A85 A Fast STR Genotyping Process for Time-Sensitive Situations

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After attending this presentation, attendees will learn about different approaches aimed at reducing the time to generate STR profiling results, including a short lysis step, extraction on the Promega Maxwell 16 System and rapid PCR protocols, and about their integration into a single accelerated analytical process.

This presentation will impact the forensic science community by providing options and feasible alternatives for laboratories interested in gaining analytical efficiencies or to expedite sample processing, offering strategies compatible with standard forensic laboratory equipment.

Significant efforts are being devoted to the development of methods enabling rapid generation of short tandem repeat (STR) profiles in order to reduce turnaround times for the delivery of human identification results from biological evidence. Moreover, in some circumstances, the need for rapid human identification might be critical for police investigations. Different approaches were investigated to achieve flexibility for processing biological evidence. Combining these accelerated protocols into a single analytical process enables generation of STR profiles for human identification within five hours.

Reduced incubation times at 56°C for the lysis of biological samples were evaluated (30 min to 4 hours) and the results were compared to the usual overnight incubation. A quick DNA extraction method using the Promega Maxwell 16 System (27 min/16 samples) was also explored and the results were compared to the RCMP automated DNA IQ extraction protocol on TECAN robotic workstations. A variety of single-source and two-person mixture samples were subjected to different lysis conditions and extraction methods. With the exception of old blood on FTA cards, a 30-min incubation was sufficient to obtain similar or higher DNA yields compared to the longer incubation times. DNA yields were enhanced for some challenging fabrics, likely due to a reduced quantity of dye/chemicals leaching out of the fabric and interfering with the extraction by DNA IQ. While comparable STR profile quality was usually obtained, improvement in inter-loci balance was noted for the 30-min lysis step for saliva and buccal samples. Similarly, DNA yields and profile quality were shown to be equivalent using the quick DNA extraction method on the Promega Maxwell 16 when compared to the current RCMP DNA IQ method.

By modifying the cycling conditions and combining the use of a DNA polymerase optimized for high-speed PCR (SpeedSTAR HS) and of a more efficient thermal cycler instrument (Bio-RAD C1000), it was possible to reduce the amplification process time to 26 minutes. No modification to the commercial AmpFℓSTR® Profiler Plus or Identifiler primer mix was required. Mock and casework single-source and two-person mixture samples were used. Compared to standard amplification protocols, the fast amplification procedure demonstrated similar sensitivity, peak height ratios, and overall profile balance. Minor alleles in mixtures were reliably typed. An increase in the n-4 stutter ratio (2.2% on average for all loci) for profiles amplified with the fast protocol was noted compared to the standard protocols. Complete concordance was obtained with profiles previously generated with a standard amplification protocol for mock case samples and for casework samples.

Together, these protocol modifications provide interesting options to current laboratory processes to expedite the generation of STR results, especially in circumstances requiring quick actions. Other strategies to further reduce turn-around time included the evaluation of the Qiagen Investigator HYres quantification kit and profile generation on an Applied Biosystems 3500/3500xl Genetic Analyzer. The cycling procedure of the Investigator HYres cuts off approximately 30 minutes in the quantification step compared to the Applied Biosystems Quantifiler Duo. Comparable human and male DNA yield values were obtained for both systems from forensically relevant DNA samples. Excellent STR profile quality was demonstrated from biological samples submitted to the entire accelerated analytical process in less than five hours.

DNA, STR, Accelerated Process

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