

## Semi-automatic preparation of biological database samples for STR typing

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**Abstract.** We have implemented a laboratory automation system (LAS) for the preparation of database samples. The LAS encompass registration of samples in a LIMS database, export of a work-list to a puncher, punching of FTA-cards by a BSD600-duet puncher, electronic sample check by means of barcodes, washing of punches by the THEONYX liquid-handler from MWG, PCR amplification and electrophoresis of amplicons. We validated the system using fresh blood and saliva immobilized on FTA-cards as sources of biological material. The use of the LAS led to:

- Correct STR types with a quality equal to or higher than that of the profiles produced using chelex-extracted DNA.
- A reduction of approximately 35% in the number of reanalysed samples.
- A reduced risk of mix-up of samples during the laboratory procedure.
- Fewer sample transfers.
- A reduction in the number of PCR cycles from 28 to 24 compared to chelex-extracted DNA from blood.

During the validation process, we did not observe mix-up of samples, loss of FTA-card punches, contamination from external sources, or cross-contamination between samples. © 2005 Published by Elsevier B.V.

*Keywords:* Database samples; Short tandem repeats; Laboratory automation system

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### 1. Introduction

Application of laboratory automation systems (LASs) for the preparation of forensic crime-case and database samples is important to support the increasing demand for fast, reliable and cheap forensic genetic analyses. The LAS must support an undisputed handling of samples, sample identity and laboratory protocols. Here, we present a

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validated (ISO 17025) laboratory semi-automated system for STR analyses of biological material immobilized on FTA-cards.

**2. Material and methods**

Sample- and data-flow of the LAS was tested by isolating one blood and one saliva sample from 37 individuals. All 74 samples were subdivided in two preparations and tested independently (Fig. 1). DNA was purified manually from blood samples using the chelex extraction method. DNA from saliva samples was immobilized on FTA-indicating cards (Whatman). A 1.2-mm disk was punched out of the FTA-card directly into a microtiterplate (Eppendorf) containing 10 µl of ddH<sub>2</sub>O using a BSD600-duet puncher (BSD Robotics). After each sample-punch, a cleaning-punch was performed. The arrangement of the FTA-punches in the wells of the microtiterplate was controlled using a work-list exported from the LIMS to the puncher. The FTA-punches in the microtiterplate were washed by a THEONYX liquid handler (MWG) using the following procedure: 150 µl of ddH<sub>2</sub>O was added to each well and was mixed by aspirating and dispensing five times. The H<sub>2</sub>O was left in the wells for a minimum of 10 min and mixed by aspirating and dispensing five times followed by removal of app. 130 µl liquid. Again, 150 µl of ddH<sub>2</sub>O was added to each well and mixed by aspirating and dispensing five times. The H<sub>2</sub>O was left in the wells for a minimum of 10 min, and mixed by aspirating and dispensing five times followed by removal of as much of the remaining liquid as possible. All punches were dried at 60 °C for 45 min. All chelex and FTA preparations were typed using the AmpF/STR® Identifiler® PCR amplification kit (Applied Biosystems) in 10 µl reaction volumes on a GeneAmp® PCR System 9700 (Applied Biosystems). One punch of each sample immobilized on FTA-cards was amplified using 24 PCR cycles and 1 µl of chelex extract of each blood sample was amplified using 28 PCR cycles. All PCR products were analysed in an ABI Prisms® 3100 Genetic Analyzer.

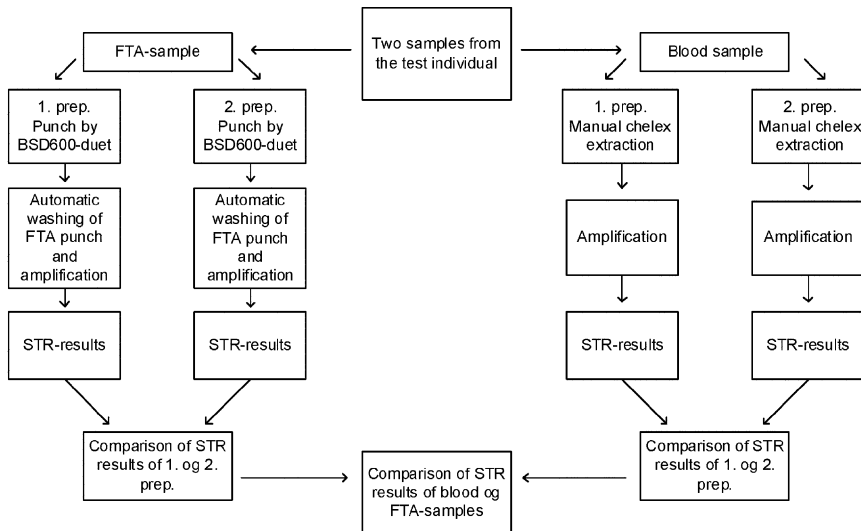


Fig. 1. Flowchart of the saliva (□) and blood (□) sample preparation steps in the laboratory.

### 3. Results

By comparing the two DNA profiles generated from the same FTA-card (the two preparations) it is evident that:

1. The BSD600-duet puncher transfers the FTA-disks into the correct wells.
2. No punch was lost during the laboratory preparation, the punching process or the preparation by the THEONYX liquid handler.
3. No contamination was observed.

By comparing the DNA profiles generated from the saliva sample and the blood sample of the same individual (Table 1) it is evident that:

1. Consistent STR profiles were obtained.
2. None of the samples were swapped during the LAS handling.
3. 9% of the FTA-card preparations had to be reanalysed while 14% of the chelex preparations had to be reanalysed.
4. The overall quality of the STR typing (allele and system balance) using FTA samples was equal to or better than that of the profiles produced using chelex-extracted DNA.

### 4. Discussion

It is clear that the STR profiles generated using the LAS is of equal or better quality than that of STR profiles generated using chelex purified DNA. Furthermore the implementation of the LAS has lead to

1. a reduction of approximately 35% in the number of reanalysed samples,
2. reduced risk of mix-up of samples during the laboratory procedure by introduction of automatic control of sample numbers,
3. fewer sample transfer steps in the laboratory procedures, and
4. a reduction in the number of PCR cycles from 28 to 24 compared to chelex-extracted DNA from blood.

We have implemented the LAS for STR typing of reference samples, and we intend to develop similar automated methods for some kinds of crime scene samples such as cigarette buds and bloodstains secured on cotton swabs.

Table 1

Results of STR profiles generated from saliva samples stored on FTA-cards and blood samples extracted with the chelex method

	FTA				Chelex			
	First preparation	Second preparation	Total	Frequency	First preparation	Second preparation	Total	Frequency
Full profile	32	35	67	0.91	29	34	63	0.85
Partial profile	3	0	3	0.04	7	3	10	0.14
Missing profile	2	2	4	0.05	1	0	1	0.01
Total	37	37	74	1.00	37	37	74	1.00

A total of  $2 \times 37$  samples were used. Each sample was prepared twice.