fecal Microbiome DNA

Purify DNA suitable for next-generation sequencing (NGS) of microbial populations from cat and dog 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: Cat or dog feces

Input: 200-250mg

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 Industries, 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

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 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, fresh dog feces, or frozen cat 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.1-3). 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 average Bray-Curtis dissimilarity for taxonomic distribution between purification replicates averaged 0.030 for the ZymoBIOMICS® Gut Microbiome Standard, indicating a high level of reproducibility in microbial purification and library preparation using this method. Example microbial distributions from dog feces (Fig.2) and cat feces (Fig.3) are also shown, with highly variable microbial populations between individuals. In addition, one dog fecal DNA sample was used for Oxford Nanopore long-read sequencing and is shown in Figure 4.

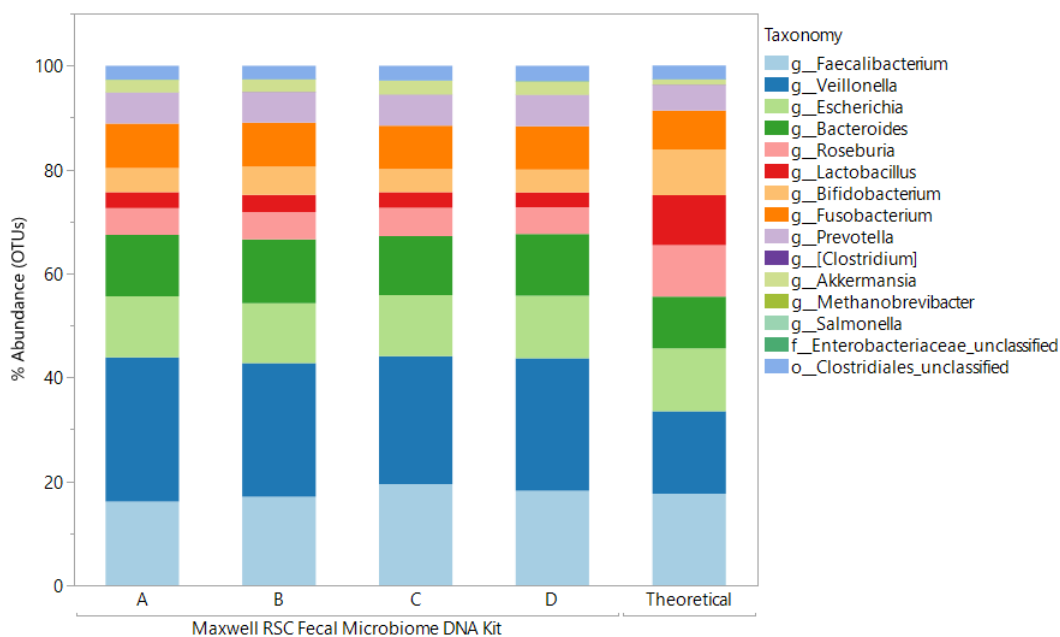


Figure 1. Taxonomic distributions from 16S V3/V4 metagenomic sequencing of the ZymoBIOMICS® Gut Microbiome Standard purified using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating. DNA was purified in quadruplicate from 50µl of the ZymoBIOMICS® Microbiome Standard (Zymo Research, Cat.# D6331) 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 Guide¹ 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.

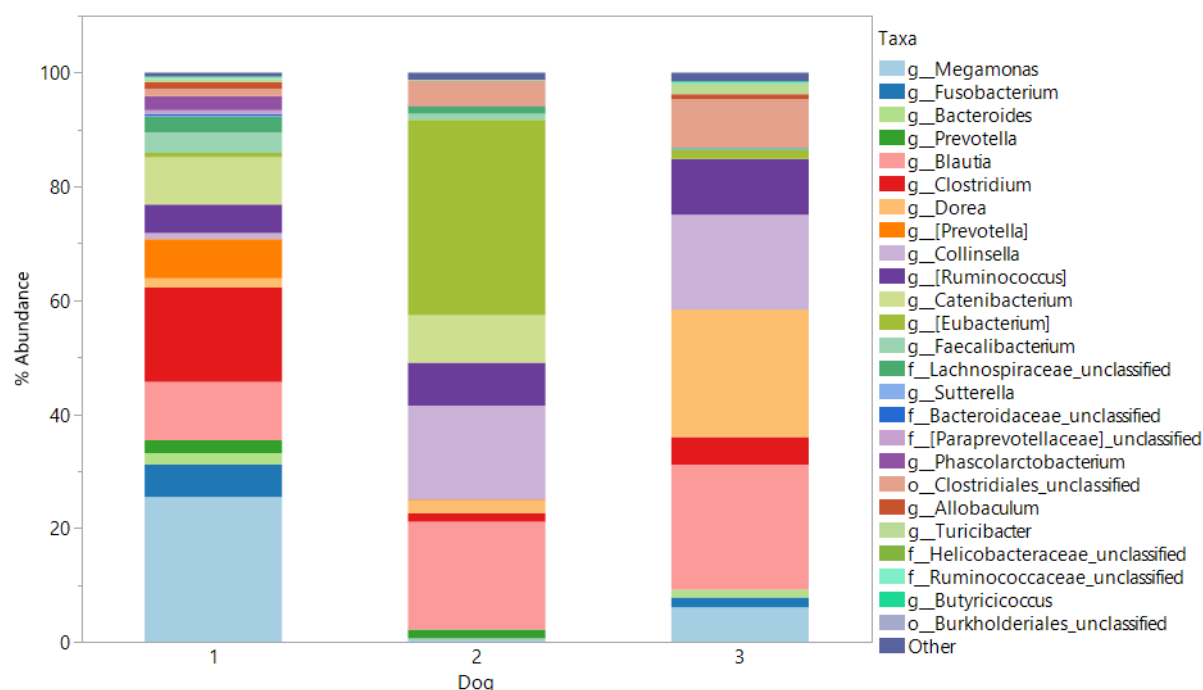


Figure 2. Canine microbiome taxonomic distributions from 16S V3/V4 metagenomic sequencing of dog fecal DNA purified using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating. DNA was purified from 200-250mg of fresh (<12 hour) dog feces from three animals using the above protocol. Microbial DNA was sequenced over the V3 and V4 variable regions of the 16S gene following the Illumina 16S Metagenomic Sequencing Library Preparation Guide¹ 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.

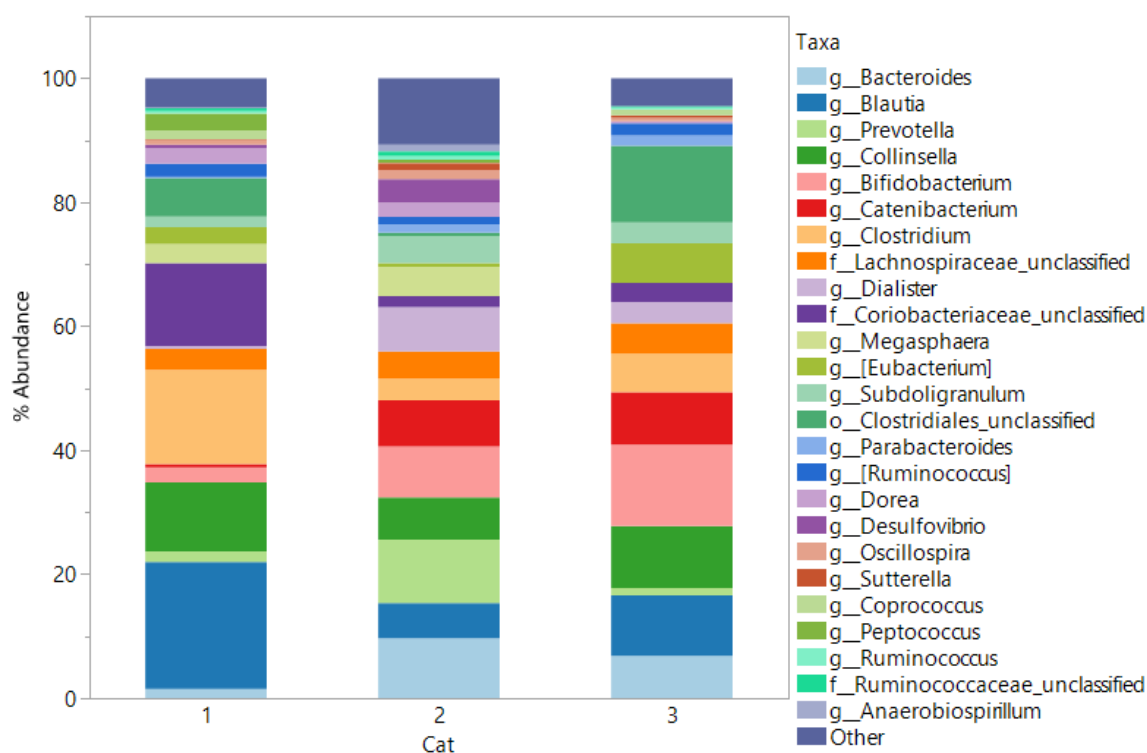


Figure 3. Feline microbiome taxonomic distributions from 16S V3/V4 metagenomic sequencing of cat fecal DNA purified using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating. DNA was purified from 200-250mg of frozen cat feces from three animals using the above protocol. Microbial DNA was sequenced over the V3 and V4 variable regions of the 16S gene following the Illumina 16S Metagenomic Sequencing Library Preparation Guide¹ 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.

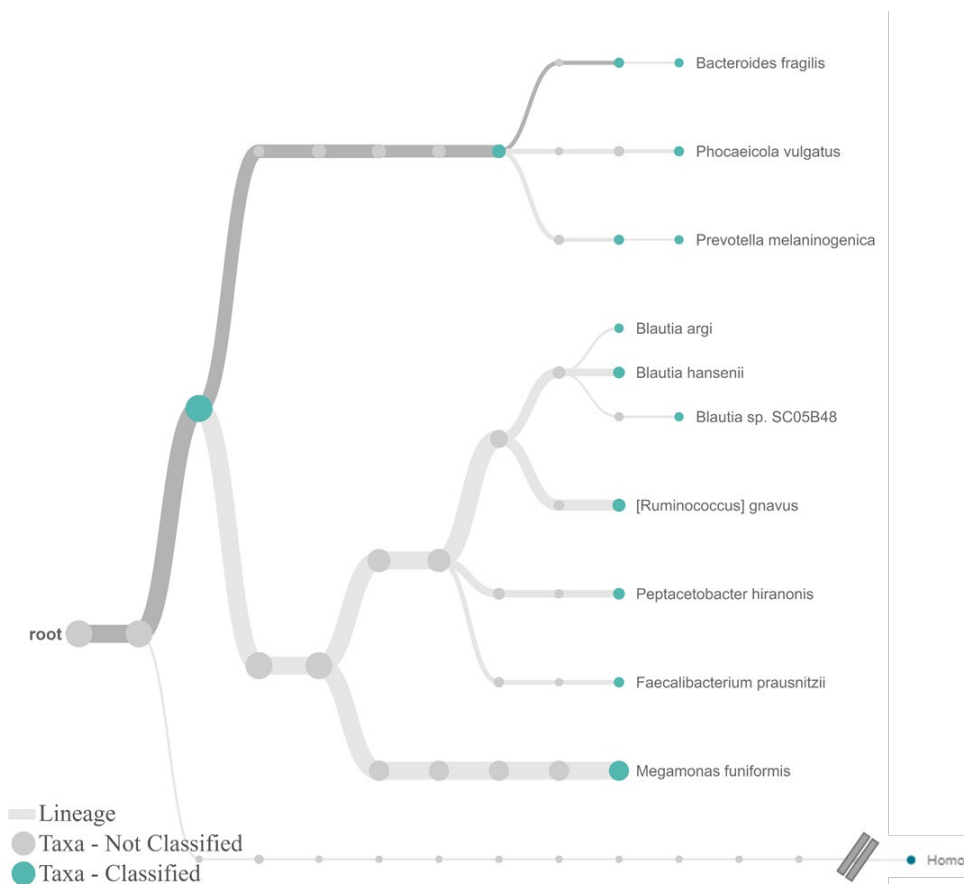


Figure 4. Oxford Nanopore Genomic DNA by Ligation sequencing results for dog fecal microbiome. 500ng of dog fecal DNA purified using the Maxwell® RSC Fecal Microbiome DNA Kit with bead beating was sequenced according to manufacturer's protocol using the Ligation Sequencing Kit (SQK-LSK109)^{3,4} and 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. The species level subtree is shown, representing the NCBI taxonomy associated to the most common assignments (minimum 0.5% abundance cutoff).

Table 1. Sequencing metrics for Oxford Nanopore Genomic DNA by Ligation sequencing results for dog fecal microbiome. See Fig.4 for details.

Library Prep Kit	SQK-LSK109
Flow Cell Type (# Pores)	Flongle (96)
BaseCaller Model	High Accuracy (Guppy v4.3.4)
EPI2ME Analysis	Fastq WIMP r2021.03.05
Reads Analyzed	341,949
Total Yield	257.3 Mb
Avg Quality Score	12.33
Classified	114,184
Unclassified	227,765

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-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.
3. Oxford Nanopore Genomic DNA by Ligation (SQK-LSK109) Protocol. Version GDE_9063_v109_revY_14Aug2019 Last update 21/04/2021.
4. ProNex® Chemistry-Based Clean-up in the Oxford Nanopore Ligation Sequencing Kit. [PA411](#). 10/19.