FailSafe™ PCR System

1. What is the FailSafe PCR System, and how does it work?

The FailSafe PCR System consists of several components that are designed to provide dependable, consistent PCR results from every template and primer set (Figure 1).

a) For the first PCR experiment with a new template or primer set, purchase the FailSafe PCR PreMix Selection Kit (Cat. No. FS99060). It contains the FailSafe PCR Enzyme Blend and 12 different 2X PreMixes (A–L).

b) Perform PCR using your template, primers, FailSafe PCR Enzyme Blend, and each of the 12 FailSafe PreMixes in separate reactions.

c) Analyze the PCR products by agarose gel electrophoresis. Visually determine which FailSafe PreMix produced the best amplification (e.g., a single, sharp band of the expected size, or multiple bands of the expected sizes for multiplex PCR).

d) Use the optimal 2X PreMix, identified previously, for all future PCR amplifications with the same template and primer set.

e) To reduce subsequent costs, purchase a FailSafe PCR System with PreMix Choice (Cat. No. FS99100, FS99250, or FS9901K; ≥40, ≥100, or ≥400 reactions, respectively), instead of buying the enzyme and 2X PCR PreMixes separately. These kits provide sufficient enzyme and the appropriate amount of your favorite 2X PCR PreMix(es).

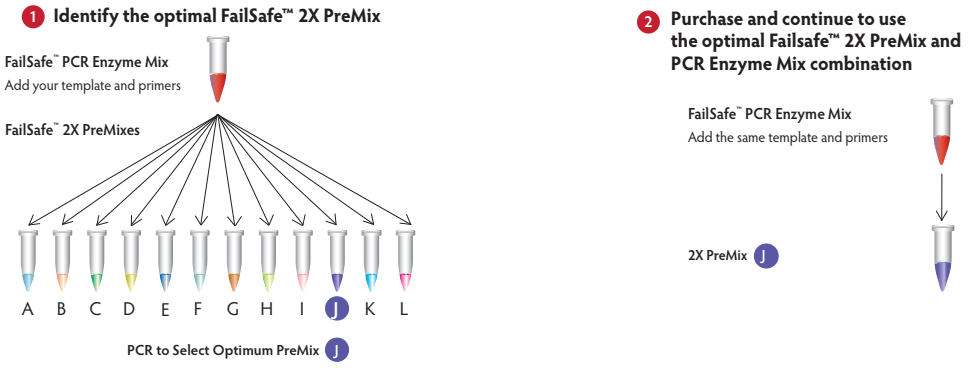

Figure 1. Never fail at PCR again with the FailSafe™ PCR System.

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2. What do the FailSafe PreMixes contain?

The FailSafe PreMixes include buffer, deoxyribonucleotides (dNTPs), salt, magnesium, and FailSafe PCR Enhancer. The concentrations of these components vary with each PreMix.

3. What DNA Polymerase is used in the FailSafe System?

The FailSafe PCR Enzyme Mix is a proprietary blend of thermostable DNA polymerases and includes 3'→5' exonuclease (proofreading) activity. This combination of enzymes provides excellent PCR efficiency and greater fidelity than systems that only use *Taq* DNA polymerase, and it allows amplification of even difficult templates, such as those with high GC content.

4. What is the fidelity of the FailSafe PCR Enzyme Mix?

The fidelity of the FailSafe PCR Enzyme Mix is approximately three times better than *Taq* DNA polymerase. The error rate for *Taq* DNA polymerase is approximately 1 in 9,000 bases, and that of the FailSafe PCR Enzyme Mix is approximately 1 in 27,000–30,000 bases. Although other proofreading enzymes (such as *Pfu*, *Pwo* or Phusion DNA polymerase) may have even higher fidelity rates, the fidelity of FailSafe is more than sufficient for use in amplifying RNA-seq or DNA-seq libraries for next-generation sequencing.

5. Which FailSafe PreMix should I use for my GC-rich template? I don't want to test all of the PreMixes.

It is nearly impossible to determine, without experimentation, which FailSafe PCR PreMix will work best with a given template/primer combination. We strongly recommend using all of the PreMixes for optimization. Without testing the complete set of FailSafe Premixes, you may not be able to determine the optimal PCR conditions for the specific amplicon that you need.

6. I want to amplify a template that is ~20 kb. Should I use the FailSafe PCR System or the MasterAmp™ Extra-Long PCR Kit?

The FailSafe PCR System can amplify templates ~20 kb. For templates >10 kb, we recommend using 2.5 units of the FailSafe PCR Enzyme Mix (for templates <10 kb, use 1.25 units) in 50 µL reactions.

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7. Can I use the PCR products from the FailSafe PCR System with TOPO® or TA Cloning®?

Yes. The FailSafe PCR System generates three different types of amplicons:

a) amplicons with non-template A nucleotides on both ends that can be used in TA Cloning applications using T vectors, or with TOPO Cloning products;

b) amplicons with blunt ends on both ends;

c) amplicons with a non-template A nucleotide on one end and a blunt end on the other.

The relative amounts of the PCR amplicons will vary based on the template. We have not determined specific reaction conditions that will favor one amplicon type over another.

8. What should I do if I get very little or no amplification after PCR?

You can adjust several reaction parameters, such as lowering the annealing temperature or increasing the initial template denaturation time. An important consideration is designing primers that are not self-complementary. You can also perform touchdown or stepdown PCR, or use a hot-start protocol. You can find further information in the *FailSafe PCR Kits Technical Manual #MA136E*.

9. What should I do if I get multiple amplicons or a smear of products after PCR?

These results typically indicate nonspecific amplification or that too much starting material was used. You can decrease the amount of template, increase the annealing temperature, or decrease the number of PCR cycles. You can also perform touchdown or stepdown PCR, or use a hot-start protocol. You can find further information in the *FailSafe PCR Kits Technical Manual #MA136E.*

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