

The SoilMaster™ DNA Extraction Kit Provides PCR-Ready Soil DNA in Less Than an Hour

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Introduction

The analysis of DNA from microbial populations in soil and sediment samples has been fraught with difficulties. The direct lysis of cells within the soil matrix, often results in the coextraction of other soil components, including potent organic inhibitors such as humic and fulvic acids. These components can prevent the amplification of DNA by the polymerase chain reaction (PCR).^{1,2}

The SoilMaster™ DNA Extraction Kit provides a reliable, simple method for producing PCR-ready DNA from soil and sediment samples. This method is based on hot-detergent lysis methods^{3,4} and incorporates an inhibitor removal chromatography step.

Larger size and more intact DNA

Genomic DNA was purified from soil samples that included forest, marsh, and cave soil using the SoilMaster™ DNA Extraction Kit following the kit's protocol. The DNA isolated with the SoilMaster Kit was compared to the DNA purified with two other soil DNA kits incorporating bead beating or vortex mixing in the presence of beads. Extracted DNA was examined by agarose gel electrophoresis (Figure 1). The DNA extracted with the SoilMaster Kit was of larger size and contained more intact DNA than DNA purified by other methods.

Difficult DNA extractions

The SoilMaster Kit extracts DNA from difficult-to-extract soil and sediment samples. Cave sediment DNA was successfully extracted using the SoilMaster Kit, but no visible DNA was purified in attempts with two other kits, as shown when examining proportional amounts of DNA preparations by agarose gel electrophoresis (Figure 2).

Amplification of diverse organisms

Purified soil DNA was amplified and the PCR results illustrate the diverse set of organisms represented in the extracted DNA. DNA from cave sediment, forest soil, and marsh soil was extracted and specific targets were subsequently amplified with the FailSafe PCR System. The extracted genomic DNA was amplified by a series of DNA primers with different specificities, including 1) two sets of consensus bacterial primers, 2) fungi, pro-

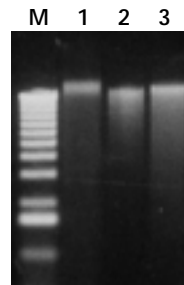


Figure 1. The SoilMaster™ DNA Extraction Kit extracts high molecular weight intact DNA from compost soil sample. Lane M, kb DNA ladder; Lane 1, soil DNA extracted with the SoilMaster Kit; Lanes 2 and 3, DNA purified using other soil kits.

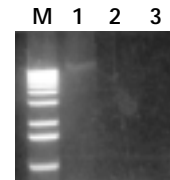


Figure 2. The SoilMaster™ DNA Extraction Kit extracts DNA from difficult-to-extract soil and sediment samples. Lane M, kb DNA ladder; Lane 1, cave sediment DNA extracted with the SoilMaster Kit; Lanes 2 and 3, purification attempts of cave sediment DNA using other soil kits.

tists, and green algae primers, 3) plant primers, 4) primers to high G+C, gram positive bacteria, and 5) *Bacillus* primers. Amplification products were obtained from all 5 primer sets using the extracted DNA from all samples tested (Figure 3).

PCR product cloning and RFLP analysis

The DNA amplified with 16S bacterial consensus primers was cloned into pCC1™ with the CopyControl™ PCR Cloning Kit. Clones containing the 1.3 kb PCR product were examined by RFLP with *Rsa I* to examine sequence variations in the cloned fragments. The RFLP analysis of clones demonstrated the diversity of 16S sequences amplified from the extracted soil DNA (data not shown). This indicates that a wide variety of organisms and species are represented in the extracted soil DNA.

Discussion

The SoilMaster™ DNA Extraction Kit efficiently extracts PCR-ready DNA from a wide variety of organisms from soil including difficult-to-extract sediments. DNA from soil and sediments can be effectively amplified by FailSafe PCR amplification and subsequently cloned for further characterization.

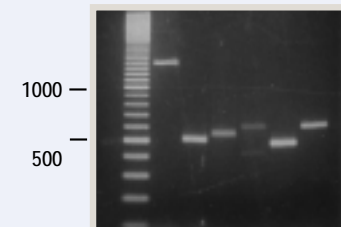
References

1. Tsai, Y.-L and Olson, B.H. (1992) *Appl. Environ. Microbiol.* **58**, 754.
2. Tebbe, C.C. and Vahjen, W. (1993) *Appl. Environ. Microbiol.* **59**, 2657.
3. Selenska, S. and Klingmuller, W. (1991) *Letters in Appl. Microbiol.* **13**, 21.
4. Zhou, J. et al. (1996) *Appl. Environ. Microbiol.* **62**, 316.

www.epicentre.com/soilmaster.asp

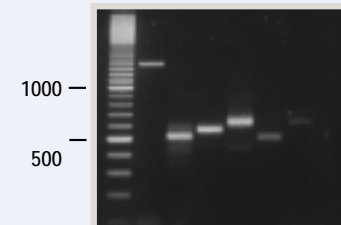
SoilMaster™ DNA Extraction Kit
SM02050 50 Reactions

3A M 1 2 3 4 5 6



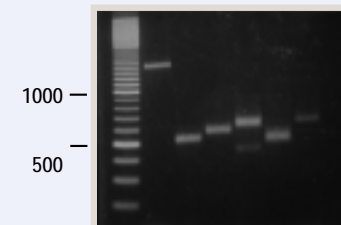
Cave Sediment

3B M 1 2 3 4 5 6



Forest Soil

3C M 1 2 3 4 5 6



Marsh Soil

Figure 3. FailSafe™ PCR amplification of extracted soil DNA. DNA was extracted from 3 distinct soil types including cave sediment (Panel A), forest soil (Panel B), and marsh soil (Panel C). The extracted soil was amplified using the following primers: Lanes 1 and 2, consensus bacterial primers to the 16S ribosomal RNA gene; Lane 3, fungi, protists, and green algae primers; Lane 4, plant primers NS3/NS4; Lane 5, high G+C gram positive bacterial primers; Lane 6, *Bacillus* primers.