

# EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research

## The FailSafe™ Real-Time PCR Capillary System: Now Available for Use with Capillary-Based Real-Time PCR Instruments

New

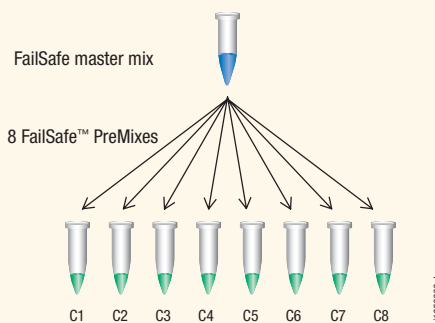
Haiying Gunenwald and Gordon Hunter, EPICENTRE

Recently EPICENTRE introduced the FailSafe™ Real-Time PCR System with SYBR® Green I dye for the detection and quantification of real-time PCR products.<sup>1</sup> This popular real-time PCR system ensures successful quantitative PCR the first time and every time, just like the widely-accepted standard FailSafe™ PCR System, which has been available since 1999.

Now we introduce the FailSafe™ Real-Time PCR Capillary System for quantitative PCR thermal cyclers that use glass capillary reaction vessels, such as the Roche LightCycler® instrument. The FailSafe Real-Time PCR Capillary System contains the FailSafe™ PCR Enzyme Mix and 8 unique FailSafe™ PCR PreMixes. Initially, use your template and primer pair with each Pre-Mix to determine which of the 8 mixes gives the best quantitative PCR results for your template/primer combination. Then use the selected PreMix with the tested template/primer combination for future quantitative PCR reactions.

The FailSafe PCR Enzyme Mix contains a unique blend of thermostable enzymes capable of amplifying even the most difficult DNA templates with high sensitivity and high fidelity. The 8 optimized FailSafe PCR PreMixes in the system contain the patented FailSafe™ PCR Enhancer (with betaine\*) to improve the specificity and consistency of every PCR reaction. In addition to the FailSafe PCR Enhancer, the PreMixes contain: SYBR® Green I dye, dNTPs, buffer, salt, different concentrations of MgCl<sub>2</sub>, stabilizer, and proprietary reagents specific for efficient real-time PCR with thermal cyclers using glass capillaries. Experimental data presented here compares the sensitivity and specificity of EPICENTRE's new FailSafe System to a real-time PCR kit from another leading supplier of reagents for glass capillary systems, designated "Supplier R" in this report.

An overview on how to use the FailSafe™ Real-Time PCR Capillary PreMix Selection Kit.



**Step 1. Prepare a master mix containing FailSafe™ PCR Enzyme Mix and your template and primers, then add to each of the 8 FailSafe™ Real-Time PCR Capillary PreMixes (C1-C8) and perform PCR.**

**Step 2. Analyze PCR results and choose the best PreMix for subsequent amplifications.**

**Repeat steps 1 and 2 for each new template/primer pair.**

### Methods

To test the performance of the FailSafe Real-Time PCR Capillary Kit, PCR products were amplified from 2 different DNA templates: lambda DNA and human genomic DNA. The reactions amplified a 100-bp product from lambda DNA or a 289-bp product from the human cystic fibrosis transmembrane conductance regulator (CFTR) exon 20. PreMix C1, which gave the best PCR results with both the lambda DNA and the human genomic DNA templates in the initial round of amplifications, was used in all subsequent reactions. Each 20-µl FailSafe Real-Time PCR capillary reaction contained: 0.2 pg of lambda DNA or 4 ng of human genomic DNA as template, 500 nmol each of the appropriate forward

and reverse primers, 10 µl of the C1 2X PreMix, and 1 µl of the FailSafe Real-Time PCR Enzyme Mix. Supplier R reactions for the two templates were optimized by titrating the MgCl<sub>2</sub> concentration from 1 to 5 mM, by 0.5 mM increments.

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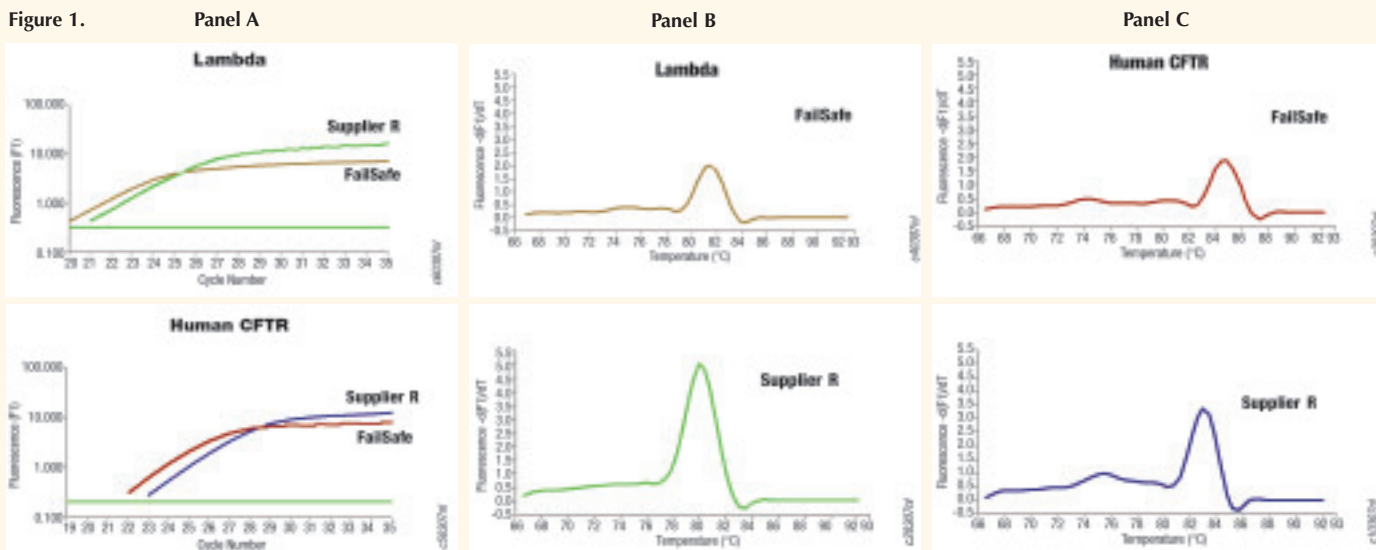
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**Panel A. Faster threshold cycle values with the new FailSafe™ Real-Time PCR Capillary System.** Real-Time PCR quantification graphs produced using the FailSafe™ Real-time PCR Capillary System or Supplier R reagents with lambda and human genomic DNA templates.

**Panel B. No non-specific amplicon or primer/dimer formation with the lambda DNA template.** Melt curve analysis graphs for real-time PCR using the new FailSafe™ Real-Time PCR Capillary System or Supplier R reagents and the lambda template.

**Panel C. No non-specific amplicon or primer/dimer formation with the human genomic DNA template.** Melt curve analysis graphs for real-time PCR using the new FailSafe™ Real-Time PCR Capillary System or Supplier R reagents and the human CFTR template.

The best amplification for both templates with Supplier R reagents had a final concentration of 2.5 mM MgCl<sub>2</sub>. All reactions were amplified on Roche's LightCycler® instrument. Initial denaturation was 95°C for 2 minutes for the FailSafe Real-Time PCR System and 95°C for 10 minutes for Supplier R. After the initial denaturation, all reactions went through 35 cycles of 95°C (10 seconds), 60°C (10 seconds), and 72°C (30 seconds).

**Results**

As indicated by Figure 1, Panel A, the FailSafe Real-Time PCR Capillary System gave threshold cycle (C<sub>T</sub>) values of 1 to 1.5 cycles faster for both the lambda and human CFTR amplifications compared to Supplier R. (Some of the 8 FailSafe PCR PreMixes demonstrated similar C<sub>T</sub> values, but only data from PreMix C1 are shown here). Supplier R reactions gave slightly higher fluorescent signals, but the height of the fluorescence peak does not correlate with the quantity of PCR products generated. High fluorescent signals can be an indication of higher SYBR® Green I concentrations in the reaction, which has been shown to inhibit PCR amplifications, resulting in delayed cycle thresholds.<sup>2</sup>

Figure 1 also gives the melt curve analysis of both sets of real-time PCR data for lambda DNA (Panel B) and human CFTR gene (Panel C) amplifications. The similar melt curves show no non-specific amplicon or primer/dimer formation, demonstrating comparable sensitivity and specificity for

both the FailSafe Real-Time PCR Capillary System and Supplier R with these template/primer combinations. Specific melt curve analysis graphs were obtained for all PCR amplifications, but only two representative graphs are shown here.

**Conclusion**

The FailSafe Real-Time PCR Capillary System provides better or equal real-time PCR performance when compared to another leading supplier of PCR reagents made specifically for thermal cyclers that use glass capillary reaction vessels. The real-time quantification and melt curve graphs presented here demonstrate excellent real-time PCR sensitivity and specificity with the new FailSafe Kit using two different template/primer combinations. The FailSafe™ PCR Real-Time Capillary PreMix Selection Kit enables quick and easy optimization of the reaction conditions, compared to the alternative method of only optimizing the MgCl<sub>2</sub> concentration in reactions by titrating from a stock solution. Unlike Supplier R, the FailSafe Real-Time PCR Capillary System PreMixes come ready to use, so no final mixing is required, and are stable at -20°C for at least 6 months.

**References**

1. Grunewald, H. (2003) *EPICENTRE Forum* 10(1), 1.
2. Nath, K et al. (2000) *J. Biochem Biophys Methods*. 42(1-2), 15.

[www.epicentre.com/realtimepcr.asp](http://www.epicentre.com/realtimepcr.asp)

**New!**

**FailSafe™ Real-Time PCR Capillary PreMix Selection Kit**  
 FSRC3832                      32 Reactions

*Contents:*  
 FailSafe™ PCR Enzyme Mix  
 8 FailSafe™ Real-Time PCR Capillary 2X PreMix

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**FailSafe™ Real-Time PCR Capillary System**  
 FSRC3896                      96 Reactions  
 FSRC38384                    4 X 96 Reactions

*Contents:*  
 FailSafe™ PCR Enzyme Mix  
 Choice of one FailSafe™ Real-Time PCR Capillary 2X PreMix

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