

FailSafe™ PCR: A New System For Reliable and Consistent Amplification of Both Routine and Challenging Templates

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Introduction

Editor's note: This article is being reprinted due to the popularity of the FailSafe PCR System.

Successful PCR depends on a variety of factors including the quality of the template, choice of enzyme, and primer design, as well as the amplification conditions used. The ideal PCR system would be able to: 1) consistently amplify a wide variety of templates including difficult (e.g., high GC content or secondary structure) and long sequences, 2) amplify with high fidelity, and 3) achieve amplification with little optimization. While enzymes for high fidelity PCR exist, as do methods for difficult and long PCR, no one system exists that addresses all of these factors in an easy-to-use format.

Here, we introduce FailSafe™ PCR. The FailSafe PCR System ensures successful and consistent PCR results with both routine and challenging templates, including long templates (up to approximately 20 kb in length) and templates with high GC content (>80% GC). The FailSafe PCR System consists of two components. The FailSafe PCR Enzyme Mix is a unique enzyme blend containing a 3'→5' proofreading enzyme for high fidelity. PCR products generated by the FailSafe PCR Enzyme Mix are readily cloned with high efficiency in TA or blunt-end vectors. The second component of the FailSafe PCR System is a set of FailSafe PCR PreMixes. The FailSafe PCR PreMixes contain buffer, dNTPs, and various amounts of MgCl₂ and FailSafe PCR Enhancer (with betaine).* The user simply adds template, primers, and the FailSafe PCR Enzyme Mix to the FailSafe PCR PreMixes and amplifies. This single-step protocol is used for all templates, no tedious optimization is required. The FailSafe PCR Enhancer included in the PreMixes increases PCR specificity, sensitivity, and consistency.

In this article, we compare the fidelity of the FailSafe PCR System to other commercially available PCR enzymes and enzyme blends. We also demonstrate amplification of long and difficult templates and multiplex PCR using the FailSafe PCR System.

Methods and Results

Fidelity of the FailSafe PCR Enzyme Mix

Applications of PCR such as cloning, expression, mutation analysis, and long amplification require the use of enzymes with low error rates. We compared the fidelity of the FailSafe PCR Enzyme Mix with enzymes and enzyme

*Patents issued and pending.

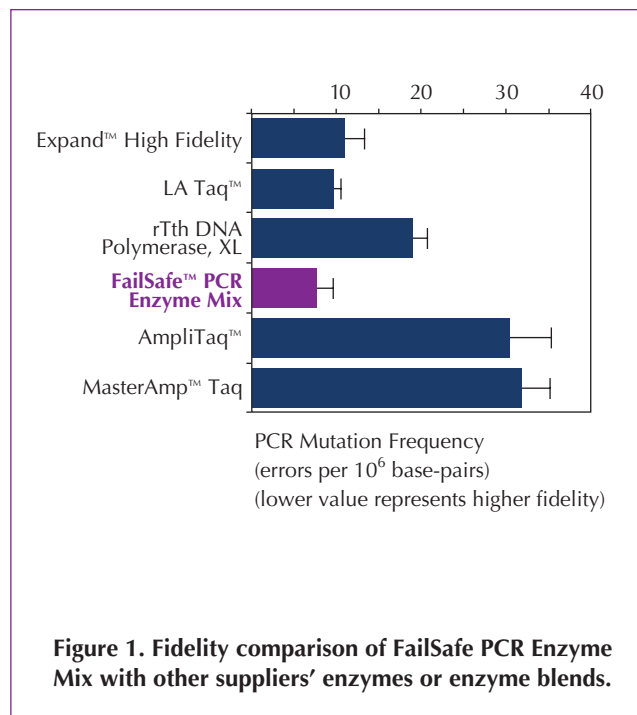


Figure 1. Fidelity comparison of FailSafe PCR Enzyme Mix with other suppliers' enzymes or enzyme blends.

blends from other suppliers using a PCR-based forward mutation assay. The method is similar to that used by Cline *et al.*¹ and measures PCR fidelity by amplifying the α -complementing portion of the lacZ gene and assessing its sequence integrity using a blue/white colony screening assay. Amplification reactions were performed using the reagents and protocols supplied by each respective manufacturer and fidelity assays were performed side by side. As shown in Figure 1, the fidelity of FailSafe PCR Enzyme Mix was at least three times better than both standard Taq polymerases tested and was equivalent to or better than the other high fidelity enzyme blends tested. Although the FailSafe PCR Enzyme Mix exhibits slightly lower fidelity than has been reported for Pfu DNA polymerase, the FailSafe PCR System is much more robust and is able to achieve more specific and consistent amplification of difficult templates (e.g., with high GC content) and long templates on which Pfu fails.

Amplification of long sequences using the FailSafe PCR System

Amplification of long templates often requires tedious optimization of reaction conditions including the addition of PCR additives. To demonstrate that the FailSafe PCR System amplifies long templates, as well as standard templates,

without tedious optimization of individual reaction components, we amplified lambda, human, and *E. coli* genomic DNA targets ranging in size from 5 kb to 21.5 kb.

For each template/primer pair combination, PCR reactions were performed using the FailSafe PCR PreMixes. Each 50 µl reaction contained 1-500 ng of genomic DNA (depending on the template), 10-50 pmoles of each primer (depending on the template), 2.5 U of FailSafe PCR Enzyme Mix, and 25 µl of a FailSafe PCR 2X PreMix (A-L). The lambda template was amplified with the following cycling profile: 94°C for 1 minute, followed by 20 cycles at 98°C for 20 seconds, 56°C for 1 minute (5 kb and 10 kb only), and 68°C for 5 minutes (5 kb), 10 minutes (10 kb), or 20 minutes (20 kb). The *E. coli* genomic DNA was amplified with the following cycling profile: 94°C for 1 minute, followed by 20 cycles (except 30 cycles for the 6 kb template) of 98°C for 20 seconds and 68°C for 5 minutes (6 kb), 10 minutes (10 kb), or 20 minutes (18 kb). The human genomic DNA was amplified with the following cycling profile: 94°C for 1 minute, followed by 14 cycles of 98°C for 20 seconds and 68°C for 20 minutes, and then another 10 cycles where the extension time is lengthened by 15 seconds for each subsequent cycle.

Figure 2 shows the PCR products amplified using the optimal FailSafe PCR PreMix for each template/primer pair combination. All PCR products were amplified in high yield and with high specificity.

Amplification of GC-rich templates using the FailSafe PCR System

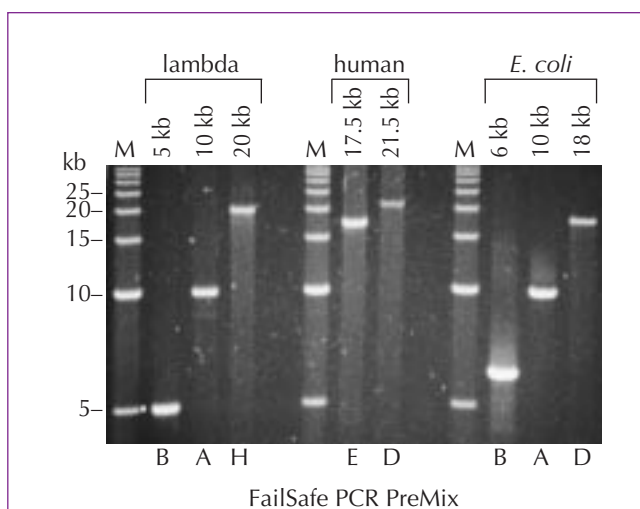


Figure 2. Amplification of various sequence lengths using the FailSafe PCR System. PCR amplification was performed as described in the text. The results using the optimal FailSafe PCR PreMix determined from each set of reactions is shown. M, 5 kb ladder.

As with long PCR, amplification of difficult templates, such as those with high GC content or secondary structure, often requires extensive optimization. To demonstrate that the FailSafe PCR protocol can be used for amplification of GC-rich templates without extensive optimization, we amplified a 250-350 bp region of the human FMR1 gene, which has a GC content of 80-85%.² An expansion of a triplet repeat (CGG) region in this gene is associated with fragile X syndrome. Human genomic DNA was purified from blood with the MasterPure™ DNA Purification Kit (Epicentre). PCR reactions were performed using the FailSafe PCR PreMixes. Each 50 µl reaction contained 50 pmoles of each primer, 100 ng of genomic DNA, 1.25 U of FailSafe PCR Enzyme Mix, and 25 µl of a FailSafe PCR 2X PreMix (A-L). After an initial denaturation at 94°C for 2 minutes, the Enzyme Mix was added and the reaction was amplified at 94°C for 4 minutes, followed by 30 cycles of 98°C for 30 seconds, 65°C for 1 minute, and 72°C for 1 minute. Using a single set of 12 reactions, FailSafe PCR PreMix J was determined to be optimal for amplification of the high GC region of the FMR1 fragile X gene (Figure 3).

Amplification of this region of the fragile X gene from four separate individuals was performed with FailSafe PCR PreMix J and the FailSafe PCR Enzyme Mix. The results are shown in Figure 4, p. 8. As seen in the Figure, the FailSafe PCR System consistently amplified this 80-85% GC-rich region. Because the number of CGG repeats varies among different individuals, the size of the resulting PCR product varies slightly, ranging between 250 bp and 350 bp.

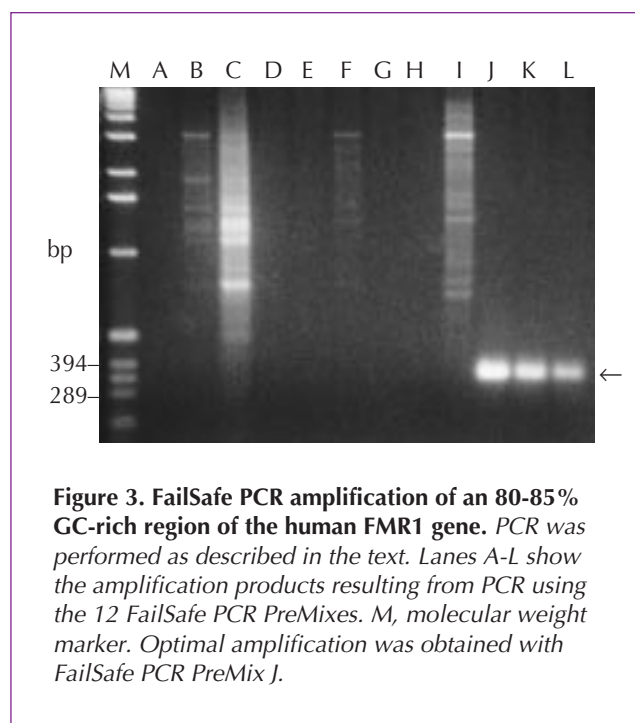


Figure 3. FailSafe PCR amplification of an 80-85% GC-rich region of the human FMR1 gene. PCR was performed as described in the text. Lanes A-L show the amplification products resulting from PCR using the 12 FailSafe PCR PreMixes. M, molecular weight marker. Optimal amplification was obtained with FailSafe PCR PreMix J.

continued

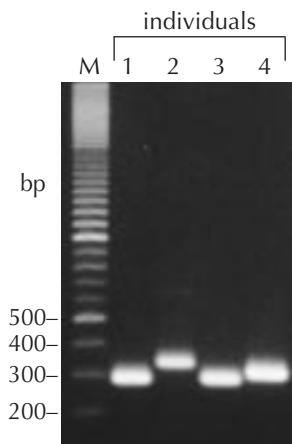


Figure 4. FailSafe PCR amplification of the FMR1 region from four different individuals. PCR was performed using FailSafe PCR PreMix J as described in the text. M, 100 bp ladder.

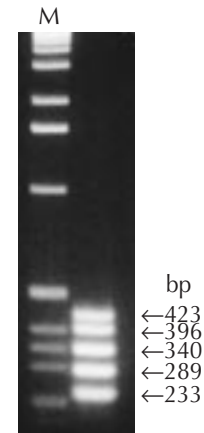


Figure 5. Multiplex PCR amplification of the CFTR gene. Multiplex PCR was performed as described in the text. The results using FailSafe PCR PreMix C are shown. M, molecular weight marker.

Multiplex amplification using the FailSafe PCR System

The FailSafe PCR System was also tested for multiplex amplification. Five exons from the cystic fibrosis transmembrane conductance regulator (CFTR) gene³ were amplified using the FailSafe PCR PreMixes. The 50 µl multiplex PCR reactions contained 25 pmoles of each primer,² 500 ng of human genomic DNA, 2.5 U of FailSafe PCR Enzyme Mix, and 25 µl of a FailSafe PCR 2X PreMix. After an initial denaturation at 94°C for 2 minutes, the Enzyme Mix was added and the reaction was amplified for 30 cycles at 94°C for 10 seconds, 53°C for 10 seconds, 74°C for 10 seconds, followed by a final extension step at 74°C for 5 minutes.

The sizes of the PCR products from the CFTR gene exons 4, 10, 11, 20, and 21 are 423, 340, 233, 289, and 396 bp respectively.² Optimal amplification was achieved using FailSafe PCR PreMix C (Figure 5). The multiplex analysis resulted in the correct size PCR product for each exon. The 5-band multiplex PCR from the CFTR gene was successfully obtained with one set of reactions using the FailSafe PCR System.

Summary

FailSafe PCR ensures successful PCR results for a variety of applications including amplification of templates at least 20 kb in length, amplification of GC-rich templates, and multiplex PCR. The high fidelity of the FailSafe PCR Enzyme Mix is important for making PCR products for cloning, expression, and mutation analysis. The convenient kit format enables easy, single-step amplification of any template without tedious optimization, and no change in protocol is required for different templates. These

advantages make the FailSafe PCR System suitable for both routine and challenging PCR amplifications.

References

1. Cline, J. *et al.* (1996) *Nucl. Acids Res.* **24** (18), 3546.
2. Fu, Y.H. *et al.* (1991) *Cell* **67** (6), 1047.
3. Richards, B. *et al.* (1993) *Human Molecular Genetics* **2** (2), 159.

FailSafe™ PCR PreMix Selection Kit

FS99060-F72

Contains the FailSafe™ PCR Enzyme Mix and the 12 FailSafe™ PCR PreMixes.

FailSafe™ PCR System

FS99100-F72	100 Units*
FS99250-F72	250 Units**
FS9901K-F72	1,000 Units*** (4x250 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).

For more information, please circle reader service number N724 on the reply card found in the center insert or visit our website at www.epicentre.com/catalog/failsafe.htm

