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A Time Course Study on STR Profiles Derived from Human Bone, Muscle and Bone Marrow

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ABSTRACT: The use of the polymerase chain reaction (PCR) to define deoxyribonucleic acid (DNA) types at several loci was investigated. PCR was used to amplify nine short tandem repeat (STR) loci along with the amelogenin locus on the X and Y chromosomes using the AmpF/STR Profiler Plus PCR amplification kit (Perkin Elmer). Rib bones were collected from 12 individuals. Five cm portions were buried at a depth of approximately 30 cm and 5 cm portions were left on the surface of the ground. Samples were exposed to the environment for periods of time ranging from two weeks to 17 months. Dried blood standards were prepared for use as reference standards for each rib sample.

Bone, muscle, and bone marrow were collected from each sample. DNA from each tissue type was extracted. Complete profile results were obtained from the surface bone samples out to an exposure time of 17 months. None of the muscle or bone marrow samples produced complete profile results beyond eight weeks. All DNA typing results from complete or incomplete profiles were consistent with DNA typing results of the corresponding blood standard. Results suggest that using the AmpF/STR Profiler Plus PCR Amplification Kit is a valid way to establish the DNA profile of tissue types from human remains.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction human remains, short tandem repeat, bone, muscle, Profiler Plus

Identification of individuals by fingerprints or dental records is not always possible. Human remains which have been exposed to the environment, thermal, or chemical extremes for long periods of time, may not be suitable for fingerprinting. Dental records may not be available or may not be informative enough to establish the identity of an individual. Decomposition can eliminate the possibility of a visual identification by an examination of facial features.

The potential to isolate and profile DNA from compact bone samples by restriction fragment length polymorphism (RFLP) or PCR analysis was evaluated by Hochmeister (1) and Lee (2). Hochmeister reported that after approximately three months of exposure to environmental conditions, samples would no longer contain sufficient high molecular weight DNA for RFLP analysis. However, the same samples exposed to environmental conditions were suitable for PCR analysis directed at polymorphic loci whose alleles were less than 1000 base pairs in length. Conclusive results were identified for the loci HLA-DQ alpha (3), D1S80 (4), D17S5 (5), apoB (6), and Co12A1 (7). A sample's D1S80 type can be defined using a commercially available amplification kit (8). The HLA-DQ alpha locus can now be multiplexed with five other loci using the Amplitype PM + DQA1 Kit (9).

The STR loci vWA (JOE), THO1 (FAM), F13A (JOE), and FESFPS (FAM) labeled with the indicated fluorescent dyes were grouped as a multiplex (10). Genomic DNA isolated from human remains, which had been exposed to high temperature, prolonged incineration and chemical insult, was successfully amplified using this multiplex. PCR product generated for these four loci was successfully typed. The STR loci D3S1358, vWA, and FGA, each labeled with the fluorescent dye FAM, were grouped as the AmpF/STR Blue PCR Amplification Kit. Genomic DNA isolated from human remains from an airline crash was successfully amplified using this multiplex (11). The PCR product generated for these three loci was also successfully typed.

The AmpF/STR Profiler Plus PCR Amplification Kit (Perkin Elmer) has the potential to amplify nine tetrameric STR loci located on nine separate chromosomes plus the amelogenin locus on the X and Y chromosomes for sex determination (12). Fragments amplified range from approximately 107 to 341 base pairs. The power of discrimination of these 10 loci is approximately 1.6×10^7 higher than the combination of the HLA-DQA1/Polymarker and D1S80 loci and 1.9×10^7 higher than the AmpF/STR Blue loci. This paper describes the potential for obtaining DNA typing results using the AmpF/STR Profiler Plus PCR Amplification Kit from human remains which had been exposed to the environment for periods of time ranging from two weeks to 17 months.

Materials and Methods

Biological Samples

Twelve rib bone samples approximately 10 cm in length were obtained from 12 deceased individuals within one day of death. Each sample was cut into two equal pieces and enclosed with a sample identifying tag in a screen wire package. Inside a fenced area, one sample from each set was buried at a depth of approximately 30 cm and one sample was placed on the surface of the ground fixed in place to a portion of screen wire. All sample treatments started on May 1, 1996. Average monthly temperatures recorded during the first year are described in Table 1 (13). Samples were frozen at −20°C after collection. Whole blood was collected at autopsy into an EDTA tube. Approximately 1 mL of the liquid blood was dried onto sterile cotton cloth within two days of sample collection to preserve as a reference standard.

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TABLE 1—*Average monthly temperatures recorded during the first year.*

Month/Year	Average Temperature
May 1996	63.3°F
June 1996	72.5°F
July 1996	72.1°F
August 1996	73.6°F
September 1996	64.2°F
October 1996	55.6°F
November 1996	35.3°F
December 1996	31.5°F
January 1997	20.4°F
February 1997	34.4°F
March 1997	43.4°F
April 1997	48.2°F

DNA Extraction

Muscle tissue remaining on the samples was removed using a sterile scalpel blade then stored at −20°C in a 15 mL plastic centrifuge tube. Rib bones were cut open and any marrow was removed and the samples stored at −20°C in a 15 mL plastic centrifuge tube. Rib bones were placed in a 15 mL plastic centrifuge tube and filled with distilled water. The tubes were put on a rocking platform at 4°C and rocked overnight to remove any remaining soft tissue. The next day, the water was decanted, the samples were rinsed one time with a 5% bleach solution and three times with distilled water. Each rinse step was approximately 1 min. The rib bones were cut into approximately 1 mm^2 pieces, weighed, and 0.7 g was placed into 15 mL plastic centrifuge tubes. The tubes were filled with 500 mM EDTA, placed on a rocking platform at 4°C, and rocked overnight. After 24 h the samples were spun in a centrifuge at 2500 rpm for 10 min and the EDTA was decanted (1). The EDTA chelation of the bone samples was repeated six times. Following EDTA treatment, samples were washed three times with distilled water. Samples were extracted overnight with 2 mL of an extraction buffer composed of 10 mM Tris, 10 mM (Na_2)EDTA, 100 mM NaCl, 2% SDS, and 2 mg Proteinase K. The following morning an additional 2 mg of Proteinase K was added and the extraction was allowed to proceed overnight again.

Muscle and bone marrow samples were extracted using the same extraction buffer and protocol without the EDTA chelation procedure. Approximately 0.1 g of muscle was extracted from the soft tissue recovered from the rib samples. All of the material recovered from the marrow cavity of each sample was extracted. Dried blood reference standards were extracted using the same extraction buffer. A single overnight extraction was performed using approximately 0.5 cm^2 of sample.

Extractions of bone, muscle, bone marrow, and blood standard samples were performed at separate times. A sample with no DNA was carried through each extraction as a sample manipulation/reagent blank control. Manipulation/reagent blank samples were amplified to monitor the extractions for the presence of any contaminant DNA.

DNA Purification

Bone, muscle, and bone marrow samples were subjected to two phenol/chloroform/isoamyl alcohol (PCI) (25:24:1) extractions followed by three separate chloroform/isoamyl alcohol (CI) (24:1) extractions. Organically extracted DNA's were purified and concentrated to a volume of approximately 100 μL using a Centricon 100 concentrator (Amicon). Concentrated samples were re-extracted with 400 μL of the extraction buffer. Reextracted samples were purified with one PCI extraction followed by one CI extraction. Samples were isolated using a Microcon 100 microconcentrator (Amicon) in conjunction with a Micropure EZ (Amicon) filter (14). Bloodstain samples were purified using one PCI and one CI organic extraction. Bloodstain DNA was isolated following the organic extraction using a Microcon 100 filter. All sample extractions were resolubilized in a volume of 100 μL TE buffer (10 mM Tris-HCl, 0.1 mM EDTA).

DNA Quantitation

DNA quantity was estimated by gel electrophoresis against known quantity high molecular weight standards. A 123 bp marker (BRL) and a HIND III digest of lambda DNA (BRL) were also included as standards to grade fragment sizes in the degraded DNA fraction. Following the agarose gel estimate, a chemi-luminescent slot-blot assay (ABI) was performed using the primate specific human alpha satellite D17Z1 as a probe to determine the amount of human DNA in the samples. Table 2 lists the recovery of human DNA from each sample.

PCR Amplification

When available, 2 ng of human DNA from each sample was amplified. If less than 2 ng of human DNA was detected, the entire sample volume was reconcentrated using a Microcon 100 filter and resolubilized in 20 μL TE buffer. The 20 μL sample was then amplified. A Perkin-Elmer 480 Thermocycler was used to amplify the samples using the cycling parameters and reagents provided in the AmpF/STR Profiler Plus Amplification Kit and Users Manual (15). The amplified product was analyzed on an Applied Biosystems 310 Capillary Electrophoresis instrument (ABI 310). Genotype results were defined using *Genotyper* (17) software. The program utilizes migration and fragment size data collected using a ROX labeled in-lane standard to define the genotypes of each sample based on the results of an allelic ladder.

Results

All 10 of the loci amplified using the AmpF/STR Profiler Plus PCR Amplification Kit were detectable in bone samples left on the surface of the ground up to 17 months (Fig. 1). DNA typing results obtained from the extraction of bone, muscle and bone marrow samples show loss of the higher molecular weight loci occurring before loci defined by shorter fragments. Additionally, peak heights obtained for the smaller loci were generally greater than the peak heights obtained for the larger loci. As peak height measured in relative fluorescence units is a measure of the concentration of PCR product generated, both observations are consistent with degradation causing fragmentation of the larger loci before the smaller, when compared with results from the reference blood standard (Fig. 2). All DNA typing results obtained from the reference blood standard samples matched the results obtained for the surface treatment and buried treatment samples.

In each sample where allele drop-out was noted, both peaks of the heterozygote could be seen, but one peak fell below the 150 rfu threshold set for allele definition. In each sample demonstrating allele loss, degradation was noted in comparison with the reference standard (Fig. 3). Allele drop-out was noted in three muscle surface treatment samples (Fig. 4). Four of the samples amplified produced

TABLE 2—*Human DNA yields.*

Sample		Bone	Total	Muscle	Total	Marrow	Total
CC-56-95	-buried	0.06ng/mL	4.2ng	0.05ng/mL	3.5ng	5ng/mL	350ng
	-surface	0.75ng/mL	52.5ng	30ng/mL	2.1ug	25ng/mL	1.75ug
CC-62-95	-buried	0.02ng/mL	1.4ng	ND		2ng/mL	140ng
	-surface	4ng/mL	280ng	25ng/mL	1.75ug	100ng/mL	7ug
CC-57-95	-buried	0.075ng/mL	5.2ng	ND		25ng/mL	1.75ug
	-surface	0.75ng/mL	52.5ng	10ng/mL	700ng	0.025ng/mL	1.7ng
CC-63-95	-buried	0.05ng/mL	3.5ng	ND		inc	
	-surface	1ng/mL	70ng	30ng/mL	2.1ug	50ng/mL	3.5ug
CC-64-95	-buried	0.02ng/mL	1.4ng	ND		ND	
	-surface	1ng/mL	70ng	ND		0.04ng/mL	2.8ng
CC-65-95	-buried	0.03ng/mL	2.1ng	ND		ND	
	-surface	3ng/mL	210ng	3ng/mL	210ng	20ng/mL	1.4ug
CC-66-95	-buried	ND		ND		ND	
	-surface	0.2ng/mL	14ng	0.2ng/mL	14ng	ND	
CC-67-95	-buried	0.025ng/u	1.7ng	ND		ND	
	-surface	0.15ng/mL	10.5ng	10ng/mL	700ng	0.02	1.4ng
CC-68-95	-buried	ND		ND		ND	
	-surface	3ng/mL	210ng	0.075ng/mL	5.2ng	ND	
CC-69-95	-buried	ND		ND		ND	
	-surface	2ng/mL	140ng	0.3ng/mL	21ng	5ng/mL	350ng
CC-70-95	-buried	0.015ng/mL	1ng	ND		ND	
	-surface	0.3ng/mL	21ng	0.015ng/mL	1ng	ND	
CC-71-95	-buried	ND		ND		ND	
	-surface	0.6ng/mL	42ng	0.015ng/mL	1ng	ND	

ND = not detected.

Total = the amount of DNA remaining after quantitation.

Inc = inconclusive due to background activity on the film.

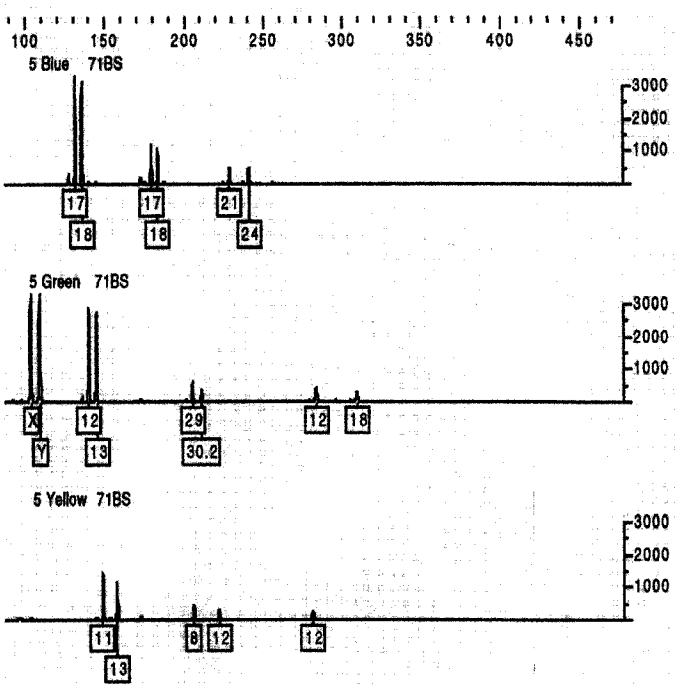

FIG. 1—*Bone sample #71: surface treatment.*

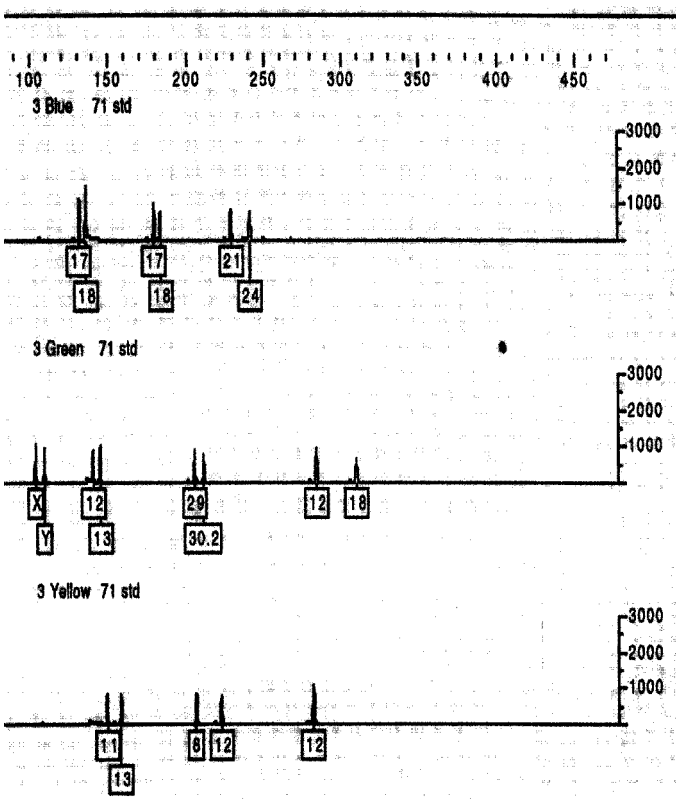

FIG. 2—*Blood standard sample #71.*

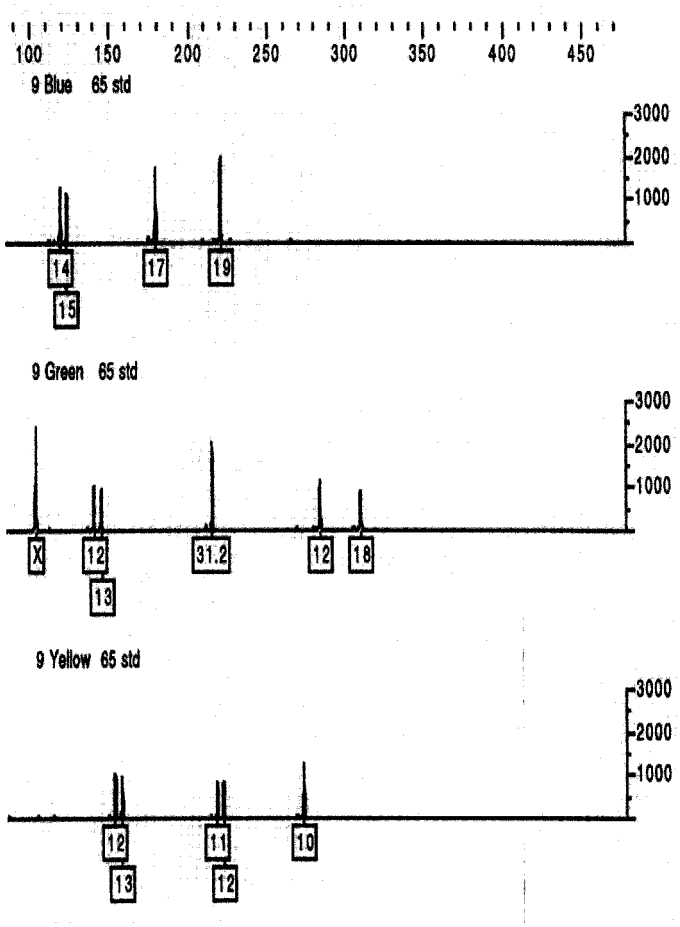

FIG. 3—*Blood standard sample #65.*

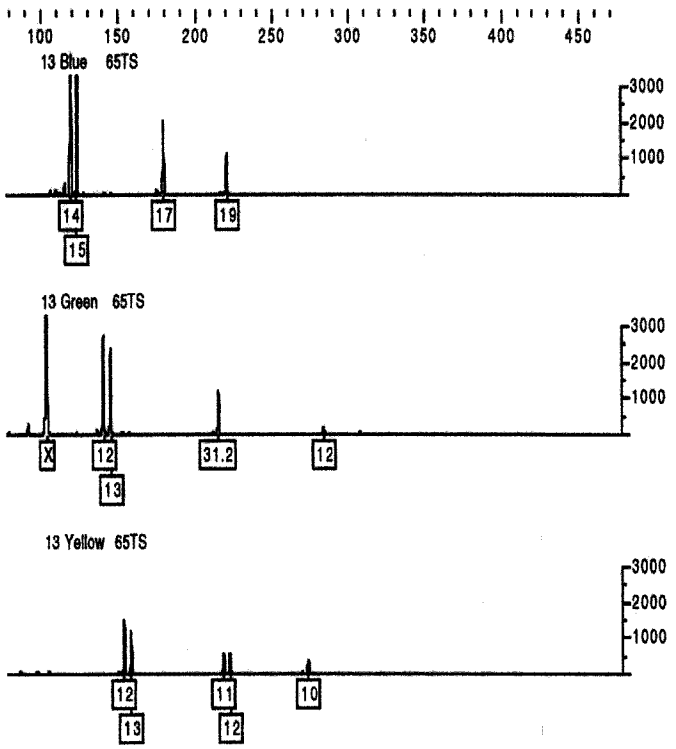

FIG. 4—*Muscle tissue sample #65: surface treatment.*

no results at the D3S1358 locus but were successfully typed at the vWA and D8S1179 loci. Each tissue type evaluated was represented in this observation. Since the D3S1358 locus is smaller than either the vWA or D8S1179 loci, degradation would not account for these incomplete results.

The fragment range reported by Perkin-Elmer (14) for the alleles defined by the three dyes, NED, 5-FAM and JOE, overlap and cover approximately the same size range of fragments. The reported size ranges are as follows: NED (yellow) 135–294 bp; 5-FAM (blue) 114–267 bp; and JOE (green) 107–341 bp. Overall, 49% of loci labeled with NED (yellow), 43% of the loci labeled with 5-FAM (blue) and 42% of the loci labeled with JOE (green) were not detected. The NED (yellow) dye was shown to be the least sensitive of the three dyes.

Discussion

In cases where human remains cannot be identified by fingerprint analysis, dentition or visual evaluation, DNA analysis accomplished through RFLP or PCR analysis may be helpful. The state of decomposition of the remains will determine which method of analysis may be most successful (1), as well as which body tissue is the most suitable for DNA analysis. Hochmeister *et al.* found that beyond a three month period severe decomposition resulted in destruction of DNA targeted for either RFLP or PCR in soft tissues. The results of this study are similar.

At 10 weeks, no results were obtained from the analysis of the AmpF/STR Profiler Plus loci in any buried soft tissue samples, and at most, 35% of the loci were detected from samples maintained on the surface of the ground. However, buried bone samples provided results for 75% of those loci at 10 weeks and surface treated samples provided results for 85% of the loci tested. At 17 months, no results were obtained for any of the soft tissue samples examined in this study. DNA recovered from surface treated bones exposed for 17 months gave results for 95% of the loci tested. DNA recovered from bone samples which had been buried for 17 months gave results for 15% of the loci tested. Bone was found to be the tissue least affected by environmental exposure. In this study conclusive results were obtained out to 17 months. Differences in samples and/or environmental exposure might increase or decrease this time. At the point in time where nuclear DNA analysis can not be completed mitochondrial DNA analysis might still be a viable option (16).

Both surface exposure and burial resulted in DNA degradation. Degradation was identified on agarose gels by a loss of high molecular weight DNA. Degradation was indicated on the slot-blot results by a decrease in the concentration/proportion of human DNA isolated. The result of degradation could be seen on electropherograms as a decrease in peak height for increasingly longer loci. Degradation also resulted in incomplete or no results at heavier loci. Degradation alone could not account for all incomplete results. Inhibition of the PCR was indicated in four samples. DNA isolated from two bone samples, one muscle sample and one bone marrow sample showed no amplification at the D3S1358 locus, but complete results for the vWA and D8S1179 loci. As D3S1358 is a smaller locus than vWA or D8S1179, degradation would not be expected to cause this result. Wallin (18) amplified the loci D3S1358,

vWA and FGA in the presence of the PCR inhibitor hematin. Inhibition of D3S1358 was effected before vWA with the addition of 17 uM hematin to the PCR reaction. The pattern of inhibition noted by Wallin *et al.* was identified in this study.

A comparison of genotypes determined for the 12 reference blood standards and genotypes identified for the corresponding bone, muscle and bone marrow samples identified no interpretable differences. Three instances of allele drop-out were observed in the surface treatment muscle samples. Examination of the electropherograms showed that both peaks of the heterozygotes were present at the loci for which allele drop-out was reported. The incomplete types reported for individual loci within a profile were found to be the result of one peak falling below the threshold defined for allele definition. Increased degradation with increased environmental exposure time allowed for an evaluation of the sensitivity of the primer sets labeled with the dyes 5-FAM (blue), JOE (green), and NED (yellow). Evaluation of the percentage of loci detected as exposure time was increased identified the NED (yellow) dye −41% as the least sensitive of the three. The percentage of loci defined by primers labeled with either 5-FAM (blue) −47% or JOE (green) −46% over time, was approximately equal.

The loci defined by the AmpF/STR Profiler Plus PCR Amplification Kit were reliably typed in each aged and environmentally exposed sample. This study supports the use of the AmpF/STR Profiler Plus PCR Amplification Kit and capillary electrophoresis for STR analysis in tissue samples associated with forensic cases.

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