EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research

Extract PCR-Ready Soil DNA in Less Than an Hour with the New SoilMaster™ DNA Extraction Kit

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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 (Figure 1). Genomic DNA was extracted from different soil and sediment samples varying in composition and origin. The integrity of the purified DNA is demonstrated by agarose gel electrophoresis. The diversity of the extracted PCR-ready genomic DNA is demonstrated by amplification of 1 µl of the extracted DNA (<1% of the total), using the FailSafe™ PCR System, with a series of primers with different target specificities.

The bacterial DNA was also amplified with consensus bacterial 16S ribosomal PCR primers and cloned using the CopyControl™ PCR Cloning Kit. The diversity of the cloned DNA was examined by restriction fragment length polymorphism (RFLP) analysis of the 16S clones.

Methods

Soil samples

Soil samples included forest soil and marsh soil from Madison, WI. Cave soil was obtained from Dr. Tina Robach, Department of Biology, at the University of Louisville.

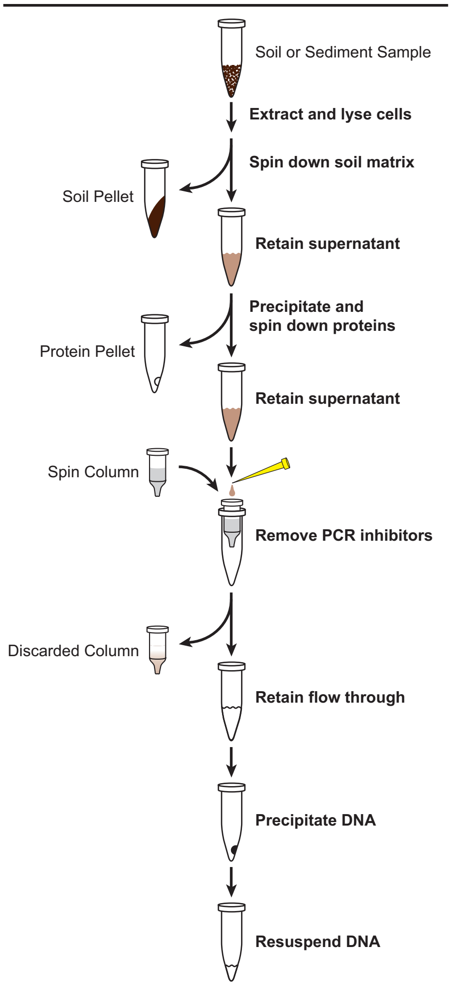

Figure 1. SoilMaster™ DNA Extraction Kit protocol.

DNA extraction

Genomic DNA was purified from soil samples using the SoilMaster™ DNA Extraction Kit. The protocol is illustrated in Figure 1. Briefly, 250 µl of Soil DNA Extraction Buffer and 100 µg of Proteinase K were added to 100 mg of soil, followed by the addition of 50 µl of Soil Lysis Buffer. The sample was vortexed, heated to 70°C for 10 minutes, vortexed again, and the soil matrix was spun down. Protein Precipitation Reagent (60 µl) was added and the sample was held on ice. The proteins were spun down and the supernatant was spun through an Inhibitor Removal Spin Column to remove enzymatic inhibitors from the soil DNA. The genomic soil

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DNA present in the column flow-through was precipitated with DNA Precipitation Solution, and washed twice with Pellet Wash Solution. The DNA was resuspended in 300 µl of TE Buffer.

PCR amplification

The primers used for PCR amplification are listed in Table 1. Purified soil DNA was amplified with the FailSafe™ PCR System. Briefly, 50 µl reactions contained 25 µl of the appropriate FailSafe™ 2X PreMix, 2.5 U FailSafe™ Enzyme Mix, 10 pmoles of each primer and 1 µl of purified soil DNA. The thermocycling parameters were: 92°C for 2 minutes, followed by 30 cycles of 92°C for 45 seconds, a variable annealing temperature for 45 seconds and 72°C for 60 seconds. Ten to twenty percent of the amplification reaction product was analyzed by agarose gel electrophoresis.

PCR product cloning

PCR products were cloned using the CopyControl™ PCR Cloning Kit with TransforMax™ EPI300™ Electrocompetent *E.coli*. Briefly, 1.3-Kb PCR products amplified with 16S bacterial consensus primers (Table 1) were precipitated with the PCR Precipitation Solution. The purified PCR product was then treated with the PCR End-Repair Enzyme Mix to generate blunt-ended and 5′-phosphorylated PCR products for cloning. The PCR product was ligated into the CopyControl™ pCC1™ (Blunt-Cloning Ready) Vector using Fast-Link™ DNA Ligase. The ligation reaction was then transformed into TransforMax™ EPI300™ Electrocompetent *E.coli* by electroporation. The resulting clones were quick screened for inserts by the direct lysis of colonies and the size selection of the DNA using EpiLyse™ Solution and EpiBlue™ Solution.

Restriction Fragment Length Polymorphism (RFLP) was performed on a series of 16S pCC1™ clones restricted with *Rsa* I (6). The fragments were separated by agarose gel electrophoresis.

Results

Larger size and more intact DNA

The DNA isolated with the SoilMaster DNA Extraction 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 2). The DNA extracted with the SoilMaster Kit was of larger size and contained more intact DNA than DNA purified by other methods.

Table 1. PCR Amplification Primers

Organism Group	Primer Pair (reference)	DNA Sequence	Product Size (bp)
Bacteria consensus	63f/1387r (5)	CAGGCCTAACACATGCAAGTC GGGCGGWGTGTACAAGGC	1325
Plants	NS3/NS4 (6)	GCAAGTCTGGTGCCAGCAGCC CTTCCGTCAATTCCTTTAAG	597
Fungi, Protists, and Green Algae	NS1/NS2 (7)	GTAGTCATATGCTTGTCTC GGCTGCTGGCACCAGACTTGC	555
High G+C Gram Positive Bacteria	Actino F/R (8)	GGCCTTCGGGTTGTAAACC CTTTGAGTTTTAGCCTTGCGGC	542
Bacteria consensus	P4/P5 (9)	AACGCGAAGAACCTTAC CGGTGTGTACAAGGCCCGGGAACG	450
Bacillus and relatives	BacF/BacR (8)	AGGGTCATTGGAAACTGGG CGTGTTGTAGCCCAGGTCATA	600

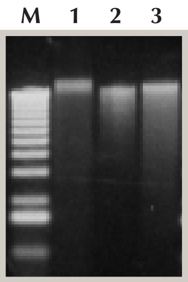

Figure 2. The SoilMaster™ DNA Extraction Kit extracts high molecular weight intact DNA from compost soil sample. Lane M, Kb DNA ladder; Lane 1, soil DNA extracted with the SoilMaster Kit; Lanes 2 and 3, DNA purified using other soil kits.

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 3).

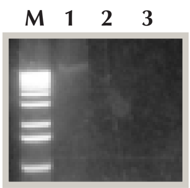

Figure 3. The SoilMaster™ DNA Extraction Kit extracts DNA from difficult-to-extract soil and sediment samples. Lane M, Kb DNA ladder; Lane 1, cave sediment DNA extracted with the SoilMaster Kit; Lanes 2 and 3, purification attempts of cave sediment DNA using other soil kits.

Amplification of diverse organisms

PCR amplification 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, protists, and green algae primers, 3) plant primers, 4) primers to high G+C, gram positive bacteria, and 5) *Bacillus* primers (Table 1). Amplification products were obtained from all five primer sets

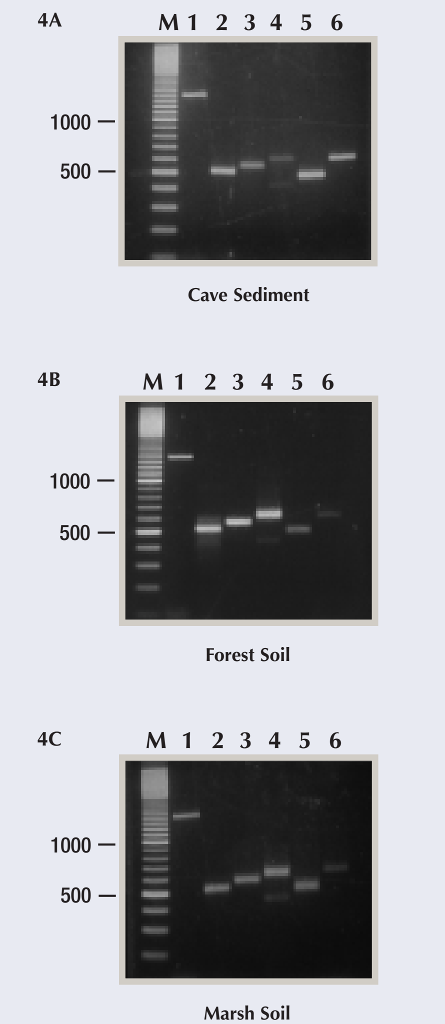

Figure 4. FailSafe™ PCR amplification of extracted soil DNA. DNA was extracted from 3 distinct soil types including cave sediment (Panel A), forest soil (Panel B), marsh soil (Panel C). The extracted soil was amplified using the following primers: Lanes 1 and 2, consensus bacterial primers to the 16S 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.

using the extracted DNA from all samples tested (Figure 4).

Successful cloning and RFLP analysis

The DNA amplified with 16S bacterial consensus primers was cloned into pCC1™ with the CopyControl™ PCR Cloning Kit (see p.4 and center insert). 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 demonstrates the diversity of 16S sequences amplified from the extracted soil DNA (Figure 5). This indicates that a wide variety of organisms and species are represented in the extracted soil DNA.

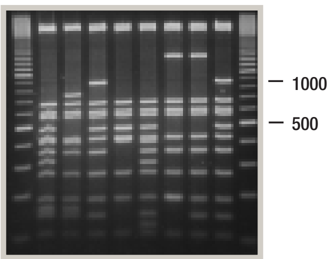

Figure 5. RFLP analysis of clones containing 1.3 Kb 16S ribosomal RNA gene PCR products. Eight clones were restricted with *Rsa* I, and the resulting fragments were separated by agarose gel electrophoresis. The varied banding patterns demonstrate the diversity of the 16S ribosomal gene sequences that were amplified and cloned into pCC1™.

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

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Here is How to Never Fail at PCR

The FailSafe™ PCR System combines a unique blend of high fidelity thermostable enzymes with an extensively tested set of FailSafe PCR PreMixes to provide a new standard for PCR performance and reliability. The FailSafe System gives consistent amplification of any template up to about 20 Kb in length, even difficult templates, such as those with high GC content or secondary structure, and multiplex PCR reactions.

FailSafe PCR is an easy 3-step process

STEP 1
Perform PCR with your template and primers using the FailSafe™ PCR PreMix Selection Kit.

STEP 2
Select the FailSafe™ PCR PreMix that provides the best amplification.

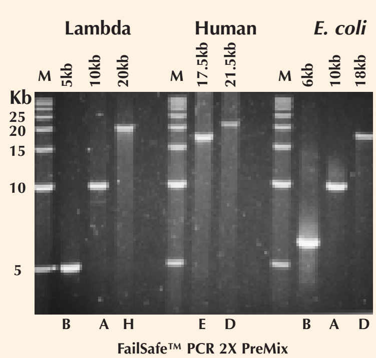
STEP 3
Use the selected PreMix with the FailSafe™ PCR Enzyme Mix for consistent amplification of your template/primer pair.

Use these three steps for each template/primer pair you wish to amplify.

Obtain high fidelity PCR with no loss in sensitivity

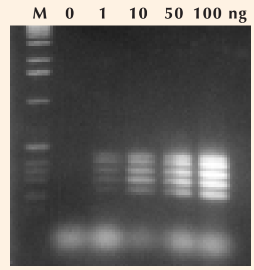
The FailSafe™ PCR Enzyme Mix contains a 3′-5′ proofreading enzyme that delivers fidelity at least three times higher than *Taq* DNA polymerase.

Amplify templates up to 20 Kb long



Amplification of a wide range of sequence sizes from different sources using the FailSafe™ PCR System.

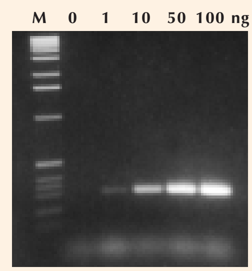
Get successful multiplex amplification



High sensitivity multiplex PCR amplification of five exons of CFTR from as little as 1 ng of human genomic DNA using the FailSafe™ System.

CFTR 5 Band Multiplex

Easily amplify GC-rich templates



Amplification of a segment of human DNA with 85% GC content: Fragile X FMRI was amplified using 1 ng of human genomic DNA by FailSafe™ PCR.

See the back cover to learn what researchers using the FailSafe PCR System are telling us.