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HEALTH SCIENCE CENTER AT FORT WORTH
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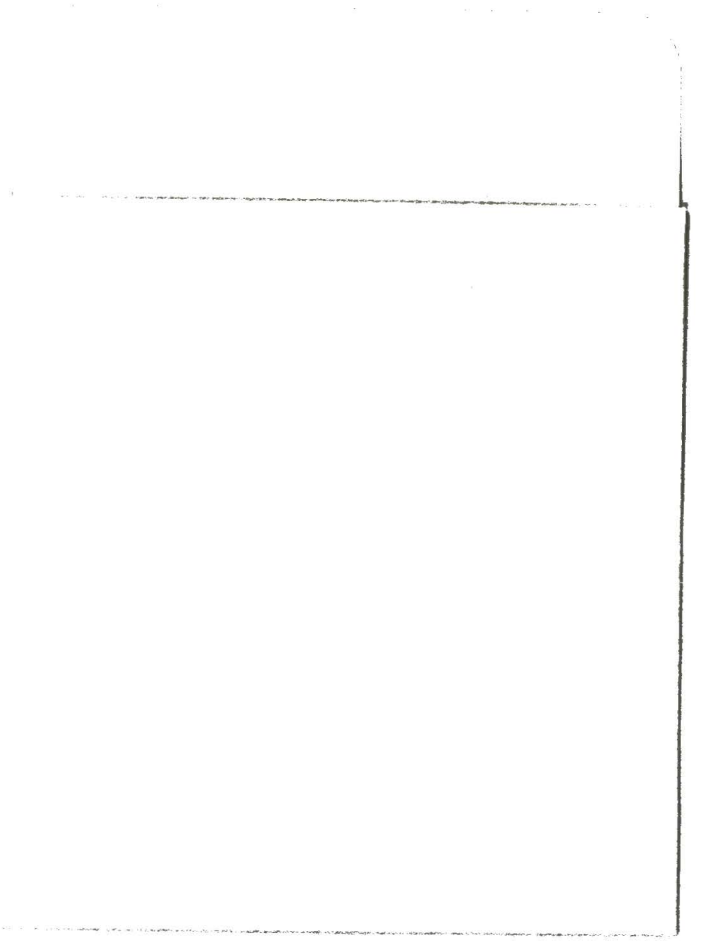

Aranda, Xavier G., Novel Methods For Processing Samples Stored Onto FTA® Paper. Masters of Science (Biomedical Sciences), May, 2003, 76 pp., 10 tables, 17 illustrations, bibliography, 30 titles.

The goal was to develop methodologies to facilitate the processing of samples stored on FTA® paper for paternity and forensic testing. The research was divided into three major areas. The first was an evaluation of the stability of the different FTA® matrices, and the development of a process which would maximize the amount of genetic information that could be obtained from a single 1.2 mm FTA® punch. The second was the development of a process for the efficient elution of the DNA trapped within the FTA® matrix. This would facilitate the use of FTA® technology with high throughput robotic processing systems. The third was the development of methodologies which would allow the integration of FTA® technology with a non-invasive buccal swab collection process. The methods developed from these studies are now used in the DNA Identity Laboratory at UNTHSC and many other laboratories throughout the world for routine casework analysis.

NOVEL METHODS FOR PROCESSING

BIOLOGICAL SAMPLES STORED

ONTO FTA® PAPER

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NOVEL METHODS FOR PROCESSING BIOLOGICAL SAMPLES STORED ONTO FTA® PAPER

THESIS

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For the Degree of

Masters of Science

By

Xavier G. Aranda, B.S.

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I would like to dedicate this thesis to my immediate and extended family. Without their encouragement and belief, my achievement could not have been possible. They were always ready and willing to help when I needed them the most. I say to everyone: "Ab uno disce onmes" – "From one learn to know all."

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INTRODUCTION

For many years, field biologists, whose research required the collection of blood samples, had to carry all of their phlebotomy supplies plus a means of preserving the samples in the field until they returned to the laboratory. Although this method of sample collection and short-term storage has worked, most have found it extremely cumbersome and usually not reliable for longer periods of time. An alternative method for the collection of blood samples has been utilized in neonatal screening programs throughout the United States for many years (Fingerhut et al., 1997). All newborns are required by law to undergo a series of diagnostic tests in order to detect genetic diseases such as Phenylketonuria (PKU) (Wang et al., 1997). A sample is collected by a heel stick and applied to a cotton paper card commonly referred to as a Guthrie Card (Makowski et al, 1996; McEwen et al., 1994). A similar method for the collection and storage of blood samples has been adopted by the Department of Defense at the Armed Forces Institute of Pathology (AFIP) (McEwen et al., 1994; McEwen, 1995). The United States Military made the decision to collect a blood sample from all personnel beginning in the late 1980's, to serve as a reference source of DNA to aid in the identification of human remains. Currently the AFIP DNA Repository has between 2 to 3 million samples from military personnel stored on bloodstain cards. The blood is spotted onto a cotton card designed specifically for the military. The card contains a unique barcode, a space for the individual's name, and a section comprised of cotton paper. This is the same cotton

paper used in neonatal screening programs (McEwen et al., 1994). The blood is spotted within target circles printed on the cotton paper, air-dried, placed in a nylon pouch with a desiccant, and sent to the repository. The drying conditions (time, temperature, and humidity), the potential biohazard exposure to the collectors, and the transport time may vary considerably. When the samples are received at the repository, they are removed from their transport packaging and ultimately repackaged in an ultra barrier pouch with a fresh desiccant. With the blood-stained cotton card in the pouch, the air is evacuated and the bag heat-sealed. The samples are then stored indefinitely at -20°C in large walk-in freezers (McEwen, 1995).

The preparation of the bloodstain cards may pose a potential health risk to those individuals involved in the overall process. Universal precautionary measures when followed in collecting and storing samples confer a high degree of protection for the collector and any individuals handling the bloodstain card (Allen et al., 1997). However, these measures are both cumbersome and complicated, and are often overlooked. In addition, universal precautionary measures are not always the most favorable to maintaining the integrity of macromolecules such as DNA. Thus, the protection and the long-term integrity of the blood stain/DNA sample may often be overlooked at the point of collection (Makowski et al., 1996). Depending on the volume of blood applied to cotton cards, varying lengths of time are required for their complete drying. During the drying period, it is possible to transfer pathogens from the bloodstain card to the sample prep personnel. Even after drying, cotton-based paper is potentially hazardous, as pathogens differ in their susceptibility to drying. Additionally, ambient temperature

fluctuations promote susceptibility of blood-stained cotton paper to microbial contaminants. Paper spotted in high humidity will dry considerably slower, thus facilitating microbial growth. The presence of these organisms can result in the degradation and loss of DNA (Makowski et al., 1996). Protocols for the collection and storage of biological samples need to accomplish multiple goals. Specifically they must address the security and integrity of both the handler and sample, while also facilitating a safe system for rapid DNA isolation and analysis (Allen et al., 1997; Caggana et al., 1998; Holden et al., 1996; Tsongalis et al., 1999).

In the late 1980's, Leigh Burgoyne from the School of Biological Science at Flinders University, Adelaide, Australia, developed a simple and more practical method of sample collection, storage, and transportation. He was able to combine the ease of use of bloodstain cards with a series of reagents that were designed to protect those individuals handling and processing the sample along with the DNA contained within the sample (Burgoyne et al., 1994). His invention has been given the name FTA® paper. FTA® paper is a cotton-based matrix that is impregnated with a unique, patented reagent mixture. The reagent mixture contains a strong buffer, protein denaturants, chelating agents, and an ultraviolet (UV) light absorbing free-radical trap. These reagents were chosen for incorporation into the cotton paper because each was known to catalyze a predictable reaction when in contact with biological samples. Once a biological sample is applied to the FTA® matrix, capillary action pulls the cells into the cotton lattice matrix, where the components of the reagent mix act upon them. A strong anionic detergent is responsible for the chaotropic properties of the FTA® paper. The detergent disrupts

cellular membranes, allowing for the release of the cell contents into the matrix. Simultaneously, most common blood-borne pathogens and heterotropic microorganisms are inactivated (Rogers et al., 1997; Rogers et al., 2000). As the nuclear envelope is disrupted, the genomic DNA explodes out, becoming tightly entangled within the matrix. A chelating agent within the paper neutralizes endogenous nuclease activity. A strong buffer allows for long-term storage of the sample by protecting the DNA from atmospheric acid deposition, UV damage, and free-radical damage. The use of FTA® paper eliminates the need for expensive sample storage equipment such as refrigerators or freezers (Burgoyne et al., 1994; Whatman BioScience, 2000). Samples can be stored and transported at ambient temperatures. In addition, it provides an efficient means of DNA isolation for PCR when compared to conventional techniques (Burgoyne et al., 1994; Whatman BioScience, 2000).

Traditional methods used for the isolation of DNA from blood or buccal swabs are often labor-intensive and utilize caustic organic solvents or expensive proprietary reagent systems (Eisenberg, 2000; Hoff-Olsen, et al. 1998; Klintschar et al., 2000). The purified DNA is usually stored at 4°C for short periods of time or at -20°C for more extended periods. The potential for sample switching or sample loss is significant with these DNA isolation methods. Centrifugation, vortex mixing, and boiling are the types of processes that make sample throughput difficult without the availability of a large staff of trained personal. In contrast, the purification of DNA from samples stored on FTA® paper is safe and rapid. Since the DNA is tightly bound within the matrix of the paper, inhibitors of the PCR process, such as heme, are easily removed by a simple wash

procedure. A small 1.2 mm punch, containing the biological sample is washed, dried, and added directly to the PCR reaction (Burgoyne et al., 1994; Caggana et al., 1998). The purified DNA remains bound within the punch during the amplification process.

Our laboratory specializes in the analysis of polymorphic regions of DNA for the purpose of parentage testing or human identification. The genetic loci that we analyze contain a Short Tandem Repeat (STR) sequence of DNA. STR loci consist of short repetitive sequence elements 3 to 7 base pairs in length. STR loci are well distributed throughout the human genome and are a rich source of highly polymorphic markers that may be detected using PCR (Budowle et al., 1997; Budowle et al., 1999; Entrala et al., 1999). Variations are detected based upon the length of the amplified products. Allele designations are based upon the number of repeats contained within the amplified region. For each STR loci, one of the PCR primers is labeled with a fluorescent tag to facilitate the visualization of the products. The amplified products are separated by electrophoresis on 4.9% denaturing polyacrylamide gels, and visualized on a Hitachi FMBIO® II Fluorescent Scanner.

Following amplification, the PCR product is in solution, and can be removed and placed in a new tube. If the FTA® process was used, the original 1.2 mm punch can potentially be washed to remove any residual amplification product. The punch can then dried and other loci can be amplified using the same DNA template bound in the paper matrix. However, the FTA® punch rapidly begins to deteriorate with additional physical manipulation, such as washing, and repetitive high temperature PCR cycling. Traditionally, FTA® paper has been manufactured with a cotton matrix similar to the type

used for the original Guthrie PKU card. Whatman BioScience has the ability to incorporate the FTA® Reagent into different types of matrices with varying tensile strengths (**Figure 1**). It is not known if a more rigid matrix will effectively retain the DNA template and how accessible it will be for the PCR process. In this study, different matrices will be evaluated for their reliability in the PCR process, as well as, their ability for repetitive re-amplification. A process will be developed that maintains the structural integrity of the FTA® punch, minimizes the loss of template DNA, and ultimately maximizes the amount of genetic information obtainable from a limited amount of biological sample (Lorente et al., 1997; Tsongalis et al., 1999).

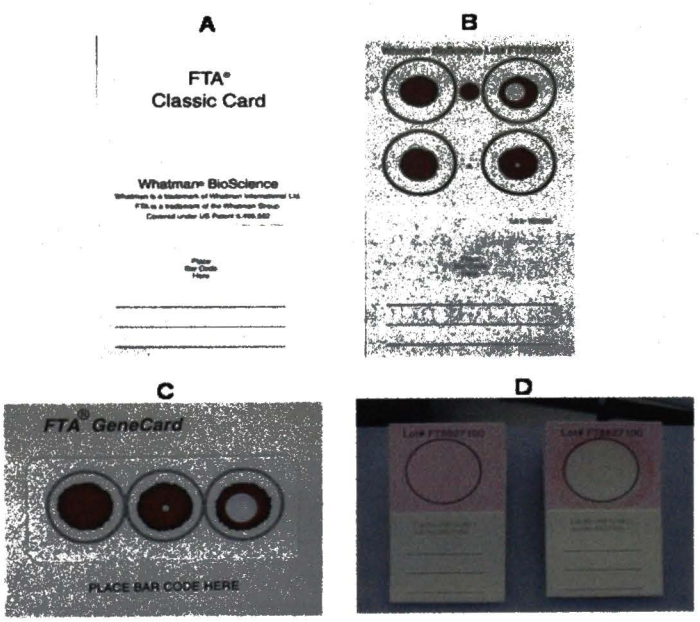


Figure 1. Whatman FTA® Card Formats. FTA® matrices of various tensile strengths with incorporated FTA® Reagent. 'A' represents FTA® Classic Card composed BFC 180 matrix. 'B' Aliquots of 25 μl of whole blood spotted within target circles of FTA® Classic Card. 'C' Aliquots of whole blood spotted within target circles of FTA® GeneCard composed of 31-ET matrix. 'D' Indicating FTA® Micro Cards. composed of 31-ET matrix before and after applying saliva. Each matrix type constructed differently creating unique tensile strengths.

Since the inception of the CODIS (Combined DNA Index System) program by the FBI (Federal Bureau of Investigation) in the United States, hundreds of thousands of samples have been collected from convicted offenders (Eisenberg, 1999). A large number of these samples have been stored on a variety of FTA® matrices. As more samples continue to be collected, the traditional manual processing of such a large number of samples becomes extremely laborious and time consuming. The most cost effective and efficient means of analyzing these samples will require the use of automation (Belgrader et al., 1995; Smit et al., 2000; William et al., Fitzco Inc.). High throughput systems usually incorporate a pipetting station along with a robotic arm capable of manipulating microtiter plates. Although automated punching devices are available, the manipulation of small 1.2 mm punches becomes difficult. The elution of DNA from the FTA® paper would greatly simplify the process of automation (Carducci et al., 1992; Smit et al., 2000). Whatman BioScience has generated electron micrographs demonstrating that the DNA trapped within the FTA® paper is tightly entangled within the fibers of the paper matrix **(Figure 2)** (Whatman BioScience, 2000)).

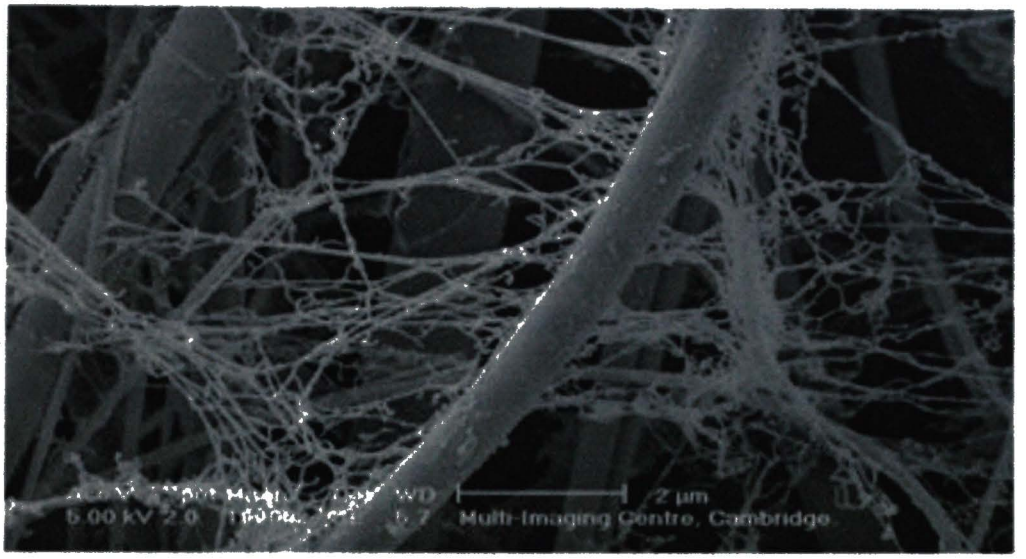


Figure 2. Electron Micrograph of DNA Trapped Within the FTA® Matrix. Cells lyse upon contact with the FTA® Reagent, releasing the intracellular contents into the matrix. The nuclear envelope is disrupted and the DNA explodes out of the nucleus becoming trapped with in the matrix lattice forming a 'web'-like structure.

Several methods, including boiling in water, a 5% Chelex solution, organic reagents, or other common methods used for DNA extraction, do not efficiently and reproducibly elute the DNA from the FTA® paper. Previous work in our lab has shown that the DNA can be released after digestion with the restriction enzyme *Hae* III (Rowan Campbell, unpublished results). However, when the *Hae* III restricted DNA is amplified with primers for the 13 core CODIS STR loci, 3 of the loci fail to amplify. These 3 loci each contain a *Hae* III restriction site between the primer binding sites, and therefore cannot be amplified. Based upon these observations, different methods for nicking or cleaving the DNA will be tested. Other restriction enzymes will be tested in order to determine if the DNA can be efficiently eluted and amplified at the 13 core CODIS STR loci.

The methodology used for the collection of biological material for paternity testing has changed significantly over the past few years. The most recent annual report of the American Association of Blood Banks has shown that over 500,000 buccal swab samples were collected in the year 2000 for parentage testing. This represented nearly 80% of all samples collected. Patients prefer the non-invasive buccal swab collection compared to traditional invasive venipuncture technique for blood. In addition, the conversion to a buccal swab collection procedure has begun to make significant inroads in the CODIS Offender sample programs in many states. Numerous types of swabs have been used to collect the buccal epithelial cells (Roger et al., 1997). They include cotton or dacron tipped swabs, pressed cotton paper resembling a serrated knife, cytology brushes, and sterile foam-tipped applicators in a variety of different sizes. After collection, the swabs are air-dried and placed in an envelope or other types of packaging for transport. Depending upon an individual's oral hygiene, the human mouth can contain a significant amount of bacteria. If the swabs are packaged while they are still moist, bacteria can grow and destroy the epithelial cell DNA (Hochmeister et al., 1998). Once received at the laboratory, DNA must be extracted from the swab device. The isolation of genomic DNA from buccal swabs is time-consuming, labor intensive, and does not lend itself easily to automation (Hoff-Olsen et al., 1999). The integration of a non-invasive buccal swab collection procedure with the storage and processing capabilities of FTA® paper would be highly advantageous. Buccal epithelial cells can be collected by vigorously rubbing the inside of both cheeks. The cells can then be manually transferred onto FTA® paper by blotting or rolling the swab over the paper

(Harty et al., 2000). The amount of cells that are transferred is variable, and is dependent upon both the type of swab used and the manner in which the individual has been swabbed. When working with blood, the number of white blood cells per mL typically varies by more than 2-3 fold in most adults or children. However, there can be considerable variability in the number of epithelial cells obtained by rubbing the inside of the cheek. Therefore, all aspects of the collection and transfer procedure must be optimized.

Using conventional FTA® matrices, it is impossible to detect where the transfer has taken place. Unlike blood, the buccal epithelial cells do not have a detectable color. To make the transfer process more efficient and reliable, we will evaluate the use of an indicator dye, phenol red, that can be incorporated into the FTA® paper. The indicating FTA® paper has a pinkish red color when dry, and will turn white where the moist buccal swab has made contact. A 1.2 mm punch taken from this area should have a much higher likelihood of containing sufficient DNA for amplification. Experiments will be done to demonstrate that the indicator dye will not inhibit the PCR process. Although several different types of swabs have been used for buccal collection, preliminary experiments have indicated that foam tipped swabs may be the most reliable. Each swab type will be evaluated for ease of use and reproducibility in the transfer and amplification process.

An alternative to the blotting or rolling method of transfer of epithelial cells to the FTA® paper will be evaluated. Following collection, the buccal swab device can be placed in a solution to elute the cells from the swab head. A solution can be developed to both elute the cells and prevent microbial growth during transport back to the lab. The

efficient entrapment of the DNA within the FTA® paper matrix appears dependent upon the addition of cells or intact nuclei. The nuclear membrane must rupture on the paper so that when the DNA explodes out of the nuclei it effectively entangles within the matrix. Therefore, the reagent must maintain the integrity of the cells or the nucleus. When the samples are received at the lab, the cells or nuclei can be concentrated by centrifugation, re-suspended in a small volume and directly applied to the FTA® paper for long-term storage or processing. The success of the transfer process will be evaluated through the amplification of the core 13 STR loci.

MATERIALS AND METHODS

Samples

Whole blood samples were collected in EDTA vacutainer tubes from volunteers within the DNA Identity Laboratory, Department of Pathology and Anatomy (UNTHSC). Following collection, a white blood cell count (CBC) was obtained for each sample using a Beckman Coulter Max M Cell Counter. A normal adult has a CBC between 5,000,000 to 10,000,000 white blood cells per mL or 5,000 to 10,000 white blood cells per mL. The CBC analysis was required to determine the total amount of DNA per volume of blood. It is estimated that there is approximately 7 *pg* of genomic DNA per white blood cell. Therefore, the theoretical amount of DNA applied to the FTA® paper is equal to the [CBC X the volume of blood (mL) X 7 *pg* (per white blood cell)]. On average, the theoretical amount of DNA contained within 25 mL of blood is 875 to 1,750 *ng*.

Buccal swab samples were collected from the same individuals who donated a blood sample. Additional buccal swab samples were obtained from individuals participating in paternity testing. A variety of buccal swab collection devices were used throughout this study. They included a sterile Cotton swab with a 6" plastic shaft (Catalogue #: 25-806 PC, Puritan Hardwood Products Company, LLC, Guilford, Maine); a sterile Dacron polyester-tipped swab with a 6" plastic shaft (Catalogue #: 25-806 PD, Puritan Hardwood Products Company, LLC, Guilford, Maine); a sterile Foam -tipped

swab with a 6" polypropylene shaft (Catalogue #: 25-1616 2PF, Puritan Hardwood Products Company, LLC, Guilford, Maine), the foam tip was similar in size to both the Cotton and Dacron tip; and a sterile, Round Foam-tipped swab (approx 29/32" wide by 1-1/8" long) with a 6" polypropylene shaft (Catalogue #: 25-1805 PF RND, Puritan Hardwood Products Company, LLC, Guilford, Maine) (often referred to as a paddle swab). The Round Foam-tipped swab was used primarily with the direct transfer of buccal cells to the FTA® paper matrix.

The buccal epithelial cell collection procedure was consistent for all buccal swab types. Each swab was carefully taken out of the packaging by grasping the plastic handle and the tip placed directly into the patient's mouth. The swab was vigorously rubbed against the inner cheek for a minimum of 20 up and down strokes per swab and the second swab used on the other inner cheek. During collection, the buccal swabs were used to soak up as much saliva as possible and then removed from the mouth. An identifying sticker was placed onto the plastic handle of the swab for referencing purposes and verification of collection.

FTA® Paper Matrices and Card Formats

FTA® paper is composed of a filter matrix which has been impregnated with a unique mixture of strong buffers, protein denaturants, chelating agents and UV absorbing, free-radical trap. The unique reagent formulation has been incorporated into several different types of filtration matrices. The matrix for the original commercially available FTA® paper was comprised of S&S 903 paper (Schleicher & Schuell BioScience, Keene,

NH). S&S 903 paper is comprised of a 100% pure cotton cellulose matrix. S&S 903 paper has been the standard specimen collection device used in newborn screening programs throughout the world. Several years ago, Whatman developed a cellulose matrix equivalent of S&S 903 which is referred to as Whatman BFC 180 (Whatman, Kent, United Kingdom). Whatman BFC 180 has also gained approval by the FDA as a collection device for newborn screening. Whatman acquired the rights to the FTA® technology and manufactured FTA® paper using their own proprietary matrices. For several years, Whatman BFC 180 was used exclusively in the manufacture of FTA® paper. Within the past few years, Whatman has manufactured FTA® paper utilizing a different cellulose-based filter matrix, 31-ET. The 31-ET pure cellulose filter matrix is thicker than BFC 180 and has the highest flow rate of all the Whatman cellulose-based papers. The 31-ET matrix has three times the tensile strength of Whatman BFC 180. Whatman has developed an Indicating FTA® paper, which incorporates a dye, phenol red, along with the standard FTA® Reagent mixture into the 31-ET matrix. The Indicating FTA® paper is used primarily with a buccal swab collection device. The indicating dye changes color from pink to white when in contact with a foam-tipped swab containing saliva and the buccal epithelial cells. In addition, Whatman has developed a FTA® paper referred to as Elute. The FTA® Elute paper utilizes a glass-fiber matrix as opposed to the more conventional cellulose-based filter matrix. The Elute paper is not commercially available at this time.

FTA® paper is available in a number of formats depending upon the specific application. Four card formats were used primarily for these studies. The Classic Card

was the original design and contains four target circles (Cat No. WB12 0205; Whatman, BioScience). Each target circle will hold approximately 125 mL of spotted blood. This card is used primarily for storage of blood from a single individual. The GeneCard was designed for automated processing using a robotic workstation (Cat No. WB12 0208; Whatman, BioScience). The card has three target circles, each will hold approximately 75 mL of spotted blood. The GeneCard has a cardboard frame surrounding the FTA® paper which allows for more rigorous handling. The Micro Sheet™ (renamed as the CloneSaver Card when impregnated with Indicating FTA® Reagent) was originally designed for high throughput processing of blood samples for PCR amplification (Cat No. WB12 0028; Whatman, BioScience). The card has 96 target circles, each holding approximately 12 mL of spotted blood. The Indicating MicroCard™ was designed for direct transfer of buccal epithelial cells (Cat No. WB12 0211; Whatman, BioScience). It has a single target circle and a color indicator to identify the area on the paper in contact with the Round Foam-tipped swab.

Application of Blood Samples to the FTA® Matrix

Blood samples were directly spotted onto the FTA® paper using either a micropipettor or a disposable transfer pipette. For the elution studies, 25 mL of blood was spotted directly onto a 7 mm punch of FTA® paper. Usually a 12 mL aliquot of blood was spotted within the target circles of the Micro Sheet™. Alternatively, a device known as a Diff-Safe® (Alpha Scientific; Malvern, PA) was placed into the rubber septum of the vacutainer tube. The Diff-Safe® was originally designed to make blood

smears on glass slides. The Diff-Safe® has a small needle which pierces the septum, and eliminates the need to remove the stopper of a blood tube. This provides an additional level of safety when handling blood samples. When the Diff-Safe® is pressed against one of the target circles on the Micro Sheet™, approximately 3-15 mL of blood is spotted onto the paper, **Figure 3**.

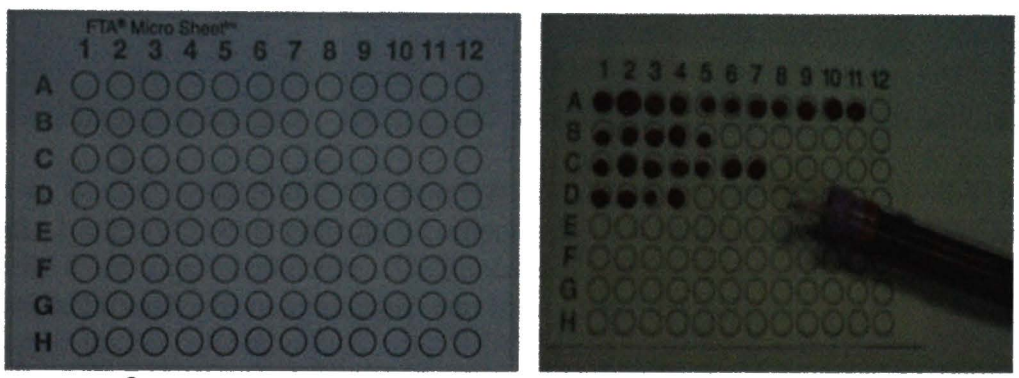


Figure 3. FTA® Micro Sheet™, Whatman 31-ET Matrix in a 96-well Format. FTA® Micro Sheets™ before and after spotting whole blood in the target circles using a Diff-Safe®.

Application of Buccal Swab Samples to the FTA® Matrix

Press and Roll Transfer

Buccal epithelial cells were collected by vigorously rubbing the inside of both cheeks using a sterile Round Foam-tipped swab (Paddle swab). The cells were then manually transferred onto FTA® paper by blotting or rolling the swab over the paper. Using conventional FTA® matrices, it is impossible to detect where the transfer has taken place. The region of the Indicating FTA® Micro Card™ changes from pink to white when in contact with the Round Foam-tipped swab containing the saliva and buccal epithelial cells.

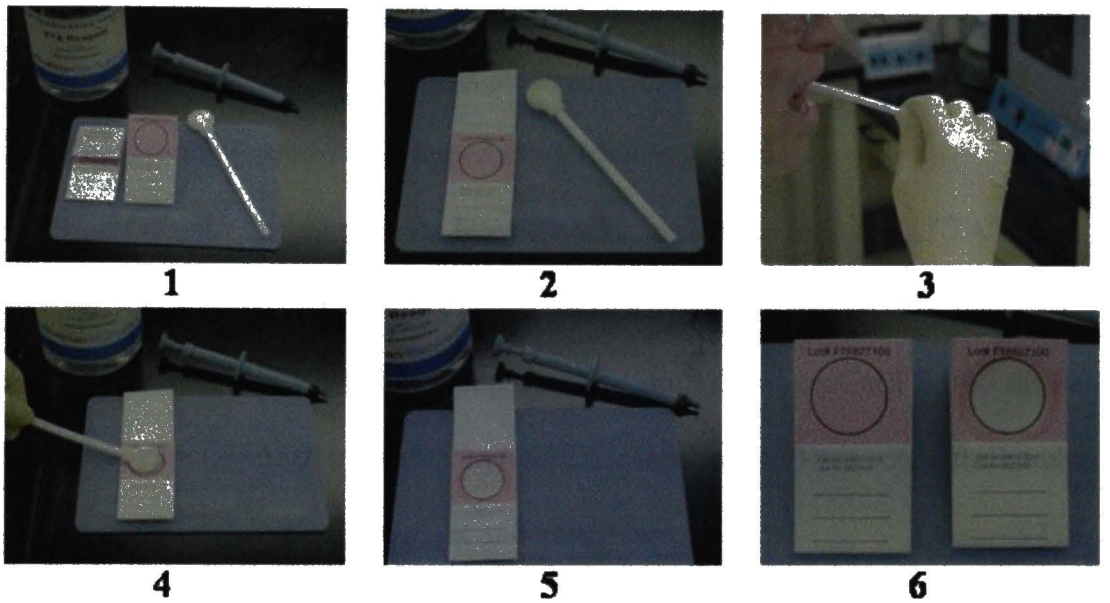


Figure 4. Buccal Epithelial Collection System Utilizing the Press and Roll Transfer Method. 1 = FTA® buccal collection components. 2 = Indicating FTA® Micro Card™ and sterile foam tipped applicator. 3 = Buccal collection using sterile foam applicator. 4 = Buccal cell transfer from applicator onto Indicating FTA® Micro Card™. 5 = Transferred buccal cells onto Indicating FTA® Micro Card™. 6 = Indicating FTA® Micro Cards™ before and after buccal cell collection and transfer.

The Indicating FTA® Micro Card™ makes it easy to visualize where the buccal epithelial cells have been transferred onto the paper. The dye is washed out during processing and will not inhibit the analysis of the immobilized or eluted DNA. The optimized procedure for the press and role method is as follows:

1. Taking care not to touch the foam tip, remove the sterile Round Foam-tipped swab from its packaging.
2. Grasping the plastic handle, place the foam tip in the mouth. Vigorously rub inside both cheeks for a minute (A minimum of 20 up and down strokes per each cheek. Use the opposite side of the sterile Round Foam-tipped swab for the

second cheek). Move the swab between the gum-line and fold of the cheek, and under the tongue, soaking up as much saliva as possible.

3. Remove the Round Foam-tipped swab from mouth.
4. Carefully lift the paper cover from the pink area of the Indicating FTA® MicroCard™. Press the flat circular swab head within the circle of the MicroCard™. Without removing the swab from the MicroCard™, roll the handle approximately 180° to the left and hold a few seconds. Then rotate the handle approximately 180° to the right and hold a few seconds.
5. Continue pressing until the circle is saturated with saliva and the pink area within the circle area turns white.
6. Discard the swab in an appropriate receptacle.
7. Do not touch the pink area of the MicroCard™. Allow the card to dry for at least one hour or overnight at room temperature.

In addition to the press and role procedure, a simplified press transfer was also tested. The press method is identical to the press and role with the omission of the rolling of the handle 180° to the left and right.

Buccal Cell Elution and Transfer

An alternative to the press and role (or simplified press) transfer of the buccal cells from the swab onto the FTA® paper was also evaluated. The buccal cells were first eluted from the swab in a buffer, pelleted by centrifugation, resuspended in a small volume, and spotted onto the FTA® paper. Two different reagents were tested for the

elution and resuspension of the buccal cells. Both solutions were isotonic to prevent the lysis of the cell and nuclear membranes. The first solution was composed of 0.9% NaCl and 1% Triton X-100, and the second solution was a commercially available reagent Isoton® III (Anhydrous Sodium Sulfate, NaCl, Procaine HCl, and Methylolurea; Beckman Coulter, Fullerton, CA). The Isoton® III reagent is a commonly used diluent in automated blood cell analytical instruments. The elution of buccal cells was evaluated with each of the four swabs previously described. A 1.0 mL aliquot of the reagent was added to a 2.0 mL Dolphine (nipple) microcentrifuge tube. The nipple design was useful in the centrifugation and resuspension of the epithelial cells. The swab head was submerged in the reagent for 15 seconds. By grasping the handle of the swab, the swab head was vigorously rubbed against the wall of the tube for an additional 15 seconds. The elution of the cells can be visualized as the solution becomes slightly cloudy. A 15 μL aliquot of Coomassie Blue (1 mg/mL) was added to the eluted cells. The tubes were centrifuged for 1 minute at 4,000 rpm in a microcentrifuge. The supernatant was decanted into a 10% bleach solution. The buccal epithelial cells were resuspended using a micropipettor in the residual reagent. A 15 μL aliquot was then spotted onto the target circles on FTA® Micro Sheet™. The FTA® Micro Sheet™ was air dried for a minimum of 1 hour prior to processing.

Sampling of Biological Material within the FTA® Matrix

In order to obtain a sample for DNA analysis, a punch and cutting mat have been designed to allow the quick and easy recovery of biological material trapped within the FTA® matrix. The Harris Micro Punch contains a sharp cutting edge which guarantees fast, precise punching and ejection, uniform sampling and no carry-over of paper from punch to punch. The cutting tip on the Harris Micro Punch is precision-tooled polished steel, that has been heat-treated, is rust resistant and case hardened. The tip is available in two sizes, 1.2 mm and 2 mm, **Figure 5**.

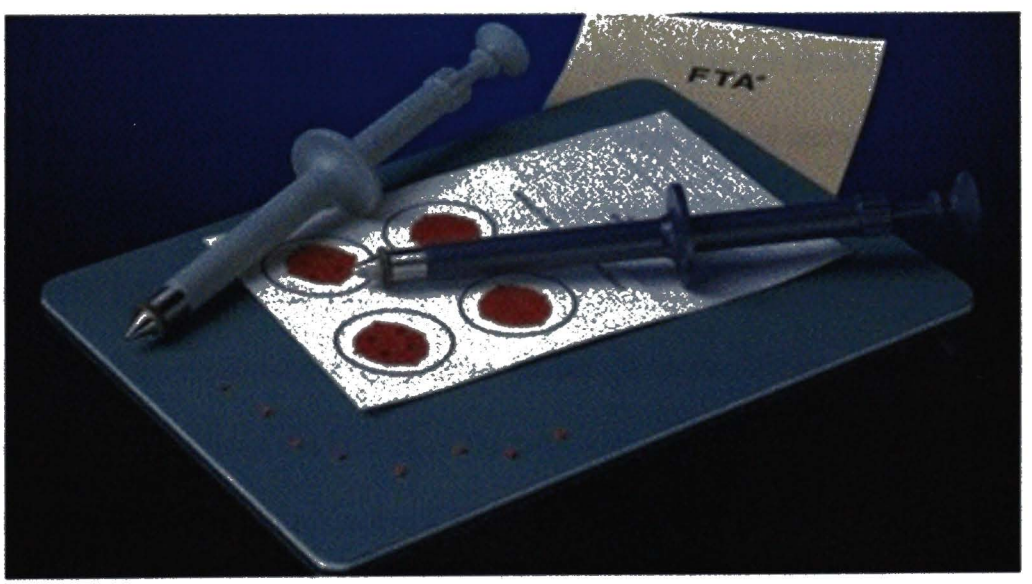


Figure 5. Harris Micro Punches, 1.2 mm and 2 mm, With Self-Healing Cutting Mat. The 1.2 mm punch is primarily used for PCR amplification of DNA entrapped with FTA® paper. The punches and the cutting mat are obtained from Whatman BioScience, Newton, MA.

The 1.2 mm punch has been used exclusively for PCR amplification studies (Catalogue Number WB10-0005, Whatman BioScience, Newton, MA). The Harris

Micro Punch is used in conjunction with a self-healing inert cutting mat that ensures clean sample cuts and protects and extends the life of the micro punch tip. A standard 7 mm hole punch was used to remove a sample of paper for the elution studies.

Preparation of DNA From Blood Samples Within the FTA® Paper Matrices for PCR Amplification

A simple washing procedure was utilized for the preparation of DNA from samples spotted onto the FTA® paper matrix for PCR amplification. For each sample analyzed, a MicroAmp® tube (200 μL capacity) was labeled. A 1.2 mm punch was removed from the FTA® Card using the Harris Micro Punch. A 100 μL aliquot of FTA® Purification Reagent (Catalogue Number: WB12 0204, Whatman BioScience) was added to each tube containing the FTA® punch. The tubes were capped and inverted repetitively for one minute. The tubes were then incubated at room temperature for three minutes. The tubes were then inverted for another minute. The FTA® Purification Reagent was carefully removed from the tubes using a disposable pipette. It is essential that the punches remain at the bottom of the tube when removing the FTA® Purification Reagent. A second 100 μL aliquot of FTA® Purification Reagent was added to the punches. The tubes were capped and inverted repetitively for a total of one minute. The tubes were then incubated at room temperature for three minutes and inverted for another minute. The FTA® Purification Reagent was carefully removed from the tubes using a disposable pipette. The punches were washed for a third and final time with 100 μL of FTA® Purification Reagent. During the third wash, the tubes were incubated for five

minutes at room temperature. The tubes were not inverted during the third wash in order to avoid the fragmentation of the FTA® paper punches. The FTA® Purification Reagent was removed and 100 μL of TE^{-4} (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) was added to the punches. The tubes were inverted several times and incubated at room temperature for 5 minutes. The TE^{-4} solution was removed with a disposable pipette and a second 100 μL aliquot of TE^{-4} was added to the punches. The punches were incubated at room temperature for five minutes. After the TE^{-4} was removed, and the tubes were placed in a 37°C incubator for two hours with the caps open to ensure adequate drying of the punches. The tubes were capped and immediately vortexed to ensure that the punches were not bound to the side of the MicroAmp® tubes (Eisenberg, 2000).

An alternative to washing individual 1.2 mm punches was to wash an entire card or FTA® Micro Sheet™ containing many different samples. If necessary individual blood spots were circled using a permanent marker to later identify their position after processing. The FTA® paper was placed into a small plastic tube (Hybriboat™ tray 5.2 x 3.7 x 1.45 inches, Life Technologies Inc. (Invitrogen™ Life Technologies)) and 35 mL of FTA® Purification Reagent was added. The tray was placed gently agitated on a shaker for 5 minutes. After the initial 5 minute incubation, the reagent was removed and discarded into a 10% bleach solution. An additional 35 mL of FTA® Purification Reagent was added and gently agitated at room temperature for 5 minutes. The wash process was repeated for a total of three washes. Following the last wash with FTA® Purification Reagent, 35 mL of TE^{-4} Buffer was added to the tray and was gently agitated for 5 minutes at room temperature. The wash with the TE^{-4} Buffer was repeated for a

total of three times. After the final TE^{-4} wash, the buffer was decanted and the FTA® paper was placed on a sheet of absorbent filter paper and dried in an incubator at 60°C for 10 minutes. After the paper was dried, it was kept at room temperature for 15 minutes. This helped reduce the amount of static electric charge after sampling with the 1.2 mm punch. As a result, the ability to eject the 1.2 mm punch into a 0.2 mL thin-walled MicroAmp® reaction tube was improved.

A vacuum assembly was created to facilitate the washing procedure for samples spotted onto the FTA® Micro Sheet™. The procedure utilized a Gast vacuum pump (Model 0211-V45N-G8CX), a vacuum manifold (Uni Vac 1, Whatman BioScience), and a modified Nunc Omni Tray (86 mm x 128 mm polystyrene, P/N 242811). The Nunc Omni Tray was reinforced with a piece of Plexi-Glass cut to the same dimensions. The Plexi-Glass was placed within the tray and fixed with rubber cement. A total of 96 holes (diameter of 1/32 of an inch) were drilled into the bottom of the tray. The modified Nunc Omni Tray was then placed on the vacuum manifold. A piece of gel blot filter paper (S&S GB 002) was cut to size to fit inside the modified Nunc Omni Tray and was moistened with 15 mL of FTA® Purification Reagent prior to use. The FTA® Micro Sheet™ was placed into a Hybriboat and 35 mL of FTA® Purification Reagent was added. The paper was incubated for 5 minutes at room temperature. The FTA® Micro Sheet™ was transferred to the vacuum assembly. The vacuum pump was set at 200 mm Hg. The FTA® Micro Sheet™ was washed under vacuum with 35 mL of FTA® Purification Reagent for a total of 3 times. After the FTA® Purification Reagent had been removed, the FTA® Micro Sheet™ was washed under vacuum a total of 3 times with 35

mL of TE^{-4} buffer. The vacuum washes appeared more effective at removing the heme from the paper. The FTA® Micro Sheet™ was placed onto a filter paper and dried at 60°C for 15 minutes.

Preparation of DNA from Buccal Swab Samples Transferred Onto FTA® Paper Matrices for PCR Amplification

DNA was prepared from buccal swab samples that were directly transferred to FTA® paper by either the press and roll or the simplified press method and were processed identical to that of blood samples. However, those buccal cell samples that were first eluted from the swabs, concentrated, and then spotted onto the FTA® paper had an additional step. These samples had an initial Proteinase K digestion step. The majority of these samples had been spotted onto the FTA® Micro Sheet™. The FTA® Micro Sheet™ was placed into the Hybriboat and 35 mL of FTA® Purification Reagent containing 10 μg/mL of Proteinase K was added and incubated at 65°C for 15 minutes. The reagent was discarded and the FTA® Micro Sheet™ was washed either manually or in the vacuum manifold as previously described for blood samples.

PCR Amplification

Following purification, amplification of DNA entrapped within the FTA® matrix was carried out utilizing a 1.2 mm punch. A 1.2 mm punch, containing the purified entrapped DNA, was placed into an individual 0.2 mL thin-walled MicroAmp® reaction tube or in a well within a 96-well Amp Plate (Harrison et al., 1998). The STR systems

amplified included the Promega GenePrint™ PowerPlex® 1.1, 2.1, 16 and 16 Bio Systems and the Applied Biosystems AmpF*l*STR® Profiler Plus *ID*™, COfiler™ and SGM Plus™ Systems. The amplification reactions were carried out with the manufacture's recommended conditions with the following exceptions: the Promega GenePrint™ PowerPlex® 1.1 and 2.1 Systems were carried out in a 10 μL reaction for a total of 26 cycles; the Promega GenePrint™ PowerPlex® 16 and 16 Bio Systems were carried out in a 12 μL reaction for a total of 28 cycles; the Applied Biosystems AmpF*l*STR® Profiler Plus *ID*™, COfiler™, and SGM Plus™ Systems were carried out in a 10 μL reaction for a total of 24 cycles. The amplification reactions were carried out in either a GeneAmp® 9600 Thermal Cycler (Perkin Elmer, Applied Biosystems), or the GeneAmp® 9700 Thermal Cycler run in 9600 emulation mode (Applied Biosystems, 1999). The amplification of the DNA eluted from the various FTA® matrices was performed using the same reaction volumes as previously described except 1 μL of the eluted DNA was amplified in place of the 1.2 mm punch. A positive control was included with each set of amplification reactions. Approximately 5 ng of K562 DNA or 9947A DNA was used as the positive amplification control. A negative amplification control was included with each set of amplification reactions. The negative amplification control included 1 μL of dH_2O in place of the 1.2 mm punch or the 1 μL of eluted DNA.

The Promega GenePrint™ Fluorescent STR MonoPlex Systems were used in the experiments to determine the durability of the FTA® punches with multiple amplification reactions. The individual MonoPlex Systems used for re-amplification experiments were those for the loci D16S539, D7S820, D13S317, and D5S818. A 10 μL reaction volume

was optimized for each MonoPlex System with a 1.2 mm FTA® punch (7.5 μL sterile dH_2O, 1μL STR 10X Buffer, 0.4 μL locus-specific 10X Primer Set, 1 μL BSA Fraction V (final concentration 160 μg/mL), and 0.08 μL Platinum *Taq* (GIBCO BRL INC., 5 units/ per μL). The DNA was amplified with the recommended conditions using the GeneAmp® PCR System 9600 Thermal Cycler, with the exception that a total of 26 cycles was used.

Stability of the FTA® Matrix After Re-Amplifcation

The PCR product was removed from the 0.2 mL thin-walled MicroAmp® reaction tube following the amplification with the first Promega Fluorescent STR MonoPlex System, D16S539. A 100 μL aliquot of ultra-filtered dH_2O was added to the 1.2 mm FTA® punch. The punch was gently washed with a micropipettor to remove any residual amplification product. The 1.2 mm punch was carefully transferred into a 1.5 mL tube. A 1 mL aliquot of TE^{-4} which had been previously heated to 95°C was added to the punch. The tube was gently agitated for a 1 minute. The TE^{-4} buffer was discarded and a second 1 mL aliquot of TE^{-4} buffer was added to the punch. The tube was gently agitated at room temperature for 2 minutes. The TE^{-4} buffer was discarded and the 1.2 mm FTA® punch was transferred into a new 0.2 mL thin-walled MicroAmp® reaction tube and allowed to dry at room temperature. The punch was then amplified for a second time with the next Promega Fluorescent STR MonoPlex System, D13S317. Following amplification the PCR product was removed and the wash procedure repeated. The re-amplification process was repeated with the MonoPlex Systems, D7S820 followed by

D5S818. Once the fourth amplification was completed, the cycle was repeated with the amplification of D16S539 locus. The PCR product after each successive round of amplification was visualized on a denaturing 4.9% polyacrylamide gel. The integrity of the 1.2 mm punch was recorded for each type of matrix.

Analysis of STR Amplification Products

The amplification products from the Promega GenePrint™ PowerPlex® 1.1, 2.1, 16 Bio, and the individual Fluorescent STR MonoPlex Systems were separated by electrophoresis on a 4.9% denaturing Polyacrylamide gel. A 3 μL aliquot of amplified product was mixed in a 0.2 mL MicroAmp® reaction tube with 3 μL of Bromophenol Blue / Xylene Cyanol sample loading buffer. The samples were heated for 2 minutes at 95°C and quick-cooled on wet-ice. The tubes were centrifuged at 3,000 x g for 30 seconds to bring the liquid to the bottom of the tubes. The samples were kept at 4°C until they were loaded onto the instrument. In addition to the amplified products, an aliquot of an allelic ladder specific for the Promega GenePrint™ System was prepared in a similar manner. A 0.2 mm mini-flex microcapillary flat tip was used to load a 4 μL aliquot of either sample or an allelic ladder into the wells of the 4.9% Polyacrylamide gel. The PCR products from the Promega GenePrint™ PowerPlex® 1.1 and MonoPlex Systems were separated on a 32 cm x 1 cm x 0.4 mm gel on a SA-32 Vertical Gel Electrophoresis Unit (Life Technologies, Gaithersburg, MD). The 32 cm gel was run in 1X TBE buffer at 2,000 volts, 300 milliamps, and 50 watts for 1.1 hours. The PCR products from the Promega GenePrint™ PowerPlex® 2.1 and 16 Bio Systems were separated on a 43 cm x 1

cm x 0.4 mm gel on a SA-32 Vertical Gel Electrophoresis Unit with the 11 cm extension plate (Life Technologies, Gaithersburg, MD). The 43 cm gel was run in 1X TBE buffer at 2,000 volts, 300 milliamps, and 60 watts for 1.5 hours. Following electrophoresis, the gel was removed from the apparatus, the gel spacers were carefully removed, and the glass plates were rinsed with dH_2O to remove any acrylamide from the surface of the glass plates. The gel plates were dried with and placed into either a Hitachi's FMBIO® II or FMBIO® IIe Fluorescent Scanner. The Promega GenePrint™ PowerPlex® 16 Bio System required the FMBIO® IIe Fluorescent Scanner for three-color detection. The scanning parameters were those specified in Promega's Technical Manual (TMD006). The Hitachi FMBIO® Analysis program (versions 6.0 or 8.0) was used for the visualization of the fluorescently labeled fragments.

The amplification products from the Applied Biosystems AmpF*l*STR® Profiler Plus™ *ID*, COfiler™, SGM Plus™ Systems and the Promega GenePrint™ PowerPlex® 16 System were analyzed on the ABI Prism® 310 Genetic Analyzer. For each sample, 23.5 μL of HI-DI Formamide (Applied Biosystems) plus 0.5 μL of ROX 500 (Applied Biosystems or ILS (Promega) internal lane standard was added to a new 0.2 mL MicroAmp® tube. A 1μL aliquot of the amplified products or allelic ladder was added to each tube. The samples were denatured for 5 minutes at 95°C in a GeneAmp® 9700 Thermal Cycler. The tubes were centrifuged at 3,000 x g for 30 seconds to bring the liquid to the bottom of the tubes. The samples were kept at 4°C until they were loaded onto the instrument.

The run parameters for the Applied Biosystems AmpF*l*STR® Profiler Plus™ *ID*, COfiler™, SGM Plus™ Systems on the ABI Prism® 310 Genetic Analyzer were set using the GeneScan Run Module F (GS STR POP-4 (1mL) F module) (Applied Biosystems, 1999). The default settings were used. The run parameters for the Promega GenePrint™ PowerPlex® 16 System on the ABI Prism® 310 Genetic Analyzer were set using the GeneScan® Run Module Z (GS STR POP-4 (1mL) Z module). The default settings were used. The analysis of the amplified STR products was performed using ABI GeneScan® Analysis software version 3.1.2 and Genotyper® software version 2.5.2 on a Macintosh operating system. The detection threshold was set at 150 relative fluorescent units (RFU).

Elution of DNA From FTA® Paper

Heat Elution

A 25 μL blood sample was spotted onto a 7 mm FTA® punch and dried at room temperature. The 7 mm sample punch was washed with 500 μL of FTA® Purification Reagent for five minutes at room temperature. A total of three washes with the FTA® Purification Reagent were followed by three washes with 500 μL of TE^{-4} buffer. Following the third TE^{-4} wash, 200 μL of either dH_2O or TE^{-4} buffer was added to the 7 mm punch. The microcentrifuge tube was placed in a 100°C heat block for 10 minutes. The microcentrifuge tube was centrifuged at 10,000 rpm for 2 minutes to pellet the residual paper debris. The supernatant containing the single-stranded DNA was removed and quantified using Molecular Probe's Oli-Green™ Fluorescent Assay.

Chelex Elution

A 25 μL blood sample was spotted onto a 7 mm FTA® punch and dried at room temperature. The 7 mm punch was placed into the basket portion of a Spin-EASE Extraction Tubes (Life Technologies Inc). The spin basket was placed into the microcentrifuge collection tube. The FTA® punch was pushed down to the bottom of the basket with the wide end of a small pipette tip. A 500 μL aliquot of FTA® Purification Reagent was placed on top of the FTA® punch in the spin basket. The FTA® Purification Reagent caused the punch to swell and create a seal within the spin basket. After 30 seconds the Spin-EASE Extraction Tube was centrifuged at 2,600 rpm for 30 seconds. The reagent was discarded into a 10% bleach solution. The process was repeated with an additional 2 washes with FTA® Purification Reagent. A 500 μL aliquot of TE^{-4} buffer was placed on top of the FTA® punch in the spin basket. After 30 seconds, the Spin-EASE Extraction Tube was centrifuged at 2,600 rpm for 30 seconds. The process was repeated with an additional 2 washes with TE^{-4} buffer. The FTA® punch was removed from the spin basket and placed into a 1.5 mL nipple tube. A 200 μL aliquot of a 5% Chelex-100 (Bio-Rad, Hercules, CA) solution was added to the punch. The tube was vortexed for 15 seconds and then placed in a 56°C heat block for 20 minutes. The tube was removed from the 56°C heat block and vortexed for 15 seconds. The tube was then placed in a 100°C heat block for 8 minutes. The tube was vortexed for 15 seconds and then centrifuged at 2,600 rpm for 10 seconds. The supernatant was transferred into a new 1.5 mL microcentrifuge tube, the FTA® punch is discarded. The tube was centrifuged at 14,000 rpm for 2 minutes to pellet any residual Chelex-100 resin and any of the punch

that may have been carried over. The supernatant was transferred into a new 1.5 mL microcentrifuge tube. Care should be taken not to remove any of the pelleted Chelex-100 resin. The tube containing the Chelex-100 resin is discarded. The single-stranded DNA was then quantified using Molecular Probe's Oli-Green™ Fluorescent Assay.

Restriction Endonuclease Elution

A 25 μL blood sample was spotted onto a 7 mm FTA® punch and dried at room temperature. The 7 mm punch was placed into the basket portion of a Spin-EASE Extraction Tubes (Life Technologies Inc) and washed with the FTA® Purification Reagent and the TE^{-4} buffer exactly as described for the Chelex elution procedure. Alternatively, an additional step between the FTA® Purification Reagent and TE^{-4} buffer was evaluated. A 500 μL aliquot of FTA® Purification Reagent containing Proteinase K (final concentration 100 μg/mL) was placed on top of the FTA® punch in the spin basket. The Spin-EASE Extraction Tube was incubated at 65°C for 1 hour. The Spin-EASE Extraction Tube was centrifuged at 2,600 rpm for 30 seconds. The washes with the TE^{-4} buffer proceeded as previously described. Following the last TE^{-4} buffer wash, a 50 μL reagent (40 μL of dH_2O, 5 μL of the specific 10X restriction digestion buffer, and up to 5 μL of a restriction enzyme) was placed on top of the FTA® punch in the spin basket. Thirteen restriction enzymes were tested: *Apa* I, *Bam* HI, *Dra* I, *Eco* RI, *Eco* RV, *Hae* III, *Hind* III, *Hinf* I, *Pst* I, *Kpn* I, *Sal* I, *Xba* I, and *Xho* I. The amount of restriction enzyme varied from 10 to 50 units. The FTA® punch was incubated for 30 to 120 minutes at

37°C. Following the restriction digestion, the Spin-EASE Extraction Tube was centrifuged at 13,000 rpm for 2 minutes. Approximately 50 μL of eluted DNA was recovered in the 1.5 mL collection tube. The spin basket insert containing the FTA® punch was discarded. The double-stranded DNA was quantified using Molecular Probe's PicoGreen® Fluorescent Assay.

DNA Quantitation

PicoGreen® Quantification of Double Stranded DNA

In order to determine the amount of eluted DNA following restriction endonuclease digestion, the PicoGreen® dsDNA quantitation reagent (Molecular Probes, 1996) was utilized. A series of known DNA quantitation standards were prepared through the dilution of a 100 μg/mL stock of Bacteriophage Lambda DNA. Lambda DNA was diluted in TE buffer (10mM Tris-HCL, 10 mM EDTA, pH 8.0) to a final concentration of 80 ng/μL, 40 ng/ μL, 20 ng/μL, 10 ng/μL, 5 ng/μL, 2.5 ng/μL, 1.25 ng/μL, 0.625 ng/μL, and 0 ng/μL. The assay was carried out in a 96-well, flat-bottomed, translucent microtiter plate (Whatman/Polyfiltronics). To each of the wells, either a 5 μL sample of the DNA standards or a 5 μL sample of the eluted DNA was added. After the standards and unknown samples were added to the appropriate wells, a solution containing 94 μL of TE buffer and 1 μL of PicoGreen® reagent was added. The samples were thoroughly mixed and allowed to incubate for 5 minutes in the dark. The microtiter plate was then scanned in a Molecular Dynamics Fluorimager (Molecular Dynamics). The Molecular Dynamics Fluorimager was calibrated at a power setting of 500V using a

530nm (D) wavelength filter. The densitometric analysis of the microtiter plate was performed using the ImageQuant™ 5.0 software package supplied with the instrument. The optical density (OD) values obtained for the DNA standards and the unknown samples were exported into a Microsoft Excel worksheet. The OD values from the DNA standards were used to generate a calibration curve. The calibration curve was then used to calculate the amount of amplified dsDNA product in each amplified sample.

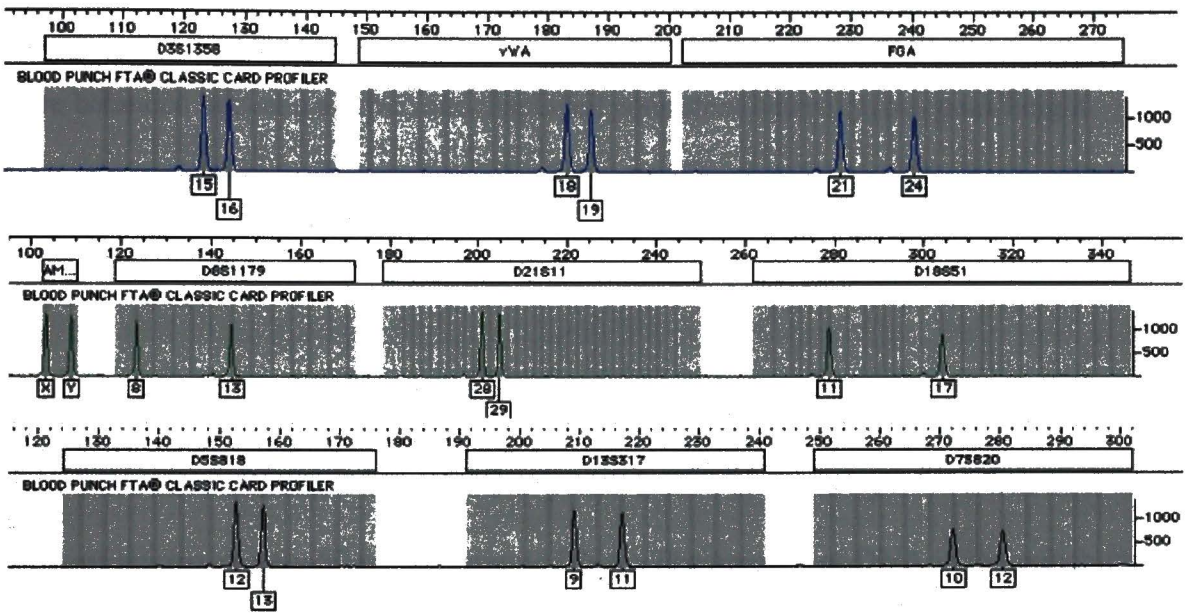
OliGreen™ Quantification of Single Stranded DNA

In order to determine the amount of DNA recovered following either the heat or Chelex elution procedures, the OliGreen™ ssDNA quantitation kit (Molecular Probes, 1999) was utilized. A series of known ssDNA quantitation standards was prepared according to the Molecular Probes protocol through the dilution of a 100 μg/mL synthetic single stranded 24-mer oligonucleotide M13 sequencing primer. The ssDNA oligomer was diluted in TE buffer and OliGreen™ reagent to a final concentration of 1 μg/mL, 500 ng/ μL, 100 ng/μL, 10 ng/μL, and 0 ng/μL. The assay was carried out in a 96-well, flat-bottomed, translucent microtiter plate (Whatman/Polyfiltronics). A 200 μL aliquot of each of the ssDNA standards was added to the appropriate wells in the microtiter plate. A 5 μL aliquot of the eluted ssDNA sample was mixed with a solution containing 95 μL of TE buffer and 100 μL of diluted OliGreen™ reagent was added. The samples were thoroughly mixed and allowed to incubate for 5 minutes in the dark. The microtiter plate was then scanned in a Molecular Dynamics Fluorimager as previously described in the PicoGreen® procedure.

RESULTS

FTA® paper is composed of a filter matrix which has been impregnated with a unique mixture of strong buffers, protein denaturants, chelating agents and UV absorbing, free-radical trap. The unique reagent formulation has been incorporated into several different types of filtration matrices. The matrices included the original S&S 903 paper, Whatman BFC 180, Whatman 31-ET, and a glass-fiber matrix referred to as Whatman Elute. In addition, Whatman has developed an Indicating FTA® paper, which incorporates a dye, phenol red along with the standard FTA® Reagent mixture into the 31-ET matrix. The various support matrices have varying fiber densities and tensile strengths. Blood samples were spotted onto each of these different FTA® paper matrices. The ionic detergent within the FTA® paper causes the lysis of the cell membrane, releasing the cell contents into the matrix. The nuclear envelope is immediately disrupted; the genomic DNA explodes out, becoming tightly entangled within the matrix. Since the DNA is tightly bound within the matrix of the paper, inhibitors of the PCR process, such as heme, are easily removed by a simple wash procedure. A small 1.2 mm punch, containing the biological sample is washed, dried, and added directly to the PCR reaction. The amplification of a 1.2 mm punch from each of the FTA® paper matrices was compared in order to determine if the matrix composition had any effect on the efficiency of the PCR process. The amplification of the punches was examined with several of the commercially available STR kits used for human identification.

Panel 1 Applied Biosystems AmpF*l*STR® Profiler Plus *ID*™ System



Panel 2 Applied Biosystems AmpF*l*STR® COfiler™ System

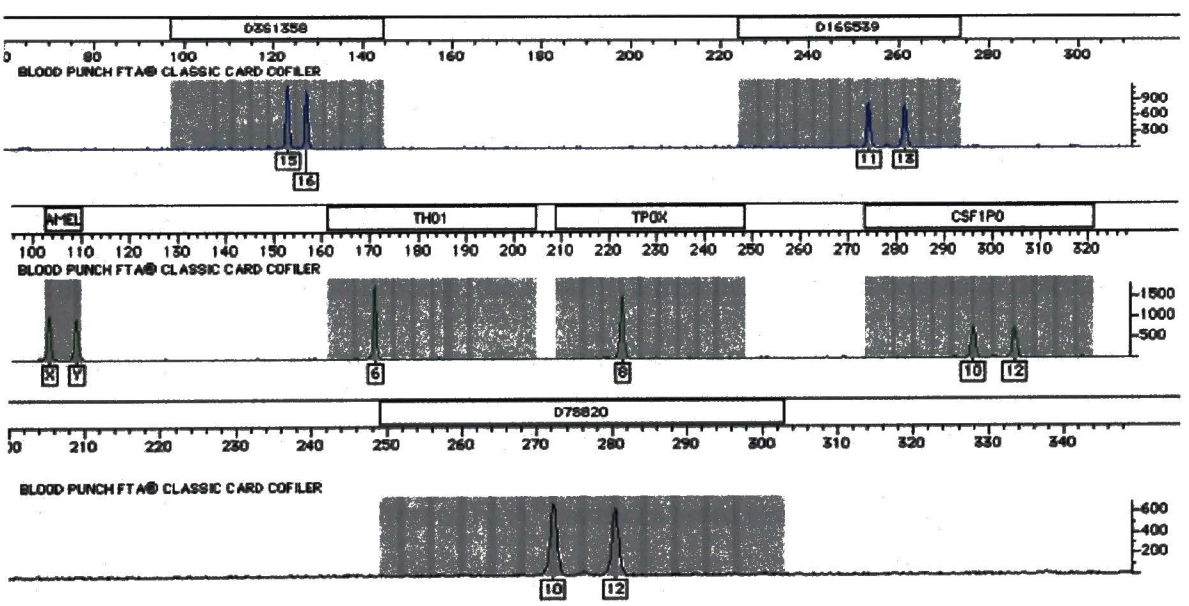


Figure 6. AmpF*l*STR® Profiler Plus *ID*™ and COfiler™ Amplification of a 1.2 mm Punch From a Blood Sample Spotted Onto a Whatman FTA® Classic Card. The punch was washed using the standard purification procedure. The PCR products from the Applied Biosystems AmpF*l*STR® Profiler Plus *ID*™ and COfiler™ Systems were analyzed on the ABI Prism® 310 Genetic Analyzer.

The results for each of the different matrices were identical. The STR amplification results were comparable to the highly-purified control DNA. The profiles were balanced between all loci, and displayed consistent heterozygous peak height balance within a locus. An example of the amplification of a 1.2 mm punch from a blood sample spotted onto a Whatman BFC 180 FTA® Classic Card is shown (**Figure 6**).

Matrix Stability

We have estimated that a 1.2 mm punch from a blood sample from a normal adult contains approximately 60 ng of genomic DNA. The amount of DNA in the punch far exceeds the recommended amount of DNA (0.5 to 2 ng) for the STR multiplex systems. The number of cycles used for amplification has been reduced on average four cycles for each of the Applied Biosystems AmpF*l*STR® and the Promega GenePrint™ PowerPlex® systems. The template DNA remains bound within the FTA® punch throughout the amplification process. However, we have observed that during physical manipulation and the PCR amplification process, the small 1.2 mm paper punch begins to deteriorate. As a result, the DNA template entangled within the matrix is gradually lost. The loss of DNA template would limit the amount of genetic information that could be obtained from an individual punch. The different FTA® matrices were evaluated in order to determine their stability during physical manipulation and repetitive PCR amplification reactions. A more resilient FTA® matrix would be advantageous for the storage and processing of a biological sample especially when the sample quantity is limiting. Blood samples from two different individuals were spotted onto the four different matrices. A 1.2 mm punch

from each person and from each matrix was processed as described in triplicate. The punches were amplified with the D16S539 Promega Fluorescent STR MonoPlex System. The PCR product was removed from the MicroAmp® reaction tube and was visualized on a denaturing 4.9% polyacrylamide gel. The punch was washed as described and re-amplified with the D13S317 Fluorescent STR MonoPlex System. This process was repeated with the D7S820 Fluorescent STR MonoPlex System followed by D5S818 system. The cycle was then repeated starting with D16S539 locus. An amplification reaction was successful if the entire profile for the locus was visualized. The results displayed in **Table 1** are the average of the three separate experiments. The Whatman 31-ET matrix proved to be the most successful throughout the re-amplification process.

	PCR Amplification Reactions
S&S 903	3
Whatman BFC 180	4
Whatman Elute	4
Whatman 31-ET	6

Table 1. Stability of Different FTA® Matrices After Re-Amplification. A 1.2 mm punch from a blood sample spotted onto each of the different FTA® matrices was amplified with the D16S539 Promega Fluorescent STR MonoPlex System. The PCR product was removed and was visualized on a denaturing 4.9% polyacrylamide gel. The punch was washed as described and re-amplified with the D13S317 Fluorescent STR MonoPlex System. This process was repeated with the D7S820 Fluorescent STR MonoPlex System followed by D5S818. The cycle was then repeated with D16S539. An amplification reaction was successful if the entire profile was visualized. The entire process was done in triplicate.

The Whatman 31-ET punch could be re-amplified a total of six times before the DNA template was either degraded or lost. The 1.2 mm 31-ET punch did not deteriorate as fast as the S&S 903 or Whatman BFC 180 cellulose based matrices. The 1.2 mm Elute punch was physically the most durable, however, the template DNA was apparently lost faster than the 31-ET matrix. The Elute punch was successfully amplified a total of four times. The 31-ET matrix is currently used for the manufacture of the different FTA® collection cards.

Elution of DNA From FTA® Paper Matrices

The simplified processing of the FTA® paper punches is advantageous when limited numbers of samples must be analyzed. The manipulation of individual 1.2 mm punches and/or the manipulation of numerous FTA® collection cards, such as the FTA® Micro Sheet™ does not lend itself to automation. The ability to elute the DNA trapped within the FTA® matrix would facilitate the use of high throughput automated robotic systems which have been designed for liquid handling. The efficiency of the elution procedure was assessed by quantitating the amount of recovered DNA. Based upon the CBC of the blood sample, it was possible to determine the theoretical amount of DNA applied to the FTA® matrix. An assumption was made that a 25 μL aliquot of blood would not saturate a 7 mm punch and that the lysis and the entrapment process was 100% efficient. A quantitation method specific for the structure of the DNA following the elution procedure was chosen. The amount of DNA recovered following either the heat or Chelex elution procedures was determined using the OliGreen™ ssDNA quantitation

kit. The OliGreen™ reagent does not exhibit fluorescent enhancement when bound to dsDNA. The PicoGreen® dsDNA quantitation reagent was used to quantify the DNA recovered following restriction endonclease digestion. Similarly, the PicoGreen® reagent shows a greater than one thousand-fold fluorescent enhancement when bound to dsDNA as opposed to ssDNA. The quality of the eluted DNA was verified through the amplification and detection of core 13 CODIS loci. The amplification of the 13 CODIS loci was verified with the multiplex amplification systems from both Promega and Applied Biosystems. This was necessary since the companies utilized different primer sets for the amplification of the CODIS loci.

Heat Elution

A simple heat elution method was evaluated first. Blood spotted on a 7 mm FTA® punch from each of the different matrices was purified as described in the materials and methods section. Following the third TE^{-4} wash, 200 μL of either dH_2O or TE^{-4} buffer was added to the 7 mm punch. The microcentrifuge tubes were placed in a 100°C heat block for varying lengths of time. The tubes were centrifuged and the supernatant was removed, and an aliquot quantified. The amount of DNA eluted using either the dH_2O or TE^{-4} buffer was extremely low with each of the four matrices. The amount of eluted DNA was typically less than 10% of the theoretical recovery. The eluted DNA was successfully amplified with each of the multiplex amplification systems. Although the process was inexpensive, the heat elution with either the dH_2O or TE^{-4} buffer was not pursued do to the poor recovery.

An alternative to the heat elution with either the dH_2O or TE^{-4} buffer was through the use of a Chelex-100 resin. Chelex-100 is a reagent currently used in many forensic laboratories to extract DNA specifically for PCR amplification from a variety of biological samples including whole blood or bloodstains. Chelex-100 is a synthetic resin, which sequesters excess divalent ions (Mg^{++}), which are required for nuclease activity and can also inhibit PCR amplification. The 7mm blood spots were processed with the FTA® Purification Reagent and TE^{-4} buffer as previously described. The FTA® punches were then transferred to a 2.0 ml nipple-microcentrifuge tube and 200μl of a 5% Chelex solution was added. The samples were processed as described in the materials and methods section. The ssDNA should be released into solution and any residual PCR inhibitors should remain bound to the Chelex-100 resin. The supernatant containing the ssDNA was removed, and quantitated using the Oli-Green™ Fluorescent Assay. The results of the Chelex-100 elution procedure are shown in **Table 2**.

	DNA Recovered ng/μL		Total DNA Recovered ng		Percent Theoretical Recovery (%)
	n=12		n=12		n=12
BFC 180	0.73		147		10.8
S&S 903	1.32		265		19.4
31-ET	1.09		217		15.9
Elute	1.08		217		15.9

Table 2. Elution of DNA Using Chelex-100 Resin. DNA was eluted from blood samples spotted onto 7 mm FTA® paper Punches from each of the four matrices. The amount of DNA recovered was quantitated using an OliGreen™ ssDNA quantitation kit on the Molecular Dynamics Fluorimager.

A 1 μL aliquot of the DNA eluted with the Chelex-100 resin was amplified. The 1 μL aliquot contained approximately 1 ng of ssDNA regardless of the matrix processed. The results of the Promega GenePrint™ PowerPlex® 2.1 system is shown in **Figure 7**. The amount of eluted DNA ranged between 10 and 20% of the theoretical recovery. The differences in the yield may not be significant due to the relatively small number of replicate samples. The recovery with the Chelex-100 resin was significantly better than the heat elution with dH_20 or TE^{-4}.

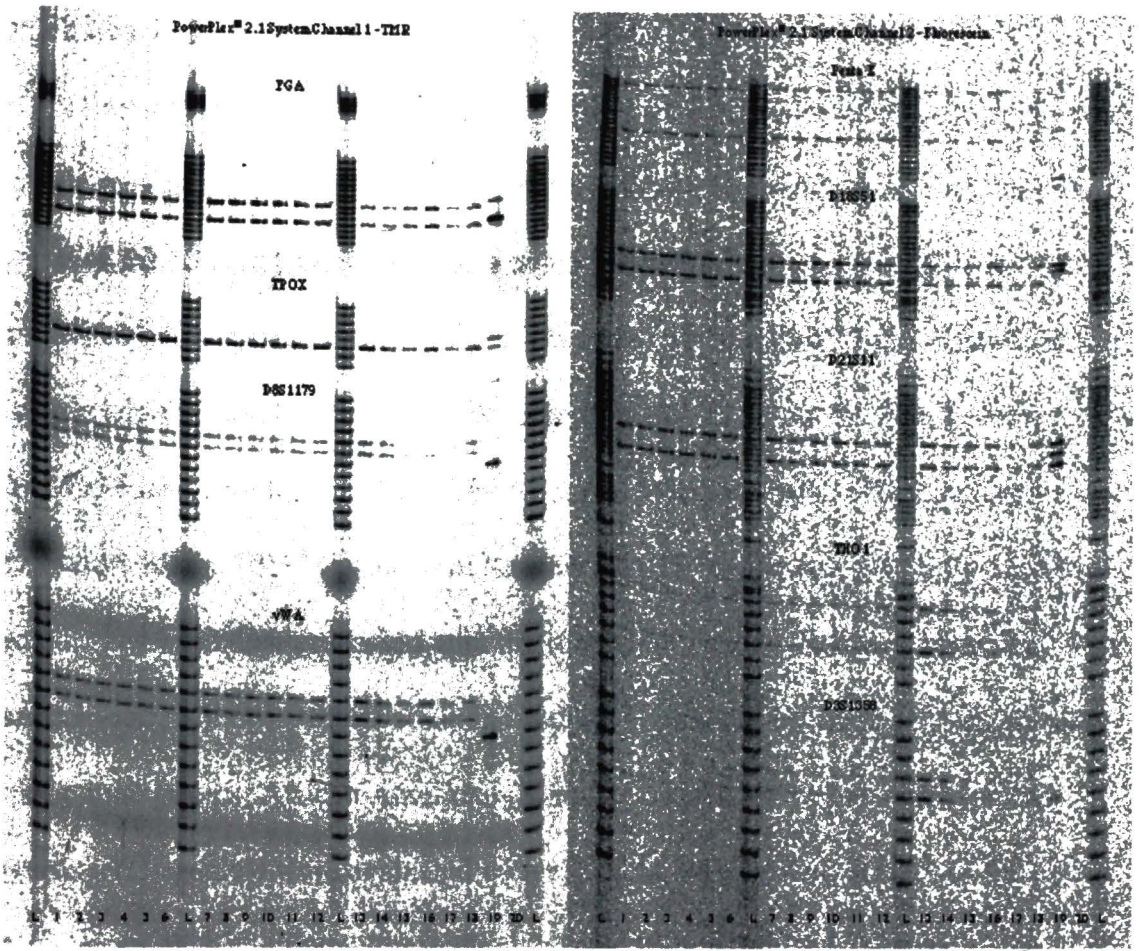


Figure 7. PowerPlex 2.1 Amplification of DNA Eluted From Various FTA® Matrices Following Chelex-100 Extraction. PowerPlex® 2.1 Fluorescent STR System was used to amplify 1 μl of DNA eluted with Chelex-100 from 7 mm punches spotted with blood. Lanes L contain PowerPlex® 2.1 Allelic Ladders. Lanes 1-3 BFC 180; Lanes 4-6 S&S 903; Lanes 7-9 31-ET; Lanes 10-12 Elute; Lanes 13 and 14 Control 1.2 mm FTA® paper Punches (BFC 180); Lanes 15-18 Chelex extraction of whole blood, and Lanes 19 and 20 Positive and Negative amplification controls.

All of the 13 CODIS loci were amplified from each of the four matrices. The gel image shows weak amplification of the Penta E, THO1, and D3S1358 loci. The same weak amplification signal was seen with the Chelex-100 and positive controls. The reduced amplification cycles used with 1.2 mm FTA® punches were not optimized for a 1 ng DNA sample.

Restriction Endonuclease Elution

Previous work in our lab has shown that the DNA can be released after digestion with the restriction enzyme *Hae* III (Rowan Campbell, unpublished results). However, several of the 13 core CODIS STR loci failed to amplify with the *Hae* III eluted DNA. The restriction digestion elution process was tested with total of 13 different restriction enzymes (*Apa* I, *Bam* HI, *Dra* I, *Eco* RI, *Eco* RV, *Hae* III, *Hind* III, *Hinf* I, *Pst* I, *Kpn* I, *Sal* I, *Xba* I, and *Xho* I,). The 7mm (FTA® paper, Whatman BFC 180) blood spots were processed with the FTA® Purification Reagent and TE^{-4} buffer as previously described. The punches were then processed with the restriction endonucleases as described in the materials and methods. A total of six individuals [2 individuals from each of the three major ethnic groups; African American (Black), Caucasian (White), and Mexican American (Hispanic)] were evaluated with each enzyme. A 1 μL aliquot of the eluted DNA was amplified with the PowerPlex® 1.1 and PowerPlex® 2.1 systems. The amplification products from DNA eluted with the following restriction endonucleases: *Hae* III, *Hind* III, *Bam* HI, *Eco* RI, *Pst* I, and *Xho* I are shown on **Figures 8 and 9**. As previously seen, the loci CSF1PO, TPOX, and THO1 failed to amplify with the *Hae* III eluted DNA. In addition, the PowerPlex® 2.1 system demonstrated the loss of the Penta E locus with the *Hae* III eluted DNA. Examples of weak amplification can be seen at the locus THO1 with the endonucleases *Hind* III, *Bam* HI and *Eco* RI; and the vWA locus with the enzyme *Xho* I. The restriction enzyme *Pst* I gave the most consistent results of the initial 6 enzymes tested. An additional set of 7 enzymes (*Apa* I, *Dra* I, *Eco* RV,

Hinf I, *Kpn* I, *Sal* I, and *Xba* I) were used to elute DNA from 7mm FTA® blood spots. A 1 μL aliquot of the eluted DNA was amplified with the PowerPlex® 1.1 and PowerPlex® 2.1 Systems.

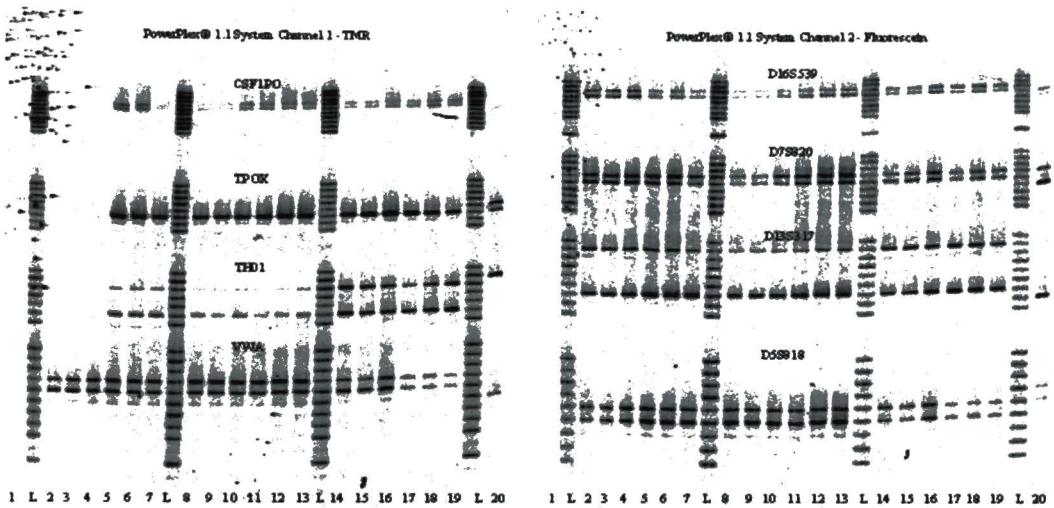


Figure 8. PowerPlex® 1.1 Amplification of DNA Eluted From FTA® Matrix (With Proteinase K) Following Restriction Digestion With Various Enzymes. PowerPlex® 1.1 Fluorescent STR System was used to amplify 1 μl of DNA eluted following a 2 hour digestion with 50 Units of various endonucleases from 7 mm punches (FTA® BFC 180 paper) spotted with blood (single individual). Lanes L contain PowerPlex® 1.1 Allelic Ladders. Lane 1 Negative Amplification Control; Lanes 2-4 *Hae* III Digestion; Lanes 5-7 *Hind* III Digestion; Lanes 8-10 *Bam* HI Digestion; Lanes 11-13 *Eco* RI Digestion; Lanes 14-16 *Pst* I Digestion; Lanes 17-19 *Xho* I Digestion; and Lane 20 K562 Positive Amplification Control.

The amplification products were analyzed on a 4.9% denaturing polyacrylamide gel (data not shown). The results for each of the 13 restriction endonucleases are summarized in **Table 3**. Each of the enzymes was evaluated with six individuals from the 3 major racial groups in order to identify any common restriction site polymorphisms that may exist. The presence (+) or absence (-) of an amplification product for each of the 13 core CODIS loci plus the Penta E locus was denoted. The band intensity (relative

amount of amplified product) at each locus was also evaluated. If a locus showed a strong band intensity a designation of ++ was given. A designation of +/- was used to indicate a reduced band intensity or the loss of one or both alleles in at least one of the six individuals tested.

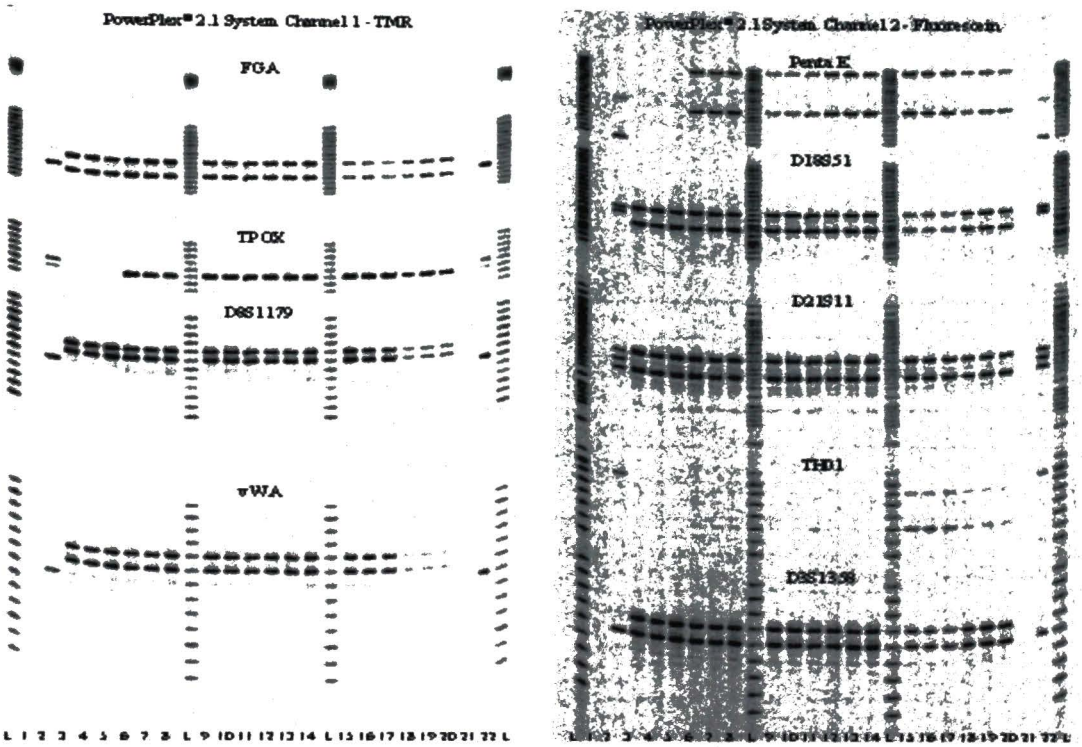


Figure 9. PowerPlex® 2.1 Amplification of DNA Eluted From FTA® Matrix (With Proteinase K) Following Restriction Digestion With Various Enzymes. PowerPlex® 2.1 Fluorescent STR System was used to amplify 1 μl of DNA eluted following a 2 hour digestion with 50 Units of various endonucleases from 7 mm punches (FTA® BFC 180 paper) spotted with blood (single individual). Lanes L contain PowerPlex® 2.1 Allelic Ladders. Lanes 1 and 21 Negative Amplification Control; Lanes 3-5 *Hae* III Digestion; Lanes 6-8 *Hind* III Digestion; Lanes 9-11 *Bam* HI Digestion; Lanes 12-14 *Eco* RI Digestion; Lanes 15-17 *Pst* I Digestion; Lanes 18-20 *Xho* I Digestion; and Lanes 2 and 22 K562 Positive Amplification Control.

Of the 13 enzymes evaluated, only *Dra* I, *Pst* I, *Sal* I, *Xba* I, and *Xho* I allowed the amplification of each of the CODIS core loci and the Penta E locus. These 5 enzymes

were ranked based upon the band intensity between the 14 loci evaluated with the Promega GenePrint™ PowerPlex® 1.1 and 2.1 systems. The enzyme *Sal* I appeared to give the most consistent band intensities across all the loci for the six individuals tested. The rank order of these 5 enzymes was *Sal* I, *Xba* I, *Dra* I, *Pst* I, followed by *Xho* I. However, the band intensities were not significantly different between any of these 5 enzymes. The amount of DNA eluted from the 7 mm (FTA® paper, Whatman BFC 180) blood spots from each of the six individuals was measured for the 5 enzymes.

	Apa I	*Bam* HI	*Dra* I	*Eco* RI	*Eco* RV	*Hae* III	*Hind* III	*Hinf* I	*Kpn* I	*Pst* I	*Sal* I	*Xba* I	*Xho* I
CSF1PO	+	+/-	++	+	+	-	+	+	+	+	++	+	+
TPOX	+	++	++	++	++	-	++	+	++	++	++	++	++
TH01	+	+/-	+	+/-	+	-	+/-	+/-	++	+	++	++	+
VWA	-	++	++	++	++	++	++	++	++	++	++	++	+
D16S539	+	+	++	+	-	++	++	+/-	+	+	++	++	+
D7S820	++	+	++	++	++	++	++	++	++	++	++	++	+
D13S317	++	++	++	++	++	++	++	+	+	++	++	++	++
D5S818	+	++	+	++	++	++	++	++	++	++	++	++	+
FGA	++	++	++	++	++	++	++	++	++	+	++	++	++
D8S1179	++	++	++	++	++	++	++	++	++	++	++	++	+
D18S51	++	++	+	++	++	++	++	++	++	+	++	++	++
D21S11	++	++	++	++	++	++	++	+	++	++	++	++	++
D3S1358	++	++	++	++	++	++	++	++	++	+	++	++	++
Penta E	++	+	++	++	++	-	+	+/-	-	++	++	+	+

Table 3. Amplification of Core CODIS 13 STR Loci Plus Penta E Locus Using DNA Eluted With 13 Different Endonucleases. DNA was eluted from 7 mm (FTA® paper, Whatman BFC 180) blood spots (from six individuals) following a two hour digestion with 50 units of 13 different restriction endonucleases. A 1 µl aliquot of eluted DNA from each restriction enzyme was amplified using Promega's GenePrint™ PowerPlex® 1.1 and 2.1 systems. The presence (+) or absence (-) of an amplification product for each of the 13 core CODIS loci plus the Penta E locus was denoted. The band intensity (relative amount of amplified product) at each locus was also evaluated. Strong band intensity is indicated with "++", while "+" indicates moderate band intensity. A designation of +/- was used to indicate a reduced band intensity or the loss of one or both alleles in at least one of the six individuals tested.

The results in **Table 4** show the average recovery following restriction digestion with the enzymes *Dra* I, *Pst* I, *Sal* I, *Xba* I and *Xho* I. The average recovery with *Dra* I, *Pst* I, *Xba* I and *Xho* I was not significantly different. However, the restriction endonuclease elution with *Sal* I was approximately 6-fold less efficient.

RESTRICTION ENZYME	RECOVERY n=12 ng	RECOVERY n=12 %
Dra I	816	68.5
Pst I	824	69.1
Sal I	135	11.3
Xba I	761	63.9
Xho I	880	73.8

Table 4. Recovery of DNA Eluted From 7 mm FTA® BFC 180 Punches Following Restriction Endonuclease Digestion. DNA was eluted from the 7 mm punches from six different individuals after a two hour digestion with 50 units of each of the restriction enzymes. The average amount of DNA recovered (ng) and the amount recovered as a percentage of the theoretical amount of DNA entrapped (%) are displayed.

The cost of the 5 enzymes was used in the selection process. The cost per unit of each of these 5 enzymes (list price from the Life Technologies Inc. 2000 catalogue) is shown in **Table 5**. The enzyme *Pst* I was between 1.5 to 8 fold less expensive than *Xho* I, *Sal* I, *Xba* I, and *Dra* I. Based upon the amount of DNA recovered and the per unit cost, the enzyme *Pst* I was chosen for the restriction endonuclease elution procedure.

	Cost Per Unit (US Dollars)
***Dra* I**	0.03
***Pst* I**	0.0085
***Sal* I**	0.07
***Xba* I**	0.027
***Xho* I**	0.0128

Table 5. Per Unit Cost of the Restriction Enzymes Which Allowed the Amplification of Each of the CODIS Core Loci and the Penta E Locus. The price per unit was based on the list price in the Life Technologies, GIBCO BRL 2000 catalogue.

The consistency of the DNA eluted following a *Pst* I restriction enzyme digestion was demonstrated following the amplification of a 1 μL aliquot with the Promega's GenePrint™ PowerPlex® 1.1 and 2.1 Systems (**Figures 10 & 11**).

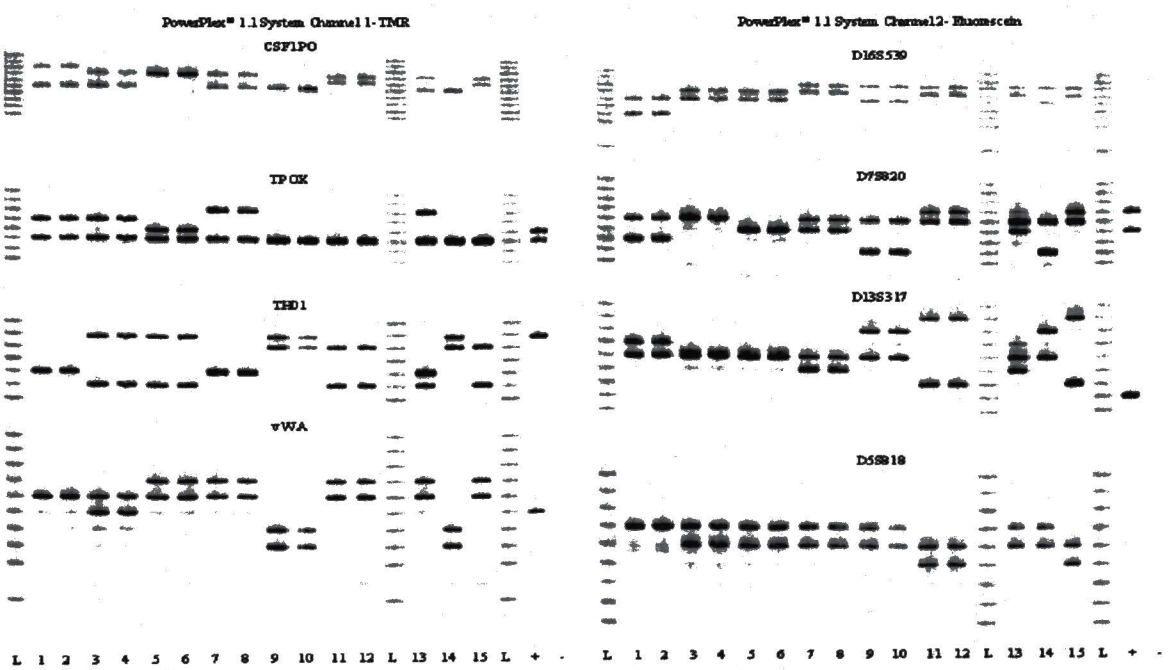


Figure 10. PowerPlex® 1.1 Amplification of DNA Eluted From Six Individuals (With Proteinase K) Following a 2 Hour Digestion With 50 Units of *Pst* I. Lanes L contain the PowerPlex® 1.1 Allelic Ladder: Lanes 1 and 2 Individual 1; Lanes 3 and 4 Individual 2; Lanes 5 and 6 Individual 3; Lane 7 and 8 Individual 4; Lanes 9 and 10 Individual 5; Lanes 11 and 12 Individual 6; Lanes 13-15 1.2 mm FTA® Punch Individuals 4-6, "+" Positive Amplification Control K562, "-" Negative Amplification Control.

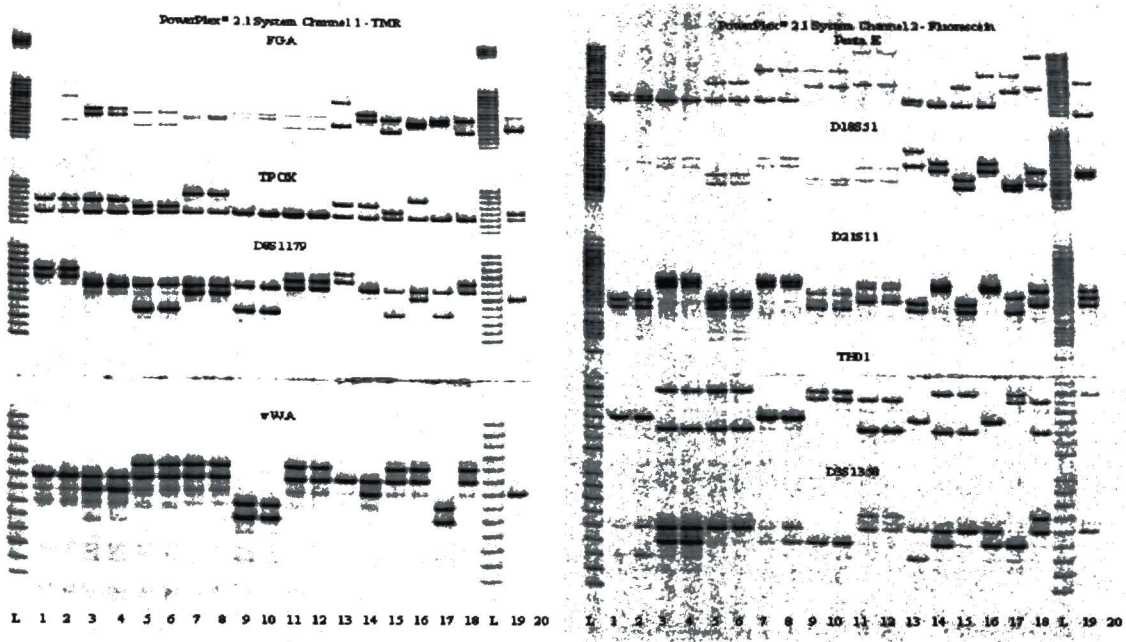


Figure 11. PowerPlex® 2.1 Amplification of DNA Eluted From Six Individuals (With Proteinase K) Following a 2 Hour Digestion With 50 Units of *Pst* I. Lanes L contain the PowerPlex® 2.1 Allelic Ladder: Lanes 1 and 2 Individual 1; Lanes 3 and 4 Individual 2; Lanes 5 and 6 Individual 3; Lane 7 and 8 Individual 4; Lanes 9 and 10 Individual 5; Lanes 11 and 12 Individual 6; Lanes 13-18 1.2 mm FTA® Punch Individuals 1-6 , "+" Positive Amplification Control K562, "-" Negative Amplification Control.

Several parameters were evaluated in order to optimize the restriction endonuclease elution procedure using *Pst* I. These included the number of units of *Pst* I, the time of digestion, and whether or not a Proteinase K digestion is required prior to restriction digestion. For each of these parameters, the amount of DNA recovered, the amount recovered as a percentage of the theoretical amount of DNA trapped in the 7 mm FTA® punch, and the quality of the eluted DNA was determined. A 1 μL aliquot of the eluted DNA samples was amplified with the PowerPlex® 1.1 and PowerPlex® 2.1 Systems. The amplified products were visualized using a Hitachi FMBIO® II Fluorescent Scanner after electrophoresis on a 4.9% denaturing Polyacrylamide gel. DNA was eluted from 7 mm FTA® (Whatman BFC 180) punches from six different individuals after a one

hour Proteinase K digestion followed by a two hour digestion with varying amounts of *Pst* I. **Table 6** shows that by increasing the number of units of *Pst* I from 3 units to 50 units significantly increased the amount of DNA eluted from the 7 mm punches.

FTA MATRIX	BFC 180	BFC 180	BFC 180	BFC 180	BFC 180	BFC 180	31-ET
UNITS *Pst* I	3	6	12.5	25	35	50	50
	n=12	n=12	n=12	n=12	n=12	n=12	n=12
RECOVERY (ng)	97.9	198.6	247.5	522.2	515.4	930.2	646.0
RECOVERY (%)	7.2	14.6	18.2	38.4	37.9	68.4	47.5

Table 6. Elution of DNA (With Proteinase K) Following Restriction Digestion With Varying Amounts of *Pst* I. DNA was eluted from 7mm FTA® punches from six different individuals after a one hour Proteinase K digestion followed by a two hour digestion with varying amounts of *Pst* I. The average amount of DNA recovered (ng) and the amount recovered as a percentage of the theoretical amount of DNA entrapped (%) are displayed.

With 50 units of *Pst* I, 68.4% of the theoretical amount of DNA within the FTA® punches was recovered. Following a one hour Proteinase K digestion, DNA was then eluted from 7 mm FTA® punches (Whatman BFC 180 and 31-ET paper) by restriction digestion with *Pst* I for 15 to 120 minutes. **Table 7** shows that using a 60 minute digestion with either 25 or 50 units of *Pst* I released greater than 60% of the total amount of DNA entrapped within the punches. A two hour restriction digestion with 25 units of *Pst* I with both the BFC 180 and 31-ET resulted in less DNA eluted than a one hour digestion.

FTA MATRIX UNITS *Pst* I		15 (min)	30 (min)	60 (min)	120 (min)
		n=12	n=12	n=12	n=12
BFC 180 25 UNITS	RECOVERY (ng)	720.8	908.5	983.3	722.2
	RECOVERY (%)	53.0	66.8	72.3	53.1
31-ET 25 UNITS	RECOVERY (ng)	565.8	722.2	967.0	897.6
	RECOVERY (%)	41.6	53.1	71.1	66.0
BFC 180 50 UNITS	RECOVERY (ng)	NT	476.0	802.4	923.4
	RECOVERY (%)	NT	35.0	62.3	67.9

Table 7. Elution of DNA (With Proteinase K) Following Restriction Digestion With *Pst* I For Varying Lengths of Time. DNA was eluted from 7mm FTA® punches from six different individuals after a one hour Proteinase K digestion followed by digestion with 2 different amounts of *Pst* I for varying lengths of time. The average amount of DNA recovered (ng) and the amount recovered as a percentage of the theoretical amount of DNA entrapped (%) are displayed.

The necessity for a Proteinase K digestion prior to the *Pst* I restriction digestion was evaluated next. Initially, it was thought that wash procedure developed for the preparation of samples spotted onto the FTA® paper for PCR amplification would not be sufficient for the efficient restriction digestion of the DNA trapped within the matrix. Following the third FTA® Purification Reagent wash, a 500 μL aliquot of FTA® Purification Reagent containing Proteinase K (final concentration 100 μg/mL) was placed on top of the FTA® punch in the spin basket. The Spin-EASE Extraction Tube was incubated at 65°C for 1 hour. The punch was then washed with the TE^{-4} buffer as previously described. The Proteinase K digestion step was included to remove any

proteins bound to the DNA, making the DNA more accessible to the *Pst* I restriction enzyme.

The addition of the Proteinase K digestion step did not significantly increase the quantity of DNA eluted from the 7mm FTA® punches (**Table 8**). Therefore, the Proteinase K digestion step was eliminated from the *Pst* I restriction endonuclease elution procedure. The *Pst* I restriction time was then optimized in the absence of the Proteinase K digestion. For both the FTA® BFC 180 and 31-ET matrixes, a 60 minute digestion with 25 units of *Pst* I, resulted in the elution of greater than 65% of the total DNA trapped within the punch (**Table 9**). The quality of the eluted DNA was demonstrated following amplification of a 1 μL aliquot with the Promega GenePrint™ PowerPlex® 1.1 and 2.1 Systems (**Figures 12 and 13**).

		PRO K	NO PRO K	PRO K	NO PRO K	PRO K	NO PRO K
FTA Matrix UNITS *Pst* I		30 (min)	30 (min)	60 (min)	60 (min)	120 (min)	120 (min)
		n=12	n=12	n=12	n=12	n=12	n=12
BFC 180 25 UNITS	RECOVERY (ng)	908.5	823.3	983.3	907.1	707.2	722.2
	RECOVERY (%)	66.8	61.2	72.3	66.7	52.0	53.1
31-ET 25 UNITS	RECOVERY (ng)	722.2	697.7	967.0	869.0	897.6	678.6
	RECOVERY (%)	53.1	51.3	71.1	63.9	66.O	49.9

Table 8. Elution of DNA (With and Without Proteinase K) Following Restriction Digestion With 25 Units of *Pst* I For Varying Lengths of Time. DNA was eluted from 7mm FTA® punches from six different individuals with and without a one hour Proteinase K digestion followed by digestion with 25 units of *Pst* I for varying lengths of time. The average amount of DNA recovered (ng) and the amount recovered as a percentage of the theoretical amount of DNA entrapped (%) are displayed.

FTA Matrix UNITS *Pst* I		30 (min)	60 (min)	120 (min)
		n=12	n=12	n=12
31-ET 10 UNITS	RECOVERY (ng)	486.9	680.0	667.8
	RECOVERY (%)	35.8	50.0	49.1
		n=24	n=24	n=24
31-ET 25 UNITS	RECOVERY (ng)	775.2	922.1	829.6
	RECOVERY (%)	57.2	67.8	61.0
		n=24	n=24	n=24
BFC 180 25 UNITS	RECOVERY (ng)	787.4	907.1	708.6
	RECOVERY (%)	57.9	66.7	52.1

Table 9. Elution of DNA (Without Proteinase K) Following Digestion With *Pst* I For Varying Lengths of Time. DNA was eluted from 7mm FTA® punches from six different individuals without a Proteinase K digestion. Restriction digestion with either 10 or 25 Units of *Pst* I for varying lengths of time. The average amount of DNA recovered (ng) and the amount recovered as a percentage of the theoretical amount of DNA entrapped (%) are displayed.

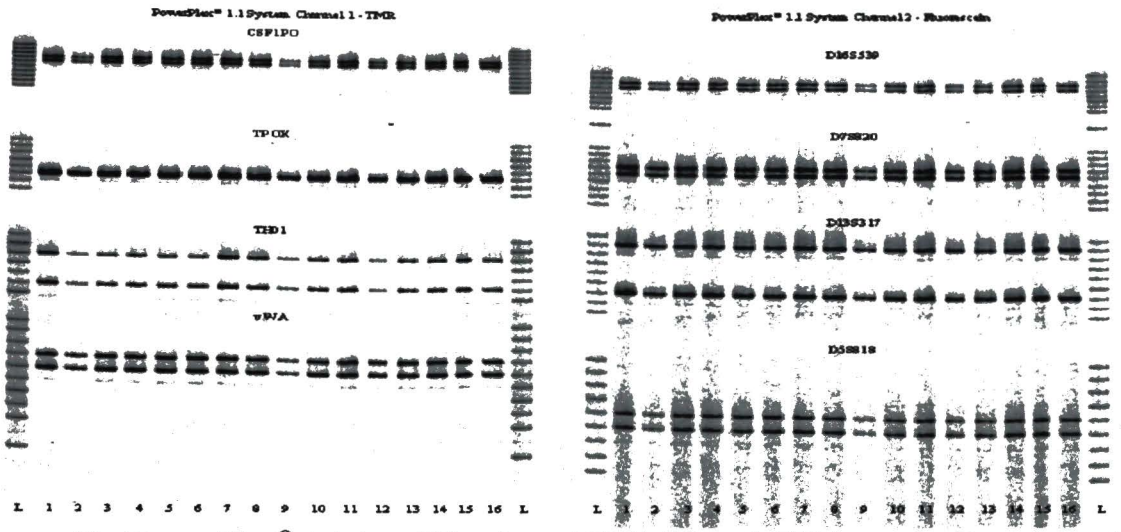


Figure 12. PowerPlex® 1.1 Amplification of DNA Eluted (Without the Proteinase K) Following a *Pst* I Restriction Digest. Restriction digestion was done with 10 and 25 Units of *Pst* I for 60 Minutes. Lanes L contain the PowerPlex® 1.1 Allelic Ladder; Lanes 1-4 BFC 180 matrix digested with 10 Units of *Pst* I; Lanes 5-8 BFC 180 matrix digested with 25 Units of *Pst* I, Lanes 9-12 31-ET matrix digested with 10 Units of *Pst* I, Lanes 13-16 31-ET matrix digested with 25 Units of *Pst* I.

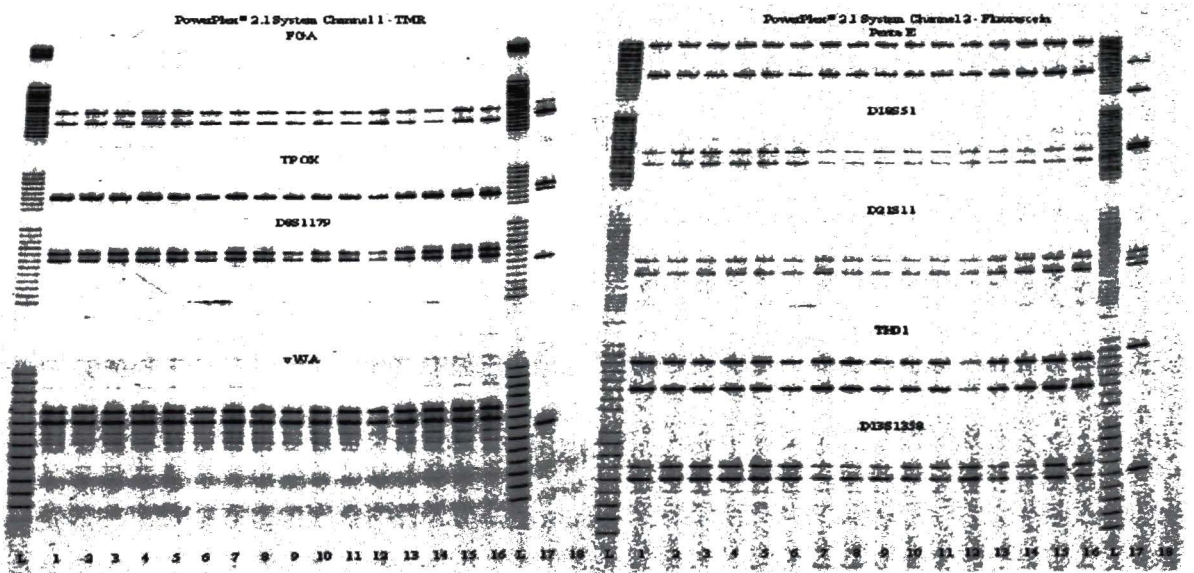


Figure 13. PowerPlex® 2.1 Amplification of DNA Eluted (Without the Proteinase K) Following a *Pst* I Restriction Digest. Restriction digestion was done with 10 and 25 Units of *Pst* I for 60 Minutes. Lanes L contain the PowerPlex® 2.1 Allelic Ladder; Lanes 1-4 BFC 180 matrix digested with 10 Units of *Pst* I; Lanes 5-8 BFC 180 matrix digested with 25 Units of *Pst* I, Lanes 9-12 31-ET matrix digested with 10 Units of *Pst* I, Lanes 13-16 31-ET matrix digested with 25 Units of *Pst* I.

Buccal Epithelial Cell Transfer Methods

The integration of a non-invasive buccal swab collection procedure with the storage and processing capabilities of FTA® paper was investigated using two different methodologies. The first involved the direct transfer of the buccal epithelial cells from the swab onto the FTA® paper at the time of collection, and the second involved the elution of the buccal epithelial cells from the swab, followed by the spotting of the cells onto the FTA® matrix. The elution and spotting method would be done in the laboratory upon receipt of the swab samples.

Direct Transfer Method

Two different procedures were tested for the direct transfer of the buccal swab samples onto to the FTA® matrix. With both methodologies, the buccal epithelial cells were collected by vigorously rubbing the inside of both cheeks using a sterile Round Foam-tipped swab (Paddle swab). The cells were then manually transferred onto a Indicating FTA® MicroCard™ (Whatman 31-ET matrix) by either pressing (blotting) or by both pressing and then rolling the swab over the paper. At the point of transfer, the Indicating FTA® MicroCard™ changes from pink to white. The efficiency of the transfer process was evaluated by the amplification of a 1.2 mm punch taken from the center and periphery of the transfer site with the Promega GenePrint™ PowerPlex® 1.1 system. The band intensity (relative amount of amplified product) at each of the 8 loci was evaluated (**Table 10**). The punches with either a "++" or "+" band intensity were summed together to assess the success of the transfer method. For the press transfer method, 88.3% of the

punches taken from the center gave a successful amplification, whereas, 92.4% of the punches taken from the periphery gave a successful amplification. For the press and roll transfer method, 87.6% of the punches taken from the center gave a successful amplification, whereas, 85.5% of the punches taken from the periphery gave a successful amplification. The results indicated that both transfer methods were comparable and the location of the punch used for amplification did not significantly affect the quality of the results.

TRANSFER METHOD	PUNCH LOCATION	BAND INTENSITY ++	BAND INTENSITY +	BAND INTENSITY -
PRESS	CENTER	57.3%	31.0%	11.7%
PRESS	PERIPHERY	63.4%	29.0%	7.6%
PRESS & ROLL	CENTER	56.6%	31.0%	12.4%
PRESS & ROLL	PERIPHERY	57.2%	28.3%	14.5%

Table 10. Amplification Efficiency of the Press and Press & Roll Direct Buccal Swab Transfer Methods. The efficiency of the transfer method was assessed by the band intensity of the alleles following the amplification of a 1.2 mm punch with the Promega GenePrint™ PowerPlex® 1.1 System. Punches were taken from either the center or the periphery of the transfer site. A total of 290 punches (145 punches from both the center and the periphery) were evaluated for each transfer method. If a locus showed strong band intensity a designation of "++" was given. A designation of "+" was used to indicate a reduced band intensity at one or two loci. A designation of "-" was used to indicate a reduced band intensity at more than two loci or allelic dropout. The number of punches for each location and transfer method showing each amplification pattern were counted and expressed as a percentage of the total number of punches evaluated.

Elution of DNA From Buccal Cells Transferred Onto FTA® Paper

The ability to efficiently elute DNA from the Indicating FTA® MicroCard™ following the direct transfer of buccal epithelial cells was evaluated. Swabs were collected from six individuals at different times of the day and transferred onto Indicating FTA® MicroCards™ by the press and roll method. A 7 mm punch was removed from the transfer site (white area on MicroCard™) and placed in the basket portion of a Spin-EASE Extraction Tube and processed in an identical manner as a 7 mm blood punch. The *Pst* I restriction endonuclease elution procedure, optimized for recovering DNA from blood spots, was used with buccal epithelial punches. The average recovery of DNA from the 7 mm punch was 108.4 ng (n=24) or approximately 2 ng/μl. A comparison of the amplification of a 1.2 mm punch taken from the periphery of the card, and the amplification of 1 μl aliquot of DNA eluted from a 7 mm punch from the same Indicating FTA® MicroCard™ using the *Pst* I restriction endonuclease procedure is shown in **Figures 14 and 15**. The amplification of a 1.2 mm punch and DNA eluted from buccal epithelial cells transferred onto an Indicating FTA® MicroCard™ with Applied Biosystems AmpF*l*STR® Profiler Plus *ID*™ and COfiler™ Systems resulted in comparable, high quality STR profiles. The amplification products showed balanced peak heights and minimal stutter across the different loci. The 1 μl aliquot which contained approximately 2 ng of DNA gave consistently much higher relative fluorescent units (RFU).

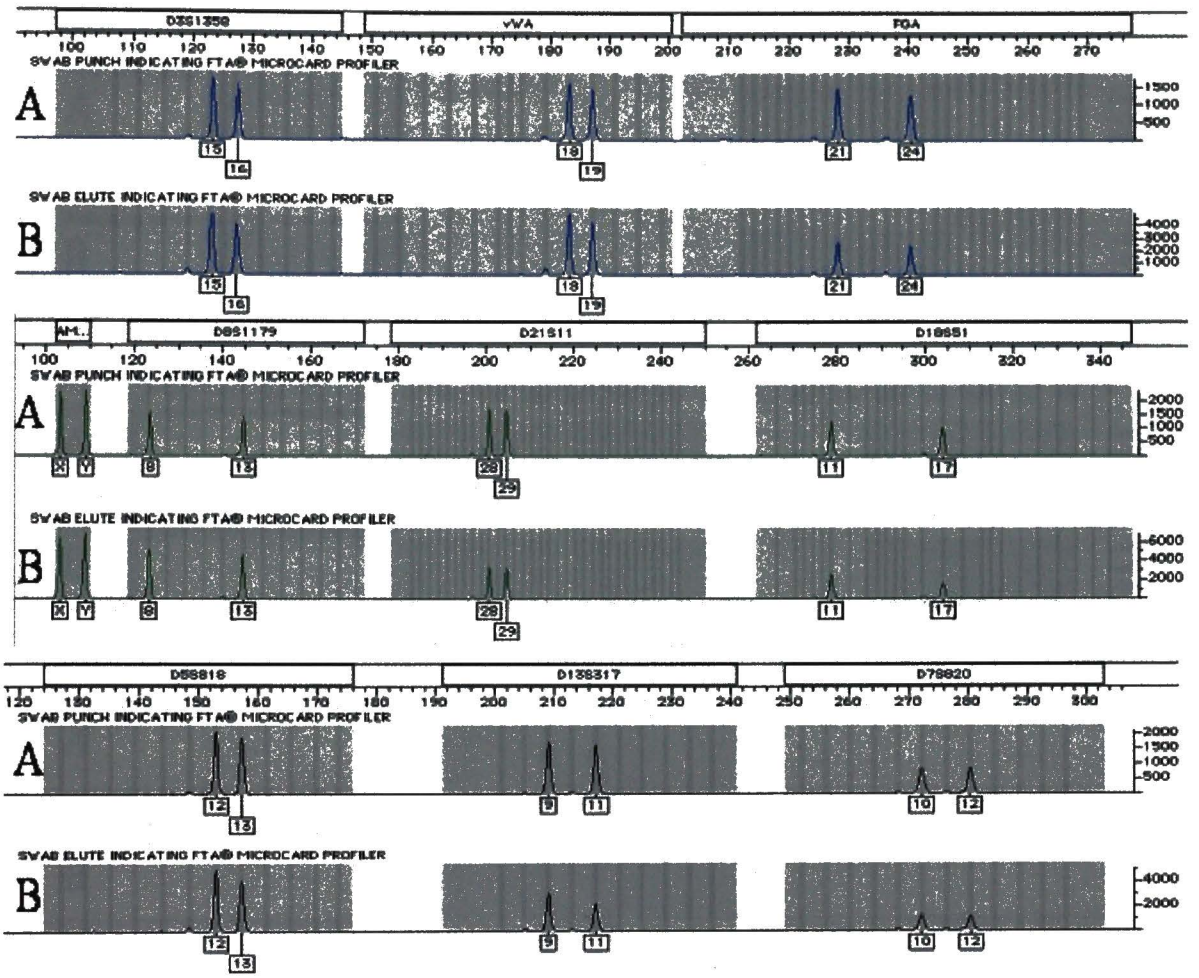


Figure 14. AmpF*l*STR® Profiler Plus *ID*™ Amplification of a 1.2 mm Punch and DNA Eluted from Buccal Epithelial Cells Transferred Onto Indicating FTA® MicroCards™ by Press and Roll Method. Panel A is the amplification of a 1.2 mm punch taken from the periphery of the card. Panel B is the amplification of 1 μl aliquot of DNA eluted from a 7 mm punch from the Indicating FTA® MicroCard™ using the *Pst* I restriction endonuclease procedure.

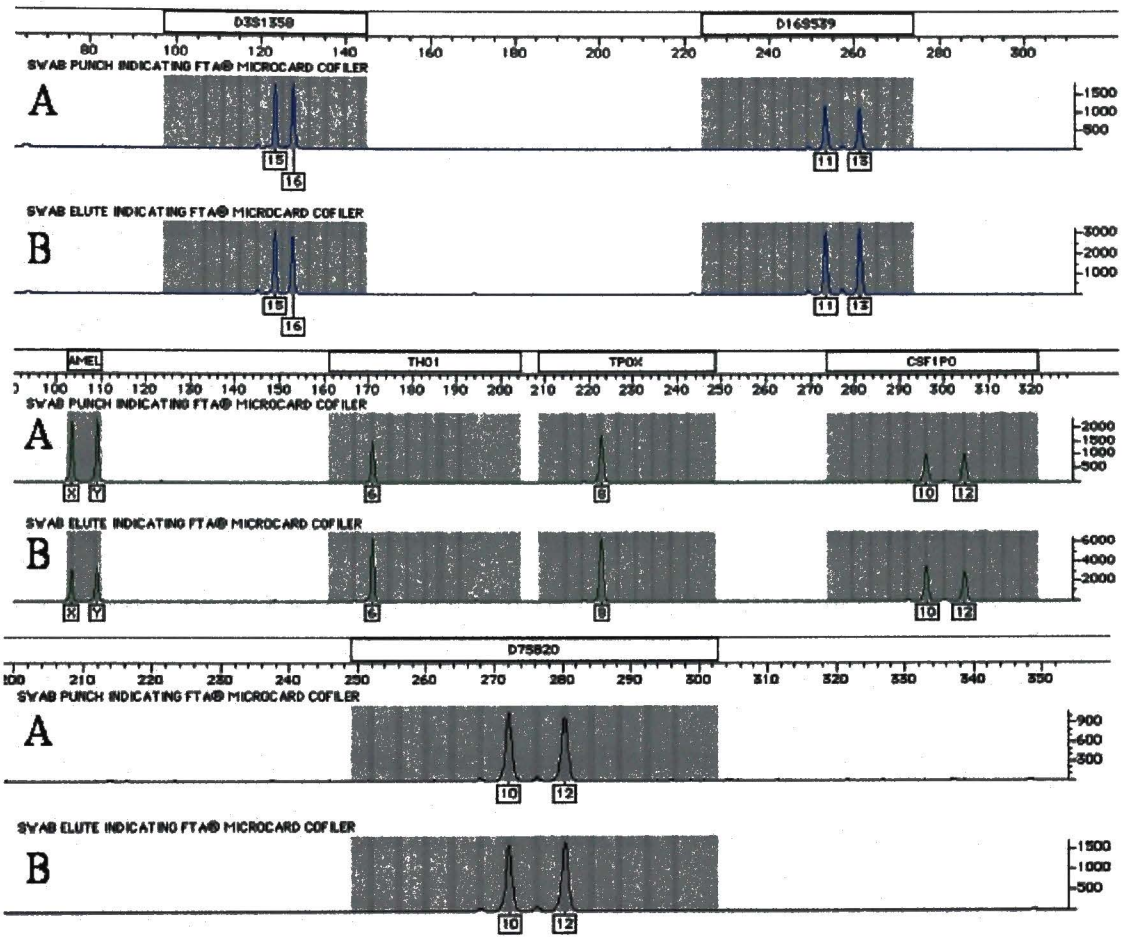


Figure 15. AmpF*l*STR® COfiler™ Amplification of a 1.2 mm Punch and DNA Eluted from Buccal Epithelial Cells Directly Transferred Onto Indicating FTA® MicroCards™ by Press and Roll Method. Panel A is the amplification of a 1.2 mm punch taken from the periphery of the card. Panel B is the amplification of 1 μl aliquot of DNA eluted from a 7 mm punch from the Indicating FTA® MicroCard™ using the *Pst* I restriction endonuclease procedure.

Elution of Buccal Cells From Swabs

Buccal epithelial cells were eluted from various types of swabs and spotted onto an FTA® Matrix. Two isotonic solutions were tested to facilitate the elution and spotting of the buccal cells. The first cell elution solution (0.9% NaCl, 1.0% Triton X-100) contained a wetting agent designed to facilitate removal of the cells from the swab head

material. The second cell elution solution tested was Isoton® III, a commercially available reagent sold as a diluent for automated blood cell analytical instruments. The initial experiments demonstrated that buccal cells were lysing with the first elution solution and that the DNA from the epithelial cells was not being trapped within the FTA® matrix. This solution was not used for any further studies. The Isoton® III reagent preserves the buccal cells and was used for all buccal cell elution and spotting experiments.

Several different types of swabs with a variety of head materials were tested. These included the Round Foam-tipped swab (Paddle swab), Regular Foam-tipped swab, Dacron swabs, and Cotton swabs. The Regular Foam-tipped swabs were found to give the most consistent STR profiles. The Regular Foam-tipped swabs were submerged in 1.0 mL of the Isoton® III reagent for 15 seconds, the swab head was then vigorously rubbed against the wall of a 2.0 mL nipple tube for an additional 15 seconds. A 15 μL aliquot of Coomassie Blue (1 mg/mL) was added to the eluted cells. The epithelial cells were then pelleted by centrifugation, and the Isoton® III reagent was decanted off. The buccal epithelial cells were then re-suspended using a micropipettor in the residual reagent. A 15 μL aliquot was then spotted onto the target circles on an FTA® Micro Sheet™. The Coomassie Blue allowed the visualization of the buccal cell spot on the FTA® Micro Sheet™. Alternatively, an Indicating FTA® Card™ could be used to visualize the spotted cells. The FTA® Micro Sheet™ was then washed with the FTA® Purification Reagent and the TE^{-4} buffer as previously described. For each sample, a standard 1.2 mm punch was removed after processing of the FTA® Micro Sheet™ and

amplified with the commercially available multiplex STR systems (**Figures 16 and 17**). The amplified products give consistent results with good heterozygous peak height balance between each of the different fluorescent dyes. This procedure is now used routinely for parentage testing in our laboratory.

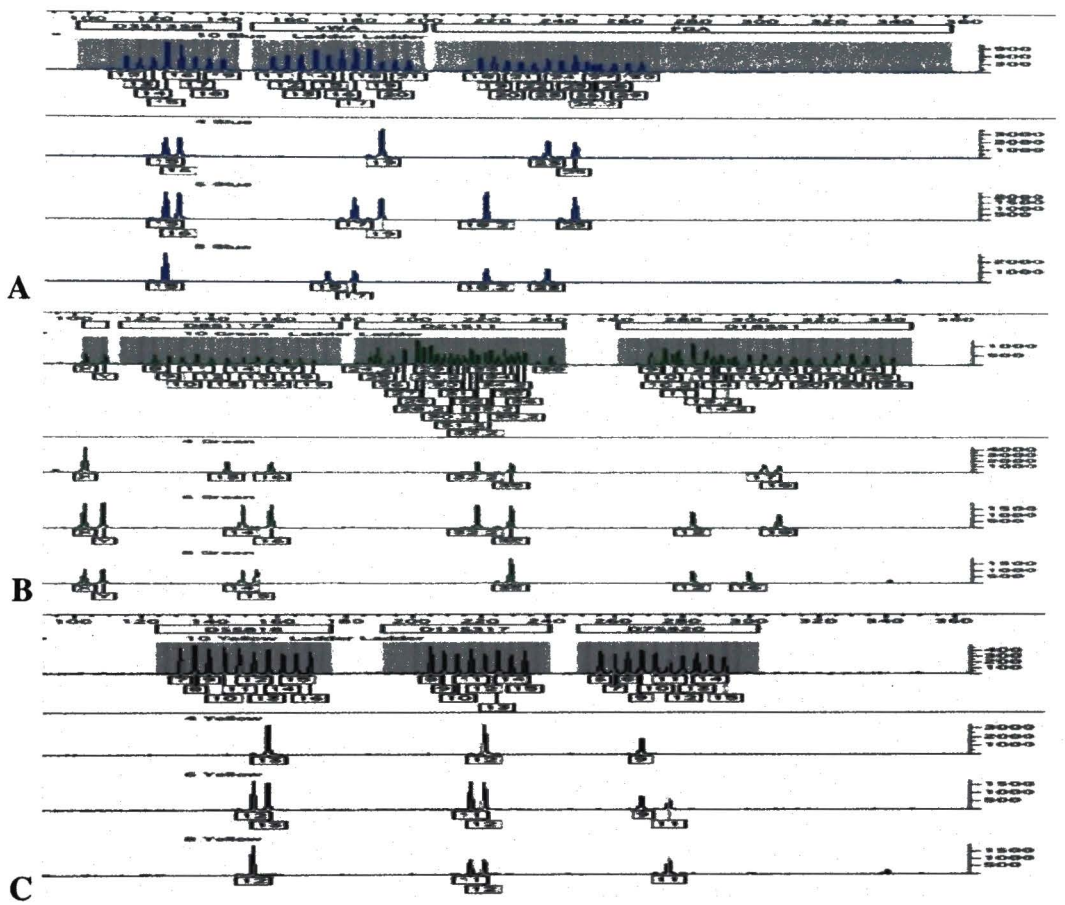


Figure 16. AmpF*l*STR® Profiler Plus *ID*™ Amplification of a 1.2 mm Punch From FTA® Paper Spotted With Buccal Cells Eluted From Foam Swabs. Swabs from a mother, child, and an alleged father were collected, and the buccal cells were eluted from each swab and spotted onto an FTA® Micro Sheet™. The FTA® Micro Sheet™ was processed for PCR amplification and a 1.2 mm punch was taken for each sample. In each panel the top electropherogram is the allelic ladder, then the mother, child, and alleged father. Panel A is the Blue electropherograms representing the loci with FAM labeled primers (D3S1358, vWA, and FGA). Panel B is the Green electropherograms representing the loci with JOE labeled primers (Amelogenin, D8S1179, D21S11, and D18S51). Panel C is the Yellow electropherograms (seen as Black) representing the loci with NED labeled primers (D5S818, D13S317, and D7S820)

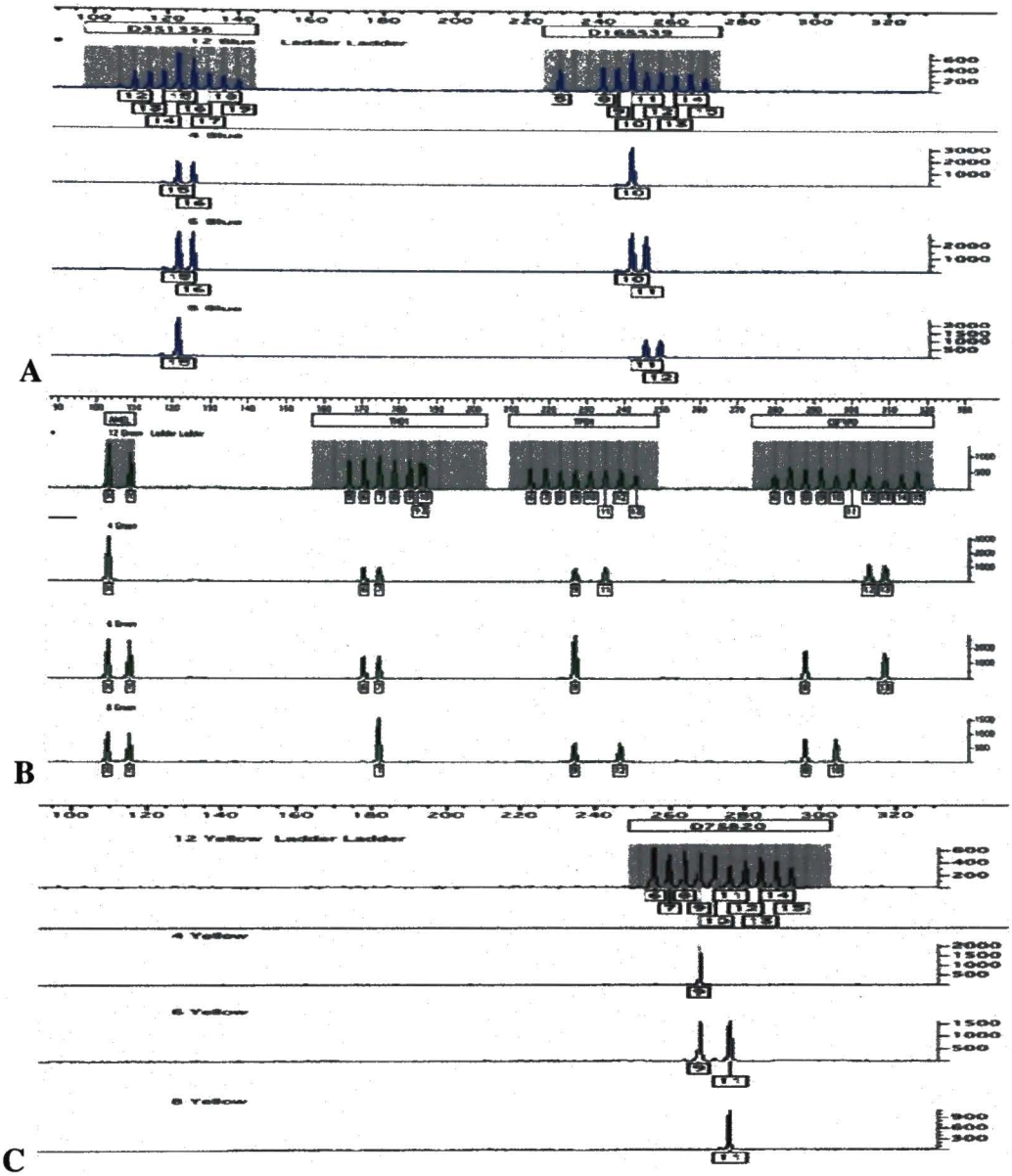


Figure 17. AmpF*l*STR® COfiler™ Amplification of a 1.2 mm Punch From FTA® Paper Spotted With Buccal Cells Eluted From Foam Swabs. Swabs from a mother, child, and an alleged father were collected, and the buccal cells were eluted from each swab and spotted onto an FTA® Micro Sheet™. The FTA® Micro Sheet™ was processed for PCR amplification and a 1.2 mm punch was taken for each sample. In each panel the top electropherogram is the allelic ladder, then the mother, child, and alleged father. Panel A is the. Blue electropherograms representing the loci with FAM labeled primers (D3S1358, and D16S539). Panel B is the Green electropherograms representing the loci with JOE labeled primers (Amelogenin, THO1, TPOX, and CSF1PO). Panel C is the Yellow electropherograms (seen as Black) representing the loci with NED labeled primers (D7S820).

DISCUSSION

The goal of this thesis project was to develop novel methodologies for the processing of FTA® paper in order to facilitate its use in the fields of paternity testing, forensic testing, and human identification. The research was divided into three major areas. The first was an evaluation of the stability of the different FTA® matrices, and the development of a process which would maximize the amount of genetic information that could be obtained from a single 1.2 mm FTA® punch. The second was the development of a process for the efficient elution of the DNA trapped within the FTA® matrix. This would allow laboratories to incorporate the advantages of FTA® storage with high throughput robotic processing. The third area included the development of methodologies which would allow the integration of FTA® technology with a non-invasive buccal swab collection process.

Matrix Stability

A variety of FTA® treated matrices have been developed for sample collection, archiving, and transport. The cotton-based paper matrices included the original S&S 903 paper, Whatman BFC 180, and Whatman 31-ET. An experimental glass-fiber matrix referred to as Whatman FTA® Elute has also been developed. The various support matrices have varying fiber densities and tensile strengths. Following amplification, the PCR product is in solution, and can be removed and placed in a new tube. The original

1.2 mm punch can be washed to remove any residual amplification product. The punch is then dried and other loci can be amplified using the same DNA template bound in the paper matrix. During physical manipulation and the PCR amplification process, a 1.2 mm paper punch begins to deteriorate. As a result, the DNA template entangled within the matrix is gradually lost. The loss of DNA template limits the amount of genetic information that could be obtained from an individual punch. The different FTA® matrices were evaluated in order to determine their stability during physical manipulation and repetitive PCR amplification reactions. The number of successful amplification reactions for the cotton based matrices was directly proportional to the tensile strength of the matrix. The Whatman 31-ET matrix proved to be the most durable throughout the re-amplification process. The Whatman 31-ET matrix is thicker than BFC 180 and has the highest flow rate of all the Whatman cellulose-based papers. The 31-ET matrix has three times the tensile strength of Whatman BFC 180 paper. A 1.2 mm 31-ET punch could be re-amplified on average 6 times before the DNA template was completely lost. The Whatman Elute was originally designed to facilitate the release of the DNA entangled within the matrix. The Elute paper (glass-fiber based filter matrix) has a higher tensile strength as compared to the cellulose based matrices. However, a 1.2 mm Elute punch could only be amplified on average 4 times before the DNA template was completely lost. In this study, a process was developed that maintains the structural integrity of the 1.2 mm FTA® punch, minimizes the loss of template DNA, and ultimately maximizes the amount of genetic information obtainable from a limited amount of biological sample. In

addition, the data generated by this study supported Whatman's decision to use the 31-ET matrix for the manufacture of all the different FTA® collection card.

Elution of DNA From FTA® Paper

It has been estimated that over the past 5 years, several hundred thousand blood samples have been stored on a variety of FTA® collection cards. The most cost-effective and efficient means of analyzing these samples will require the use of automation. High throughput systems usually incorporate a pipetting station along with a robotic arm capable of manipulating microtiter plates. Although automated punching devices are available, the manipulation of small 1.2 mm punches has been difficult. The elution of DNA from the FTA® Paper would greatly simplify the process of automation. Three different methods to elute the DNA from an FTA® matrix were tested, a simple heat elution in dH_2O or TE^{-4} buffer, a heat elution with Chelex-100 resin; and a restriction endonuclease elution. For each method, 7 mm FTA® punches were spotted with a 25 μL aliquot of blood. Blood samples from six different individuals with known white cell counts were used for the elution studies. At a minimum, the elution from each individual's blood spots was done in duplicate.

Heat Elution

For the simple heat elution method, 200 μL of either dH_2O or TE^{-4} buffer was added to the 7 mm punch after the standard processing with the FTA® Purification Reagent and TE^{-4} buffer. The samples were placed in a 100°C heat block for varying lengths of time, they were then centrifuged and the supernatant containing the eluted

DNA was quantitated. The amount of DNA eluted using either the dH_2O or TE^{-4} buffer was extremely low with each of the four matrices. The amount of eluted DNA was typically less than 10% of the theoretical recovery.

Chelex-100 resin was used as an alternative to the heat elution with either the dH_2O or TE^{-4} buffer. Following the standard processing with the FTA® Purification Reagent and TE^{-4} buffer, 200 μL of a 5% Chelex-100 solution was added, and processed as described in the materials and methods section. Using the Chelex-100 resin, ssDNA is released into solution and any residual PCR inhibitors remain bound to the Chelex-100 resin (Walsh et al., 1991). The amount of DNA eluted after heating with the Chelex-100 resin ranged between 10 and 20% of the theoretical recovery. The quality of the DNA was assessed through the amplification of a 1 μL aliquot of the eluted DNA. Several of loci on the gel images displayed weak band intensities. Retrospectively, we know that this was due to the reduced cycle numbers used with the amplification of the 1.2 mm punches. The 1.2 mm punches contained significantly more DNA than does a 1 μL aliquot (approximately 1 ng) of the eluted DNA. The Chelex-100 heat elution method results in DNA which produces good quality STR profiles. Although this elution procedure is relatively inexpensive, the sub-optimum recovery diminishes its utility.

Restriction Endonuclease Elution

Previous work in our lab had shown that DNA can be eluted from an FTA® matrix after digestion with the restriction enzyme *Hae* III (Rowan Campbell, unpublished results). When the *Hae* III restricted DNA was amplified with primers for the 13 core

CODIS STR loci, three of the loci (CSF1PO, TPOX, and THO1) failed to amplify. These 3 loci each contain a *Hae* III restriction site within the primer binding sites, and therefore cannot be amplified. Twelve additional restriction enzymes (*Apa* I, *Bam* HI, *Dra* I, *Eco* RI, *Eco* RV, *Hind* III, *Hinf* I, *Kpn* I, *Pst* I, *Sal* I, *Xba* I, and *Xho* I) were tested in order to determine if the eluted DNA would amplify each of the 13 core CODIS STR loci. Of the thirteen (including *Hae* III) restriction enzymes tested only *Dra* I, *Pst* I, *Sal* I, *Xba* I, and *Xho* I allowed the amplification of each of the CODIS core loci and the Penta E locus. The enzyme *Sal* I appeared to give the most consistent band intensities across all the loci for the six individuals tested. However, the band intensities were not significantly different between any of these five enzymes. The amount of DNA eluted from the 7 mm FTA® blood spots from the six individuals and the cost for each of the five enzymes was used to select the optimum restriction endonuclease. The average recovery of DNA with *Dra* I, *Pst* I, *Xba* I, and *Xho* I was not significantly different. However, the restriction endonuclease elution with *Sal* I was approximately 6-fold less efficient. The cost of the enzyme *Pst* I was between 1.5 to 8 fold less expensive than *Dra* I, *Sal* I, *Xba* I, and *Xho* I. Based upon the amount of DNA recovered and the per unit cost, the enzyme *Pst* I was chosen for the restriction endonuclease elution procedure.

Several parameters were evaluated in order to optimize the restriction endonuclease elution procedure using *Pst* I. These included the number of units of *Pst* I, the time of digestion, and whether or not a Proteinase K digestion step was required prior to restriction digestion. For each of these parameters, the amount of DNA recovered and the quality of the STR profiles following the amplification of a 1 μL aliquot of the eluted

DNA was evaluated. The results indicated that a one-hour Proteinase K digestion step was not required prior to restriction digestion since it did not significantly increase the quantity of DNA eluted from the 7mm FTA® punches. The elimination of the Proteinase K digestion reduced both the cost and the time of the restriction endonuclease elution procedure. The optimized procedure included a 60 minute restriction digest with 25 units of *Pst* I. With both the FTA® BFC 180 and 31-ET matrixes, a 60 minute digestion with 25 units of *Pst* I, resulted in the elution of greater than 65% of the total DNA trapped within the punch. The restriction endonuclease elution procedure resulted in the greatest recovery of DNA trapped within the different FTA® matrices. The quality of the eluted DNA was verified by the amplification of a 1 μL aliquot of the eluted DNA with each of the commercially available multiplex STR systems used in human identity testing. The amplification products showed balanced peak heights and minimal stutter across the different loci. The *Pst* I restriction endonuclease eluted DNA produces consistent high quality STR profiles.

Buccal Cell Transfer Onto FTA® Matrices

Over the past 5 years there has been a consistent trend towards a buccal swab collection method for all forms of genetic testing. Patients much prefer a non-invasive buccal swab collection as compared to the traditional invasive venipuncture technique for blood. As a result of the increased use of swabs for the collection of a biological sample, the integration of a non-invasive buccal swab collection procedure with the storage and

processing capabilities of FTA® paper was necessitated. Two different approaches were investigated to transfer the buccal epithelial cells from a swab onto the FTA® matrix.

The first involved the direct transfer of the buccal epithelial cells from the swab onto the FTA® paper at the time of collection. The written instructions describing the collection and transfer would need to be very simple and straight forward. This was mandated by the large number of individuals that are routinely collecting buccal swab samples for both paternity and forensic testing. The advantage of the direct transfer method is that the DNA within the buccal epithelial cells is protected immediately upon contact with the FTA® matrix and is therefore, not susceptible to bacterial degradation during shipping or transfer to the lab for testing. A potential disadvantage of the direct transfer method is that each sample would be on an individual FTA® Card. This could limit the laboratories ability for high throughput processing of the samples. The second involved the elution of the buccal epithelial cells from the swab, followed by the spotting of the cells onto the FTA® matrix. The elution and spotting method would be done in the laboratory upon receipt of the swab samples. The elution from the swab and the spotting onto the FTA® matrix within the lab would provide a greater flexibility in the processing of the DNA for PCR analysis.

Direct Transfer Method

Two different procedures were tested for the direct transfer of the buccal swab samples onto to the FTA® matrix. The buccal epithelial cells were collected by vigorously rubbing the inside of both cheeks using a sterile Round Foam-tipped swab

(Paddle swab). The sterile Round Foam-tipped swabs were chosen because they provided the greatest surface area for transferring onto the FTA® matrix. The epithelial cells were then manually transferred onto a Indicating FTA® MicroCard™ by either pressing (blotting) or by both pressing and then rolling the swab over the paper. Using conventional FTA® matrices, it is impossible to detect where the transfer has taken place. Unlike blood, the buccal epithelial cells do not have a detectable color. To make the transfer process more efficient and reliable, Indicating FTA® MicroCards™ were used. At the point of transfer, the Indicating FTA® MicroCard™ changes from pink to white, allowing the easy visualization of the transfer sight. There is considerable variability in the number of epithelial cells obtained by rubbing the inside of the cheek from person to person. In addition, there is no reliable procedure for quantifying the number of epithelial cells recovered on the swab head. The press method and the press and roll method gave comparable results. Punches taken from either the center or periphery of the Indicating FTA® MicroCards™ using either method also gave similar results. The amplification of a total of 290 punches for each of the direct transfer methods resulted in approximately 8 to 15% of the STR profiles having reduced band intensity at more than two loci or the loss of one or multiple alleles. This appears to be directly related to the amount of cells that were transferred from the swab onto the FTA® matrix.

The ability to elute DNA from the Indicating FTA® MicroCard™ following the direct transfer of buccal epithelial cells by the press and roll method was evaluated. A 7 mm punch was removed from the transfer site and processed using the *Pst* I restriction endonuclease elution procedure. The average recovery of DNA from the 7 mm punch

was 108.4 ng (n=24) or approximately 2 ng/μL. A comparison of the amplification of a 1.2 mm punch taken from the periphery of the card, and the amplification of a 1 μL aliquot of eluted DNA produced comparable STR profiles.

Elution and Transfer Method

It was originally thought that an alternative to the direct transfer of the epithelial cells onto the FTA® paper would be to place the swab head in a solution immediately following collection and then transport the samples back to the laboratory. The epithelial cells would be eluted during transport. An isotonic solution would be developed to both elute the cells and prevent microbial growth during transport back to the lab. An isotonic solution is required for the efficient entrapment of the DNA within the FTA® paper matrix. The nuclear membrane must rupture on the FTA® Paper so that when the DNA explodes out of the nuclei it entangles within the matrix. When the samples were received at the lab, the epithelial cells would be concentrated by centrifugation, resuspended in a small volume and directly applied to the FTA® paper for long-term storage or processing. This process was abandoned for several reasons. This method would require the lab to provide the collectors with individual tubes containing the elution and transport solution. The collectors would then ship the solution containing a biological sample back to the lab using an overnight carrier service. The transport of liquid samples is significantly more expensive and fraught with potential problems, such as the leakage of the tubes during shipment. Alternatively, a procedure for the elution

and spotting of the epithelial cells in the lab upon receipt of the buccal swabs was ultimately developed.

A total of four different swabs types and two different isotonic solutions were evaluated for the elution of the buccal epithelial cells and the spotting onto the FTA® matrices. The swabs included a Round Foam-tipped swab (Paddle swab), a Regular Foam-tipped swab, a Dacron swab, and a Cotton swab. They were each tested with the two isotonic elution solutions. The first cell elution solution (0.9% NaCl, 1.0% Triton X-100) contained a wetting agent designed to facilitate removal of the cells from the swab head material. The second cell elution solution tested was Isoton® III, a commercially available reagent sold as a diluent for automated blood cell analytical instruments. The solution containing 0.9% NaCl, 1.0% Triton X-100 was eliminated since it caused premature cell lysis which prevented the DNA from entangling within the FTA® matrix. The combination of the Isoton® III reagent and the Regular Foam-tipped swab was the most efficient at eluting the buccal cells and spotting onto the FTA® matrix. Once the epithelial cells were eluted from the Regular Foam-tipped swabs, Coomassie Blue (1 mg/mL) was added to the eluted cells. The epithelial cells were then pelleted by centrifugation, and resuspended in the residual Isoton® III reagent. The Coomassie Blue allowed the visualization of the buccal cells spotted onto those FTA® matrices that did not include an indicating dye. Alternatively, an Indicating FTA® Card™ could be used to visualize the spotted cells. For increased throughput, up to 96 samples can be spotted onto a FTA® Micro Sheet™. After processing the FTA® Micro Sheet™, a standard

1.2 mm punch is amplified for each sample. The results obtained using the combination of the Isoton® III reagent and the Regular Foam-tipped swab provide consistent high quality STR profiles. This procedure is now used routinely for parentage testing in our laboratory.

The advantages of FTA® paper for sample collection, storage, and simplified processing of blood samples for PCR have been known for several years. However, labs requiring high throughput processing or those that were using a non-invasive buccal swab sample collection method have been slower to incorporate FTA® technology. The methods developed from these studies are now used in the DNA Identity Laboratory at the University of North Texas Health Science Center and many other laboratories throughout the world for routine casework analysis.

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