

Unlike Other PCR Products, the FailSafe™ PCR System Amplifies a GC-Rich Template and a 20-Kb Template on the First Try

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Introduction

The FailSafe™ PCR System provides consistent and dependable amplifications 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 amplifications. The FailSafe PCR System combines a unique blend of high-fidelity thermostable enzymes with an extensively tested set of FailSafe™ PCR PreMixes that include dNTPs, buffer, MgCl₂, and the patented FailSafe™ PCR Enhancer Technology. In this report we compare how the FailSafe PCR System performs the first time used with GC-rich and long templates compared with results using seven other significant suppliers of PCR enzymes and systems.

Methods and Results

Amplification of a GC-rich template

A 268-bp region (GC content = 75%) of the human apolipoprotein E gene was amplified. The reactions were set up according to each of the manufacturer's standard protocol instructions. In brief, for each 50- μ l PCR reaction, the following components were included: 25 pmole of each of the forward and reverse primers, 100 ng of human genomic DNA, 200 μ M of dNTPs, 1X reaction buffer, and 1.25 U of appropriate DNA polymerase. The FailSafe™ PreMix Selection Kit was used with the 12 FailSafe PCR PreMixes

First Time
Perform PCR with your template and primers using the FailSafe™ PCR PreMix Selection Kit and choose the PreMix that provides the best amplification.

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and Every Time
Get the selected PreMix with the FailSafe™ PCR System and use it for consistent amplification of your template/primer pair.

provided. Cycling conditions were 5 minutes at 94°C, followed by 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute. As shown in Figure 1, only the FailSafe PCR System using PreMix K successfully amplified the *apoE* gene.

Amplification of a 20-kb template

A 20-kb region of lambda DNA was amplified. The reactions were set up according to each of the manufacturer's standard protocol instructions. In brief, for each 50- μ l PCR reaction, the following components were included: 50 pmole of

each of the forward and reverse primers, 1 ng of lambda DNA, 200 μ M of dNTPs, 1X reaction buffer with MgCl₂, and 2.5 U of appropriate DNA polymerase. For the FailSafe System, the 12 PCR PreMixes in the FailSafe PreMix Selection Kit were used as directed. Cycling conditions were 1 minute at 94°C, followed by 20 cycles of 98°C for 20 seconds and 68°C for 20 minutes. As shown in Figure 2, both FailSafe and Supplier "6" successfully amplified the 20-kb PCR, however about 2-fold more PCR product was obtained from the FailSafe reaction using PreMix D.

Summary

This report demonstrated how the FailSafe PCR System amplified a high-GC template and a 20-kb long template the first time on the first try. Other PCR products did not perform as well when the standard protocol was used. It is important to note that each of these other PCR products have optimization recommendations to try in case a PCR reaction does not amplify the first time. However, as demonstrated, with the FailSafe PCR System, no time or effort were needed to amplify these templates successfully the first time. The same FailSafe PCR PreMixes that amplify a given template give consistent PCR results in all subsequent amplification reactions. Due to the system's performance, reliability, and ease of use, the FailSafe™ PCR System is the method of choice.

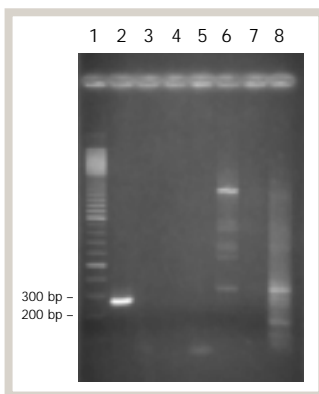


Figure 1. Amplification of a high-GC content template using the FailSafe™ PCR System and other methods. Lane 1, marker; Lane 2, FailSafe™ PCR System using PreMix K; Lane 3, Supplier 1 PCR Master Mix; Lane 4, Supplier 2 *Taq*; Lane 5, Supplier 3 *Taq*; Lane 6, Supplier 4 *Taq* (with GC-rich template amplification claims); Lane 7, Supplier 5 enzyme for use with GC-rich templates; Lane 8, Supplier 6 *Taq* for hot start PCR.

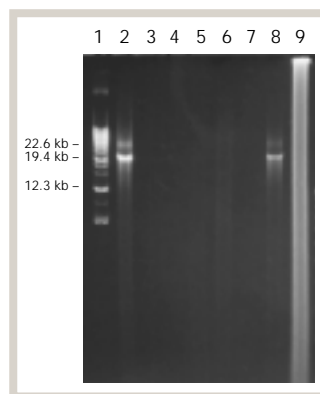


Figure 2. Amplification of a 20-kb PCR using the FailSafe™ PCR System and other methods. Lane 1, marker; Lane 2, FailSafe™ PCR System; Lane 3, Supplier 1 PCR Master Mix; Lane 4, Supplier 2 *Taq*; Lane 5, Supplier 3 *Taq*; Lane 6, Supplier 4 *Taq*; Lane 7, Supplier 5 enzyme for long PCR amplification; Lane 8, Supplier 6 enzyme for long PCR amplification; Lane 9, Supplier 7 enzyme for long PCR amplification.

www.epicentre.com/failsafe.asp

FailSafe™ PCR PreMix Selection Kit

FS99060 60 Units
Includes 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.