Promega

Product Application

Total Nucleic Acid Purification from Human Feces using the Maxwell® RSC Fecal Microbiome DNA Kit

Purify total nucleic acid from human feces using the Maxwell® RSC Fecal Microbiome DNA Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC Fecal Microbiome DNA Kit (Cat.# AS1700)

Analyses: qPCR, RT-qPCR

Sample Type(s): Human Feces

Input: Up to 300mg

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)
- Screw-cap microcentrifuge tubes, 2ml
- Heat block
- Microcentrifuge
- Vortex
- Optional: Bead lysing tubes (e.g. ZR BashingBead Lysing Tubes, 0.1 & 0.5mm, Zymo Research Cat.# S6012-50)
- Optional: Vortex horizontal multitube adapter

Protocol:

1. Place 100mg of sample into a 2ml screw-cap microcentrifuge tube. If performing the optional bead beating step, use bead lysing tube.
2. Add 1ml of Lysis Buffer and 40µl Proteinase K. Vortex for 30 seconds.
3. Heat at 95°C for 5 minutes. Cool for 2 minutes. Vortex for 1 minute. If performing the optional bead beating step, bead beat for 30 minutes at maximum speed on a vortex, using a horizontal multitube adapter.
4. Heat at 56°C for 5 minutes.
5. Centrifuge at maximum speed at room temperature for 5 minutes.
6. Transfer 300µl of lysate into well #1 of the cartridge.
7. Prepare cartridge and deck tray according to Technical Manual (TM640).
8. Add 300µl of Binding Buffer into well #1. Note: when performing RNA purification, do not add the RNase A Solution to the cartridge.
9. Run the Fecal Microbiome DNA method on the Maxwell® RSC Instrument.

Results:

Total nucleic acid can be purified from human feces using the Maxwell® RSC Fecal Microbiome DNA Kit on the Maxwell® RSC Instrument. Purified DNA (viral and bacterial) and RNA can be amplified by qPCR or RT-qPCR. The addition of a bead beating step may improve recovery for some bacterial strains.

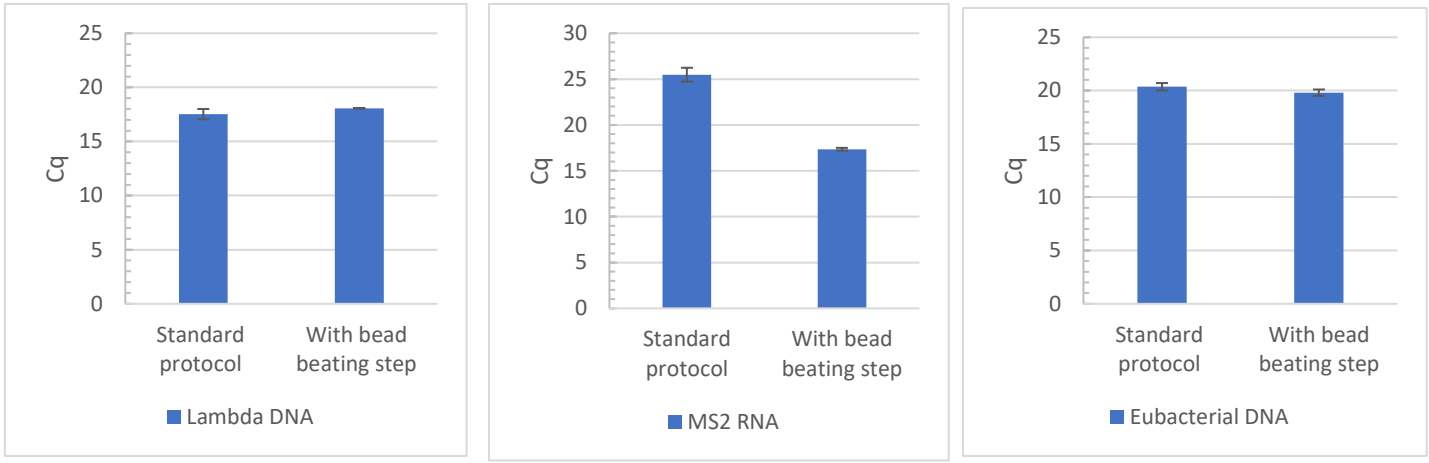

Figure 1. Total nucleic acid purification from 100mg of human feces using the Maxwell® RSC Fecal Microbiome DNA Kit on the Maxwell® RSC Instrument. 1ml of Lysis Buffer and 40µl of Proteinase K were added per sample. Lambda bacteriophage and MS2 bacteriophage were spiked at 10^7 PFU (per 300µl of lysate). Two different extraction conditions were tested (standard protocol, and with bead beating step) using 300µl of lysate per extraction (equivalent to 30mg of human feces). **Left.** Lambda DNA was amplified using the GoTaq® Probe qPCR System (Cat.# A6101) and specific primers and probe. Results shown are N=3 extraction replicates with single amplification reactions. **Center.** MS2 RNA was amplified using the GoTaq® Probe 1-Step RT-qPCR System (Cat.# A6121) and specific primers and probe. Results shown are N=3 extraction replicates with single amplification reactions. **Right.** Eubacterial DNA was amplified using the GoTaq® qPCR Master Mix System (Cat.# A6001) and specific 16S rRNA eubacterial primers. Results shown are N=6 extraction replicates for the standard protocol and N=4 for the protocol with bead beating, with single amplification reactions. All amplifications were performed on the Bio-Rad CFX96 qPCR Instrument.