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EVALUATION OF A NOVEL DUAL BIOMETRIC DEVICE USED

TO OBTAIN DNA FROM FINGERPRINTS

Michael L. Mann, B.S.

APPROVED:

Major Professor

Committee Member

Committee Member

Chair, Department of Cell Biology and Genetics

Dean, Graduate School of Biomedical Sciences

EVALUATION OF A NOVEL DUAL BIOMETRIC COLLECTION DEVICE TO OBTAIN DNA FROM FINGERPRINTS

INTERNSHIP PRACTICUM REPORT

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Michael Mann, B.S.
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Xavier G. Aranda, M.S.

Suzy Gonzalez, Ph.D.

Flavio Anderson

Bill Bailey

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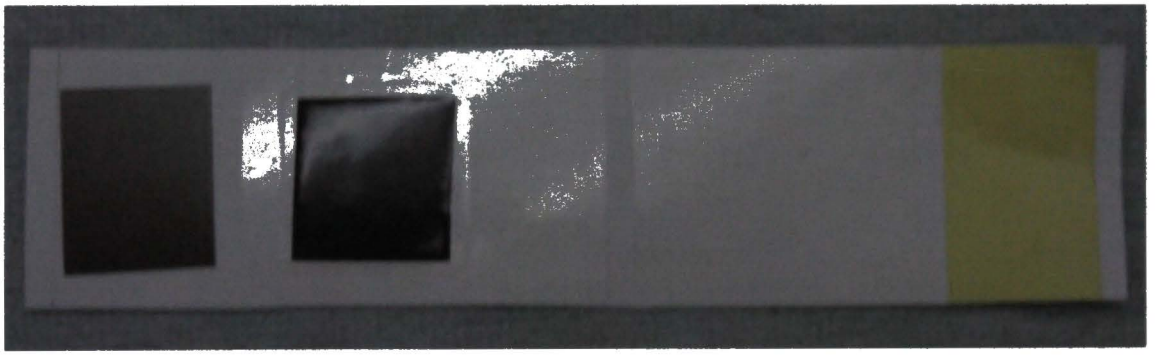
I. Summary:

MICROFIELD COMPANY S.A. is currently developing a non intrusive method of obtaining a reference DNA sample known as Lift & Rub (Figure 1). The Lift & Rub is a self-adhesive security stamp designed to collect a single fingerprint from a known individual that can be used for DNA analyses. Lift & Rub is composed of an adhesive material with a protective film. The Lift & Rub is used in conjunction with an abrasive strip and a product named Dry-Ink, a graphite laminate. Together these items allow for an alternative to the traditional fingerprint collection technique and improve on it by allowing for DNA analysis. The objective of this research is to test and maximize the performance of Lift & Rub.

Figure 1-1: MICROFIELD's Lift & Rub Fingerprinting Card



II. Problem/Hypothesis:

Microfield's Lift & Rub is designed to collect a single fingerprint from a known individual to be used for DNA analyses. The Lift & Rub is intended to provide a full genetic profile of a known individual. This project aims to: 1) establish a standard collection protocol by evaluating empirical data to determine the average DNA yields using various collection techniques; 2) evaluate the reproducibility of DNA profiles obtained; and 3) gauge the overall success rate of obtaining a complete profile.

III. Significance:

In 2005, Congress passed the DNA Fingerprint Act, an amendment to an existing federal law to authorize the collection of DNA samples from any person arrested or detained under federal authority [1]. Currently Louisiana and South Dakota are collecting DNA samples from all felony arrests and other states have passed bills to begin collection in the future such as California, complete listing can be seen in Appendix A [2]. California will begin collection of DNA samples from all felony arrests starting January, 2009. In 2006, California had over 534,000 felony arrests that would have been subject to DNA sampling under this current bill [3]. With the necessity to collect samples from such a large number of individuals, it is necessary to have a quick reliable method for obtaining collections that can also be easily stored for long periods of time.

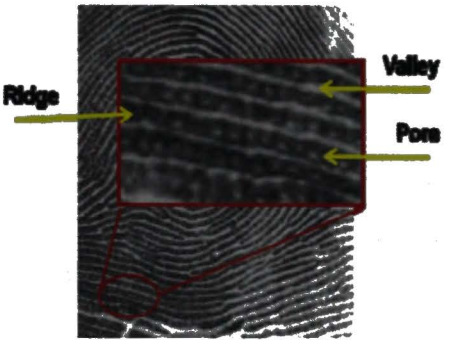
The current methods of DNA reference sampling such as buccal swabs, vena puncture and finger prick pose a number of problems when processing large batches. These methods are time consuming and can be dangerous. Buccal swabs requires a 20 minute drying time prior to packaging in which it is subject to contamination. Vena puncture requires a trained individual. With any method that requires the use of blood, vena puncture and finger prick, the possibility of blood borne pathogens is a health concern. A new method that can stream line collection in a safe manner is needed and Lift & Rub is designed for such a task.

The Lift & Rub has been designed to effectively obtain a usable fingerprint for individualization along with a DNA profile from a known individual. Fingerprint samples collected using the Lift & Rub can be easily filed for extended storage, scanned and digitized into an electronic database, and ultimately used to obtain a genetic profile. The dual biometric capability of the Lift & Rub also increases throughput by collecting two sample types during a single collection process. If shown to be reliable the Lift & Rub will be a valuable tool for forensic reference sampling.

IV. Background:

Fingerprints are a representation of the fingertip epidermis left behind when a finger is pressed to an object. These representations are viewed as patterns of ridges and valleys. The ridges are the dark lines seen when viewing a typical fingerprint while the valleys are the white spaces between the ridges (Figure 4-1).

Figure 4-1: Anatomy of a fingerprint



The ridges left behind are formed from globules of secretions from eccrine and sebaceous sweat glands. These globules have been described as being 0.05um in diameter and 600-800um in height meaning that they are thousands of times higher as they are wide. The major component is water which ranges from 98.5% to 99.5%. The remainder is a mixture of organic and inorganic materials [4]. The organic materials consist of twelve amino acids (e.g. alanine, glycine, valine, leucine, isoleucine, threonine, serine, aspartic acid, glutamic acid, phenylalanine, lysine, and tyrosine) ten fatty acids (e.g. octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid, pentadecanoic acid, hexadecanoic acid, octadecanoic acid, cis-9, cis-12 octadecadienoic acid, nonadecanoic acid, and tetracosanic acid) ammonia, and urea. The inorganic materials

consist of sodium chloride, bromide, iodide and fluoride. Sebum may also be present, which is a secretion from the sebaceous sweat glands. These glands are not found on the palmar surfaces but are transferred to the fingers by touching other areas of the body [5] (Figure 4-2).

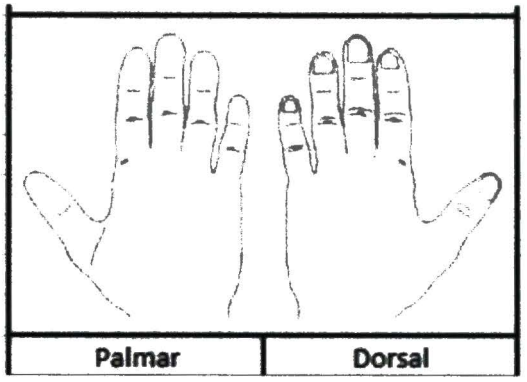


Figure 4-2: Illustration of hand surfaces

The ridge patterns of the fingerprint are formed prior to birth and remain throughout life, only to be altered by physical scaring. There are a number of theories that can contribute to the formation of these ridge patterns. A review article written by Michael Kücken [6] compiles three differing theories as to the exact process of the pattern formation. The first of these theories is the buckling hypothesis. The buckling hypothesis explains that pressure builds up from rapid proliferation of basal cells that causes a periodic movement of cells towards the soft dermis that is easier to penetrate than the more rigid upper epidermal layers. This folding of the basal layer leads to the formation of ridge patterns at the epidermis. A second theory suggests that the networking of nerves and blood vessels cause the ridge patterns through nerve fiber growth cones. It is claimed that the ridges follow the nerve capillaries. The nerve fiber

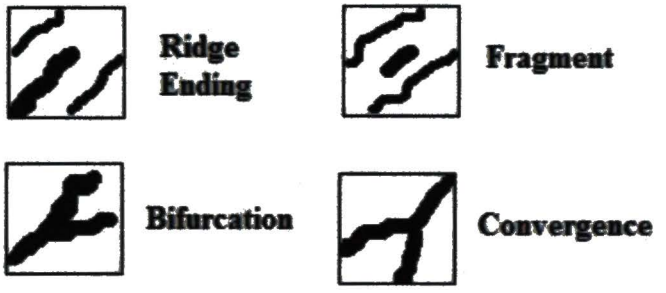
growth cones that project towards the epidermis are separated by 40mm which correlates to the distance of separation between the primary ridges [7]. A third theory describes that fibroblasts found in the dermis will form patterns in culture that take on a ridge structure. It is this fact that leads some to believe that the fibroblasts found at the fingertips is what causes the formation of ridge patterns. Kücken concludes that the buckling of the basal layer of cells is the most likely reason for the formation of ridges [6].

Although the origins of the ridge patterns are ambiguous, there is consistency throughout the literature indicating fingerprints form prior to birth. It is the variability at which the ridge patterns form that give fingerprints their individualizing characteristics. The current guidelines set by The Scientific Working Group on Friction Ridge Analysis, Study and Technology (SWGFAST) state that there are three levels of detail that a ridge pattern can be classified under [8]. 1) The friction ridge flow and general morphological information. Ridge lines form identifiable shapes with in regions of a fingerprint called singularities or singular regions. Singular regions are classified into three basic typologies: loop, arch, and whorl [9] (Figure 4-3). 2) The individual friction ridge paths. Ridge lines form distinct paths that lead to identifiable marks known as minutia. Minutia come in many forms the most common being bifurcations - a splitting of a ridge line, convergence - a joining of two ridge lines, ending ridges - an abrupt end to a ridge line, and dots - fragments of a ridge line. Examples of the common types of minutia and some of their variations can be seen in (Figure 4-4). 3)

The friction ridge dimensional attributes. Third level classification includes the shape of the ridge along with pore observation. The pores in the skin will cause deviations in the ridge line detail. The presence of pores will cause white dots to appear with in the dark lines of the ridges (Figure 4-1). In preliminary studies the Lift & Rub has shown to be capable of producing all 3 levels of detail.

Figure 4-3: Fingerprint typologies

Figure 4-4: Common minutia types



There is also a general agreement with in the scientific community that fingerprints are unique to each individual even differentiating identical twins [10, 11, 12, 13]. Fingerprints have been accepted in the court of law for over a century. It wasn't until US v. Byron Mitchell in 1999 when fingerprints were challenged under the Daubert standards on the basis that fingerprint uniqueness has not been objectively tested and

there is no known potential error rate for matching [14]. From this case through 2005, there have been 41 cases that challenged fingerprints under Daubert, none of which have been successful [15]. With fingerprints continually being attacked on the basis that there is no scientific backing of the individualizing uniqueness and the difficulty of identifying low quality prints, the incorporation of the dual biometric capabilities of the Lift & Rub can greatly enhance the power of discrimination.

V. Research Design and Methodology:

Sample Collection:

Fingerprint samples used during the testing were collected from employees and student volunteers of the University of North Texas Center for Human Identification (UNTCHI) in Fort Worth, Texas. Fingerprint samples were taken from the left and right thumbs of the participants following guidelines described by the FBI (16). An employee reference profile database kept in the quality control office was used as a control for samples collected from employees. Samples collected were given a coinciding unique identifier in order to keep all participants anonymous.

The palmar side of the finger was rubbed across a strip of abrasive in a rolling fashion ten times to remove dead skin cells from the surface of the epithelium. The finger was then rolled on the Dry-Ink for contrast when forming the fingerprint. The finger was then pressed to the adhesive portion of the Lift & Rub to form the fingerprint.

A series of collection techniques were used to determine the best approach for maximizing DNA yields. The series tested two variables at three stages of collection preprinting, printing, and post printing. The preprinting stage compared the use of an alcohol wipe to an alcohol free wipe. Subjects cleaned their hands with a hand wipe and were allowed to air dry prior to printing. The wiping of the hands prior to fingerprinting was intended to minimize the presence of inhibitors or contaminants from trace

elements picked up during routine daily activities on the fingertips of the subject. The printing stage compared two commonly used impression techniques, the rolled impression versus the plain impression (Figure 5-1).

Figure 5-1: Fingerprint impression techniques

A rolled impression is taken by pressing the finger on to the collection surface and rolling the fingertip from nail to nail. The plain impression is a flat impression of the fingertip made by a straight up and down pressing of the fingertip to the collection surface. It is believed a rolled fingerprint will result in higher DNA yields due to an increased surface area and the possibility that the skin cells on the sides of the finger will be more viable due to a lack of callusing. The post printing stage compared the performance of using a SpinEze™ Polyester Push-Off Swab (Item# 08228) swab moistened with sterile distilled water versus 70% EtOH for the removal of the cells from the adhesive.

The variables for every stage were considered in conjunction with each other resulting in a total of 8 different collection pathways, as follows:

Table 5-1: Collection Pathways			
Pathway	Stage 1	Stage 2	Stage 3
1	Alcohol	Plain	dH2O
2	Alcohol	Plain	70% EtOH
3	Alcohol	Rolled	dH2O
4	Alcohol	Rolled	70% EtOH
5	Alcohol Free	Plain	dH2O
6	Alcohol Free	Plain	70% EtOH
7	Alcohol Free	Rolled	dH2O
8	Alcohol Free	Rolled	70% EtOH

Three individuals had a different pathway tested per hand over a four day period so that each individual is tested by all possible pathways. Day 1 tested pathways 1 and 2, Day 2 pathways 3 and 4, Day 3 pathways 5 and 6, and Day 4 pathways 7 and 8. The data generated from these samples was evaluated based on total DNA yields and the quality of profiles obtained. The best collection technique was assessed and used for a reproducibility study.

Prior studies within our lab using a product similar to Lift & Rub suggested that taking a swabbing from the abrasive will yield higher DNA yields. For an additional study five samples were chosen at random during the reproducibility study and the abrasive portion of the Lift & Rub was swabbed using a SpinEze™ Polyester Push-Off Swab moistened with sterile distilled water. These samples were then run alongside the samples from the reproducibility study and compared directly to the swabbings obtained from the corresponding fingerprint portion of the Lift & Rub.

DNA Extraction:

The fingerprint samples were extracted using the DNA IQ™ Casework Sample Kit for the Maxwell® 16 Low Elution Volume (LEV). The Maxwell®16 is an automated DNA purification system setup for use with the DNA IQ™ chemistry (Figure 5-2). The DNA IQ™ chemistry incorporates a paramagnetic resin that binds to DNA and allows for the DNA to be subject to a series of wash steps removing possible inhibitors. In order to maximize the amount of extracted DNA, the "Trace Sample Preprocessing Protocol by Sample Type" as described in Promega's Technical Bulletin (Part# TB354) was followed. This protocol requires the user to supply the following materials: Tissue and Hair Extraction Kit (Cat.# DC6740) which consists of Incubation Buffer, Proteinase K, and DTT; Proteinase K (Cat.# V3021); 56°C heat block or oven; 1.5ml Microtubes (Cat.# V1231); DNA IQ™ Spin Baskets (Cat.# V1221); Nuclease Free Water (Cat.# P1193);and aerosol-resistant micropipette tips.

The protocol is as follows. The head of the SpinEze™ Polyester Push-Off Swab used for recovery of DNA from the fingerprint was ejected into a 1.5ml microtube. To each tube 90ul of Incubation Buffer, 10ul of 18mg/ml Proteinase K and 2ul was added. The tube was then briefly vortexed to completely soak the swab and incubated at 56°C for 1 hour. 200ul of Lysis Buffer was added to each sample for a total volume of 300ul. The sample and Lysis Buffer was then vortexed briefly. The swab was then transferred to a DNA IQ™ Spin Basket and placed back into the 1.5ml microtube with the Lysis Buffer and centrifuged at 1300RCF for 2 minutes. The DNA IQ™ Spin Basket containing

the swab was then removed from the 1.5ml microtube and discarded. The 1.5ml microtube containing the sample was then closed and stored at room temperature until ready for DNA extraction using the Maxwell®16 LEV instrument.

The Casework Sample Cartridge was placed into the Maxwell®16 rack once the cartridge is secure in the rack, the seal was then removed. A Low Elution Volume (LEV) plunger was placed into well #8 of the cartridge and a 0.5ml Elution Tube was placed in the elution tube holder on the Casework Sample Cartridge. 45ul of Elution Buffer was added to the Elution Tube and 300ul of sample was then transferred into well #1 of the cartridge as can be seen in (Figure 5-3). Turning on the Maxwell®16 the instrument proceeded through its diagnostics. The instrument was set at "LEV" operational mode. The rack with prepared Casework Sample Cartridges was loaded onto the base-rack of the Maxwell®16 and the "RUN" command was executed. When the run was complete the elution tube containing the extracted DNA was capped and stored at 4°C.

Figure 5-2: Maxwell®16 Instrument

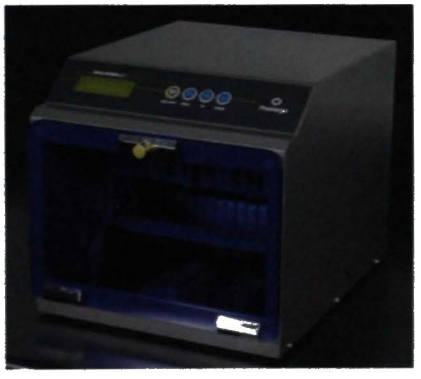
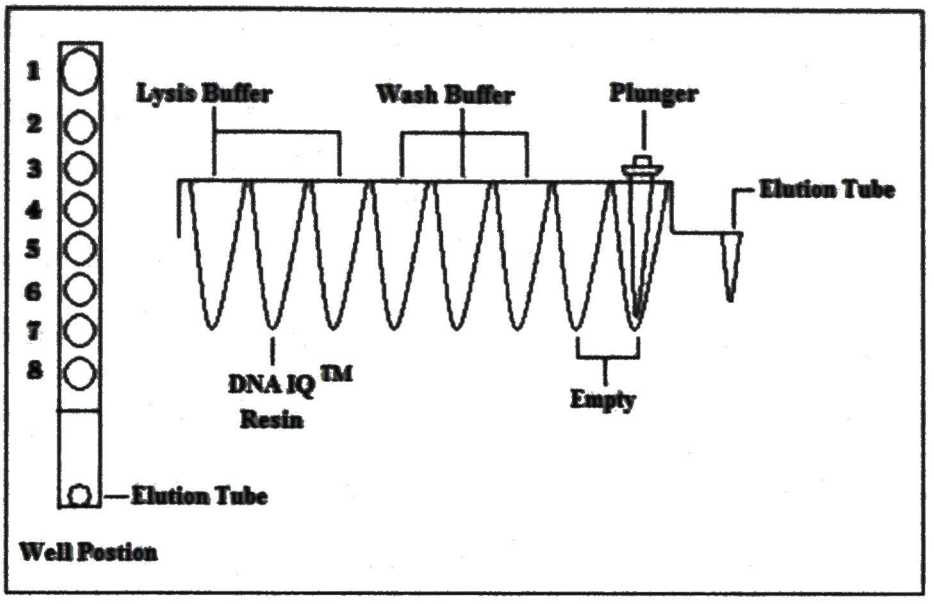


Figure 5-3: Maxwell®16 DNA IQ™ Casework Sample Cartridge



Quantification:

To determine the quantity of the DNA obtained from the various fingerprint samples was quantified using the Quantifiler(R) Human DNA Quantification Kit from Applied Biosystems using an ABI 7500 Real-Time PCR System and software. The quantification was performed as described by the protocol set forth by UNTCHI. A quantification standard dilution curve was prepared by serial dilutions of 200ng/ul stock solution to: 50, 16.7, 5.56, 1.85, 0.62, 0.21, 0.068 and 0.023ng/µl in TE-4 Buffer (10mMTris-HCl pH 8.0, 0.1mM EDTA) and was run in duplicate. The quantification Master Mix was prepared by adding 10.5ul of Primer Mix and 12.5ul of Quantifiler® PCR Reaction Mix for each sample. The Master Mix was distributed to an AB 96-well Optical

Reaction Plate by adding 23ul to each well per reaction. 2ul of standard and sample DNA extracts were added to the appropriate wells. The optical plate was then sealed using a clear AB Optical Adhesive Cover. The plate was then centrifuged briefly and placed in an ABI 7500 Real-Time PCR instrument. The software for the RT-PCR instrument was set for a 25ul reaction with the following cycle parameters: a 10 min. hold at 95°C, 40 cycles of 95°C for 15 seconds and 60°C for 1 min. The results of the quantification were evaluated and for any sample that exceeded the optimal amount (1ng/ul) of DNA for amplification were diluted with Nuclease Free Water. The total DNA yield for each sample was determined by multiplying the total volume of elution (45ul) to the results obtained from quantification.

Amplification:

The samples were amplified using AmpFℓSTR® Identifiler ® PCR Amplification Kit (Applied Biosystems) and PowerPlex®16 System (Promega) on an AB GeneAmp® PCR 9700 Thermocycler. The total reaction volume for amplifications was 25ul in 0.2 MicroAmp® reaction tubes. Both systems had an equal input volume of extracted DNA and a direct comparison was made of resulting profiles for the quality of the DNA and the number of interpretable alleles.

AmpFℓSTR® Identifiler®

The total reaction volume for amplifications were 25ul in 0.2 MicroAmp® reaction tubes which consisted of 15ul of PCR Master Mix and up to 10ul of extracted DNA product, not to exceed 1.5ng of total DNA. The PCR Master Mix was made by

mixing 10.5ul of AmpFℓSTR® Identifiler ® PCR Reaction Mix, 5.5ul of AmpFℓSTR® Identifiler ® 10X Primer Mix, and 0.5ul of AmpliTaq Gold® DNA Polymerase. 15ul of Master Mix was added to each 0.2 MicroAmp® reaction tube. Up to 10ul of DNA product was added to the appropriate reaction tubes, not to exceed 1.5ng of total DNA product, Nuclease Free Water was used to fulfill the remainder of the 10ul requirement. The amplification cycle parameters were the following: a hot start at 95°C for 11 mins.; followed by 28 cycles of denaturation at 94°C for 1 min., primer annealing at 59°C for 1 min., and an extension at 72°C for 1 min.; then an extension hold at 60°C for 1 min.; and finally a 4°C hold. The amplified product was then transferred and stored at 4°C until they were needed for analysis by capillary electrophoresis.

PowerPlex® 16 System

The total reaction volume for amplifications was 25ul in 0.2 MicroAmp® reaction tubes. Each reaction contained: 2.5ul of Gold STAR 10X Buffer; 2.5ul of PowerPlex® 16 10X Primer Pair Mix; 0.8ul AmpliTaq Gold® DNA Polymers; up to 10ul of extracted DNA, not to exceed a total of 1.5ng; and Nuclease Free Water to meet the 25ul reaction volume. The amplification cycle parameters that were followed are: a hot start at 95°C for 11 minutes followed by a hold at 96°C for 1 minute; followed by 10 cycles of denaturation at 94°C for 30 seconds, primer annealing at 60°C for 30 seconds, and an extension at 70°C for 45 seconds. For an additional 22 cycles, a denaturation at 90°C for 30 seconds, a primer annealing at 60°C for 30 seconds, and an extension at 70°C for 45 seconds. Then an extension hold at 60°C for 30 minutes, followed by a final

hold at 4°C. The amplified product was then transferred and stored at 4°C until they were needed for analysis by capillary electrophoresis.

Electrophoresis & Analysis:

The genetic profiles were generated using capillary electrophoresis on the ABI3130xl Genetic Analyzer instrument with Gene Mapper® ID v. 3.2 software. To prepare samples for analysis that were amplified with AmpFℓSTR® Identifiler® a Master Mix was prepared by mixing 8.75ul of HiDi Formamide with 0.25ul of GeneScan™ Liz 500™ Size Standard. 9ul of the Master Mix was dispensed into each well of a MicroAmp® Optical 96-well reaction plate. 1ul of amplified product and 1.5ul of AmpFℓSTR® Identifiler® Allelic Ladder was added to the appropriate wells.

For samples that were amplified with the PowerPlex® 16 System a Master Mix containing 9ul of HiDi Formamide and 1ul of Internal Lane Size Standard 600 was prepared. 10ul of the master mix was dispensed into each well of a MicroAmp® Optical 96-well reaction plate. 1ul of amplified product and 1.5ul of Allelic Ladder was added to the appropriate wells.

The remaining unused wells in a group of 16 were filled with 10ul of HiDi Formamide in order to keep the unused capillaries from drying out. The plate was fitted with a gray plate septa and briefly centrifuged to remove any air bubbles. Samples were denatured for 5mins at 95°C and snap cooled on ice for 5mins. The plate was then fitted in a black base plate and locked together with a white plate retainer prior to placement on the ABI3130xl Genetic Analyzer. The DNA fragments were electrophoresed in AB

POP-6 polymer. The genetic profiles generated from the fingerprint samples were compared to the coinciding reference sample to ensure that the correct profile was generated and to assess the quality of the DNA.

VI. Results:

The initial stage of this research was to determine the best method for the collection of the fingerprints. Eight different pathways were evaluated that tested variables in 3 different stages of collection preprinting, printing, and post printing. Each of the 8 pathways was evaluated for the overall amount of DNA recovered and the quality of the genetic profile from the 3 subjects. Of the 8 pathways the most successful was pathway 3. Pathway 3 had the highest DNA yields for subject #1 with 28.53ng of DNA and subject #3 with 0.977ng of DNA and third highest for subject #2 with 2.214ng of DNA. The average DNA concentrations for the three samples were 0.235ng/ul as can be seen in Table 6-1. The second highest average DNA concentration for the three samples was that of pathway 4 which trailed by 79.45% resulting with 0.483ng/ul. The lowest average DNA concentration was pathway 2 with an average 0.0051ng/ul. The highest yield from a single sample was from pathway 3 with 28.53ng. The lowest yield from a single sample was from pathway 6 with an undetermined amount of DNA. The majority of the samples fell below the optimal range of 0.5 – 1ng of input DNA when the maximum allowable amount was added (10ul). Also the accuracy of the Quantifiler® results is questionable due its lower end sensitivity being 23 picograms. The low DNA yields lead to a large number of stochastic effects which was an issue when the samples were analyzed.

Table 6-1: Pathway Quantification Results										
Pathway	Subject 1			Subject 2			Subject 3			Average DNA Concentration (ng/ul)
	DNA Concentration (ng/ul)	DNA Yield[1] (ng)	Input DNA[2] (ng)	DNA Concentration (ng/ul)	DNA Yield[1] (ng)	Input DNA[2] (ng)	DNA Concentration (ng/ul)	DNA Yield[1] (ng)	Input DNA[2] (ng)	
1	0.0022	0.099	0.022	0.0694	3.123	0.694	0.0062	0.279	0.062	**0.0259**
2	0.0034	0.153	0.034	0.0046	0.207	0.046	0.0072	0.324	0.072	**0.0051**
3	0.6340	28.530	1.268[3]	0.0492	2.214	0.492	0.0217	0.977	0.217	**0.2350**
4	0.1040	4.680	1.040	0.0320	1.440	0.320	0.0089	0.401	0.089	**0.0483**
5	0.0082	0.369	0.082	0.0791	3.560	0.791	0.0064	0.288	0.064	**0.0312**
6	0.0173	0.779	0.173	0.0168	0.756	0.168	UND	0.000	0.000	**0.0114**
7	0.0081	0.365	0.081	0.0276	1.242	0.276	0.0036	0.162	0.036	**0.0131**
8	0.0073	0.329	0.073	0.0213	0.959	0.213	0.0032	0.144	0.032	**0.0106**

[1] DNA recovered in a 45ul elution volume. [2]10ul of eluted DNA added per STR amp reaction. [3]Only 2ul of DNA was added per STR amp reaction

The data generated for the STR markers was evaluated using only the 13 core CODIS loci (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, THO1, D13S317, D16S539, VWA, TPOX, D18S51, D5S818, FGA) and amelogenin (AMEL). The interpretation guidelines of UNTCHI were applied to all samples using an interpretation threshold of 100 relative fluorescent units (rfu) for heterozygote peaks which must meet a 70% Peak Height Ratio (PHR) and 200rfu for homozygote peaks for profiles obtained with Identifiler®. For profiles obtained with PowerPlex®16 all peaks had to meet an interpretation threshold of 150rfu.

For the initial stage of the study in which the 8 fingerprint collection pathways were evaluated, PowerPlex®16 failed to produce any STR profiles. These samples were reinjected to try and resolve the issue however, the results did not improve. Due to time constraints the results from the 8 collection pathways was based only on the profiles obtained from Identifiler®. The electropharagrams of the samples from the 8 collection pathways were evaluated based on the number of correct allele calls when compared to the reference sample. Of the 24 samples; there was a total of 5 full profiles, 9 partial profiles, 4 inconclusive profiles, and 6 mixed profiles. The allele calls for each of the 8 collection pathways can be seen in Appendix B. Pathway 3 had the highest number of correct allele calls over the 3 samples with 71 of a possible 76 alleles, resulting in a 93.42% allele call recovery. Pathway 3 was the only pathway to have more than one full profile. Pathway 3 had full profiles for subject #1 and subject #2 (Figure 6-

1). The profile generated from subject #3 had 20 correct alleles called but showed evidence of contamination with multiple genetic profiles detected (Figure 6-2). All profiles generated for subject #3 showed evidence of a mixture with the exception of a single partial profile (sample 3-2). The electropharagrams of 2 of the mixed samples can be seen in (Figures 6-3, 6-4)

Figure 6-1: Electropharagrams for samples 1-3 and 2-3

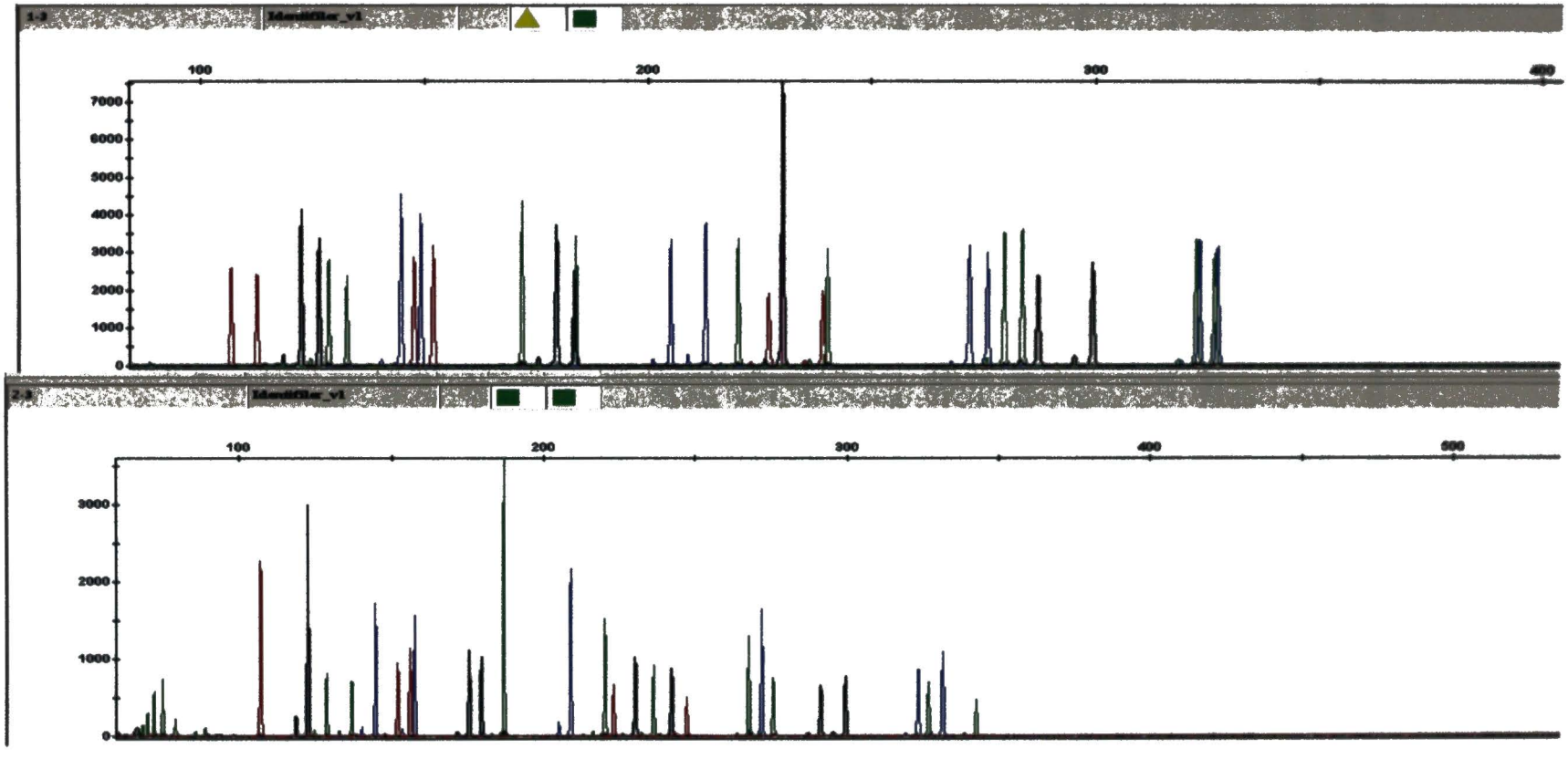


Figure 6-2: Electropharagram for sample 3-3 showing evidence of possible PCR contamination

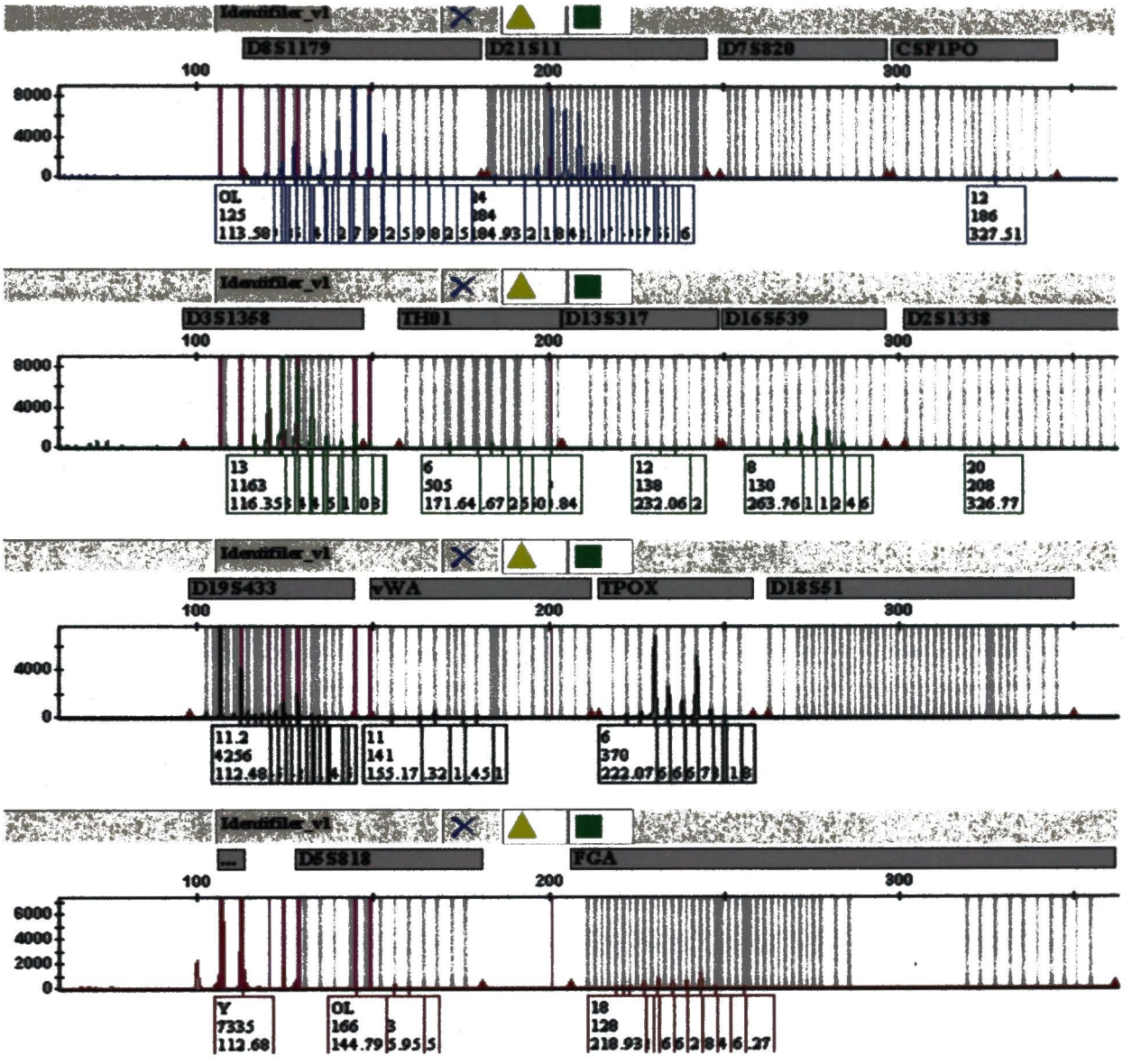


Figure 6-3: Electropharagram of mixed sample 3-1

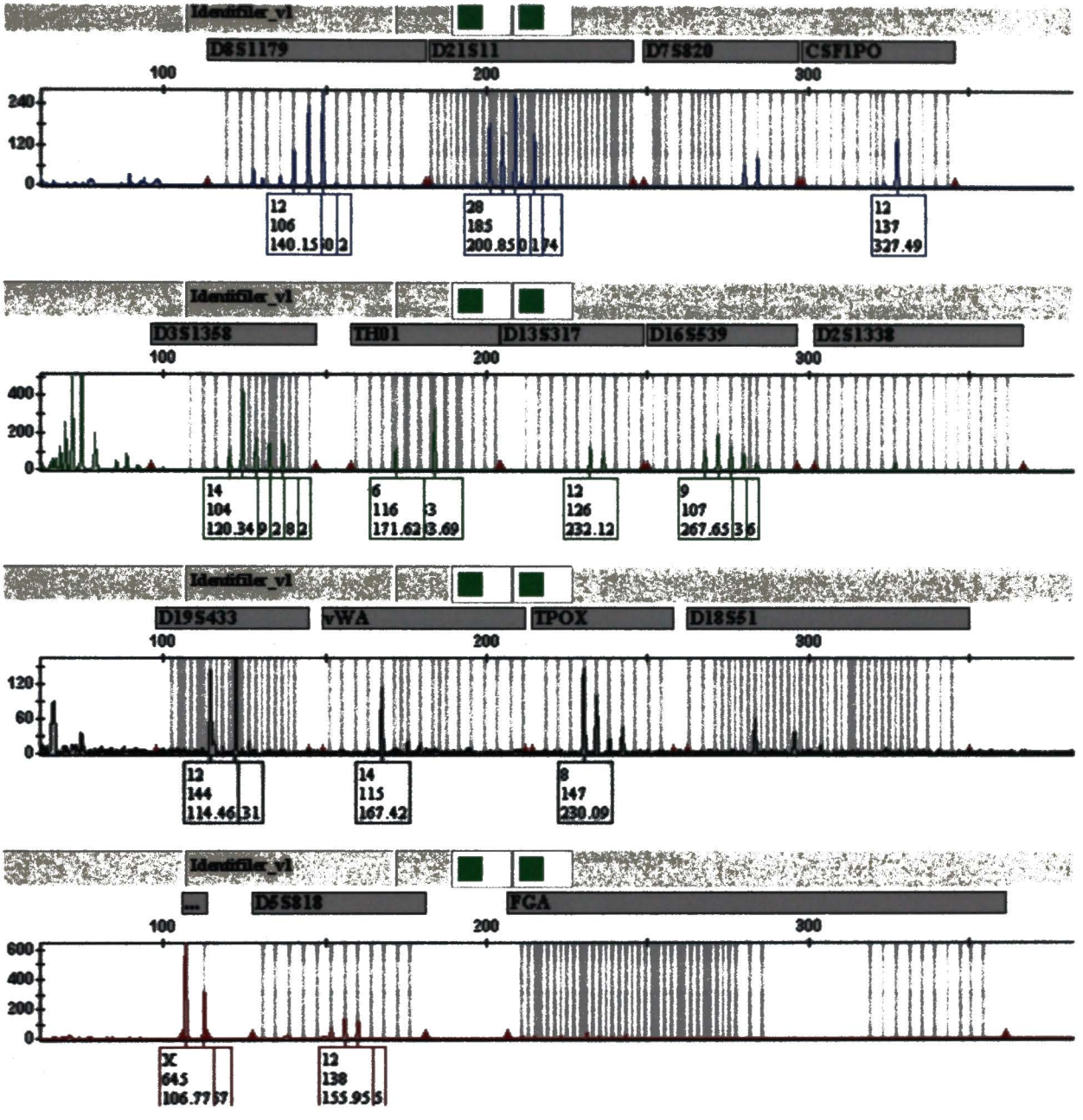
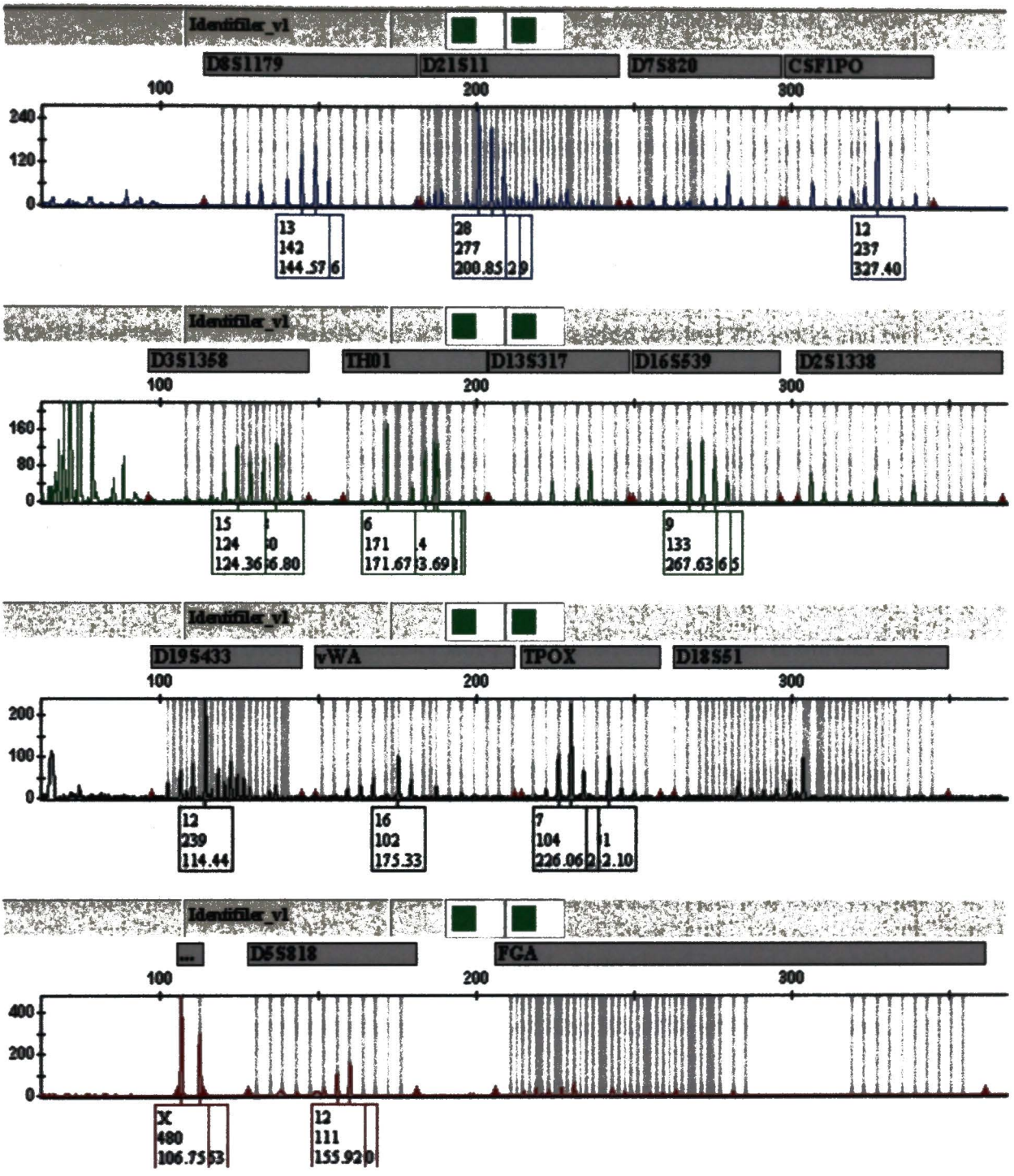


Figure 6-4: Electropharagram of mixed sample 3-4



Reproducibility Study:

Pathway 3 was the collection method used for the reproducibility study portion of the research. There were 3 samples taken (1 from each thumb and one from an index finger) from 13 subjects. The total average DNA concentration from each sample was 0.0129ng/ul significantly lower than the 0.235ng/ul average of the preliminary results for pathway 3. Only one of the 39 samples had a DNA concentration higher than 50pg/ul (sample 10-2, 0.0674ng/ul), which would be needed to meet the lower end of the optimal range for input DNA when the max allowable of 10ul was used. 20 of the samples fell into the low copy number range of less than 100pg of input DNA. 2 of the 39 samples (3-2 and 11-3) recovered an undetermined amount of DNA. A complete listing of DNA recovery can be seen in Table 6-2.

The samples of the reproducibility study were analyzed with both Identifiler® and PowerPlex®16 systems. The Identifiler® system was able to obtain only 2 full profiles (samples 8-1 and 13-1A) resulting in a 4.54% success rate. The other profiles resulted with 12 partial, 26 inconclusive and 3 mixed. PowerPlex®16 overall produced higher quality samples obtaining 5 full profiles at 11.36% success rate. The other profiles resulted with 12 partial, 4 inconclusive and 22 mixed. PowerPlex®16 produced a higher number of correct interpretable alleles with 662 of 1085 (61.01%) while the samples processed with Identifiler® only produced 257 (23.68%) correct interpretable alleles. A complete listing of interpretable alleles for the reproducibility study can be seen in Appendix C and D.

Table 6-2: Reproducibility Fingerprint Sample DNA Recovery Results										
Subject	Sample 1			Sample 2			Sample 3			Average DNA Concentration (ng/ul)
	DNA Concentration (ng/ul)	DNA Yield[1] (ng)	Input DNA[2] (ng)	DNA Concentration (ng/ul)	DNA Yield[1] (ng)	Input DNA[2] (ng)	DNA Concentration (ng/ul)	DNA Yield[1] (ng)	Input DNA[2] (ng)	
1	0.0159	0.716	0.159	0.0027	0.122	0.027	0.0104	0.468	0.104	0.0097
2	0.0194	0.873	0.194	0.0103	0.464	0.103	0.0051	0.230	0.051	0.0116
3	0.0008	0.036	0.008	UND.	0.000	0.000	0.0050	0.225	0.050	0.0019
4	0.0043	0.194	0.043	0.0164	0.738	0.164	0.0146	0.657	0.146	0.0118
5	0.0366	1.647	0.366	0.0032	0.144	0.032	0.0012	0.054	0.012	0.0137
6	0.0247	1.112	0.247	0.0251	1.130	0.251	0.0059	0.266	0.059	0.0186
7	0.0020	0.090	0.020	0.0020	0.090	0.020	0.0016	0.072	0.016	0.0019
8	0.0260	1.170	0.260	0.0046	0.207	0.046	0.0133	0.599	0.133	0.0146
9	0.0021	0.095	0.021	0.0009	0.041	0.009	0.0112	0.504	0.112	0.0047
10	0.0301	1.355	0.301	0.0674	3.033	0.674	0.0370	1.665	0.370	0.0448
11	0.0054	0.243	0.054	0.0052	0.234	0.052	UND.	0.000	0.000	0.0035
12	0.0212	0.954	0.212	0.0181	0.815	0.181	0.0240	1.080	0.240	0.0211
13	0.0149	0.671	0.149	0.0070	0.315	0.070	0.0084	0.378	0.084	0.0101

[1]DNA recovered in a 45ul elution volume. [2]10ul of eluted DNA added per STR amp reaction.

Abrasive Study:

The 5 samples (1-3, 2-2, 5-2, 8-3, 13-1) used for the abrasive study showed that the swabbings of the abrasive overall produced higher DNA yields than the swabbings of the corresponding fingerprints (Figure 6-5). The average DNA concentration for the 5 swabs obtained from the abrasive was 0.05422ng/ul, an increase of 521.34% when compared to the swabs obtained from the corresponding fingerprints (0.0104ng/ul) (Table 6-3). The abrasive sample 2-2A recovered the highest DNA concentration of the abrasive samples with 0.137ng/ul an increase of 1330.09% from the corresponding fingerprint sample 2-2 at 0.0103ng/ul.

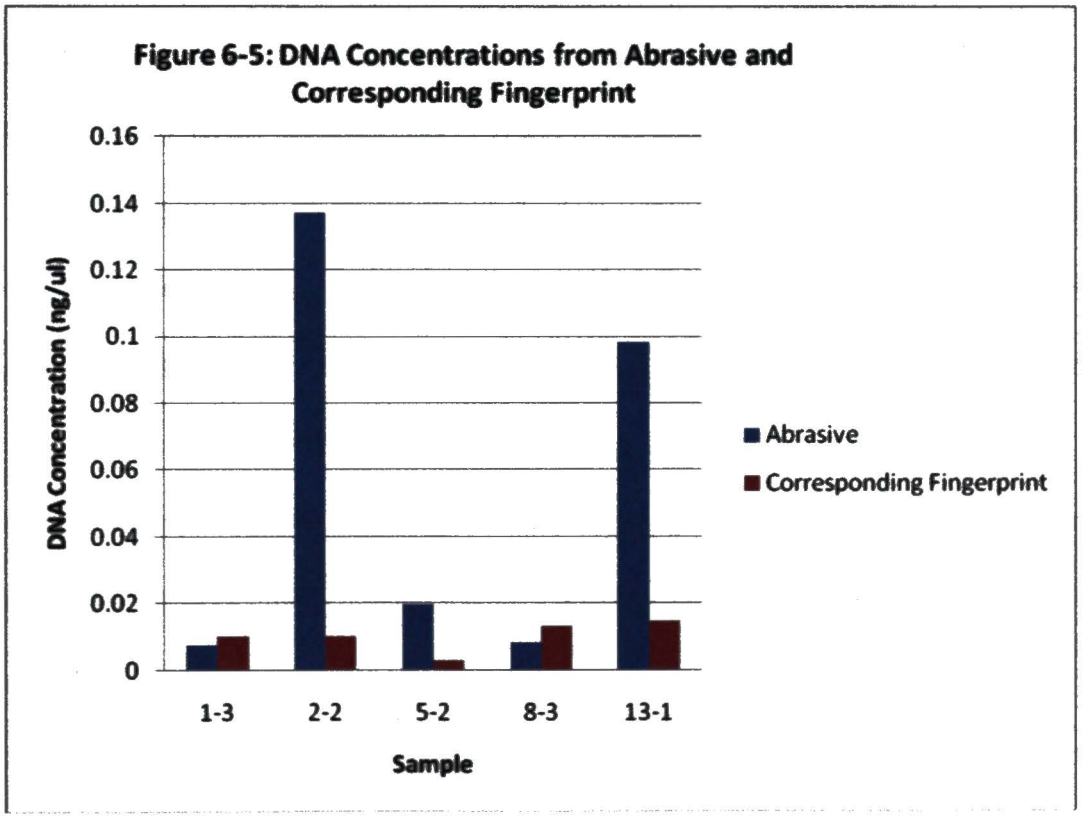


Table 6-3: DNA Recovery from Abrasive and Corresponding Fingerprint							
Sample	Abrasive			Corresponding Fingerprint			Percent Change
	DNA Concentration (ng/ul)	DNA Yield[1] (ng)	Input DNA[2] (ng)	DNA Concentration (ng/ul)	DNA Yield[1] (ng)	Input DNA[2] (ng)	
1-3	0.0076	0.342	0.076	0.0104	0.4680	0.1040	73.07
2-2	0.137	6.165	1.37	0.0103	0.4635	0.1030	1330.09
5-2	0.0198	0.891	0.198	0.0032	0.1440	0.0320	618.75
8-3	0.0085	0.3825	0.085	0.0133	0.5985	0.1330	63.90
13-1	0.0982	4.419	0.982	0.0149	0.6705	0.1490	659.00
[1]DNA recovered in a 45ul elution volume. [2]10ul of eluted DNA added per STR amp reaction							

Overall, the profiles obtained from the swabs collected from the abrasive had fewer incidences of stochastic effects and produced a slightly greater number of interpretable alleles.The sample with the clearest results for both systems was sample 13-1. The abrasive swab for sample 13-1 when processed with Identifiler® had 8 more interpretable alleles resulting in a full profile. There was also a 25% decrease in the occurance of peak hight imbalance amongst heterozygous peaks. For the same sample when processed with PowerPlex®16 the abrasive swab had an increase of 2 interpretable alleles also resulting in a full profile. Again there was a decrease in the occruance of peak hight imbalance amongst heterozygous peaks by 50%.

Figure 6-6: Sample 13-1 profile obtained from the swabbing of the adhesive using Identifiler®. ★ Denotes peak height imbalance.

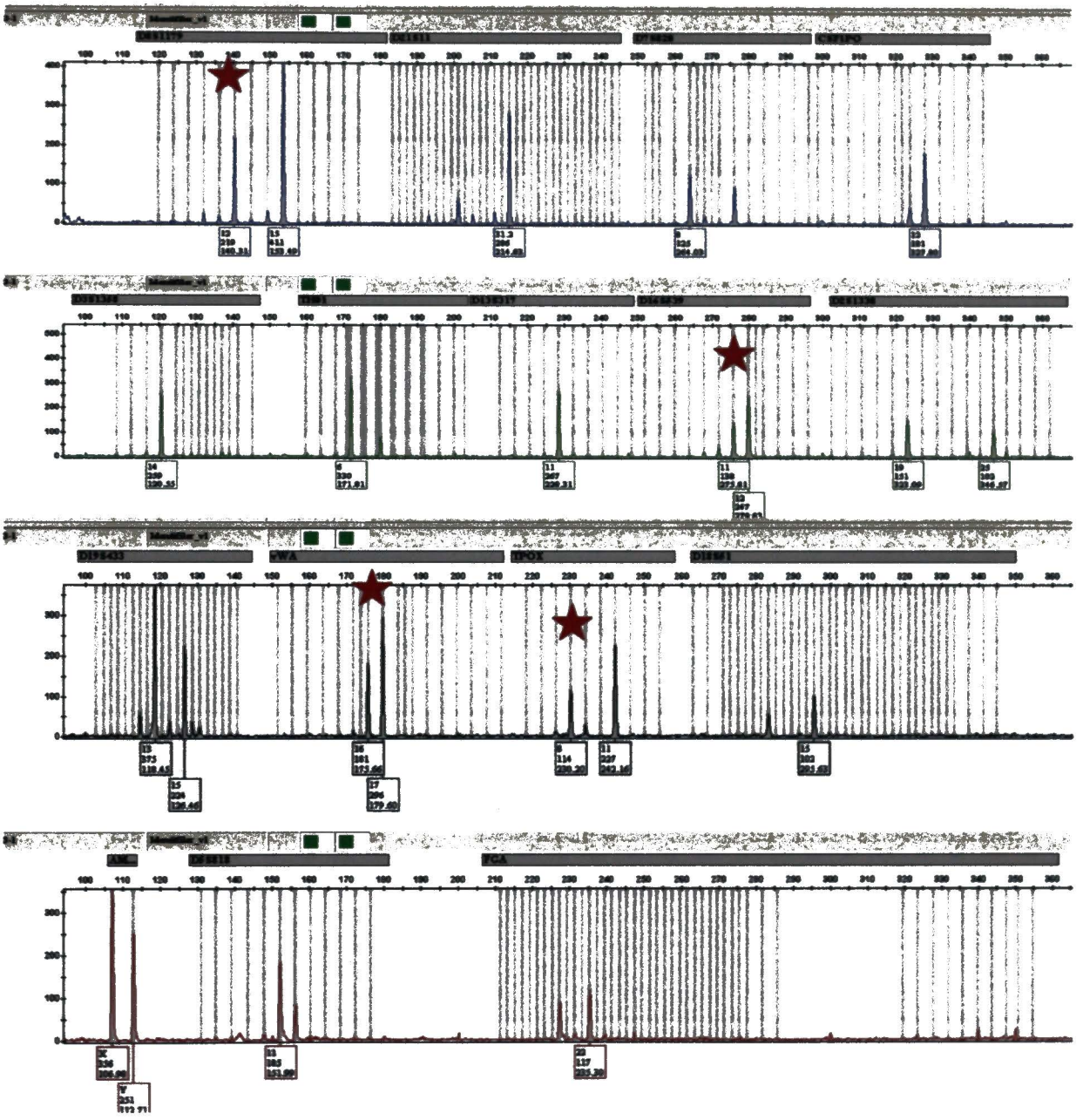


Figure 6-7: Sample 13-1 profile obtained from the swabbing of the abrasive using Identifiler®. ★ **Denotes peak height imbalance.**

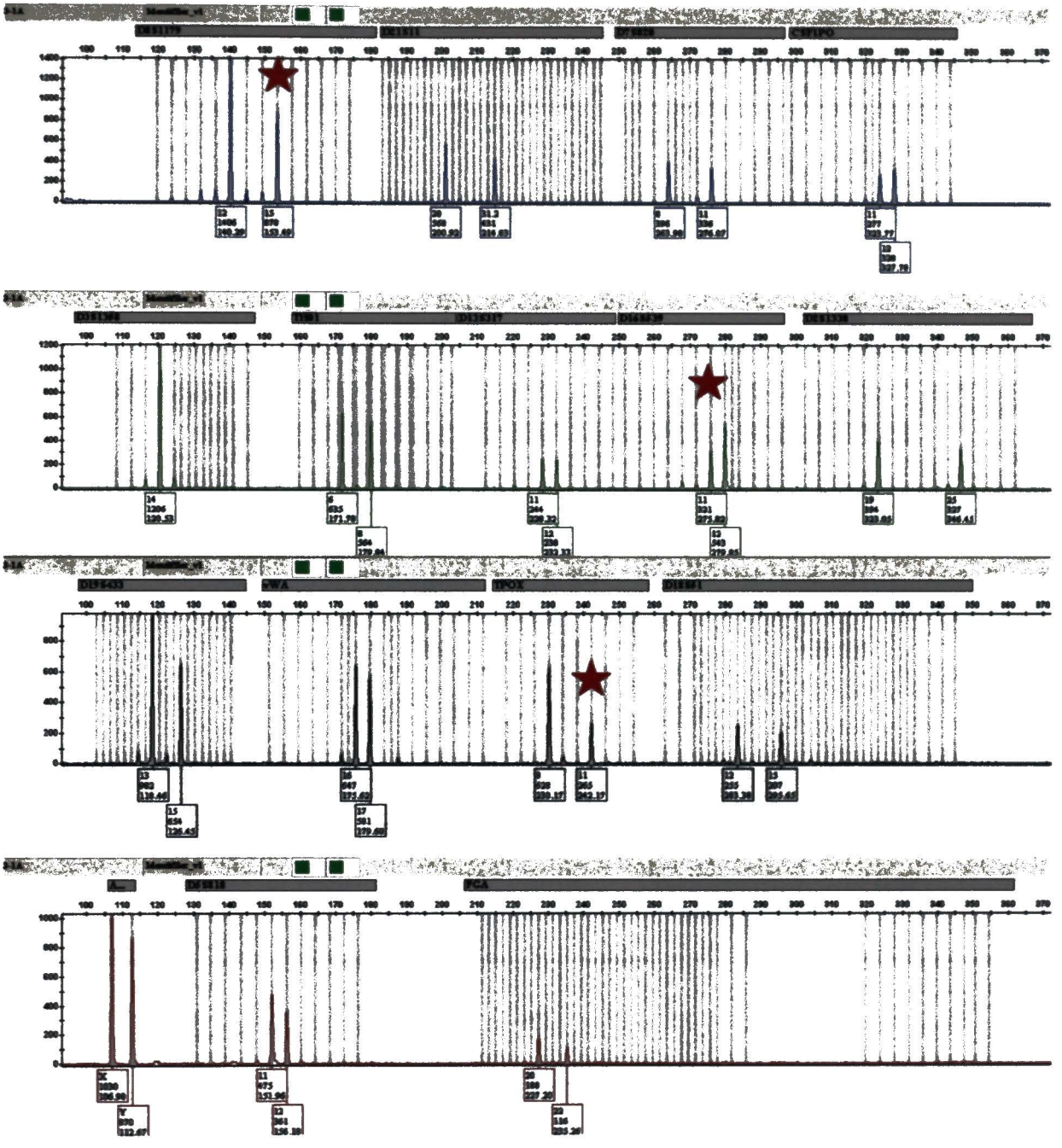


Figure 6-8: Sample 13-1 profile obtained from the swabbing of the adhesive using PowerPlex®16. ★ Denotes peak height imbalance.

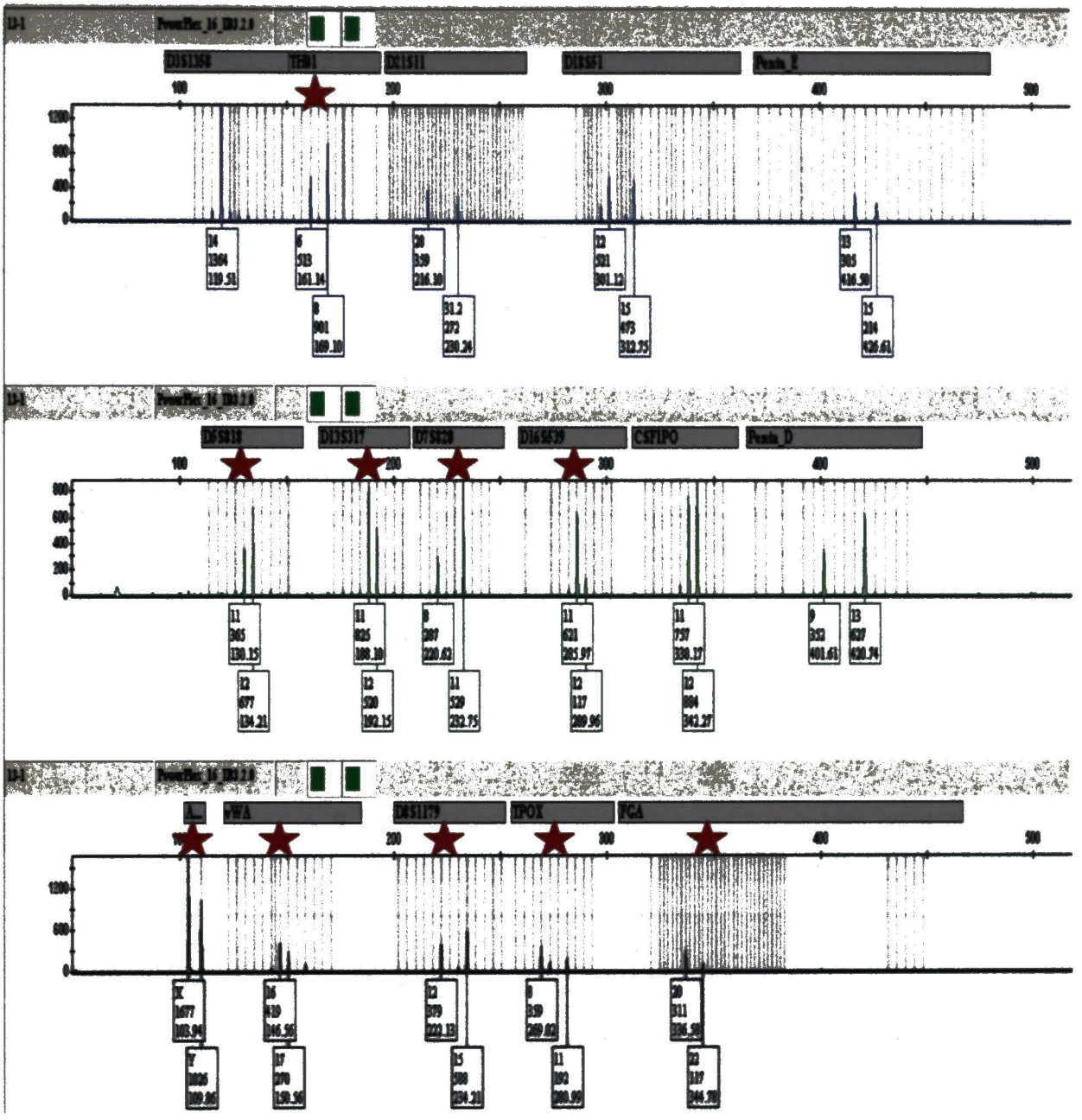
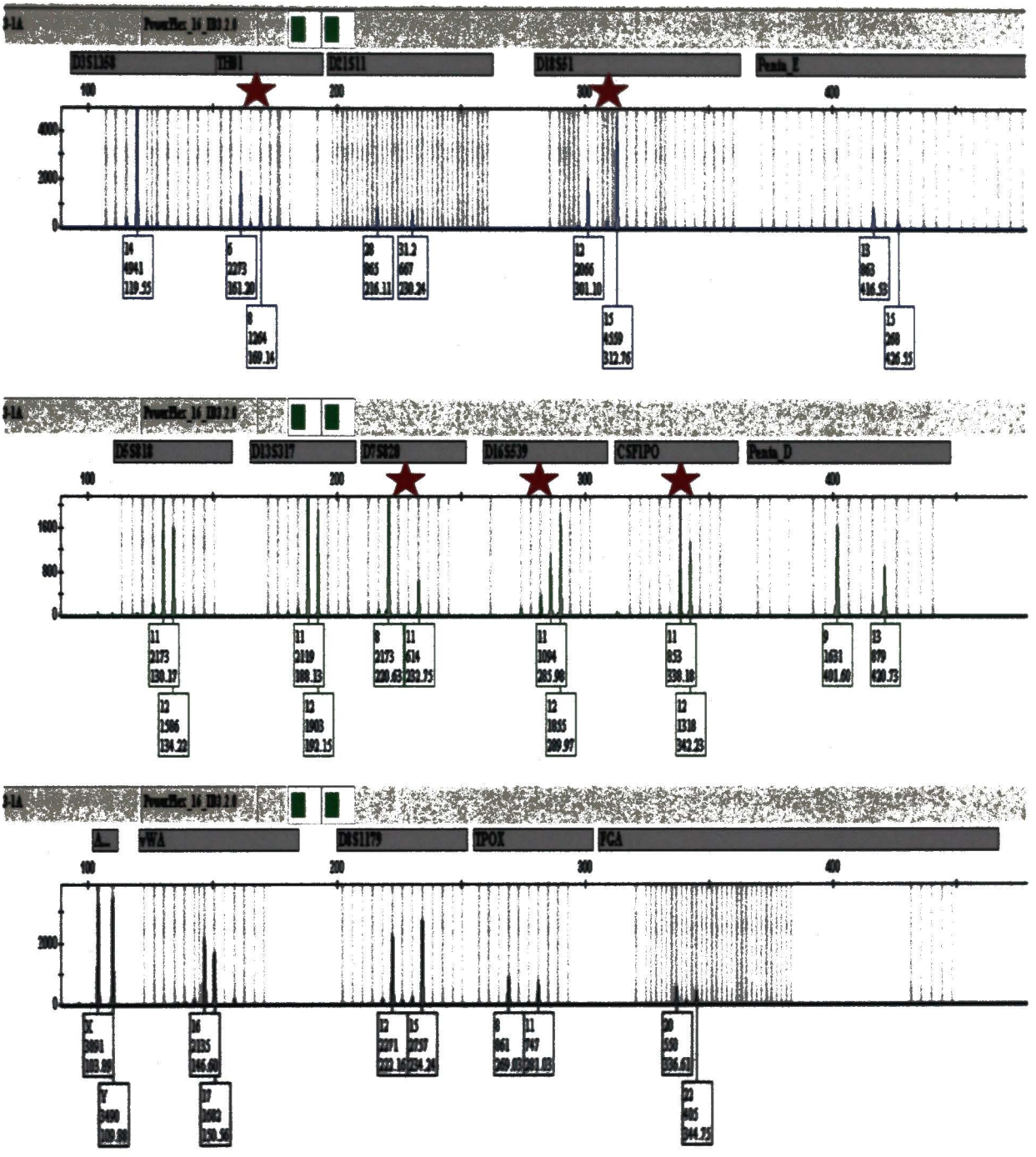


Figure 6-9: Sample 13-1 profile obtained from the swabbing of the abrasive using PowerPlex®16. ★ **Denotes peak height imbalance.**



VII. Discussion

The samples that were used to obtain genetic profiles during this research were fingerprints. Fingerprint samples yielded trace amounts of DNA which varies depending on the shedder type of the individual [22]. However, the use of the abrasive prior to printing with MICROFIELD's Lift & Rub is intended to increase the amount of viable cells that can be deposited. It has been previously reported that a fingerprint is not going to yield more than a few nanograms of DNA, with yields typically in the <0.01ng to 0.3ng range [23 & 24]. In general this study has shown this to be true with the exception of one sample that had a yield of 28.5ng DNA. Even with the use of the abrasive prior to printing the amount of DNA recovered from the fingerprints was typically below 1ng.

The majority of the samples within this study were low copy number (LCN) samples. The STR profiles generated from LCN samples typically display increased artifacts due to stochastic affects. Stochastic effects such as peak height imbalance, allele dropout and increased sizes of stutter were evident with these samples. Figure 6-8 is a good example of the large number of occurrences of peak height imbalance issues that were present throughout most of the samples. Another concern within a laboratory setting is the possibility of contamination through secondary transfer when working with fingerprints [25]. Due to these known problems when working with fingerprint samples a number of precautions were taken to avoid such issues. Fingerprints were collected in the morning prior to potential exposure to amplified DNA.

Prior to printing, the subjects had cleaned their hands with hand wipes and allowed them to air dry. The Lift & Rub fingerprinting kits were treated with UV using a StrataLinker® 2400 (Stratagene®). This was done to crosslink any DNA that may have been introduced during the manufacturing of the kits. The forceps used during sample processing were cleaned with a series of 10% bleach, distilled water, and 70% ethanol washes before and after each use. However, a number of samples demonstrate profiles from multiple donors and possible contamination by PCR product (Figure 6-2).

Based upon the results obtained, PowerPlex®16 provided the best results. Overall, PowerPlex®16 produced a greater number of full profiles, 5 resulting in an 11.36% success rate. Identifiler® produced a total of 2 full profiles resulting in a 4.54% success rate. The difference in the success rates may be attributed to the difference between the amplification parameters of the two systems. The PowerPlex®16 kit uses 32 cycles for the PCR amplification process, while Identifiler® uses 28 cycles. Identifiler® allows for a maximum volume of 10ul of input DNA, while PowerPlex®16 allows for up to 19ul of input DNA. During this study a volume of 10ul of input DNA was used as a standard for comparison between the systems. By utilizing an additional 9ul of input DNA with PowerPlex®16, may lead to a greater number of full profiles obtained.

VIII. Conclusion

MICROFIELD's Lift & Rub fingerprinting card is a novel device that provides a dual biometric means for the identification of an individual. The collection method to obtain both a fingerprint and a DNA sample is fast and simple and has great potential for future forensic use. As a result of this study the most effective protocol to obtain an STR profile is to initially have the individual clean their hands with an alcohol wipe and allow the fingers to air dry. The fingertip is then rubbed on the abrasive strip ten times in a rolling fashion. The fingertip is then applied to the Dry-Ink, and then using a rolling or plain impression, the fingertip is pressed on to the adhesive pad. To obtain a DNA sample, a sterile swab moistened with distilled water is gently pressed and rolled on the abrasive pad. The original idea behind the Lift & Rub fingerprint card was to obtain the DNA from the adhesive portion of the device. However, the new protocol of swabbing the abrasive as opposed to the adhesive appears to provide greater results.

The STR profiles obtained from the new Lift & Rub device does not suggest that this method is currently capable of replacing the conventional methods used to obtain a reference sample from a known individual. Further research needs to be done to improve the reliability and reproducibility of this product. With the limited number of samples obtained for this study there was little consistency. The STR profiles generated varied considerably within an individual and between individuals. The quality of the STR profiles showed day to day variation as well as variation from samples collected on the same day. A number of factors could contribute to these variations such as the shedder

status, handedness, and pressure applied during the brushing of the finger on the abrasive. Research has shown that classifying an individual as a good shedder or poor shedder is not easily determined and that any one individual is capable of producing a spectrum of results depending on the day [26]. Handedness of the subjects was not taken into consideration during the study. Perhaps the utilization of one hand versus the other for an individual could account for the differences observed from the samples collected on the same day. In addition, it was not possible to standardize the amount of pressure applied to the individual's finger during the abrasive rubbing step. Any one of these factors, or a combination of them, could account for the variations in the STR profiles obtained. Based upon the limited amount of testing done, it is difficult to attribute any one of these factors to the overall success rate of obtaining a complete STR profile.

Further research using the Lift & Rub technology is necessary before this device could be considered for the collection of a reference DNA sample. Larger studies with greater number of participants and more samples from each are needed in order to obtain more statistically sound results. An improved method for cleaning the hands and fingers may be necessary to reduce the amount of contamination resulting in a mixed STR profile. This could be done by implementing a bleach wipe prior to the alcohol wipe or having the individuals wash their hands with soap and water. The amount of DNA recovered from the Lift & Rub card may have been reduced due to DNA being trapped within the . fibers of the swab. Utilizing a swab moistened with dH_2O, the cells removed

from the abrasive may be lysed and physically entrapped within the swab head. Utilizing a swab moistened with isotone could prevent the cells from lysing and the DNA entrapping within the fibers of the swab. In addition by reducing the elution volume of the sample from 45ul to 25ul would increase the final DNA concentration and would allow more DNA to be added to the PCR amplification reaction. The reduction in the elution volume the DNA would provide a more optimal amount of input DNA for amplification. During this study a volume of 10ul of input DNA was used as a standard for comparison between the Identifiler® and PowerPlex®16 systems. The PowerPlex®16 System allows for up to 19ul of input DNA. Utilization of the maximum volume of DNA would also increase the amount of input DNA and may ultimately result in a higher number of interpretable alleles. This could potentially result in an increased percentage of full profiles obtained. With these adjustments the Lift & Rub could become the standard for law enforcement DNA reference sample collection.

APPENDIX A

State	All Felonies	Some Juveniles	Some Misdemeanors	Some Arrestees	Not Guilty By Mental Defect or Guilt	Other
Alabama	X					
Alaska	X	X		X -- Violent felonies.		
Arizona	X	X		X -- Many serious felonies.		Includes residential and criminal burglary.
Arkansas	X	X -- Violent crimes only.	X -- Some sexual offenses.		X	
California	X	X		X -- Expansion to all felon arrestees starts in 2009.		Includes those convicted of terrorist activity in violation of weapons of mass destruction provisions; and those convicted of a qualifying offense in another state.
Colorado	X	X				Includes any person who has a duty to register as a sex offender, including probationers, habitual offenders as condition of parole, and those released without parole supervison.
Connecticut	X				X	Includes persons on probation or parole prior to discharge from supervision.
Delaware	X		X -- Certain child endangerment or abandonment crimes.			

State Laws on DNA Data Banks Qualifying Offenses, Printed with permission from National Conference of State Legislatures, Criminal Justice Program, Denver Colorado [2].

State	All Felonies	Some Juveniles	Some Misdemeanors	Some Arrestees	Not Guilty By Mental Defect or GBMI	Other
Kentucky	X	X				Includes those convicted of unlawful transaction with a minor, promoting sexual performance of a minor, Burglary I and II and Class A and B felonies involving death or serious injury to the victim.
Louisiana	X	X		X -- Authorized to extent funding is available.		
Maine	X	X	(May include a lesser included offense if a qualifying offense was originally charged.)			Includes all Class A, B, C serious crimes and Class D and E convictions if the person had prior felony conviction for which DNA not collected.
Maryland	X	X				Includes some misdemeanors.
Massachusetts	X	X				
Michigan	X	X				
Minnesota	X	X	(May include offenses "arising out of same set of circumstances.")	X -- Specified serious crimes upon judicial finding of probable cause.		
Mississippi	X					
Missouri	X					
Montana	X	X				
Nebraska						

State Laws on DNA Data Banks Qualifying Offenses, Printed with permission from National Conference of State Legislatures, Criminal Justice Program, Denver Colorado [2].

State	All Felonies	Some Juveniles	Some Misdemeanors	Some Arrestees	Not Guilty By Mental Defect or GBMI	Other
Nevada			X -- Failure to register as a convicted person.			Includes all Class A or B felonies, or a Category C felony that involved use or threatened use of force; also includes some drug offenses.
New Hampshire		X				Includes violent crimes.
New Jersey	X	X	X -- Any crime for which a sentence of imprisonment of 6 months or more is imposed.		X	
New Mexico	X	X		X -- Specific violent felonies.		
New York	X		X -- Many misdemeanors.			Includes many serious felonies and some controlled substance offenses.
North Carolina	X				X	Includes persons on community supervision.
North Dakota	X			X -- All felonies - effective 01/09.		Many serious felonies, including burglary.
Ohio	X	X	X -- Certain child victim offenses.			
Oklahoma	X					2001 law requires planning to incrementally add qualifying felonies to the database, to include all felony offenses by 2006.
Oregon	X	X				
Pennsylvania		X				Includes violent and sexual offenders.
Rhode Island	X					

State Laws on DNA Data Banks Qualifying Offenses, Printed with permission from National Conference of State Legislatures, Criminal Justice Program, Denver Colorado [2].

State	All Felonies	Some Juveniles	Some Misdemeanors	Some Arrestees	Not Guilty By Mental Defect or GBMI	Other
South Dakota	X	X		X -- Felonies punishable by 5 years or more in prison.		
Tennessee	X	X		X -- Violent felonies, upon the finding of probable cause.		Includes those persons seeking transfer to the state under interstate compact who have committed qualifying offense.
Texas	X	X	(May be required by court order for any offense.)	X -- Post-indictment only in certain sex crimes.		Expanding to all felons contingent upon federal funds.
Utah	X	X	X -- Class A misdemeanors. Others may qualify if convicted on lower degree of qualifying offense.		X	Includes persons convicted in another state of a qualifying offense. Includes all persons who must register as a sex offender.
Vermont	X		(Only if as part of a plea agreement.)			
Virginia	X	X		X -- Violent felonies, including attempts.		
Washington	X	X				Includes those who have been convicted out of state or under federal law of a violent offense. Includes all persons who must register as a sex offender.
West Virginia	X					
Wisconsin	X	X	X -- Some misdemeanors for which sex offender registration is required.		X	
Wyoming	X	X				Includes all persons required to register as a sex offender.

State Laws on DNA Data Banks Qualifying Offenses, Printed with permission from National Conference of State Legislatures, Criminal Justice Program, Denver Colorado [2].

APPENDIX B

Pathway Results Allele Calls

Subject 1

	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,14	29,31	10,11	11,12	16,17	6,9	9,14	12,13	17,18	8,8	13,16	10,11	20,23	X,Y
PW														
1	13,14	31	INC	INC	INC	INC	9,14	12	17	8	INC	11	INC	X
2	13,14	INC	INC	INC	INC	6	INC	INC	INC	INC	INC	INC	INC	INC
3	13,14	29,31	10,11	11,12	16,17	6,9	9,14	12,13	17,18	8	13,16	10,11	20,23	X,Y
4	13,14	29,31	10,11	11,12	16,17	6,9	9,14	12,13	17,18	8	13,16	10,11	20,23	X,Y
5	13,14	29,31	11	11,12	16,17	6,9	9,14	INC	17,18	8	13	10,11	INC	X,Y
6	13,14,[12]	29,31,[28,30]	11	11	16,17,[14,15]	6,9	14	12,[10]	17	8	INC	10	INC	X,Y
7	13,14	29,31	10,11	11,12	16,17	6,9	9,14	12,13	17,18	8	13,16	10,11	20,23	X,Y
8	13	29,31	INC	INC	16,17	6,9	9,14	12	17	(8)	13	10,11	INC	X,Y

Subject 2

	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,16	30,30	10,10	11,13	16,18	9.3,9.3	9,13	9,11	16,17	8,11	14,16	11,12	19,25	X,X
PW														
1	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	(X)
3	13,16	30	10	11,13	16,18	9.3	9,13	9,11	16,17	8,11	14,16	11,12	19,25	X
4	13,16	30	10	11	16,18	9.3	9,13	9,11	16,17	8,11	14,16	11,12	19,25	X
5	13,16	30	10	11,13	16,18	9.3	9,13	9,11	16,17	8,11	14,16	11,12	19,25	X
6	13,16	30	10	11,13	18	9.3	9,13	11	16,17	11	16	11,12	INC	X
7	13,16	30	10	11	16,18	9.3	9,13	11	16,17	8,11	14	11,12	19	X
8	13,16	30	10	INC	16,18	9.3	9	9	16	8	INC	11,12	INC	X

PW - Pathway, INC - inconclusive, (#) - allele detected but below interpretation threshold, [#] - called allele foreign to donor profile

Pathway Results Allele Calls

Subject 3														
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	14,14	30,31.2	12,13	12,12	18,18	6,9	12,13	9,10	14,17	8,8	12,17	12,13	21,24	X,X
PW														
1	14,[12,13]	30,31.2,[28,29]	INC	(12)	18,[14,15,16,17]	6,9	12	9,10,[11]	14	(8)	INC	12,13	INC	X,[Y]
2	14	30,31.2	INC	12	18	6,9	13	9,10	14,17	8	INC	13	24	X
3	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
4	13,14	30,[28,29]	INC	12	18,[15]	6,9,[9.3,10]	INC	9,10,[11]	[16]	8,[7,11]	INC	12,13	INC	X,[Y]
5	14	INC	INC	INC	[15]	INC	INC	INC	INC	(8)	INC	INC	INC	X
6	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
7	14,[15]	[28,29]	INC	INC	[15]	INC	INC	INC	INC	INC	INC	INC	24	X,Y
8	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC

PW - Pathway, INC - inconclusive, (#) - allele detected but below interpretation threshold, [#] - called allele foreign to donor profile

APPENDIX C

Reproducibility and Abrasive Study Identifiler® Allele Calls

	Subject 1													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	14,14	28,29	10,10	10,12	14,15	6,8	12,13	11,13	14,15	8,10	14,17	11,12	19,20	X,X
Sample														
1	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
3	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
1-3A	14,[10]	INC	INC	INC	15	INC	INC	INC	INC	INC	INC	INC	INC	INC

	Subject 2													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,14	29,31	10,11	11,12	16,17	6,9	9,14	12,13	17,18	8,8	13,16	10,11	20,23	X,Y
Sample														
1	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
3	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
2-2A	13,14	29	INC	INC	16,17	6,9	INC	12,13	17,18	8	13,16	10	INC	X,Y

	Subject 3													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,14	28,32.2	11,12	11,11	16,18	7,9	8,13	9,14	14,18	8,9	12,13	11,13	19,25	X,X
Sample														
1	INC	INC	INC	INC	INC	INC	[12]	INC	INC	INC	INC	INC	INC	INC
2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
3	INC	INC	INC	(11)	INC	INC	INC	INC	INC	9	INC	11	INC	INC

PW - Pathway, INC - inconclusive, (#) - allele detected but below interpretation threshold, [#] - called allele foreign to donor profile

Reproducibility and Abrasive Study Identifiler® Allele Calls

	Subject 4													
.	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,16	30,30	10,10	11,13	16,18	9.3,9.3	9,13	9,11	16,17	8,11	14,16	11,12	19,25	X,X
Sample														
1	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
3	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
	Subject 5													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	14,14	30,31.2	12,13	12,12	18,18	6,9	12,13	9,10	14,17	8,8	12,17	12,13	21,24	X,X
Sample														
1	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
3	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
5-2A	14	31.2	INC	INC	18	6	INC	10	14,17	8	INC	INC	INC	X
	Subject 6													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,15	29,30	12,12	INC	15,17	INC	8,13	INC	15,16	8,8	14,14	9,11	21,24	X,X
Sample														
1	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
3	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC

PW - Pathway, INC - inconclusive, (#) - allele detected but below interpretation threshold, [#] - called allele foreign to donor profile

Reproducibility and Abrasive Study Identifiler® Allele Calls

	Subject 7													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
.	8,14	29,29	9,9	10,12	15,16	9.3,9.3	10,12	11,13	17,19	8,8	12,13	12,13	20,25	X,X
Sample														
1	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
2	INC	INC	INC	INC	INC	(9.3)	INC	INC	INC	(8)	INC	INC	INC	(X)
3	INC	INC	INC	INC	INC	(9.3)	INC	INC	INC	(8)	INC	INC	INC	(X)

	Subject 8													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	12,13	27,29	9,9	10,10	14,14	7,9.3	11,11	11,12	15,18	11,12	14,15	12,13	21,24	X,X
Sample														
1	12,13	27,29	9	10	14	7,9.3	11	11,12	15,18	11,12	14,15	12,13	21,24	X
2	13	INC	INC	INC	INC	[8]	(11)	INC	INC	INC	INC	INC	INC	(X)
3	12,13	INC	INC	(10)	14	7,9.3	(11)	11	INC	INC	INC	INC	INC	(X)
8-3A	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC

	Subject 9													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	12,13	30,31.2	8,8	11,11	16,16	8,9.3	11,12	9,11	14,19	8,8	15,15	11,13	19,22	X,X
Sample														
1	INC	30	INC	INC	(16)	INC	INC	INC	19	(8)	INC	INC	INC	INC
2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
3	12,13	INC	(8)	(11)	16	8	INC	11	14	8	15	INC	22	X

PW - Pathway, INC - inconclusive, (#) - allele detected but below interpretation threshold, [#] - called allele foreign to donor profile

Reproducibility and Abrasive Study Identifiler® Allele Calls

	Subject 10													
.	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	12,13	29,32.2	7,10	10,11	15,18	6,9	12,14	10,10	16,18	9,10	13,14	12,12	21,24	X,Y
Sample														
1	12,13	29,32.2	7	10,11	15,18	6,9	INC	10	16,18	9,10	13,14	12	INC	X,Y
2	12,13,[16]	29,32.2,[30,31.2]	7,10	10,11	15,18,[16]	6,9,[8]	12,14,[11]	10	16,18,[17]	9,10	13,14,[11]	12,[11]	21,24	X,Y
3	12,13	32.2	7,10	11	15,18	6,9	12	10	16,18	10	14	12	INC	X,Y
	Subject 11													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	10,12	30,31	7,13	9,10	14,16	7,9.3	11,12	11,12	16,17	8,9	15,17	9,13	21,24	X,X
Sample														
1	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
3	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
	Subject 12													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,13	30,31.2	9,9	11,11	15,16	8,9.3	11,12	11,12	14,14	8,8	13,15	11,12	19,22	X,Y
Sample														
1	13	30,31.2	9	INC	15,16	INC	INC	11,12	14	8	INC	12	INC	X,Y
2	13	30,31.2	9	11	15,16	8,9.3	12	11,12	14	8	15	11,12	19	X,Y
3	13	30,31.2	9	11	15,16	8,9.3	11,12	11	14	8	INC	INC	INC	X,Y

PW - Pathway, INC - inconclusive, (#) - allele detected but below interpretation threshold, [#] - called allele foreign to donor profile

Reproducibility and Abrasive Study Identifiler® Allele Calls

	Subject 13 D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	12,15	28,31.2	8,11	11,12	14,14	6,8	11,12	11,12	16,17	8,11	12,15	11,12	20,22	X,Y
Sample														
1	12,15	31.2	8	12	14	6	11	11,12	16,17	8,11	15	11	22	X,Y
2	12,15	INC	11	11,12	14	8	INC	11,12	16,17	11	12	11	INC	X,Y
3	12	INC	INC	INC	14	INC	INC	11	16,17	8	INC	11	INC	Y
13-1A	12,15,[10,11,13]	28,31.2	8,11	11,12	14	6,8	11,12	11,12	16,17	8,11	12,15	11,12	20,22	X,Y

PW - Pathway, INC - inconclusive, (#) - allele detected but below interpretation threshold, [#] - called allele foreign to donor profile

APPENDIX D

Reproducibility and Abrasive Study PowerPlex16® Allele Calls

	Subject 1													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	14,14	28,29	10,10	10,12	14,15	6,8	12,13	11,13	14,15	8,10	14,17	11,12	19,20	X,X
Sample														
1-1	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
1-2	INC	INC	INC	INC	14,(15)	(6),8	12	INC	(14)	INC	INC	(11,12,)[(10)]	INC	X,[Y]
1-3	14	(28)	10	10	14,15	6,8	12,13	11,13	14,15	8,10	14	11,(12)	INC	X
1-3A	INC	INC	10	10	15	8	[(9)]	13	INC	INC	17	12	[21]	X

	Subject 2													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,14	29,31	10,11	11,12	16,17	6,9	9,14	12,13	17,18	8,8	13,16	10,11	20,23	X,Y
Sample														
2-1	13,(14)	29,31	10,11	11,12	16,17	6,9	9,14	12,13	17,18	8	13,16	10,11	(23)	X,Y
2-2	13,14,[10]	29,31	10,11	11,12	16,17	6,9	9,14	12,13	17,18	8	13,16	10,11	23	X,Y
2-3	13,14	29,(31)	10,11	(12)	16,(17)	6,9	9,14	12	17,18,[19]	8	13,(16)	10,11	(20)	X,Y
2-2A	13,14	29,31	10,11	12	16,17	6,9	9,14	12,13	17,18	8	13,16	10,11	(20)	X,Y

	Subject 3													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,14	28,32.2	11,12	11,11	16,18	7,9	8,13	9,14	14,18	8,9	12,13	11,13	19,25	X,X
Sample														
3-1	(14),[(16)]	INC	(11)	INC	16	INC	INC	9,14	(14),18,[17]	8	[17]	11,(13)	INC	X
3-2	(13)	INC	INC	(11)	(16)	7,(9)	(8),[12]	9,[(11)]	INC	8	INC	(11)	INC	X
3-3	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC

	Subject 4													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,16	30,30	10,10	11,13	16,18	9.3,9.3	9,13	9,11	16,17	8,11	14,16	11,12	19,25	X,X
Sample														
4-1	13,16	30	10	11,13	16,18	9.3	9,13	(9),11,[(12)]	16,17	8,11	14,16	11,12	19	X,[Y]
4-2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
4-3	13,16,[10]	30	10	11,13	16,18,[15,17]	9.3	9,13	9,11	16,17	8,11	14,16,[13]	11,12	19,25	X

PW - Pathway, INC - inconclusive, (#) - allele detected but below interpretation threshold, [#] - called allele foreign to donor profile

Reproducibility and Abrasive Study PowerPlex16® Allele Calls

	Subject 5													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	14,14	30,31.2	12,13	12,12	18,18	6,9	12,13	9,10	14,17	8,8	12,17	12,13	21,24	X,X
Sample														
5-1	14	30,31.2	12,13	12	18	6,9	12,13	9,10	14,17	8	12,17	12,13	21,24	X
5-2	14	30	(12),13	12	18	6,9	12,13	9,10	14,17	8	12,17	12,13	21	X
5-3	14	INC	12	12	18	(6),9	12	9,(10)	14,17	8	(17),[(19)]	(12)	INC	X
5-2A	14	30,31.2	12,13	12	18	6,9	12,13	9,10	14,17	8	12,17	12,13	21,(24)	X
	Subject 6													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	13,15	29,30	12,12	INC	15,17	INC	8,13	INC	15,16	8,8	14,14	9,11	21,24	X,X
Sample														
6-1	13,15	29,30	12,	[7,13]	15,17	[7,9]	8,13	[9,14]	15,16	8	14	9,11	21,24	X
6-2	13,15	29,30	12	[7,13]	15,17	[7,9]	8,13	[9,14]	15,16	8	14	9,11,[12]	21,24	X,[Y]
6-3	15,[10]	29,30	12	[7,13,12]	15,17,[16]	[9,(7),(8)]	8,13	[9]	15,16	8	14	9,11,[13]	24	X,Y
	Subject 7													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	8,14	29,29	9,9	10,12	15,16	9.3,9.3	10,12	11,13	17,19	8,8	12,13	12,13	20,25	X,X
Sample														
7-1	[12]	INC	INC	INC	16	INC	INC	(11)	INC	INC	INC	(12)	INC	(X)
7-2	8,14,[(13)]	INC	(9)	10	15,16,[(17)]	9.3	10	11,[10]	(17),19	8	INC	(12),[11]	INC	X,[Y]
7-3	(8),14,[12]	INC	(9)	INC	(15),16	9.3,[7]	(12)	13	19	8	(13),[14]	(13)	INC	X
	Subject 8													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	12,13	27,29	9,9	10,10	14,14	7,9.3	11,11	11,12	15,18	11,12	14,15	12,13	21,24	X,X
Sample														
8-1	12,13	27,29	9	10	14	7,9.3	11	11,12	15,18	11,12	14,15	12,13	21,24	X
8-2	12,13,[10]	(29)	[(8)]	10	14	9.3	INC	INC	INC	11	14,(15)	(13),[11]	21	X,[Y]
8-3	12,13,[15]	27	INC	10,[OL]	14	7,9.3	11,[8,9]	11,12,[10]	18,[17]	INC	15	12,13	INC	X,[Y]
8-3A	INC	INC	INC	INC	14	INC	INC	INC	INC	INC	INC	INC	INC	X

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Reproducibility and Abrasive Study PowerPlex16® Allele Calls

	Subject 9													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	DSS818	FGA	AMEL
	12,13	30,31.2	8,8	11,11	16,16	8,9.3	11,12	9,11	14,19	8,8	15,15	11,13	19,22	X,X
Sample														
9-1	(12),13[8,14,15]	(30),(31.2)	8,[(10),11]	[(10)]	16,[(14),15]	(9.3),[(6)]	12	11	[18]	(8),[11]	15,[13]	11,13	(19)	X,[Y]
9-2	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC	INC
9-3	12,13,[10]	(30),31.2	8	11,[10]	16,[15,(17)]	8,9.3	11,(12)	9,11,[(10)]	14,19,[(16),18]	8	15,[13,14]	11,13	INC	X

	Subject 10													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	DSS818	FGA	AMEL
	12,13	29,32.2	7,10	10,11	15,18	6,9	12,14	10,10	16,18	9,10	13,14	12,12	21,24	X,Y
Sample														
10-1	12,13	29,32.2	7,10	10,11	15,18	6,9	12,14,[11]	10,[(11)]	16,18,[17]	9,(10)	13,14	12	21,24	X,Y
10-2	12,13,[16]	29,32.2,[(30)]	7,10,[8]	10,11,[(12)]	15,18,[16]	6,9,[8]	12,14,[8,11]	10,[13]	16,18,[17,19]	9,10,[8]	13,14,[11]	12,[10,11]	21,24	X,Y
10-3	12,13	(29),32.2	7,10,[8]	10,11	15,18,[16]	6,9,[(8)]	12,14	10	16,18	9,[8]	13,14,[15]	12,[11]	(21),24	X,Y

	Subject 11													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	DSS818	FGA	AMEL
	10,12	30,31	7,13	9,10	14,16	7,9.3	11,12	11,12	16,17	8,9	15,17	9,13	21,24	X,X
Sample														
11-1	10,12,[(16)]	INC	13,[(10)]	INC	INC	7,9.3	11,12	INC	INC	[(10)]	15	INC	INC	X
11-2	INC	INC	[(10)]	INC	14,[(15)]	(7)	INC	INC	(17)	INC	(15),17,[(14)]	INC	INC	X
11-3	12,[14]	INC	INC	INC	16	INC	(12)	[9,10]	(16)	INC	INC	INC	[(19)]	X,[Y]

	Subject 12													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	DSS818	FGA	AMEL
	13,13	30,31.2	9,9	11,11	15,16	8,9.3	11,12	11,12	14,14	8,8	13,15	11,12	19,22	X,Y
Sample														
12-1	13	INC	9	INC	15,16	8,9.3	11,12	INC	14	INC	INC	11,12	INC	X,Y
12-2	13,[(17)]	30,31.2	9	11,[12]	15,16	8,9.3	11,12	11,12,[(13)]	14	8	13,15,[16]	11,12	19,22	X,Y
12-3	13	30,31.2	9	11	15,16	8,9.3	11,12	11,12	14	8	13,15	11,12	19,22	X,Y

PW - Pathway, INC - inconclusive, (#) - allele detected but below interpretation threshold, [#] - called allele foreign to donor profile

Reproducibility and Abrasive Study PowerPlex16® Allele Calls

	Subject 13													
	D8S1179	D21S11	D7S820	CSFIPO	D3S1358	THO1	D13S317	D16S539	VWA	TPOX	D18S51	D5S818	FGA	AMEL
	12,15	28,31.2	8,11	11,12	14,14	6,8	11,12	11,12	16,17	8,11	12,15	11,12	20,22	X,Y
Sample														
13-1	12,15	28,31.2	8,11	11,12	14	6,8	11,12	11,(12)	16,17	8,11	12,15,[11]	11,12	20,(22)	X,Y
13-2	12,15,[10,(13)]	28,31.2	8,11	11,12,[10]	14,[(16)]	(6),8,[(7)]	11,(12)	11,12	16,17	INC	12,15	11,12	20,22	X,Y
13-3	12,15	INC	(8),11	11,12	14,[(15)]	6,8	11,12	11,[(10)]	16,(17),[(15)]	(8),11	12,15	11,12	20	X,Y
13-1A	12,15,[11,13,14]	28,31.2	8,11	11,12	14,[13,15,16]	6,8,[7,(9.3)]	11,12	11,12,[(8),10]	16,17,[15,19]	8,11	12,15,[14,20]	11,12,[(10)]	20,22	X,Y

PW - Pathway, INC - inconclusive, (#) - allele detected but below interpretation threshold, [#] - called allele foreign to donor profile

REFERENCES

1. Violence Against Women and Department of Justice Reauthorization Act of 2005. 109th Congress; Public Law 109-162 – Jan. 5, 2006.

2. State Laws on DNA Data Banks Qualifying Offenses, Others Who Must Provide Samples. Denver, CO: National Conference of State Legislatures Criminal Justice Program; June 2008. Available from: http://www.ncsl.org/programs/cj/dnadatabanks.htm

3. Crime in California 2006. California: California Department of Justice, Division of California Justice Information Services Bureau of Criminal Information and Analysis CRIMINAL JUSTICE STATISTICS CENTER; 2006. Available from: http://ag.ca.gov

4. Victoria Police Department. Victoria Forensic Science Centre Fingerprint Branch. Structure of the Skin. FPB Training 2002;3:1-11

5. Croxton et al. Development of a GC-MS Method for the Simultaneous Analysis of Latent Fingerprint Components J Forensic Sci 2006; 51:1329-1333

6. Kücken M. Models for fingerprint pattern formation. Forensic Sci Int 2007;171:85-96

7. D.A. Dell, B.L. Munger, The early embryogenesis of papillary (sweat duct) ridges in primate glabrous skin. The dermatotopic map of cutaneous mechanoreceptors dermatoglyphics, Comp. Neurol. J. 1986; 244:511–532.

8. Scientific Working Group on Friction Ridge Analysis, Study and Technology. Friction Ridge Examination Methodology for Latent Print Examiners 8/22/2002 ver. 1.01

9. Maltoni D, Cappelli R, Advances in fingerprint modeling. Image Vis. Comput. 2007; doi:10.1016/j.imavis.2007.01.005.

10. Jain AK, Prabhakar S, Pankanti S. On the similarity of identical twin fingerprints. Pattern Recognition 2002; 35:2653-2663.

11. Pounds CA, Jones RJ. Physicochemical techniques in the development of latent fingerprints. Trends in Analytical Chemistry 1983;2:180-183

12. Czekanski P, Fasola M, Allison J. A Mechanistic Model for the Superglue Fuming of Latent Fingerprints. J Forensic Sci 2006;51:1323-1328

13. Wickenheiser RA. Trace DNA: A Review, Discussion of Theory, and Application of the Transfer of Trace Quantities of DNA through Skin Contact. J Forensic Sci 2002;47:442-450

14.US v Byron Mitchell, Criminal Action No. 96-407, US District Court for the Eastern District of Pennsylvania. July 1999

15. Latent Print Examination, Fingerprints, Palmprints, and Footprints; Available from: http://onin.com/fp/, 2002

16. Taking Legible Fingerprints. Federal Bureau of Investigation. Available from: http://www.fbi.gov/hq/cjisd/takingfps.html

17. DNA IQ™ Casework Sample Kit for Maxwell® 16. USA: Promega Corporation; 2007;TB354. Available from: www.promega.com.

18. Tissue and Hair Extraction Kit for use with the DNA IQ™ Protocol. USA: Promega Corporation; 2006; TB307. Available from: www.promega.com.

19. Maxwell® 16 instrument operating manual. Madison, WI: Promega Corporation; 2007;TM274.

20. AmpFISTR® Identifiler® PCR Amplification Kit . User's Manual. Applied Biosystems; 2006

21. PowerPlex® 16 system. USA: Promega Corporation; 2008;TMD012. Available from: www.promega.com.

22. Lowe A, Murray C, Whitaker J, Tully G, Gill P. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. Forensic Sci Int 2002;129:25-34

23. Alessandrini F, Cecati M, Pesaresi M, Turchi C, Carle F, Tagliabracci A. Fingerprints as Evidence for a Genetic Profile: Morphological Study on Fingerprints and Analysis of Exogenous and Individual Factors Affecting DNA Tying. J Forensic Sci 2003;48:586-592

24. Schulz MM, Reichert W. Archived or directly swabbed latent fingerprints as a DNA source for STR typing. Forensic Sci Int 2002; 127:128-130

25. Lowe A, Murray C, Whitaker J, Tully G, Gill P. The propensity of individuals to deposit DNA and secondary transfer of low level DNA from individuals to inert surfaces. Forensic Sci Int 2002;129:25-34

26. Phipps M, Petricevic S. The tendency of individuals to transfer DNA to handled items. Forensic Sci Int 2007;168:162-168