Transitioning from Standard to Fast PCR on the Applied Biosystems 9800 Fast PCR System

Introduction

Researchers who wish to transition from standard to faster PCR may now do so on their Applied Biosystems 9800 Fast PCR System. The 9800 Fast PCR System features the 9800 Fast Thermal Cycler, GeneAmp® Fast PCR Master Mix (2X), and a 96-Well Fast Thermal Cycling Plate. The system delivers fast turnaround and unsurpassed convenience.

Conversion of Standard Methods to Fast Methods on the 9800 Fast PCR System

Using the easy, four-step method below, any laboratory interested in speeding up its PCR process can easily adapt the 9800 Fast Thermal Cycler to fast PCR.

- Ensure T_m of primers are > 62°C.
- Combine the annealing and extension steps into one.
- Determine the starting point by averaging the annealing and extension temperatures. Use a 25-second/kb rule to determine the annealing/extension time.
- Reduce the denaturing step to 0 seconds at 95°C (touch-and-go protocol).

If the GC content is high, lengthen the time required, or revert to the three- step method. Shorten the annealing/extension times if required by the complexity of the background (e.g., plasmids).

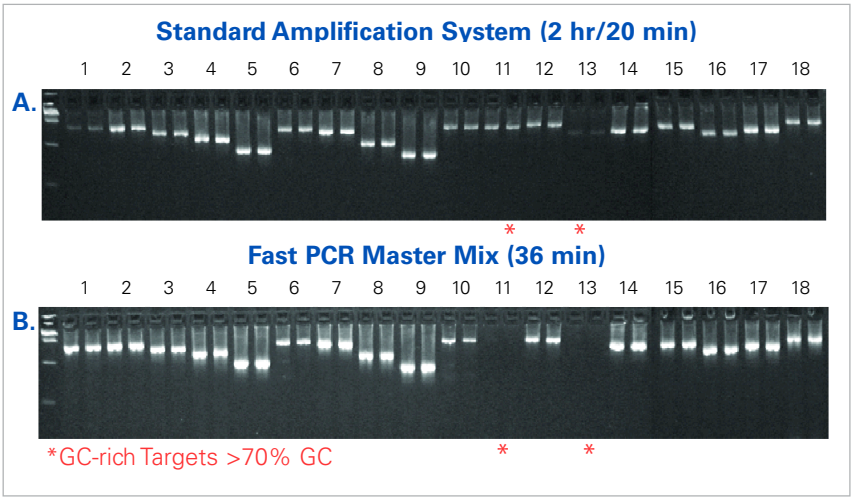


Figure 1. Data from a standard PCR system and from the 9800 Fast PCR System using a suite of Applied Biosystems products. **A.** Amplicons with different GC content were amplified on the GeneAmp® PCR System 9700 with a standard amplification system, using a three-step protocol. PCR run time: 2 hr, 20 min. **B.** The same amplicons were generated using the Applied Biosystems 9800 Fast PCR System and GeneAmp® Fast PCR Master Mix, using a two-step protocol (94°C, 0 seconds; 64°C, 35 seconds). The PCR run was shortened to 36 minutes. Amplicons 11 and 13, with approximately 72% GC content, (Table 1) did not amplify with the two-step protocol. Each amplicon above was amplified from 10 ng of human genomic DNA.

Table 1. Human PPARG Amplicons with Varied GC Content

Amplicon Name	Amplicon GC Content
Human PPARG (16) 580 bp	54%
Human PPARG (17) 570 bp	72%
Human PPARG (32) 460 bp	75%

Targets with High GC Content

The fast three-step protocol improves yield for targets that have a higher GC content. In Figure 1B above, amplicons 11 and 13 with approximately 72% GC content did not amplify using the GeneAmp Fast PCR Master Mix. A fast two-step protocol with an increased denaturation time of 5 seconds results in a successful amplification. However, a fast three-step protocol with GeneAmp® Fast PCR Master Mix increased the yield for amplicons 11 and 13 (Figure 2B).

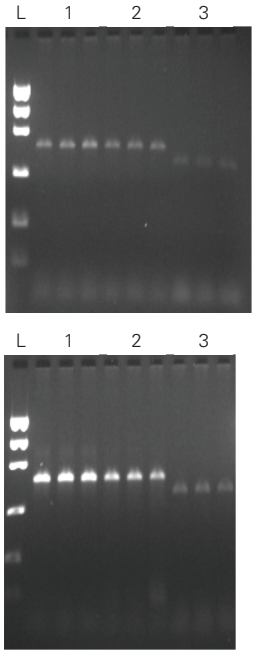
Low-Complexity Targets

Amplification of a 500 bp target usually requires approximately 20 minutes on the 9800 Fast PCR System. However, the 9800 Fast PCR System can amplify targets with smaller genomes (e.g. plasmid/phage) in approximately 10 minutes (Figure 3).

Conclusion

Transferring existing standard protocols to the 9800 Fast PCR System is easy for a wide range of targets. Most targets are successfully amplified using the recommended protocol of a denaturation step of 94°C, 0 seconds and a combined annealing/extension step using 25 second/kb of amplicon length. The remainder of targets may be adopted to the 9800 Fast PCR System by minor modifications to the basic protocol.

In conclusion, the Applied Biosystems 9800 Fast PCR System is a robust solution designed to significantly decrease your time to result.



Fast (two-step) Protocol using GeneAmp® Fast PCR Master Mix

Fast (two-step) Protocol:

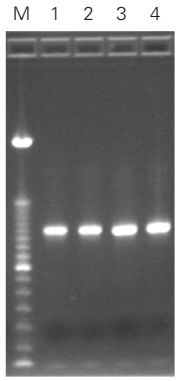
95ºC, 10 seconds
(95ºC, 5 seconds; 62ºC, 45 seconds)
35 cycles, 72ºC, 10 seconds
Total time: 47 minutes

Fast (three-step) Protocol using GeneAmp® Fast PCR Master Mix

Fast (three-step) Protocol:

95ºC, 10 seconds
(95ºC, 5 seconds; 60ºC 20 seconds; 72ºC, 25 seconds)
35 cycles, 72ºC, 10 seconds
Total time: 51 minutes

Figure 2. Amplicons 10, 11, and 13 were amplified on the Applied Biosystems 9800 Fast Thermal Cycler with GeneAmp® Fast PCR Master Mix (A and B) using a two-step or three-step protocol. These amplicons have varied GC content (Table 1). **A.** Amplification using GeneAmp Fast PCR Master Mix and a standard two-step PCR protocol. **B.** Yields from amplicons 11 and 13 were higher with the three-step fast PCR protocol. Each amplicon above was amplified from 10 ng of human genomic DNA.



Touch-and-Go Protocol for Low-Complexity Targets

94ºC, 0 seconds; 64ºC, 0 seconds, 25 cycles
Total time: 10 minutes

Figure 3. Amplification of a 500 bp Lac Z target in 10 minutes. This target was amplified using lambda phage DNA with a starting concentration of 0.25 pg/µL and using a fast touch-and-go protocol from 94°C, 0 seconds to 64°C, 0 seconds for 25 cycles.

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