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Forensic Mitochondrial DNA Analysis of 116 Casework Skeletal Samples*

ABSTRACT: Between February 1999 and May 2005, 116 DNA extractions were completed on skeletal remains from routine casework. Overall, at least a partial mitochondrial DNA (mtDNA) profile was obtained on 83.6% of samples. Skeletal remains fell into two general categories: (1) samples for body identifications submitted by law enforcement and (2) samples submitted to answer historical or family identity questions. Body identification cases were more likely to yield full mtDNA profiles, whereas historical cases were more likely to result in partial profiles. Overall, the ability to obtain a full or partial profile primarily reflects the difference in the average age and condition of the samples in these two categories and thus, difference in the quantity and quality of the DNA. Cremated remains were uniformly unsuccessful, whereas infant/fetal remains were uniformly successful. Heteroplasmy in skeletal remains was observed at a rate similar to that in hair (~10%). For body identification cases, skeletal remains had the same mtDNA profile as the accompanying reference sample in 50% of cases.

KEYWORDS: forensic science, mitochondrial DNA, skeletal remains, hypervariable regions 1 and 2, small amplicons, degraded DNA, burned bone

With thousands of unidentified skeletal remains in the United States, there has been increased forensic application of current DNA technology and kinship analyses to solve missing persons cases. A critical factor in the success of the National Missing Person DNA Database Program will be the ability to obtain nuclear STR profiles and/or mitochondrial DNA (mtDNA) profiles from diverse skeletal remains. To assess our laboratory's success with skeletal remains and provide a benchmark for the forensic community involved in identification of these remains, we retrospectively examined our ability to develop mtDNA profiles from the remains submitted to our laboratory in the last 6 $\frac{1}{2}$ years. We describe here the approximate age and type of remains we have received, whether a full, partial, or no profile was obtained, rate of use of small amplicons, frequency of site heteroplasmy and mixtures, and frequency of different case outcomes.

Our laboratory focuses exclusively on mtDNA, a powerful tool in missing persons cases due to its persistence in skeletal material for centuries if not millennia. Because mtDNA is maternally inherited without recombination (1,2), a single maternal reference sample, such as a buccal or blood sample, is all that is required for comparison with skeletal remains. Candidate maternal relatives can be closely related to the missing person, such as mothers and siblings, or they can be quite distant in the maternal family tree. In historical cases, living maternal relatives can provide mtDNA references for skeletal remains many generations removed (3,4). Mitochondrial DNA is used to support other information that is available to make an identification, such as physical anthropology, dental or other radiographic data, location and time of body recovery, personal effects, and associated grave artifacts (1,5).

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Materials and Methods

Samples and DNA Extraction

Mitochondrial DNA profiles were generated from bones and teeth submitted for routine casework. Samples were processed individually and extracted in parallel with a reagent blank control that accompanied the sample throughout testing. First, the outside layer of exposed bone was removed with a silicon carbide grinding stone attached to a rotary tool (Sears Craftsman, Hoffman Estates, IL; teeth were not sanded). When the submitted bone was larger than *c.* 2 cm^2, a portion of bone was cut from the main sample using a rotary tool cutting wheel. Cross-sections of the shaft portion of long bones were used. The cut sample was cleaned in at least three 30-min 5% (w/v) Terg-a-zyme washes (Alconox, New York, NY) in an ultrasonic water bath, followed by a 5-min 10% (v/v) ultrasonic bleach wash, a 70% ethanol rinse, and air-drying. The resulting sample was pulverized in a sterilized stainless-steel Waring blender cup (Waring, Torrington, CT). Approximately 0.25–0.4 g of pulverized sample was incubated at least 2 h at 56°C in extraction buffer (6) with occasional agitation. Total DNA was isolated from skeletal remains by a silica-based method (6) in which nucleic acids were bound to silica and the silica was washed to remove any impurities. The nucleic acids were eluted from the silica at 56°C in water and DNA extraction products were either amplified immediately or stored at −20°C.

Family reference samples accompanying the skeletal remains were usually buccal swabs or dried blood samples. Total DNA was isolated from these samples with the QIAamp® DNA Mini Kit (QIAGEN Inc., Valencia, CA) according to the manufacturer's instructions.

PCR Amplification and Sequencing

PCR amplification was carried out using 5–15 μL of extraction product in a 50 μL reaction volume containing final concentrations of 1 × PCR Gold buffer with 2 mM $MgCl_2$ (Applied Biosystems, Foster City, CA), 0.2 μM of each primer (custom synthesized, QIAGEN Inc.), 400 μM of dNTPs (Amersham Biosciences,

Piscataway, NJ), 0.4 mg/mL BSA (bovine serum albumin, Sigma Aldrich, St. Louis, MO), and 1.25 U AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA). Positive (HL-60; American Type Culture Collection, Manassas, VA or K562; GibcoBRL, Gaithersburg, MD) and negative PCR controls accompanied the sample and its reagent blank. Thermal cycling was performed using GenAmp PCR 2400, 2700, or 2720 thermal cyclers (Applied Biosystems) starting with 12 min at 95°C, followed by 36–40 cycles of 15 sec at 95°C, 30 sec at 45–56°C, and 45 sec at 72°C, ending in a 4°C hold. Amplicon yield was evaluated by running 10 μL of PCR product on a 1–2% SeaKem agarose gel (Cambrex Bio Science, Rockland, ME) and visualized with ethidium bromide and a UVP gel photodocumentation system (Upland, CA). In general, primer pairs used on samples followed those published previously (7), which amplify hypervariable regions 1 (HV1) and 2 (HV2) in two overlapping segments each, with an average amplicon size of 283 base pairs (bp). In addition to these primer pairs, some samples required amplification in smaller amplicons (mini-primer set strategy (8,9)). This strategy targets double-stranded DNA that may be abundant in quantity but reduced to small fragments as the result of exposure to heat, acid, bacterial or fungal growth, or moisture over time. In this type of case, HV1 and HV2 were amplified in four overlapping segments each with an average amplicon size of 151 bp (9). Multiple overlapping amplifications generate redundancy that provides internal control on the authenticity of the results. Family reference samples were amplified as a single amplicon each for HV1 and HV2. PCR products were purified using Rapid PCR Purification Systems (Marligen Biosciences, Philadelphia, PA) according to the manufacturer's instructions.

Cycle sequencing of both strands (light and heavy strands) was carried out according to the manufacturer's instructions using the Big Dye® Terminator Cycle Sequencing Kit (v1.0 or v1.1; Applied Biosystems), with the PCR primers and internal primers as necessary. Cycle sequencing products were purified using Performa DTR Gel Filtration Cartridges (Edge Biosystems, Gaithersburg, MD). Samples were electrophoresed on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and the resulting DNA sequence data were analyzed by two forensic examiners using the LASERGENE SeqMan software package (DNASTAR, Madison, WI). The reagent blank and positive and negative amplification controls were sequenced on the light strand.

In this laboratory, a full forensic mtDNA profile consists of nucleotide positions 15,998–16,400 (HV1) and nucleotide positions 30–407 (HV2). Partial profiles are some portion of HV1, HV2, or both. In addition to the standard HV1 and HV2 regions, in some cases two additional regions are examined. These regions comprise nucleotide positions 16,471–16,561 (variable region 1; VR1) and nucleotide positions 424–548 (variable region 2; VR2, which has also been designated HVIII (10)). These additional regions may be examined when only a single nucleotide difference is observed between skeletal remains and the family reference or when the skeletal remains and family reference sample have a profile that is relatively frequent in the human population. In the former case, an additional nucleotide difference may be identified that allows an exclusion rather than an inconclusive result; in the latter case, the identification of a rare polymorphism that is common to both questioned and known samples may give additional confidence in a failure to exclude. Because many primers are available that are spaced throughout the control region, nearly any region can be targeted for analysis with a variety of amplicon sizes. All nucleotide polymorphisms are numbered with respect to the revised Cambridge Reference Sequence (rCRS) (11,12).

TABLE 1—*Type of skeletal remains and mtDNA profiles obtained for 116 extractions.*

	mtDNA Profile Obtained		
Sample	Full	Partial	None
Femur	25	8	3
Tibia	2	—	—
Fibula	—	—	1
Metatarsal	3	—	—
Pelvis	1	—	—
Humerus	6	—	—
Radius	1	—	—
Scapula	1	—	—
Rib	3	—	2
Clavicle	—	1	—
Skull	4	2	—
Mandible	—	—	1
Tooth	23	12	4
Unidentified fragments	—	5	8
TOTAL	69	28	19

mtDNA, mitochondrial DNA.

Results and Discussion

Sample Types

Between February 1999 and May 2005, 116 DNA extractions were completed on skeletal remains. Table 1 illustrates the range of skeletal remains and whether a full, partial, or no mtDNA profile was obtained. Full profiles were obtained on 69 samples (59.5%), partial profiles were obtained for 28 samples (24.1%), and no profiles were obtained for 19 samples (16.4%). Overall, at least a partial mtDNA profile was obtained for 83.6% of samples, which is comparable with the rate reported by the Armed Forces DNA Identification Laboratory (AFDIL) (8).

Femurs and teeth were the most common samples, and if available, these were requested from clients, due to an overall high rate of obtaining profiles. Ninety-two percent of femurs and 90% of teeth provided full or partial profiles. At AFDIL, femurs have the second highest success rate at 95% (surpassed only by ribs at 96%), while 80% of teeth provide reportable profiles (8). Sometimes, only small anatomically unidentifiable bone fragments were submitted, and these samples did not have a high success rate. While 38% provided a partial profile, 62% yielded insufficient mtDNA to develop a profile. Most (80%) of the partial profiles that were recovered from these small bone fragments were mixtures, which further complicated the conclusions that could be drawn from these samples.

Many failed samples were accompanied by descriptions of challenging environmental exposures, especially burning (11 of 16 failures of body identifications). Although the sample size was small ($n = 5$ cases), cremains (cremated remains) were uniformly unsuccessful. Exposure to high heat for long periods resulting in bone fragmentation virtually ensures that these samples will fail. In controlled experiments of exposure of human bone fragments to high (800–1200°C) temperatures for 20 min, Cattaneo et al. (13) were unable to amplify mtDNA from the heat-exposed bones although all unexposed controls amplified. In this lab, seven extraction attempts of skeletal remains from two cases of criminal burning, where the intent was to destroy the body, failed. In contrast, several cases of remains recovered from water such as rivers or the ocean resulted in full profiles, and it was rare for a water-collected sample to fail to provide any data. This result is consistent with observations by other practitioners (J. E. B. Stewart, personal communication).

While burned remains are typically brittle, blackened, and friable, and the failure to DNA-type them is not surprising, appearances may be deceiving. Of particular interest was a case where only partial mtDNA results were obtained for an anatomically complete and perfectly preserved modern skeleton with a history of having been in an enclosed barrel in moderate heat for only several months. As noted by other authors, the appearance of bone is not necessarily a good predictor of successful DNA typing (14).

Four cases involved the identification of infant or fetal remains. These remains were an *c*. 7 cm femur, a 4.5 cm long bone, a scapula from a 30-year-old burial, and a neural arch from a 40-year-old burial. Although the remains were minimal in both size and weight, the quantity and quality of mtDNA from infant/fetal remains were outstanding. With the exception of the neural arch, the other remains provided a full profile. Although very small (*c*. 1 cm × 3 mm), the neural arch had abundant degraded mtDNA and a partial profile was obtained with small amplicons. In all four cases, the infant/fetal remains had the same mtDNA profile as the putative mothers. We speculate that high levels of mitotic activity may make fetal remains especially good samples.

Site Heteroplasmy

There were 12 independent observations of site heteroplasmy among the 116 sample extractions (10.3%). Heteroplasmy was defined as a single nucleotide position within the analyzed region where two nucleotides were observed at a single position. In two cases, different skeletal remains thought to have originated from the same individual shared a common profile with a site heteroplasmy. These were not considered as independent observations and thus were counted only once. This rate of observation of heteroplasmy is comparable with the rate of observed heteroplasmy in hairs from casework in this laboratory of 11.4% (15). The observed heteroplasmic sites and the frequency of observation are listed in Table 2. Sites 16,069, 16,179, 16, 192, and 151 were not previously reported for hairs by this laboratory but are all sites known to vary in human populations. The most commonly observed heteroplasmic site was 16,093, which is known to have a high rate of heteroplasmy (8,15–17). In one case, the site heteroplasmy observed in the skeletal remains was also present in the maternal reference sample (mother), providing additional support for an identification.

TABLE 2—*Observed heteroplasmic positions in skeletal remains.*

Heteroplasmic Position*	Other Matched K or Q Sample in Case For Comparison?	Nucleotide Present in Comparison Sample	Relationship of K to Skeletal Remains
16,069C > T	K buccal	C; rCRS	Unknown
16,093C = T??	–		
16,093C > T	Q tooth	C > T heteroplasmy	
16,093C > T	K blood	C; substitution from rCRS	Mother
16,093C > T	K blood	C; substitution from rCRS	Mother
16,093C > T	K buccal	C > T heteroplasmy	Mother
16,129A > G	K blood	A; substitution from rCRS	Half-sibling
16,179C > T	–		
16,192C > T	Q tooth	C > T heteroplasmy	
16,399G > A	K buccal	A; rCRS	Unknown
151C > T	K buccal	C; rCRS	Half-sibling
189A > G	K buccal	A; rCRS	Daughter

*(>) indicates the relative peak heights of the two nucleotides on the light and heavy strand.

A, adenine; C, cytosine; G, guanine; T, thymine; rCRS, revised Cambridge Reference Sequence; Q, questioned; K, known.

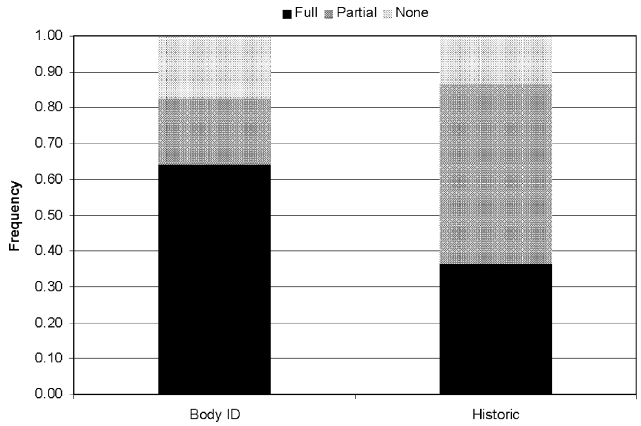

FIG. 1—*Frequency of full profiles (15,998–16,400 and 30–407), partial profiles, and no profiles among samples submitted for body identification versus historic/private casework.*

Body Identification Cases Versus Historical Cases

Skeletal remains submitted to this laboratory can generally be divided into two categories: (1) samples for body identifications submitted by law enforcement and (2) samples submitted to answer historical or family identity questions. Of the 116 DNA extractions, 92 (79.3%) were submitted for body identification, 22 (19.0%) for questions of historical interest or by private individuals, and two (1.7%) were submitted as known references. Overall, the success of obtaining at least a partial profile was nearly the same among categories but body identification cases were more likely to yield a full profile whereas historical cases were more likely to result in partial profiles ($p > 0.008$; Fig. 1). The average age of samples in historical cases is far greater than that of body identification cases (Fig. 2). Most samples in historical cases were greater than 50 years of age (86.4%), whereas most of the samples in body identifications were less than 25 years of age (87%). This suggests that skeletal remains older than about 50 years are more likely to contain minimal and degraded DNA and require an approach like that used for "ancient" DNA (18), our small amplicon analysis. A notable example of this was a historical case involving the skeletal remains analysis of "Wild Bill Longley," a renowned Texas gunfighter (4).

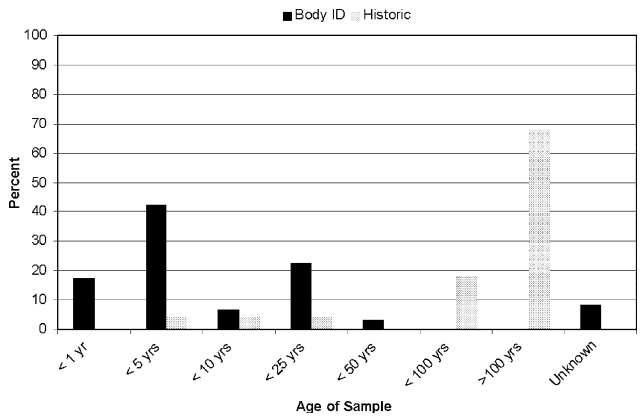

FIG. 2—*Approximate age of skeletal remains among samples submitted for body identification versus historic/private casework.*

Among historical cases, small amplicon analysis was necessary for most samples. For those historical samples that yielded full or partial profiles, 13 of 19 samples (68%) required amplification with at least one amplicon less than 200 base pairs. AFDIL noted that over 50% of their cases require the use of at least one "mini-primer" set (8). In contrast, only 17 of 76 samples (22%) from body identification cases required small amplicon analysis. Without small amplicon analysis for these samples, four cases of body identification would not have been resolved. The mtDNA profile from all four of these degraded samples had profiles matching the accompanying reference samples. Overall, among body identification cases 46 of 92 samples (50%) had the same mtDNA profile as the submitted reference sample.

Mixtures and Damaged DNA

Mixed profiles are defined by our laboratory as profiles where two or more sites have two or more nucleotides present on both light and heavy strands and are observed by the appearance of overlapping peaks on the electropherograms. Mixed profiles were obtained on seven of 22 samples (31.8%) associated with historical cases and nine of 92 samples (9.8%) associated with body identifications.

Among body identification cases, all samples that had mixed sites were analyzed with small amplicons and only partial profiles were obtained; this result was an indication of the compromised quality of the mtDNA authentic to the samples. Five of the samples were small fragments of unidentified bones, several of which produced only enough powdered bone for a single extraction. Four of these five were from a single case where the fragments had been sifted from the dirt floor of a garage. The remaining four samples with mixture profiles were teeth. Each of these mixtures was likely to be a true mixture of DNA authentic to the sample plus contaminating DNA, as the mixed sites were those that are known to vary in populations and the possible derived haplotypes have been observed in human populations. In this laboratory, mixed profiles may be used for exclusions but important caveats are given regarding the challenges of interpreting mtDNA mixtures.

Among historical cases, each of the samples that contained a mixture was 200 years old or older. Again, for these samples, small amplicon analysis was necessary and only partial profiles were obtained. Some of the mixture profiles reflect minimal quantities of authentic templates originating from the sample (based on presumptive matches with associated reference samples) plus contaminating contemporary DNA. Historic samples often have a history of handling by other individuals; teeth and long bones are often subjected to extensive manual examination in an archeological context. A recent study suggests that longer bleach washes with a more concentrated solution than we utilize may remove surface-contaminating DNA from ancient skeletal remains without jeopardizing the authentic DNA (19), but another study warns that bleach use will reduce availability of authentic nuclear but not mtDNA (20). Given this information, we recommend modest use of bleach on environmentally compromised samples.

Along with these true mixtures, most historical samples also showed evidence of damaged DNA (21). These damaged DNA profiles have one or more nucleotide substitutions with no evidence of an alternative underlying nucleotide (unmixed polymorphic sites) plus several to many sites with a background alternative peak present on both strands (mixed sites). Overlapping amplicons or repeat PCRs show that the "clean" polymorphisms can be replicated but the mixed sites cannot be replicated. These mixed positions are often observed to be invariant or rarely variable within human populations. Postmortem DNA modifications may be occurring in these samples; a combination of low template number plus the occasional single nucleotide change that is picked up in an early round of PCR may explain their detectability (22). In one sample, the amplification of a known nuclear pseudogene contributed to a very complex mixture.

Taq polymerase error has been reported to create singleton variants that may increase to a detectable level in sequenced DNA. However, we have amplified and sequenced over three megabases of positive control DNA (HL60) in parallel with our samples, and have never seen mixed nucleotides in these positive control electropherogram data, which are analyzed along with questioned or known sample data. This observation, along with the observation that most mixed nucleotide sites occur in old and compromised samples, suggests that most mixed sites arise from damaged template rather than from polymerase error.

Very small bone fragments in particular appear much more likely to either fail to yield any mtDNA or yield minimal amounts of degraded DNA from multiple sources. In the ancient DNA community, cloning from multiple extractions and multiple amplifications can provide a picture of the authentic DNA sequence relative to sites that result from damaged DNA (21). Cloning within a forensic laboratory is not currently standard protocol due to concerns about contamination. Methods to sort mixtures are greatly needed in the forensic mtDNA community. The application of DHPLC Transgenomic Wave^{TM} technology to separate and characterize mixtures is one hope for the future of forensic mtDNA analyses (23). However, even with the ability to sort out the contributing profiles, determining the authentic profile for small samples that cannot be replicated will remain problematic.

Conclusion

Some recognizable patterns have emerged from our experience with a limited sample size of mtDNA skeletal cases. The age and condition of the sample are generally correlated with success in developing an mtDNA profile, but the occasional surprise (a well-preserved bone with little DNA or a poorly preserved bone with abundant degraded DNA) means that practitioners should not generalize as to which samples are deserving of analysis based on their appearance. The exception to this is cremated remains, which have not, to date, been successfully analyzed in this laboratory. We routinely dissuade clients with crematorium-processed remains who are in search of an identity confirmation. In criminal burning cases, we now require anatomically recognizable bones for analysis.

Although water-collected remains might seem to be poor samples, they have worked well in our hands, as have fetal or infant remains. The age of the sample need not be a discouraging factor; the oldest historical sample typed by this laboratory was a tooth archeologically dated to 1000 years. However, when copy number is low in a historical sample, the forensic lab increasingly will be concerned about amplification competition between authentic DNA, contaminating modern DNA, and the occasional damaged postmortem DNA template. What is clear is that the small amplicon approach is highly successful at capturing degraded but abundant mtDNA from challenging samples and should be part of the testing repertoire for all missing persons programs.

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References

1. Holland MM, Fisher DL, Roby RK, Ruderman J, Bryson C, Weedn VW. Mitochondrial DNA sequence analysis of human remains. Crime Lab Digest 1995;22:109–15.
2. Budowle B, Allard MW, Wilson MR, Chakraborty R. Forensics and mitochondrial DNA: applications, debates, and foundations. Ann Rev of Genom Hum Genet 2003;4:119–41.
3. Gill P, Kimpton C, Stoneking M, Melton T. Anastasia and the tools of justice. Nat Genet 1994;8:205–6.
4. Owsley D, Ellwood B, Melton T. Search for the grave of William Preston Longley, hanged Texas gunfighter. Hist Arch 2006;40:50–63.
5. Kemkes-Grottenthaler A. The reliability of forensic osteology—a case in point. Forensic Sci Int 2001;117:65–72.
6. Hoss M, Pääbo S. DNA extraction from Pleistocene bones by a silica-based purification method. Nucleic Acids Res 1993;21:3913–4.
7. Isenberg AR, Moore JM. Mitochondrial DNA analysis at the FBI laboratory. http://www.fbi.gov/hq/lab/backissue/july1999/dnatext.htm
8. Edson SM, Ross JP, Coble MD, Parsons TJ, Barritt SM. Naming the dead—confronting the realities of rapid identification of degraded skeletal remains. Forensic Sci Rev 2004;16:63–90.
9. Gabriel MN, Huffine EF, Ryan JH, Holland MM, Parsons TJ. Improved mtDNA sequence analysis of forensic remains using a "mini-primer set" amplification strategy. J Forensic Sci 2001;46:247–53.
10. Lutz S. Is it possible to differentiate mtDNA by means of HVIII in samples that cannot be distinguished by sequencing the HVI and HVII regions? Forensic Sci Int 2000;113:97–101.
11. Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. Nature 1981;290:457–65.
12. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 1999;23:147.
13. Cattaneo C, DiMartino S, Scali S, Craig O, Grandi M, Sokol R. Determining the human origin of fragments of burnt bone: a comparative study of histological, immunological, and DNA techniques. Forensic Sci Int 1999;102:181–91.
14. Edson SM, Thew HA, Damann FE, Boyer CA, Barritt-Ross SM, Smith BC. Success rates for recovering mitochondrial DNA (mtDNA) from 4000 "ancient" human skeletal remains. Proceedings of The 17th Meeting of the International Association of Forensic Sciences; August 21-6 2005; Hong Kong, A0606. Philip SL Beh, ed. Hong Kong: International Association of Forensic Sciences/Hong Kong Forensic Science Society, 2005: 58.
15. Melton T, Dimick G, Higgins B, Lindstrom L, Nelson K. Forensic mitochondrial DNA analysis of 691 casework hairs. J Forensic Sci 2005;50:73–80.
16. Tully LA, Parsons TJ, Steighner RJ, Holland MM, Marino MA, Prenger VL. A sensitive denaturing gradient-gel electrophoresis assay reveals a high frequency of heteroplasmy in hypervariable region 1 of the human mtDNA control region. Am J Hum Genet 2000;67:432–43.
17. Holland MM, Parsons TJ. Mitochondrial DNA sequence analysis—validation and use for forensic casework. Forensic Sci Rev 1999;11:21–50.
18. Krings M, Geisert H, Schmitz RW, Krainitzki H, Pääbo S. DNA sequence of the mitochondrial hypervariable region II from the Neanderthal type specimen. Proc Natl Acad Sci USA 1999;96:5581–5.
19. Kemp BM, Smith D. Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci Int 2005;154:53–61.
20. Steadman DW, DiAntonio LL, Wilson JJ, Sheridan KE, Tammariello SP. The effects of chemical and heat maceration techniques on the recovery of nuclear and mitochondrial DNA from bone. J Forensic Sci 2006;51:11–7.
21. Hofreiter M, Jaenicke V, Serre D, von Haeseler A, Pääbo S. DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. Nucleic Acids Res 2001;29:4793–99.
22. Gilbert MTP, Hansen AJ, Willerslev E, Rudbeck L, Barnes I, Lynnerup N, Cooper A. Characterization of genetic miscoding lesions caused by postmortem damage. Am J Hum Genet 2003;72:48–61.
23. Danielson PD, Kristinsson R, Shelton R, LaBerge GS. Separating human mtdna mixtures using denaturing high-performance liquid chromatography (DHPLC). Exp Rev Mol Diagn 2005;5:53–63.

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