International Congress Series 1239 (2003) 621–625

The National DNA Data Bank of Canada—a laboratory bench retrospective on the first year of operation

Chantal J. Frégeau*, Benoît Leclair, Kathy Bowen, Frances Porelle, Ron Fourney

Royal Canadian Mounted Police, Forensic Laboratory Services, National DNA Data Bank of Canada, 1200 Vanier Parkway, Ottawa, Ontario, Canada K1G 3M8

Abstract

In this report, we review the concepts and practical framework behind the design and operation of the National DNA Data Bank of Canada. We emphasize the automated approach developed for tracking biological samples through each step of the process and for ensuring complete control over sample traffic.

Keywords: Data bank; Automation; Robotics; Sample tracking; Membrane comb

1. Introduction

In June 2000, the National DNA Data Bank of Canada, located within the RCMP Forensic Laboratory in Ottawa, was officially launched and mandated to process biological samples from criminal offenders convicted of serious crimes. The success of the data bank as an investigative tool became readily apparent by virtue of the great number of cold hits recorded during the first year of operation (53 convicted offender to crime scene matches and 10 crime scene to crime scene matches). Such a level of performance is attributable, in turn, to the approach elaborated during the 18 months prior to its opening. Several issues were addressed during this initial phase: (1) the collection of samples (including the commercial development of collection kits) and the training of law enforcement officers responsible for collecting biological samples from offenders, (2) the development and optimization of extraction and purification procedures using robotic workstations, (3) the enhancement of

* Corresponding author.

0531-5131/03
PII: S0531-5131(02)00317-5

sample throughput at analytical genotyping workstations (i.e. accommodation for the use of 96-tooth membrane combs), (4) the development of a training strategy for personnel responsible for the performance of the analytical work, (5) the development of a specialized software suite to manage sample flow and (6) the implementation of a local and national CODIS network. The following overview of our experience for our first year of operation is therefore provided to assist other countries that may be contemplating the implementation of their own national DNA data bank.

2. Materials and methods

2.1. Collection of convicted offender samples

According to the current legislation in Canada, blood, buccal or hair samples from convicted offenders can be submitted to the National DNA Data Bank. All three sample types are deposited on FTA Collection Cards included in each of the three authorized RCMP Collection Kits for Blood, Buccal or Hair designed by the RCMP in collaboration with Fitzco/Whatman. Two distinct series of commercial collection kits were manufactured. One series was produced specifically for the training of police officers responsible for collecting biological samples from offenders. The second set of kits was designed for the actual collection of biological samples from offenders in the courtroom or in prison destined for submission to the data bank. To prevent ''training'' samples from inadvertently being accepted by the data bank, training kits bore bright yellow barcodes with five digits. The convicted offender collection kits, on the other hand, were issued with white seven-digit barcode labels. Prior to the opening of the national data bank, over 1200 police officers had received training across Canada through presentations, handouts and collection workshops. During these sessions, attendees learned how to collect blood, buccal and hair samples properly using colleagues as volunteer donors. A web site showing step by step the entire collection process has been created to facilitate the training of future law enforcement personnel.

Over 69,000 collection kits were sent across Canada to federal and municipal police forces and detachments. Approximately 10,390 samples were submitted to the National DNA Data Bank during the first year of operation. Of those samples received, 1% were rejected either because they were collected for a nondesignated offence, were collected using a training kit instead of the official collection kit or were deemed inadequate for analysis. Accepted samples were issued a kit reception (KR) locator number, and were forwarded to the laboratory for analysis and stored at room temperature in dedicated and secure filing cabinets while being processed. Meanwhile, all personal documentation regarding the convicted offender was separated from the FTA card (biological sample) and sent outside the data bank to the criminal records section.

2.2. Automated DNA typing process

Although only 10,392 submissions were received in the first year of operation, it was anticipated that between 30,000 and 97,000 samples could be submitted to the data bank

on a yearly basis. Processing each sample using both the AmpF/STR® Profiler Plus™ and AmpF/STR® COfiler™ megaplexes could, in turn, require 60,000 to 194,000 amplification reactions to be potentially carried out annually. Automation of the entire DNA typing process became the ultimate choice in order to meet the highest standards of quality control, as well as to achieve enhanced efficiency and reduced operational cost. In order to streamline the processing of all convicted offender samples submitted to the data bank, a protocol was developed to transfer the cellular portion of hair roots onto a FTA collection card. Using this protocol, blood, buccal and hair samples deposited on FTA cards can be processed simultaneously on the robotic workstation using a single routine, thereby facilitating and enhancing the efficiency of the entire analytical process. The RSP-150/8 Liquid Handling System from TECAN was selected to process convicted offender samples as this robotic workstation met all five of our preestablished criteria: (1) the station is equipped with eight teflon-coated tips capable of working independently in order to allow flexibility in protocol design and "cherry-picking" of samples required for rework anywhere in the process queue, (2) the station provides integrated barcode reading capability in any direction from any position on the robot worktable, (3) the station has software allowing the customization of every tip and barcode reading device movement, (4) the station accepts ASCII-type worklists specifying all tip and barcode reading device movements and all source and destination vessels, and (5) the station produces log and export files documenting every tip movement, source-destination links.

New protocols were developed and optimized to accommodate both 96 well-plate/15 μl PCR reactions and 384 well-plate/5 μl PCR reactions. The use of oil to prevent evaporation during amplification dictated that extra purification protocols had to be developed and incorporated into the post-amplification stage. Currently, two TECAN robotic workstations are utilised at the pre-PCR stage and two stations at the post-PCR stage. The pre-PCR workstations are essentially duplicates of each other and share the tasks of washing the 2 mm punched sample disks and preparing and dispensing the AmpF/STR® Profiler Plus™ and AmpF/STR® COfiler™ amplification cocktails in appropriate wells, along with the oil. The post-PCR workstations are also a duplicate of each other and are responsible for purifying the amplicons (butanol extraction, ethanol precipitation), preparing amplicons for electrophoresis (resuspension in formamide/Genescan500) and loading samples on 96-tooth membrane combs (0.9 μl aliquots per tooth). The pre-PCR process takes approximately 1 h 15 min for a full 96 well-plate while the post-PCR process requires approximately 2 h.

The analysts process samples in batches. A batch consists of 84 samples (either all new or a combination of new samples and samples that need to be reworked), plus controls (blank FTA card, blood internal standard, positive and negative amplification controls, allelic ladders) for a total of 96 samples. Samples that fail to produce a complete profile are sent for rework at the punch step. Samples that produce complete profiles of low intensity can be sent for rework at the post-PCR step where a larger aliquot of amplified product is used for precipitation prior to comb loading. In the first year of operation, 6% of samples required rework but the success rate after rework was 100%. All blood (10,044), buccal (334) or hair (14) samples that were processed yielded complete profiles; hence, there was no need to request a second sample from any offender.

2.3. Managing sample flow

A barcode-based tracking system tasked with sample traffic management, sample rework, genotype compilation and CODIS CMF file export was written in Visual Basic. This software suite was further expanded to provide the details of any batch to a worklist generator, which was itself built to control the robotic workstations through worklist commands. This original sample tracking software was further developed and expanded into a commercial software application called STaCS (Sample Tracking and Control System). This sophisticated software application tracks and controls the sequence of events involved during the analysis of offender samples to ensure the integrity of the entire process. It captures all relevant information pertaining to each sample and process from the moment samples are received at kit reception through to the upload of the genetic profiles to CODIS. It is also an inventory system for all reagents used in the process.

At kit reception, STaCS captures statistical information pertaining to the sample collection and submission to the data bank. STaCS also provides the history and current status for each sample entered in the system and uses a first-in-first-out approach to assign priority. The FTA cards are received in the laboratory by the DNA analysts and documented in STaCS by scanning each unique barcode. STaCS creates batches of samples to be processed, including samples that need to be reworked. AmpF*l*STR® COfiler™, AmpF*l*STR® Profiler Plus™ or hybrid plate formats can be selected by the analyst as the plate type. A unique barcode is created by STaCS for each batch of samples. STaCS interfaces with the punch instrument and provides the list of samples to be punched for each specific plate created. STaCS incorporates the rework command given by the analysts after gel analysis (that is, to use one or two punches for the rework). STaCS retrieves and archives the record entry file produced by the puncher detailing the specific well assignment for each sample. STaCS interfaces with the pre- and post-PCR robots to calculate the volumes required for each reagent. It ensures that the required chemicals and plates are on the robot worktable and generates the worklist or the list of commands to be executed by the robot specifying all tip and scanner movements, as well as all source and destination vessels specific for a batch. STaCS archives all log files and output files produced by the robot and parses the information to ensure that the robot performed its routine according to the worklist provided. During amplification, STaCS retrieves and archives the log files produced by the thermal cycler documenting the block temperature through the cycling steps. At the ABI 377 DNA Sequencer, STaCS records the electrophoretic parameters and archives the run folders. The analysis of the genetic profiles is performed independently by two analysts before the data are imported into STaCS, which then compares both the Profiler Plus and COfiler profiles for agreement at the three redundant loci. Once compared, the data are merged and converted into CMF files for upload to CODIS.

3. Conclusion

The success of the National DNA Data Bank of Canada is, in large measure, attributed to the efforts that were deployed by many individuals during the period of 18

months prior to its official launch. The fully integrated and automated approach designed and developed to process criminal offender samples requires very little human intervention. It makes the most efficient use of samples, costly reagents, personnel time and robots, while ensuring that all samples are processed error-free in the shortest possible time.