Promega

Product Application

Automated Purification of RNA from Soil

Purify RNA from soil using the Maxwell® RSC Instrument and the Maxwell® RSC simplyRNA Tissue Kit.

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

Analyses: Dye-based quantitation; RT-qPCR of bacteria 16S rRNA

Sample Type(s): Soil

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)
- Centrifuge
- Bead beater (e.g: FastPrep 24 from MP Biomedicals)
- Bead beating tubes (e.g: lysing matrix E from MP Biomedicals)
- Isopropanol

Protocol:

1. Place 250mg of soil into a bead-beating tube.
2. Add 1ml of CTAB buffer with 2% 1-Thioglycerol. Vortex for 15 seconds.
3. Bead-beat twice at 5.5m/s for 30 seconds.
4. Centrifuge samples for 5 minutes at 12,000 x g at 4°C.
5. Prepare cartridges.
    a. Place cartridges in the Maxwell® RSC cartridge rack and remove foil seals.
    b. Add 15µl of blue DNase I solution into well #4.
    c. Add 60µl of Elution Buffer to Elution Tubes and place tubes in cartridge rack.
    d. Place plungers into well #8.
6. In a 1.5ml tube, mix 300µl of lysate with 300µl of lysis buffer
7. Add 400µl of the mix into well #1.
8. Run Maxwell® RSC with simplyRNA Tissue method.

Results:

RNA was purified from 250mg of 3 different soil samples: soil 1 (agricultural soil, pH 7.24), soil 2 (organic soil, pH 4.5), soil 3 (organic soil from a peatland, pH 4.5) using the protocol below (n=3).

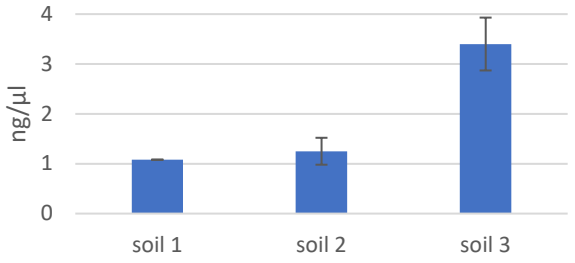

Figure 1: Concentration of RNA purified from soil. RNA was quantified using a fluorescent-dye based method (QuantiFluor® RNA System, Cat.# E3310). Mean ± standard deviation is shown, n=3.

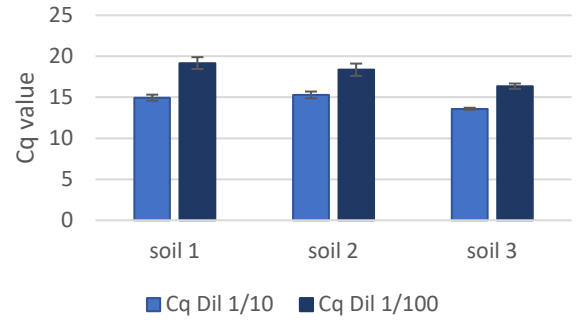

Figure 2: qPCR analysis of 16S rRNA purified from 250mg of soil. Cq values for 2µl of 1:10 and 1:100 dilutions of RNA amplified using the GoTaq® 1-Step RT-qPCR System (Cat.# A6020) and 16S rRNA gene primers. Mean ± standard deviation is shown, n=3.

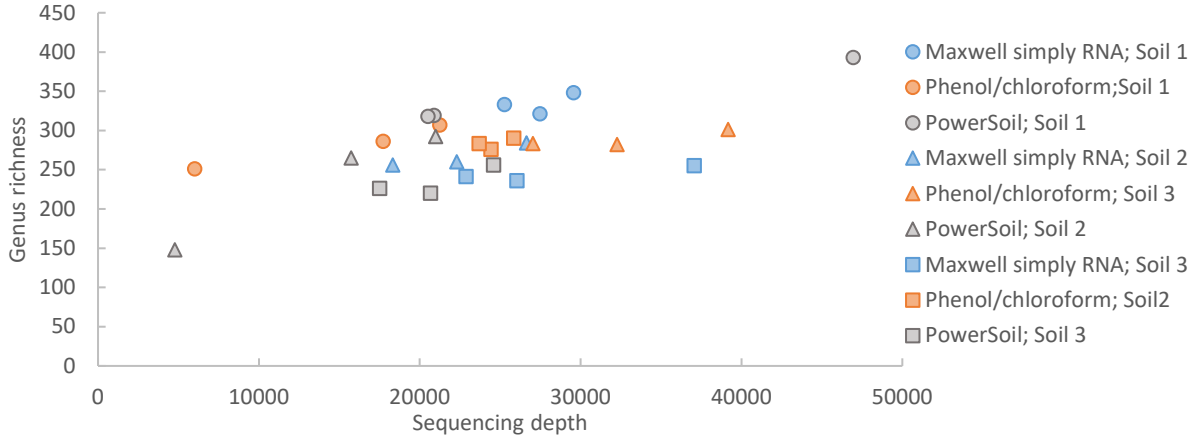

Figure 3: Genus richness of soil bacteria. RNA was extracted from 250mg of 3 different soil samples: soil 1 (agricultural soil, pH 7.24), soil 2 (organic soil, pH 4.5), soil 3 (organic soil from a peatland, pH 4.5) using the protocol described above and two additional methods: phenol/chloroform and Qiagen RNeasy® PowerSoil® Total RNA Kit. The V3-V4 hypervariable region of the 16S rRNA cDNA was sequenced using the MiSeq™ System (Illumina). Genus richness (number of OTUs detected per sample at the genus level of the taxonomic classification) by each method was plotted as a function of sequencing depth. At similar sequencing depths, samples extracted from the same soil using different RNA purification methods showed a similar genus richness.