Promega

Product Application

RNA purification from human feces

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

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

Analyses: NanoDrop™, QuantiFluor® RNA System, RT-qPCR

Sample Type(s): Human fecal samples

Materials Required:

- CTAB Buffer (Cat. #MC1411)
- 1-Thiogylcerol (Cat. #A208B)
- Benchtop centrifuge
- Bead beating device (ex: FastPrep 24™ 5G Instrument from MP Biomedicals)
- Beads (ex: Lysing Matrix E from MP Biomedicals)
- Maxwell® RSC Instrument (Cat. #AS4500)

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.

Further information can be found in Technical Manual #TM416, available at: www.promega.com/protocols

or by e-mailing technical services at techserv@promega.com

Protocol:

Precautions should be taken to keep samples cool (ex: on ice) during pre-processing steps.

1. Add 250mg of feces into a bead beating tube (ex: 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. Well #1 is the largest well of the 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, 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).

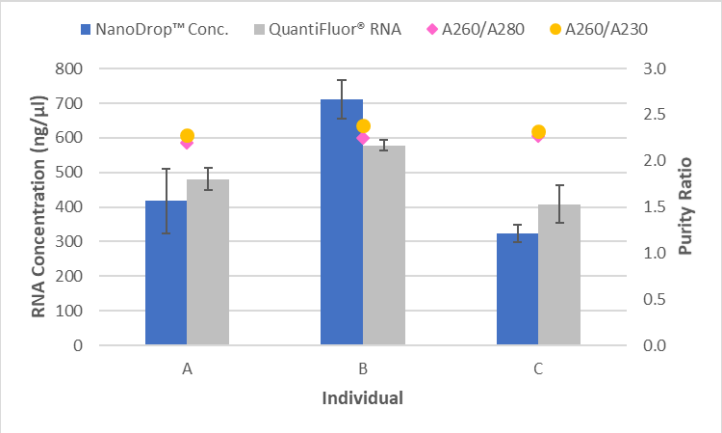

Figure 1. RNA Quantitation using NanoDrop™ and QuantiFluor® RNA System. Average eluate RNA concentration for 250mg of feces purified with the Maxwell® RSC simplyRNA Tissue Kit using 10 bead beating cycles. N=3. High yield and high purity ratios were obtained for all three samples tested.

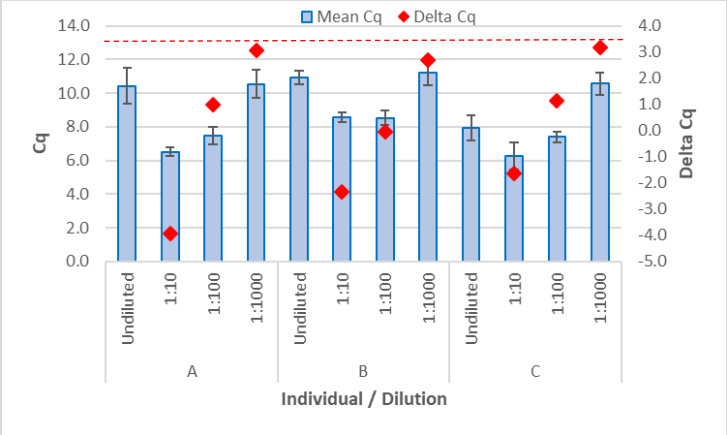

Figure 2. Average bacterial 16S rRNA RT-qPCR Cqs. 2µl of undiluted, diluted 1:10, diluted 1:100 and diluted 1:1000 eluate was amplified in 1-step RT-qPCR to calculate a delta Cq value (the red dashed line at 3.3 indicates no PCR inhibition). N=3. PCR inhibition is overcome at the 1:1000 dilution.

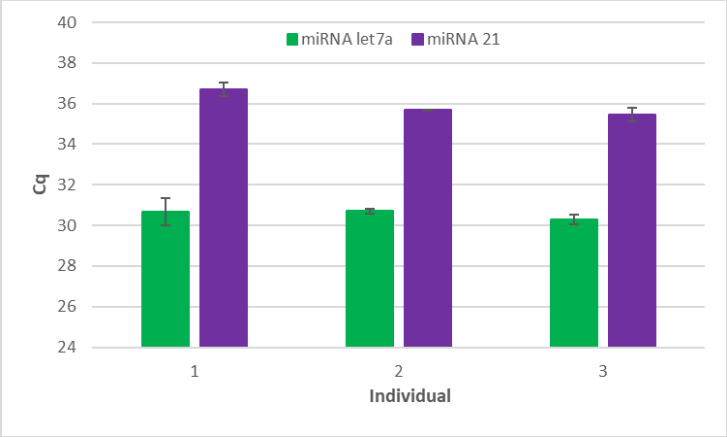

Figure 3. Average Taqman® miRNA RT-qPCR Cqs. TaqMan® assays for miRNA-21 and miRNA-let7a were run in duplicate using 1.3µl of cDNA from the Taqman® miRNA RT Kit RT reaction using 5µl of purification eluate with GoTaq® Probe qPCR Master Mix. N=3. miRNA Cqs were observed for all three individuals.