APPLICATION NOTE

Detection of the Bacterial 16S rDNA Gene from Soil Samples using the Omni Soil DNA Purification Mini Kit

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Introduction

Soil microbiome research seeks to understand the diversity and abundance of microorganisms in various soil types as a function of environmental conditions. As a first step toward this goal, microbe nucleic acids must be extracted from the soil substrate. A major obstacle toward defining the soil microbiome is the ability to first culture these microorganisms to gain a better understanding of their ecology, diversity and species richness. Currently microbial cell culture media is selective and only certain isolates can be determined by this approach. As an alternative to the cell culture approach, a popular determination method is to directly extract and amplify microbial DNA from soil samples. Although this alternative method has given promising results, there remains hurdles that must be overcome. Most notably, soil is natively rich in substances such as humic acids, that inhibit polymerases and restriction enzymes making the amplification of DNA difficult. Fortunately, methods have been established to separate microbial DNA from inhibitors prior to DNA purification and amplification. One such approach is available in the Omni Soil DNA Purification kit and is demonstrated in this application note.

Herein, we evaluate the Omni Soil DNA Purification Mini kit's ability to extract DNA from Georgia red clay using the Bead Ruptor 24 Elite for mechanical dissociation of the soil/microbe samples prior to PCR inhibitor removal and DNA purification.

Materials and Methods

Equipment

- Bead Ruptor Elite (Cat#: 19-040E)
- 2 mL tube carriage kit (Cat#: 19-010-310)
- Omni Soil DNA Purification Mini Kit (Cat#: 26-013G)
- Molzyme GmbH & Co. Mastermix 16S Complete (Cat#: S-020-0100)

Bead Ruptor Elite

Sample Prep and DNA Extraction

211 mg of Georgia red clay was obtained and placed into a nuclease free pre-filled bead tube containing 0.7 mm garnet beads as provided in the Omni Soil DNA Purification Kit (26-013G). 750 µL of XLSM buffer was added to the tube. Sample were then mechanically dissociated on the Bead Ruptor Elite Bead Mill Homogenizer at 5 m/s for 45 seconds. The protocol was followed henceforth as per the manufactures' directions. DNA was eluted in 60 µL of EB buffer and concentration was determined on the NanoDrop 2000 Spectrophotometer (ThermoFisher Scientific).

PCR Analysis

Extracted DNA was diluted to 10 fg/µL. The Molzyme Mastermix 16S Complete PCR protocol was carried out per the manufactures' instructions for a total reaction volume of 26 µL. Amplification was carried on a T100 Thermal Cycler (Biorad) as per settings in table 1. PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide and visualized on the Gel Doc EZ System (Biorad).

	Temperature	Time
Denaturation	95°C	1 min
40 cycles	95°C	5 sec
	55°C	5 sec
	72°C	25 sec

Table 1. PCR Program

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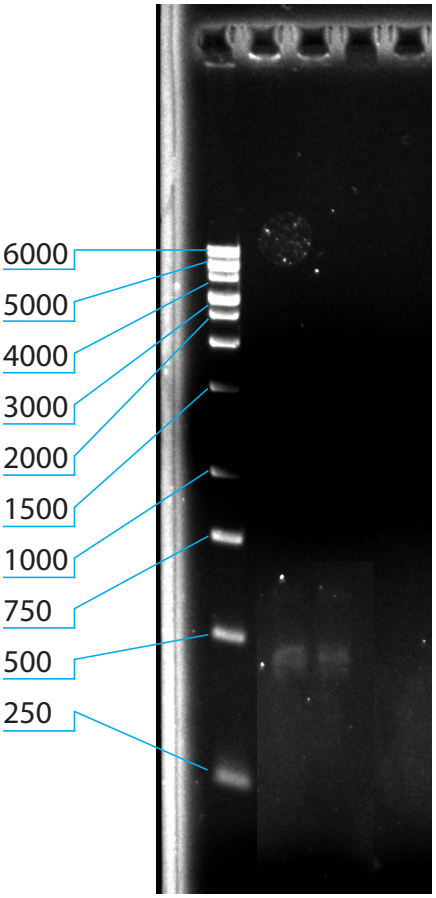
Results

Herein, we evaluated the capability of the Omni Soil DNA Purification Mini Kit to extract genomic DNA from a typically challenging soil sample type, Georgia red clay. The Omni Soil DNA Purification Kit was used to first mechanically dissociate the soil and microbial DNA, PCR inhibitors were removed and the DNA was purified using the silica spin capture columns. This process enabled the amplification of the 16S rDNA bacterial gene through PCR. Genomic DNA was quantified via spectrophotometry. The DNA yield averaged 97.8 ng/µL. The amplicon from the soil sample was analyzed on a 2 % agarose gel. The expected size of the 16S bacterial rDNA gene was 450 base pairs and as seen in figure 1, the amplicon is in the desired base pair length.

Figure 1: Bacterial 16S rDNA Detection from Soil
Lane 1: Molzyme 1 kb Ladder, Lane 2-3: PCR product



Soil DNA Purification Mini Kit

2 mL tube carriage kit

Conclusion

The Omni Soil DNA Purification Mini Kit in conjunction with the Bead Ruptor Elite is able to extract microbial DNA from challenging soil samples such as Georgia red clay. High yields of genomic DNA was observed and the Omni Soil DNA Purification Mini Kit was able to successfully remove PCR inhibitors as seen from the amplification of the bacterial 16S rDNA gene.

References

Picard, Christine et al. "Detection and Enumeration of Bacteria in Soil by Direct DNA Extraction and Polymerase Chain Reaction." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, 58.9 (1992): 2717-722. Print.

Braid, Michael D. et al. "Removal of PCR inhibitors from Soil DNA by Chemical Flocculation." Journal of Microbiological Methods 52.3 (2003): 389-93. Print.

Zhou, Jizhong et al. "DNA Recovery from Soils of Diverse Composition." APPLIED AND ENVIRONMENTAL MICROBIOLOGY 62.2 (1996): 316-22. Print