Promega

Product Application

Automated Purification of Fecal Microbial DNA on the Analytik Jena CyBio FeliX

Purify fecal microbial DNA with the Maxwell® HT Fecal Microbiome DNA Kit on the Analytik Jena CyBio FeliX.

Kit: Maxwell® HT Fecal Microbiome DNA Kit (Cat.# A6040)

Analyses: Next-generation sequencing (NGS)

Sample Type(s): human feces

Input: ≤100mg

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 or for details on how to obtain the method described herein, contact Technical Services at: **techserv@promega.com**

Materials Required:

- Maxwell® HT Fecal Microbiome DNA Kit (Cat.# A6040)
- Isopropanol, Molecular Biology Grade
- ZR BashingBead™ Lysis Tubes (Zymo Cat.# S6003-50)
- Horizontal Vortex Adapter for 1.5/2.0ml Tubes (e.g. Qiagen Cat.# 13000-V1-24)
- Vortex
- Heat Block set to 56°C
- CyBio FeliX with Extraction Set (Analytik Jena Cat.# OL5015-24-100, OL5015-25-120)
- Deep Well Plate, 96 square well, 2ml (Analytik Jena Cat.# 845-FX-8500025)
- CyBio TipRack 96/1000µl, PCR-certified, pre-sterilized, filter (Analytik Jena Cat.# OL3811-25-939-F)
- Protective Plate (Analytik Jena Cat.# OL3317-25-126)

Protocol:

1. Prepare the following plates. Reagent mixtures can be prepared as a master mix and aliquoted into each well of a 2ml deep-well plate.

   Note: 70% isopropanol and 80% ethanol solutions should be prepared fresh daily.

Plate	Reagent	Volume per Sample
Processing Plate	Binding Buffer	250µl
	Isopropanol	350µl
	Maxwell® HT C Resin	18µl
	Preprocessed fecal lysate in Lysis Buffer	*250µl*
Reagent Plate - Wash 1	Wash Buffer A (WBA)	400µl
	RNase A	20µl

Reagent Plate - Wash 2	Wash Buffer A (WBA)	320µl
Reagent Plate - Wash 3	70% Isopropanol	320µl
Reagent Plate - Wash 4	80% Ethanol	250µl
Reagent Plate - Elution	25mM Tris-HCl (pH 8.0)	110µl
Final Elution Plate	Empty	

2. Aliquot ≤100mg of feces into a ZR BashingBead™ Lysis Tube.
3. Add 1ml of Lysis Buffer and 40µl of Proteinase K to the sample in the lysis tube. Vortex at maximum speed (~3,000 rpm) for 30 minutes on a lab vortex fitted with a horizontal tube adapter for the vortex.
4. Incubate the sample at 56°C for 5 minutes.
5. Centrifuge the sample at maximum speed (>13,000 x *g*) in a microcentrifuge for 5 minutes.
6. Transfer 250µl of cleared lysate to the prepared Processing Plate.
7. Run the HT Fecal Microbiome method on the CyBio FeliX, and set up the deck of the CyBio FeliX Instrument as directed in the setup screens.

Summary of processing steps for purification of fecal microbial DNA wtih the Maxwell® HT Fecal Microbiome DNA Kit on the CyBio FeliX Automated Liquid Handler.

- Binding:
  - User loads the Processing Plate containing pre-processed fecal lysates, Binding Buffer, isopropanol, and Maxwell® HT C Resin.
  - Nucleic acid is captured on the magenetic resin by shaking and tip mixing to keep the resin suspended in the sample.
  - The resin is magnetized, and the lysate is removed and discarded.
- Wash 1: Wash Buffer A (WBA) and RNase A.
  - 420µl of Wash Buffer A/RNase A solution is added.
  - The resin is resuspended by shaking and tip mixing.
  - The resin is magnetized, and the wash buffer is removed and discarded.
- Wash 2: Wash Buffer A (WBA)
  - 320µl of Wash Buffer A is added.
  - The resin is resuspended by shaking and tip mixing.
  - The resin is magnetized, and the wash buffer is removed and discarded.
- Wash 3: 70% Isopropanol
  - 320µl of 70% Isopropanol is added.
  - Resin is resupended by shaking and tip mixing.
  - The resin is magnetized and the wash buffer is removed and discarded.

- Wash 4: 80% Ethanol
    - 250µl of 80% Ethanol is added.
    - The resin is resuspended by shaking and tip mixing.
    - The resin is magnetized, and the wash buffer is removed and discarded.
- Resin Dry:
    - The resin is dried with heating and shaking.
- Elution:
    - 110µl of 25mM Tris-HCl (pH 8.0) is added.
    - The resin is resuspended by tip mixing and shaking, and the sample is heated.
    - The resin is magnetized, and the eluate is transferred to the final elution plate.

Results:

Microbial DNA purified from 50mg and 100mg of human feces with the Maxwell® HT Fecal Microbiome DNA Kit was purified in sufficient quantity and quality for metagenomic sequencing using an Illumina-based 16S V3/V4 next-generation sequencing protocol.

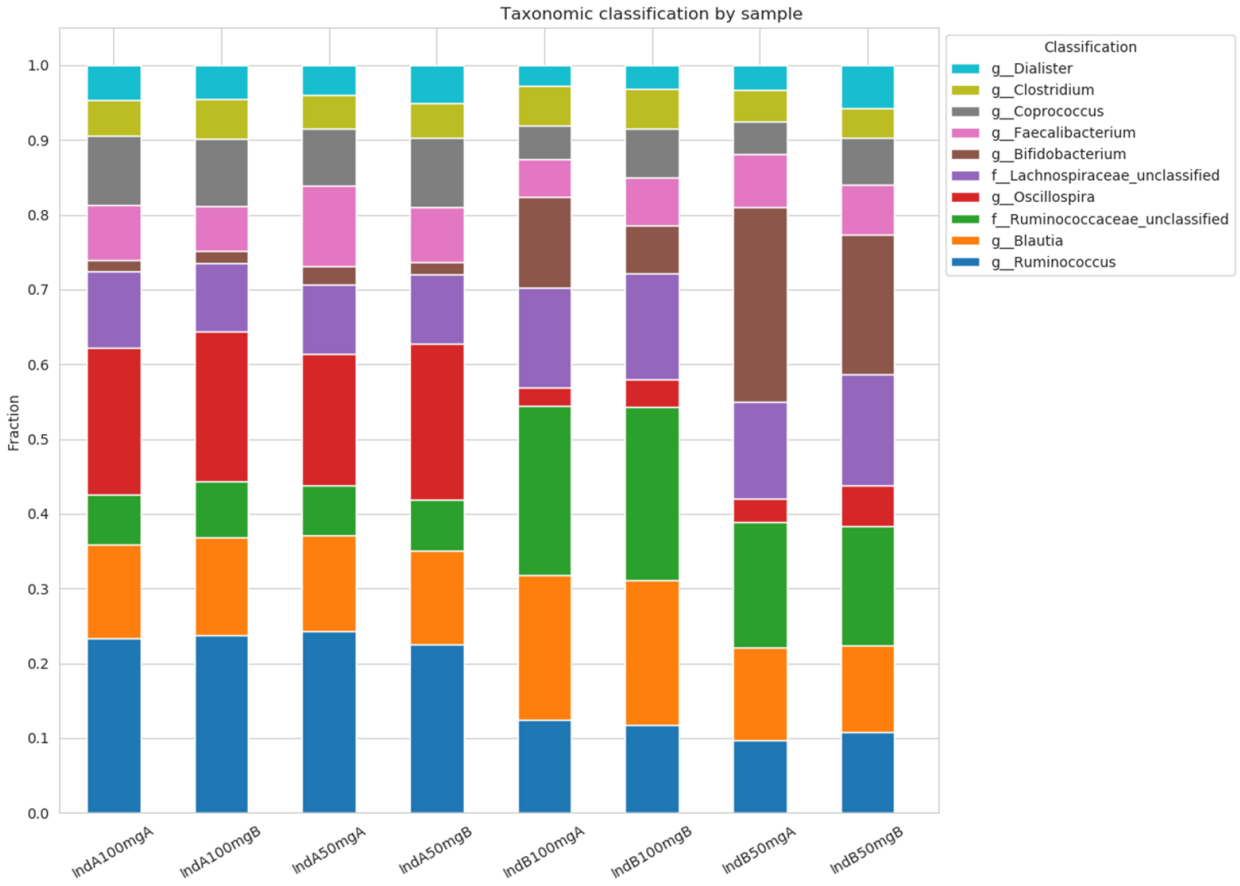

Figure 1. 16S V3/V4 metagenomic sequencing of fecal DNA purified with the Maxwell® HT Fecal Microbiome DNA Kit on the CyBio FeliX Instrument. DNA was purified from 50mg or 100mg of fecal material from two individuals using the Maxwell® HT Fecal Microbiome DNA Kit on the CyBio FeliX Automated Liquid Handler as described above. Bacterial 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 modifications: the 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. The libraries were normalized and pooled based on quantification with the ProNex® Library Quant Kit (Cat.# NG1201) and 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]. For each sample, a taxonomic bar plot showing the classification of the top 10 bacterial genera is shown. Duplicate samples were purified for each individual (indA and indB) and input amount (100mg and 50mg).

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.