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Extraction of Single-Copy Nuclear DNA from Forensic Specimens with a Variety of Postmortem Histories*

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ABSTRACT: Specimens of human bone, teeth and dried blood spots from 3 months to 91 years old, with a variety of postmortem histories, were used in a comparative study of recovery of single-copy nuclear DNA sequences from forensic material. Sequences of the amelogenin and HLA-DPB1 genes were chosen for their value in sexing and identification. Sequences of the mitochondrial non-coding region V were also amplified to compare the recovery of mitochondrial and single-copy nuclear DNA. A variation of the silica method for DNA extraction was refined for application to the forensic specimens in this sample. Single-copy nuclear DNA was amplified from 100% of recent postoperative bone specimens (n = 6), 80% of forensic teeth and bone specimens (n = 10), 78% of recently extracted teeth (n = 18), 78% of exhumed bone up to 91 years old (n = 37) and 69% of 15 year old bone specimens fixed in 10% formalin (n = 20). Amelogenin sexing was correct in 85% of cases (n = 74) in which the sex of the donor had been recorded. There was no correlation between the age of the specimen and the extent of DNA preservation.

KEYWORDS: forensic science, HLA-DPB1, amelogenin, DNA extraction, PCR; mtDNA, bone; teeth, dried blood

The sensitivity of the PCR technique is well known and its effectiveness in forensic analysis is well established (1). Applications to a variety of forensically-interesting material, such as hair (2), fingernails (3), dried blood (4), wax-embedded tissues (5,6), excrement (7), bone (8–11) and teeth (12,13) have been demonstrated. Forensic DNA analysis may target nuclear loci (8–11,14,15) or mitochondrial DNA (10,12), which occurs in multiple copies in the cell. Sequences from functional nuclear genes are a difficult target due to their low copy-number, but remain important in forensic analysis due to the high degree of polymorphism occurring in parts of the genome, notably in the HLA complex (13,16,17), and because of the importance of loci on the sex chromosomes in DNA sexing techniques (18).

In this study we have sought to compare the effectiveness of one method for single-copy nuclear DNA recovery and analysis on a variety of forensic specimen types, focusing on the polymorphic HLA-DPB1 locus and on the X-Y homologous amelogenin gene. We have also amplified the mitochondrial non-coding region V to provide a comparison of mitochondrial and nuclear DNA survival. The sequences we target are all of a size which would reasonably be expected to survive in forensic specimens. Human skeletal material comprised bone recovered post-operatively (n = 6); teeth extracted during dental treatment or shed deciduous teeth (n = 18); bone fixed in formalin (n = 20); and teeth and bone recovered from forensic cases (n = 10) or as a result of exhumation (n = 37). There was a variable degree of decomposition and skeletonization represented in the sample, and some specimens had been subjected to preparative chemical procedures or buried in quick-lime. Dried blood spots (n = 28) were collected on gauze. Because of its value in removing PCR inhibitors, we used a variation of the silica method of DNA extraction (19,20), which we have adapted to a range of forensic material. Negative controls were included throughout in order to allow an assessment of the extent and influence of contamination with extraneous DNA.

Materials and Methods

Equipment Preparation

Precautions were taken against contamination with intrusive DNA (21). Bone extraction equipment was cleaned by soaking in 5.0% sodium hypochlorite solution for 1 h. Pre- and post-PCR activities were conducted in separate rooms and a laminar flow cabinet was used during extraction and purification steps. Surfaces of bone or teeth were cleaned by washing with 0.5% sodium hypochlorite solution or by abrasion with a grinding tip or drill bit, which was then discarded.

Sample Preparation

Post-operative fragments of femur and tibia were recovered following routine orthopaedic surgery. Specimens of shaved bone were pulverized prior to extraction, except for one specimen, which was extracted from a femoral head by drilling with a flamed bit. Exhumed material came from three cemeteries. Burials had taken place between 1904 and 1984. Specimen *A1* originated from a grave treated with quick-lime (CaO) and subsequently contaminated with automotive lubricating oil. Powdered bone from 16 of the exhumed skeletons was generated during the removal of segments from the shafts of long-bones by hacksaw for nitrogen analysis (see below). Cross-sections of tibia had been removed from donated medical cadavers during 1980 and fixed in 10% formalin. Bone powder was extracted from the cross-sections by drilling with a flamed bit, material from the outer surface being

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discarded. Forensic material originated from cases arising between 1986 and 1994. Not all material was skeletonized when recovered. Specimens *SF* and *SM* had been cleaned by immersion in hot water (95°C) for several days. Specimens *Y*, *Y1*, and *Y2* had been fixed in 10% formalin prior to cleaning in papain. Other bone surfaces were cleaned by abrasion or by washing in 0.5% sodium hypochlorite solution and the bone ground to a fine powder in a coffee mill (Philips HR2811) or in liquid nitrogen using a pestle and mortar. Extracted permanent teeth had been collected following dental treatment and stored at 4°C. Specimens *ET4* to *ET8* had been fixed in 10% formalin. Specimen *ES* was a shed deciduous tooth stored at room temperature for 2 years. Intact whole teeth were prepared by washing in 0.5% sodium hypochlorite solution and fractured longitudinally to expose the pulp cavity. Fragments of impact-fractured dentine were used in the extraction procedure. Dried blood spots had been collected on sterile gauze over a 26-year period from 1969 and stored in sealed containers at room temperature. Ethical Committee approval and appropriate consent for each specimen was obtained for the study.

Extraction Rationale

We use a variation of the silica method for DNA extraction (19,20) which we have refined using material of the kind described in this study as an experimental model. In refining the method, we have endeavored to retain its simplicity and rapidity, and not to increase the number of steps. We have made the following preliminary observations (22) (data not shown).

Use of a detergent (Triton X-100 or sodium dodecyl sulfate) makes no difference to the effectiveness of the method, but addition of proteinase K is marginally advantageous. Use of 0.5 M Na_2EDTA increases DNA yield, but also results in the extract containing a plethora of proteins and other biomolecules. DNA yield improves with up to 48 h of mixing in extraction buffer at room temperature, after which further gains are marginal. Extraction temperature makes little difference: increased release of DNA may be countered by increased DNA degradation and reduced proteinase activity. We found 4 M guanidine isothiocyanate (GuSCN) superior to 6 M sodium iodide as the DNA/silica binding agent and a 2 h binding time at room temperature to be optimal—others have found a higher binding temperature more effective, however (23). The GuSCN solution should be freshly prepared and stored in a light-proof container. A thorough washing step, prior to elution of DNA from the silica, is also important. We use a reduced silica volume (20 μL), which we consider adequate for the DNA quantities involved, and elute once into a large aliquot (115 μL) of sterile filtered distilled water, taking off a slightly smaller aliquot (105 μL) to avoid inadvertent removal of silica. Presence of 160 μg/mL bovine serum albumin (BSA) in the PCR reaction made no difference to product yield, perhaps reflecting the effectiveness of the silica method in removing inhibitors. A degree of random contamination of extraction, purification, and amplification blanks was experienced. When the amplifications were repeated using a layer of 25 μL mineral oil to prevent evaporation of the reaction mixture this problem diminished. Preliminary experiments with extended PCRs (of up to 50 cycles) increased the frequency of positive results obtained, but an increased frequency of contamination was also experienced. However, extended-cycle PCRs may be necessary if DNA is to be detected in especially difficult forensic specimens. PCRs of 35 or 40 cycles were therefore employed and blank controls were included in all recovery and amplification steps.

Although our method is effective in releasing DNA from bone specimens and removing inhibitors, there is an "overhead" of DNA loss which may be greater than that involved when the phenol-chloroform method (24) is used. The beneficial effects of proteinase K, a high Na_2EDTA molarity and lower extraction temperature may be a reflection of the types of substrates we have examined, which we expect contain a relatively high proportion of fixed proteins and other biomolecules, and their break-down products. These preliminary results were used as guidelines for the amendment of our extraction and PCR protocols, which are those given below.

DNA Extraction and Purification

Quantities of ~1.0 g of bone, ~0.1–0.7 g of tooth fragments or ~0.5 cm^2 of dried blood spot (on gauze) were combined with 2.0 mL 0.5 M Na_2EDTA pH 8.0 and 25 μL proteinase K (20 mg/mL) in 3.5 mL polystyrene tubes and mixed on a rotary mixer for 48 h at room temperature. Substrate residues were pelleted by centrifugation at 4000 g for 5–15 min. Aliquots of 0.5 mL extract supernatant were bound to 20 μL silica suspension using 1.0 mL 4 M. GuSCN by mixing for 2 h in 1.5 mL Eppendorf tubes on a rotary mixer at room temperature. DNA/silica matrix was pelleted by microcentrifugation for 20 s at 13,000 g and washed twice in 1.5 mL 70% ethanol and once in 1.5 mL acetone. The pellet was dried at 56°C for 5 min. DNA was eluted from the silica into 115 μL sterile filtered distilled water by heating at 56°C for 15 min, and vortexing every 5 min, to aid solution of the DNA. Silica was pelleted by centrifugation at 13,000 g for 2 min. Volumes of 105 μL DNA solution were taken off to avoid inadvertent removal of unwanted silicate. Specimens were stored in 0.5 mL Eppendorf tubes at −20°C prior to PCR analysis.

PCR Analysis

All PCRs are of the sequence specific primer (SSP) type and were carried out using a Perkin-Elmer GeneAmp 9600 thermal cycler. PCR reaction mixtures and programs were optimized using modern samples by D.S. Standard precautions against contamination were taken (25); Primer sequences are given in Table 1. The *Amel* PCR targets the X-Y homologous amelogenin gene. The X homologue contains a 6 bp deletion, generating 106 and 112 bp products from the X and Y chromosomes, respectively (18). Aliquots of 5 μL of DNA sample were amplified in a 10 mL reaction mixture consisting of Tris-HCl pH 8.3 (10 mM), KCl (50 mM), $MgCl_2 \cdot 6H_2O$ (1.5 mM), 1% Triton X-100, dNTPs (200 μM), *Taq* polymerase (1U) and oligonucleotide primers Amel-A and Amel-B (1.0 μM). The PCR program consisted of an initial strand separation step (94°C, 120 s), followed by a biphasic amplification with a 10 cycle stringent phase (94°C, 10 s, 65°C, 60 s) and a 30 cycle non-stringent phase (94°C, 10 s; 62°C, 40 s; 72°C, 30 s). The PCR products were visualized by electrophoresis. Aliquots of 4 μL DNA solution were added to 1 μL by 5 gel loading buffer

TABLE 1—*Oligonucleotide primer sequences.*

5′CCC TGG GCT CTG TAA AGA ATA GTG3′ (Amel-A)
5′ATC AGA GCT TAA ACT GGG AAG CTG3′ (Amel-B)
5′GAG AGT GGC GCC TCC GCT CAT3′ (DPB-AmpA)
5′GCC GGC CCA AAG CCC TCA CTC3′ (DPB-AmpB)
5′ATG CTA AGT TAG CTT TAC AG3′ (A)
5′ACA GTT TCA TGC CCA TCG TC3′ (B)

(40% sucrose, 0.1% bromophenol blue, 50 mM. Na_2EDTA, 50 mM. Tris-HCl pH 7.6 and 5% SDS) and run on a 5% agarose gel (125 V, 40 min) containing 10 μL ethidium bromide (10 μg/mL).

The HLA-DPB1 primers amplify a 327 bp sequence of the polymorphic second exon (26). The mtDNA analysis, included to provide a comparison of nuclear and mtDNA survival, used primers which target a 121 bp segment of the non-coding region V (27). In both cases, aliquots of 5 μL of DNA sample were amplified in a 10 μL reaction mixture consisting of Tris-HCl pH 8.3 (10 mM), KCl (50 mM), $MgCl_2 \cdot 6H_2O$ (1.5 mM), gelatin (100 μg/mL), dNTPs (200 μM), *Taq* polymerase (1 U) and 1.0 μM oligonucleotide primers (DPB-AmpA and DPB-AmpB for HLA-DPB1; A and B for mtDNA region V) each at 0.5 μM. The PCR program consisted of an initial strand separation step (95°C, 5 min), a 35 cycle amplification phase (95°C, 1 min; 55°C, 1 min; 72°C, 1 min) and a final elongation step (72°C, 5 min). The PCR products were visualized by electrophoresis (100 V, 20 min) on a 1.5% agarose gel.

Blank controls were included in each extraction, purification and amplification step to allow detection of contamination with extraneous DNA and to provide an indication of its origin. Positive controls were included in extraction (50 μL modern blood in 450 μL sterile filtered distilled water), purification (0.5 mL/DNA solution at 10 ng/μL) and amplification (5 mL DNA solution at 10 ng/μL) steps.

Results

The *Amel* PCR yields two bands, corresponding to X and Y, for a male, but only the X band for a female (Fig. 1). Single bands are generated by the amplification of HLA-DPB1 sequences (Fig. 2) and sequences of the mitochondrial non-coding region V (Fig. 3). The relative intensity of region V mtDNA sequences amplified was subjectively rated on a 3 point scale corresponding to products generated from >200 pg/μL, 2–200 pg/μL and <2 pg/μL of modern DNA. Concentrations of <0.2 pg/μL do not amplify reliably using our system (22). Results are given in the appendix and are summarized in Table 2. Some samples were used in methodological development (see above), leaving insufficient DNA for all three subsequent PCR analyses (these are given as "not attempted" in the appendix).

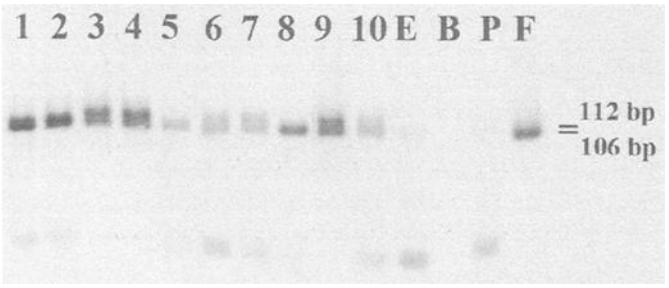

FIG. 1—*Photograph of 5% agarose gel showing typical results for Amelogenin analysis. Aliquots of 4 μL DNA solution were added to μL by 5 gel loading buffer (40% sucrose, 0.1% bromophenol blue, 50 mM Na_2EDTA, 50 mM Tris-HCl pH 7.6 and 5% SDS) and run on a 5% agarose gel (125 V, 45 min). Normally, 2 bands are generated for a male (112 and 106 bp) and 1 for a female (106 bp only). Lanes 1–10: DNA extracted from forensic specimens; 1: F31, 2: F32, 3: F33, 4: F34, 5: F35, 6: F36, 7: F37, 8: F38, 9: F39, 10: F40, E: extraction blank, B: silicate binding (purification) blank, P: PCR blank, F: PCR positive control DNA (female, 10 ng/μL). Faint contamination can be distinguished in blanks E and P (see discussion).*

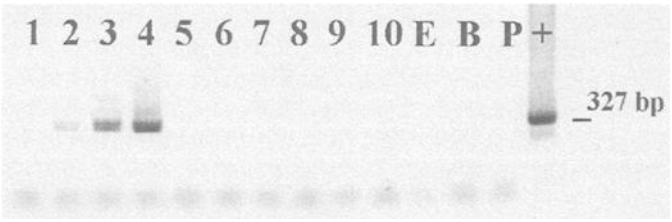

FIG. 2—*Photograph of 1.5% agarose gel showing typical results for HLA-DPBI analysis run on a 1.5% agarose gel (100 V, 20 min). Single bands are generated by the amplification of the 372 bp target sequence. Lanes 1–10: DNA extracted from forensic specimens; 1: F31, 2: F32, 3: F33, 4: F34, 5: F35, 6: F36, 7: F37, 8: F38, 9: F39, 10: F40, E: extraction blank, B: silicate binding (purification) blank, P: PCR blank, +: PCR positive control DNA (10 ng/μL).*

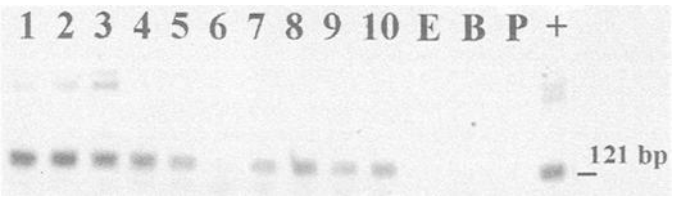

FIG. 3—*Photograph of 1.5% agarose gel showing typical results for mtDNA region V analysis run on a 1.5% agarose gel (100 V, 20 min). Single bands are generated by the amplification of the 121 bp target sequence. Lanes 1–10: DNA extracted from forensic specimens; 1: F31, 2: F32, 3: F33, 4: F34, 5: F35, 6: F36, 7: F37, 8: F38, 9: F39, 10: F40, E: extraction blank, B: silicate binding (purification) blank, P: PCR blank, +: PCR positive control DNA (10 ng/μL). Although sensitive to low DNA substrate concentrations (~250 fg/μL), the PCR may generate high molecular weight artefacts at higher concentrations (lanes 1–3 and +).*

Discussion

As expected, shorter PCR products predominated, with the relative length of the mtDNA product (compared with the amelogenin products) being compensated for by its higher copy-number. Similar success was achieved with amplification of mitochondrial and amelogenin sequences. HLA-DPB1 sequences were successfully amplified roughly half as commonly as the other two products. This trend tended to prevail irrespective of the source material, but in fixed bone cross-sections and teeth, HLA-DBP1 and mtDNA region V sequences were relatively more prevalent than might have been predicted. Relatively high HLA-DPB1 recovery in bone cross-sections may be a consequence of formalin treatment. Fixing in 10% formalin may preserve longer DNA strands, but at the same time hinder DNA purification, especially in teeth. In 5 cases absence of *Amel* sequences did not preclude amplification of HLA-DPB1 and in 10 cases *Amel* DNA was amplified, but not mitochondrial. In 4 of the latter cases an incorrect DNA sex was obtained, however.

Amelogenin sexing was correct in 71 out of 84 cases (85%) where the sex of the donor of the specimen was known. The sex of individual donors of the bone cross-sections was not known, but the sexing results obtained (6 male and 6 female) were consistent with the known proportion of males to females in the sample (31 male and 27 female). Sexing errors were exclusively "false females." We have observed apparent female results from known male material in previous experiments (results not shown) and attribute these to preferential amplification of one target strand, normally of the shorter (X-chromosome) product, in highly degraded or "dirty" samples. Stone et al. (28) suggest that in some

TABLE 2—*Table of results.*

Specimen type	*Amel*					HLA-DPB1			mtDNA			Total		
	n	Correct Sex	Incorrect Sex	+	%	*n*	+	%	*n*	+	%	*n*	+	%
Exhumed bone	37	21	8	29	78	26	6	23	26	16	62	89	51	57
Cross-sections	20	?	?	12	60	16	11	69	16	14	88	52	37	71
Dried blood spots	28	28	0	28	100	28	11	39	28	27	96	84	66	79
Forensic bone	8	3	3	6	75	7	4	57	7	6	86	22	16	73
Forensic teeth	2	0	2	2	100	2	2	100	2	2	100	6	6	100
Extracted teeth	18	13	?	14	78	7	5	71	7	6	86	32	25	78
Post-operative bone	6	6	0	6	100	3	2	67	3	2	67	12	10	83
Total	119	71	13	97	82	89	41	46	89	73	82	297	211	71

Results of PCR analysis for Amelogenin (*Amel*), HLA-DPB1 and mtDNA region V (mtDNA) in forensic specimens. Total number of specimens (*n*), positive samples (+), generating a PCR product of appropriate size, and % of positive samples are given.

samples amplifiable DNA quantity may fall to a level approaching equivalence to a haploid genome. In such cases, a great deal of chance variation will influence whether the X or Y sequence tends to be amplified in males (in their experimental comparison it again tends to be the X): in some samples there may simply be too little DNA to confidently sex the individual. In all but one of the "false female" cases we encountered in this study HLA-DPB1 sequences have failed to amplify and mitochondrial region V sequences are also absent or faint, confirming the degraded nature of the samples and the low, or effectively low, concentration of DNA. We have found that samples extracted by this method rarely contain sufficient inhibitors to prevent amplification of a modern DNA sample. Nevertheless, we note that "false-female" results predominated in the partially-decomposed forensic material or those bones and teeth which had been fixed in 10% formalin. We found that one forensic bone specimen (from a two-year old partially-skeletonized body) gave such a result, but when the DNA sample was put through a second purification step, a clear male DNA sex was obtained. Two other specimens gave the correct result when the extraction process was completely repeated (others could not be repeated due to lack of material). In some cases, however, a second purification step resulted in the loss of any amplifiable DNA-probably due to the "overhead" inherent in the silicate method. Stone et al. avoided the potential problem of preferential amplification of the shorter strand by amplifying a different 112 bp region of amelogenin occurring without deletion on both the X and Y chromosomes. Dot-blots were used to test for the X and Y homologues, which have slightly different sequences.

Dried blood spots on gauze were an especially useful substrate and all of our specimens, which were up to 26 years old, yielded amplifiable nuclear DNA allowing amelogenin sexing, which was confirmed to be correct from records. Not surprisingly, all recent post-operative bone specimens were also amenable to amelogenin sexing, although a single sample did not yield results from HLA-DPB1 and mtDNA region V analysis.

Good results were obtained from unfixed tooth fragments, but fixed teeth yielded poorer results. Although the DNA content of teeth may be relatively low compared with bone, the tooth structure itself offers physical protection to DNA preserved inside. The proportion of organic material resulting from diagenesis is less and will interfere less with DNA purification. Treatment with 10% formalin inhibits DNA recovery to a greater extent in teeth than in bone, perhaps reflecting a greater extent of fixing. Skeletal material included specimens which had been subjected to a variety of chemical processes as a result of the burial environment or postmortem preservation procedures. Burial in quick-lime, which might be expected to accelerate DNA degradation by oxidation, does not seem to have too adversely affected DNA preservation. In fact, burial in quick-lime may enhance DNA preservation (see below). Results were also obtained from skeletal material which had been cleaned by immersion in papain solution, fixed in formalin or cleaned by prolonged immersion in hot water (95°C) and stored at room temperature for up to 15 years.

Nitrogen content has been considered an indicator of DNA preservation in archaeological material (29) and may provide an indicator of DNA survival in forensic specimens. Jarvis (30) has measured the nitrogen content of 16 specimens of the exhumed skeletal material, up to 91 years old, included in this study. We recovered DNA from 15 of the 16 samples. It was not possible to associate the failed sample with diminished nitrogen content. However, the relationship between DNA preservation and nitrogen content may not be straight-forward, (31). One specimen (*A1*) had been buried in a grave with quick-lime and later contaminated with engine oil. DNA was recovered, nevertheless, and Jarvis (30) points out that lime treatment may enhance organic preservation by inhibiting microbial and biochemical activity. Age is not the determining factor of single-copy nuclear DNA survival, an observation which Richards et al. (32) have made with reference to mtDNA survival in archaeological material.

Previous studies of forensic specimens have indicated that soiled materials tend to be poorer sources of suitable DNA than clean specimens and that putrefied biological material may be unsuitable for analysis (33–37). The contrasts between results obtained from dried blood spots (stored on clean gauze), fixed specimens, and forensic skeletal material examined in this study are consistent with these observations.

A high level of random contamination of extraction and PCR blanks was experienced in the study, with contamination evident in 38% of all blank controls analyzed. This raises the question "can the positive results be explained by a pattern of random contamination?" It is not conceivable that 71 specimens sexed correctly by chance. Furthermore, a statistical analysis using generalized linear interactive modeling (38) indicated that positive results from forensic DNA samples were independent of the presence or level of contamination detected in the blank controls (22, Evison et al., forthcoming). Also, although we sometimes experience false female results with degraded forensic or archaeological material (and male control DNA diluted to below 25 pg/μL), false male results (i.e., with two bands) are extremely rare (22). In this study there were no "false males," which would be predicted if random contamination of the forensic DNA samples was occurring.

We are presently investigating the sequences and derivation of the apparent contamination.

Conclusions

This study demonstrates a single method capable of recovering single-copy nuclear DNA from a variety of forensic specimens from a few months to 91 years old. Amelogenin sequences were recovered at a similar frequency to mtDNA region V sequences, but DNA sexing results were compromised in fixed or putrefied samples. HLA-DPB1 sequences were recovered at about half the frequency of the other two products. Fixing in 10% formalin reduces the frequency of DNA recovery, especially from teeth, but does not preclude it. Increasing the sensitivity of recovery and amplification protocols led to a concomitant increase in the frequency of apparent positive results detected in blank controls. However, comparison of DNA sexing results with the recorded sex of the donor, and a statistical analysis, exclude contamination as an explanation for the results obtained from forensic specimens.

Acknowledgments

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APPENDIX

APPENDIX—*Results of amelogenin* (Amel), *HLA-DPB1 (DPB1) and mtDNA region V (region V) PCR analysis of forensic specimens.*

Specimen	Age (yr.)	Sex	Result: *Amel*	*DPB1*	*Region V*	Comments
Dried Blood Spots						
A	1	F	1	+	+	
B	1	M	2	+	+	
F23	5	M	2	+	+++	
F23F	5	M	2		+	
F25	0	F	1	+	+++	
F26	0	M	2	+	+++	
F27	0	M	2		+++	
F28	5	F	1	+	+++	
F29	0	F	1	+	+++	
F30	0	M	2		+++	
F31	0	F	1		+++	
F32	0	F	1	+	+++	
F33	0	M	2	+	+++	
F34	?	M	2	+	++	
F35	26	F	1		++	
F36	26	M	2			
F37	20	M	2		+	
F38	22	F	1		+	
F39	11	M	2		+	
F40	11	M	2		+	
F41	15	M	2		+++	
F42	12	F	1		+++	
F43	5	F	1		+++	
F44	5	F	1		+++	
F45	0	F	1		+++	
F47	5	F	1		+++	
F48	5	M	2	+	+++	
F49	5	M	2		+++	
Post-Operative Bone						
POB1	1	M	2	na	na	
POB2	1	F	1	na	na	
POB3	1	F	1	+	+	
POB4	1	F	1	na	na	
POB5	1	M	2	+	+	
POB6	1	F	1			
Forensic Teeth						
Y1	1	M	1*	+	+	10% formalin
Y2	1	M	1*	+	+	10% formalin
Extracted Teeth						
ET1	1	F	1	na	na	
ET2	1	M	2	na	na	
ET3	1	M	2	na	na	
ET4	2	?		na	na	10% formalin
ET5	2	?		na	na	10% formalin
ET6	2	?	1	na	na	10% formalin
ET7	2	?		na	na	10% formalin
ET8	2	?		na	na	10% formalin
ET9	1	F	1	na	na	
ET10	1	M	2	na	na	
ET11	1	M	2		+	
ET12	1	F	1		+	
ET13	1	M	2	na	na	
ET14	1	M	2	+	+	
ET15	1	M	2	+	+	
ET17	1	M	2	+	++	
ET18	1	M	2	+		
ES	2	F	1	+	+	
Exhumed Bone						
2396X	5	F	1	+	+++	
A1	91	M	1*	na	na	Lime/Oil
AB17	58	M	1*	na	na	
AB18	39	M	2		++	
AB19	38	M	2	na	na	
AB20	49	M	2	na	na	
AB21	48	M	1*	na	na	
AB22	45	M	2	+	+++	
AB24	47	M	1*	+	+++	
AB32	44	M		na	na	
AB38	37	M	2		+++	
LG15	40	M	2	na	na	
LG17	34	M	2	na	na	
LG19	29	M	2	na	na	
LG20	37	M	2	na	na	
LG25	52	M	2	na	na	
F50	26	F	1		+	
F51	44	F				
F52	44	F	1		++	
F53	24	F				
F54	27	F	1		++	
F55	28	M			+	
F56	27	M	1*		+	
F57	27	M				
F58	67	M				
F59	48	M				
F60	17	M				
F61	41	M	2		+++	
F62	53	M	2		+	
F63	23	M	2	+	+++	
F64	58	M	1*			
F65	46	M	1*			
F66	30	M	2		+++	
F67	11	M	2	+	+++	
F68	44	M	2	+		

APPENDIX—*Continued*

Specimen	Age (yr.)	Sex	Result			Comments
			Amel	*DPB1*	*Region V*	
F69	31	M	1*			
F70	49	M	2		+++	
Forensic Bone						
CC1	2	M	2	na	na	
F71	4	M	2	+	+++	
F72	?	?		+	++	
F73	3	M	1*		+	
F74	1	M			+	
SF	6	F	1	+	+	
SM	6	M	1*	+	+	
Y	1	M	1*			10% formalin
Cross-sections						
S.A1	15	?	1	+	++	10% formalin
S.A2	15	?		na	na	10% formalin
S.A5	15	?	1	+	+	10% formalin
S.A7	15	?	1	+	+++	10% formalin
S.A8	15	?		na	na	10% formalin
S.B1	15	?	2	+	+	10% formalin
S.B3	15	?		+	+	10% formalin
S.B4	15	?	1	+	+	10% formalin
S.B5	15	?	1			10% formalin
S.B6	15	?	2			10% formalin
S.B7	15	?		na	na	10% formalin
S.B8	15	?	2	na	na	10% formalin
S.B9	15	?	2	+	+	10% formalin
S.B10	15	?		+	+	10% formalin
S.C1	15	?	2		+	10% formalin
S.C2	15	?			+	10% formalin
S.C3	15	?		+	+	10% formalin
S.C4	15	?	1	+	+	10% formalin
S.C6	15	?		+	+	10% formalin
S.D2	15	?	2		+	10% formalin

Positive results (1,2,+), sexing errors (*), tests not attempted (na), number of bands (*Amel* results only) and intensity of mtDNA product (+,++,+++; see text) are indicated. Unusual preservation is given in the comments column.

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