NEW ANALYTICAL PROCESS FOR CONVICTED OFFENDER SAMPLES SUBMITTED TO THE CANADIAN NATIONAL DNA DATA BANK

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Potential changes in Canadian legislation could substantially increase the number of convicted offender (CO) biological samples that are collected on FTA® cards (blood, buccal or extracted hair roots) for the National DNA Data Bank of Canada (NDDB). In preparation, the DNA analytical processes currently used for CO samples have been modified to ensure optimal efficiencies with the implementation of new equipment and STR kits.

Currently, biological samples collected on FTA® cards are sampled using a 1.5 mm paper disk punched from the cards which is subsequently washed three times using robotics before STR amplification using AmpF/STR® Profiler Plus® and COfiler®. Recently, amplification conditions were optimized and validated using MJ Research PTC-200 DNA Engine thermal cyclers to perform direct amplification of single source blood, buccal and extracted hair roots directly from FTA® paper without the need for sample purification prior to amplification. Validation was performed using the AmpF/STR® Identifiler® Direct from Applied Biosystems and PowerPlex® 16 HS from Promega. The evaluation and validation of both STR megaplexes was carried out for three reasons: 1) to ensure complete compatibility with CO DNA data currently contained in the NDDB of Canada as well as the crime scene DNA samples developed by its clients, 2) to facilitate CO DNA profile exchange/comparison with national and international agencies such as Interpol with an increase in the power of discrimination by including four additional loci (D2S1338 and D19S433 for AmpF/STR® Identifiler® Direct and Penta D and Penta E for PowerPlex® 16 HS), and 3) to ensure continued operations by enabling a redundancy in the supply of STR kit reagents in the event of circumstances such as quality control issues which could reduce the availability of reagents from a single manufacturer.

Different avenues have been explored to reduce the overall sample processing cost without compromising the quality of the DNA profiles generated. Key to the successful processing of forensic samples using a high-throughput automated DNA approach has been the optimal control of DNA available for PCR amplification. For direct amplification protocols, the amounts of DNA and PCR inhibitors are two elements that must be tightly controlled for optimal STR results. The design of a smaller punch head for our FTA punching unit (DBS Wallac puncher) allowed a significant reduction in the reaction volume required for direct amplification and produced high quality profiles. Conditions were also optimized for the separation and detection of amplicons on the high-throughput AB 3730 DNA Analyzer (48 capillaries).

A wide collection of blood, buccal and extracted hair roots collected on FTA® paper from volunteer donors was used to optimize and validate the new automated high-throughput analytical process. Studies such as cross-contamination, precision, reproducibility, robustness, sample stability and concordance were carried out.

The validation of a more efficient high-throughput analytical process is pivotal in meeting the additional processing capacity requirements anticipated for the NDDB of Canada. In addition, protocols developed for the NDDB will serve as a successful foundation for the enhancement of future operational casework requirements and could also prove useful for identification of victims of mass disasters or missing persons.