Promega

Product Application

Automated Purification of Total RNA from Human Feces

Purify total RNA, including miRNA, from human feces using the Maxwell® RSC simplyRNA Tissue Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC simplyRNA Tissue Kit (Cat.# AS1340)

Analyses: UV absorbance, Dye-based quantitation, and RT-qPCR

Sample Type(s): Human fecal samples

Input: 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 TM416, available at: **www.promega.com/protocols**

or contact Technical Services at **techserv@promega.com**

Materials Required:

- Maxwell® RSC simplyRNA Tissue Kit (Cat.# AS1340)
- Maxwell® RSC Instrument (Cat.# AS4500)
- CTAB Buffer (Cat.# MC1411)
- 1-Thioglycerol (Cat.# A208B)
- QuantiFluor® RNA System (Cat.# E3310)
- GoTaq® 1-Step RT-qPCR System (Cat.# A6020)
- Benchtop centrifuge
- Bead beating device (e.g., FastPrep 24™ 5G Instrument from MP Biomedicals)
- Beads (ex: Lysing Matrix E from MP Biomedicals)
- NanoDrop™ 8000 Spectrophotometer (Thermo Fisher Scientific)

Protocol:

Precautions should be taken to keep samples cool (e.g., on ice) during pre-processing steps.

1. Add 250mg of feces into a bead beating tube (e.g., Lysing Matrix E).
2. Add 1ml of chilled CTAB + 2% 1-Thioglycerol buffer.
3. Vortex for 30 seconds.
4. Bead beat at 5.5m/s for 20 seconds.
5. Place sample on ice for 30 seconds after each bead beating cycle. Return tube holder to FastPrep-24™ 5G Instrument for the next cycle. Alternatively, a Fast Prep-24™ CoolPrep™ Adapter or similar could be used. Repeat for a total of 10 bead beating cycles.*
6. Centrifuge at 4°C for 5 minutes at maximum speed.
7. Transfer CTAB supernatant to a new 1.5ml tube.
8. Add 200µl of Lysis Buffer to 200µl of CTAB supernatant. Vortex vigorously for 15 seconds to mix. Transfer all 400µl of lysate to well #1 of the Maxwell® RSC Cartridge.
9. Add 10µl of blue DNase I solution (See Section 3.A of TM416) to well #4 of the Maxwell® RSC simplyRNA Tissue cartridge.
10. Proceed to Section 4.B of TM416, Maxwell® RSC simplyRNA Cartridge Preparation.

**Reducing bead beating cycles will reduce the total RNA yield*

Results:

Total RNA, including miRNA, can be purified from fecal samples using the Maxwell® RSC simplyRNA Tissue Kit with 10 cycles of bead beating. A_{260}/A_{280} and A_{260}/A_{230} purity ratios are greater than 2.0, however RNA eluates contained PCR inhibitors, which can be overcome by eluate dilution(s).

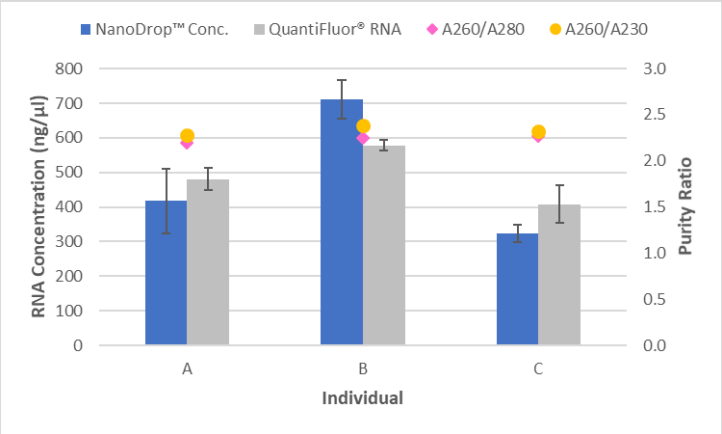

Figure 1. RNA Quantitation using NanoDrop™ and QuantiFluor® RNA System (Cat.# E3310). Average eluate RNA concentration for 250mg of feces purified with the Maxwell® RSC simplyRNA Tissue Kit on the Maxwell® RSC Instrument using 10 bead beating cycles. N=3. High yield and high purity ratios were obtained for all three samples tested.

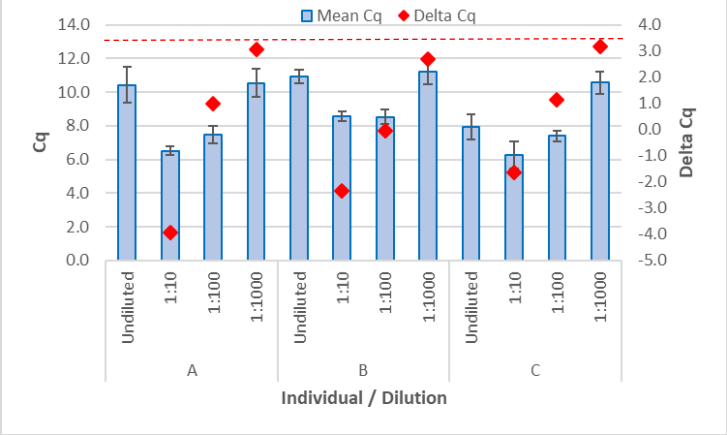

Figure 2. Average bacterial 16S rRNA RT-qPCR Cqs. 2µl of undiluted, diluted 1:10, diluted 1:100 and diluted 1:1000 eluate was amplified using GoTaq® 1-Step RT-qPCR System (Cat.# A6020) on a Bio-Rad CFX96™ Real-Time System to calculate a delta Cq value (the red dashed line at 3.3 indicates no PCR inhibition). N=3. PCR inhibition is overcome at the 1:1000 dilution.

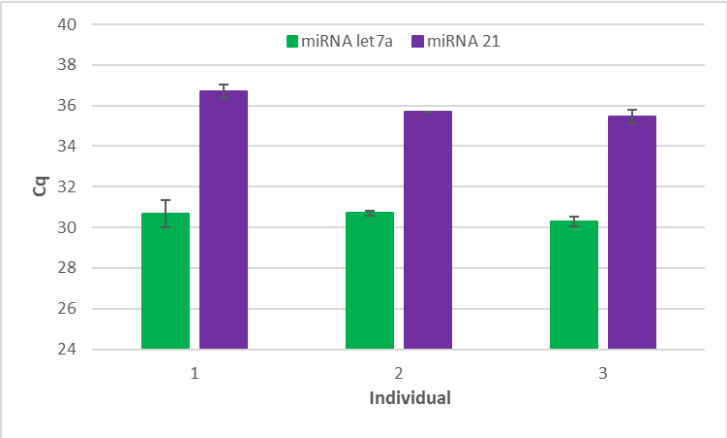

Figure 3. Average TaqMan™ miRNA RT-qPCR Cqs. cDNA was prepared using the Taqman™ miRNA RT Kit (ThermoFisher) with 5µl of purification eluate and amplified using the GoTaq® Probe qPCR Master Mix (Cat.# A6101). TaqMan™ assays for miRNA-21 (ID 000397) and miRNA-let7a (ID 000377) were run in duplicate using 1.3µl of the cDNA. N=3. miRNAs were detected from all three individuals.