A FAST-CYCLING PROTOCOL FOR RAPID GENERATION OF AmpFlSTR IDENTIFILER PROFILES FOR HUMAN IDENTIFICATION

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Traditional PCR methods for forensic STR genotyping require approximately 2.5 to 4 hours to complete, contributing a significant portion of the time required to forensically process DNA samples. A fast-cycling protocol has recently been developed and validated that enabled amplification of AmpFℓISTR® Profiler Plus® loci in less than 26 min. The purpose of this study was to adapt this fast-cycling protocol for the amplification of the 16 loci targeted by the AmpFℓISTR® Identifiler® primer set. This could allow for increased sample throughput and decreased processing times.

Fast-cycling conditions were achieved by: 1) adopting a two-step approach and modifying cycling parameters, 2) trading the traditional *Taq polymerase* for SpeedSTAR™ DNA polymerase which is designed for fast PCR, and 3) upgrading to a thermocycler with greater efficiency. Total time required for the optimized protocol is 26 minutes.

Profiles produced from DNA extracted from 133 forensically relevant samples were complete and interpretable. These profiles were examined for peak height ratios, stutter ratios and other artifacts. Heterozygote peak height ratios were not affected by fast-cycling and the profiles produced with the fast-cycling protocol exhibited median n-4 stutter percentages ranging from 2.3% ± 0.9% to 9.9% ± 2.7%. Full profiles were produced for single-source DNA template amounts between 2.0 and 0.125ng. Primer specificity and mixture studies were also performed.

The fast-cycling protocol presented offers a feasible alternative to current amplification methods and could reduce overall time in STR profile production or could be incorporated into a fast STR genotyping procedure for time-sensitive situations.