ADOPTING FASTER DNA EXTRACTION AND PCR AMPLIFICATION PROTOCOLS TO ENHANCE STR CASEWORK PROCESSING

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The automated STR DNA processing of casework samples at the RCMP currently requires two overnight incubations: one at 56°C for the lysis of samples prior to the DNA extraction using an automated DNA IQ protocol and one at room temperature following DNA amplification in order to further promote the 3' terminal transferase activity of the AmpliTaq Gold DNA polymerase. We investigated ways to significantly decrease sample processing and decrease the PCR cycling time using two approaches: 1) optimization of the lysis of biological samples and subsequent DNA recovery, 2) development of a robust fast amplification protocol for high quality AmpF/STR Profiler Plus profiles.

Different incubation times for the lysis of biological samples were assessed (30 min, 1 hr, 2 hr, 3 hr, 4 hr) and the total DNA recovered from biological samples was compared to DNA recovery using the current overnight incubation. Different volumes of blood on different substrate types were tested as well as different biological sample types. An incubation of 30 min was sufficient to obtain comparable or higher DNA levels to the overnight incubation.

By modifying the cycling conditions in addition to combining the use of a DNA polymerase optimized for high speed PCR (SpeedSTAR HS) and use of a more efficient thermal cycler instrument (Bio-RAD C1000), we were able to reduce the amplification process to 26 minutes. It is noteworthy that no modification to the commercial AmpF/STR Profiler Plus primer mix was required. Compared to the current RCMP amplification protocol, no differences were noted using our fast amplification procedure on the specificity, sensitivity, peak height ratios and overall profile balance. Moreover, complete concordance was obtained with profiles previously generated with the standard amplification protocol and minor alleles in mixture samples were reliably typed. Two differences were noted: an increase in the n-4 stutter ratio (2.2% on average for all loci) for profiles amplified with the fast protocol and the sporadic observation of low peak artifacts in the D21S11 region in a minority of profiles (9%).

Our results demonstrate the efficacy of a reduced lysis step and show that comparable AmpF/STR Profiler Plus profiles can be obtained in substantially less time using a fast amplification protocol. These protocol changes, when incorporated into a re-organized workflow, could considerably reduce the overall time required for the generation of STR profiles from forensic samples and as such, increase efficiencies. In addition, these modifications provide an interesting option to current laboratory processes to expedite time-sensitive cases.