Direct PCR from a Single Bacterial Colony Without DNA Extraction Using the FailSafe™ PCR System

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Introduction

PCR template preparation that requires DNA purification can be tedious, costly, and time consuming, especially with large numbers of samples. In previous reports,[1,2] we demonstrated that the FailSafe™ PCR System enabled direct PCR amplification using whole blood and dried blood spots collected on Guthrie cards or glass slides, all without prior DNA purification. This article reports reproducible PCR amplification directly from single *E. coli* colonies using the FailSafe PCR System.

Methods and Results

Direct PCR from bacterial colonies using the FailSafe PCR System

The FailSafe™ PCR PreMix Selection Kit contains the unique FailSafe™ PCR Enzyme blend and 12 PreMixes (A to L), each representing a different PCR condition. A PCR amplification will succeed under at least one condition for any template and primer pair combination.

In order to evaluate the ability of the FailSafe PCR System to amplify directly from bacterial cells, 10 single *E. coli* colonies, 1 mm in diameter, were first picked from the plate and suspended in 10 µl of water. Each suspension was used for PCR in separate reactions with four different sets of PCR primer pairs that amplify different regions of the *E. coli* genome. Primer Pair A generates a PCR product of 2 Kb in size, Primer Pair B 4 Kb, Primer Pair C 6 Kb, and Primer Pair D 8 Kb. PCR was carried out with each primer pair using the FailSafe PCR PreMix Selection Kit in a 50 µl volume that contained 1 µl of the resuspended *E. coli* cells, 50 pmoles of each primer, 25 µl of the optimal FailSafe™ 2X PCR PreMix, and 2.5 Units of FailSafe PCR Enzyme Mix. PCR cycling profile was: 3 minutes at 99ºC, followed by 30 cycles of 20 seconds at 98ºC and 3 minutes (for Primer Pair A and B) or 5 minutes (for Primer Pair C and D) at 68ºC.

The PCR amplifications were successful for all four sets of PCR primers using the *E. coli* cell suspensions from the ten colonies. Direct PCR amplification of *E. coli* with Primer Pair A using PreMix C is shown in Figure 1.

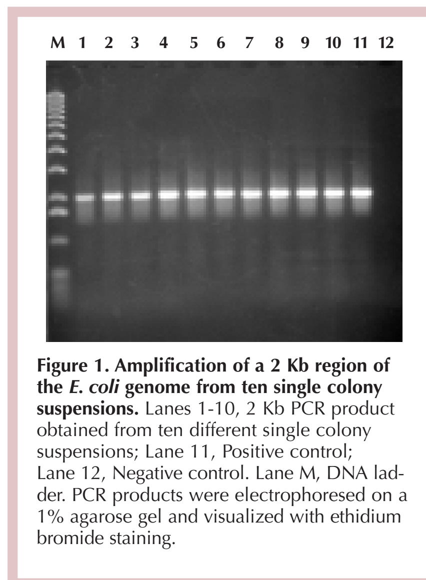

Figure 1. Amplification of a 2 Kb region of the *E. coli* genome from ten single colony suspensions. Lanes 1-10, 2 Kb PCR product obtained from ten different single colony suspensions; Lane 11, Positive control; Lane 12, Negative control. Lane M, DNA ladder. PCR products were electrophoresed on a 1% agarose gel and visualized with ethidium bromide staining.

Direct PCR using *E. coli* cell suspensions from an overnight cell culture was also successful (data not shown). The cell suspension was prepared by centrifuging 1 ml of a saturated *E. coli* culture and resuspending the cell pellet in 1 ml of water. One microliter of resuspended cells was used per PCR reaction as indicated above.

Comparison of direct PCR from an E. coli colony using FailSafe and the leading Taq DNA Polymerase

All PCR amplifications using the FailSafe System were performed as indicated above. PCR amplifications using the market-leading *Taq* DNA Polymerase were performed as directed using the supplied 10X Buffer provided by the manufacturer. Cycling conditions were identical to those used for FailSafe. The differences in PCR results between the FailSafe System and the market leader are shown in Figure 2. FailSafe consistently amplified *E. coli* DNA using all of the primer pairs tested, while the leading *Taq* Polymerase amplified *E. coli* DNA with only 2 of the 4 primer pairs. Also, of the two positive PCR reactions using the leading *Taq* DNA Polymerase, the yield of the PCR products was visibly lower than that obtained using the FailSafe PCR System.

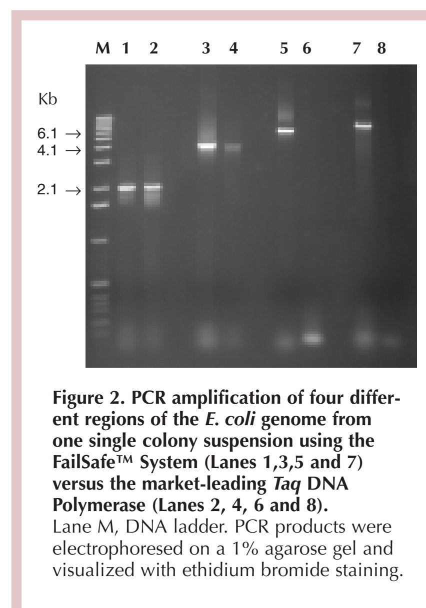

Figure 2. PCR amplification of four different regions of the *E. coli* genome from one single colony suspension using the FailSafe™ System (Lanes 1,3,5 and 7) versus the market-leading *Taq* DNA Polymerase (Lanes 2, 4, 6 and 8). Lane M, DNA ladder. PCR products were electrophoresed on a 1% agarose gel and visualized with ethidium bromide staining.

Conclusions

The FailSafe PCR System reliably amplified DNA from *E. coli* colonies without a DNA extraction step.

References

1. Grunenwald, H. (2000) *EPICENTRE Forum* **7**(4), 10.
2. Grunenwald, H. (2001) *EPICENTRE Forum* **8**(2), 4.

FailSafe™ PCR PreMix Selection Kit

FS99060-F83
Contains the FailSafe™ PCR Enzyme Mix and the 12 FailSafe™ PCR PreMixes.

FailSafe™ PCR System

FS99100-F83	100 Units*
FS99250-F83	250 Units**
FS9901K-F83	1,000 Units*** (4 x 250 U)

*Includes your choice of one FailSafe™ PCR 2X PreMix (2.5 ml).

**Includes your choice of two FailSafe™ PCR 2X PreMixes (2.5 ml each).

***Includes your choice of eight FailSafe™ PCR 2X PreMixes (2.5 ml each).

See the center insert for more information on the FailSafe™ PCR System.