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The AmpFLSTR® Identifiler® Plus PCR Amplification Kit (Life Technologies, Carlsbad, CA), and the recently released GlobalFiler™ PCR Amplification Kit (Life Technologies) were compared to determine which provided the greater power of discrimination from DNA extracted from skeletal remains. I hypothesized, STR profiles obtained using the GlobalFiler™ kit for analysis of skeletal remains would result in an increased number of reportable genetic loci, and provide greater power of discrimination as compared to the Identifiler® Plus Kit. The results of this study showed GlobalFiler™, along with an automated extraction, could produce comparable or greater genetic typing results and discriminatory power from skeletal remains without the use of a second amplification kit.

Typing Success of Short Tandem Repeats on Skeletal Remains: Comparison of AmpFLSTR® Identifiler® Plus PCR Amplification Kit and GlobalFiler™ PCR Amplification Kit

Stephanie A. Garcia, B.S.

APPROVED:

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Major Professor

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Committee Member

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Committee Member

_____

University Member

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Arthur Eisenberg, Ph.D., Chair, Department of Forensic and Investigative Genetics

_____

Meharvan Singh, Ph.D., Dean, Graduate School of Biomedical Sciences

Typing Success of Short Tandem Repeat Loci on Skeletal Remains: Comparison of AmpFLSTR® Identifiler® Plus PCR Amplification Kit and GlobalFiler™ PCR Amplification Kit

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Stephanie A. Garcia, B.S.

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CHAPTER I

INTRODUCTION

There have been many mass disasters that have occurred in the United States and around the world. Many individuals often think of the terrorist attacks on the World Trade Center on September 11th, 2001, the Tsunami that devastated Southeast Asia in 2004, Hurricane Katrina that affected New Orleans in 2005, and the Tsunami that affected Japan in 2011, which killed over 230,000 individuals. However, few think of the large number of missing persons in the United States in the past five decades as a mass disaster (1). In the United States, it is reported in the National Crime Information Center's (NCIC) Missing Person File that there are between 85,000 to 100,000 active missing persons records annually, 639 of those being deceased unidentified bodies (2). Skeletal remains that cannot be identified by conventional means, such as fingerprints, dental, or anthropological, are either buried in graves labeled as Jane/John Doe, cremated with or without the retention of a biological sample for DNA analysis, or stored in medical examiners evidence rooms (1). With continued advances in DNA technology, the storage of biological samples for future testing is required to help the process of human identification.

Due to the structure of bones there are areas of mineralization within each bone that act as physical barriers to the extraction reagents, preventing release and recovery of DNA molecules (3). Obtaining DNA from bone samples is often challenging due to low levels of endogenous DNA, environmental, bacterial, and post-mortem DNA damage, as well as the presence of inhibitors that can co-purify with DNA (3). Unfortunately, often these samples are

the only source of biological material available for human identification. Because of its inherent variability, nuclear DNA is the initial choice for forensic examination (4). However, nuclear DNA recovered from skeletal remains is often limited and may be degraded. A study investigating the success rate of short tandem repeat (STR) typing from different types of bone samples observed a higher STR success rate in dense cortical bone, of weight bearing leg bones, compared to long arm bones (5). They concluded that the success of STR typing is often related to the type of bone samples available (6). However, a recent paper by Mundorff *et al*. contradicts that finding, and established a ranking of skeletal elements according to each bone's capacity to provide usable genetic information for identification (7). The research demonstrated that small, predominantly cancellous bone, or spongy bone, out-performed the dense cortical bones upon which many previously relied.

DNA testing examines highly polymorphic regions of DNA, which can vary in length, and this analysis provides the ability to differentiate individuals. The field of forensic genetics testing performs identification and comparisons of STRs with a technique known as the polymerase chain reaction (PCR). The PCR amplification process allowed forensic scientists to generate STR profiles starting from small amounts of DNA. The polymorphic regions of DNA detected contain STR loci that vary in length based upon the number of tandem repeats present within a fragment (8). These STR loci are highly variable between individuals. The amplified products generated are typically between 100 to 400 base pairs (bps) in length. The small amplicon size makes them very effective for human identification, specifically from compromised evidentiary samples such as bone (8).

By the early 1990s analysis of STR loci was starting to appear in forensic DNA testing (9). With the success of STR typing technology in the United Kingdom, the Federal Bureau of

Investigation (FBI) laboratory led the U.S efforts to establish a core group of STR loci. The development of these core STR loci became the backbone for the Combined DNA Index System (CODIS) (10). In April of 1996, 21 DNA typing laboratories along with the FBI began the evaluation of potential suitable STR loci for the CODIS National DNA Database (10). In 1997 the 13 core CODIS loci, CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S359, D18S51, and D21S11 were established. They were mandated for entry of known reference samples and were required to be attempted on all evidentiary samples for entry into the CODIS database. Commercial manufacturers developed PCR amplification kits to incorporate these 13 core loci.

There have been many generations of amplification kits developed to meet the requirements set forth by the FBI. In 2010, the AmpFLSTR® Identifiler® Plus PCR Amplification Kit (Life Technologies) was released, to perform a single amplification of all 13 core loci. With a single amplification reaction this kit analyzed the 13 core loci plus an additional two autosomal loci (D2S1338 and D19S433), as well as the Amelogenin sex-determining locus for a total of 16 (Figure 1). This kit uses a 5-dye chemistry: 4-dye channels for the STR loci and one for the size marker. This kit was also developed to assist case working laboratories in addressing the need for greater sensitivity, and a better tolerance to PCR inhibitors (11). The Identifiler® Plus kit required consumption of less DNA from evidentiary samples as compared to the past generation of kits. In only a single amplification reaction, the Identifiler® Plus kit generated a probability of identity of 10^{-18} for African-American, Caucasian, and Hispanic populations and 10^{-17} for Native Americans (12). Probability of identity is the probability that two individuals selected randomly would have an identical genetic profile. The Identifiler® Plus kit became one of the most widely used amplification reagents worldwide.

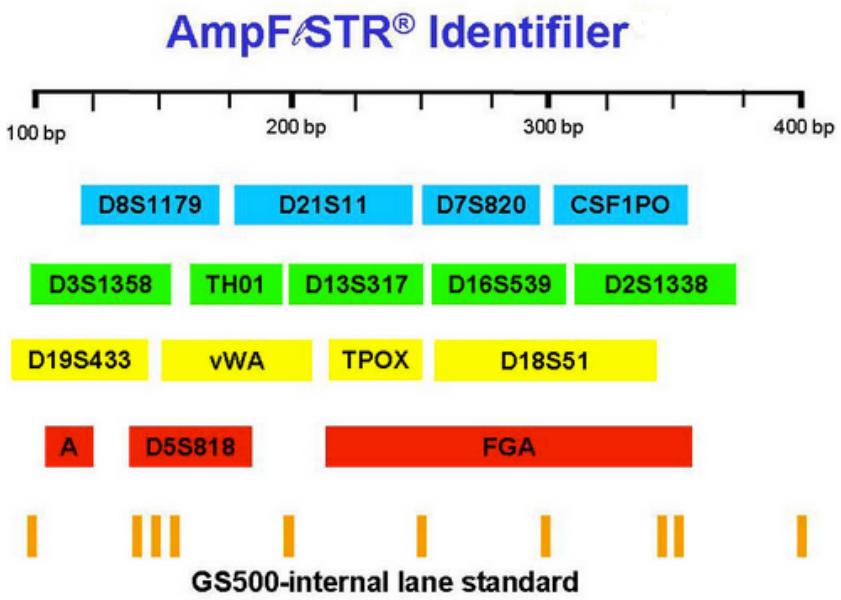


Figure 1: AmpFLSTR® Identifiler® Plus Loci. The 16 STR loci typed in the Identifiler® Plus kit along with the fluorescent dye label color and relative PCR product size ranges (13).

Within the past few years, the FBI has begun to re-evaluate the choice of core loci for the National DNA Index System (NDIS) working group. In May 2010, the FBI formed a CODIS Core Loci Working Group to determine which core loci would be advantageous (14). The group found three major reasons for expanding the number of core loci: (1) to reduce the likelihood of adventitious matches as the number of profiles in the database continues to grow, (2) to increase the number of concordant loci internationally, and (3) to increase discrimination power to aid missing persons cases (14). The working group gave strong consideration for the inclusion of the original core loci in the revised set, primarily due to the large number of genetic profiles already uploaded in the NDIS database. They also considered a number of loci that have been routinely typed internationally (14). Ultimately the CODIS Core Loci Working Group came up with a composite list. Section A, which the FBI considered the new minimum required CODIS core loci, and section B, which contained additional loci that were highly recommended for inclusion

in the amplification kits if possible (14). The FBI required kit manufacturers produce these new STR kits and complete required developmental validation studies specified by national standards.

In 2014, Life Technologies released a new kit known as the GlobalFiler™ PCR Amplification Kit to meet the recommendation of the Working Group. Life Technologies developed GlobalFiler™ as a 6-dye kit, which allows more STR loci to be typed in a single amplification reaction. This kit amplifies 21 autosomal loci, one Y-STR (DYS391), an insertion/deletion marker on the Y chromosome (Y-indel), and the sex-determining locus Amelogenin (15) (Table 1). Thirteen of the autosomal loci are from the original core and an additional seven loci are from the European Standard Set of Loci (ESSL). The GlobalFiler™ kit contains a total of 10 mini-STR loci, with amplicon size falling below 220 bps, which was designed to maximize performance on degraded samples (15) (Figure 2).

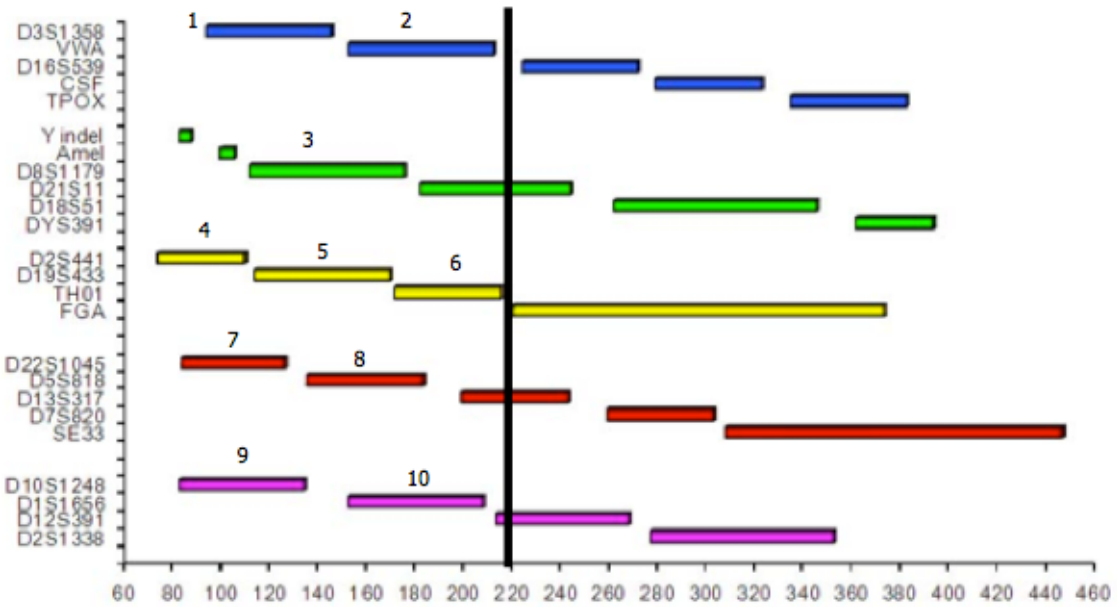


Figure 2: GlobalFiler™ Loci. The 24 loci typed in the GlobalFiler™ Amplification kit along with the fluorescent dye label color and relative PCR product size ranges. The black line that divides the graph is placed at 220 bps; the ten labeled loci left of the black line reflect the mini-autosomal STRs in this kit (16).

GlobalFiler™ contains an additional Y-STR locus to account for Amelogenin null Y alleles that can occur in some populations (14). The Amelogenin locus amplifies a region on the short arm

of the Y chromosome whereas the Y-indel and the DYS391 STR loci are located on the long arm of the chromosome. The additional Y-chromosome loci can aid in determining gender when the Amelogenin locus fails to amplify. Having these extra gender specific markers helps eliminate the need to use a second gender confirmatory test. The GlobalFiler™ Kit contains the same modified buffer system that was originally developed for the MiniFiler™ kit and used in the Identifiler® Plus kits. This buffer has been optimized to overcome PCR inhibitors that could co-purify with extracted DNA. Life Technologies has indicated that the GlobalFiler™ kit offers a probability of identity of about 10^{-26} for major population groups and can amplify DNA in approximately 80 minutes with results in approximately 2 hours. The salient difference between the Identifiler® Plus Kit and the GlobalFiler™ Kit is that the GlobalFiler™ can amplify additional STRs, including the mini-STR loci in a single amplification reaction, potentially helping with human identification.

Table 1: A Comparison of the Loci Typed with Identifiler® Plus and GlobalFiler™. Shows the core loci amplified in Identifiler® Plus and GlobalFiler™. Also shows the common loci amplified between the two kits.

Amplification Kits	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA	D10S1248	D22S1045	D2S441	D1S1656	D12S391	SE33	AMEL
Identifiler® Plus	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X							X
GlobalFiler ™	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

With all of the amplification kits used in laboratories today, full genetic profiles are not always obtainable. The UNT Center for Human Identification (UNTCHI) currently uses the AmpFLSTR® Identifiler® Plus kit to obtain genetic information from skeletal remains. In those cases where a full profile cannot be obtained with the Identifiler® Plus kit, another kit known as the AmpFLSTR® MiniFiler™ PCR Amplification Kit is then attempted. The combination of

both of these two kits can provide a probability of identity of 10^{-18}, with the major population groups. However, the use of a second kit requires an additional DNA aliquot, which in turn increases cost, time, and labor in order to obtain a comparable genetic profile. In contrast, the GlobalFiler™ kit combines the 13 core CODIS loci with the analysis of 10 mini-STR loci, in a single amplification. Therefore, the Federal Bureau of Investigation (FBI) has suggested that the Combined DNA Index system (CODIS) should expand the current 13 core loci to 24 loci. A kit that can analyze 24 loci will provide a greater power of discrimination and provide DNA analysts with greater confidence in associations made between skeletal remains and family reference samples. The use of a single amplification system, such as GlobalFiler™, could provide greater amounts of genetic information from challenged bone samples.

Therefore, the **purpose** of this study was to determine if STR profiles obtained from DNA extracted from bones with the AutoMate Express™, and then amplified with the GlobalFiler™ PCR Amplification kit could routinely provide a greater power of discrimination than the Identifiler® Plus kit. This study will assess the utility of the mini-STRs in the GlobalFiler™ Kit to enhance the overall power of discrimination from skeletal remains from unidentified decedents. I hypothesized that the STR profiles obtained using the GlobalFiler™ kit for the analysis of bone samples, would result in an increase number of reportable genetic loci, and provide a greater power of discrimination as compared to the currently used Identifiler® Plus Kit. GlobalFiler™ could potentially be the optimum amplification choice for the limited amounts of DNA obtained from challenged bone samples.

CHAPTER II

MATERIALS AND METHODS

Bone Sample Selection

Nine bone samples from the UNTCHI Missing Persons Laboratory were selected based on the amount of genetic information provided for this comparative study. A range of bone samples that had been previously processed using the standard demineralization and organic extraction methodology by the UNTCHI laboratory were used. Three different groups of bones were selected based on amount of genetic information the bones provided with Identifiler® Plus: samples that gave poor profiles (0-5 loci), partial profiles (6-11 loci), and full or nearly full profiles (12-15 loci) (Table 2). Each of the bone samples was amplified in duplicate.

Bone Sample Preparation

The bones were prepared following the protocol "Preparation of Skeletal Remains and Teeth for DNA Extraction" from UNTCHI Missing Persons Laboratory. All tools used in the preparation of bone samples were cleaned and UV cross-linked for one hour prior to use, and between samples. An area of each bone was cleaned in a negative airflow hood with a Dremel tool and sanding cone. The sanded bones were then cut into thin sections, using the Dremel tool with a cutting disk, so they would ultimately fit into a SPEX Freezer Mill cylinder. After the bone samples were initially cleaned they were then put into a labeled conical tube, and gently agitated with a 50% bleach solution for 5 minutes. The bleach solution was disposed of into a waste container. The bone fragments were rinsed several times in distilled water. This was

repeated until the water was clean and there was no scent of bleach. An ethanol wash was then performed using 100% ethanol and the waste was decanted. The cleaned samples were placed in a weigh boat and set aside to dry overnight.

Table 2: Bone Samples used in This Study Based on Their Groups. Samples provided by UNTCHI Missing Persons Laboratory. The number of loci generated with DNA extracted using their standard demineralization and organic extraction protocol and amplified using Identifiler® Plus Amplification Kit.

Poor Profiles (0-5 loci)

Sample Number	Bone Type	Amount of Genetic Information (loci) From Identifiler® Plus
0072-14	Femur	5
0075-14	Femur	1
0078-14	Femur	0

Partial Profiles (6-11 loci)

0077-14	Femur	10
0079-14	Femur	9
0080-14	Femur	7

Full or nearly full profiles (12-15 loci)

0073-14	Femur	15
0074-14	Femur	15
0076-14	Femur	15

Grinding of Bone with SPEX CentriPrep 6750 Freezer/Mill® Grinder

The cleaned, dry bone samples were grounded using the SPEX Freezer Mill® Grinder. The samples where placed into a polycarbonate tube, a metal impactor was placed into the tube and tube closed with metal end caps. The reservoir of the SPEX Freezer Mill® was filled with liquid nitrogen and, after an initial chill period of 7 minutes, additional liquid nitrogen was added. The cylinder containing the bone sample was inserted into the Freezer Mill and pulverized for 7 minutes. The bone samples were then visually inspected for proper pulverization. After samples were pulverized they were left overnight allowing them to warm to

room temperature. The end caps and impactor were removed and the bone powder was weighed and placed in labeled 50 mL conical tube. The bone powder was stored in -20°C (24).

DNA Extraction Using the AutoMate Express™

The manufacturer's recommended protocol for the AutoMate Express™ DNA Extraction System (Life Technologies) was followed for extraction of DNA from bone powder. Approximately 100 mg of powdered bone was transferred from the conical tube to a PrepFiler™ Bone and Tooth Lysate tube. Fresh PrepFiler BTA™ lysis solution was prepared using 220 µL PrepFiler™ BTA Lysis solution, 3 µL 1 M dithiothreitol (DTT), and 7 µL Proteinase K for each sample. The freshly prepared lysis solution, 230 µl, was added to the tube containing the bone powder, then mixed and centrifuged briefly. The tubes were then placed in a Multi-Therm™ shaker (Benchmark Scientific, Inc. South Plainfield, NJ) and incubated at 56°C at 1,100rpm for 2 hours. After the incubation the samples were centrifuged for 90 seconds at 10,000 x g and the clear lysate was transferred into a new PrepFiler™ Sample Tube. The volume was adjusted to 230 µL with fresh PrepFiler BTA™ Lysis Solution. The volume was critical in order to effectively bind the DNA to magnetic particles, allow for proper mixing, and prevent formation of air bubbles in the tip during the automated extraction run. The samples were gently mixed, and centrifuged, 230 µL of the PrepFiler BTA™ Lysis Solution was transferred from the lysate tubes to appropriate sample tubes. The AutoMate Express™ was assembled by adding the correct number of cartridges (Figure 3) into the cartridge rack and loaded into the instrument. The PrepFiler™ sample tubes containing lysate, AutoMate Express™ Tips, and elution tubes were placed into the tip and tube rack which was set into the machine in front of the cartridge rack. The AutoMate Express™ was run for approximately 30 minutes. After the run was

complete, the purified DNA was eluted in a volume of 50 µL of elution buffer and stored at 4°C (17).

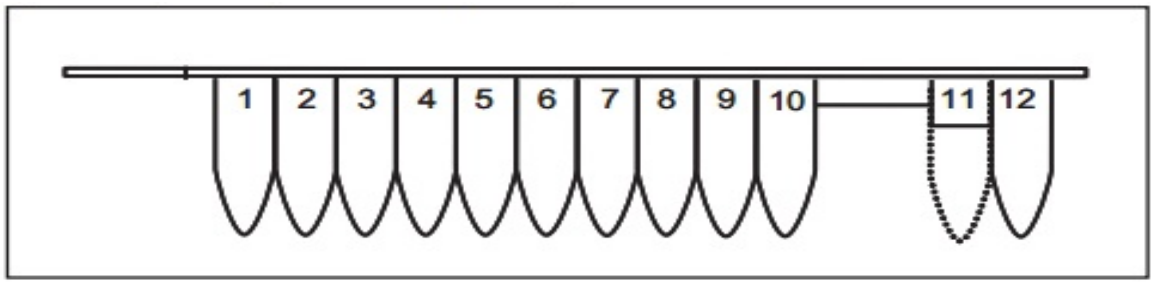


Figure 3: Illustration of the PrepFiler Express™ Cartridge. During the AutoMate Express™ run, tube 1 contains the lysis buffer, tube 2 contains magnetic particle suspension, tube 3 has the binding solution, tube 4 through 6 contains the wash buffer, tube 7 has the elution buffer, and tube 12 is placed in a heated chamber for elution (17). Tubes 8 through 11 are not used in this protocol.

Comparison between Standard Organic Extraction and Automated Extraction

The UNTCHI Missing Persons Laboratory previously performed the demineralization and organic extraction method, to obtain DNA, on the bones provided for this study. The standard demineralization and organic extraction method, performed by the Missing Persons Laboratory was compared to the PrepFiler BTA™ Forensic DNA Extraction Kit (Life Technologies) and the AutoMate Express™ (Life Technologies) extraction method. This was performed to determine which method recovered a greater amount of total DNA.

Quantification

DNA extracts were quantified using the Quantifiler® Duo Human DNA Quantification Kit (Life Technologies). The UNTCHI protocol for the "Human and Male DNA Quantification using Applied Biosystems Quantifiler® Duo Kit" was followed. Reactions were carried out on the 7500 Real-Time PCR System (Life Technologies). A standard dilution series was run with the samples to compare the results and determine the quantity of DNA to be used for further applications.

STR Amplification

Amplification was performed using the AmpFLSTR® Identifiler® Plus PCR Amplification Kit (Life Technologies) and the GlobalFiler™ PCR Amplification kit (Life Technologies). All bone samples were amplified in duplicate. Up to 10 µl of input DNA was used for the PCR reaction with both amplification kits. Thermal cycling conditions for the Identifiler® Plus Kit were as follows: 1) 95°C for 11 minutes, 2) 29 cycles alternating 94°C for 20 seconds and 59°C for 3 minutes, 3) 60°C for 10 minutes, and 4°C indefinitely. Thermal cycling conditions for the GlobalFiler™ Kit was as follows: 1) 95°C for 1 minutes, 2) 29 cycles alternating 94°C for 10 seconds and 59°C for 90 seconds, 3) 60°C for 10 minutes; 4°C for up to 24 hr.

Capillary Electrophoresis and Data Analysis

Amplified products from the Identifiler® Plus and GlobalFiler™ Amplification Kits were then electrophoresed on a 3500*xl* Genetic Analyzer (Life Technologies). Products obtained from the two amplification kits were run on the instrument following the manufacturer's recommendations on the 3500*xl* Genetic Analyzer. Data was analyzed using GeneMapper® *ID-X* v. 1.2 software (Life Technologies). The 3500*xl* has not been previously validated for use with forensic casework samples. The thresholds used for analysis of generated products were as follows: an analytical threshold of 100 relative fluorescence units (RFU) and a stochastic threshold of 200 RFU were used for both Identifiler® Plus and GlobalFiler™ kits. STR profiles, typed with both amplification kits, were categorized as full or nearly full profiles (12-15 loci), and partial profiles (anything under 12 loci).

Statistics

For statistical calculations, 15 common loci in both amplification kits were compared. Power of discrimination (PD) was calculated for both kits to determine which kit has greater statistical power for the purpose of human identification. Power of discrimination is related to the random match probability (RMP), which is the probability that two randomly selected individuals have identical phenotypes/genotypes. Power of discrimination is determined by the equation shown below:

$$P_D = 1 - P_i \quad (1)$$

The P_i is the random match probability and the random match probability is the sum of the squares of observed phenotype/genotype frequencies in a database. Random match probabilities were calculated using CODIS PopStats v.7.0.57.3.

CHAPTER III

RESULTS

DNA Extraction using the AutoMate Express™

The initial goal of this study was to compare extracted DNA from bone samples using the PrepFiler Express BTA™ Forensic DNA Extraction Kit (Life Technologies) and the AutoMate Express™ (Life Technologies) instrument to the DNA profiles generated from the UNTCHI Missing Persons Laboratory, which used the conventional organic extraction method. Total DNA recovered using the automated extraction method was compared to the total DNA recovered using the conventional organic extraction method from 100 mg of bone powder. The amount of DNA recovered was determined from the average of the quantification values obtained from each sample using the Quantifiler® Duo Human DNA Quantification Kit (Life Technologies). The DNA recovered using the AutoMate Express™ was greater for 7 out of the 9 bone samples with the exception of samples 0074-14 and 0076-14 (Table 3). The quality of the DNA extracted from the AutoMate Express™ System and the conventional organic extraction were further evaluated by examining the STR profiles generated with the Identifiler® Plus Amplification System.

The Identifiler® Plus STR profiles produced using the AutoMate Express™ where compared to the STR profiles produced using the conventional organic extraction method. In 6 of the 9 bone samples tested, the DNA extracted with the AutoMate Express™ gave more reportable loci (Table 4). Three samples, (0073-14, 0074-14, and 0076-14) from each extraction method produced full STR profiles. Overall, samples extracted with the AutoMate Express™

recovered a greater amount of DNA and generated higher quality profiles when compared to the conventional organic extraction.

Table 3: Comparison of DNA Recovered using the AutoMate Express™ vs. Organic Extraction. The total DNA recovered from 100 mg of bone powder for the 9 samples tested with different extraction methods.

Sample	AutoMate Express™ (ng)	Conventional Organic Extraction (ng)
0072-14	1.215	0.115
0073-14	4.480	1.050
0074-14	0.206	0.498
0075-14	3.835	0.402
0076-14	0.755	9.675
0077-14	0.331	0.007
0078-14	0.237	0.021
0079-14	0.650	0.243
0080-14	0.354	0.189

Table 4: Number of Reportable Loci using AutoMate Express™ vs. Organic Extraction. The number of loci reported using DNA obtained with UNTCHI's standard organic extraction method with the Identifiler® Plus kit (excluding the Amelogenin locus) versus the number of loci reported using DNA extracted with the AutoMate Express™ and amplified with Identifiler® Plus. Numbers reported reflect the profiles with the lesser number of loci from the duplicate samples.

Samples	Identifiler® Plus Loci Reported with UNTCHI Standard Organic Extraction	AutoMate Express™ loci with Identifiler® Plus
0072-14	5	15
0073-14	15	15
0074-14	15	15
0075-14	1	11
0076-14	15	15
0077-14	10	12
0078-14	0	14
0079-14	9	13
0080-14	7	15

Comparison of the Quality of the STR Profiles between Identifiler® Plus and GlobalFiler™ PCR Amplification Kits

The 15 loci in common between the Identifiler® Plus and the GlobalFiler™ kit were compared to assess the performance of each amplification system. The nine bone samples were extracted with the AutoMate Express™ and then amplified in duplicate using the Identifiler® Plus and GlobalFiler™ PCR Amplification Kits. The results showed that in 6 of the 9 samples, the Identifiler® Plus gave a greater number of loci producing better STR profiles (Table 5). In 3 of the 9 samples, both amplification kits gave a complete, 15 locus profile for the common loci (0072-14, 0073-14, and 0076-14). For those loci that were not reported, one allele was above the detection threshold but below the stochastic threshold. The assumption was that one allele dropped out at that locus.

Table 5: Number of Common Reportable Loci between Identifiler® and GlobalFiler™ Amplification Kits. The number of loci reported using DNA extracted with the AutoMate Express™ and amplified with Identifiler® Plus and GlobalFiler™ kits. The loci counted were those in common between the two kits minus the Amelogenin locus. Numbers reported reflect the profiles with the lesser number of loci from the duplicate samples.

Samples	Identifiler® Plus	GlobalFiler™
0072-14	15	15
0073-14	15	15
0074-14	15	13
0075-14	11	6
0076-14	15	15
0077-14	12	9
0078-14	14	11
0079-14	13	11
0080-14	15	12

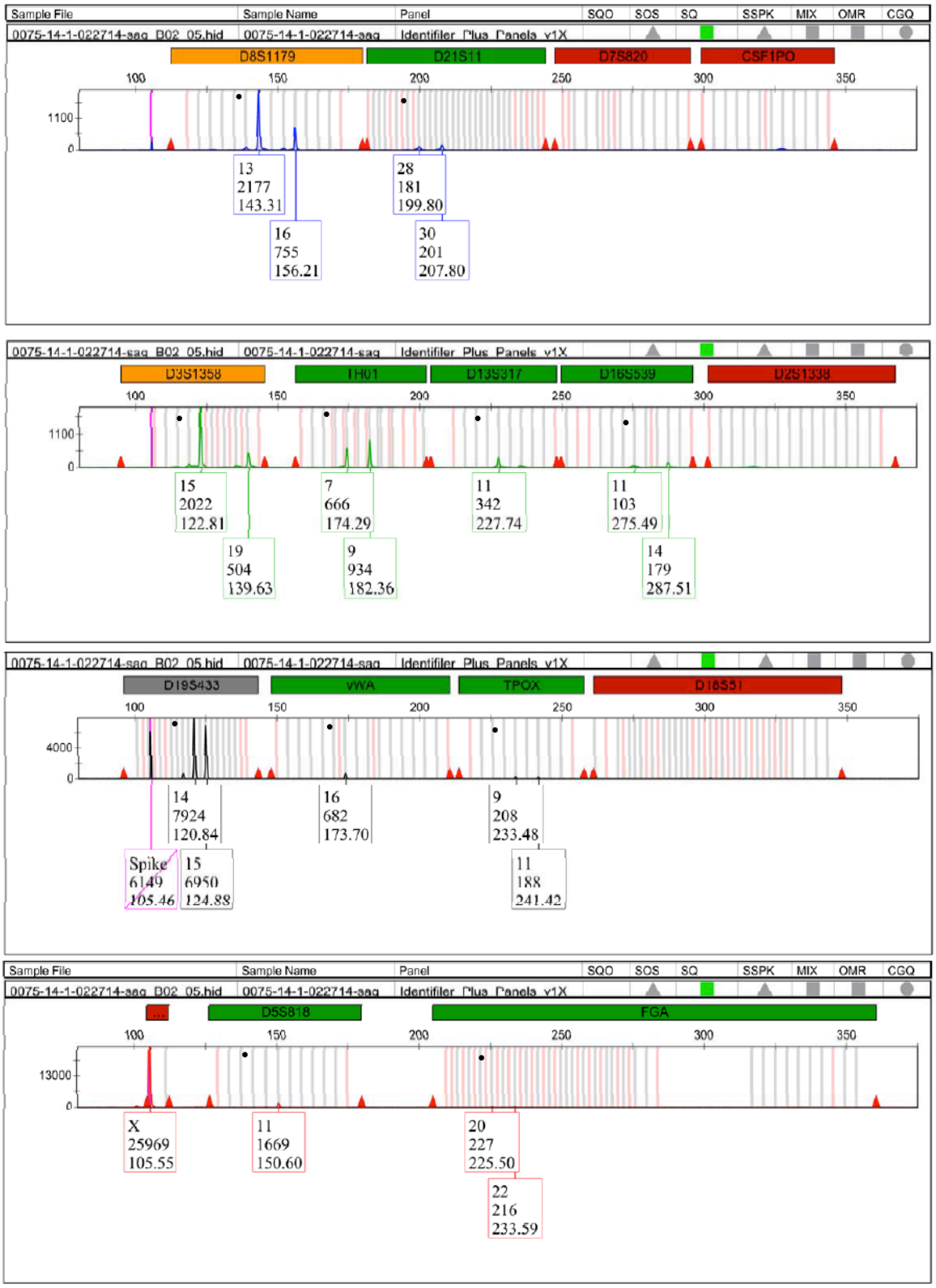
A comparison of the total number of reportable alleles generated between the common loci can be seen in table 6. For samples 0072-14, 0073-14, 0074-14, and 0076-14 the number of reportable alleles were identical between Identifiler® Plus and GlobalFiler™. As for the

remaining 6 samples, the total number of reportable alleles was greater when the samples were amplified with Identifiler® Plus Kit. Figure 4 shows two electropherograms that were generated from sample 0075-14. Figure 4A is an electropherogram from sample 0075-14 when amplified with Identifiler® Plus. When the same sample was amplified with GlobalFiler™ kit, there were less reportable loci (Figure 4B). Reportable loci are marked with dots.

Table 6: Total Number of Reportable Alleles Detected from the Bone Samples Amplified with Identifiler® Plus and GlobalFiler™ Amplification Kits. The number of alleles recovered from the replicates of both amplification kits. Loci that are in common were used to count the number of recovered alleles. Amelogenin was not included in the counts.

Sample	Identifiler® Plus		GlobalFiler™	
0072-14	27	27	27	27
0073-14	29	29	29	29
0074-14	27	27	25	27
0075-14	19	23	10	11
0076-14	25	25	24	25
0077-14	22	21	15	17
0078-14	26	26	23	23
0079-14	27	29	25	24
0080-14	26	26	22	24

A.)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SQ
SSPK
MIX
OMR
CGQ
0075-14-1-022714-sag_B02_05.hid
0075-14-1-022714-sag
Identifiler_Plus_Panels_v1X
D5S818
FGA
100
150
200
250
300
350
13000
0
X
25969
105.55
11
1669
150.60
20
227
225.50
22
216
233.59

B.)

Figure 4: STR Profiles of Sample 0075-14 Amplified using Identifiler® Plus and GlobalFiler™. 4A is an electropherogram of DNA extracted from bone sample 0075-14 amplified using Identifiler® Plus. 4B is an electropherogram of DNA extracted from bone sample 0075-14 amplified using the GlobalFiler™ Amplification kit. Reportable loci are marked with dots.

Quality of the STR profiles with GlobalFiler™ PCR Amplification Kit

The GlobalFiler™ kit was capable of amplifying more loci in a single amplification than the Identifiler® Plus kit. There is a total of 21 autosomal STR markers amplified with the GlobalFiler Kit, plus Amelogenin, a Y-indel, and a DYS391. We were only concerned with categorizing the 21 autosomal STR loci. In 8 of the 9 bone samples (0072-14, 0073-14, 0074-14, 0076-14, 0077-14, 0078-14, 0079-14, and 0080-14) full or nearly full STR profiles were produced. One of the samples (0075-14) produced a partial profile. When comparing the average number of reportable loci, which included the mini-STR loci and SE-33, amplification with GlobalFiler™ increased the number of reportable loci (Figure 5). The GlobalFiler™ mini-STRs increased three samples (0072-14, 0073-14, and 0076-14) from 15 loci to 21 loci, which would be considered a full profile (minus the Amelogenin and Y-indel, and Y-STR). Samples 0074-14 and 0080-14 increase by 5 loci, 0078-14 and 0079-14 increased by 4 loci, and 0075-14 and 0077-14 increased by 3 loci. We can also see from table 7 that there was an increase in recoverable alleles. All nine of the bone samples increased in the amount of alleles recovered. This increase in reportable loci and alleles recovered was expected to occur because of the extra STRs amplified.

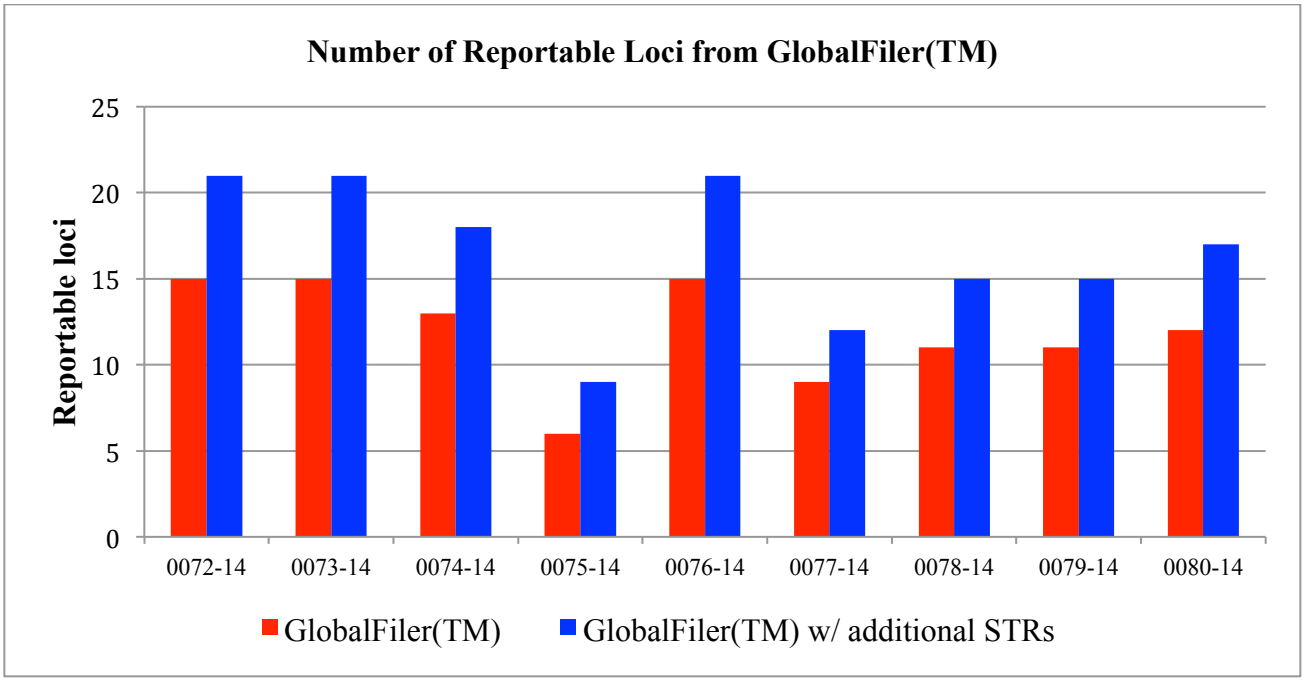

Figure 5: Average Number of Reportable Loci from GlobalFiler™. Average number of reportable loci with samples amplified with the GlobalFiler™ Amplification Kit. The reported loci are with and without the additional autosomal mini-STR loci. Averages were rounded down. Reportable loci excluded the sex-determining marker Amelogenin, the Y-indel, and DYS391. Numbers reported reflect the profiles with the lesser number of loci from the duplicate samples.

Table 7: Number of Alleles Generated with the GlobalFiler™ Amplification Kit. The number of alleles recovered from the replicates of GlobalFiler™ Amplification Kit without and with the mini-STRs. The Amelogenin, Y-indel, and DYS391 STR loci were not included in the counts.

Samples	GlobalFiler™ w/o additional STRs		GlobalFiler™ w/additional STRs	
0072-14	27	27	39	39
0073-14	29	29	38	38
0074-14	25	27	37	37
0075-14	10	11	15	16
0076-14	24	25	35	36
0077-14	15	17	22	23
0078-14	23	23	30	30
0079-14	25	24	32	32
0080-14	22	24	29	34

Comparison of Power of Discrimination between Identifiler® Plus and GlobalFiler™ PCR Amplification Kits

The main purpose of this research was to determine if STR profiles obtained from DNA extracted from the nine bone samples and then amplified using the GlobalFiler™ Amplification Kit would provide greater power of discrimination, as compared to the STR profiles obtained using Identifiler® Plus Amplification Kit. The random match probabilities were calculated for all nine of the bone samples with both amplification kits (Data Shown in Appendix Table 1 and 2). The power of discrimination was reported for the 15 common loci in the three major population groups (Table 8). Samples (0072-14, 0073-14, and 0076-14) each generated full profiles for the 15 common loci, and therefore produced the same power of discrimination. For those bone samples that did not produce identical profiles, the power of discrimination for the Identifiler® Plus Amplification kit was greater compared to the GlobalFiler™ kit.

Table 8: Power of Discrimination between Identifiler® Plus and GlobalFiler™ Amplification Kits. The power of discrimination was calculated for the three major population groups between the 15 common loci in the two amplification kits.

	Identifiler® Plus			GlobalFiler™		
	Major Populations			**Major Populations**		
Samples	**Caucasian**	**African American**	**Southwest Hispanics**	**Caucasian**	**African American**	**Southwest Hispanics**
0072-14	3.20E+20	4.31E+17	2.03E+23	3.20E+20	4.31E+17	2.03E+23
0073-14	4.16E+20	4.78E+21	3.49E+23	4.15E+20	4.78E+21	3.49E+23
0074-14	4.50E+20	8.71E+20	3.78E+19	2.29E+19	1.58E+19	2.06E+18
0075-14	7.14E+17	1.04E+18	8.01E+17	5.12E+08	7.13E+08	4.04E+07
0076-14	5.64E+18	1.61E+19	2.01E+18	5.64E+18	1.61E+19	2.01E+18
0077-14	9.96E+17	3.06E+18	1.16E+15	1.15E+14	3.54E+13	1.33E+11
0078-14	7.48E+23	9.94E+24	2.88E+21	1.60E+19	8.01E+20	5.60E+17
0079-14	2.82E+24	4.66E+25	1.92E+22	4.45E+19	2.71E+21	4.83E+17
0080-14	1.52E+21	5.29E+22	8.29E+18	4.10E+16	1.74E+18	4.63E+14

The utility of mini-STRs plus the SE33, in the GlobalFiler™ Kit where assessed to determine what role they play in the overall power of discrimination from the skeletal remains of

unidentified decedents. Random match probabilities were also calculated for the nine bone samples amplified with GlobalFiler™, using the additional loci (Data shown in the Appendix, Table 2). When adding the additional loci, the power of discrimination for all nine samples became significantly greater (Table 9). The sample that produced the highest power of discrimination was 0072-14 in the Southwest Hispanic population, with 10^{37}. This number is 10^{28} times greater than the world's population. The sample that yielded the smallest power of discrimination was sample 0075-14 with $7.47x10^{9}$ in the Caucasian population. This is equal to approximately the world's Caucasian population.

Table 9: Power of Discrimination for the GlobalFiler™ Amplification Kit. The power of discrimination was calculated for the three major population groups with results obtained from the GlobalFiler™ Amplification Kit; Amelogenin, Y-indel, and DYS391 are not included in this calculation.

	GlobalFiler™ w/additional loci		
	Major Populations		
Samples	**Caucasian**	**African American**	**Southwest Hispanics**
0072-14	9.46E+33	2.68E+28	1.35E+37
0073-14	1.08E+27	4.68E+29	8.20E+31
0074-14	5.91E+26	1.61E+27	5.16E+25
0075-14	7.47E+09	1.31E+11	9.51E+09
0076-14	1.51E+31	1.52E+33	2.11E+31
0077-14	2.83E+16	2.18E+17	2.32E+14
0078-14	1.68E+22	1.01E+26	5.15E+21
0079-14	1.91E+25	1.98E+31	4.19E+23
0080-14	2.27E+25	2.56E+30	2.54E+24

CHAPTER IV

CONCLUSIONS

There have been a great number of mass disasters that have occurred around the world, with many individuals killed, and leaving families with missing loved ones. The skeletal remains that are found usually end up at medical examiner, coroner, or law enforcement agencies. Without collection or submission of bone samples, the potential to identify an individual is eliminated. The identification of skeletal remains often relies on the analysis of mitochondrial DNA (mtDNA); however, the discriminatory power of mtDNA is significantly less than that of nuclear DNA. Quite often skeletal remains will remain unidentified, they are cremated without the retention of the biological sample, or buried as Jane or John Doe without any sample submission. Nuclear DNA testing plays a vital role in the identification of skeletal remains.

Identification of skeletal remains requires the extraction of DNA from bones in order to obtain a genetic profile. The nuclear profile from the remains can be then compared to pedigree trees, which are made up of profiles derived from close relatives. There are many factors that can play a role in the success of STR typing from skeletal remains. Obtaining DNA from skeletal remains can be challenging due to limited amounts of DNA available, which may be fragmented or degraded, and the presence of inhibitors that could co-purify with extracted DNA and prevent amplification by the polymerase chain reaction (PCR). Each bone that is received at UNTCHI is unique in terms of how degraded the bone may be, the length of time each bone was exposed to environmental conditions, and the quantity of DNA that is available.

The UNTCHI Missing Person's Laboratory uses a conventional organic extraction method. The organic extraction method has been used for many years and is considered a reliable technique for DNA extraction. This method however is time consuming, involves the use of hazardous chemicals, and contains multiple tube transfers, which could lead to errors, contamination, or complete sample loss. In this study an automated extraction method was utilized. The DNA extraction using the AutoMate Express™ typically recovered more DNA compared to the organic extraction for all but two samples. The quality of the DNA profiles was also significantly better; producing more reportable loci compared to the profiles produced using the organic extraction method. Although the AutoMate Express™ platform has sample limitations, it typically produced enough DNA extract that could be effectively used for sample amplification. Overall the AutoMate Express™ recovered more DNA that generated better quality profiles, when compared to the conventional organic extraction.

After extraction of the DNA it must be amplified to visualize the DNA profiles. Laboratories such as the UNTCHI have used the AmpFLSTR® Identifiler® Plus PCR Amplification Kit (Life Technologies) to obtain genetic information from the STR loci from challenging bone samples. In cases where partial or no profiles are obtained, UNTCHI has relied on the AmpFLSTR® MiniFiler™ PCR Amplification Kit to acquire additional genetic information from degraded or inhibited bone samples. Use of a second amplification kit is costly and requires consumption of an additional aliquot of DNA extracts. With advances in DNA technology, storage of biological samples for future testing is required to assist in the process of human identification.

A new amplification system, the GlobalFiler™ PCR Amplification Kit (Life Technologies), has recently been developed and released which can maximize the amount of

genetic data from challenging samples. This new single amplification system combines traditional STRs in the Identifiler® Plus kit and a new set of mini-STRs, similar to those in the MiniFiler™ kit. The purpose of this project was to evaluate the performance of the GlobalFiler™ Amplification Kit, and compare it with that of the AmpFLSTR® Identifiler® Plus PCR Amplification Kit. We wanted to examine the quality of the amplified product and determine if it could generate more genetic information and produce an amplified sample with a greater power of discrimination.

The two amplification kits have proven to be reliable for STR typing of skeletal remains when extracted with the AutoMate Express™. When analyzing STR profiles produced by Identifiler® Plus and GlobalFiler™ all nine bone sample were able to produce either a partial or a full STR profile. When evaluating only the 15 common loci, Identifiler® Plus generated more complete STR profiles than the GlobalFiler™ kit. When comparing the total number of alleles, Identifiler® Plus and GlobalFiler™ produced similar results. The exceptions were sample 0075-14 and 0077-14, where the Identifiler® Plus generated more alleles (Table 6). When only evaluating the performance of the common loci, Identifiler® Plus outperforms the GlobalFiler™ Amplification kit. However, there are more primers in the GlobalFiler™ kit and competition between the primers may reduce the overall amplification efficiency for the 15 common loci between the two kits.

The GlobalFiler™ Amplification kit can amplify additional genetic markers as compared to the Identifiler® Plus kit. The GlobalFiler™ Amplification kit included the ability to amplify the same 15 loci, plus a number of additional mini-STR loci, a highly polymorphic loci SE-33, Amelogenin, a Y-Indel, and the Y-STR marker DYS391, all in a single amplification. When evaluating the additional loci in the GlobalFiler™ kit, it generated STR profiles with greater

quality. All nine of the bone samples increased in the amount of reportable STR loci. Three of the samples, 0077-14, 0078-14, and 0079-14, that were considered partial profiles without the additional loci, became considered full profiles. Sample 0075-14 was still considered a partial profile, but the amount of reportable loci increased. When comparing the total number of alleles, the profiles with the additional loci recovered a greater amount compared to the alleles recovered from the 15 loci. Overall the GlobalFiler™ Kit can produce greater quality profiles from skeletal remains compared to the Identifiler® Plus kit.

All of the nine bone samples were used to calculate the power of discrimination for the 15 common loci and the additional mini-STR loci (Tables 8 and 9). With the addition of the mini-STRs and SE-33 the GlobalFiler™ kit, was now capable of generating more discriminating STR typing results for the nine bone samples tested (Table 9). Since the additional loci were comprised mostly of new mini-STRs, the smaller template required for amplification increases the chance that the recovered DNA would be amplified. The three samples that produced full profiles with both the Identifiler® Plus and the GlobalFiler™ Amplification Kits (0072-14, 0073-14, and 0076-14) reported the same power of discrimination for the 15 common loci. However, when the additional GlobalFiler™ loci are co-amplified within the same tube, the Discrimination Power for the GlobalFiler™ kit is increased substantially.

When assessing additional loci (D10S1248, D22S1045, D2S441, D1S1656, D12S391, SE33) typed in GlobalFiler™, calculations were done by hand as they are not represented in CODIS PopStats v.7.0.57.3. Allele frequencies were used to calculate the locus probability, which was then used to calculate the random match probability and the power of discrimination. A theta value of 0.01 (θ=0.01) was incorporated when appropriate. Values of the random match probabilities become significantly smaller when compared to the random match probabilities

with just the 15 loci. These numbers would make these DNA profiles very unlikely to be seen within the three major population groups. As an example, sample 0072-14 had a power of discrimination that is 10^{27} times greater than the world's population for Southwest Hispanics. Without the additional loci, the sample would have a power of discrimination of one trillion (10^{12}) times greater than the world's population. Although this number is very significant, having a greater statistical power would provide the DNA analysts with greater confidence in any associations made between skeletal remains and reference samples.

Advanced extraction techniques were found to be an essential tool for obtaining sufficient amounts of DNA from bone samples. Extraction with the AutoMate Express™ combined with a more sensitive and robust amplification kit can reduce the challenges that are typically associated with obtaining amplifiable DNA from challenged bone samples. Use of the GlobalFiler™ kit produced more genetic information from the nine skeletal remains. This ultimately will improve the DNA analysis and lead to the identification of more missing individuals. The GlobalFiler™ Amplification Kit generated a more robust STR profile for all nine bone samples compared to Identifiler® Plus. With this ability to obtain increased genetic information from the skeletal remains, comparisons with pedigree trees could result in additional associations with greater statistical significance. If the UNTCHI Missing Persons Laboratory implemented the AutoMate Express™ in conjunction with the GlobalFiler™ Amplification kit, they could utilize a single amplification system, to acquire more genetic information, save money, time, and retain additional DNA for newer testing methods.

APPENDIX

TABLES

Table A-1: Tables showing the alleles used to calculate the random match probabilities of the 15 common loci for the nine bone samples.

	0072-14		0073-14		0074-14	
Loci	**Identifiler® Plus**	**GlobalFiler ™**	**Identifiler® Plus**	**GlobalFiler ™**	**Identifiler® Plus**	**GlobalFiler ™**
D8S1179	14, 15	14, 15	11, 14	11, 14	10, 15	10, 15
D21S11	28, 29	28, 29	28, 30	28, 30	27, 31.2	27, 31.2
D7S820	9	9	8, 12	8, 12	11	11
CSF1PO	8, 12	8, 12	11	11	12	12
D3S1358	16	16	13, 14	13, 14	15	15
TH01	7, 8	7,8	9, 9.3	9, 9.3	7, 9	7, 9
D13S317	11, 12	11, 12	11, 12	11, 12	9, 12	9, 12
D16S539	11,12	11, 12	11, 13	11, 13	11, 12	
D2S1338	23, 25	23, 25	18, 25	18, 25	22, 23	22, 13
D19S433	13, 13.2	13, 13.2	14, 17.2	14, 17.2	13,15.2	13, 15.2
vWA	15, 18	15, 18	17, 18	17, 18	14, 18	14, 18
TPOX	8, 9	8, 9	9, 10	9, 10	8, 11	
D18S51	18, 20	18, 20	15, 16	15, 16	13, 15	13, 15
D5S818	12	12	11, 12	11, 12	10, 12	10, 12
FGA	21, 24	21, 24	20, 22	20, 22	21,24	21, 24

	0075-14		0076-14		0077-14	
Loci	**Identifiler® Plus**	**GlobalFiler ™**	**Identifiler® Plus**	**GlobalFiler ™**	**Identifiler® Plus**	**GlobalFiler ™**
D8S1179	13, 16	13, 16	13, 15	13, 15	14	14
D21S11	28, 30		30, 32.2	30, 32.2	29	29
D7S820			10, 11	10, 11		
CSF1PO			10, 11	10, 11	11, 12	
D3S1358	15, 19	15, 19	15, 17	15, 17	15	15
TH01	7, 9	7, 9	9	9	7, 9.3	7, 9.3
D13S317	11,13		12	12	12, 13	
D16S539	11, 14		11	11	10	10
D2S1338	18		19, 25	19, 25		
D19S433	14, 15	14, 15	13, 14	13, 14	14, 16	14, 16
vWA	16	16	16	16	16, 18	16, 18
TPOX	9, 11		8, 11	8, 11	8, 11	
D18S51			13, 16.2	13, 16.2	14	
D5S818	11	11	11	11	7, 12	7, 12
FGA	22		24, 27	24, 27	19, 25	19, 25

	0078-14		0079-14		0080-14	
Loci	**Identifiler® Plus**	**GlobalFiler ™**	**Identifiler® Plus**	**GlobalFiler ™**	**Identifiler® Plus**	**GlobalFiler ™**
D8S1179	14, 16	14, 16	14, 16	14, 16	12, 14	12, 14
D21S11	30, 33.2	30, 33.2	30, 33.2	30, 33.2	29, 30	29, 30
D7S820	11		11, 12	11, 12	10	10, 11
CSF1PO			10, 12		10, 12	
D3S1358	15	15	15	15	15	15
TH01	9.3, 10	9.3, 10	9.3, 10	9.3, 10	6, 9	6, 9
D13S317	8, 9	8, 9	8, 9	8, 9	14	14
D16S539	10, 12		10, 12		11, 12	11, 12
D2S1338	17	17, 24	17, 24		19	19
D19S433	15, 15.2	15, 15.2	15, 15.2	15, 15.2	14, 16	14, 16
vWA	15, 17	15, 17	15, 17	15, 17	16, 18	16, 18
TPOX	6, 8		6, 8	8	11, 12	
D18S51	12, 13	12, 13	12, 13	12, 13	17, 19	
D5S818	10, 11	10, 11	10, 11	10, 11	7, 11	7, 11
FGA	18.2, 22	18.2, 22	18.2, 22	18.2, 22	20, 21	20, 21

Table A-2: Tables showing the random match probabilities of the two amplification kits with the common loci and the additional loci in the GlobalFiler™ Kit.

	Identifiler® Plus			GlobalFiler™		
	Major Populations			Major Populations		
Samples	**Caucasian**	**African American**	**Southwest Hispanics**	**Caucasian**	**African American**	**Southwest Hispanics**
0072-14	3.13E-21	2.32E-18	4.92E-24	3.13E-21	2.32E-18	4.92E-24
0073-14	2.41E-21	2.09E-22	2.86E-24	2.41E-21	2.09E-22	2.86E-24
0074-14	2.22E-21	1.15E-21	2.65E-20	4.36E-20	6.31E-20	4.85E-19
0075-14	1.40E-18	9.65E-19	1.25E-18	1.95E-09	1.40E-09	2.47E-08
0076-14	1.77E-19	6.21E-20	4.98E-19	1.77E-19	6.21E-20	4.98E-19
0077-14	1.00E-18	3.27E-19	8.60E-16	8.71E-15	2.83E-14	7.52E-12
0078-14	1.34E-24	1.01E-25	3.47E-22	6.26E-20	1.25E-21	1.79E-18
0079-14	3.55E-25	2.15E-26	5.20E-23	2.25E-20	3.69E-22	2.07E-18
0080-14	6.58E-22	1.89E-23	1.21E-19	2.44E-17	5.74E-19	2.16E-15

GlobalFiler™ w/additional loci			
	Major Populations		
Samples	**Caucasian**	**African American**	**Southwest Hispanics**
0072-14	1.06E-34	3.73E-29	7.42E-38
0073-14	9.28E-28	2.14E-30	1.22E-32
0074-14	1.69E-27	6.20E-28	1.94E-26
0075-14	1.34E-10	7.61E-12	1.05E-10
0076-14	6.64E-32	6.59E-34	4.75E-32
0077-14	3.54E-17	4.58E-18	4.31E-15
0078-14	5.94E-23	9.90E-27	1.94E-22
0079-14	5.23E-26	5.05E-32	2.39E-24
0080-14	4.41E-26	3.91E-31	3.94E-25

Table A-3: Table showing the alleles used to calculate the random match probabilities for the GlobalFiler™ Kit with its additional loci for all nine bones.

Loci	0072-14	0073-14	0074-14	0075-14	0076-14	0077-14	0078-14	0079-14	0080-14
D8S1179[4]	14, 15	11, 14	10, 15	13, 16	13, 15	14	14, 16	14, 16	12, 14
D21S11[4]	28, 29	28, 30	27, 31.2		30, 32.2	29	30, 33.2	30, 33.2	29, 30
D7S820[4]	9	8, 12	11		10, 11		11,12	11, 12	10, 11
CSF1PO[4]	8, 12	11	12		10, 11				
D3S1358[4]	16	13, 14	15	15, 19	15, 17	15	15	15	15
TH01[4]	7,8	9, 9.3	7, 9	7, 9	9	7, 9.3	9.3, 10	9.3, 10	6, 9
D13S317[4]	11, 12	11, 12	9, 12		12		8, 9	8, 9	14
D16S539[4]	11, 12	11, 13		14	11	10	10		11, 12
D2S1338[5]	23, 25	18, 25	22, 23		19, 25				19
D19S433[5]	13, 13.2	14, 17.2	13, 15.2	14, 15	13, 14	14, 16	15, 15.2	15, 15.2	14, 16
vWA[4]	15, 18	17, 18	14, 18	16	16	16, 18	15, 17	15, 17	16, 18
TPOX[4]	8, 9	9, 10			8, 11			8	
D18S51[4]	18, 20	15, 16	13, 15		13, 16.2	14	12, 13	12, 13	
D5S818[4]	12	11, 12	10, 12	11	11	7, 12	10, 11	10, 11	7, 11
FGA[4]	21, 24	20, 22	21, 24		24, 27	19, 25	18.2, 22	18.2, 22	20, 21
D10S1248[1,2,3]	12,15	15,16	13, 14	15	9,12	14	13	13	13, 14
D22S1045[1,2,3]	10,12	16	15,16		13,15	15	15,16	15, 16	15, 17
D2S441[1,2,3]	12,13	11	11, 14	11	13,14	11,11.3	11	11	10
D1S1656[1,2,3]	15,16.3	14,15	13, 15		16		16,17.3	16, 17.3	12
D12S391[1,2,3]	18,21	18	17, 21		20, 22				19, 23
SE33[1,2,3]	16,21	19,27.2	19,29.2		19, 31.2			36	10.2, 14

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A theta value of 0.01 was incorporated when appropriate.

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