Philisa® Thermal Cycler Application Note

DNA Sequence Verification of PCR Product Fidelity Produced by a Rapid Method Using the Streck Philisa Thermal Cycler

Joel R. TerMaat, Ph.D., M. Rohan Fernando, Ph.D., and Sheila E. Norton, M.S.

Overview

The purpose of this study was to demonstrate that the Philisa Thermal Cycler maintains high sequence fidelity when PCR products are produced by a rapid method. As compared to traditional protocols, rapid thermal cycling minimizes thermal damage to DNA by limiting exposure to high temperatures as well as saving time.

A 787 bp fragment of the GAPDH gene of human genomic DNA (GenBank accession no. NG_007073.2) was amplified in triplicate in the Philisa Thermal Cycler in less than 15 minutes. Following purification, the amplicons were evaluated by DNA sequencing. The resulting data showed exact sequence matches of the amplified products to the reference GenBank sequence.

Materials and Methods

PCR Composition: Each 25 µl reaction mixture consisted of 3.5 mM $MgSO_4$, 0.2 mM dNTPs, 0.4 µM each forward and reverse primers (F: 5'-CTTCATACCCTCACGTATTCCC-3'; R: 5'-CCAGTCTTGGATGAGAAAGG-3'), 1x reaction buffer, 0.5U of KOD Hot-Start-Polymerase (Novagen), and 50 ng human genomic DNA (Promega). Triplicate samples and a no template control were processed in the Philisa Thermal Cycler.

Protocol: 30-second hot-start at 95°C; followed by 30 cycles [95°C for 6 sec, 56°C for 6 sec, and 72°C for 8 sec]; and a final extension at 72°C for 16 sec. Runtime: 14.5 min.

Gel Electrophoresis Detection: 8 µl of each PCR product was electrophoresed on a 1.5% agarose gel and stained with ethidium bromide along with a 100 bp reference ladder.

DNA Sequencing: The remaining PCR reactions (17 µl) were purified using a GENECLEAN Turbo Kit (MP BioMedicals). The products were sequenced at the DNA Sequencing Facility at the University of Nebraska Medical Center using an ABI 3730 48-capillary electrophoresis analyzer. Bi-directional sequencing was performed with the forward and reverse primers used for PCR amplification. Using ChromasPro V1.5 software (Technelysium Pty, Ltd), the inner 650 bp of the aligned sequencing data was compared for fidelity to the GenBank reference GAPDH sequence. Any ambiguities resulting from the bi-directional data were resolved by visual inspection of the electropherograms.

Results and Discussion

Figure 1 shows the gel electrophoresis results for the triplicate PCR samples and no template control. The total thermal cycler run time required for the 787 bp amplification was 14.5 minutes.

Figure 2 **(see reverse side)** shows an excerpt of the ChromasPro sequencing data analysis. For all three PCR products generated in the Philisa Thermal Cycler, analysis of the bi-directional sequencing data showed the targeted nucleotide sequences exactly matched the human GAPDH reference sequence.

The results clearly demonstrate that rapid cycling in the Philisa Thermal Cycler maintains high fidelity of amplified products and is suitable for DNA sequence verification or similar downstream applications.

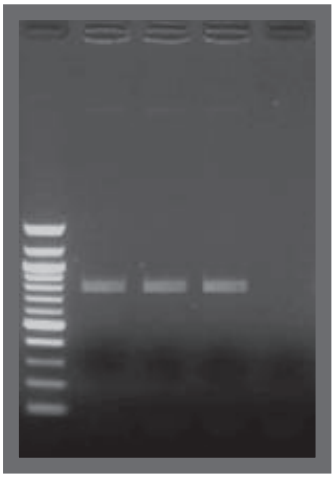

Figure 1 - 787 bp PCR products amplified in 14.5 minutes.

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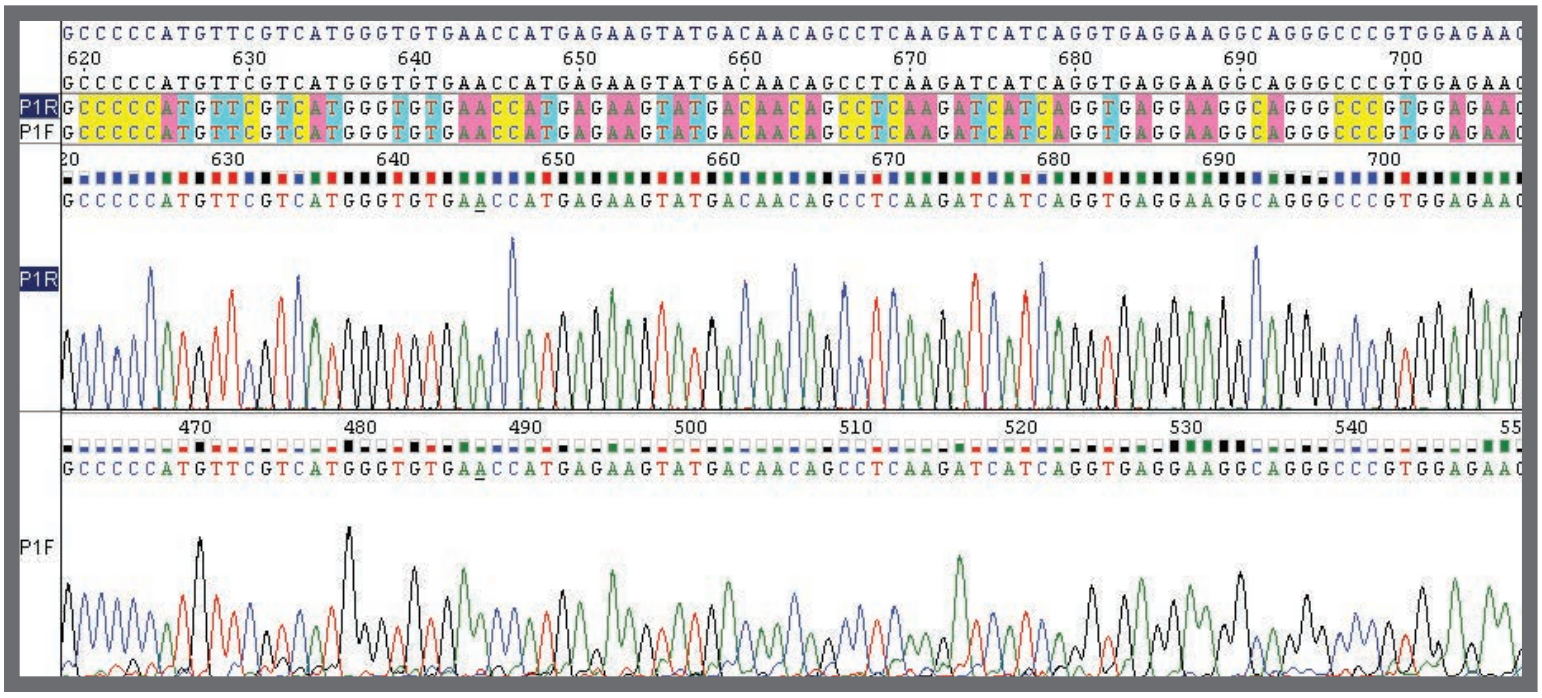

Figure 2 - Excerpt of the ChromasPro sequencing data analysis. The reference sequence is located at the top.