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Adoption of automated DNA processing for high volume DNA casework: A combined approach using magnetic beads and real-time PCR

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Abstract. In February 2004, an initiative was created between the RCMP Evidence Recovery and Biology Services and the National DNA Data Bank (NDDB) to increase the number of profiles contained in the crime scene index of the data bank in order to enhance the number of matches made with serious unsolved crimes. The magnetic bead extraction technology from Promega (DNA IQ™) was extensively modified to optimize DNA recovery from highly challenged soiled samples using TECAN robotic workstations. The DNA yields obtained using the magnetic bead-based approach were equivalent to conventional processes and 3–4 fold higher for samples compromised with soil, based on the Quantifiler™ Human Quantification Assay developed by Applied Biosystems. A full batch of 84 samples plus controls (96 in total) can be extracted in 2 h and 15 min following an overnight cell lysis, quantified in 2 h and 15 min (30 min for real-time PCR set up on the robot and 1 h 46 min for amplification and detection in the ABI Prism® 7000 instrument) and set up for STR amplification in 1 h using the output file from the ABI Prism® 7000 instrument. The original Sample Tracking and Control System (STaCS™) created for the National DNA Data Bank of Canada was amended to accept "break and enter (B&E)" type samples. © 2005 Published by Elsevier B.V.

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1. Introduction

Trends in Canada have indicated that 44% ($N=1078$ as of June 21st 2005) of non-suspect B&E cases are resolved or assisted by the National DNA data bank. Twelve

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percent (12%) of matches established by the data bank in Canada originate from criminals charged with designated secondary offences such as B&E. For this offence alone, the matches assisted in 55 murder and 100 sexual assault investigations (as of June 21st, 2005). The use of automation was recognized, early in 2002, as a key means to assist in the processing of non-suspect B&E cases and augment the solution for limited human resources. In addition, processing large numbers of B&E cases should promote the number of matches to serious unsolved crimes. The following provides an overview of that automated process.

2. Materials and methods

2.1. The B&E DNA processing unit

The RCMP Biology Services-NDDB initiative involves four different teams. The Case Receipt Unit (CRU) controls the type and number of exhibits submitted for analysis (a maximum of 2 samples per case is accepted). The Evidence Recovery Unit (ERU) searches and prepares the biological samples (in tubes) for the analysts of the NDDB who process the samples robotically. Samples with insufficient DNA are forwarded to Biology Services for manual processing.

2.2. The method

The Promega DNA IQ™ protocol was extensively modified and adapted for our TECAN robotic workstations to produce reliable and consistent results for swabs contaminated with soil. A modification of the extraction protocol was initially used by the RCMP Forensic Laboratory in Vancouver for processing over 142,000 soiled samples collected for a very high profile serial murder case.

Current high throughput processing uses pre-PCR robotic platforms equipped with 16 position-tube racks for sample lysates, 60 mL and 200 mL troughs for the various buffers used during extraction, a flat magnet to isolate the magnetic beads, a TECAN te-shake unit to optimize mixing and collisions between the beads and DNA in the lysate and two wash stations for the robot non-disposable tip cleanup.

2.3. The process

Samples are received from the ERU by the data bank sample kit receptionists and entered into STaCS™. They are then transferred to the NDDB analysts who then proceed with the creation of the batch plate and sample lysis on day 1 (i.e. lysis buffer addition to samples and lysis overnight), DNA extraction, DNA quantification and STR amplification setup on day 2 (i.e. clippings or swabs are transferred to baskets, centrifuged and DNA is extracted from the resulting lysates (2 h 15 min), then quantified (30 min for assay setup and 1 h 46 min for 40 cycles amplification)). Following quantification, the output file from the ABI 7000 SDS unit is imported directly into STaCS™ which provides the robot with the list of samples that need to be diluted before proceeding to PCR setup and those that will be used directly without dilution. STaCS™ also alerts the analysts to remove from the robotic worktable samples with insufficient DNA (<3.3 ng). DNA extracts of less than 3.3 ng are forwarded to Biology Services via the CRU for manual processing. To streamline the B&E process, no option was incorporated for the use of vacuum or filtration units to

concentrate DNA eluates. Samples with sufficient DNA are amplified under oil (45 min for assay setup using the robotic workstation and 4 h for 28 cycles amplification) and amplicons are moved to the post-PCR robotic workstation for further treatment on day 3. The post-PCR process (2 h) includes a butanol extraction, an ethanol precipitation, a formamide/GS500 cocktail addition, followed by comb loading and finally the development of the STR profiles on the ABI Model 377 Gene Sequencer (2 h). Profiles are analysed independently by two analysts (1 h), verified by STaCS™ and uploaded to the B&E index in CODIS (Combined DNA Index System) (30 min).

Potential concerns over cross-contamination were extensively evaluated with B&E samples extracted with magnetic beads since DNA in the cell lysate or as an eluate travels through tips and tubing and is present in concentrations varying from a few nanograms to hundreds of nanograms. Currently, two washing stations are used and a 2% bleach washing step has been incorporated strategically during the extraction, as well as after the extraction session. The robot tips are flushed into the waste, then directed to the bleach trough for a few mixes to expunge any remaining traces of DNA then directed to the "dirty" shallow reservoir and immediately to the "clean" shallow reservoir to get rid of potential traces of bleach remaining inside or outside each tip.

The use of 2% bleach within the extraction process was not accompanied by a loss in DNA yield nor a loss in the quality of the DNA as the STR profiles derived from the DNA extracted were as intense and as balanced as those detected for the "no bleach" approach.

3. Results and discussion

Processing B&E samples robotically offers major time savings and a substantial increase in productivity. One analyst normally processes 27–30 samples through phenol/chloroform extraction and slot blot quantification in 1 day using manual protocols. With automation, one analyst could process 88 samples through magnetic bead extraction and real-time PCR quantification in 1 day. This represents a 3-fold increase in sample number. With full sample tracking integration and robots executing the commands, samples are processed error-free under the highest quality control standards.

As of June 21st, 2005, 1607 exhibits had been processed representing 1078 cases. From those cases, 685 valid profiles were entered into CODIS. B&E samples hit with profiles from the various data bank indexes 69% of times. In approximately 22% of the time, the profile matches with other profiles from the B&E index. Almost one B&E sample in 4 matched with a profile contained in the convicted offender index and 19% of the time, the B&E profile matched a profile from the crime scene index. This data corroborates the trends established by convicted offender DNA data banks of various countries and reinforce the importance of processing high volume non-suspect property crime samples.