

EPICENTRE Forum

Tools & Techniques for Genomics, Proteomics & RNA Research



The FailSafe™ Real-Time PCR System Provides Highly Sensitive and Specific Real-Time PCR Analysis

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The FailSafe™ Real-Time PCR System extends the unsurpassed specificity, sensitivity, and consistency of the FailSafe™ PCR System to quantitative PCR applications. The FailSafe™ Real-Time PCR System incorporates SYBR® Green I dye for the detection and quantitation of PCR products without the expense of labeled PCR primers, molecular beacons, or other labeled probes. Like the standard Fail-Safe™ PCR System, this new real-time PCR kit ensures successful quantitative PCR the first time and every time.

The FailSafe Real-Time PCR System uses the FailSafe PCR Enzyme Mix, a unique blend of thermostable enzymes that is capable of amplifying the most difficult DNA templates with extremely high sensitivity and high fidelity. In addition, the patented FailSafe PCR Enhancer (with betaine)* greatly improves the specificity and consistency of PCR.

The final key components of the system are 12 unique FailSafe PCR PreMixes. Each FailSafe PCR PreMix contains everything else you need for a successful quantitative PCR except your own template and primers: SYBR Green I dye, dNTPs, buffer, and varying amounts of MgCl₂ and EPICENTRE's patented FailSafe PCR Enhancer. A separate container of ROX, a fluorescent passive reference dye that is required for signal normalization in SYBR Green dye reactions assayed using ABI real-time PCR instruments, is also provided.

In this report, we compare the specificity, sensitivity, and dynamic range of the FailSafe Real-Time PCR System with real-time PCR kits of leading suppliers.

Methods and Results

Higher specificity with the FailSafe™ Real-Time PCR System

To test the specificity of real-time PCR reactions, a 357-bp fragment of human

First Time

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



Every Time

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

Duchenne Muscular Dystrophy (DMD) exon 43 was amplified using 100 ng of human genomic DNA, 500 nmol each of the forward and reverse primers, and either the FailSafe Real-Time PCR System or another supplier's kit according to the manufacturer's directions. FailSafe PreMix E was found to be optimal in the first round of PCR and was used in all subsequent FailSafe Real-Time PCR reactions with this template and primer set. All reactions were set up at room temperature. Prior to thermocycling, hot-start enzymes of other suppliers were reactivated according to each of the other manufacturer's instructions. The FailSafe System does not use a hot-start enzyme and no reactivation step was required. The PCR cycling program

consisted of 50 cycles of: 10 seconds at 95°C; 10 seconds at 55°C; and 30 seconds at 72°C.

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Figure 1. Higher specificity with the FailSafe™ Real-Time PCR System. Real-time PCR amplification of a 357-bp fragment from human DMD exon 43 was performed with FailSafe Real-Time PCR System and 5 major hot-start real-time PCR suppliers' kits. **Panel A**, PCR quantification graph. **Panel B**, Melt curve analysis. **Panel C**, Agarose gel electrophoresis. Real-time PCR amplification was carried out on MJ Research's Opticon® 2 Real-Time PCR Detection System.

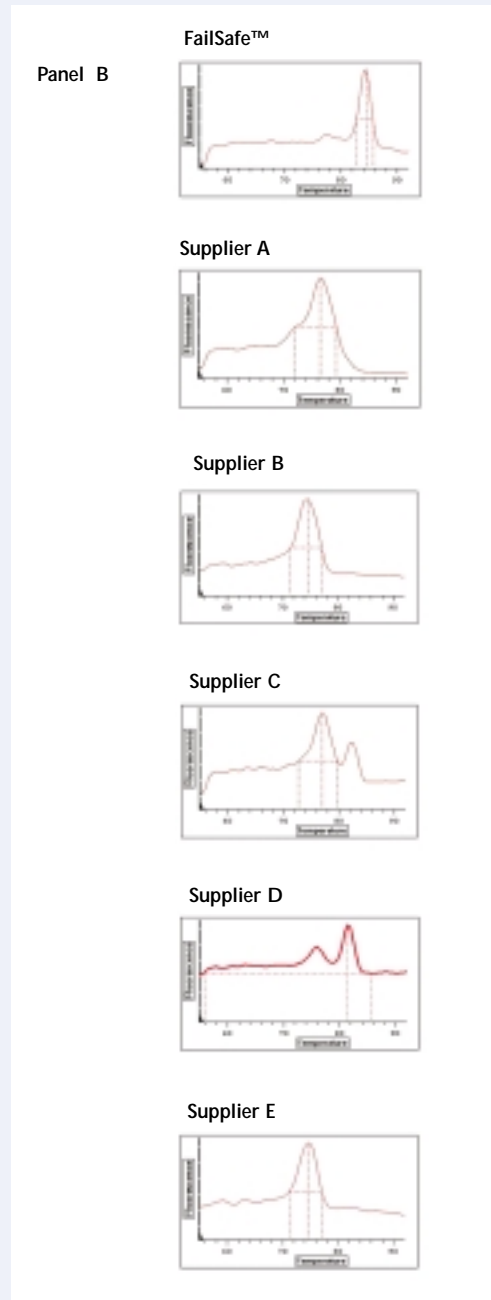
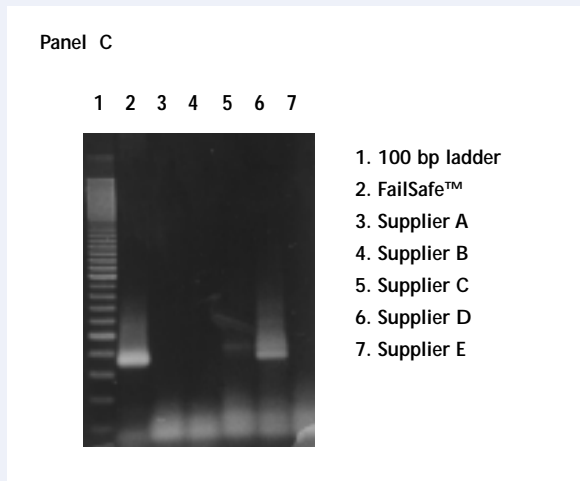
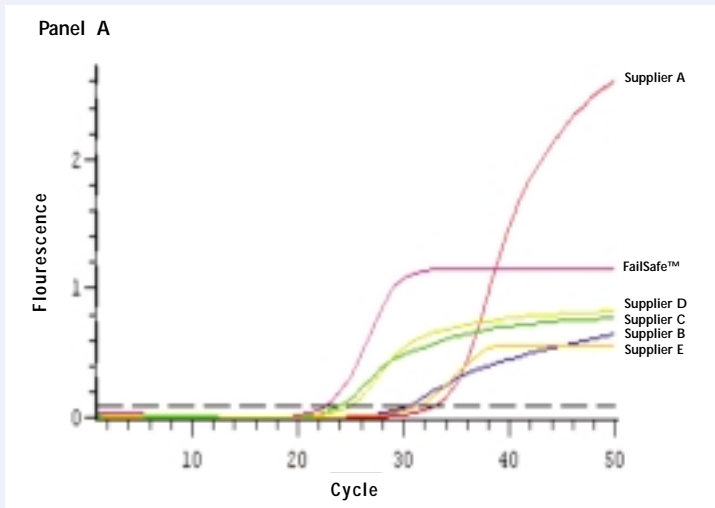


Figure 1, Panel A shows real-time PCR results obtained using the various kits. A significantly faster cycle threshold (C_T) was obtained using the FailSafe Real-Time PCR System compared to all of the other kits tested. Figure 1, Panel B illustrates melt curve analysis. Although some kits resulted in higher overall fluorescence than the FailSafe System, the melt curve analysis demonstrated that this higher fluorescence was due to non-specific or primer-dimer amplification rather than specific primer-dependent amplification. The fact that the FailSafe Real-Time PCR System provided the best true

amplification was also apparent by agarose gel electrophoresis of the PCR products, as shown in Figure 1, Panel C.

Higher sensitivity with the FailSafe™ Real-Time PCR System

To compare the sensitivity of quantitative PCR, a 181-bp fragment of human DMD exon 47 was amplified using the FailSafe Real-Time PCR System and five other suppliers' kits using 10 ng of human genomic DNA and 500 nmol each of the forward and reverse primers according to the manufacturer's directions. FailSafe™ Real-Time PreMix B was found to be opti-

mal with this template and primer set in the first round of PCR and was used in all subsequent FailSafe Real-Time PCR reactions. All reactions were set up at room temperature. Again, prior to thermocycling, the hot-start enzymes of each of the other suppliers were reactivated according to the other manufacturer's instructions, while no reactivation step was required or used for the FailSafe System. The PCR cycling program consisted of 50 cycles of: 10 seconds at 95°C; 10 seconds at 55°C; and 30 seconds at 72°C. As seen in Table 1, the FailSafe Real-Time PCR System provided the fastest C_T value.

Supplier	C _T Value (Average of Triplicate Reactions)
FailSafe™ Real-Time PCR System	24.5
Supplier A	26.1
Supplier B	30.1
Supplier C	28.0
Supplier D	27.4
Supplier E	29.8

Table 1. Higher sensitivity and faster C_T value with the FailSafe™ Real-Time PCR System. Real-time PCR amplification of a 181-bp fragment from human DMD exon 47 was performed with 10 ng of human genomic DNA using the FailSafe Real-Time PCR System and 5 major hot-start real-time PCR suppliers' kits. C_T values obtained from triplicate reactions of each PCR kit were averaged. Real-time PCR amplification was carried out on Bio-Rad's iCycler iQ™ Real-Time PCR Detection System.

Broader dynamic range with the FailSafe™ Real-Time PCR System

In order to evaluate the FailSafe Real-Time PCR System and five other suppliers' kits for dynamic range in quantitative real-time PCR, a 460-bp sequence was amplified using from as little as 1 molecule to 10⁶ molecules of bacteriophage lambda DNA as a template. Each 50- μ l real-time PCR was carried out using lambda DNA ranging from 1 molecule to

10⁶ molecules and 500 nmol each of the forward and reverse primers according to the manufacturer's directions. FailSafe™ Real-Time PreMix E was found to be optimal in the first round of PCR and was used in all subsequent FailSafe Real-Time PCR reactions with this template and primer set. All reactions were set up at room temperature. Each manufacturer's instructions were followed on reactivating their hot-start PCR enzyme, a step not required with the FailSafe Real-Time PCR

System. The PCR cycling program consisted of 45 cycles of: 10 seconds at 95°C; 10 seconds at 55°C; and 30 seconds at 72°C. As demonstrated in Figure 2, the FailSafe Real-Time PCR System provided more sensitive quantitative PCR amplification, as well as a broader dynamic range.

Conclusions

The FailSafe Real-Time PCR System provides highly specific and sensitive quantitative PCR data with broad dynamic range. The reported quantitative PCR analysis was performed on Bio-Rad's iCycler iQ™ and MJ Research's Opticon® 2. The FailSafe™ Real-Time PCR System has also been tested on ABI Prism 7700.

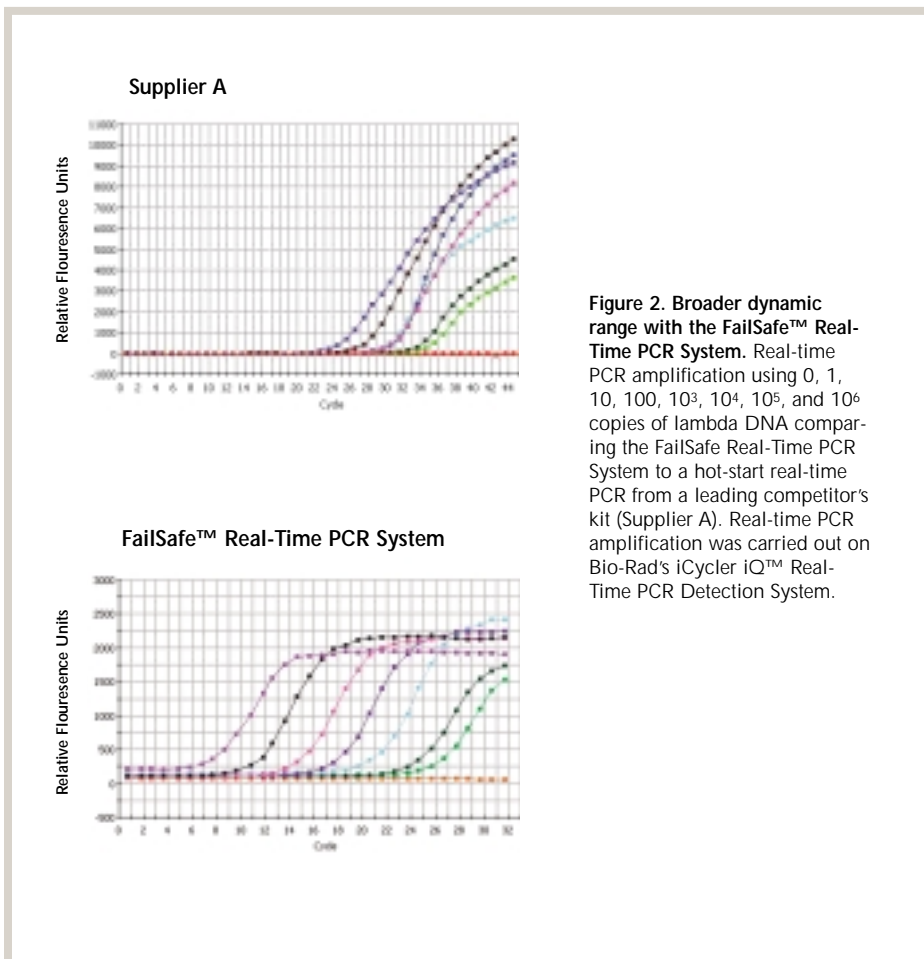


Figure 2. Broader dynamic range with the FailSafe™ Real-Time PCR System. Real-time PCR amplification using 0, 1, 10, 100, 10³, 10⁴, 10⁵, and 10⁶ copies of lambda DNA comparing the FailSafe Real-Time PCR System to a hot-start real-time PCR from a leading competitor's kit (Supplier A). Real-time PCR amplification was carried out on Bio-Rad's iCycler iQ™ Real-Time PCR Detection System.

www.epicentre.com/realtimempcr.asp

FailSafe™ Real-Time PCR PreMix Selection Kit

FSR0360 48 Reactions

Contents:

FailSafe™ PCR Enzyme Mix, 12 FailSafe™ Real-Time PCR 2X PreMixes, and Passive Reference Dye.

FailSafe™ Real-Time PCR System

FSR03200 200 Reactions

Contents:

FailSafe™ PCR Enzyme Mix, choice of two FailSafe™ Real-Time PCR 2X PreMixes, and Passive Reference Dye.

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SYBR is a registered trademark of Molecular Probes, Inc. SYBR® Green I Dye is covered by patents.

This product is accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) and RT-PCR for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or purchased, i.e., an authorized thermal cycler.