APPLICATION NOTE

Detection of the bacterial 16S rRNA gene from soil samples using the Omni Bead Ruptor Elite bead mill homogenizer.

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. The bacterial communities within soil also present a significant hurdle when preparing samples for DNA extraction. The tough outer cell wall present in these bacteria, specifically, is the lynchpin for sample prep as time consuming enzymatic digestions remain a common method for bacterial lysis. High-energy, bead-based homogenization presents a time-saving solution for bacterial lysis from soil matrices prior to DNA extraction.

Herein, we evaluate the efficacy of the Omni Bead Ruptor Elite bead mill homogenizer in a soil DNA extraction workflow for downstream 16S rRNA detection via endpoint PCR.

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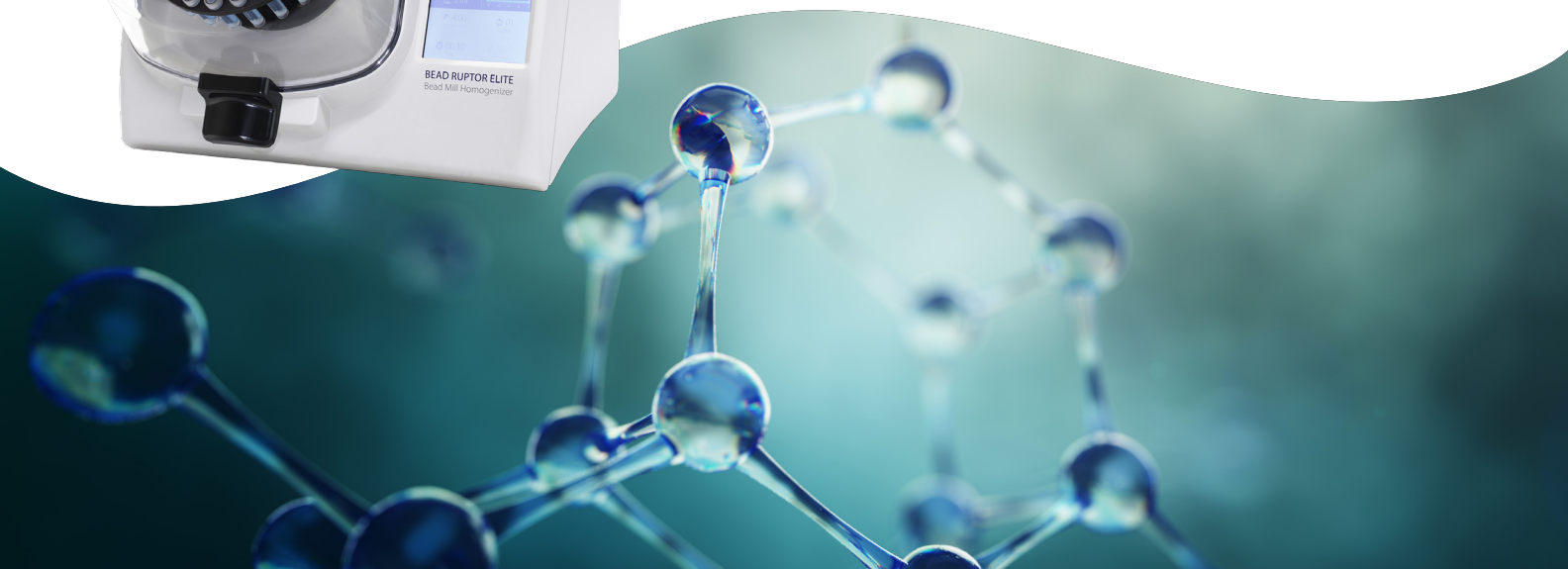
Omni Bead Ruptor Elite bead mill homogenizer



Materials and methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite 2 mL Tube Carriage (Cat # 19-373)
- Hard & Fibrous Tissue Mix (0.7 mm Garnet) (2 mL) (Cat # 19-624)

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 (Cat # 19-624) along with 750 µL of commercially provided lysis buffer specialized for soil matrices. Sample were then mechanically dissociated on the Omni Bead Ruptor Elite bead mill homogenizer at 5 m/s for 45 seconds. A commercially available soil DNA extraction kit protocol was followed henceforth as per the manufactures' directions. DNA was eluted in 60 µL of elution buffer and concentration was determined on the NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific).

PCR analysis

Extracted DNA was diluted to 10 fg/µL. The Molzym Mastermix 16S Complete PCR protocol (Molzym, Cat # S-020-0100) was carried out per the manufactures' instructions for a total reaction volume of 26 µL. Amplification was carried out on a T100 Thermal Cycler (Bio-Rad) as per settings in Table 1. PCR products were separated on a 2 % agarose gel, stained with ethidium bromide and visualized on the Gel Doc EZ System (Bio-Rad).

Table 1. PCR Program

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

Results

Herein, we evaluated the capability of the Omni Bead Ruptor Elite bead mill homogenizer to lyse bacteria contained in soil samples for DNA extraction and PCR amplification of the 16S rRNA gene. 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.

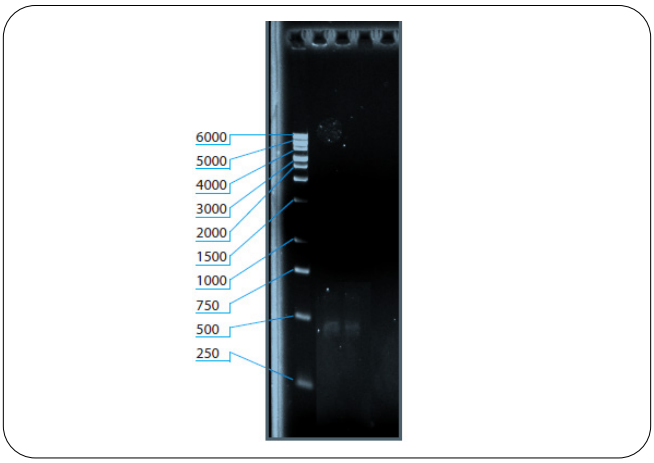


Figure 1. Bacterial 16S rDNA Detection from soil
Lane 1: Molzym 1 kb Ladder, Lane 2-3: PCR product

Conclusion

The Omni Bead Ruptor Elite bead mill homogenizer is able to extract microbial DNA from challenging soil samples such as Georgia red clay. High yields of genomic DNA was observed and the commercially available soil DNA extraction kit was able to successfully remove PCR inhibitors as seen from the amplification of the bacterial 16S rDNA gene.

References

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