Egyptian Journal of Forensic Sciences (2015) 5, 30–35

HOSTED BY

Contents lists available at ScienceDirect

Egyptian Journal of Forensic Sciences

journal homepage: http://www.journals.elsevier.com/egyptian-journal-of-forensic-sciences

ORIGINAL ARTICLE

Evaluation of techniques for human bone decalcification and amplification using sixteen STR markers

Ajay Balayan, Abhilasha Kapoor, Garima Chaudhary, Anupuma Raina *

DNA Profiling Lab., Department of Forensic Medicine, All India Institute of Medical Sciences, New Delhi, India

Received 30 July 2013; revised 21 March 2014; accepted 5 May 2014
Available online 14 June 2014

KEYWORDS

Decalcification;
Sternum bone;
Short tandem repeats;
Multiplex PCR;
Genotyping

Abstract Efficient DNA extraction procedures, as well as accurate DNA amplification, are critical steps involved in the process of successful DNA analysis of skeletal samples. Unfortunately, at present there is no infallible method to recover DNA from highly degraded samples due to variations in DNA yield from larger bone fragments, which may be attributed to heterogeneity within bones. We evaluated two different protocols for bone decalcification in the DNA extraction procedure for bones. This study is important for analysis of challenging forensic samples.

1. Introduction

Since inception of the DNA fingerprinting technique by Jeffreys et al. in 1985 it has become a powerful tool in medico-legal cases. The development and validation of new technology for detection of DNA polymorphisms have been very rapid. Over the last twenty years DNA profiling has become an important method for forensic human identification, in particular by introducing the study of microsatellite regions – Short Tandem Repeat (STR) loci – in criminal cases as well as in civil cases.[1]

In cases like missing personal identification, mass disaster and ancient DNA investigation, bone and teeth are the most commonly available biological samples. Bone is a complex, highly organized and specialized connective tissue. The majority of DNA in the bone is located in the osteocytes; a microgram quantity of DNA could potentially be extracted from a gram of bone.[2,3]

We extract DNA using different decalcification protocols for the sternum bones, which are more than 20 years old. This method consists of separation of DNA from proteins and waste material by using a phenol–chloroform mixture. Moreover, the recovery of information from these degraded samples is enhanced by the use of STR (Short Tandem Repeats) typing by multiplex PCR.[4]

2. Materials and methods

The present study was conducted on ten sternum bone samples. All the samples were cleaned thoroughly using sandpaper

* Corresponding author. Tel.: +91 9868397147.
E-mail address: anupumaraina@gmail.com (A. Raina).
Peer review under responsibility of The International Association of Law and Forensic Sciences (IALFS).

http://dx.doi.org/10.1016/j.ejfs.2014.05.002

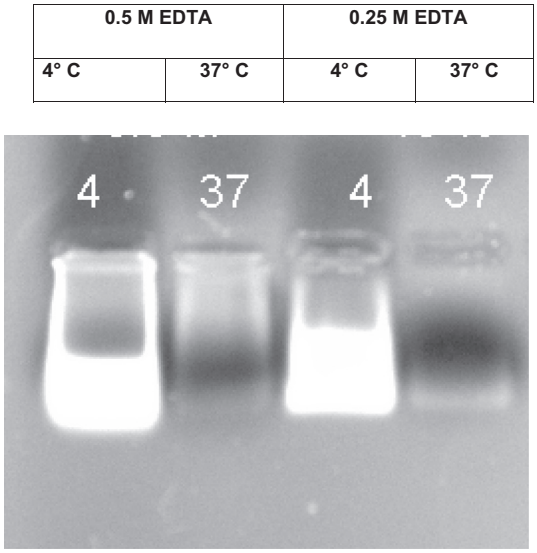

Figure 1 The agarose gel picture of extracted DNA from one sample.

to remove potential contamination. Bones were crushed to bone powder for further processing of the samples.

2.1. Decalcification

We used two different methods for decalcification of bone using two different concentrations of the ethylene diamine tetra-acetic acid (EDTA) buffer (0.5 M EDTA and 0.25 M EDTA) and two temperatures (37 °C and 4 °C) for decalcification of the bone powder. These samples were incubated at two different temperatures (37 °C and 4 °C) for 7 days with daily changes of EDTA buffer DNA extraction.

DNA extraction was performed using the organic extraction method proposed by Sambrook et al. After 7 days the tubes were centrifuged, the supernatant was discarded and the remaining decalcified pellet was extracted using the organic extraction method.[5]

Table 1 Genotype profile of sample analyzed.

Marker	0.5 M EDTA 37 °C	0.5 M EDTA 4 °C	0.25 M EDTA 37 °C	0.25 M EDTA 4 °C
D8S1179	12, 13	12, 13	*	*
D21S11	29, 31.2	29, 31.2	*	*
D7S820	8, 12	*	*	*
CSF1P0	12	12	*	*
D3S1358	15, 16	15, 16	*	*
THO1	6, 9.3	6, 9.3	6, 9.3	6, 9.3
D13S317	10	10	*	*
D16S539	9, 12	9, 12	*	*
D2S1338	23	23	*	*
D19S433	12, 15	12, 15	*	*
vWA	15, 17	17	*	*
TPOX	8, 11	8, 11	*	*
D18S51	15, 17	*	*	*
Amelogenin	X, X	X, X	X, X	X, X
D5S818	11	11	*	*
FGA	21, 23	21, 23	*	*

* No amplification at the particular locus.

2.2. DNA purification

DNA from dried bone powder samples was purified using a Nanosep centrifugal device. DNA was purified by employing centrifugation for 10 min at 10,000 rpm for 3 times to get a better yield.

2.3. Agarose gel electrophoresis

Extracted DNA from bone powder was allowed to run on 1% agarose gel with 1× TBE buffer to check the quality of the extracted DNA (Fig. 1). Quantification was performed using an automatic UV spectrophotometer (Table 3).

Table 2 Loci amplified with AmpFlSTR® Identifiler™ PCR Amplification Kit, the range of PCR products expressed in base pair and the corresponding dyes used.

Locus	Range of PCR product sizes (bp)	Dye label
D8S1179	123–169	6-FAM™
D21S11	185–240	
D7S820	255–291	
CSF1PO	305–341	
D3S1358	112–140	VIC®
TH01	163–202	
D13S317	217–245	
D16S539	252–292	
D2S1338	307–359	
D19S433	102–135	NED™
TPOX	222–250	
D18S51	262–346	
Amelogenin	106/112	PET®
D5S818	134–172	
FGA	215–355	

Table 3 Concentration of different bone samples using UV spectrophotometer.

Samples	Conc./Temp	260 nm	280 nm	Ratio	Quantity
Bone 1	0.5 M (37 °C)	7.1	29.0	1.5	235
	0.5 M (4 °C)	5.7	3.7	1.6	288
	0.25 M (37 °C)	4.0	2.4	1.6	200
	0.25 M (4 °C)	0.5	0.3	1.5	29
Bone 2	0.5 M (37 °C)	2.1	1.1	1.7	109
	0.5 M (4 °C)	2.7	1.4	1.6	135
	0.25 M (37 °C)	2.4	1.5	1.7	115
	0.25 M (4 °C)	1.8	1.5	1.5	120
Bone 3	0.5 M (37 °C)	2.2	1.4	1.7	144
	0.5 M (4 °C)	2.7	1.4	1.5	165
	0.25 M (37 °C)	3.5	2.2	1.8	179
	0.25 M (4 °C)	2.0	1.8	1.5	190
Bone 4	0.5 M (37 °C)	5.0	2.5	1.9	251
	0.5 M (4 °C)	5.7	3.9	1.4	286
	0.25 M (37 °C)	7.2	8.1	1.7	186
	0.25 M (4 °C)	3.7	1.9	1.9	189
Bone 5	0.5 M (37 °C)	0.9	0.5	1.9	496
	0.5 M (4 °C)	1.3	0.7	1.8	65
	0.25 M (37 °C)	2.9	3.4	0.8	147
	0.25 M (4 °C)	1.81	0.96	1.2	90