Promega

Product Application

Automated DNA Extraction from Soil Samples

Purify bacterial DNA from soil samples using the Maxwell® RSC PureFood GMO and Authentication Kit on the Maxwell® RSC Instrument.

Kit: Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)

Analyses: Quantification (absorbance, fluorescent dye), qPCR amplification

Sample Type(s): Soil 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.

Further information can be found in Technical Manual #TM473, available at: www.promega.com/protocols

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

Materials Required:

- Maxwell® RSC PureFood GMO and Authentication Kit (Cat.# AS1600)
- Maxwell® RSC instrument (Cat.# AS4500)
- Heat block
- Centrifuge
- Bead beater (e.g., FastPrep-24™ from MP Biomedicals)
- Beads (e.g., Lysing Matrix E from MP Biomedicals)

Protocol:

1. Place 250mg of sample into a bead-beating tube (Lysing Matrix E, MP Biomedicals).
2. Add 1ml of CTAB buffer. Vortex for 15 seconds.
3. Heat sample at 95°C for 5 minutes. Allow samples to cool down for 2 minutes.
4. Bead-beat twice at 5.5m/s for 30 seconds.
5. Add 40µl of Proteinase K and 20µl of RNase A and incubate sample at 70°C for 10 minutes.
6. Centrifuge samples for 5 minutes at 12,000 x g.
7. Prepare cartridges
    a. Place cartridges in RSC cartridge rack and remove foil seals.
    b. Add 100µl of Elution Buffer to Elution Tubes and place tubes in cartridge rack.
    c. Place plungers into well 8 of cartridge.
    d. Add 300µl of Lysis Buffer into well 1 of cartridge.
8. Add 300µl of supernatant into well 1 of cartridge.
9. Run Maxwell® RSC with PureFood GMO and Authentication Protocol.

Note: In case of amplification inhibition, an optional clean-up can be performed using ProNex® Size Selective Purification System (Cat.# NG2001) following TM508 with a 3:1 ratio of ProNex® chemistry: sample.

Results:

DNA was extracted from 250mg of 3 soil samples (soil from a forest, a corn field and potting soil) using the protocol above. DNA is amplifiable and enabled 16S rRNA sequencing to assess bacterial richness.

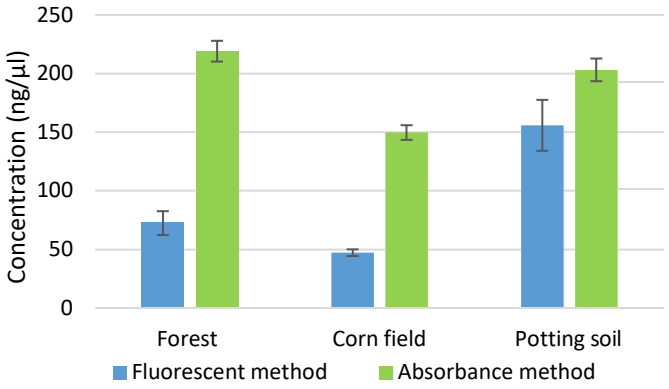

Figure 1: Concentration of DNA extracted from 250mg of soil using Maxwell® RSC PureFood GMO and Authentication Kit.
Quantitation using a fluorescent based method (QuantiFluor® ONE dsDNA System) or by absorbance using NanoDrop™ One Spectrophotometer. Data are shown as mean ± standard deviation of n=3.

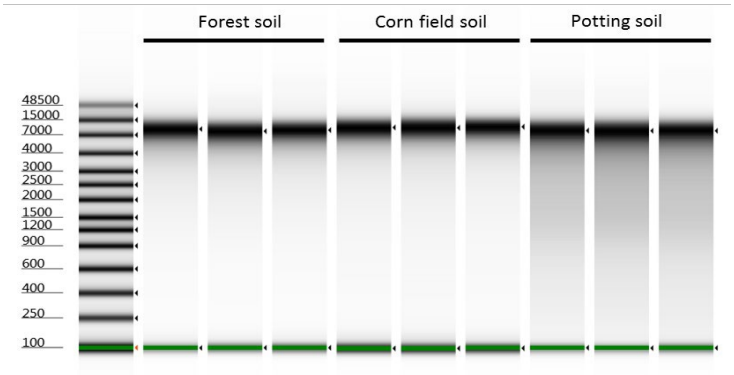

Figure 2: TapeStation analysis of DNA extracted from 250mg of soil using Maxwell® RSC PureFood GMO and Authentication Kit. DNA were analyzed using Genomic DNA ScreenTape.

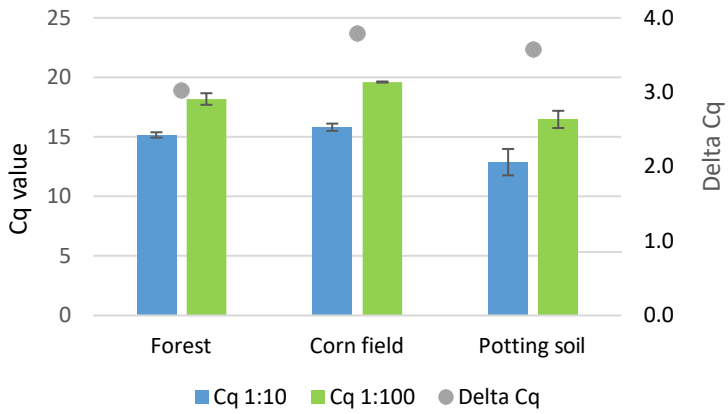

Figure 3: qPCR analysis of 16S rRNA gene of DNA extracted from 250mg of soil samples. An extra clean-up was performed using ProNex® Size-Selective Purification System. Cq and ΔCq values for 2µl of 1:10 and 1:100 dilution of the extracted DNA amplified using GoTaq® qPCR Master Mix (Cat.# A6002) and 16s rRNA gene primers in a final volume of 20µl. A ΔCq of 3.3 indicates no inhibitors are present. Data are shown as mean ± standard deviation of n=3.

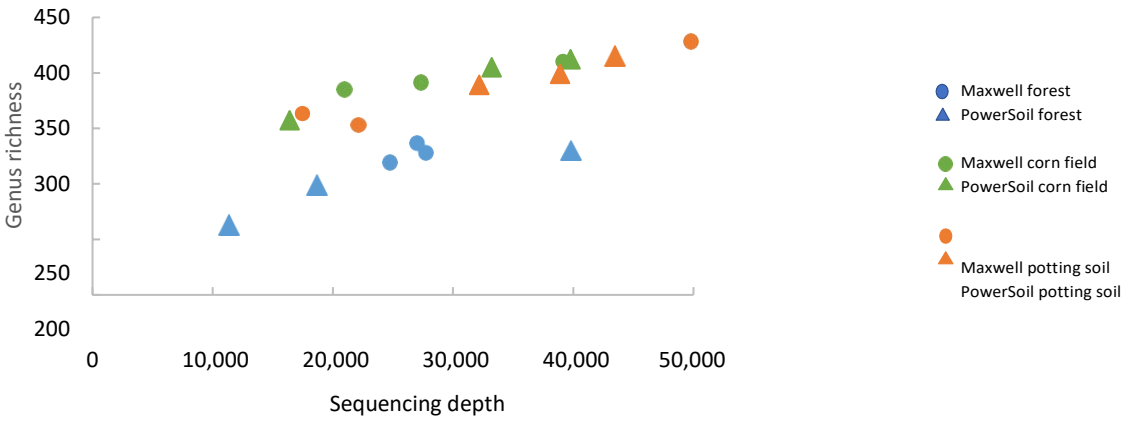

Figure 4: 16S rRNA gene sequencing. DNA was extracted from different soil samples (forest, corn field and potting soil) using either the Maxwell® RSC with the protocol described above or the DNeasy® PowerSoil® Pro Kit. 16S rRNA sequencing revealed lower bacterial richness for the forest soil than the potting soil or the soil from the corn field. No significant differences in terms of bacterial richness were observed between the automated Maxwell® extraction and the manual DNeasy® PowerSoil® Pro method.