Legal Medicine 15 (2013) 171–176

Contents lists available at SciVerse ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

The effect of an enzymatic bone processing method on short tandem repeat profiling of challenged bone specimens

Richard Li *, Stacey Klempner

Forensic Science Program, Department of Science, John Jay College of Criminal Justice, The City University of New York, New York, NY 10019, USA

ARTICLE INFO

Article history:
Received 18 August 2012
Received in revised form 21 December 2012
Accepted 26 December 2012
Available online 4 February 2013

Keywords:
Bone
Trypsin
Forensic
DNA
STR

ABSTRACT

Forensic analysis of DNA from bone can be important in investigating a variety of cases involving violent crimes and mass fatality cases. To remove the potential presence of co-mingled remains and to eliminate contaminants that interfere with forensic DNA analysis, the outer surface of the bone fragment must be cleaned. This study evaluated two methods for processing bone specimens prior to DNA isolation. Mechanical sanding and enzymatic trypsin methods were compared in this study. The effects of these methods on the yield of DNA isolated and the quality of DNA analysis were studied. It was revealed that comparable values of DNA yields between the two methods were observed. Additionally, to evaluate the capabilities of the cleaning effect of the bone processing methods, the presence of polymerase chain reaction inhibitors in the DNA extracts was monitored using the internal positive control. Similar C_t values of the internal positive control were observed as the DNA extracts of the trypsin method compared with that of the sanding method. The characterization of the effects of the trypsin treatment on the quality of DNA profiling was also carried out. To evaluate the integrity of the nuclear DNA isolated, the percentage of allele calls and the peak-height values of alleles of the short tandem repeat profiles were compared between the two methods. A paired-sample *t*-test revealed no significant difference between the two methods. Our data suggested that the trypsin method can be used as an alternative cleaning method to mechanical cleaning methods. This method can be used to process multiple samples simultaneously. This can be very important for achieving high-throughput DNA isolation through potential automation, which can be extremely valuable for situations such as the forensic DNA analysis of skeletal remains from mass fatality incidents.

© 2012 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Forensic analysis of DNA from bone can be important in investigating a variety of cases involving violent crimes and mass fatality cases [1–5]. To assure the accuracy of the forensic DNA analysis, bone samples must be appropriately processed prior to DNA isolation. Bone samples collected from crime scenes have potential contamination by presence of co-mingled remains and by physical contact of emergency dispatch personnel [6–8]. Additionally, buried bones usually contain polymerase chain reaction (PCR) inhibitors that interfere with forensic DNA analysis [9,10]. Thus, an outer surface layer (approximately 1–2 mm) of bone fragment should be removed. This is usually carried out using mechanical methods, such as sanding using sanding discs attached to a rotary tool [3,11,12] or sandpaper [13,14].

In our previous study, an enzymatic method using trypsin solution [15,16] was adapted to the sample cleaning method prior to DNA isolation from fresh bone samples [17,18]. Microscopic studies demonstrated that this trypsin method is effective for the removal of outer surface materials such as the mineralized bone connective tissue of fresh human bone samples. The yield of DNA isolated from trypsin-treated fresh bone samples was sufficient for subsequent short tandem repeat (STR) analysis. In this study, the application of the enzymatic trypsin method for DNA isolation was studied in samples that are more typically encountered in actual forensic cases. Additionally, the yield and the quality of DNA extracted from challenged bones were compared between the mechanical sanding and enzymatic trypsin method side-by-side.

2. Materials and methods

2.1. Sample preparation

Precautions were followed to eliminate possible DNA contaminants. Sampling was carried out in a sterilized laminar flow cabinet. Consumables were DNA free. Disposable laboratory coats, gloves, and masks were used throughout the procedure.

* Corresponding author. Tel.: +1 646 557 4886.
E-mail address: rli@jjay.cuny.edu (R. Li).

1344-6223/$ - see front matter © 2012 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.legalmed.2012.12.002

2.1.1. Sampling

In this study, challenged human bone specimens (non-probative) were used (Fig. 1). A total of 14 bone specimens (a similar sample size as in Loreille et al. [12]) including cranium, rib, and tibia from different individuals were selected. Aged bones including buried bones unearthed from archaeological sites were included. Bones ranging in age from approximately 50 to over 100 years post-mortem were selected for this study. Bones exposed to potential insults (such as the possibility of maceration using bleaching or boiling, or buried under high heat and humidity) were included.

A pair of bone fragments (approximately 1 g each) was dissected from each bone specimen. A pair of bone fragments was then processed using the sanding and trypsin method separately for pair-wise comparisons. A paired-sample *t*-test (two-tail) was conducted to compare the data from the sanding and trypsin methods in this study.

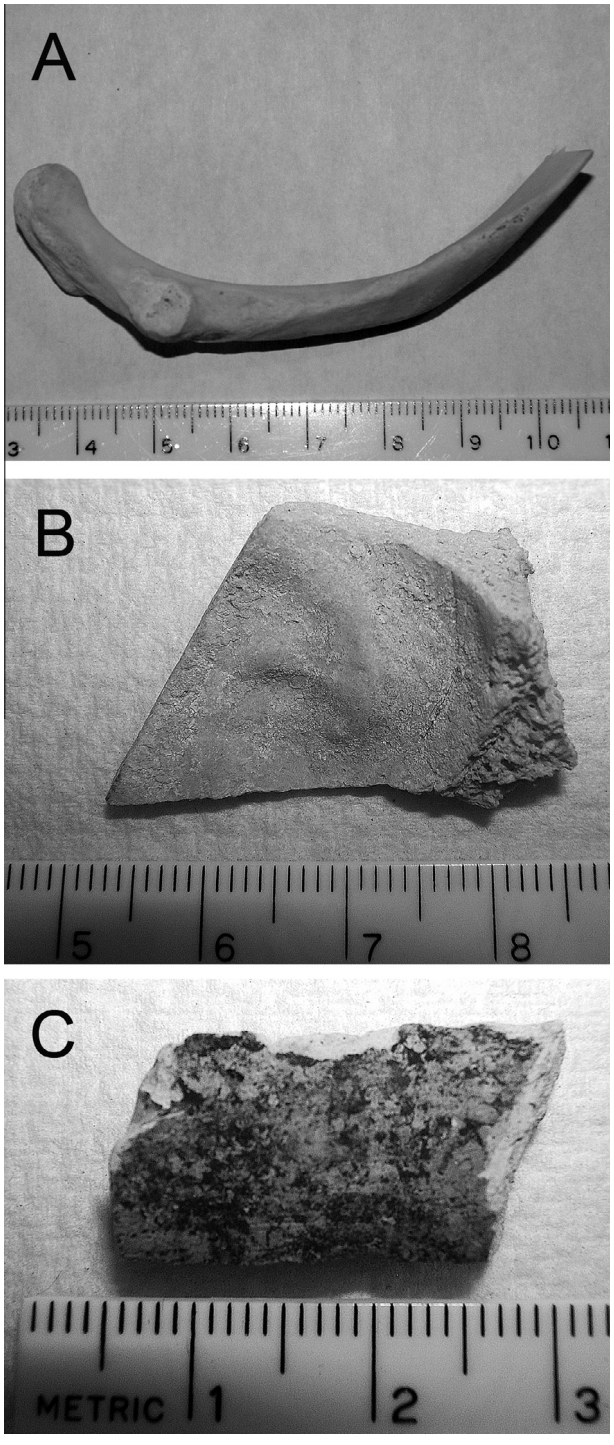

Fig. 1. Typical bone fragment specimens investigated in this study. (A) Rib, buried for approximately 50 years with possible maceration using bleach after unearthing, stored at room temperature; (B) parietal bone, autopsy specimen, possibly macerated using boiling, stored at room temperature for approximately 65 years; and (C) tibia (no DNA detected), buried for over 100 years under high heat and humidity, stored at room temperature.

2.1.2. Surface cleaning

The trypsin treatment was carried out as developed previously [17]. It was initiated by adding 5 ml of fresh trypsin (Fisher Scientific) solution (30 μg/μl, 10 mM Tris, pH 7.5) to the bone fragment and then was incubated at 55 °C with gentle agitation for 2 h. After incubation, the supernatant was removed. The sanding was carried out using a current sanding method [11] with single-use sanding discs attached to a rotary sanding tool (Dremel, Racine, WI). The outer surfaces were sanded thoroughly. The cleaned bone fragments using both methods were further processed by inversion for 30 s in distilled water, 0.5% sodium hypochloride, and 96% ethanol as described in Davoren et al. [11]. The bone fragments were then air dried.

2.1.3. Bone tissue disruption

The pulverized bone powder from each fragment was prepared using the freezer mill method [12] that utilizes a cryogenic impact grinder (SamplePrep 6770 Freezer Mill, SPEX, Metuchen, NJ). The procedure was programmed according to the manufacturer's protocols: 10 min pre-cooling followed by 2 cycles of grinding (2 min grinding at a rate of 20 impacts/s and 2 min cooling for each cycle).

2.2. DNA extraction

Demineralization of pulverized bone powder was carried out as described in Loreille et al. [12]. For each sample, 0.2 g of pulverized bone powder was decalcified by incubating in 3.2 ml of extraction buffer (0.5 M EDTA, 1% lauryl-sarcosinate) and 200 μl of 20 mg/ml proteinase K overnight at 56 °C with gentle agitation.

Table 1
Summary of pair-wise comparisons of DNA quantitation results.

Sample name	Sample type	Surface cleaning	DNA yield (ng DNA/g bone)	Number of STR allele detected
JJC12	Rib[a]	Sanding	79.5	2
		Trypsin	34.8	1
JJC34	Rib[a]	Sanding	28.71	4
		Trypsin	22.8	4
JJC56	Rib[a]	Sanding	9.09	17
		Trypsin	4.95	17
JJC78	Rib[a]	Sanding	20.64	17
		Trypsin	41.1	17
JJC910	Rib[a]	Sanding	13.17	14
		Trypsin	20.85	14
JJC1920	Rib[a]	Sanding	5541	7
		Trypsin	7341	8
JJC1516	Parietal bone[b]	Sanding	2.253	12
		Trypsin	14.61	16
JJC1718	Mandibular condyle bone[b]	Sanding	11.61	18
		Trypsin	10.5	18
JJC142	Temporal bone[b]	Sanding	3.69	13
		Trypsin	3.24	12

[a] Buried bones (buried for approximately 50 years) with possible maceration using bleach after unearthing were stored at room temperature.

[b] Autopsy specimens, possible maceration using boiling, were stored at room temperature for approximately 65 years.

Download English Version:

https://daneshyari.com/en/article/10254555

Download Persian Version:

https://daneshyari.com/article/10254555

Daneshyari.com