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TECHNICAL NOTE

CRIMINALISTICS; PHYSICAL ANTHROPOLOGY

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Full STR Profile of a 67-Year-Old Bone Found in a Fresh Water Lake

ABSTRACT: DNA extraction from and DNA typing of fresh water-exposed aged bone specimens poses a challenging task and is not very well examined. This study presents a new method to extract typable DNA from such problematic bone specimens. The procedure comprises low-heat drilling and cryogrinding, mild lysis conditions, and silica-column-based DNA cleaning. DNA quantity is assessed by quantitative PCR prior to short tandem repeat (STR) amplification. The procedure was employed with a 67-year-old tibia bone fragment recovered from a fresh water lake and succeeded to produce a full STR profile using the MPX-SP1 and MPX-SP2 mini-STR kits and a partial profile with 12 successfully amplified STRs using the Identifiler STR kit. The new method for the extraction of DNA from aged fresh water-exposed bone specimens presented herein was successfully applied to prepare DNA of sufficient quality and quantity to generate a full STR profile.

KEYWORDS: forensic science, forensic genetics, fresh water, bone, DNA extraction, DNA typing

We were assigned to generate an individualizing DNA profile of a 67-year-old fragment of a human tibia bone that had been recovered from the fresh water lake "Laacher See" in a volcanic area in Germany from a depth of 20 m (Fig. 1). The lake water is mainly phreatic and has a temperature of 4°C in 20 m depth. The bone can very probably be assigned to one of three missing crew members of a Halifax aircraft who died when the aircraft crashed into the lake in 1942.

Fresh water exposure of bone specimens has been shown to be problematic for DNA profiling (1) and we were unable to find any studies presenting protocols specifically adapted to fresh water-exposed samples in the literature.

Indeed, neither of our established methods nor protocols for DNA extraction from ancient bone specimens gathered from the literature (2–5) were suitable to produce DNA of sufficient quantity and quality from which a satisfactory short tandem repeat (STR) profile could be generated. We therefore combined and fine-tuned several parts of different methods to eventually arrive at a protocol that resulted in a complete STR profile.

Materials and Methods

Prevention of Contamination

To minimize the hazard of contamination, all procedures described herein were performed wearing fresh protective gloves and face masks and using DNA-free solutions, disposable plastic wares, and filter tips. No other samples were handled simultaneously in the same room, and all surfaces, devices, and instruments were thoroughly cleansed and decontaminated before use.

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Preparation of Bone Powder

The specimen was completely dried at room temperature. The surface of the bone was then cleansed with distilled water and ethanol. Afterward, the surface layer of the diaphysis part of the bone was abrased using two kinds of sequentially finer sandpaper. A drill was positioned onto the smoothened surface and was applied with low speed to shave off strands without generating too much warmth of friction. The strands were collected and ground to a fine powder in a mortar under liquid nitrogen. In this way, 1 g of bone powder was generated.

DNA Extraction

A 16 ml extraction buffer (0.5 M EDTA, 250 μg/mL Proteinase K, 0.5% SDS and 50 mM DDT, pH 8.0) was used per 1 g of bone powder. The buffer was prepared from 0.5 M EDTA, 20 mg/mL Proteinase K, 10% SDS, and 1 M DDT stock solutions. The bone powder was incubated in extraction buffer over night (16–18 h) at 37°C with gentle horizontal agitation (200 rpm) in a heated water bath, followed by incubation at 56°C for 3 h with gentle horizontal agitation (200 rpm). The extract was then cooled down at room temperature for 30 min.

DNA Concentration

To concentrate the DNA, the extract was centrifuged at $2000 \times g$ for 5 min. The supernatant was carefully transferred to 2 mL reaction tubes in 1.75 mL aliquots. The 2 mL tubes were then centrifuged at $13{,}000 \times g$ for 5 min. For each 2 mL reaction tube, one YM-100 Microcon filter device (Millipore Corporation, Billerica, MA) was prepared. Five hundred microliters of the supernatant from each 2 mL tube was transferred to the prepared filter device followed by centrifugation at $3000 \times g$ for 10 min (filtration

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step) and then at $3000 \times g$ for 5 min (retention step). This was repeated until all supernatant was filtered. The retentates contain the concentrated DNA and were pooled from all filter devices.

DNA Cleaning and Elution

Five vol. of PB-Buffer from the Qiaquick PCR Purification Kit (Qiagen, Hilden, Germany) was added to the pooled concentrate and mixed well. Seven hundred and fifty microliters of the mixture was then transferred to a Qiaquick column from the Qiaquick PCR Purification Kit and centrifuged at $12{,}800 \times g$ for 1 min, discarding the flow through. This was repeated using the same column until the whole mixture had been passed through that column. Then, 750 μL PE-Buffer from the Qiaquick PCR Purification Kit was pipetted into the column and centrifuged at $12{,}800 \times g$ for 1 min, discarding the flow through. Another identical centrifugation step was performed to remove the residual amounts of PE-Buffer from the column. Subsequently, 30 μL EB-Buffer from the Qiaquick PCR Purification Kit was transferred to the center of the column membrane, incubated for 1 min at room temperature and centrifuged at $12{,}800 \times g$ for 1 min. This step was repeated with another 30 μL EB-Buffer resulting in an overall eluate of about 55 μL.

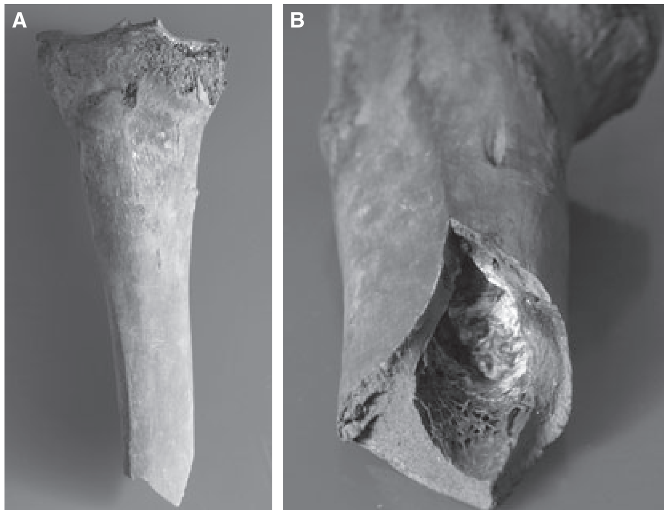

FIG. 1—*Fragment of the upper two-thirds of the left tibia. A) Total view of the recovered tibia bone fragment; B) Close up of the site of bone fracture shows complete loss of soft tissue and no adipocere formation.*

DNA Quantification

DNA quantification and assessment of possible PCR inhibitors was carried out by quantitative PCR using the Quantifiler Human DNA Quantification Kit (Applied Biosystems, Foster City, CA) with an ABI Prism 7000 system (Applied Biosystems) according to manufacturer's prescription. The successful application of this quantification method on aged bone samples has been described elsewhere (6). Only samples that were of sufficient quantity and showed no significant PCR inhibition were used in subsequent STR PCR.

STR Profiling

STR profiles were generated using the Mini-STR-Kits MPX-SP1 and MPX-SP2 (Serac, Bad Homburg, Germany) and the AmpFℓSTR Identifiler PCR Amplification Kit (Applied Biosystems), according

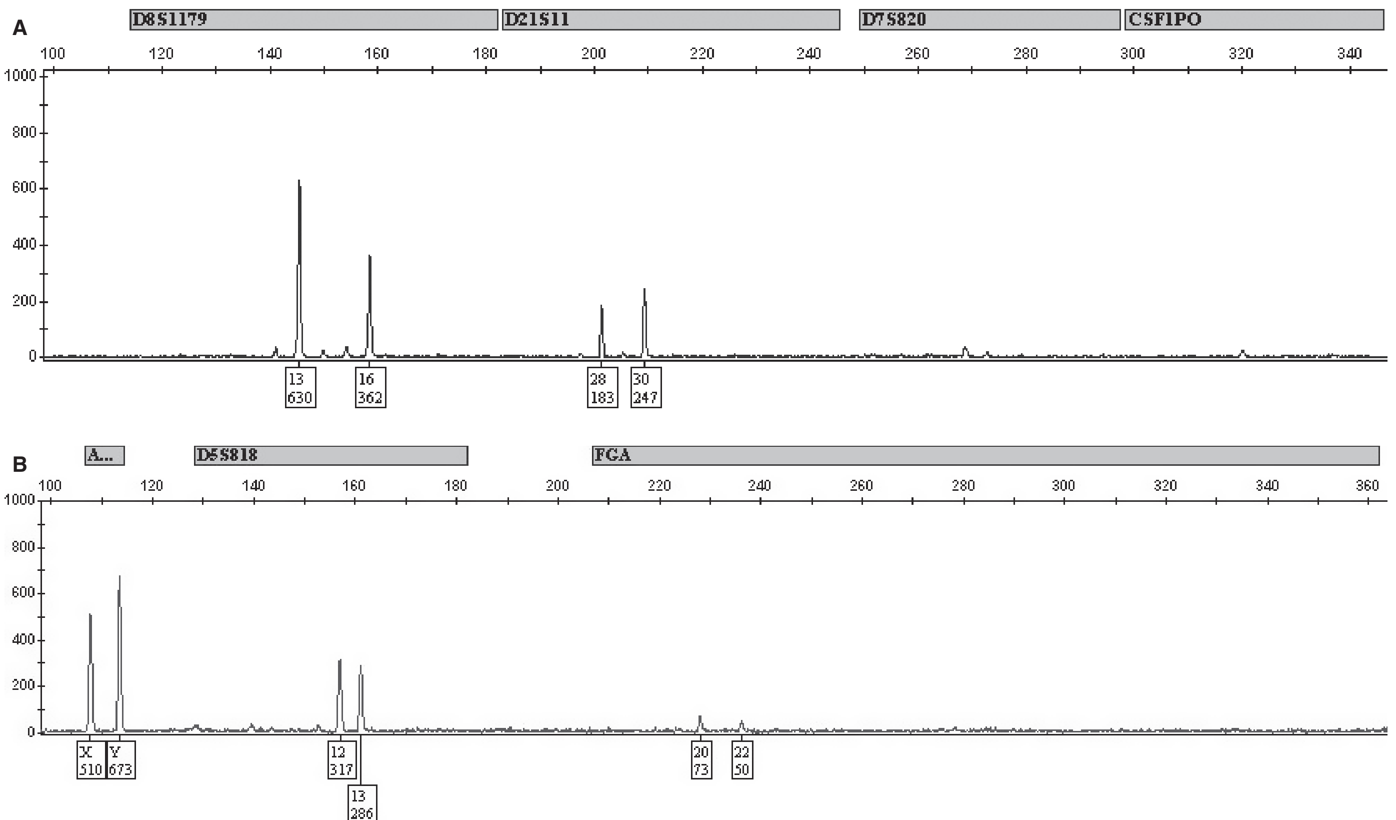

FIG. 2—*Exemplary electropherograms of several STR loci from the Identifiler kit. A) Signal loss in the STR systems D7S820 and CSF1PO; B) analyzable peaks in the STR systems Amelogenin, D5S818, and FGA, indicating a male profile. A…, Amelogenin.*

TABLE 1—*Amplification results for two STR kits.*

Kit	System	
	Successful (Alleles)	Unsuccessful
MPX-SP1, MPX-SP2	AM (X, Y) VWA (16) TH01 (9.3) D3S1358 (15, 16) D8S1179 (13, 16) SE33 (20, 20.2) D21S11 (28, 30) FGA (20, 22) D18S51 (17)	
AmpFℓSTR Identifiler	AM (X, Y) D21S11 (28, 30) D3S1358 (15, 16) TH01 (9.3) D13S317 (10, 11) D16S539 (11) D19S433 (15, 16) VWA (16) TPOX (8) D8S1179 (13, 16) D5S818 (12, 13) FGA (20, 22)	D7S820 CSF1PO D2S1338 D18S51

to manufacturer's prescription. 0.5 ng of DNA was used for each kit. Capillary electrophoresis was carried out using a 310 Genetic Analyzer System (Applied Biosystems). Data collection was conducted using the ABI 310 Genetic Analyzer Data Collection v.3.0.0 software (Applied Biosystems), and data analysis was performed using the Gene Mapper ID v.3.2.1 software (Applied Biosystems). For both kits, size calling was carried out implementing the local Southern method, and the peak amplification threshold was set to 50 rfu. Injection time was 5 sec with a sample injection volume of 5 μL and a run voltage of 15 kV.

Results

Quantification of the eluted DNA resulted in a total yield of 3 ng of DNA with no detectable PCR inhibitors. This yield was sufficient to provide the STR PCRs with adequate DNA template. A complete profile comprising the STR systems AM, VWA, TH01, D3S1358, D8S1179, SE33, D21S11, FGA, and D18S51 was produced using the MPX-SP1 and MPX-SP2 kits. This profile is compatible to the German "DNA Analysedatei" (DAD) which is a DNA analysis data file system established in 1998 at the German Federal Office of Criminal Investigation allowing for the systematic collection and comparison of DNA profiles from crime scenes and criminal offenders (http://www.bka.de/profil/faq/dna01.html, accessed November 9, 2009) (7). The amplification employing the AmpFℓSTR Identifiler Kit which encompasses the STR systems Amelogenin, D21S11, D3S1358, TH01, D13S317, D16S539, D19S433, VWA, TPOX, D8S1179, D5S818, FGA, D7S820, CSF1PO, D2S1338, and D18S51 resulted in a partial profile with 12 successfully amplified loci and a signal loss at four loci (D18S51, D7S820, CSF1PO, and D2S1338, see Fig. 2). The amplification results for all STR kits and systems are listed in Table 1. To rule out contamination as the source for the STR profiles derived from the bone, they were compared to the STR profiles of all laboratory personnel and no match was found.

Discussion

In this work, we established a DNA extraction protocol which yielded DNA from a fresh water-exposed bone fragment of 67 years that was of sufficient quantity and quality to generate a full DAD STR profile. In our hands, previously presented methods (2,4) did not produce an analyzable STR profile because of massive signal loss or lack of DNA quality.

Our protocol is composed of parts of our established protocols as well as previously published methods: we combined thorough surface decontamination (rinsing and abrasion), careful low-heat drilling and cryogrinding of drill strands (see "Preparation of bone powder" in Materials and Methods) with mild conditions for lysis incubation (see "DNA extraction" in Materials and Methods) as described by Rohland et al. (2) and Microcon filter device-based DNA concentration (see "DNA concentration" in Materials and Methods) and silica-column-based DNA washing (see "DNA cleaning and elution" in Materials and Methods) as described by Yang et al. (4).

The difficulty was to weigh and balance all parts against another and between the requirements of high yield on the one hand and low "stress," i.e., heat, chemical strain and shearing, for the probably highly degraded, susceptible DNA on the other hand, as procedures which increase yield are generally associated with higher stress and *vice versa* (8).

We therefore chose relatively mild extraction and incubation conditions that allowed for just enough yield without damaging the DNA more than necessary. The subsequent and somewhat cumbersome concentration step proved to be essential to optimize elution from the silica column although retention is never complete, and minute amounts of DNA are lost in this step. When the concentration step was provisionally left out, elution from silica columns would produce far too low DNA yields for subsequent analysis. The utilization of Qiaquick columns facilitated washing and elution of the DNA without inflicting additional damage that would preclude the following analytical procedure and just yet provided sufficient DNA yield for subsequent STR profiling.

Conclusion

Our compound method does not produce optimal yield nor optimal DNA quality but presents an optimized compromise which, in contrast to other methods, allows for the recovery of STR profilable DNA from fresh water-exposed aged bone specimens. The resulting full STR DNA profile generated from an aged, fresh water exposed bone using the method presented herein will very probably be suitable to identify the deceased person.

Conflict of interest: The authors have no relevant conflicts of interest to declare.

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