Technical Note

Ultra-Fast PCR using Philisa*FAST*™ DNA Polymerase

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Overview

The purpose of this study was to determine the fastest PCR protocol possible for amplification of targets 500 bp or less using Streck's Philisa*FAST*™ DNA Polymerase and the Philisa® Thermal Cycler. These data demonstrate that PCR protocols can be shortened to less than eight minutes, resulting in rapid amplification of a wide range of targets using ultra-fast PCR.

Materials and Methods

Control 1 from the Philisa® *amp*C ID kit (Catalog No.: 250026), Philisa*FAST* DNA polymerase (Catalog No.: 250024) and associated reagents were obtained from Streck, Inc. (Omaha, NE USA). Gene targets and template sizes are given below:

Table 1

Target	PCR Product
FOX	247 bp
ACC	346 bp
MOX	520 bp

For each 25 µl reaction, reagent volumes were added per manufacturer's instructions for the Philisa *amp*C ID kit except the indicated changes to the PCR cycling protocols. Make-up water and no template control (NTC) used molecular biology grade water (Sigma, Catalog No.: W4502, St. Louis, MO USA).

PCR was done on the Philisa Thermal Cycler using four different protocols as indicated below (Table 2). PCR amplicons were stained with ethidium bromide and resolved on a 2.5% agarose gel. A BioRad Molecular Imager® VersaDoc™ was used for PCR band detection and imaging.

Results and Discussion

Amplification using Philisa*FAST* DNA Polymerase was successful for all targets amplified under the appropriate PCR protocol (Figure 1). Protocol 1 was similar to the 15-minute protocol described in the Philisa *amp*C ID Kit IFU and was used as a control in this study. As expected, all gene targets were successfully amplified. In general, for each shortened protocol tested, reduction of PCR cycling times resulted in less PCR product for each target. However, amplicons were still visually distinct on the gel and readily identified, with one exception: MOX amplification under protocol 3 (Figure1). To address this, the denaturation temperature was elevated to 98 °C in protocol 4; we assume this addresses the reduction in cycling time by accelerating double stranded DNA denaturation allowing PCR to proceed. Data generated using protocol 4 demonstrate successful amplification of all targets, including MOX. These data demonstrate the processivity limits of Philisa*FAST* when paired with the Philisa Thermal Cycler. When protocol 4 was used, amplicons of 500 base pairs or less were detected in less than eight minutes. No modification to the reaction mixture was required; all reactions used the same amount of Philisa*FAST* DNA Polymerase.

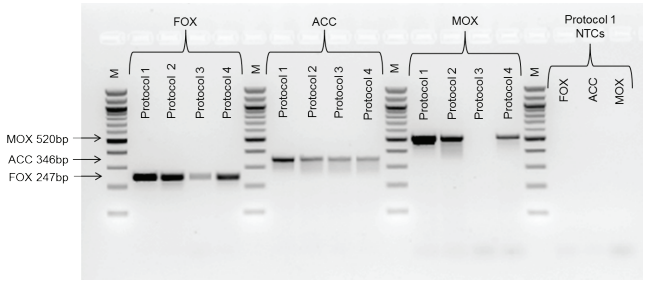

Figure 1: Decreasing PCR protocol cycling time for ultra-fast PCR using PhilisaFAST DNA Polymerase.

Table 2

Protocol	1	2	3	4
Hot-Start	96 °C for 30s	96 °C for 30s	96 °C for 30s	96 °C for 30s
30 Cycles of	96 °C for 5s 64 °C for 10s 72 °C for 7s	96 °C for 5s 64 °C for 10s	96 °C for 3s 64 °C for 5s	98 °C for 3s 64 °C for 5s
Final Extension	72 °C for 10s			
Run time	**14:50.0 mins**	**10:53.7 mins**	**7:27.6 mins**	**7:39.8 mins**

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09-2014
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