

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.

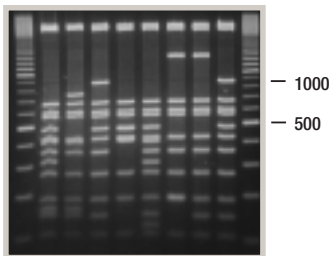


Figure 5. RFLP analysis of clones containing 1.3 Kb 16S ribosomal RNA gene PCR products. Eight clones were restricted with *Rsa* I, and the resulting fragments were separated by agarose gel electrophoresis. The varied banding patterns demonstrate the diversity of the 16S ribosomal gene sequences that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

1. Tsai, Y.-L. and B.H. Olson (1992) *Appl. Environ. Microbiol.* **58**, 754.
2. Tebbe, C.C. and W. Vahjen (1993) *Appl. Environ. Microbiol.* **59**, 2657.
3. Selenska, S. and W. Klingmuller (1991) *Letters in Appl. Microbiol.* **13**, 21.
4. Zhou, J. et al. (1996) *Appl. Environ. Microbiol.* **62**, 316.
5. Marchesi, J.R. et al. (1998) *Appl. and Envir. Microbiol.* **64**, 795.
6. Gardes, M. and T. Bruns (1993) *Mol. Ecol.* **2**, 113.
7. White, T.J. et al. p315-322. In M.A. Innis et al. (ed.), *PCR Protocols, a guide to methods and applications*. Academic Press, San Diego, CA.
8. Kuske, C.R. et al. (1998) *Appl. Environ. Microbiol.* **64**, 2463.
9. Krsek, M. and E.M.H. Wellington (1999) *J. of Microbiol. Meth.* **39**, 1.

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SoilMaster™ DNA Extraction Kit

SM02050 50 Reactions

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

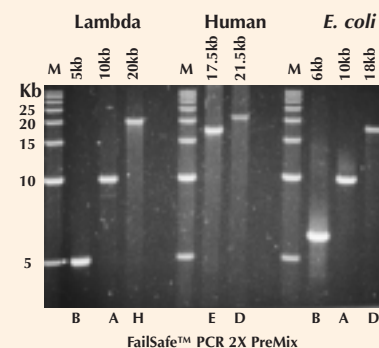
Use the selected PreMix with the FailSafe™ PCR Enzyme Mix for consistent amplification of your template/primer pair.

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

Obtain high fidelity PCR with no loss in sensitivity

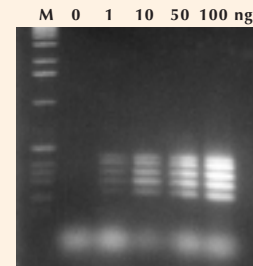
The FailSafe™ PCR Enzyme Mix contains a 3'-5' proofreading enzyme that delivers fidelity at least three times higher than *Taq* DNA polymerase.

Amplify templates up to 20 Kb long



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

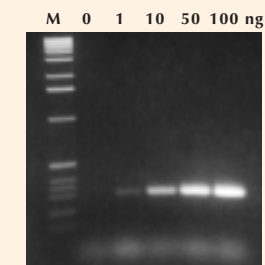
Get successful multiplex amplification



CFTR 5 Band Multiplex

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

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.

www.epicentre.com/failsafe.asp

FailSafe™ PCR PreMix Selection Kit

FS99060 60 Units
Contains FailSafe™ PCR Enzyme Mix and the 12 FailSafe™ PCR 2X PreMixes.

FailSafe™ PCR System

FS99100 100 Units
Includes FailSafe™ PCR Enzyme Mix and choice of one FailSafe™ PCR 2X PreMix.

FS99250 250 Units
Includes FailSafe™ PCR Enzyme Mix and choice of two FailSafe™ PCR 2X PreMixes.

FS9901K 1,000 Units
Includes FailSafe™ PCR Enzyme Mix and choice of eight FailSafe™ PCR 2X PreMixes.