PrimeSTAR® GXL DNA Polymerase: One Enzyme for Every Reaction

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Data generated by: Takara Bio Inc.

PrimeSTAR GXL DNA Polymerase is a revolutionary high-fidelity PCR that includes a novel elongation factor that dramatically enhances PCR performance. Because of this, PrimeSTAR GXL polymerase can be used to obtain excellent amplification from every PCR reaction.

This application note illustrates the exceptional performance and versatility of PrimeSTAR GXL polymerase for both standard reactions (section I) and for amplification of target sequences that often presents a challenge for standard PCR enzymes (sections II and III).

I. Sensitivity and Specificity Compared to other Enzymes

The amplification efficiency of PrimeSTAR GXL DNA Polymerase was compared to the efficiencies of other commercially available high fidelity PCR enzymes and standard *Taq* DNA polymerase.

Methods

A 2 kb portion of the *DCLRE1A* gene was amplified from human genomic DNA according to the protocols specified by each manufacturer. PCR products were resolved by agarose gel electrophoresis.

Results

Amplification with PrimeSTAR GXL DNA Polymerase resulted in the highest sensitivity and amplification efficiency among the PCR enzymes tested (Figure 1). In addition, PrimeSTAR GXL polymerase was able to amplify the target across a wide range of template quantities.

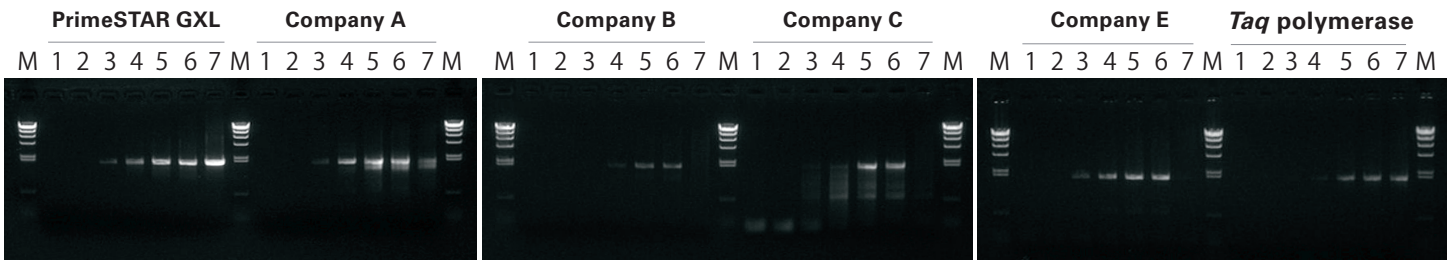

Figure 1. Amplification of *DCLRE1A* using either PrimeSTAR GXL polymerase, enzymes from other manufacturers (Companies A, B, C, D, or E), or standard *Taq* DNA polymerase. Different amounts of DNA (purified human gDNA) were included in the reaction (50 µl) as the template: lane 1, no template (negative control); lane 2, 100 pg; lane 3, 1 ng; lane 4, 10 ng; lane 5, 100 ng; lane 6, 200 ng; lane 7, 500 ng. Lane M contains a λ-*Hin*d III digest molecular size marker.

II. Long Range PCR

PrimeSTAR GXL DNA Polymerase was used for long range PCR of targets from human genomic DNA, lambda DNA, and cDNA templates.

Methods

Portions of the *TP53*, *DCLRE1A*, and beta-globin genes were amplified from 100 ng of purified human genomic DNA. Various sized fragments were amplified from 1 ng of lambda DNA. Regions of the Dystrophin, CCND2, and *TFR* genes were amplified from cDNA synthesized from 250 ng of total RNA (RT-PCR). All reactions were performed in a final volume of 50 µl according to the recommended protocol.

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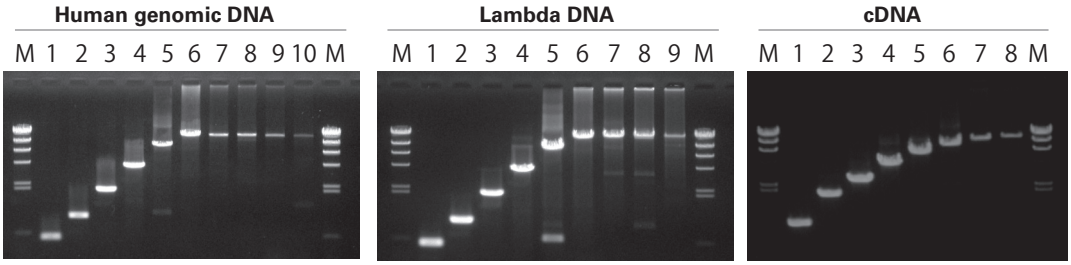
Results

With PrimeSTAR GXL polymerase, products up to 30 kb could be amplified from human genomic DNA, and products up to 40 kb were efficiently amplified from lambda DNA (Figure 2, left and middle panels). In addition, fragments up to 13.5 kb were obtained from cDNA (Figure 2, right panel).



1. *TP53*	0.5 kb	1.	0.5 kb	1. Dystrophin	1 kb
2. *DCLRE1A*	1 kb	2.	1 kb	2. Dystrophin	2 kb
3. *DCLRE1A*	2 kb	3.	2 kb	3. *CCND2*	2.8 kb
4. *DCLRE1A*	4 kb	4.	4 kb	4. *TFR*	4 kb
5. Beta-globin	8.5 kb	5.	8 kb	5. Dystrophin	6 kb
6. Beta-globin	15 kb	6.	15 kb	6. Dystrophin	8 kb
7. Beta-globin	20 kb	7.	20 kb	7. Dystrophin	12 kb
8. Beta-globin	24 kb	8.	30 kb	8. Dystrophin	13.5 kb
9. Beta-globin	27 kb	9.	40 kb		
10. Beta-globin	30 kb				

Figure 2. Amplification of long targets using PrimeSTAR GXL polymerase. Amplicons of varying lengths were amplified by PCR from either human gDNA (left), lambda DNA (middle), or synthesized cDNA (right). The expected size of each product is listed beneath the gel image. In all cases, lane M contains a λ-*Hin*d III digest molecular size marker.

III. Amplification of GC-Rich Targets

The performance of PrimeSTAR GXL polymerase was compared to several other commercially available high-fidelity polymerases and polymerases marketed as being optimized for use with GC-rich templates.

Methods

Fragments with high GC-content were amplified from either 100 ng of human genomic DNA (lanes 1 and 2) or 10 ng of *T. thermophilus* HB8 genomic DNA (lanes 3 and 4). All reactions were performed according to the protocol recommended by each manufacturer.

Results

Under the conditions used, only PrimeSTAR GXL polymerase yielded highly specific amplification of all of the GC-rich targets tested (Figure 3).

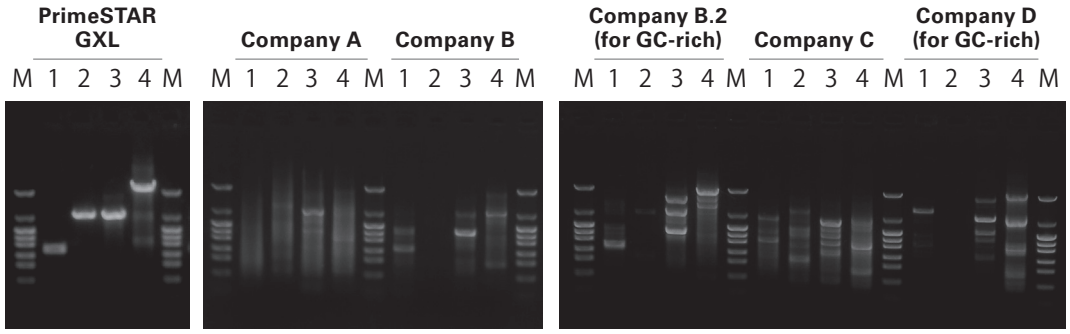


Figure 3. Amplification of GC-rich targets using PrimeSTAR GXL polymerase or enzymes from other manufacturers (Companies A, B, C, or D). The Company B.2 and Company D enzymes marketed as optimized for GC-rich templates. Amplicons of varying GC-content were amplified from either human gDNA (lane 1, APOE, 74% GC; lane 2, TBFß1, 69% GC) or *T. thermophilus* HB8 gDNA (lane 3, 2029 bp, 74% GC; lane 4 4988 bp, 74% GC). Lane M contains a pHY molecular size marker.

Conclusions

PrimeSTAR GXL polymerase outperforms other enzymes and provides highly specific amplification in a variety of challenging PCR scenarios (i.e., long range PCR and amplification of GC-rich sequences). With PrimeSTAR GXL polymerase, excellent PCR results can be obtained without the need for special buffers or modifications to the reaction conditions.