Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International: Genetics Supplement Series 1 (2008) 24–25
www.elsevier.com/locate/FSIGSS

Research article

A more efficient extraction method of human bone resulting in improved DNA profiling

Stijn Desmyter *, Christine De Greef

National Institute for Criminalistics and Criminology, Vilvoordsesteenweg 100, B-1120 Brussels, Belgium

Received 20 August 2007; accepted 10 October 2007

Abstract

Eight human bone samples, from a forensic case, were extracted in parallel using our standard protocol with and without PTB in the buffer. Both methods were sometimes inadequate for (complete) STR profiling, while the presence of PTB even decreases the DNA yield.

The complete decalcification of the bone extraction residues in an EDTA-solution with SDS recovered sufficient amounts of DNA, which resulted in complete STR profiling for all samples. Complete decalcification without SDS yielded even higher amounts of DNA and also complete STR profiling for all samples.

Similar results were obtained for the DNA extraction from a human tooth.
© 2008 Elsevier Ireland Ltd. All rights reserved.

Keywords: Bone; Teeth; DNA extraction; DNA profiling

1. Introduction

Recently two articles about the efficiency of bone DNA extraction have been published. Loreille [1] describes the significant enhanced quantity of extracted DNA after total demineralization of the bone material. In a study on a wide range of methods with ancient cave bear bones, the most promising method uses a solution without any additive [2]. The findings of both publications were tested on forensic casework samples of human remains. The results obtained from the bones were verified by comparison with the profiles of the presumed biological parents.

2. Methods

Eight human bone fragments (femur, two fibula, two os coxa, phalanges, humerus, scapula) that were recovered after 4 months from a humid, warm environment, were brushed with extraction buffer, rinsed through brushing with water, dried with paper and sanded with a rotary tool (Dremel 432). Two series of 0.5–1 g samples were recovered from the washed bone fragments using a rotary tool with diamond circle saw (Dremel 545). The bone samples were grinded in a porcelain mortar under liquid nitrogen and extracted in parallel using our standard protocol without and with 16 mM PTB in 2 ml buffer (10 mM EDTA, 0.5% SDS, 100 mM NaCl, 10 mM Tris–HCl, pH 8, 2% βME). 0.8 mg ProteinaseK was added twice and the samples were incubated with rotation at 56 °C overnight and for 2 h, respectively. The bone extraction residues from the standard protocol were decalcified in 15 ml 0.5 M EDTA, one half with and the other without 1% SDS. Four milligram ProteinaseK was added twice and the sample were incubated with rotation at 56 °C overnight and for 2 h, respectively. After organic extraction and purification (Centricon YM50) all four methods were evaluated for DNA yield (Real-Time PCR, Quantifiler, Applied Biosystems), mitochondrial DNA (HV2 sub region 35–254, 30 cycles) and STR profiling (ProfilerPlus 28 cycles, ABI3130xl – 1200 V 60 s – both Applied Biosystems).

The pulp of an intact human canine, which stayed for a month in the water, was recovered via an endodontic incision, while the remaining tooth was split by a vertical section. One half, grinded in a stainless steel bullet trilling mill with liquid nitrogen, was treated by the standard lab procedure, while the other half was decalcified with SDS. The standard extracted tooth residue was later decalcified with SDS. The different extracts were evaluated for DNA yield and STR profiling.

* Corresponding author. Tel.: +32 2 2400529; fax: +32 2 2416105.
E-mail address: stijn.desmyter@just.fgov.be (S. Desmyter).

1875-1768/$ – see front matter © 2008 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.fsigss.2007.10.092

Table 1
DNA yield, mitochondrial DNA and STR profiling of eight bone fragments extracted with four different methods

Bone	Bone type	Standard − PTB			Standard + PTB			Decal + SDS			Decal − SDS		
		ng DNA/g bone	Mito	STR	ng DNA/g bone	Mito	STR	ng DNA/g bone	Mito	STR	ngDNA/g bone	Mito	STR
A	Femur (upper leg)	4.3	+	7	0.7	+	/	23.2	+	10	33.9	+	10
B	Fibula (lower leg)	0.9	+	5	0.8	+	6	8.6	+	10	10.2	+	10
C	Os coxa (hip bone)	36.8	+	2	8.1	+	6	11.0	+	10	27.7	+	10
D	Phalanges (foot bone)	1.4	+	10	1.1	+	10	50.7	+	10	75.2	+	10
E	Humerus (upper arm)	0.0	+	/	0.0	+	/	5.3	+	10	na	na	na
F	Fibula (lower leg)	0.4	+	/	0.1	+	/	7.4	+	10	na	na	na
G	Scapula (shoulder blade)	0.0	+	2	0.0	+	/	9.1	+	10	14.8	+	10
H	Os coxa (hip bone)	69.7	+	7	33.0	+	6	67.1	+	10	90.1	+	10

Mito: double stranded sequencing of HV2 sub region (35–254). STR: number of complete ProfilerPlus STRs (Amelogenin included) > 50 rfu.

3. Results

The standard protocol yielded for six of the eight bone samples between 0.4 and 69.7 ng DNA/g bone (Table 1). For two samples, E and G, no DNA could be measured in the extract. The significant highest DNA yield was recovered from the two hip bones C and H (0.06–1.05 ng/μl), which were the only two samples showing inhibition during Real-Time PCR and hence their extracts had to be diluted for appropriate quantification. Adding PTB to the extraction buffer decreased the DNA yield up to six times. Samples C and H showed less inhibition during Real-Time PCR as compared to the extraction without PTB.

Total decalcification with SDS of half of the bone residues recuperated after extraction without PTB yielded higher amounts of DNA for six of the eight samples, including the samples E and G for which no DNA could be demonstrated in the standard protocol extracts. The DNA yield is 5.5–35 times higher for samples A, B, D and F. The two hip bone samples, which released already high amounts of DNA during the standard extraction, yielded three times less DNA for sample C and about an equal amount for sample H.

Omitting SDS from the decalcification solution further increases the DNA yield up to two times for the six samples tested (A, B, C, D, G and H). The remnant bone residues from the samples E and F were totally used for decalcification with SDS.

Nearly all undiluted decalcification extracts showed inhibition during Real-Time PCR.

Amplification and double stranded sequencing of the mitochondrial DNA fragment from position 35 to 254 was successful for all extracts. Only amplification of the standard extracts of the samples C and H was hampered by inhibitors which forced us to dilute these extracts: despite their higher amount of DNA, the standard extracts without PTB needed to be diluted more (at least 100 times) than the standard extracts with PTB (at least 50 times). All decalcification extracts were diluted 100 times.

STR profiling of the standard DNA extracts with and without PTB results in a complete profile for only sample D (phalanges). For the other seven samples, either no or an incomplete profile was obtained. The presence or absence of PTB did not seem to have an influence on the results.

After decalcification of the bones, full profiles were obtained for all samples. There was no influence of SDS on the quality of the profiles.

While the standard extraction without PTB of one dental half yielded no measurable amount of DNA and no STR profile, the decalcification with SDS of the other dental half yielded more than enough DNA (157.5 ng) for complete STR profiling. The decalcification with SDS of the remnants of the tooth already extracted according to the standard procedure without PTB yielded a sufficient amount of DNA (36.9 ng) for complete STR profiling. No inhibition was observed.

4. Conclusion

Our standard method is sometimes inadequate for (complete) STR profiling of human bones and teeth. Adding PTB to the extraction buffer cannot overcome this problem: the DNA yield even decreases and STR profiling is still incomplete or absent.

The complete decalcification in the presence of SDS not only recovers more DNA, but also results in complete STR profiling for all samples in this study. Complete decalcification without SDS yields even higher amounts of DNA. STR profiling was still of high quality.

Acknowledgments

We thank A. Velghe, N. Van Houtvinck, S. Comblez and V. Hougardy for technical assistance.

Conflict of interest

None.

References

[1] O.M. Loreille, T.M. Diegoli, J.A. Irwin, M.D. Coble, T.J. Parsons, High efficiency DNA extraction from bone by total demineralization, Forensic Sci. Int.: Genet. 1 (2007) 191–195.

[2] N. Rohland, M. Hofreiter, Comparison and optimization of ancient DNA extraction, BioTechniques 42 (2007) 343–352.