CASE REPORT

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DNA Typing of Samples for Polymarker, DQA1, and Nine STR Loci from a Human Body Exhumed After 27 Years

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ABSTRACT: A body was exhumed from the ground after 27 years. Samples of femur bone, tooth, and a fingernail were collected and successfully subjected to DNA extraction, quantitation, amplification, and subsequently typed for DQA1, polymarker, and nine STR loci. All three types of samples were typed for D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S17, D7S820, and amelogenin using ABI Prism 377 DNA sequencer.

KEYWORDS: forensic science, polymerase chain reaction, DQA1, DNA typing, bone, teeth, fingernail, exhumation, case, short tandem repeat, LDLR, GYPA HBGG, D7S8, GC, D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, amelogenin

A body was exhumed in Columbus, Ohio. The purpose of the exhumation was to acquire the DNA profile as a standard and compare it with evidentiary samples in a criminal case. When the coffin was pulled out, water was dripping from it; the coffin lining was rotted and disintegrated. The body was wet but in perfect condition. The skin looked fresh and the body was perfectly identifiable by viewing. No decomposition or bad odor was noted. The polymerase chain reaction (PCR) based typing method was used to perform the DNA comparison. These methods included analysis at DQA1 and short tandem repeat (STR) loci. The polymorphic nature of these loci makes them a powerful tool of analysis for forensic casework samples. Trimeric and tetrameric repeats in STR loci are found throughout the human genome (1,2). Due to the overall short repeat length of the repetitive region it has been possible to amplify these regions. This characteristic has made degraded DNA samples amenable to STR typing. This study reports typing of DNA samples extracted from bone, a tooth and nail from a body exhumed after 27 years.

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Materials and Methods

Extraction of DNA

Bone—DNA extractions were performed according to the FBI protocol with modifications (3–5). A 2½ in. long (6.35 cm) piece of femur bone was boiled in deionized water for about 10 min. Surface debris was cleaned with 0.5 M EDTA using a new toothbrush. The bone was let to dry and filed by placing in a vice. Approximately 2 g of bone dust were collected in a large petri dish placed underneath the bone. The bone dust was finely ground in a liquid nitrogen chilled mortar pestle and transferred to a 30 mL Falcon tube. The bone dust was washed twice by vortex mixing with 20 mL 0.5 M EDTA and spun at 3000 rpm in a medifuge (American Scientific Products, McGaw Park, IL) for 10 min at room temperature. The pellet was resuspended in 9 mL of digest buffer (pH 7.5) supplemented with 250 μL Proteinase K (20 mg/mL), 600 μL of 1 M dithiothreotol (DTT). The suspension was incubated overnight at 56°C. The next morning two aliquots of 250 μL Proteinase K (20 mg/mL) were added at 3 h intervals followed by incubation at 56°C. The suspension was spun in a medifuge at 4000 rpm for 10 min; supernatant was aliquoted in microcentrifuge tubes and extracted twice with equal volumes of phenol/chloroform/isoamylalcohol and then centrifuged at 14 000 rpm for 10 min. The aqueous phase from the microcentrifuge tubes was transferred to Centricon 100 microconcentraters, washed with 10 mL of TE^{-4} (pH 7.5) and concentrated to a final volume of 40 to 50 μL.

Tooth and Nail—One tooth and one whole nail were cleaned exactly as described above for bone. The dried tooth was placed in a five-layered plastic sleeve and crushed into small pieces using a vice. Fine grinding of the small pieces was achieved by crushing with a hammer followed by grinding in a liquid nitrogen chilled mortar and pestle. The nail was cut into fine shavings with a scalpel. DNA was extracted according to the procedure described (5).

Quantitation of DNA

Human genomic DNA was quantitated by using a Quantiblot hybridization kit according to the manufacturer's recommendations (Perkin Elmer, Foster City, CA).

Amplification and Detection of STR Loci

Twenty microliters of DNA (8.0 ng) were used to amplify D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820 STR loci, and amelogenin. AmpFℓSTR profiler plus kit was used to amplify DNA extracted from bone, tooth and nail according to the manufacturer's instructions (Perkin Elmer Biosystems, Foster City, CA).

Four microliters of the amplicons or ladder were mixed with 5.0 μL of formamide loading solution (formamide/blue dextran, 5:1), denatured at 95°C for 5 min, and snap cooled on ice for 5 min. One and a half microliters of the denatured sample were loaded in a 4.2% polyacrylamide gel. The samples were electrophoresed at 3000 V for 2 h and 15 min in a ABI Prism 377 DNA sequencer. Gel image was analyzed with Genescan and Genotyper DNA fragment analysis software.

HLA-DQA1, PM Amplification

HLA-DQA1 and PM loci were amplified from the extracted DNA using the Amplitype PM + DQA1 kit (Perkin Elmer, Foster City, CA) according to the manufacturer's instructions with minor modifications. An additional 5 units of Taq Polymerase were added to the reaction mixture at the time of PCR set up. The amplified products of Amplitype PM and DQA1 loci were detected by reverse dot blot hybridization.

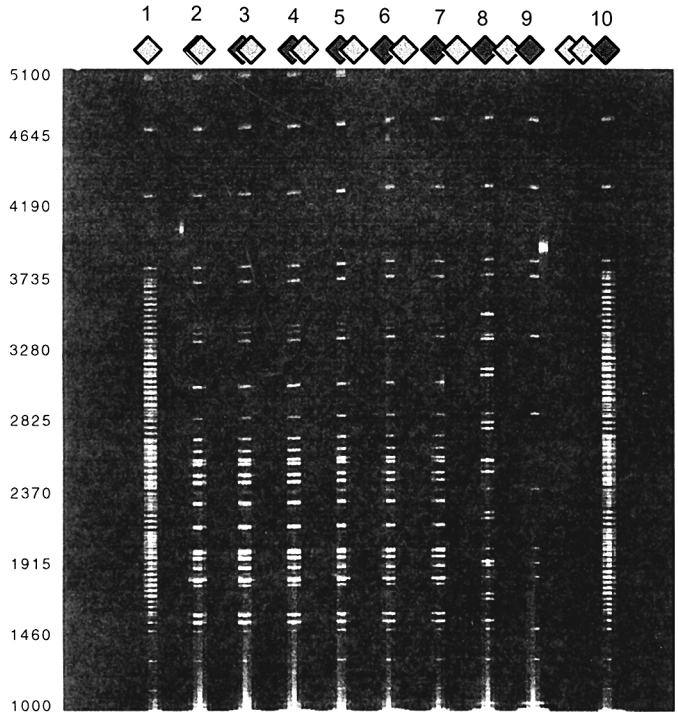

FIG. 1—*Resolution of amplified DNA fragments from STR loci on a 4.2% polyacrylamide gel using the ABI Prism 377 DNA sequencer. Scan numbers and lane numbers are shown along the* Y*-axis and* X*-axis, respectively. An internal size standard was included in all samples. Lanes 1 and 10; Allelic ladder standard, Lanes 2, 3, and 4; (1.5 μL) amplified DNA from bone, nail, and tooth, respectively, Lanes 5, 6, and 7; (1 μL) amplified DNA from bone, nail and tooth. Lanes 8 and 9; positive and negative controls. Only internal size standard is present in negative control lane.*

TABLE 1—*GeneScan analysis of the STR loci from tooth DNA.*

Sample Peak	STR Locus	Minutes	Size	Peak Height	Peak Area	Data Point
1	Pull up	41.76	106.26	1027	4074	1566
2	Pull up	43.07	112.14	369	1593	1615
3	Stutter (12.5%)	48.08	134.59	767	6215	1803
4	D3S1358	49.01	138.76	6159	70847	1838
5	Stutter (6.1%)	57.12	172.63	117	1295	2142
6	vWA	58.11	176.53	1896	26118	2179
7	Stutter (8.4%)	61.17	188.63	117	1725	2294
8	vWA	62.21	192.74	1386	22474	2333
9	FGA	71.07	227.79	921	9556	2665
10	FGA	73.12	235.88	831	8323	2742
1	X	41.81	106.50	3598	29650	1568
2	Y	43.09	112.26	3589	27946	1616
3	Pull up	48.99	138.64	1091	7210	1837
4	Stutter (6.1%)	50.11	144.04	126	1030	1879
5	D8S1179	51.07	148.53	2069	20452	1915
6	Stutter (5.6%)	52.99	156.42	87	710	1987
7	D8S1179	53.97	160.21	1567	17598	2024
8	Stutter (6.1%)	65.89	207.32	92	1104	2471
9	D21S11	66.93	211.45	1505	18293	2510
10	D21S11	69.49	221.58	1418	15518	2606
11	D18S51	91.25	305.56	230	2201	3422
12	D18S51	92.29	309.65	183	2040	3461
1	Pull up	41.79	106.38	835	3511	1567
2	Pull up	43.07	112.14	456	1883	1615
3	Pull up	48.99	138.64	1042	5297	1837
4	Stutter (4.4.%)	51.79	151.64	113	925	1942
5	D5S818	52.72	155.38	2576	26193	1977
6	D5S818	53.71	159.19	2343	25006	2014
7	D13S317	65.71	206.58	1002	13508	2464
8	D13S317	68.83	218.94	1048	11781	2581
9	D7S820	82.11	270.50	613	6297	3079

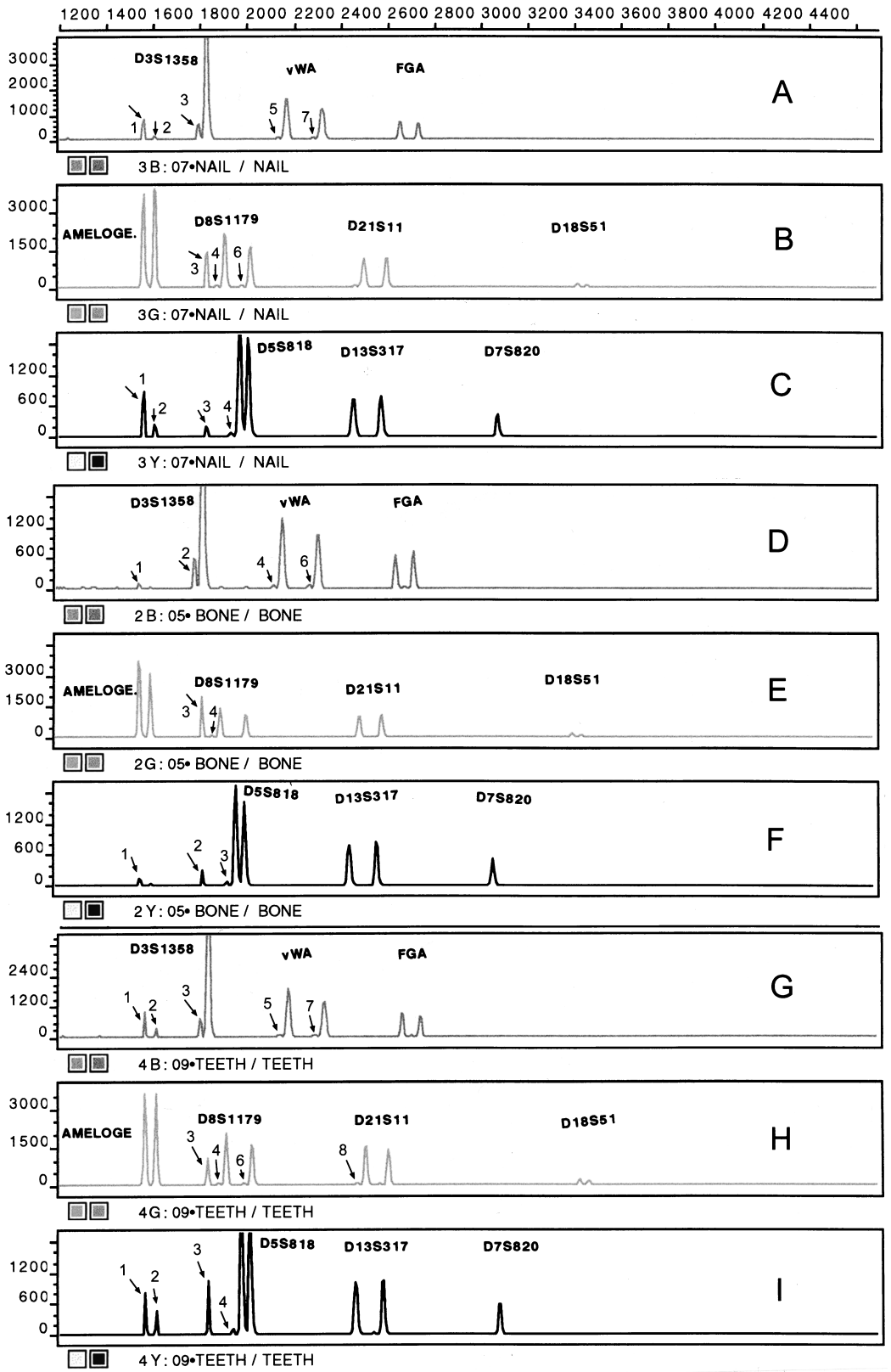

FIG. 2 (Panels A–I)—*STR typing analysis of DNA from fingernail, bone, and tooth using the ABI Prism 377 DNA sequencer GeneScan software. Fluorescence intensity (RFU,* Y*-axis) and the scan number* (X*-axis) are shown. Individual loci in each panel are labeled. For clarity only pull up and stutter product peaks are labeled with numerals and arrows. See Table 1 for identity of labeled peaks. D3S1358, vWA, FGA, primers are labeled with FAM, amelogenin, D8S1179, D21S11, and D18S51 primers are labeled with JOE, D5S818, D13S317, and D7S820 primers with NED. Detection threshold level for GeneScan analysis was 100 RFU.*

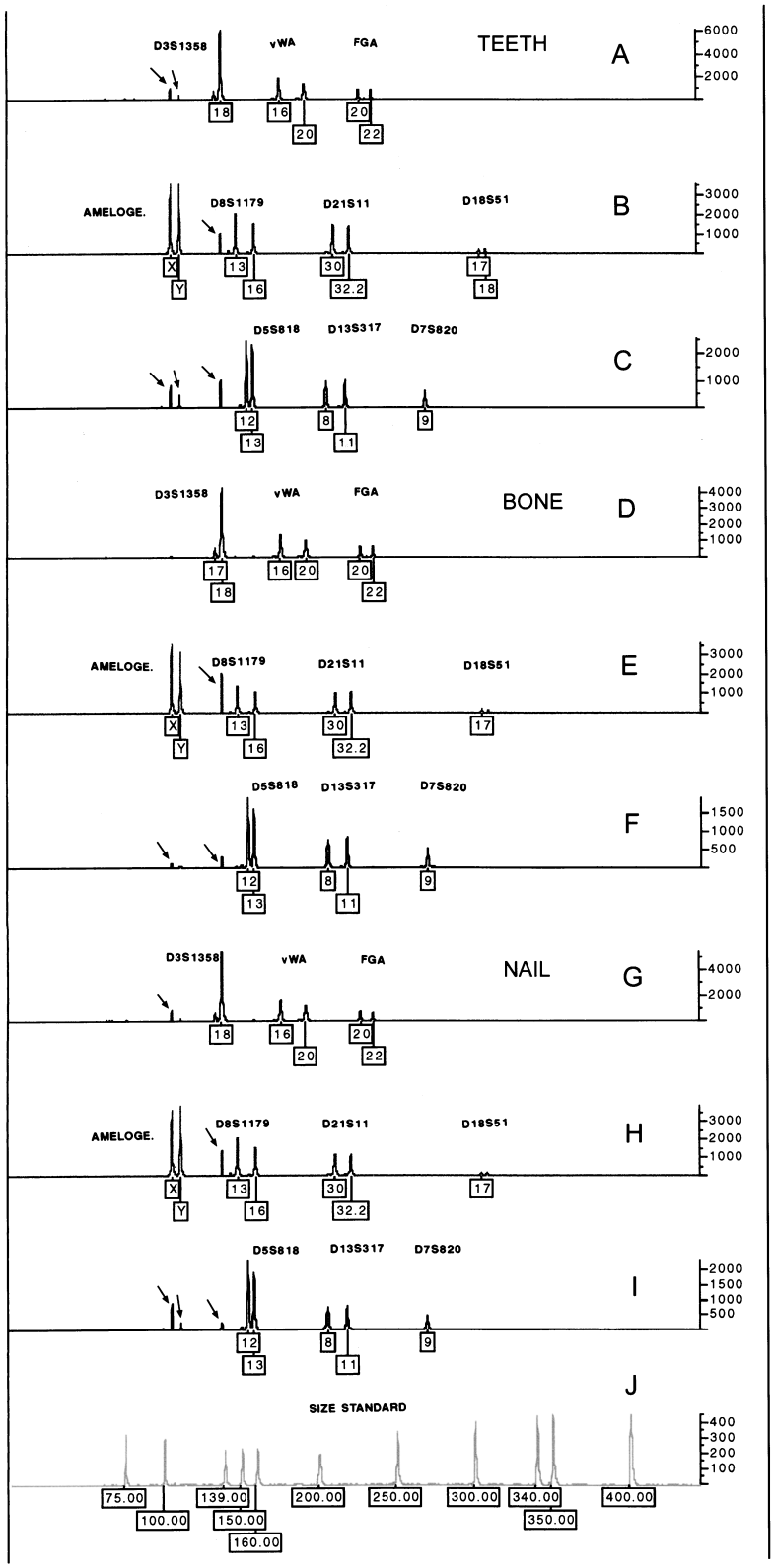

FIG. 3 (Panels A–J)—*Allele designations from tooth, bone, and fingernail DNA using the ABI Prism 377 DNA sequencer Genotyper software. Fluorescence intensity (RFU,* Y*-axis) and size standard 75 to 400 bp* (X*-axis) are shown. Individual loci and alleles in each panel are labeled. Pull ups are indicated with arrows in panels A through I.*

Ethidium bromide stained amplicons were resolved on a 4% agarose gel (3:1 high resolution blend, Amresco, Solon, OH) in 0.5x TBE. Electrophoresis was carried out for 30 min at 150 V.

Results and Discussion

DNA from bone, a nail and a tooth obtained from a body exhumed after 27 years was extracted and subjected to DQA1, PM, and STR loci typing. Yield gel analysis of the DNA from these samples showed a high degree of degradation. The DNA was quantitated with quantiblot using human specific probe D17Z1 and 6.4 and 8.0 ng of template DNA was used to conduct DQA1+ PM and STR typing, respectively.

The difference in quantity used is due to the degradation of DNA and the difference in fragment sizes for the two methods, i.e., 138 to 242 bp for DQA1+ PM and 107 to 341 bp for STR loci. Amplicons obtained for STR are shown resolved on a 4.2% polyacrylamide gel (Fig. 1). Electropherograms of the STR profile obtained from the nail, bone, and tooth DNA are shown in Fig. 2 (Panels A-I). A fairly balanced signal was observed for vWA, FGA, D8S1179, D21S11, D18S51, D5S818, and D13S317 loci. Stutter products observed from tooth DNA analysis are shown in Table 1. Such data from the nail and bone are not shown. With the exception of D3S1358 (stutter product percent of 12.4) all other stutter products are within the range observed (AmpFℓSTR, Profiler plus amplification kit, user's manual, PE biosystems, Foster City, CA). High stutter product percent for D3S1358 could be attributed to high fluorescence. Pull up peaks were observed for D3S1358, D8S1179, D5S818, and amelogenin loci (Fig. 2). Due to degradation of DNA preferential amplification of smaller intact fragments

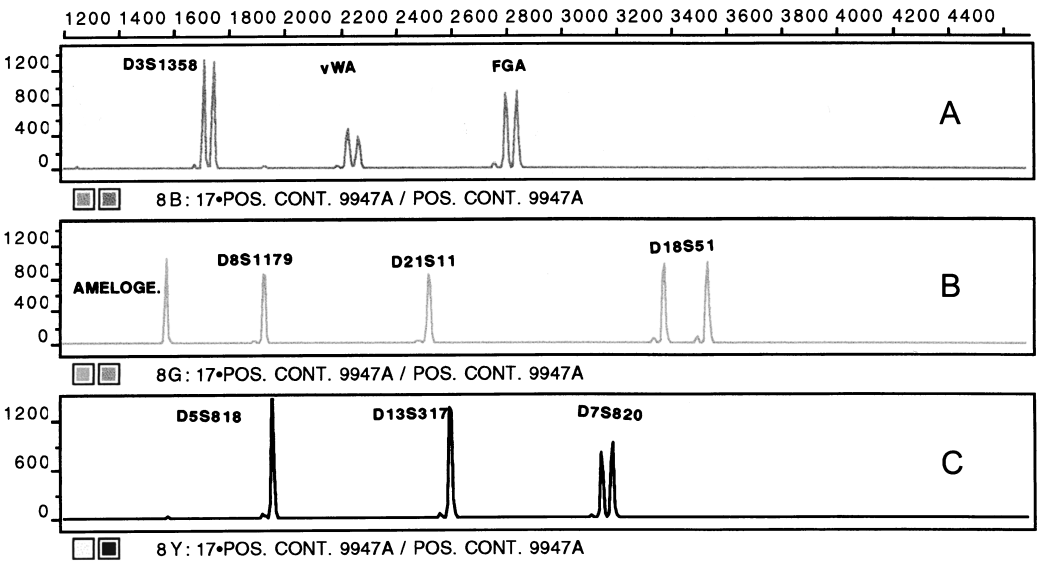

FIG. 4—*9947A DNA is included as positive control. Fluorescence intensity (RFU,* Y*-axis) and the scan number (*X*-axis) are shown. Individual loci in each panel are labeled. Primer labeling and detection threshold level for GeneScan analysis was the same as in legend to Fig. 1.*

TABLE 2—*Short tandem repeat (STR), DQA1, and Polymaker DNA profile from exhumed body.*

	STRs					
	Bone		Tooth		Nail	
Loci	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
D3S1358	18	18	18	18	18	18
vWA	16	20	16	20	16	20
FGA	20	22	20	22	20	22
Ameloge	X	Y	X	Y	X	Y
D8S1179	13	16	13	16	13	16
D21S11	30	32.2	30	32.2	30	32.2
D18S51	17	18	17	18	17	18
D5S818	12	13	12	13	12	13
D13S317	8	11	8	11	8	11
D7S820	9	9	9	9	9	9
	DQA1 and Polymarker					
	DQA1	LDLR	GYPA	HBGG	D7S8	GC
Bone	1.2, 1.3	AB	B	A	B	BC
Tooth	1.2, 1.3	AB	B	A	B	BC
Nail	1.2, 1.3	AB	B	A	B	BC

could lead to high fluorescence and pull ups for smaller sized amplicons. This is true for D3S1358 and D5S818 loci from nail, bone, and the tooth DNA samples. No pull up peaks were observed for positive control (Fig. 3).

Results of the allele designation calls by Genotyper software are shown in Fig. 3 (Panels A through I). Pull up peaks are also present in these printouts; however, they do not have any designation. Due to high fluorescence from the D3S1358, D8S1179, and D5S818 alleles the D18S51 alleles are barely visible. Both alleles at this locus are present from teeth, bone, and nail DNA. Table 2 shows the genotype obtained from bone, teeth, and fingernail DNA. In conclusion, we were able to type samples for 16 loci without any difficulty. It is clear from this study that samples as old as these, or even older, could be typed reliably for STR, DQA1, and PM.

Acknowledgments

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