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A DNA extraction method of small quantities of bone for high-quality genotyping

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ABSTRACT

DNA genotyping techniques have been used successfully in forensic science for almost three decades and represent the gold standard for individual identification. However, efficient protocols for obtaining DNA from exhumed bones suitable for genotyping are still scarce and most of them require a considerable amount of starting material, are time consuming and are inefficient for reducing inhibitor's effects. We sought to develop an optimised protocol for extracting DNA from bone samples obtained from exhumations. We tested two approaches for preparing bone samples: (a) fine powder and (b) thin slices of bone. The best ratio of bone amount to DNA yields was assessed by a titration experiment using bone powder ranging from 50 to 1000 mg. We obtained optimal DNA yields (27 pg mg^{-1} on average) when 150–200 mg of starting material were processed using a one-step demineralisation method. Better-quality profiles (determined by the number of genotyped loci) were obtained when DNA was extracted from bone slices compared to extraction from bone powder. From bone slices 83.9% and from bone powder 46.7% of the samples provided genotypes for 11 or more loci. Since bone preparation procedures were carried out at room temperature, the method developed in the present study might be an attractive alternative to the standard freeze-mill approach, being faster and more cost-efficient.

DNA-based genotyping has been used successfully for 27 years and is the gold standard for assisting in personal identification [1]. A remarkable variety of procedures have been developed for DNA extraction from diverse types of human tissues or evidentiary materials [2]. However, efficient protocols for obtaining DNA from exhumed-bone samples suitable for genotyping have not attained the state of art yet, since most of them require considerable amounts of starting bone, increasing the need of disposable material, reagents and time [3–5]; others are unable to circumvent the effects of co-extracting inhibitors that reduces amplification efficiency [6,7].

In clear contrast to DNA extraction and purification procedures from soft tissue, which typically have high DNA yields ranging from 50 to 500 ng mg^{-1} of muscle or from 20 to 40 μg ml^{-1} of blood [8], bone DNA extraction procedures can be considered challenging and most protocols are less efficient than those for fresh tissue. This is in part due to the fact that DNA, after post-mortem cellular decay, is adsorbed onto the mineral matrix structuring complex aggregates; it is exposed to a wide variety of environmental challenges and it is highly prone to degradation [9].

Given that bones are the only potential source of genetic material in many scenarios, robust protocols for DNA extraction are required. Accordingly, a large number of procedures describing DNA extraction from ancient to freshly obtained human bones for nuclear or mitochondrial DNA analysis have been published [5,7,10–17]. Variations in DNA yields from bone fragments have been observed, which may be attributed to heterogeneity between bones and inhumation conditions. Therefore it is not possible to predict DNA yield from bone macroscopic appearance [3,11,17,18]. Features of an ideal DNA extraction protocol from bone must: (a) include reduced amounts of starting material, (b) attain DNA yields suitable for obtaining reliable genetic profiles, (c) be cost-effective and (d) be rapid. At present, a number of highly effective protocols exist, but there is no unique standard method preferred for DNA extraction from exhumed bones so new methods are constantly being developed [9,19].

Aiming to achieve these goals and offer a new methodological alternative, we tested two techniques for obtaining bone samples suitable for efficient DNA extraction starting from small quantities of tissue. We developed an optimised protocol that uses bone

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slices and produces better genotype profiles compared to bone powder evaluated by an 'expert software'. The developed protocol proved to be optimal when the amounts of bone slices ranged from 150 to 200 mg.

1. Materials and methods

1.1. Sample handling

The bone samples were treated under conditions designed to minimise contamination. A variable-speed dental milling drill (Saeshin Precision Co., Strong 207S, Daegu, Korea) was used to scrape the bones for eliminating superficial contaminants using a thick milling drill. Between sample preparations, the drill was treated with a 10% bleach solution for 30 min, washed with tap water, rinsed three times in distilled water, rinsed in 100% ethanol and air-dried.

All steps, including bone cutting, surface removal, powdering and DNA extraction were carried out in a hood under negative pressure. Polymerase chain reaction (PCR) preparation and post-PCR work were carried out in separate rooms. Negative and positive controls were included throughout the entire process. The negative control consisted of reagent blanks and the positive control of a bone sample previously extracted by means of Loreille and colleague's protocol and successfully analysed [7]. All liquid handling was performed using disposable sterile filtered tips and sterile tubes (Axygen, Union City, CA, USA). All operators wore latex gloves and sterile disposable surgery clothing including a cap, chinstrap and gown. To ensure that no contamination with external genetic material occurred, all genetic profiles were compared to the profiles of our laboratory personel using GeneMapper ID-X software version 1.0 (Life Technologies, Applied Biosystems, Foster City, CA, USA). Different operators carried out the extraction and PCR amplification steps. To minimise cross-contamination, the number of DNA extractions performed simultaneously was limited to two samples and a negative and a positive control.

1.2. Exhumed-bone samples

In all cases, human bone samples were analysed as part of the judicial requirements. A total of 34 bones (three tibias, two vertebral 120 discs, one radial bone, one metacarpus and 27 femurs) with different histories of conservation and different burial periods prior to analysis (ranging from 0.2 to 64 years) were used (Table 1). All samples were stored in paper envelopes at room temperature.

1.3. DNA extraction

To determine the optimum bone mass for efficient DNA yield, a titration experiment was carried out. DNA was extracted from bone powder derived from the compact cortical diaphysis of a femur obtained from a corpse after 36 years of interment. Drilling was carried out using a thin milling drill at low speed, between 150 and 200 revolutions per minute to reduce heating of the bone. The powder sample was placed into sterile microfuge tubes and maintained at room temperature until DNA extraction was performed. Samples containing 50, 100, 150, 200, 500 and 1000 mg of bone powder were demineralised and deproteinised by overnight incubation in 0.5 M ethylenediamine tetra-acetic acid bi-sodium salt (EDTA-Na_2) pH = 8 (Promega Corp., Madison, WI, USA) in volumes of: 0.75, 1.5, 2.25, 3, 7.5 and 15.0 ml, respectively, and 100 μl of 20 mg ml^{-1} Proteinase K (Promega Corp., Madison, WI, USA) was added and the samples were incubated at 56 °C with agitation at 750 rpm in a Vort-Temp Mod. 1550 (Labnet International Inc., Edison, NJ, USA). After overnight incubation the samples were boiled for 10 min in a water bath and then two organic extractions were performed for each sample for DNA purification: one with Tris–HCl/saturated phenol:chloroform:iso-amyl alcohol (25:24:1) (Life Technologies, Foster City, CA, USA) and the other with water-saturated chloroform: iso-amyl alcohol (24:1) (Life Technologies, Foster City, CA, USA). The 500 and 1000 mg samples were concentrated to 500 μl in an AMICON-30 device (Millipore, Billerica, MA, USA). The concentrated samples and those corresponding to 50, 100, 150 and 200 mg samples were processed in Microcon YM-30 columns (Millipore, Billerica, Massachusetts, USA) for DNA purification and concentration. Three washes with 500 μl of distilled water were performed before elution. The final elution was performed in 30 μl according to the manufacturer's protocol. DNA quantification was performed to determine which starting amount would be optimal to perform a comparison of bone preparation procedures prior to DNA extraction.

1.4. Bone preparation procedures: preparing bone powder and bone slices

All bones analysed were submitted to two procedures in parallel for obtaining samples in order to extract DNA from them in a comparative way. For each bone the net amount was similar for both procedures and ranged from 150 to 200 mg. This quantity was determined in the titration experiment described previously.

One procedure for obtaining samples was drilling, as described in Section 1.3. As an alternative, small bone fragments were used as DNA sources. Once the scraping process was concluded as described above, in Section 1.1, small wedge-shaped bone fragments (approximately 3 mm long and 1 mm wide) were cut using either a variable-speed dental drill fitted with a sterile cutting disc at low speed or a handheld saw. The cutting disc or saw blade was discarded after cutting each bone specimen. Either bone powder or slices were extracted following the method described in Section 1.3 maintaining the ratio of bone weight (expressed in mg) to EDTA volume (expressed in ml). The amount of Proteinase K was not varied since the published protocols use around 2 mg of Proteinase K for 0.5 mg to 2 g of bone powder and 2 ml or even 10 ml of lysis buffer [7,11,15,19,20]

1.5. DNA quantification

All DNA extracts were quantified by real-time PCR in a Rotor Gene 6000 (Corbett Life Science, Sydney, Australia) using a Plexor HY® kit (Promega Corp., Madison, WI, USA) according to the manufacturer's instructions. Negative and positive controls were included. Election of the quantification kit was in part based on the fact that it permits detecting the presence of inhibitors in the sample.

1.6. Genotyping

A set of 15 autosomal short tandem repeats (STRs) and the gender marker Amelogenin, included in the commercial kit PowerPlex16HS® (Promega Corp., Madison, WI, USA) was used for genotyping. Samples containing between 100 pg and 1 ng of extracted DNA were used in PCR reactions of 10 μl final volume and 32 cycles in a GeneAmp® PCR System 9700 (Life Technologies, Applied Biosystems, Foster City, CA, USA). Negative and positive controls were included in the PCR reaction. One microlitre of PCR product was separated and visualised in a 3100-Avant Genetic Analyzer and analysed with GeneMapper IDX version 1.0 software (Life Technologies, Applied Biosystems, Foster City, CA, USA). This software allows qualifying the electropherograms. The quality of

Table 1
Sample characteristics, quantification results and STR amplification score.

Bone number	Date of death	Type of bone	Bone slices method			Bone powder method		
			(pg/μl)[a]	(pg mg^{-1} of bone)[b]	N° amplified nuclear markers	(pg/mμl)[a]	(pg mg^{-1} of bone)[b]	N° amplified nuclear markers
1	1976	Femur	256.9	51,4	16	147.8	29.6	12
2	1970	Femur	46.1	8.6	16	43.9	9.4	6
3	Not available	Femur	220.0	47.1	14	9.7	2.1	1
4	Not available	Femur	42.0	8.4	11	67.0	13.4	7
5	2004	Femur	460.0	86.3	16	60.0	10.0	3
6	2004	Femur	717.0	126.5	16	460.0	69.0	13
7	1948	Femur	33.8	6.8	8	32.6	6.5	7
8	2002	Femur	123.5	23.2	16	186.0	27.9	5
9	1971	Femur	782.8	146.8	16	611.0	183.3	15
10	2000	Femur	133.9	22.3	6	79.0	13.2	15
11	2005	Radial bone	351.9	66.0	14	221.2	39.0	15
12	2009	Femur	102.9	19.3	3	182.4	36.5	16
13	2006	Femur	80.0	16.0	12	83.0	13.8	8
14	1988	Femur	80.0	18.5	16	119.5	32.6	3
15	1988	Femur	435.9	76.9	14	95.4	23.9	4
16	1988	Femur	221.2	41.5	16	174.0	32.6	N/A[c]
17	2010	Vertebra	240.4	41.2	16	383.0	65.7	2
18	2010	Femur	238.4	59.6	14	37.7	9.4	15
19	2010	Femur	231.6	57.9	16	102.5	20.5	N/A[c]
20	Not available	Femur	90.0	16.9	16	20.0	3.8	15
21	2007	Femur	14.5	2.5	8	16.2	2.8	4
22	2012	Femur	143.2	21.5	16	<0.016	<0.003	–[d]
23	Not available	Femur	67.0	15.5	16	42.0	9.7	8
24	Not available	Tibia	19.8	3.3	15	11.4	2.3	15
25	Not available	Femur	61.7	10.3	16	80.4	12.1	13
26	Not available	Tibia	21.5	3.4	10	249.1	37.4	14
27	Not available	Tibia	22.1	5.1	N/A[c]	67.4	14.4	16
28	Not available	Femur	170.8	32.0	13	147.2	27.6	4
29	Not available	Femur	836.1	139.4	N/A[c]	63.1	11.1	16
30	2012	Femur	30.0	5.1	N/A[c]	80.0	13.7	14
31	2010	Metacarpus	160.0	32.0	16	70.0	11.1	9
32	Not available	Femur	20.0	4.0	15	<0.016	<0.003	–[d]
33	Not available	Vertebra	30.3	5.2	16	46.9	8.0	5
34	Not available	Femur	40.0	6.9	16	60.0	8.2	4

Table 1 Details of the analyzed bones.
[a] DNA concentration.
[b] DNA to bone mass.
[c] N/A, no–amplification; 0.016 ng/ul is the minor point of quantification standard curve.
[d] –, these samples were not amplified due they did not show DNA suitable for analysis.

each genotyped locus is displayed as colour coded flags: a green flag indicates that the sample passed the pre-defined range for the sizing quality >0.75; a red flag indicates that the sample had a low quality range <0.25 and, finally, a yellow flag appears when the value between both ranges was attained. In addition, other electropherogram features such as the peak height, peak height ratio or when more than two alleles appear are qualified by the software in yellow colour. The cut-off, established by our internal laboratory validation, was defined at 30 relative fluorescent units (RFUs) [21,22]. The autosomal markers were classified as NC (not conclusive) when locus drop-in or drop-out was observed. In cases where allele height did not reach the cut-off value, the PCR product was reanalysed either by re-injecting for an increased time or by sample concentration using columns (MiniElute®, Qiagen, Valencia, CA, USA) [23]. In all cases, the genotypes were consistent with the biological kinship determined by comparison with reference samples.

2. Results

2.1. Establishing the optimal starting bone mass

To test the relationship between DNA recovery and starting bone quantity, different amounts of bone powder were purified from a single bone, quantified and genotyped. Samples containing 50, 100, 150 and 200 mg of bone powder were completely dissolved in the EDTA-Proteinase K incubation step. The aqueous phase obtained after the first organic extraction from the 500 and 1000 mg samples was opaque; the solution turned clear after the second extraction of the 500 mg sample but not of the 1000 mg sample. When we analysed DNA yields (pg μl^{-1}) versus bone starting amount (mg), we observed that at the lower starting quantities of bone, the DNA yield increased proportionally to the starting bone quantity (Fig. 1). By quantitative PCR, DNA recovered from the 1000 mg starting material showed no amplification of the internal control indicating the presence of

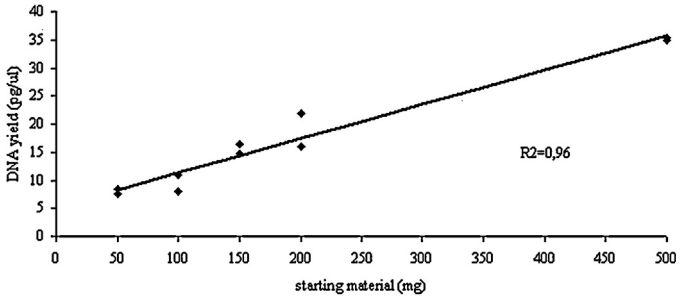

Fig. 1. DNA yields from different quantities of bone powder. DNA yields from one bone extracted starting from different quantities of bone powder. The DNA recovered from the 1000 mg starting material sample showed inhibitors and thus could not be quantified, and therefore was not plotted.

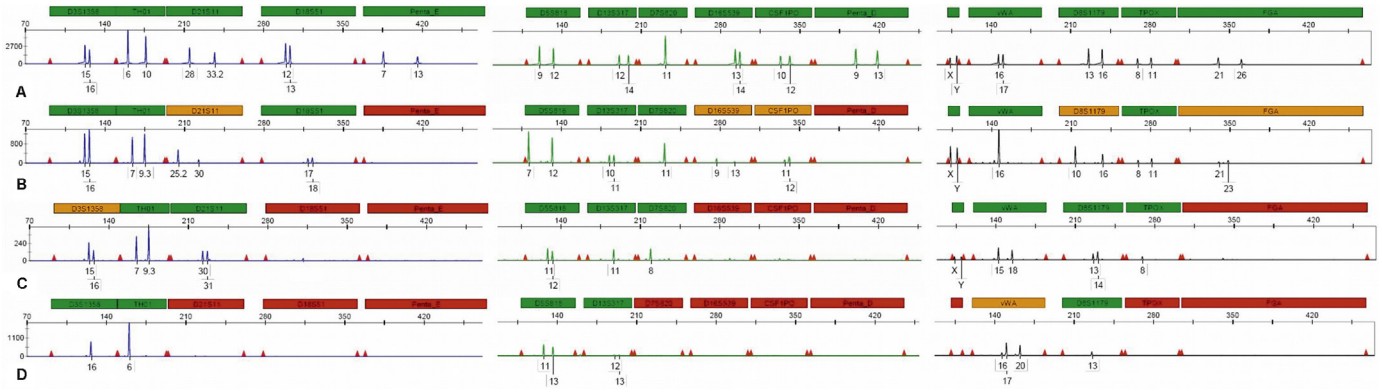

Fig. 2. Profiles quality evaluation. (A) Full electropherogram (Bone#23 slice method). (B) Acceptable electropherogram (Bone#11 slice method). (C) Quite acceptable electropherogram (Bone#26 slice method). (D) Electropherogram not suitable for analysis (Bone#15 Powder method). The expert software allows qualifying the electropherograms with different colours (see Section 2 for details).

inhibitors. We determined that 150–200 mg of starting material was the optimal starting mass of bone based on adequate DNA yields for achieving a reliable genetic profile and a handily downstream process. It is worthy to note that the average standard deviation for the DNA yield from samples of 150–200 mg of bone was only ± 0.03 ng μl^{-1} underscoring the reproducibility between extractions (data not shown). Based on the results obtained for bone powder from the titration experiment, we compared DNA recovery efficiency and electropherogram quality of 34 samples extracted from bone powder and slices using 150–200 mg starting material.

2.2. Comparison of bone powder and bone slice methods

Quantification results are summarised in Table 1. The average DNA yield was 191.9 ± 219.4 pg μl^{-1} (or expressed as DNA to bone mass 36 ± 39.3 pg mg^{-1}) for bone powder and 126.5 ± 135.1 pg μl^{-1} (or 25 ± 33.7 pg mg^{-1}) for bone slices. No inhibition was detected in the 68 studied DNA extracts. The standard deviation observed concerning DNA yields in bone preparation approaches reflects the intrinsic variation between bones; by contrast, no difference was obtained between replicates along the titration experiment (Fig. 1), where a single bone was used for establishing the optimal bone mass to be used.

In order to qualify the electropherograms, DNA profiles were classified into three categories according to the number of genotyped loci, similar to those proposed by Amory et al. and Holland et al.: full profiles (all 16 loci), acceptable profiles (between 11 and 16 loci); quite acceptable (between eight and 10 loci) and finally, profiles not suitable for analysis (less than eight loci) (Fig. 2) [16,24]. Of the profiles obtained from DNA extracted from bone slices, 54.8% were full, 29% were acceptable, 9.7% were quite acceptable, and only 6.5% were not suitable. By contrast, when profiles were obtained from DNA extracted from bone powder, 10% were full, 36.7% were acceptable, 10% were quite acceptable and 43.3% were not suitable (Table 1). In some cases, the obtained DNA yields were similar for both techniques, but there was a difference in the profile quality. Furthermore, we observed an increase in stochastic effects, such as allele/locus drop-in or drop-out, when DNA was obtained from bone powder (Fig. 3). Five DNA extracts showed no genotyping results, (they were amplified three times in different assays, with addition of extra Taq polymerase). Nevertheless, two separate quantification assays denoted similar values of DNA concentration in those samples. This could be explained by the characteristics of the Plexor HY® quantification kit that the inhibitor detecting DNA target amplifies a single locus of 99 base pairs long, shorter than the smallest STR included in the kit, suggesting a considerable degradation of the samples.

3. Discussion

The aim of this work was to optimise the procedures for extraction of DNA from reduced amounts of exhumed bones. At present, in many protocols, bone samples are often prepared using freeze-mill powdering processes, but this technique is limited by the requirement for liquid nitrogen [4,15]. One option for obtaining bone samples is to produce a fine powder by drilling at low speed or cooling to reduce excess heating [25]. As an alternative approach, we developed a method using thin slices of bone. These two strategies were compared in the present study by evaluating DNA yields and STR profile qualities.

We first evaluated DNA yield from different starting quantities of bone powder to define the smallest amount of starting material that yields sufficient DNA to allow obtaining a reliable and reproducible genotype. Based on DNA yields and ease of performing downstream processing steps, we determined that 150–200 mg of bone was the optimal starting mass. We next tested whether DNA extraction from bone powder or bone slices produced higher quality genotype profiles. At the time when this

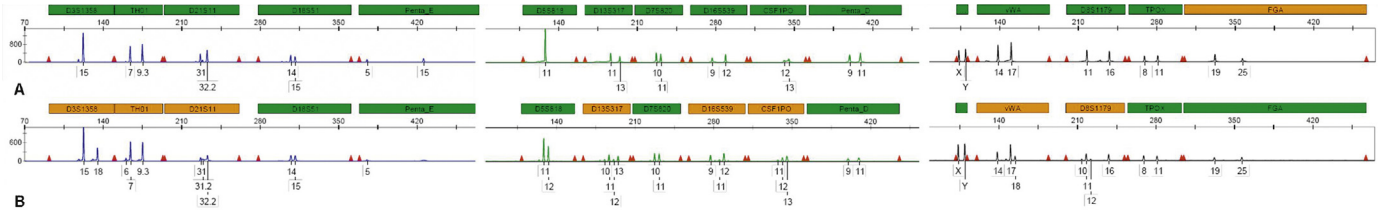

Fig. 3. Quality profile comparison: slice vs. bone powder method. Electropherogram obtained from the same bone sample (Bone#2). (A) Extracted by slice method. (B) Extracted by powder method. Note the increase of drop-in and drop-out in the electropherogram in b. The expert software allows qualifying the electropherogram with different colours (see Section 2).

research was started PowerPlex16HS was the most robust megaplex available. Now a days alternative kits, almost unaffected by inhibitors, are available such as: PowerPlex®21, PowerPlex®17ESI, PowerPlex®Fusion, AmpFiSTR® NGM™, AmpFliSTR® IdentifilerPlus® and AmpFliSTR®GlobalFiler, or approaches such as binary polymorphisms (single nucleotide polymorphisms (SNPs) or In/dels) could be considered [4].

Structurally, the major proportion of bone is matrix, consisting of both an inorganic (principally hydroxyl apatite) and an organic fraction, which is composed chiefly of type I collagen and extracellular matrix proteins, such as glycosaminoglycans and osteocalcin [17,18]. Demineralisation of bone through incubation with EDTA or EDTA coupled with Proteinase K digestion maximises the efficiency of DNA extraction [26]. Moreover, the use of EDTA reduces DNAse activity by chelating bivalent cations such as Mg^{2+} or Ca^{2+} [7]. In a one-step demineralisation protocol, potential co-purification of soil compounds and groundwater incorporated into the sample material could result in the inhibition or failure of the amplification reaction [24,27]. The inhibition of DNA amplification from bone exposed to soil could be explained by the presence of humic acid, tannins, iron, cobalt and other materials that can be incorporated into the bone after long periods of exposure to soil [28]. Moreover, collagen type I and Maillard products [29] are other inhibitory factors of successful PCR amplifications [10]. The aggregate of Dextran Blue, Biz/Na_2CO_3 and a variety of silica-based DNA binding substrates, filtration methods or microwave treatment are somewhat effective in removing PCR inhibitors resulting, sometimes, in excessive loss of DNA or are time-consuming [3,10,24,26,30–32]. In the present work, a simple additional step of sample boiling for 10 min before organic extractions was included to denature inhibitory compounds. We decided to add this step after the success in removing inhibitory substances during the DNA extraction of a femur from a formolised body (data not shown). We only detected evidence of inhibitor substances when 1000 mg of starting material was processed. The substances that act as inhibitors might be present in all of the DNA extracted fractions, but because all of the extractions were concentrated to a volume of 30 μl in the final step of purification, the concentration of inhibitors could be increased in samples containing greater quantities of starting material, at least for inhibitors that are larger than the cut-off size of the columns. As the inhibitor substances interfered in the PCR amplification, optimal DNA recovery is important not only because a sufficient quantity of DNA is necessary to obtain a reliably profile but also because too much DNA input could increase the concentration of inhibitors, reducing the number of amplified loci [16].

Schwarz et al. detected DNA in pellet and supernatant fractions following demineralisation of 150 mg bone powder treated with 0.5 M EDTA in a volume of 1.5 ml for 3 days [14]. Salamon et al., in their work on fossilised bones, reported that the major benefit of using the pellet instead of solubilised bone is that longer and better preserved DNA molecules were found in the aggregates [11]. Our method allows the collection of organic and inorganic DNA fractions in a final volume of 30 μl. The Proteinase K quantity and the ratio of bone mass to EDTA volume used in our assay allow an adequate balance between the dissolution of the inorganic bone matrix, cellular degradation, potential inhibitor concentration, and final concentration of calcium ions. Demineralisation is reported to significantly increase the proportion of full profiles but increasing the decalcification temperature or the EDTA exposure time to 48–120 h can induce DNA damage or degradation despite the time of analysis [13,15,33]. Jakubowska et al. suggested that demineralisation could be used for satisfactory analysis of both exhumed and fresh bones [33]. According to Kitayama et al., the use of ground powder samples could contribute to airborne contamination when multiple bone samples are processed simultaneously; hence, highly stringent contamination control measures must be included [34]. We found that the average DNA recovery was 1.5 times higher when bone powder was used as the starting material, although the quality profiles were lower. Theoretically, the finest powder might produce higher yields because there is more contact surface between the bone matrix fragments and the EDTA solution, but the DNA is more exposed to degradative agents, increasing the stochastic effects during PCR amplification [6,35]. This may explain the lack of correlation between yield and profile quality in some samples. Samples prepared from bone powder often showed low-quality profiles compared to those prepared from bone slices, despite equivalent or greater yields, possibly reflecting a correlation between DNA degradation and complete extraction.

In analysis of starting bone quantity, Amory et al. obtained an equivalent or greater yield from 0.5 g compared to 2 g of bone powder [16]. The use of smaller bone quantity is an advantage for many skeletal remains, when largely intact anatomical features are required for anthropological analyses or when duplicate or multiple extractions must be performed. Loreille et al., obtained on average 228 times more DNA when starting from 0.2 g than when using 1–2 g of bone powder using total demineralisation [7]. Moreover, Holland et al. used 25–50 mg or, alternatively, 125–150 mg of starting material, although from recent victims, for their identification in the World Trade Center attacks with a high percentage of successful results [24]. In addition, more frequent sample manipulation and large reagent volumes are likely to increase the risk of contamination. In our study, we determined that 150–200 mg of bone is the minimal quantity that leads to a good DNA recovery (average yield ~160 pg μl^{-1} or expressed as DNA to bone mass 27 pg mg^{-1}). A high standard deviation was observed, probably due – as we described in Sections 1 and 3 – to heterogeneity between the type of bone, its preservation and inhumation conditions. However, the obtained concentration in this assay is adequate for PCR-based genotyping and three or four PCR reactions per sample could be performed from only one extraction. Furthermore, the incorporation of a boiling step after the overnight incubation at 56 °C allows more efficient amplifications.

Regarding time and cost, the recent modified method proposed by Piglonica et al., performed on NucleoSpin DNA Trace Kit, uses 10 columns per bone and an incubation period of three days – two disadvantages since it is costly and time consuming [9]. Moreover, Karija et al., proposed an automatable method processing up to 16 samples in <30 min without regarding the 3 days of the demineralisation step and the equipment availability [19]. Most of the effective published methods using one or two steps of demineralisation are time consuming and involve incubations from 48 to almost 72 h, which is not feasible when emergency exists in obtaining results [4,9,11,13,14,31,34]. In this work, we propose an exhumation bone preparation method carried out at room temperature with overnight one-step demineralisation and incorporating an additional boiling step for denaturing inhibitors, either for quantification or genotyping. We obtained an optimal DNA yield from 150 to 200 mg of starting bone material (average yield ~160 pg μl^{-1} or expressed as DNA to bone mass 27 pg mg^{-1}) that allows obtaining a reliable genetic profile. In addition, better-quality profiles were obtained when DNA was extracted from bone slices than from bone powder. Success rate was evaluated according to the number of loci to be reliably typed for 11 or more loci. DNA samples prepared from bone slices met this criterion in 83.9% of the cases, instead of 46.7% in the case of samples prepared from bone powder.

4. Conclusions

The method proposed herein allows obtaining robust and reliable DNA profiles, minimises the chance of contamination and

reduces the time, materials and reagents required. The proposed protocol represents an alternative option for DNA extraction when only small exhumed-bone fragments are available.

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