

High specificity and robustness of AccuPrime™ *Taq* provides the ideal tool for demanding miniaturized, multiplex, and high-throughput PCR

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Introduction

In the post-sequenced genomic era, Polymerase Chain Reaction (PCR) is one of the most utilized techniques for analysis of the information encoded in the human genome. To accurately study particular stretches of genomic DNA or open reading frames in genomic DNA, sequence specificity must be maintained.

AccuPrime™ *Taq* DNA polymerase is a powerful and efficient tool proven to consistently provide the highest levels of sensitivity and specificity in demanding PCR applications. In this paper, we describe the use of AccuPrime™ *Taq* DNA polymerase to amplify DNA sequences accurately in various applications, including PCR miniaturization, multiplex PCR, and high-throughput colony screening.

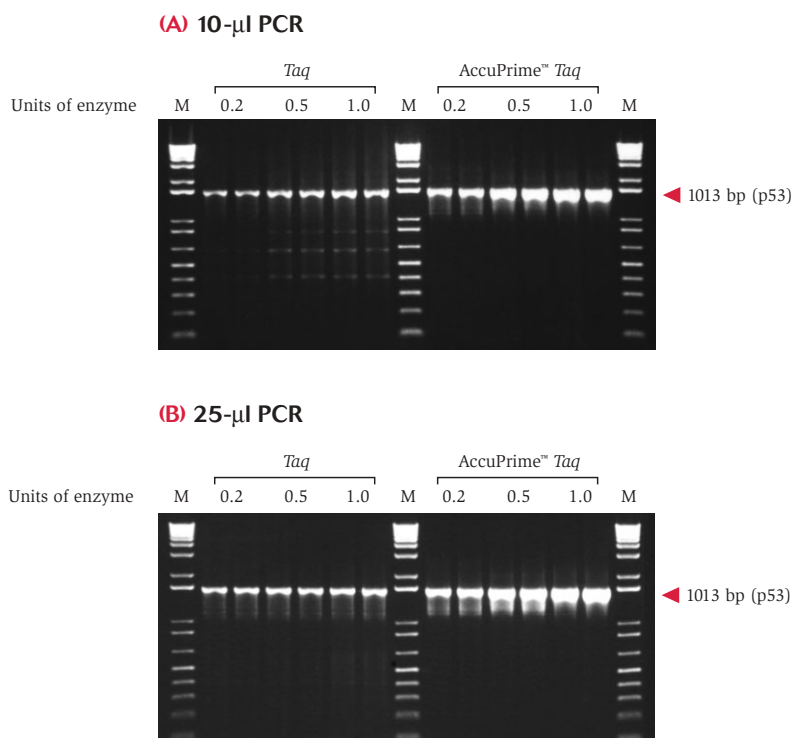
Methods

PCR miniaturization. PCR amplification reactions were performed in 10- μ l or 25- μ l volumes using 0.2, 0.5, and 1 unit of *Taq* DNA polymerase or AccuPrime™ *Taq* DNA polymerase (Invitrogen, Cat. no. 12339-016). Components were proportionally reduced in volume for each reaction, with concentrations of 0.2 μ M primers, 200 mM each dNTP, 1X PCR buffer, and 1.5 mM MgCl₂. Cycling conditions were 94°C, 2 min pre-incubation followed by 35 cycles of 94°C, 15 s; 55-60°C, 30 s; and 68°C, for 1 min/kb. Twenty percent of each amplification reaction was analyzed by electrophoresis with 0.5X TBE 1.2% agarose containing 0.5 μ g/ml ethidium bromide.

Multiplex PCR. PCR amplifications were prepared using 2, 5, or 10 units of hot-start *Taq* or AccuPrime™ *Taq* DNA Polymerase under standard PCR conditions in a 50- μ l reaction. A mix of 20 different primer pairs targeting amplicons ranging in length from 44 to 469 base pairs were prepared in a single reaction

tube and amplified. Cycling conditions were 94°C, 2 min pre-incubation followed by 35 cycles of 94°C, 15 s; 55-60°C, 30 s; and 68°C, for 1 min/kb. Twenty percent of each amplification reaction was analyzed by electrophoresis with a 0.5X TBE-3% agarose containing 0.5 μ g/ml ethidium bromide.

Figure 1 – PCR miniaturization using AccuPrime™ *Taq* DNA polymerase



Unlike *Taq* DNA polymerase alone, AccuPrime™ *Taq* DNA polymerase functions efficiently regardless of the reaction volume and the amount of the enzyme. AccuPrime™ *Taq* enzyme amounts can be lowered proportionally to the reaction volume without losing the robustness or specificity of the reaction. M: 1 Kb Plus DNA Ladder.

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High-throughput PCR. AccuPrime™ *Taq* DNA polymerase was compared to hot-start *Taq* DNA polymerase in a high-throughput colony PCR screening application. pUC19 was selected as the target DNA sequence. PCR was performed in a 96-well microtiter plate for 18 cycles using either 2 units of AccuPrime™ *Taq* DNA polymerase or 2 units of hot-start *Taq* DNA polymerase. PCR cycling parameters were 94°C for 2 min, followed by 18 cycles of 94°C for 15 s; 55°C for 30 s; and 68°C for 3 min. The PCR products were

analyzed by electrophoresis with 0.5X TBE 0.8% agarose containing 0.5 µg/ml ethidium bromide.

Results and Discussion

PCR miniaturization. A typical result from PCR miniaturization with AccuPrime™ *Taq* is shown in Figure 1 (page 11). The target was a 1013 base pair sequence of the p53 gene. As a direct comparison, equal amounts of *Taq* polymerase were used in similar reactions. In a 10-µl reaction, AccuPrime™ *Taq* polymerase produced

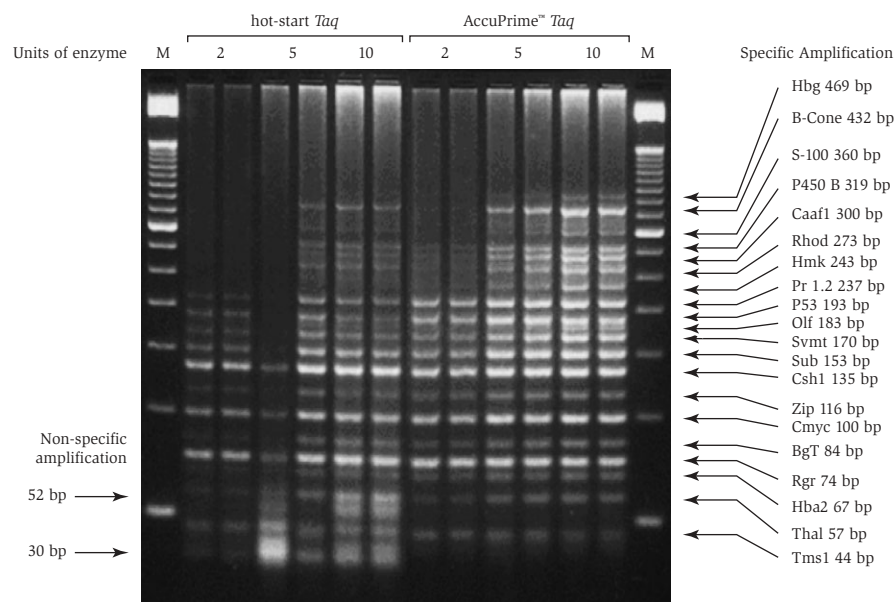
more product than *Taq* polymerase, using as little as 0.2 units, or approximately 2.5 times less enzyme than *Taq* polymerase, (Figure 1A). Even as enzyme units were gradually increased, specificity was maintained with AccuPrime™ *Taq*. However, non-specific priming was observed with increasing units of *Taq* polymerase, (Figure 1A).

The specificity of *Taq* polymerase improved with an increase in reaction volume (Figure 1, panels A and B), regardless of reaction volume. Even with this increase, AccuPrime™ *Taq* maintained its superiority over *Taq* regarding yield and specificity. Unlike *Taq*, the amount of the specific product increased proportionally to the amount of AccuPrime™ *Taq* added, indicating that the enzyme had not yet reached its performance plateau under the conditions tested.

Multiplex PCR. Multiplex PCR is a highly desirable tool for detecting several genes within a single reaction. However, due to the high degree of complexity, extensive optimization is usually required. Using standard PCR conditions, we compared the amplification of 20 different target sequences using either hot-start *Taq* or AccuPrime™ *Taq* at 2, 5, or 10 units of enzyme (Figure 2). AccuPrime™ *Taq* was able to amplify all 20 distinct targets, while hot-start *Taq* failed to amplify some of the targets and also produced non-specific bands.

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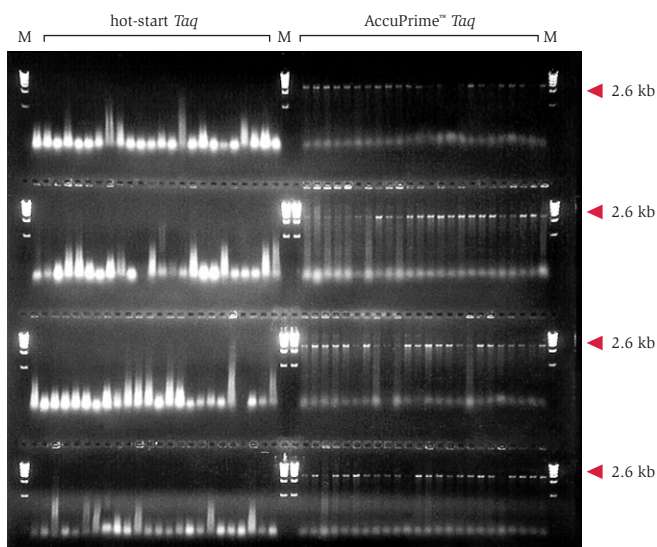
Figure 2 – Multiplex PCR results using hot-start *Taq* and AccuPrime™ *Taq* DNA polymerase



Comparison of multiplex PCR performance between hot-start *Taq* and AccuPrime™ *Taq* for 20 sets of primers with varying amounts of polymerase. Only AccuPrime™ *Taq* amplified all 20 amplicons. M: 50 bp DNA Ladder.

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Figure 3 – Comparison of high-throughput screening between hot-start *Taq* and AccuPrime™ *Taq*



Comparison of high-throughput PCR screening with hot-start *Taq* and AccuPrime™ *Taq*. Colonies from an agar plate were sampled with pipette tips and mixed with PCR amplification mixes. An 18 cycle PCR was used. Only AccuPrime™ *Taq* DNA polymerase successfully amplified the specific target in greater than 90% of the 96 colonies. The high specificity of AccuPrime™ *Taq* DNA polymerase promoted the high success rate of this high-throughput application. M: High DNA Mass™ Ladder.

Acknowledgements

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References

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AccuPrime™ *Taq* DNA Polymerase is subject to Limited Use Label Licenses 4, 14, and 33. Please refer to the Invitrogen web site or catalog for the corresponding Limited Use Label License.

High-throughput PCR. AccuPrime™ *Taq* can also be used in high-throughput colony PCR screening applications (Figure 3). We performed colony PCR screening with either hot-start *Taq* DNA or AccuPrime™ *Taq* polymerase with bacteria cells grown on a solid agar plate. The target was a 2.6 kilobase pair sequence of the pUC19 plasmid. The bacterial cells were touched with pipette tips, mixed directly with reaction mixtures, and amplified in an 18-cycle PCR. The high specificity of AccuPrime™ *Taq* poly-

merase allowed for more than 90% of the 96 reactions to be successfully amplified.

Conclusion

AccuPrime™ *Taq* DNA polymerase consistently outperforms hot-start *Taq* in all of the tested applications. Providing for unparalleled performance in sensitivity, specificity, and yield, AccuPrime™ *Taq* is the most suitable enzyme for delivering accurate and reliable performance in highly demanding applications as miniaturized PCR, multiplex PCR, or high-throughput PCR. ■