Amplification of Long Products from Human Genomic DNA

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PrimeSTAR® GXL DNA Polymerase is a high fidelity PCR enzyme that is optimized for long range PCR. With this enzyme, it is possible to amplify of products up to 30 kb from human genomic DNA templates. In this experiment, large fragments (~23 kb) of the human RHD and RHCE genes were amplified from human genomic DNA using PrimeSTAR GXL DNA Polymerase (Cat. # R050A) or a high fidelity, long range PCR enzyme from Company R.

Methods

Human genomic DNA was purified from whole blood samples collected from RHD+ donors using the DNeasy Blood & Tissue Kit (Qiagen). One hundred nanograms of gDNA were used as a template for PCR. PCR reactions were assembled and run according to the manufacturer's recommendations. The composition of PCR reaction mixtures (50 µl) are summarized in Tables 1 and 2 for PrimeSTAR GXL polymerase and Company R polymerase, respectively. PCR was performed on a BioRad C1000 thermal cycler according to the conditions in Table 3. PCR products were resolved by gel electrophoresis.

Table 1. Reaction Composition: PrimeSTAR GXL DNA Polymerase

Component	Amount
5X PrimeSTAR GXL buffer	10 µl
dNTP Mixture (2.5 mM each)	4 µl
primers, each	1 µl (10 pmol)
Template	2 µl (100 ng)
PrimeSTAR GXL DNA Polymerase	1 µl (1.25 U)
Sterile dH_2O	31 µl

Table 2. Reaction Composition: Company R DNA Polymerase

Component	Amount
5X buffer	10 µl
dNTP Mixture (10 mM each)	2.5 µl
primers, each	1 µl (10 pmol)
Template	2 µl (100 ng)
DMSO	1 µl
Enzyme	0.7 µl (3.5 U)
Sterile dH_2O	30 µl

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Table 3. PCR Cycling Conditions for PrimeSTAR GXL and Company R Polymerase Reactions.

PrimeSTAR GXL enzyme		Company R enzyme	
98°C 10 sec. 68°C 23 min.	30 cycles	92°C 2 min.	
		92°C 10 sec. 60°C 15 sec. 68°C 23 min.	10 cycles
		92°C 10 sec. 60°C 15 sec. 68°C 23 min.+20 sec./cycle	25 cycles
		68°C 7 min.	

Results

For reactions amplified with PrimeSTAR GXL polymerase, ~23 kb products were obtained for both the RHD and RHCE targets (Figure 1, lanes 1 and 4). However, neither product was detected in reactions amplified using Company R enzyme (Figure 1, lanes 3 and 5).

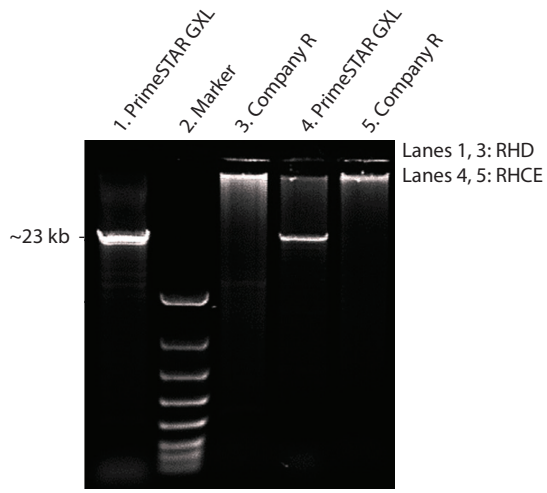

Figure 1. PCR products obtained from amplification of the RHD and RHCE regions from human gDNA using either PrimeSTAR GXL DNA Polymerase or a long range enzyme from Company R. Lanes 1 and 4 were amplified with PrimeSTAR GXL enzyme; lanes 3 and 5 were amplified with the Company R enzyme. Lane 2 contains a DNA ladder (100 bp – 4 kb).

Conclusions

PrimeSTAR GXL DNA Polymerase allowed efficient amplification of large regions of the RHD and RHCE loci (~23 kb) without requiring optimization or special cycling conditions. In addition, the PrimeSTAR GXL polymerase reactions were complete in fewer cycles and in less total time than the Company R polymerase reactions.