Sample Collection System for DNA Analysis of Forensic Evidence: Towards Practical, Fully-Integrated STR Analysis

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I. Executive Summary

The goal of this research project was to develop a sample collection system for law enforcement agents to collect, protect, and document biological evidence and to perform initial processing steps in a format compatible with rapid sample-in to results-out microfluidic DNA analysis. The sample collection system consists of an evidence collection device (a collecting swab and accompanying tube for swab insertion) and a sample processing cartridge. This "Smart Cartridge™" will lyse cells, solubilize and concentrate DNA, and transfer the DNA solution to a microfluidic biochip. The microfluidic biochip will process the DNA solution to generate purified DNA compatible with the requirements for STR analysis.

The evidence collection device has been designed to accept blood, buccal cells, saliva, and cellular samples. Cotton, modified cellulose, foam, nylon, polyester and rayon-tipped swabs were evaluated for reproducibility of cell collection, ease of collection, tolerance to collection

protocol, liquid absorption, and application in forensic applications. The Bode SecurSwab was selected for the sample collection system.

To operate the system, the user will collect a swab sample, insert the swab into the accompanying collection tube, place the tube into the Smart Cartridge, and press a start button—no further user intervention is required. The DNA generated by the sample collection system will then be transferred automatically to NetBio's fully integrated microfluidic biochip for STR typing. Several approaches to the sample processing steps were evaluated and those selected were integrated to develop an optimized Smart Cartridge.

This research has resulted in the development of a crime scene evidence collection and processing system compatible with an instrument that will generate an STR profile in 45 minutes from sample introduction with minimal operating requirements. The integrated instrument will process 16 samples in parallel and dramatically reduce the costs (including labor, space, and validation) of setting up and operating a DNA lab. The evidence collection device and Smart Cartridge are designed such that the system will be easy to operate and compatible with both forensic and microfluidic requirements. This sample collection system represents a critical addition to a fully integrated system for forensic DNA analysis.

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III. Introduction

A. Purpose, Goals, and Objectives

The use of microfluidic devices limits the samples for analysis to liquids, and the miniaturized nature of the devices limits the volume of sample that can be analyzed. *These limitations on microfluidic sample format preclude the direct insertion of biological samples collected by validated forensic collection methods directly into a microfluidic biochip.* To microfluidically process a conventionally collected biological sample, extensive manual processing would be required to extract, solubilize, and concentrate the DNA of interest.

The purpose of this project was to develop a sample collection system that will allow law enforcement agents to easily collect, protect and document biological evidence, and to perform rapid sample-in to results-out microfluidic DNA analysis in the forensics laboratory or at the crime scene. Accordingly, this research focused on the development of an evidence collection device and a sample processing cartridge (termed the “Smart Cartridge”) that will operate in tandem with a fully integrated forensic instrument and microfluidic biochip that will perform DNA extraction and purification, STR amplification, and microfluidic separation and detection.

The process flow of the fully integrated STR analysis system is shown in Figure 1. A forensic sample is collected using the evidence collection device and then placed into the Smart Cartridge. After pressing the start button, all required operations are completed, and STR results are reported. Within the cartridge, several processing steps are accomplished (cell lysis, DNA solubilization, and concentration; Figure 2). On the biochip, the DNA is subjected to

purification (Module I), and an appropriate aliquot of the remaining DNA is transferred to the PCR chamber for STR amplification (Module II). The DNA fragments are then subjected to separation and detection (Module III), resulting in an STR profile.

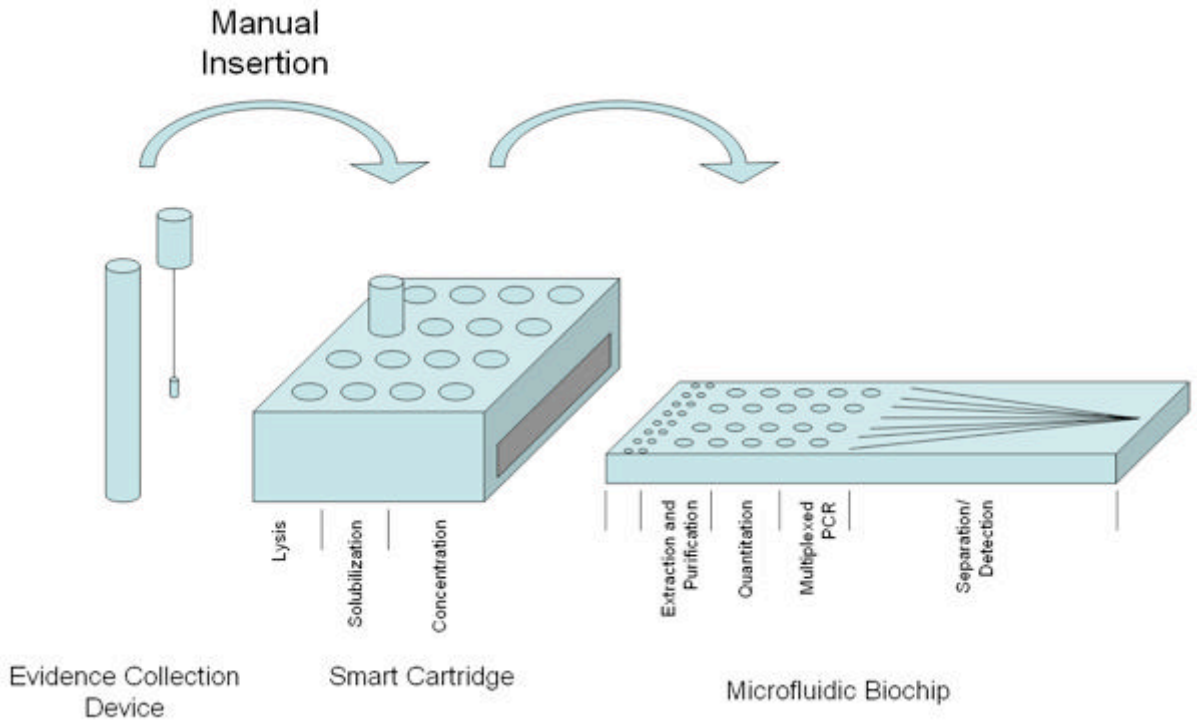

Figure 1. Process flow for sample-in to results-out STR analysis of casework samples in the fully integrated system.

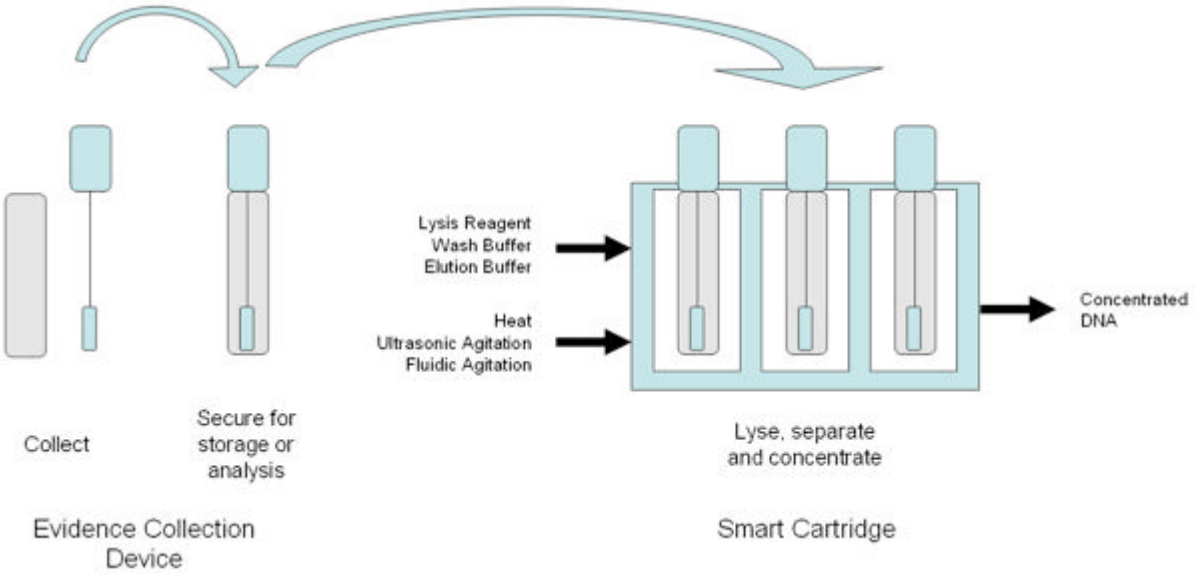

Figure 2. Process flow on the Evidence Collection Device and Smart Cartridge.

Finally, commercially available STR typing kits allow the effective generation of highly accurate STR profiles, but only when the input DNA template falls within a narrowly optimized range. The DNA advisory board to the Federal Bureau of Investigation has recommended the use of human-specific DNA quantitation prior to PCR amplification of casework samples as there is the potential for sample contamination from non-human sources including non-human mammalian, bacterial, and fungal DNA. Accordingly, a fully-integrated microfluidic system for forensic human identification should perform human-specific DNA quantitation in order to determine precisely the amount of DNA template to be subjected to STR amplification. NetBio is pursuing microfluidic quantitation under NIJ Award 2008-DN-BX-K009, "Rapid Microfluidic Human Specific DNA Quantitation."

B. NetBio Sample-in to Results-out Components

1. Sample collection and DNA recovery

Bode Technology has developed numerous highly advanced and innovative DNA collection products including the Crime Scene collector and Slider Buccal DNA Collector. The Crime Scene collector was specifically designed to provide law enforcement secure more reliable evidence from crime scenes, to protect the collected samples for shipping and storage, and to simplify documentation of biological evidence. Evaluation of sample collection methods show that swabbing is more effective for collection and recovery of DNA compared to taping. Furthermore, cotton tipped swabs are the most effective media and format for collection and recovery of DNA (Figure 3).

NetBio previously performed an initial evaluation of lysis solution formulations. Increases in the DNA extraction efficiency of 1.2 to 2 times compared to that of the unoptimized lysis solutions were achieved. Figure 4 shows the DNA purification efficiency for 10 – 300 μl of human blood in each of five lysis solution formulations. In this earlier research, optimization of the collection media and lysis buffer, in tandem over a range of sample types, was performed to maximize DNA recovery. Ultimately, formulations based on a guanidinium chloride (GuHCl) protocol were selected for further optimization.

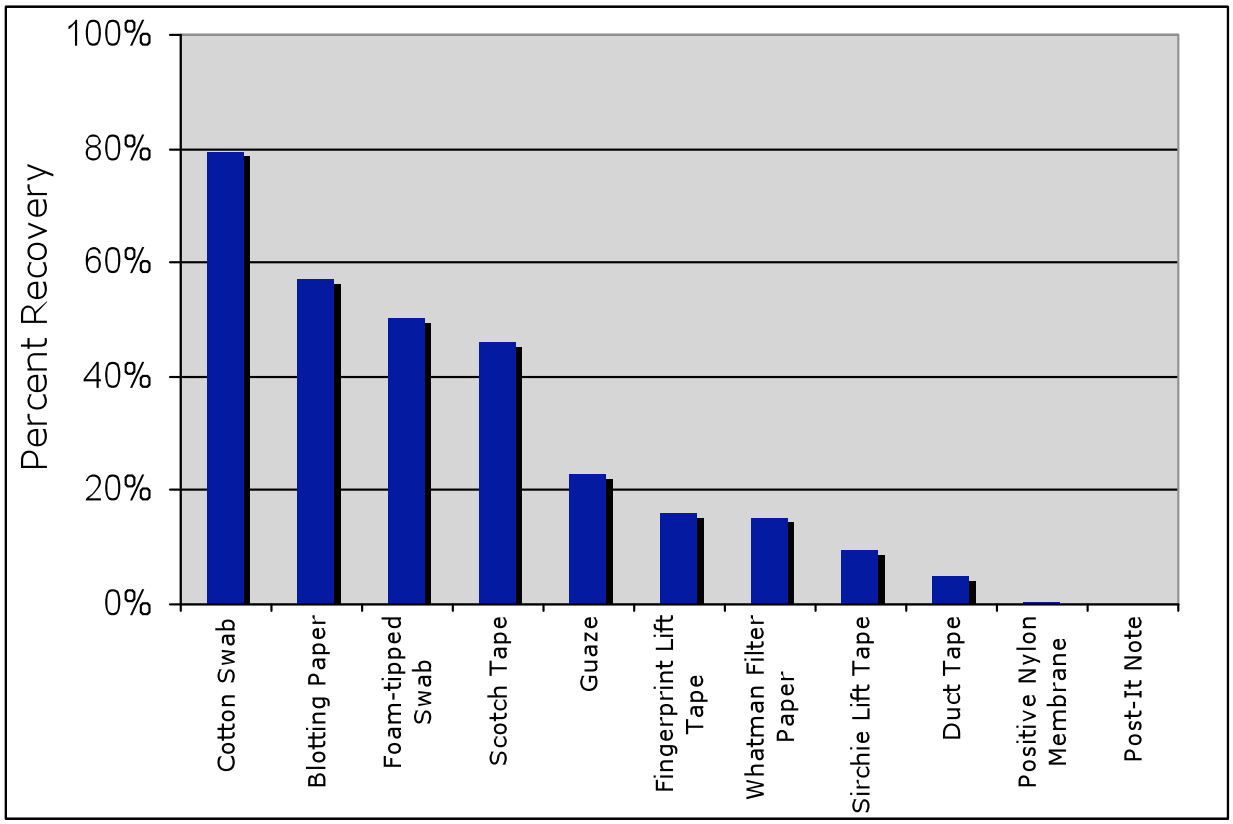

Figure 3. DNA recovery for epithelial cells on polyester blend fabric collected using various sample collection matrices and devices.

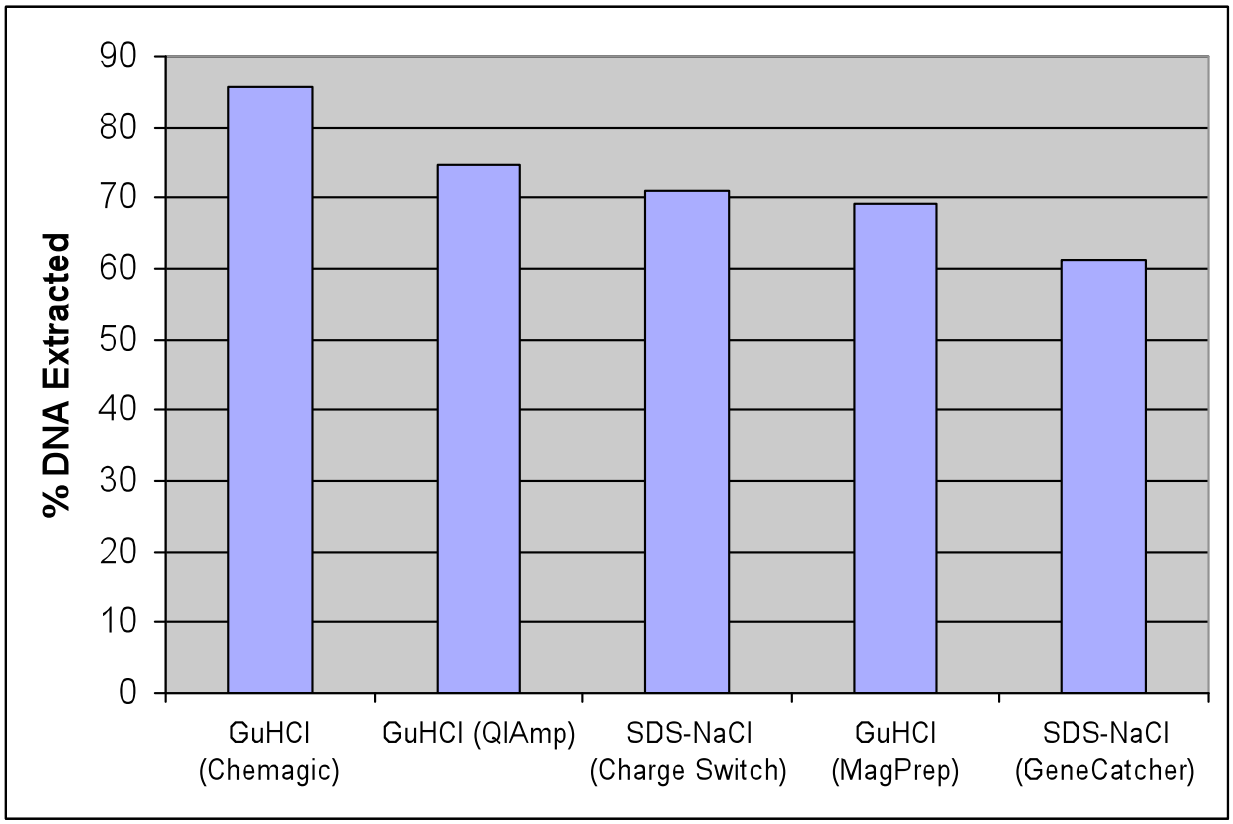

Figure 4. Comparison of GuHCl and SDS-NaCl based lysis solutions for extraction of DNA from whole blood.

2. Microfluidic DNA Extraction and Purification

Development of the microfluidic biochip-based extraction and purification module has been completed, and this work was funded in part by NIJ Award 2007-DN-BX-K184. Preliminary work has been demonstrated using 8-sample biochips incorporating a solid phase bind/wash/elute membrane, and a schematic of the current biochip is shown in Figure 5. For blood, lysis is performed with a GuHCl-based-lysis buffer, and DNA in the lysate is bound to a silica membrane. The operations to manipulate the fluids through the biochip are driven in an automated fashion by the sequential actuation of pneumatic lines. Figure 6 shows that the human genomic DNA purified on the biochip has an average length of approximately 50 kbp, a

size appropriate for STR amplification. Finally, DNA extracted using this microfluidic biochip amplifies effectively and generates STR profiles (Figure 7) that are indistinguishable from conventional tube based reactions.

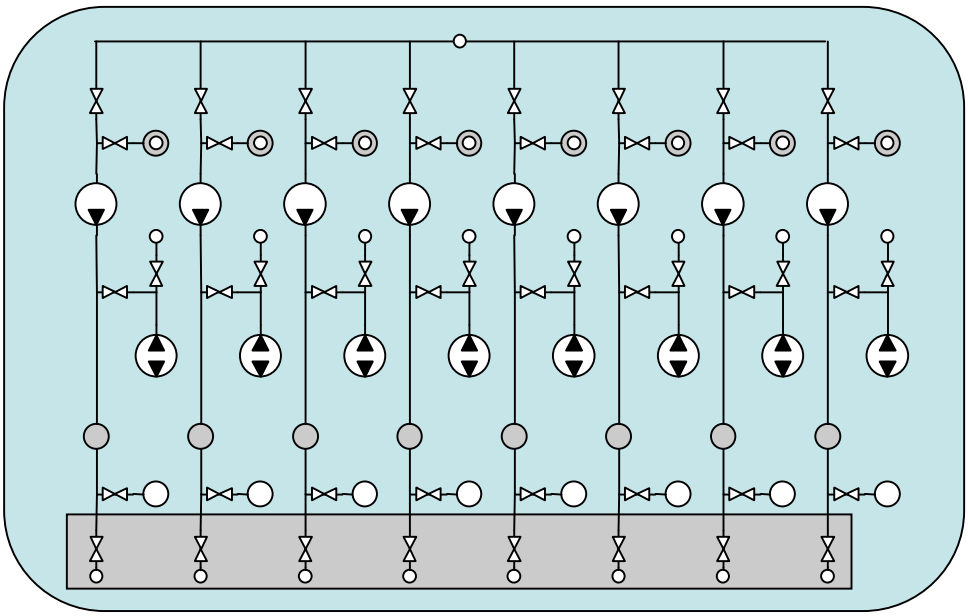

Figure 5. Schematic of an 8-sample microfluidic chip for extraction and purification of DNA.

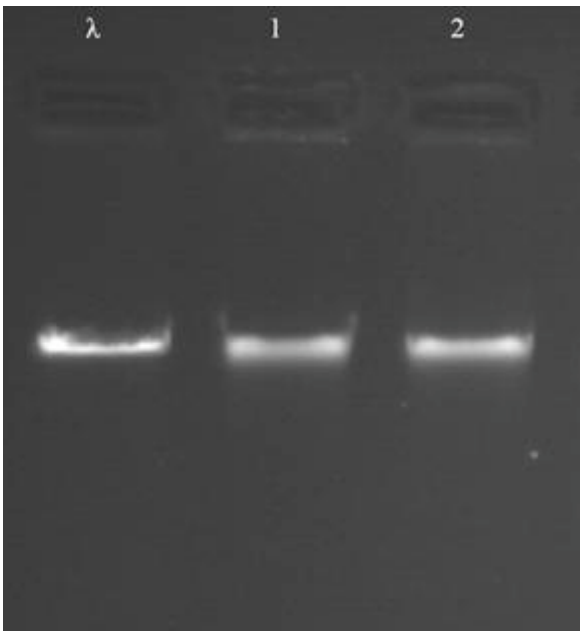

Figure 6. Agarose gel analysis of DNA extracted from whole blood with microfluidic biochip. (1:Biochip extaction, 2:Qiagen control extraction, λ:50kbp lambda DNA)

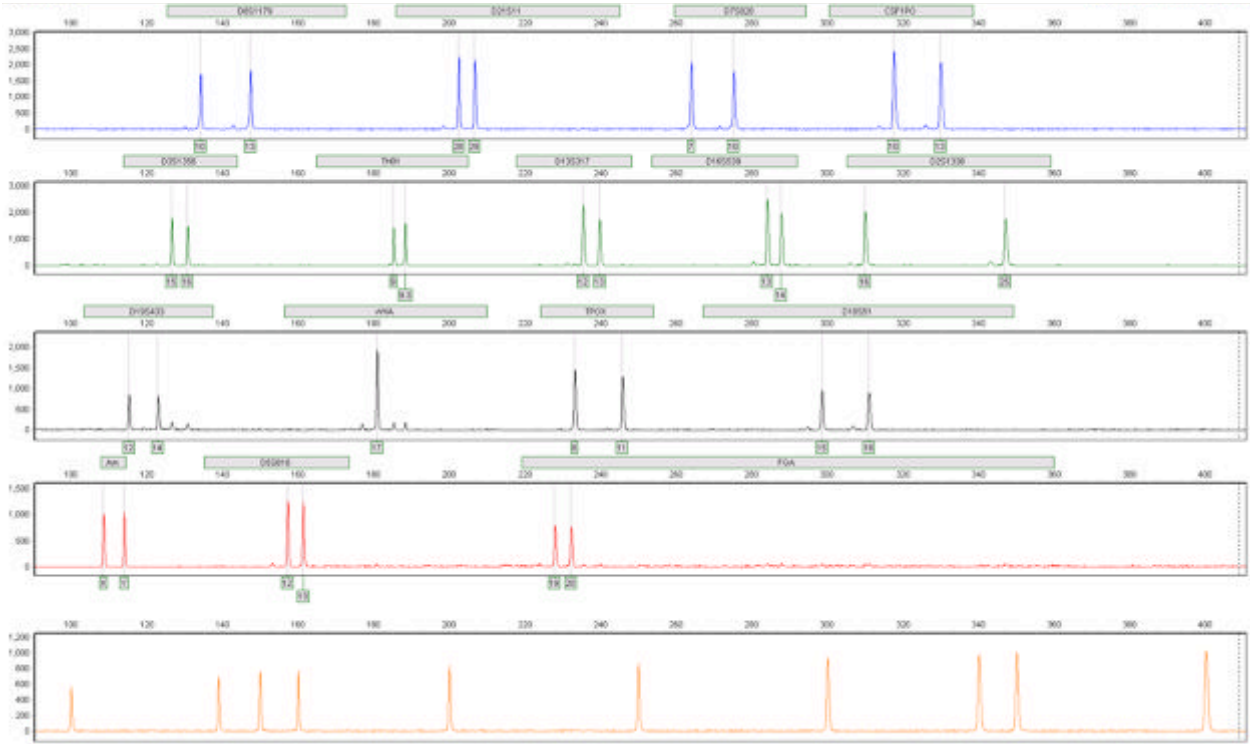

Figure 7. STR profile from DNA extracted and purified from whole blood with microfluidic biochip.

3. Rapid Multiplex PCR Amplification in a Microfluidic Chip

Rapid multiplexed STR amplification was accomplished by focusing on two major areas: rigorous optimization of all reaction mix components and cycling parameters and the development of instrumentation to allow rapid and highly-controlled temperature transitions. The custom thermal cycler shown in Figure 8 is designed with a high output thermoelectric cooler/heater mounted to a high efficiency heat sink, together referred to as the heatpump. This instrument accepts a 16-chamber microfluidic biochip (Figure 9) which is coupled to the heatpump by applying a compressive pressure with a clamping mechanism. Each PCR chamber holds 7 μl of PCR reaction solution. The 16 PCR reaction solutions are placed into individual chambers of the microfluidic biochip. The thermal cycler has the ability to heat and cool a reaction solution at rates of 15.8°C/s and 15.4°C/s respectively, much faster than commercially available cyclers. Appropriate selection of an enzymes allows a highly multiplexed PCR reaction to be performed in as little as 17 minutes (Figure 10). Figure 11 shows a representative fast STR profile using the NetBio thermal cycler and separated and detected on Genebench FX. The fast PCR profiles generated using this approach meet forensically relevant requirements including signal strength, stutter, peak-height ratio, complete non-template nucleotide addition, and inter locus balance (Giese 2009). Figure 12 shows the relationship between DNA template level and signal strength, indicating that the amplification system has a near single copy limit of detection.

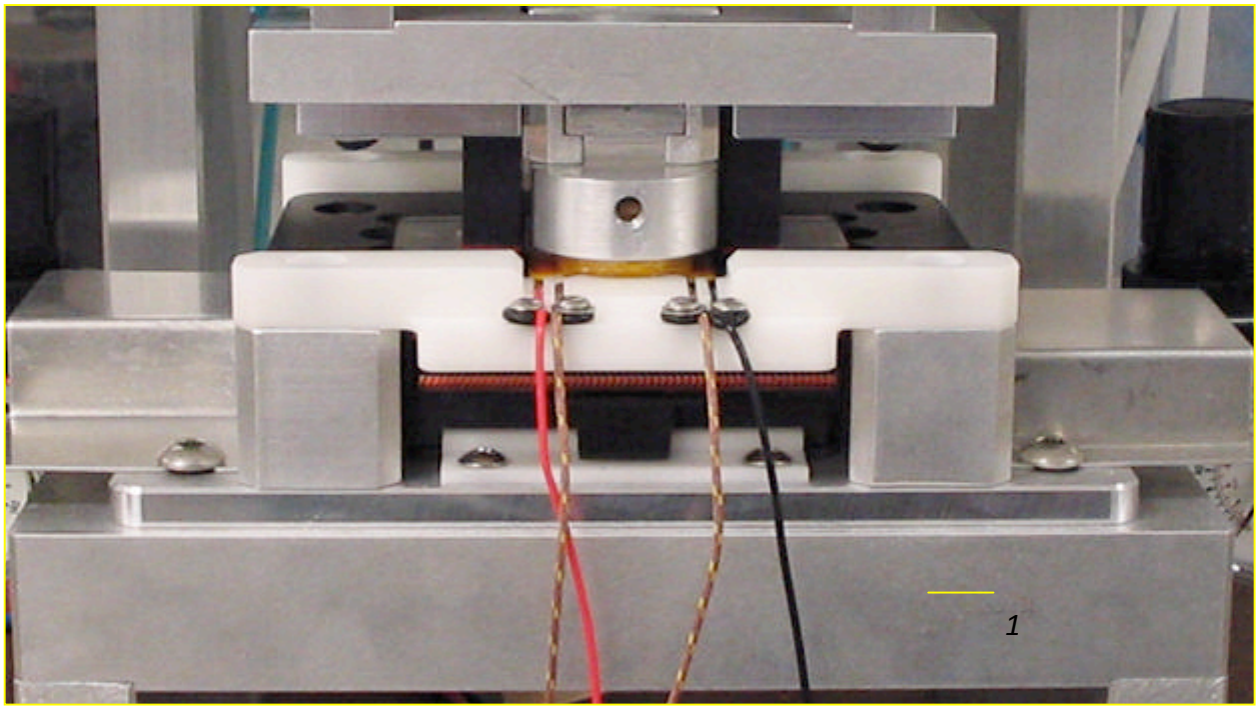

Figure 8. NetBio fast thermal cycler.

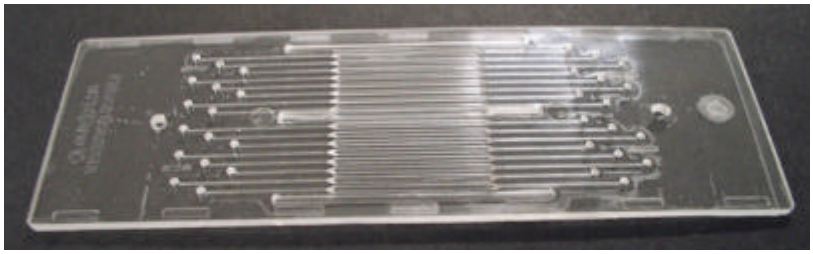

Figure 9. 16-sample PCR Biochip for rapid highly-multiplexed amplification in NetBio's fast thermal cycler.

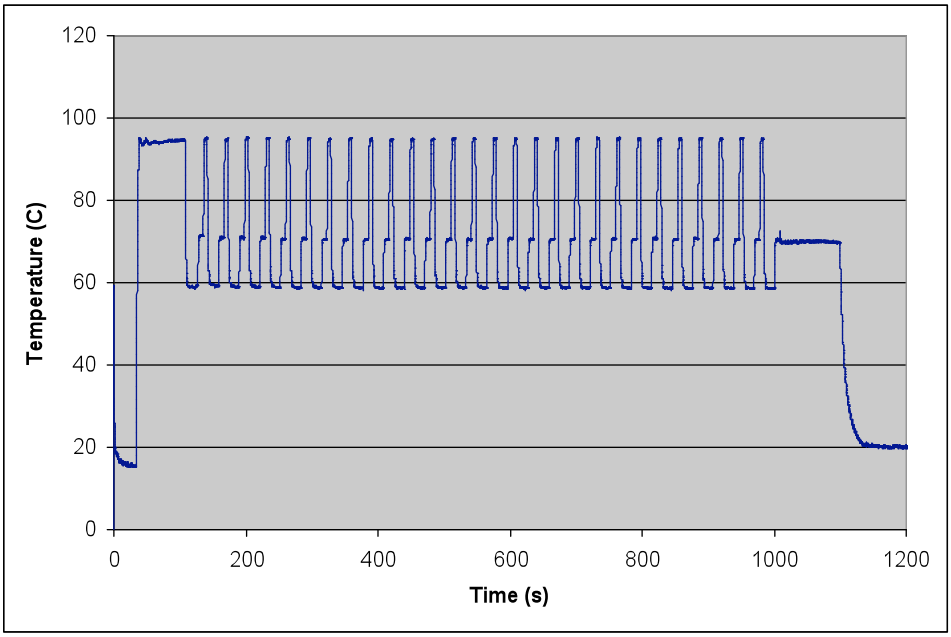

Figure 10. Thermal profile for fast multiplexed amplification of STRs in the fast thermal cycler.

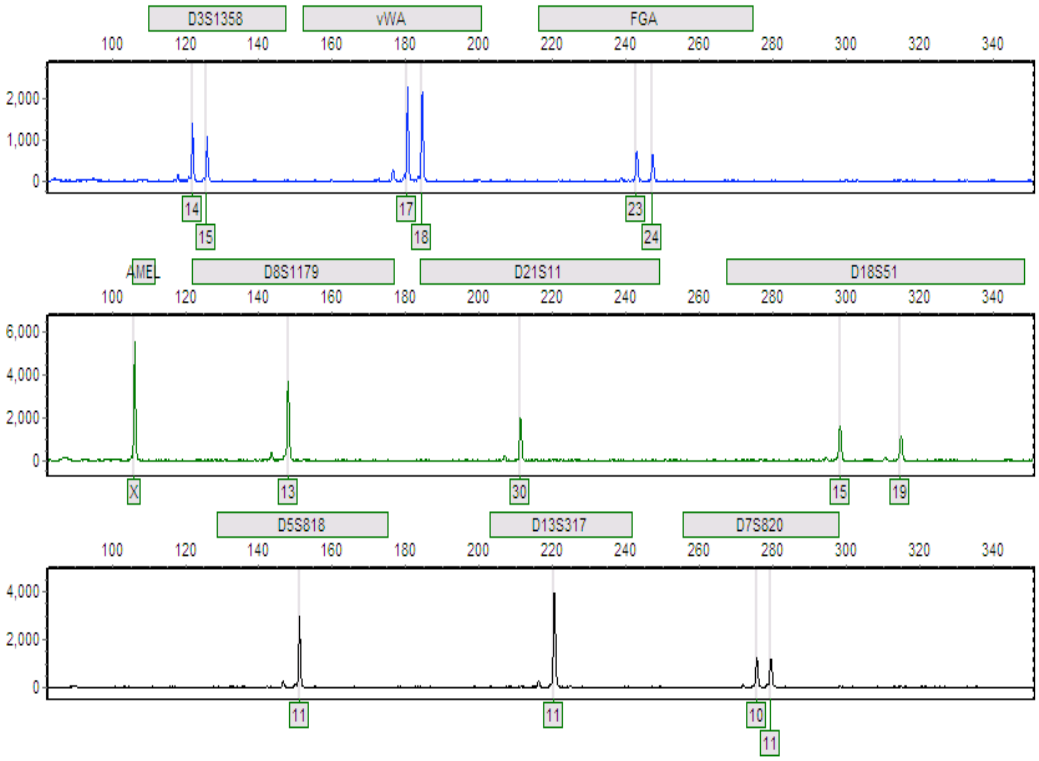

Figure 11. STR profile for 0.5 ng DNA (9947A) amplified in biochip under fast thermal cycling conditions with primers from the AmpFlSTR Profiler Plus ID PCR amplification kit.

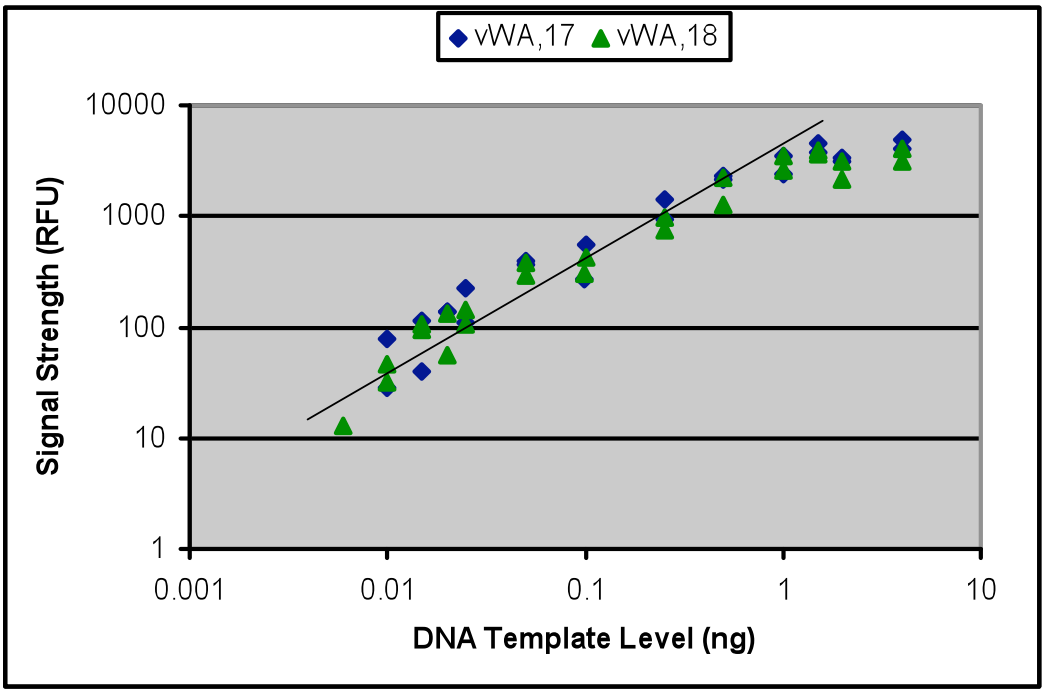

Figure 12. Signal strength of vWA 17 and 18, amplified in biochip under fast thermal cycling conditions with the fast thermal cycler and separated and detected on Genebench-FX.

4. Microfluidic Separation and Detection

Genebench FX™ is a rapid, high resolution, and high sensitivity DNA fragment sizing and sequencing instrument for laboratory and field use. The instrument separates DNA based on fragment size by electrophoresis on microfluidic biochips, and excitation and detection of labeled DNA fragments is accomplished by laser-induced fluorescence detection. Genebench FX (Figure 13) can be operated in both the forensic laboratory and in the field, has low power consumption, and is CE marked under the Low Voltage Directive 73/23/EEC. Separation of the DNA fragments take place within a microfluidic biochip that is filled with a sieving matrix. The biochip (Figure 14) accepts 16 samples to allow for simultaneous analysis of multiple samples and required control reactions.

DNA samples for analysis by Genebench-FX are prepared with conventional methods and manual pipetting into the sample reservoirs of the biochip. After loading the samples and buffers, the biochip is placed into the instrument and analysis continues without further user manipulation, with electric fields applied to electrophoretically separate the DNA and excitement and detection of the fluorophores as they pass through the detection zone. The fully integrated version of the Genebench instrument, the focus of NetBio's development efforts, will feature a fully integrated microfluidic biochip that will accept forensic samples from the user and perform all DNA manipulations, allowing for sample-in to results-out operation without the need for user intervention.

Figure 15 shows an STR profile using the SGM+ primer set (Applied Biosystems, Foster City, CA) generated on Genebench-FX. The PCR product resulted from the amplification of 1 ng of 007 DNA template under the manufacturer's recommended condition. Genebench-FX has 5 color detection capability, allowing the instrument to perform 5 color multiplexed DNA fragment sizing assays such as those required by the AmpFlSTR Identifiler PCR Amplification Kit (Applied Biosystems).

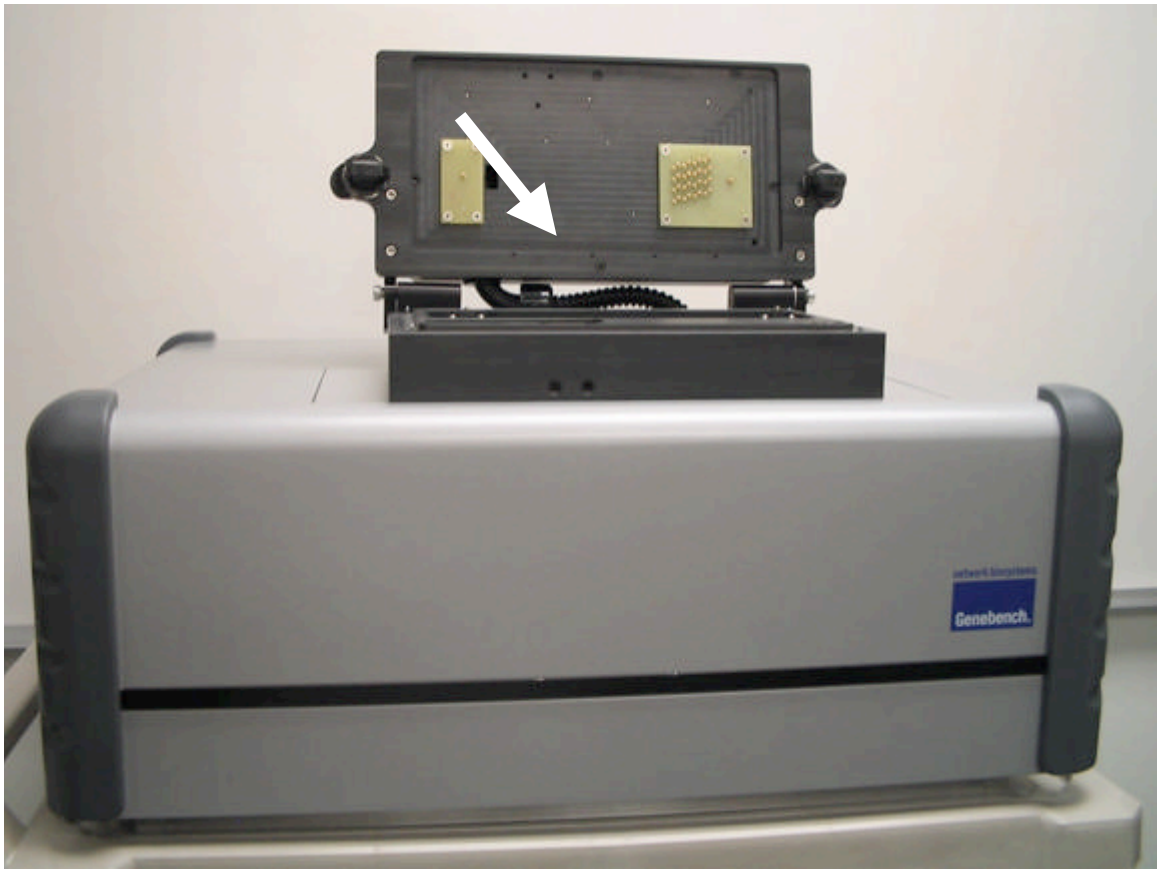

Figure 13. Genebench-FX series 100. Arrow indicates site of microfluidic biochip insertion.

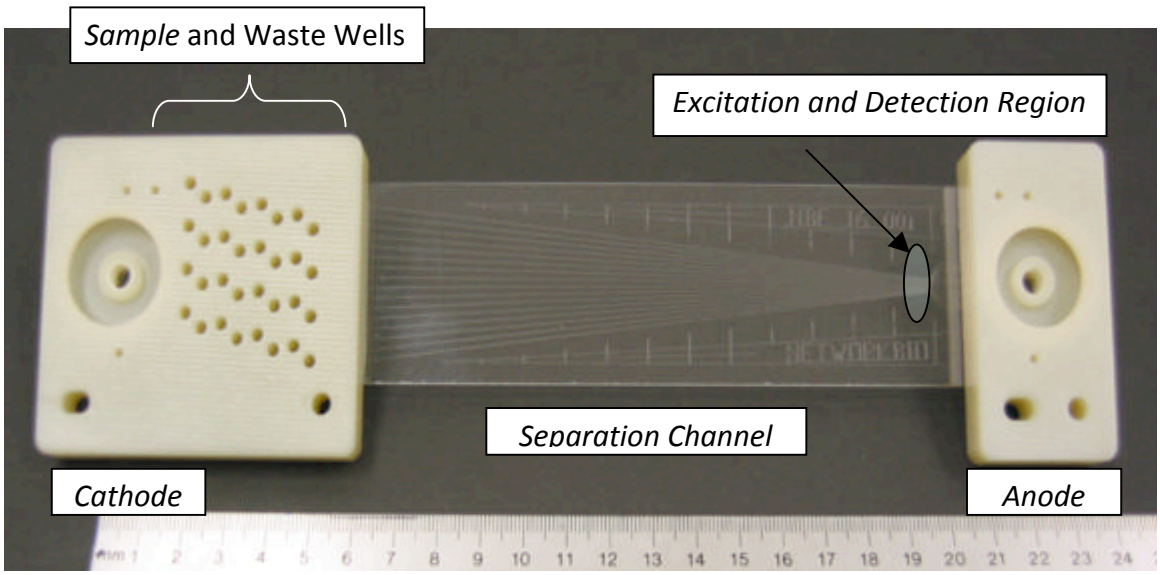

Figure 14. 16-sample microfluidic chip for separation and detection on Genebench-FX.

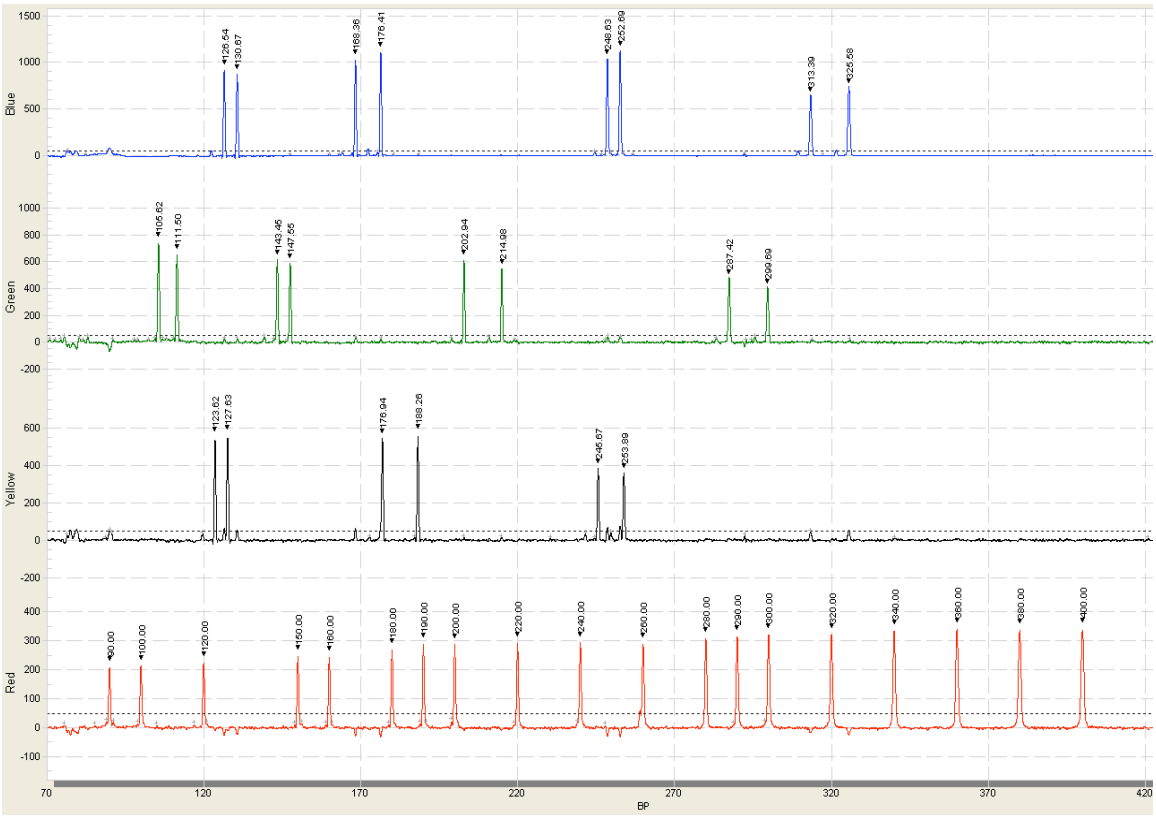

Figure 15. Genebench-FX STR profile of 1ng DNA (007) with AmpFlSTR SGM+ amplification kit.

Taken together, these results show the developmental progress of the modules required for a fully integrated, sample-in to results-out forensic DNA analysis system.

C. Review of Relevant Literature

Sample Collection and Elution. Collection of biological evidence for DNA analysis from crime scenes is a process that effectively collects cells from a variety of surfaces, preserves the collected cells to prevent molecular degradation, and releases the material for downstream processing. Blood, semen, epithelial cells, urine, saliva, bone, and various tissues can be associated with the crime scene and require careful and effective collection (Lee 1998). The age of the sample as well as the surface on which it is found are factors determining how the sample should be collected.

The most common collection technique is performed using a cotton swab. A single swab can be taken from an area or the wet-dry double swab technique can be used. The double swab technique may be the most prevalent, and a number of different fluids including water, buffered saline, or lysis buffers can be used to moisten the first swab (Leemans 2006). This technique allows for dried samples to become re-hydrated, with the majority of material collected on the first swab and the dry second swab collecting the remainder of the sample.

Swabs can be comprised of various materials including cotton or synthetic variations. Swabbing can also be performed using gauze-like materials, disposable brushes, or commercially available biological sampling kits (Lauk and Schaaf 2007). Another standard collection technique involves taking cuttings of the area of interest such as a biological fluid from clothing; however this destroys the integrity of the evidence. Adhesive tape lifts are also used on a variety of surfaces to collect trace evidence that may contain human DNA.

If samples are collected and are not processed immediately, they should be allowed to dry to prevent fungal or bacterial growth. Evidentiary samples should not be immediately sealed in plastic, which can result in microbial growth and cause degradation of the DNA. Typically, swabs or cuttings are placed in breathable containers made of paper or cardboard. Storing collected evidence in cool, dry environments also minimizes sample deterioration (Lee and Ladd 2001).

Once a sample is collected, the next challenge is to remove the biological cells from the collection matrices. Cells can be extracted directly from the collection device, as is the case in most cuttings or cotton swabs, by placing the collector into an extraction buffer. In the case of

tape lifts, swabs moistened with solutions such as xylene can be utilized to dissolve the adhesive, collecting as much material from the tape as possible prior to subjecting the swab to extraction.

In some cases, DNA from the collected cells should not be extracted directly and the biological material should be removed first for examination. When using cotton swabs to collect material, there can be problems removing biological material from the cotton matrix; as the cotton swab dries after collection, the biological material can adhere to the swab. For example, due to the saccharic composition of the spermatocyte membrane, spermatocytes stick to solid supports, especially cotton (Lazzarino 2008). In order to release the maximum amount of material from the swabs, a variety of buffers have been tested and compared to the standard differential extraction buffer. Use of detergents such as 1-2% sodium dodecyl sulfate (SDS) has shown to increase sperm cell recovery (Norris 2007). Also, the addition of low amounts of cellulase has shown to release more epithelial and sperm cells from the cotton swab matrix than buffer elution alone (Voorhees 2006).

Problematic Samples. There can be many challenges to obtaining STR profiles from biological materials including low quantity or quality of DNA. Low copy number samples (containing less than 50 picograms of DNA) as well as low quality, degraded samples require highly efficient collection, extraction, and amplification procedures. These samples are seen in a variety of forensic evidence including touch evidence and aged samples.

PCR inhibitors are another challenge and must be eliminated before downstream applications can be performed. Common inhibitors are indigo dyes from denim, heme from

blood, humic acid found in plants and soil, and collagen found in various tissues. The majority of these inhibitors are effectively eliminated using silica-based DNA extraction methods or additional purification with charge or size exclusion columns. The presence of inhibitors can be detected by performing PCR with internal positive controls. If present, some inhibitors can be neutralized by various treatments including sodium hydroxide washes or further purification with Millipore Microcon YM® columns.

Integration of Evidence Collection with Post-Collection Processing. The need to reconcile the "real world" requirements of sample collection with the microfluidic requirements of a fully integrated microfluidic DNA processing biochip can be referred to as the "macro-to-micro interface" or the "world-to-chip interface" (Fredrickson 2004). Much of the reported research on addressing this interface is focused on resolving the mismatch between the macrofluidic and microfluidic volumetric requirements, but little of no research concerning the reconciling of specific forensic sampling requirements and formats with microfluidic devices has been reported.

The (non-forensic) volumetric mismatch has been commercially addressed by Agilent in the Bioanalyzer 2100 by the use of a capillary to aspirate samples from a microtiter plate to a chip for enzyme assays (Lin 2003). Similarly, Gyros has developed a capillary dispenser for a LabCD system where samples are aspirated from a well plate into a dispensing nozzle and then directed upwards onto a rotating device (Jesson 2003). These devices, however, do not address the format incompatibility of collected forensic samples—particularly on the commonly used collection devices based on swabs.

D. Requirements of a Sample Collection System

Sample collection and initial sample processing are critical to the development of a fully integrated microfluidic system for STR typing. These steps are macrofluidic and must be conducted such that the end product—DNA in solution—can be transferred to a microfluidic biochip. The evidence collection device and Smart Cartridge developed as part of this research address the format and volumetric mismatch that exists between "real world" forensic samples that are collected and requirements for analysis by microfluidic devices, a mismatch that precludes direct insertion of biological samples collected by validated forensic collection methods directly into a microfluidic biochip. This research takes advantage of a series of forensic advances in sample collection, elution, and processing of difficult samples. These forensic advances were combined with Bode's experience with sample collection and elution and NetBio's experience with microfluidic manipulation to develop a sample collection device and Smart Cartridge compatible with the microfluidic biochip required for a fully integrated STR typing system.

The collection system was designed to possess the following properties:

Ease of use—The evidence collection system must be capable of operation by law enforcement agents with minimal training in molecular biological or chemical analysis and must protect against inter- and intra-run sample-to-sample contamination. The system will be pre-filled with all reagents to reduce labor required to operate the system.

- Sample Types—The system must accept a variety of sample types. For this project, we have focused on liquid and dried blood, buccal cells, epithelial cells, and saliva samples.

- Sensitivity—The system must be capable of accepting and processing samples with a high dynamic range of cell counts.
- Compatibility—Both the evidence collection device and Smart Cartridge must be compatible with all required reagents.
- Integration— All evidence collection matrices and sample processing reagents must be compatible with transfer using microfluidic principles.
- Time to answer—The sample processing procedure must take no more than 5 minutes.

IV. Research Design and Methods

A. Overview of Research Design

The goal of this research was to develop a sample collection and processing system consisting of an evidence collection device and a sample processing device termed a "Smart Cartridge." The Smart Cartridge will lyse and solubilize the input sample, purify DNA, and concentrate the purified DNA. The concentrated DNA will then be transferred automatically to NetBio's fully integrated microfluidic biochip for further DNA purification, DNA quantitation, STR amplification, and separation and detection. Figures 1 and 2 show schematics of the entire process, from evidence collection to generation of an STR profile.

The specific objectives of this research were to:

Develop an evidence collection device that provides for ease of biological sample collection, protection, and documentation. This includes the evaluation and selection of a collection matrix that provides efficiency of biological sample collection over a wide range of sample types and compatibility with subsequent sample processing including sample lysis, DNA solubilization, and concentration in the Smart Cartridge, and also subsequent downstream

sample processing in the integrated microfluidic biochip. The evidence collection device must protect the sample for storage and transport and prevent sample-to-sample contamination.

Develop a Smart Cartridge that accepts the evidence collection device, performs processing steps, and transfers the resulting DNA solution to the microfluidic biochip. This sample processing cartridge will incorporate sample lysis, DNA solubilization, and concentration. Appropriate mechanical and fluidic interfaces for coupling to both the evidence collection device and the biochip will also be incorporated.

B. Materials and Methods

1. Mock Casework and Reference Samples.

Buccal cell samples were obtained by moving the swabs up and down on the inside cheek of a human subject.

Fresh whole blood containing EDTA as anticoagulant was obtained on ice.

Dried blood samples on swab were prepared by allowing the blood to dry overnight.

Saliva was collected by expectorating into a 50 ml falcon tube.

Epithelial cell samples were collected by rubbing the swab heads on the palm and in between the fingers of a human subject.

A set of reference and mock casework samples was also prepared by Bode. These samples consisted o buccal, blood, dried blood, saliva, and epithelial cells that were collected and dried on SecurSwabs.

2. Vortex Extraction of DNA from Swabs.

Swabs were placed in a 2 mL microcentrifuge tube. The swab heads were separated from the shaft by cutting them off with scissors. 500 µL of NetBio lysis solution was added to the

microcentrifuge tube and the tube was vortexed for 5 seconds. The swab heads were manually removed using a pair of clean tweezers and discarded.

3. Mechanical Agitation for Extraction of DNA from Swabs.

A tube containing lysis solution was placed in a custom built mechanical agitation system. A SecurSwab was inserted into the agitation system and attached to the system by the swab cap. A miniaturized motor was used to generate vigorous vibrations and this vibrational energy is coupled to the swab tip through the cap and shaft. Mechanical agitation was applied immediately after insertion into the system.

4. Microfluidic Biochip Purification.

The microfluidic biochip contains a purification filter for DNA binding sealed within a chamber. Two microfluidic channels lead to the chamber, one to allow flow of reagents to the filter and the other to remove reagents from the filter. The lysis solution mixture was loaded into the input port and pneumatically driven through the purification filter. This was followed by pneumatically driving wash buffer through the filter. Bound DNA was eluted from the filter by pneumatically driving elution buffer through the filter.

5. Automated Microfluidic Extraction and Purification of DNA from Swabs in Smart Cartridge.

The Smart Cartridge is comprised of reagent chambers for holding preloaded solutions and process chambers. One chamber is used to hold the cotton swab with the DNA sample; four of the chambers are prefilled with purification reagents. The final two chambers are used for holding solutions during the process. The SC biochip is comprised of a network of channels,

chambers, flow control elements, and a purification filter. An automated script pneumatically manipulates solutions within the SC to extract DNA from a cotton swab that is inserted.

6. STR Amplification Reaction.

Multiplex PCR reactions were performed with the AmpFℓSTR® Identifiler® PCR Amplification Kit (Applied Biosystems, Foster City, CA). The 7 µL PCR reaction and cycling protocol was prepared as described in (Giese 2009). Amplification of 16-sample in the microfluidic biochip was completed in approximately 17 minutes and the amplified products were manually retrieved from the individual.

7. STR separation and detection instrumentation

Amplified products were separated and detected using Genebench-FX. This instrument was developed and optimized specifically for STR analysis.

V. Dissemination Strategy

The results of this research have been disseminated as follows:

Presentations. This work has been presented in an oral presentation entitled "Sample Collection System for DNA Analysis of Forensic Evidence" at the American Academy of Forensics Scientists 62nd Annual Scientific Meeting in Seattle, WA on Feb 22-27, 2010.

During and after the course of the proposed work, we have discussed our progress and asked for suggestions and comments from several forensic scientists. We have maintained and expanded contact with the NFSTC and several state crime labs. Our hope is to continue developing a close relationship with these groups to ensure that our development goals are consistent with the needs of the forensic community.

VI. Implications for Criminal Justice Policy and Practice

The availability of an STR typing instrument that combines DNA extraction and purification, amplification, and separation into a single, easy to operate instrument would represent a substantial advance in forensic DNA analysis. Significant burdens in setting up and operating a forensic DNA analysis laboratory are the costs of dedicated rooms to prevent PCR contamination, automating the procedures (either through robotics or dedicated technicians), and validating and re-validating individual instruments and the entire series of laboratory processes. A fully integrated instrument has the potential to be faster, more sensitive, less susceptible to contamination, less costly, and less labor-intensive than currently available technologies.

In developing a fully integrated system for STR typing, evidence collection and initial sample processing are critical steps, and, as such, must be incorporated into any fully integrated microfluidic STR typing system. This research has resulted in the development of a practical system for DNA analysis of forensic evidence, a system that is faster and performs better than currently available technologies. The research represents an important step towards making fully integrated forensic DNA analysis a reality.

Finally, a fully integrated microfluidic STR typing instrument would offer forensic scientists new capabilities not possible with conventional capillary instrumentation. NetBio has already ruggedized Genebench-FX and demonstrated that it withstands the rigors of transport to and operation at the crime scene. Developing an evidence collection device and Smart Cartridge capable of collecting sample and processing DNA from a variety of biological fluids

associated with a variety of evidence will also contribute towards producing reliable DNA profiles in a cost-effective manner at the crime scene. The use of a field portable, fully-integrated DNA analysis instrument will allow rapid generation of a DNA profile which could directly affect the apprehension of a suspect and thereby reduce crime and improve the public safety while decreasing the time and costs of criminal investigations.

VII. Results and Discussions

A. Evidence collection media

The objective of this work was to determine the best matrix to collect and extract DNA from a variety of cells associated with different PCR inhibitors, variety of surfaces, and from varying cell numbers. A series of collection matrices consisting of natural fiber (cotton) and synthetic matrices (modified cellulose, foam, Nylon, Polyester and Rayon) were selected for evaluation. Four buccal samples were collected from each donor with each of the collection matrices. The collection matrices were assessed based on DNA collection and extraction efficiency, lysate retention volume, and use in forensic applications.

DNA collection and extraction efficiency. DNA was extracted and purified from each of the 112 samples using a set of reagents that were optimized for purification of buccal swab samples, compatibility with biochip DNA purification, and minimal process time. DNA yield measured by UV analysis showed that a wide range of DNA (300-1900 ng) was extracted per swab with high inter- and intra- donor variation. The average recovered DNA across showed that all swab types performed similarly (Figure 16).

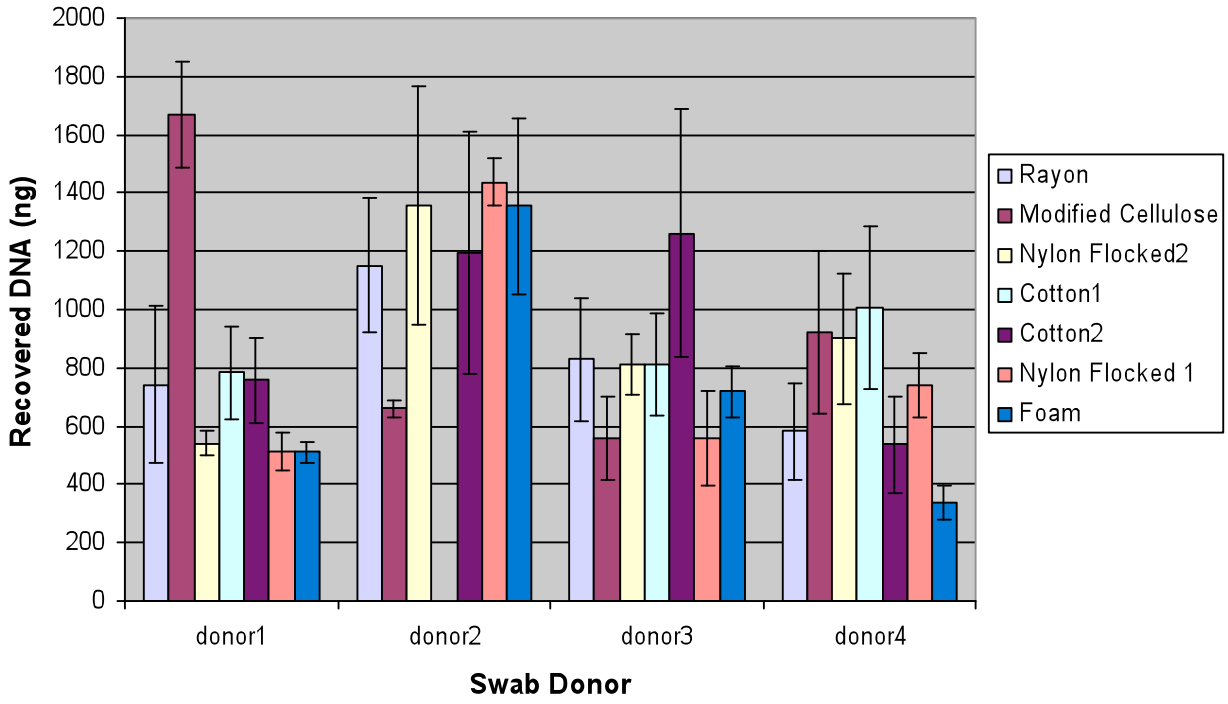

Figure 16. DNA recovered from various swab matrices.

Use in forensic applications. A survey of swab collection matrices used in forensic applications suggested that cotton was most commonly used for crime scene sample collection and for reference sample collection.

Cotton was the matrix selected for incorporation into the sample collection device because of its widespread acceptance and use within the forensics community and comparable extraction efficiency relative to other tested collection matrices.

Commercially available swabs with various swab head configurations were assessed for use in the fully integrated system. Ease of use for buccal cell collection was evaluated by having each donor swab the inside of the cheek with the candidate swab. Furthermore, each candidate swab was also used to collect mock crime scene samples with the candidate swabs. Subjective feedback from buccal cell donors and crime scene collectors were evaluated to establish the

SecurSwab (Bode) as the easiest, safest, and most convenient for use; the SecurSwab was designed for collection of DNA samples or forensic evidence and is easier to open, hold and use.

The reproducibility of the evidence collector was evaluated by collecting 4 buccal swab samples from each of nineteen donors. Two swabs were collected from the left cheek and two from the right cheek. DNA from each swab was extracted and purified. Purified DNA was quantified by UV spectrometry. Data in Figure 17 shows that DNA yielded from the 76 swabs range from 200 ng to 1850 ng, and that the swab to swab variation (%CV) for the 76 samples was 42%. The coefficient of variation (%CV) within each donor ranges from 20 to 65%. These results show that DNA collected from buccal swabbing is highly variable, however, the minimum amount that is collected by the selected evidence collection device is more than sufficient to generate the 1 ng of DNA require for STR analysis.

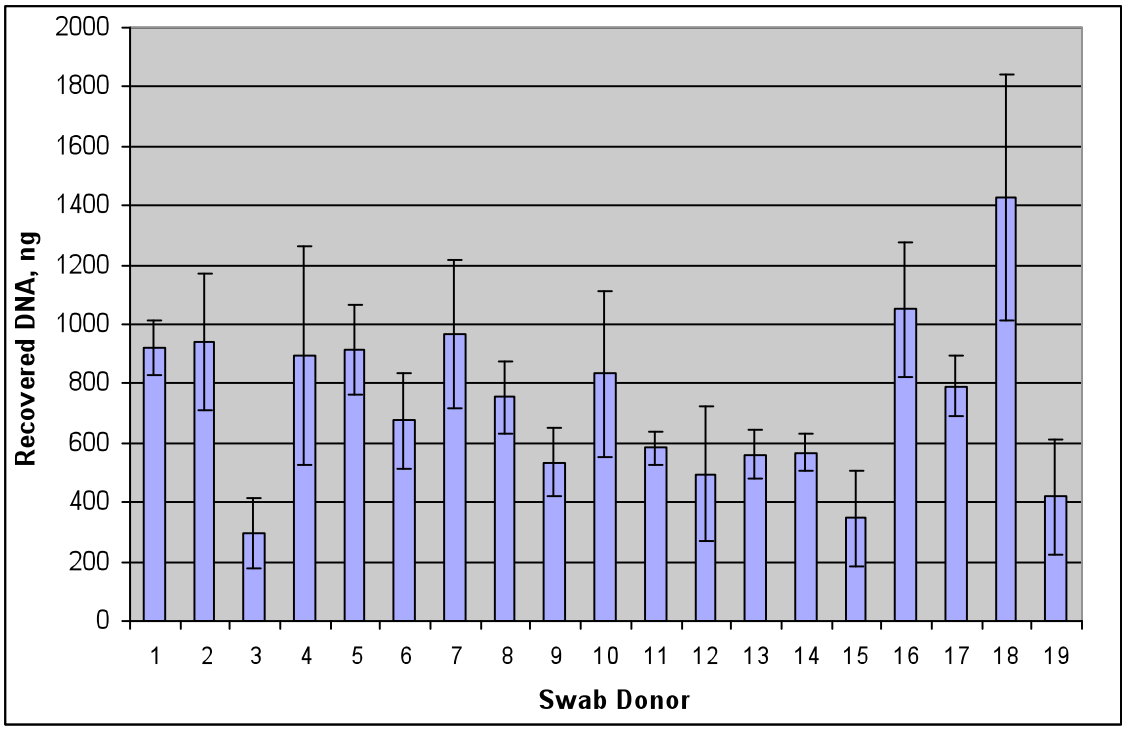

Figure 17. DNA yield from buccal swabs collected across 19 donors.

Figure 18 shows the results of an STR analysis generated by amplifying 1 ng of DNA template and the AmpFLSTR Profiler Primer set, and separating and detecting with Genebench. Template DNA was collected from buccal cells collected by the evidence collection device and extracted and purified with NetBio reagents following vortex extraction. The full STR profile that is demonstrated confirms the compatibility of the evidence collection device and the reagent set for STR analysis.

The performance of NetBio's microfluidic purification biochip was evaluated by generating sets of lysates from swabs, by vortex extraction, and purifying with NetBio's Microfluidic Biochip Purification protocol. The purified DNA was quantified and 1 ng of the purified solution subjected to amplification. The full STR profile (Figure 19) demonstrates the compatibility of the reagent set with microfluidic biochip purifications.

B. Chemical Lysis

Biological material collected with the evidence collector is lysed and DNA is solubilized within the Smart Cartridge. The solubilized DNA is then microfluidically transported for subsequent DNA purification. It is important to maximize the quantity of DNA recovered from the sample collection device, particularly in casework samples where the amount of sample collected may be limited.

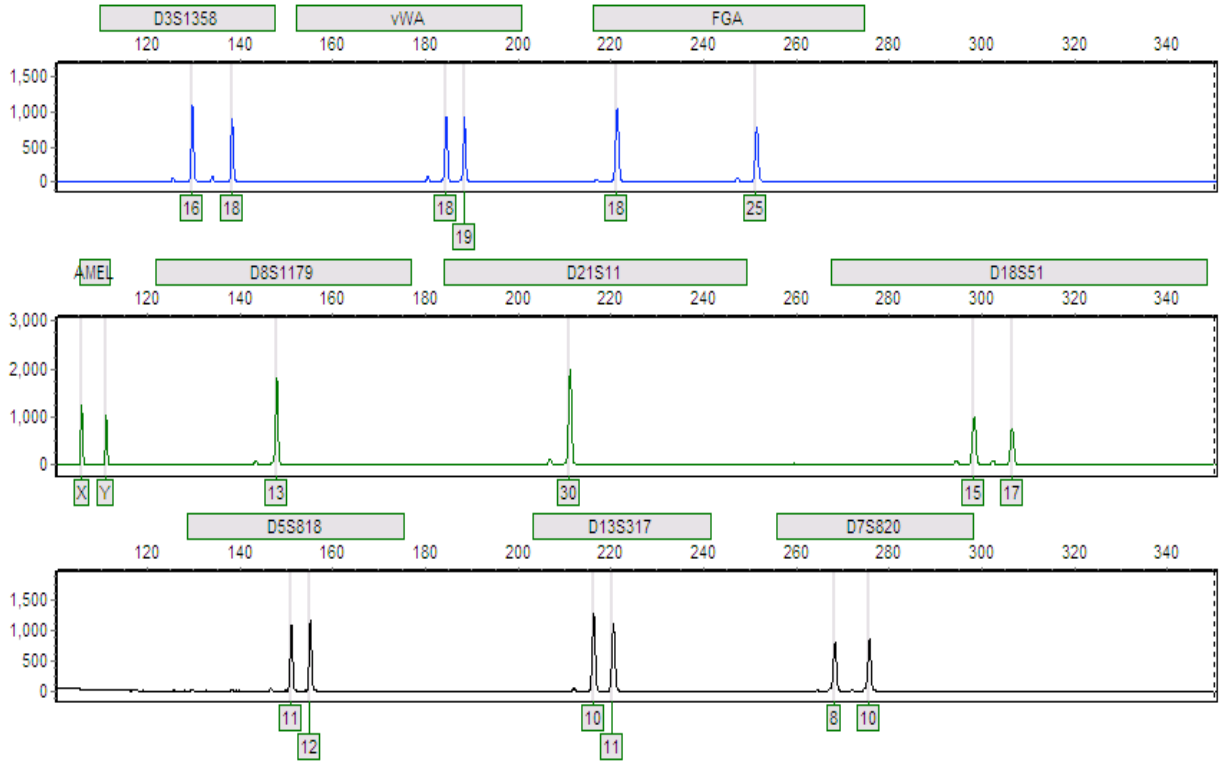

Figure 18. STR profile of DNA purified from buccal swab samples with NetBio's optimized reagent set following vortex extraction.

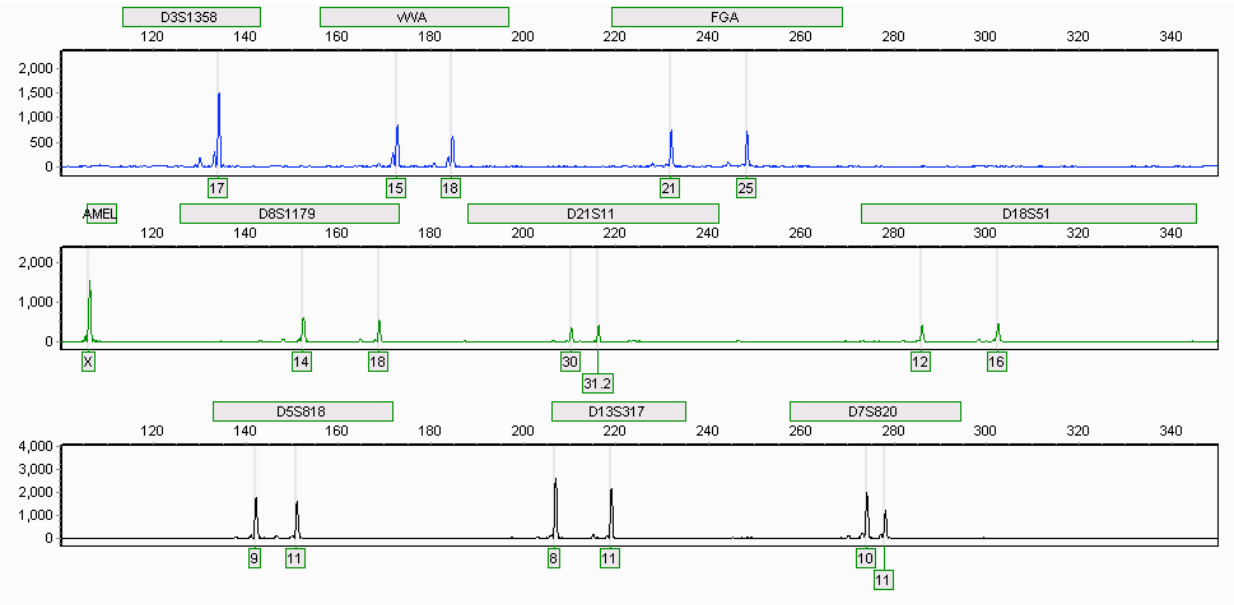

Figure 19. STR profile of DNA microfluidically purified in biochip from buccal swab samples with NetBio's optimized reagent ste following vortex extraction.

Initial work on cell lysis strongly suggested that chaotropic salts are effective lysis reagents and are compatible with microfluidic biochips. Optimization of the chemical lysis reagent components was performed to maximize DNA efficiency for sample types including blood, buccal, touch, and saliva samples, and to minimize process time. The optimization includes a systematic variation of the lysis reagent components, wash solution components, and elution buffer components. To minimize the number of iterations, an experimental matrix was developed to determine the effect of each component on DNA yield and purity, and an optimal reagent set. Three formulations (NetBio A, B and C) were evaluated by collecting 12 buccal swab samples from the same donor and purifying the DNA from the buccal swabs with each of the formulations. The DNA yielded was quantified by spectrometry shows that formulation C generates the highest average DNA yield of 900 ng compared with 850 ng and 650 ng for formulations B and A respectively.

C. Smart Cartridge Process Step 2: Selection of Silica Fiber Membrane

DNA Concentration and purification

The binding and elution properties of chaotropic salt/silica media systems allow DNA to be concentrated from relatively dilute lysates. An added advantage of using the bind/elute method for DNA concentration is the opportunity to wash the DNA while it is bound to the membrane, allowing for the removal of particulates and crude cell debris prior to elution. Silica based bind/elute media are available in several formats including silica fiber membranes and silica beads. Use of the membrane format requires the membrane to be positioned at a fixed site of the Smart Cartridge, with the attendant advantages of lower manufacturing cost and ease of containment of the silica media. The bead-based format provides the flexibility of

moving the beads to leave behind the debris associated with the collected samples and lysed cells. This format is relatively costly to implement, and the beads are somewhat difficult to contain. Accordingly, performance evaluations have been conducted on membrane based silica fiber media.

D. Smart Cartridge Development

An initial Smart Cartridge was designed and fabricated based on the optimized conditions discussed in the previous sections. This platform formed the basis for the evaluation and optimization of the three process steps in series. The initial Smart Cartridge was designed with the following features:

- Flexibility – ability to rapidly implement modifications to both the flow configurations and protocols
- Linked processing chambers – the lysis and DNA concentration are in direct communication.
- Optical imaging – the device functions with optical cameras to allow the research monitoring of reactions within each chamber and fluid flow within and between chambers.
- Fluidic drive – pneumatic and fluidic pumps are coupled to the SC to drive process fluids.
- Computer-controlled – the Smart Cartridge is computer-controlled to allow the automated execution of a process by script

A single sample smart cartridge design and associated extraction and concentration process flows has been developed. A rendering of a single SC (Figure 20) shows a macrofluidic component for accepting a Bode SecurSwab, storing reagents, and processing.

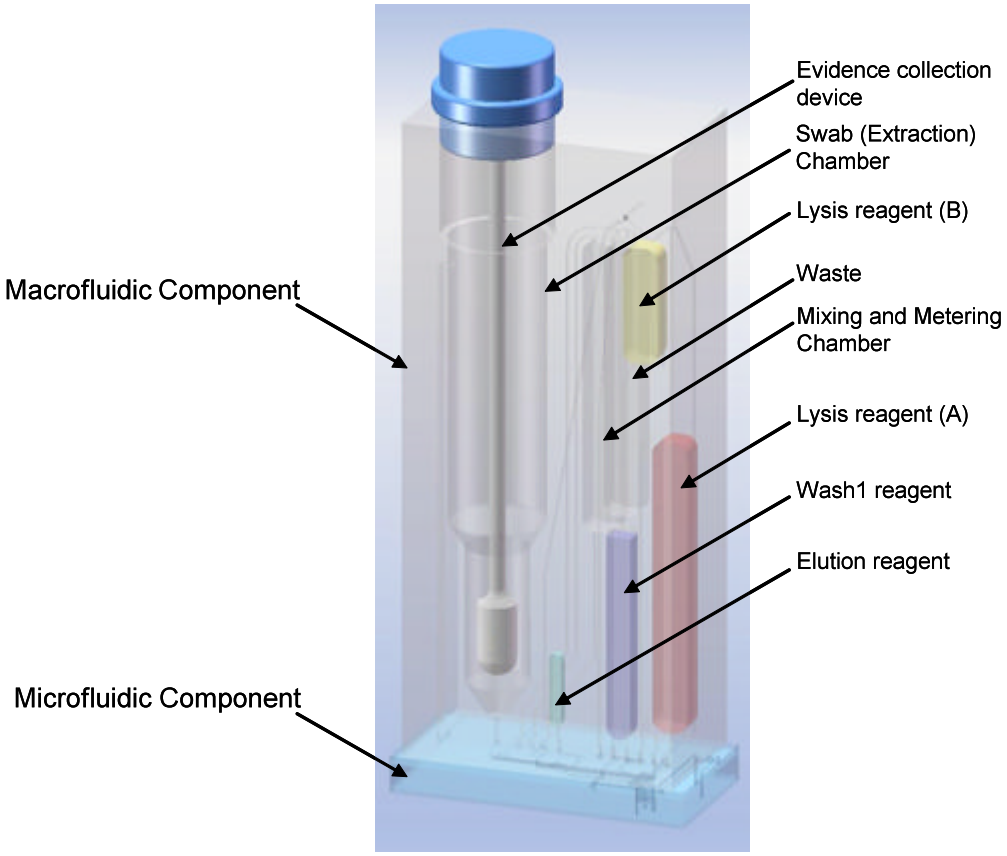

Figure 20. Rendering of a single sample smart cartridge.

E. Tandem Evaluation and Optimization of the Three Process Steps

The SC performance was characterized using buccal swabs. DNA from the process was quantified by UV absorbance shows that between 200 – 1100 ng of DNA is yielded from the 16 swab samples (Figure 21). This yield of DNA is comparable to manually processing and demonstrates the success of automated smart cartridge process.

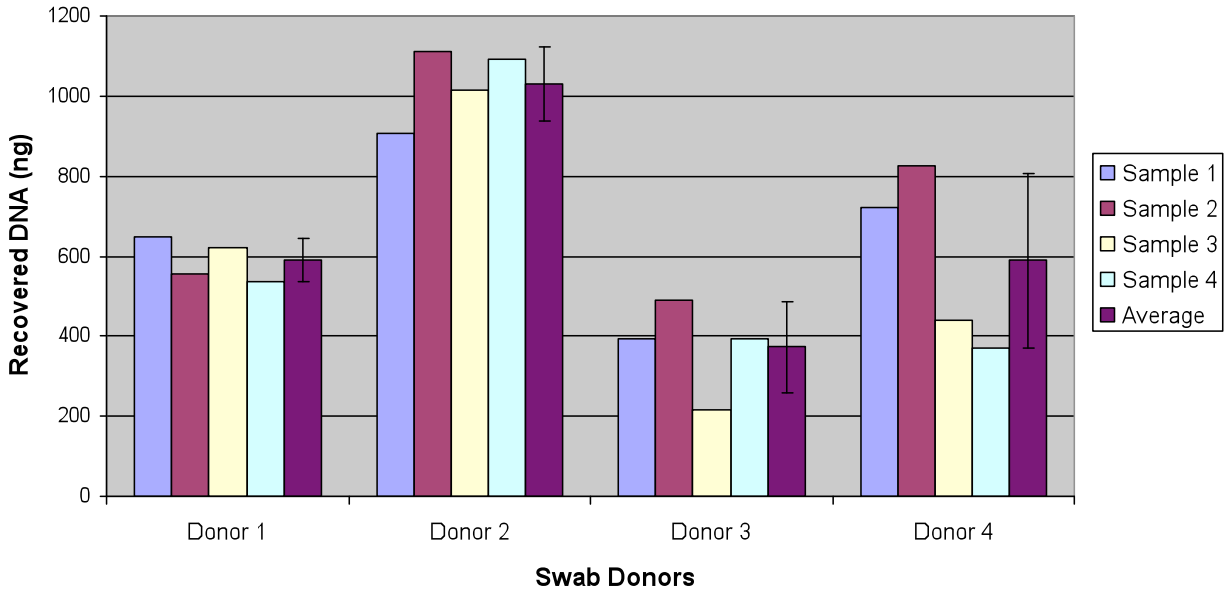

Figure 21. DNA extraction performance of the extraction component of the SC.

F. Testing of the Evidence Collection Device and Smart Cartridge

The ability of the completed evidence collection device and Smart Cartridge to collect samples and to perform the three process steps was tested. A series of mock casework samples were collected and processed as follows:

Whole blood. Two evidence collectors were used to swab the blood directly from a ceramic surface. One evidence collector was processed by automated script in the SC and the other was processed following a control Qiagen protocol. DNA from the SC process was amplified and separated and detected on Genebench. Figure 22 shows a full STR profile of the SC processed sample. This profile is similar to that generated with the control protocol and demonstrates the compatibility of the sample collection system for whole blood samples.

Dried blood. Two evidence collectors were wet with DI water and used to swab the dried blood from the surface. One evidence collector was processed by automated script in the SC,

and the other evidence collector was processed following the Qiagen protocol. DNA from the SC process and the Qiagen process was amplified and separated and detected on Genebench. Figure 23 shows a full STR profile of the SC processed sample. This profile is similar to that generated with the control protocol and demonstrates the compatibility of the sample collection system for dried blood samples.

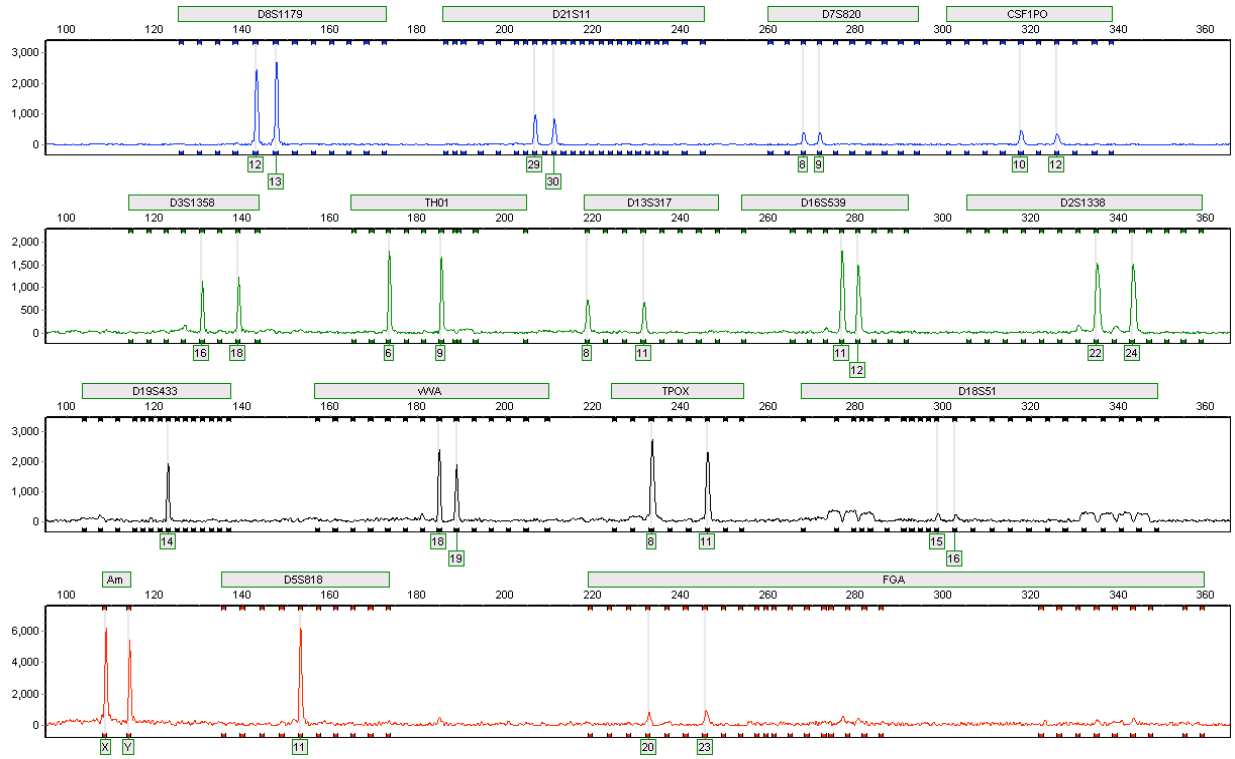

Figure 22. STR profile of DNA extracted and purified in the SC from whole blood.

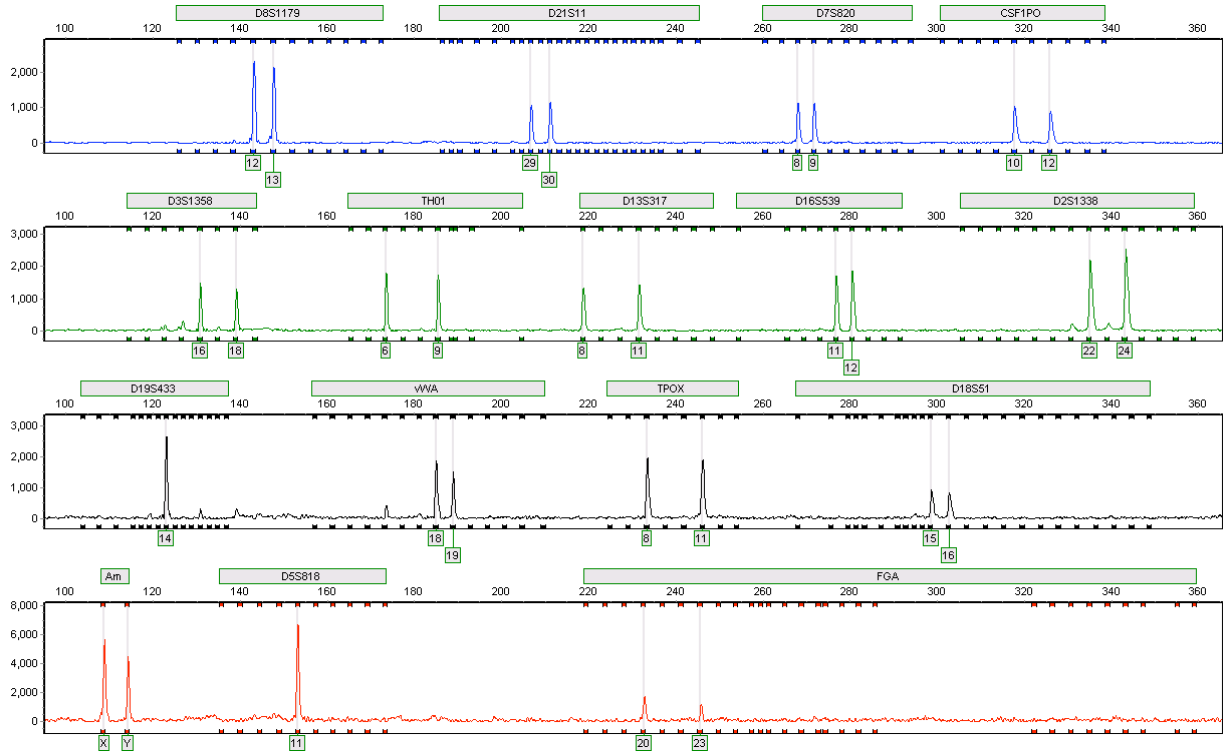

Figure 23. STR profile of DNA extracted and purified in the SC from dried blood.

Saliva samples. Pooled saliva samples were collected and spotted on a ceramic surface. Two evidence collectors were used to swab the saliva from the surface. The evidence collectors were then secured in their collection tubes overnight. One evidence collector was processed by automated script in the SC, and the other evidence collector was processed following the Qiagen protocol to serve as controls. DNA from the SC process and the Qiagen process was amplified and separated and detected on Genebench. Figure 24 shows a full STR profile of the SC processed sample.

Buccal samples. Buccal samples were collected by swabbing the inside of the cheek and then secured in their collection tubes overnight. One evidence collector was processed by automated script in the SC, and the other evidence collector was processed following the

Qiagen protocol to serve as controls. DNA from the SC process and the Qiagen process was amplified and separated and detected on Genebench. Figure 25 shows a full STR profile of the SC processed sample.

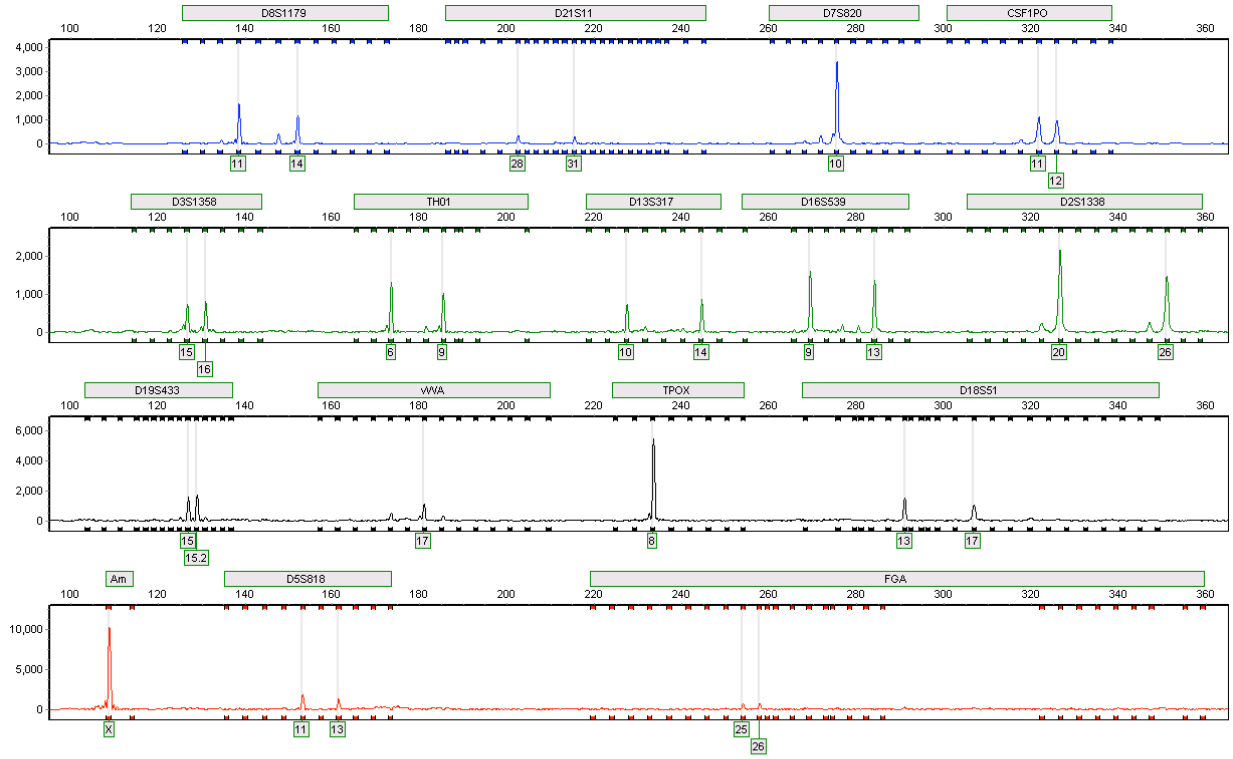

Figure 24. STR profile of DNA extracted and purified in the SC from saliva.

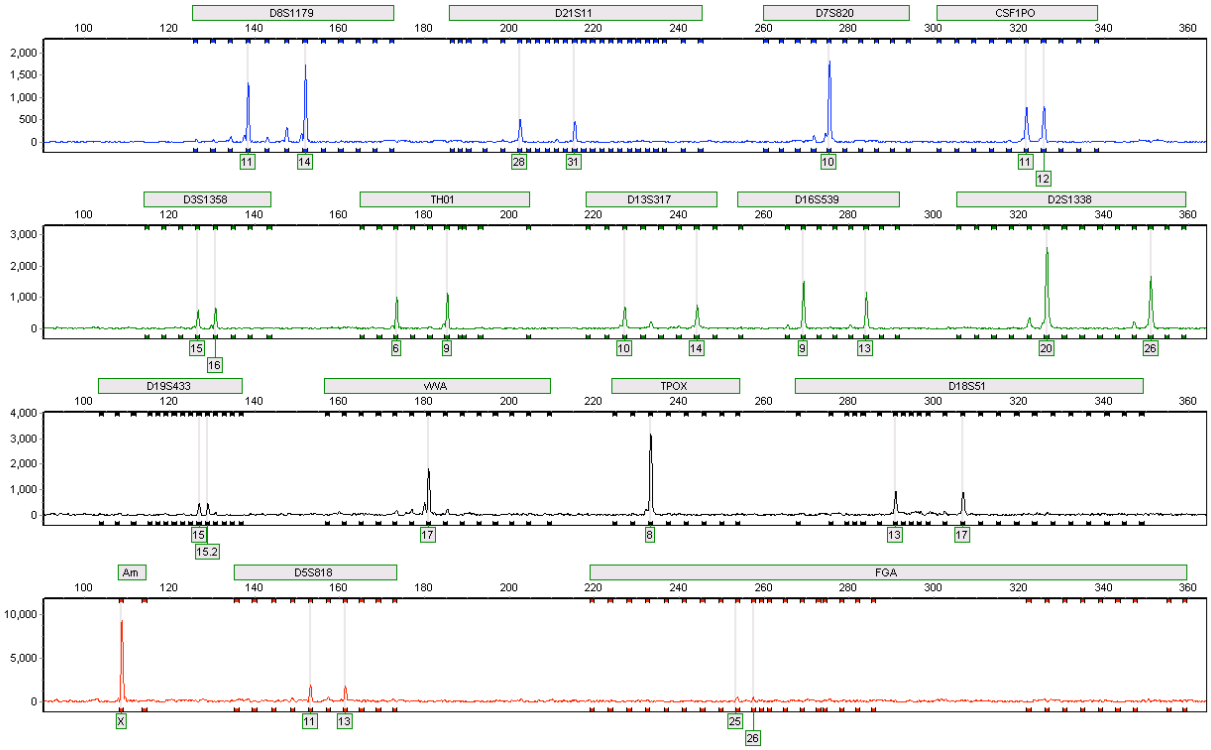

Figure 25. STR profile of DNA extracted and purified in the SC from buccal cells.

VIII. Conclusions

This report presents work demonstrating successful completion of all technical milestones of this research project.

An evidence collection device has been developed for ease of biological sample collection, protection, and documentation. The evaluation of variety sample collection matrices including cotton, modified cellulose, foam, nylon, polyester and rayon based on collection efficiency and current use in forensics applications resulted in the selection of cotton. Commercially available swabs with various swab head configurations were assessed for ease of use and resulted in the selection of the Bode SecurSwab for incorporation into the system.

A Smart Cartridge was developed to accept the evidence collection device, performs processing steps, and transfers the resulting DNA solution to the microfluidic biochip. The

optimization of a lysis and concentration reagent set, and incorporation of mechanical lysis resulted in the rapid and efficient lysis of buccal cell samples to yield 200 – 1400 ng of DNA. A Smart Cartridge was fabricated by initially designing and fabricating the macrofluidic and microfluidic components of the SC, and processing of buccal samples demonstrated that DNA of between 200 – 1100 ng of DNA is yielded by the automated SC process.

The system (evidence collector and SC) was tested by collecting mock crime scene samples including whole blood, died blood, saliva, and epithelial cell samples with the evidence collector and processing with the SC by automated script. STR profiles of DNA generated by the system were similar to those produced by conventional extraction protocols.

The results as a whole demonstrate the successful development of an evidence collector and Smart Cartridge capable of purifying DNA from a variety of sample types and substrates relevant to the forensics sciences community. The successful development of this module also represents the completion of another critical step towards the implementation of a fully integrated instrument that will generate an STR profile in 45 minutes from sample introduction with minimal operating requirements. The integrated instrument will process 16 samples in parallel and dramatically reduce the costs (including labor, space, and validation) of setting up and operating a DNA lab. The evidence collection device and Smart Cartridge are designed such that the system will be easy to operate and compatible with both forensic and microfluidic requirements.

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