A81 Protocols for Rapid Amplification of STR Typing Kits: The Use of "Non-Standard" Thermal Cyclers

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After attending this presentation, attendees will understand the principles of rapid PCR amplification of STR loci, characteristics of rapid PCR thermal cyclers, and applications of rapid DNA typing.

This presentation will impact the forensic science community by detailing protocols used for rapid PCR amplification and how these methods can be implemented into forensic laboratories and commercial integrated forensic DNA typing devices. The potential of reducing the required PCR amplification time may also benefit laboratories typing single-source reference samples.

There is a growing interest in developing methods capable of processing a single-source reference sample (e.g., a buccal swab) to an STR profile in under two hours. The ability to develop a protocol for the rapid typing of forensic STR markers is of specific interest to the forensic DNA and biometric communities. This would allow for a faster sample-in-answer-out turnaround time as well as the potential for higher throughput capabilities. One critical component of a rapid workflow involves the reduction in time required for multiplex PCR amplification of the core STR loci.

Initial tests of various commercial thermal cyclers with two- and three-step PCR cycling protocols were carried out and compared for genotype accuracy and DNA template sensitivity with a set of previously extracted single-source DNA templates. Work with various "non-standard" thermal cyclers in combination with faster processing DNA polymerases has resulted in decreasing the PCR amplification time to less than 20 minutes for a 16 locus commercial DNA typing kit.

Approximately one nanogram of DNA template was amplified in a total volume of 10 microliters. The PCR primers used for the amplification came from a commonly used commercial STR typing kit, and were used without any further modifications. Both the two- and three-step PCR protocols employed a hot start at 95 degrees for one minute for the activation of the DNA polymerase. The cycling parameters for the 2-step protocol consisted of 28 cycles of 95°C for five seconds followed by 61°C for 15 seconds. The cycling parameters for the 3-step protocol consisted of 28 cycles of 95°C for 5 seconds, 58°C for 10 seconds, followed by 72°C for 10 seconds. Both protocols employed a 72°C incubation step for one minute post cycling to promote complete PCR amplicon andenylation. The required cycling time for the two- and three-step PCR thermal cycling protocols ranged from 15-36 minutes depending on the specific thermal cycling being tested.

The rapidly generated PCR products were then diluted into a formamide solution and prepared for separation and detection by capillary electrophoresis. At least 15 unique samples were typed for the evaluation of each rapid PCR protocol on each of the thermal cyclers. The analysis of capillary electrophoresis data of the PCR products indicated good peak balance for heterozygous loci (median values were greater than 0.8), strong signal intensity (on average over 1,000 relative fluorescence units on a commonly used peltier block thermal cycler) and minor adenylation and PCR artifacts. Stutter artifacts were not significantly different when comparing the two- and three-step thermal cycling protocols. Genotyping results were concordant with PCR amplification conditions utilizing standard thermal cycling procedures (which require at least three hours).

The PCR conditions and cycling parameters developed were robust enough to routinely amplify 250pg of template DNA. These conditions can potentially be applied in a laboratory setting for faster generation of STR profiles while maintaining the robustness and reliability required by the forensic typing community.

PCR, Rapid DNA, Forensic DNA Typing

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