AccuPrime™ *Taq*: A next generation DNA Polymerase for PCR

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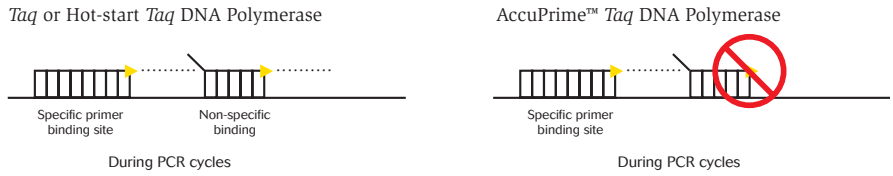
Introduction

PCR is a powerful tool widely used in molecular analysis of DNA and RNA. The drawback to PCR is that in addition to amplifying the target, it can also produce non-specific products. These undesirable by-products, resulting mainly from non-specific annealing or primer-dimer formations, decrease the yield of the target of interest and may cause reduced sensitivity in detection assays. They can also interfere with downstream processes, such as cloning or sequencing. In the past few years, a wide variety of methods have been developed to improve the specificity and sensitivity of PCR, most notably hot-start PCR (1-3). Hot-start PCR improves specificity by preventing non-specific priming during reaction set-up and the initial stages of PCR (3). However, hot-start PCR cannot eliminate non-specific products resulting from non-specific priming during PCR cycling. Currently these are dealt with by performing extensive reaction optimization procedures and/or redesigning primers, which waste time and incur additional expenses to an otherwise routine procedure.

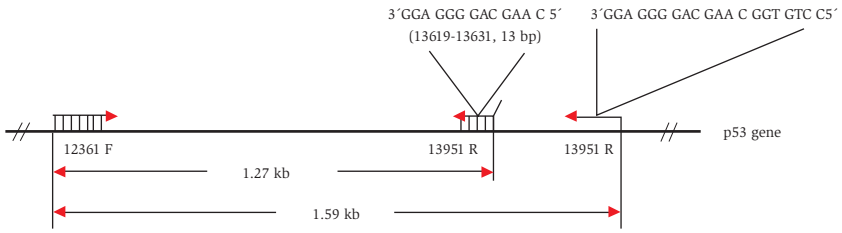
In this manuscript, we describe the introduction of a replication accessory protein (AccuPrime™ protein) to existing hot-start technology to produce a next generation polymerase, AccuPrime™ *Taq* DNA polymerase. The new technology improves specificity, sensitivity, and fidelity over automatic hot-start *Taq* DNA polymerase (4). In this platform, we find the most robust PCR enzyme, suitable for high-throughput screening and multiplex PCR.

Figure 1 – Specificity of AccuPrime™ *Taq* DNA polymerase

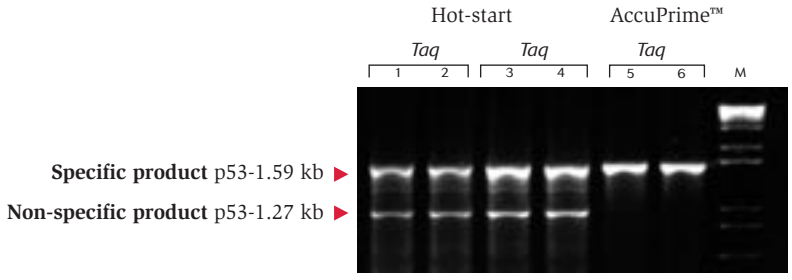
A. AccuPrime™ *Taq* mode of action



B. Schematic of amplified p53 region



C. Specificity of AccuPrime *Taq* vs. other *Taq* polymerases



1A. With AccuPrime™ *Taq*, extension does not occur at non-specific sites

1B. Schematic of defined 13 bp 3' homology p53 reverse primer sequence information and location. The 13 bp homology region located between nucleotides 12361 (p53 forward primer) and 13951 (p53 reverse primer). Detailed homology sequence and location are indicated.

1C. Specific PCR products obtained with AccuPrime™ *Taq* DNA polymerase using a defined 13 bp 3' homology primer. Twenty nanograms of K562 genomic DNA was used as template with p53 gene specific primers (Figure 1B) to amplify a 1.59 kb p53 gene fragment using *Taq*, automatic hot-start *Taq*, and AccuPrime™ *Taq* DNA polymerases. The arrows indicate the specific amplified products (1.59 kb) and non-specific amplified products (1.27 kb). Lanes 1 and 2. *Taq* DNA polymerase with room-temperature assembly. Lanes 3 and 4. Automatic hot-start *Taq* DNA polymerase with room-temperature assembly. Lanes 5 and 6. AccuPrime™ *Taq* DNA polymerase with room-temperature assembly. M: 1 Kb Plus DNA Ladder.

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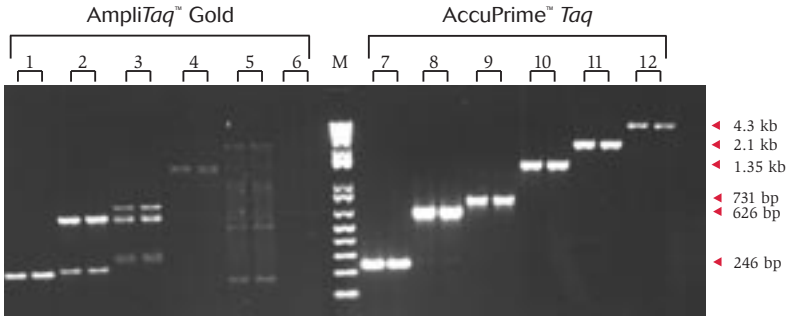
Figure 2 – Improved product specificity and yield with AccuPrime™ *Taq* DNA polymerase



Using 20-100 ng human genomic DNA as template, PCR reactions were set up at room temperature as described in Methods. Reactions were run in duplicate. The specific amplified products indicated by the arrows are as follows: Pr1.3 246 bp (lanes 1 and 7), Rhodopsin 626 bp (lanes 2 and 8), β-globin 731 bp (lanes 3 and 9), Hpfh-6 enhancer 1.35 kb (lanes 4 and 10), p53 2.1 kb (lanes 5 and 11), and p53 4.3 kb (lanes 6 and 12). Lanes 1-6 products were amplified using Ampli*Taq*™ Gold DNA polymerase with Gold buffer (Applied Biosystems, Inc.). Lanes 7-12 products were amplified using AccuPrime™ *Taq* DNA polymerase. M is the 1 Kb Plus DNA ladder. The same amount of enzyme units was used for each reaction. For Ampli*Taq*™ Gold DNA polymerase, the PCR reactions were heated to 95˚C for 10 minutes before PCR cycling.

Methods

PCR. All reagents and enzymes were obtained from Invitrogen. Amplification reactions were performed in 50 µl volumes using 2 units of recombinant *Taq* DNA Polymerase, automatic hot-start *Taq* DNA Polymerase, or AccuPrime™ *Taq* DNA polymerase (Cat. no. 12339-016). The buffer for all reactions was 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, and 200 nM of each primer. For the AccuPrime™ *Taq* reaction, the buffer also contained the thermostable AccuPrime™ protein. Reactions were assembled at room temperature, then incubated for 2 min. at 94˚C, followed by 28-35 cycles at 94˚C for 15 s., 55˚C-60˚C for 30 s., and 68˚C for 1 min/kb. PCR products (10 µl from each reaction) were analyzed on 0.8%-1.5% (w/v) agarose gels containing 0.4 µg/ml ethidium bromide.

rps*L fidelity assay.* pMOL 21 plasmid DNA (4 kb), containing the ampicillin resistance (Ap^r) and reporter (*rps*L) genes, was linearized with *Sca* I. Standard PCR was performed on the linearized product using biotinylated primers. Amplification was completed using 2 units of AccuPrime™ *Taq* DNA polymerase and 1 ng template for 25 cycles. PCR cycling parameters were 94˚C for 2 min., followed by 25 cycles at 94˚C for 15 s., 58˚C for 30 s., and 68˚C for 5 min. PCR products were purified using streptavidin-labeled magnetic beads to ascertain linearity. Purified PCR products were analyzed on an agarose gel and DNA concentration and template doubling time were estimated. The purified DNA was religated using T4 DNA ligase and transformed into MF101 competent cells. To determine the total number of transformed cells, cells were plated on LB plates containing 100 µg/ml ampicillin. To determine the total number of *rps*L mutants, cells were plated on LB plates containing 100 µg/ml ampicillin and 40 µg/ml streptomycin. Mutation frequency was determined by dividing the total number of mutations by the total number of transformed cells. The error rate was determined by dividing the mutation frequency by 130 (the number of potential mutation sites that cause phenotypic changes in the *rps*L gene) and the template doubling time.

Results and Discussion

Elimination of non-specific priming. PCR specificity is dependent on multiple factors, including primer design, magnesium concentration, and annealing temperature. Non-specific products are often prominent when the target gene is buried within an excess of other DNA, such as in genomic DNA templates. Extensive PCR condition optimization may be required to obtain specific products. Here we show that the unique combination of anti-*Taq* antibodies and the AccuPrime™ accessory protein in AccuPrime™ *Taq* DNA polymerase is able to amplify a specific target with minimal optimization (Figure 1A, page 10).

To demonstrate the enhanced specificity, a specific 13 bp 3' homology primer on the p53 gene was designed (Figure 1B, page 10). As a control, the same amount of recombinant *Taq* or hot-start *Taq* was used in side-by-side reactions. A clean, specific product of the expected size (1.59 kb) was generated using AccuPrime™ *Taq* (Figure 1C, lanes 5 and 6, page 10). Amplifications

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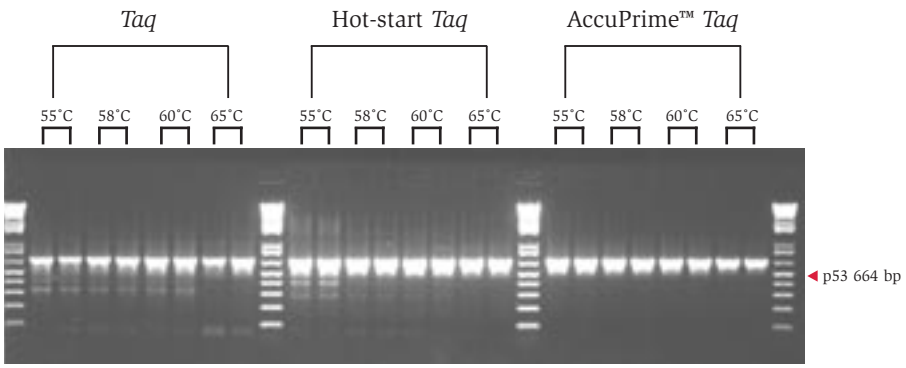
Figure 3 – Improved specificity and wide range of annealing temperature with AccuPrime™ *Taq* DNA polymerase



The arrow indicates the specific amplification product. *Taq*, automatic hot-start *Taq*, and AccuPrime™ *Taq* DNA polymerase, as well as the different annealing temperatures used, are indicated.

performed using *Taq* or automatic hot-start *Taq* produced a non-specific 1.27 kb product in addition to the 1.59 kb target (Figure 1C, lanes 1-4, page 10). The non-specific DNA fragment (1.27 kb) was gel purified and the sequence confirmed by double restriction digest assays (data not shown). These assays indicated that the 1.27 kb product was generated from a 13 bp homology sequence at the 3' end of the reverse primer. This result suggests that the AccuPrime™ accessory protein assists in specific primer-template hybridization during PCR cycles.

Statistical analysis of specificity enhancement. To demonstrate that AccuPrime™ *Taq* retains this enhanced specificity in a wide range of reactions, we compared the activities of AccuPrime™ *Taq*, recombinant *Taq*, and hot-start *Taq* in over 300 PCR amplifications. Reactions were performed using different randomly designed genomic primer sets and plasmid and cDNA, as well as linear templates. Overall, AccuPrime™ *Taq* produced higher specificity and yield than *Taq*. In addition, about 40% of the AccuPrime™ *Taq* reactions showed significant improvement and 35% showed minor improvement in specificity and/or yield compared to the hot-start *Taq*-mediated reactions (data not shown). AccuPrime™ *Taq* performance in PCR was compared to another commercially available *Taq* preparation, Ampli*Taq*™ Gold DNA polymerase with Gold buffer (Applied Biosystems, Inc.). The standard protocols for each polymerase, as indicated on product profile sheets, were followed. The same amount of enzyme, 1.5 mM Mg^{2+}, and the same annealing temperature were used in PCR. For Ampli*Taq*™ Gold DNA polymerase, the PCR reactions were heated to 95°C for 10 min. before the PCR cycling. The data in Figure 2 (page 11) clearly shows higher yields of more specific product were obtained using AccuPrime™ *Taq* than Ampli*Taq*™ Gold DNA polymerase. The non-specific products produced in the Ampli*Taq*™ reaction can be reduced or eliminated by increasing the annealing temperature and/or changing Mg^{2+} concentration. But with AccuPrime™ *Taq*, specific products are obtained without modifying or optimizing PCR conditions. We also tested the functionality of AccuPrime™ *Taq* over a wide range of annealing temperatures (Figure 3).

rps*L fidelity assay.* The fidelity of AccuPrime™ *Taq* DNA polymerase was determined using the *rps*L fidelity assay. The *rps*L fidelity assay is based on the forward mutation rate of the *rps*L gene by the polymerase of interest, developed by Sekiguchi and his colleagues (5), and successfully applied to thermostable polymerases (6). Since the assay utilizes positive selection for mutation, it can detect infrequent mutations among a very large population of wild-type copies of the gene (7). The spontaneous mutation rate, a major cause of background, of the gene was measured to be 50 times lower than the mutation rate of the highest fidelity enzyme, a replicative enzyme of *E. coli* (7). *Taq* DNA polymerase (Invitrogen) was used as a control for the *rps*L assay. The error rate of

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AccuPrime™ *Taq* DNA polymerase was determined to be 1.72 x 10^{-5}. Over the course of three independent fidelity runs, AccuPrime™ *Taq* DNA polymerase showed nearly a two-fold improvement in fidelity over *Taq* DNA polymerase (Table 1). Mutant colonies were PCR amplified with *rps*L primers to verify results.

Conclusion

AccuPrime™ *Taq* DNA polymerase has automatic hot-start capabilities, as well as exceptional ability to prevent non-specific primer annealing during each PCR cycle, dramatically improving PCR specificity. Yet, AccuPrime™ *Taq* DNA polymerase is a very robust and user-friendly DNA polymerase, requiring almost no optimization compared to all other PCR polymerases tested for target sizes up to 4.4 kb. The performance of AccuPrime™ *Taq* DNA is equal to, or better than, other hot-start *Taq* DNA polymerases in every aspect examined. Based on the above features, AccuPrime™ *Taq* DNA polymerase is an ideal enzyme for demanding PCR reactions such as high-throughput applications and multiplex PCR.n

References

1. Li, H., Cui, X., and Arnheim, N. (1990) *Proc. Natl. Acad. Sci. USA* **87**: 4580.
2. Frohman, M.A., Dush, M.K., and Martin, G.R. (1998) *Proc. Natl. Acad. Sci. USA* **85**: 8998.
3. Westfall, B., Sitaraman K., Solus J., Hughes J., and Rashtchian A. (1997) *Focus* **19**: 46.
4. Huang, L., Hu, E., Park, K., Lackovich, J., Lee, J., and Rashtchian, A. *An ultra-specific PCR DNA polymerase*. Manuscript in preparation.
5. Mo, J.Y., Maki, H., and Sekiguchi, M. (1991) *J. Mol. Biol.* **222**: 925.
6. Lackovich, J.K., Lee, J.E., Chang, P., and Rashtchian, A. (2001) *Focus*® **23**: 6.
7. Fujii, S., Akiyama, M., Aoki, K., Sugaya, Y., Higuchi, K., Hiraoka, M., Miki, Y., Saitoh, N., Yoshiyama, K., Ihara, K., Seki, M., Ohtsubo, E., and Maki, H. (1999) *J. Mol. Biol.* **289**: 835.

Table 1 – Fidelity improvement using AccuPrime™ *Taq* DNA Polymerase

Enzyme	Expt.	(Colonies on Amp plate)/ (Total Colonies)	(Colonies on Amp + Strep Plate)/ (Volume)	TD	mf (%)	Er (E-6)	Relative Fidelity
Taq DNA polymerase	1	999 (3,996)	235 (1.2 ml)	12.3	5.88	36.8	1X
	2	906 (9,060)	420 (600 µl)	12.3	4.64	29.0	
	3	731 (14,260)	616 (600 µl)	11.6	4.32	28.6	
	Avg.			12.1 ± 0.3	4.95 ± 0.62	31.5 ± 3.57	
AccuPrime™ *Taq* DNA polymerase	1	732 (7,320)	234 (1.2 ml)	11.6	3.20	21.2	1.8X
	2	942 (28,260)	633 (600 µl)	11.6	2.24	14.9	
	3	798 (15,960)	330 (600 µl)	10.2	2.07	15.6	
	Avg.			11.1 ± 0.6	2.5 ± 0.47	17.2 ± 2.63	

TD: template doubling time, mf: mutation frequency, Er: error rate. See "Methods" for an explanation of calculations.