Philisa® Thermal Cycler Application Note

Rapid Screening of Transformants Using the Streck Philisa Thermal Cycler

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Overview

Cloning is a basic molecular biology procedure that has many applications in the biomedical and industrial research fields. However, cloning protocols can be challenging and screening transformants by PCR amplification on conventional heat block thermal cyclers can take over 4 hours. A faster alternative is to use the rapid cycling capabilities of the Streck Philisa® Thermal Cycler to screen transformants for the cloned gene of interest. The purpose of this study was to use the Philisa Thermal Cycler in the evaluation of potential clones containing the β-lactamase gene, *bla*$_{CTX-M-15}$, ligated into the cloning vector, pMP220.

Materials and Methods

Template DNA from suspected clones and a clinical urinary tract isolate of *Escherichia coli* (FHM6) serving as the *bla*$_{CTX-M-15}$ positive control were prepared by resuspending a bacterial pellet obtained from 1 ml of overnight culture in 500 µl of sterile nanopure water. The resuspended pellet was heated to 100°C for 10 minutes. Two microliters of supernatant was used in the PCR reactions. A 499 bp amplicon was generated within the *bla*$_{CTX-M-15}$ structural gene using previously published primers: (F) 5'- GACGATGTCACTGGCTGAGCTTAGC-3' and (R) 5'-AGCCGCCGACGCTAATACA-3' (1). Each 50 µl PCR reaction consisted of 1X final concentration of *Taq* Buffer + KCl, 5.0 mM $MgCl_2$, 200 µM dNTPs, 2 pmols of each forward and reverse primer, and 0.25 µl of *Taq* polymerase (5U/µl) (Fermentas, ThermoFisher).

The Philisa Thermal Cycler was programmed with the following parameters: initial denaturation at 95°C for 15 seconds; followed by 30 cycles of denaturation at 95°C for 5 seconds, annealing at 55°C for 5 seconds, and extension at 72°C for 15 seconds. Amplified products were removed from the Philisa® PCR Tubes by centrifugation into 5 µl of loading dye in a microfuge tube. Ten microliters of PCR product/loading dye was analyzed by gel electrophoresis on a 1% agarose gel. The gel was stained with ethidium bromide, destained in distilled water, and photographed on a Kodak Imaging System.

Results and Discussion

Figure 1 shows the results after screening 6 potential clones for the CTX-M-15 β-lactamase gene of interest. Amplified product was observed in 3 out of the 6 reactions indicating successful vector insertion of the correct gene in these three clones (lanes 3-5). Lane 9 indicates the positive control and lanes 2 and 10 represent the negative controls (no DNA template). Amplification using the Philisa Thermal Cycler was completed in 17 minutes. Overall detection time including agarose gel electrophoresis was 1.5 hours in comparison to a total of 4 hours when using conventional or real-time thermal cyclers. Therefore, the Philisa Thermal Cycler significantly reduced the time required to screen transformants for the correct insert.

The rapid cycling conditions of the Philisa Thermal Cycler allows multiple transformants to be screened quickly, thus decreasing the time it takes to identify a correct clone.

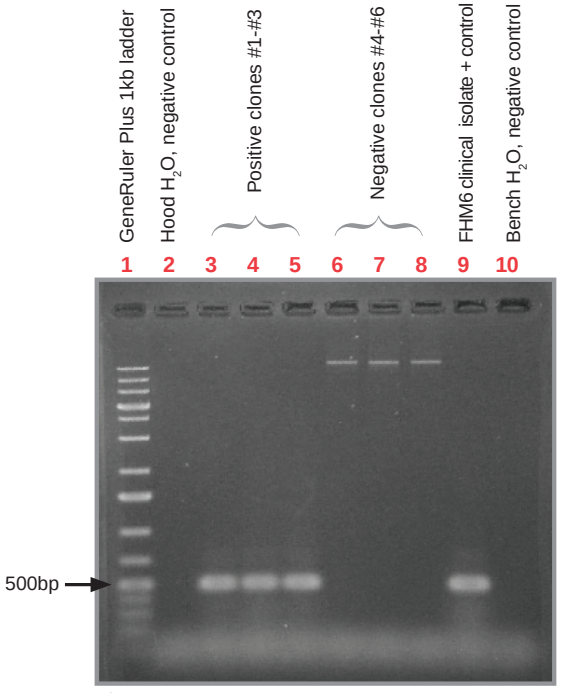

Figure 1

References

1. **Pitout, J. D. D., A. Hossain, and N. D. Hanson.** 2004. Phenotypic and molecular detection of CTX-M β-lactamases produced by *Escherichia coli* and *Klebsiella* spp. J. Clin Microbiol. **42**: 5715-5721.

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