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Powder-Free DNA Extraction from Post-Mortem Teeth

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Abstract

In order to perform human identification through DNA analysis, bones and teeth are considered the samples with the highest success rates. Regarding the protocols used to process these samples, they are also usually submitted to the same treatment, which consists of sample powdering. However, there are tissues in a tooth that have low DNA content, and thus, should not be included in DNA extraction. Recent studies have reported that sampling of dental cementum could optimize DNA typing from teeth due to its high cellularity and resilience. The objective of this article is to present an improved protocol for DNA extraction from teeth that targets cementum and its use in two real cases. After a 24-hour decalcification step, cementum was sampled and submitted to a simple DNA extraction protocol based on a commercial kit and automated platform. Real-time PCR (Polymerase Chain Reaction) quantification, PCR amplification of 27 DNA markers and capillary electrophoresis were performed. For both real cases, a different sample type had yielded a full DNA profile using a different and validated protocol. Quantification showed expected concentration and integrity of DNA in both tooth samples. After genotyping, all samples presented full and concordant profiles. This pilot study demonstrated that the presented protocol, specifically designed for teeth, was able to obtain full DNA profiles from a burnt and a skeletonized human body.

Keywords: Teeth; DNA; Post-mortem; Forensic; Human identification

Key Points

- In order to extract DNA from teeth, minimally destructive techniques should be performed.
- Cementum enriched samples have been shown to provide better results.
- We obtained full concordant DNA profiles from forensic cases that had been previously typed.
- The presented technique was able to retrieve authentic DNA profiles from burned and skeletonized human teeth.

Introduction

In forensic cases of human identification, bones and teeth are the body structures most likely to resist decomposition [1-3]. Nevertheless, studies have suggested that teeth may preserve DNA better than bones [4,5]. When it comes to the laboratory processing of samples, teeth and bones are submitted to the same treatment, which involves grinding or pulverizing whole teeth or bone pieces [1-3,6-8]. Following that, DNA in these samples can be extracted and purified through different protocols [9]. It should be highlighted that new protocols must be amenable to high-throughput purification on automated platforms [10].

Researchers have recently advocated for less destructive and powder-free protocols [11,12], which do not possess the setbacks of sample powdering. Powdering of bone and whole teeth precludes future morphological analysis, as well as destroys unique specimens. Furthermore, bone and tooth powder are affected by static from the contact with plastic ware. This phenomenon may attract particles to the outside of microtubes, promoting sample loss and contamination in the laboratory.

On a technical note, teeth powdering contribute to a "dilution effect" [13], since there are DNA-poor and DNA-rich tissues. That aspect has led researchers to advocate for the sampling of DNA-rich regions when developing DNA extraction protocols for teeth [13]. In fact, numerous specific protocols for teeth have been published. However, most of them target the pulp or require dental instruments/training [11,14-16].

Since there is evidence that dental pulp decomposes faster than the other dental tissues [17,18], what region of the tooth should be targeted? Cementum has been intensively studied recently and those reports have shown that its DNA and microstructure are preserved in decomposing and degraded samples [19]. For a review on DNA content in the different dental tissues, see Higgins and Austin [13].

These recently published results are promising, and the existence of only few protocols that have attempted to extract DNA from cementum is unsatisfactory. We hypothesize that mixing the concepts of two previously published protocols [20,21] could allow for a reliable DNA typing of teeth collected from decomposing corpses. Therefore, the aim of this study was to describe an improved automatable protocol and its application in two real cases.

Materials and Methods

This improved DNA extraction protocol was used on 2 molar teeth that were received by our laboratory along with other tissue samples of two real forensic human identification cases. In both cases, the teeth

samples were processed to confirm the results of the first analysis that had provided full profiles using validated protocols.

Case 1: A human corpse was found in the debris of a house that was completely consumed by a fire. A full DNA profile was obtained previously using a muscle sample.

Case 2: A human skeletonized corpse was found on June 2011. The person had been missing since 2009. A full DNA profile was obtained previously using a femur sample.

For the processing, teeth were cleaned with pieces of gauze embedded in 70% ethanol and distilled water, sequentially. Then, they had their crowns covered in parafilm and placed separately in 15 mL falcon tubes. The tubes were filled with EDTA (Ethylenediaminetetraacetic Acid) solution (0.5 M, pH 8) and incubated for 24 hours, at room temperature, with occasional vortexing. With the aid of a scalpel, the apical third of the roots of the teeth were scraped in order to obtain decalcified wedges. Approximately 20 mg of sample was submitted to DNA extraction through the "tissues" protocol of the EZ1® DNA Investigator kit (Qiagen, Hilden, Germany), with the addition of 10 µL of DTT (dithiothreitol) to the digestion buffer. The samples were purified and eluted in 50 µL of TE buffer using an EZ1® Advanced XL (Qiagen) platform. DNA quantification was carried out using Quantifiler™ Trio kit (Thermo Fisher Scientific, Waltham, MA, USA) in a 7500 Real-Time PCR platform. For PCR, PowerPlex® Fusion 6C kit (Promega, Madison, WI, USA) was used and for capillary electrophoresis, a 3500 genetic analyzer (Thermo Fisher Scientific) was used. Genotyping was performed using GeneMapper ID-X v.1.4 Software (Thermo Fisher Scientific). In all analyses, the manufacturers' recommendations were followed.

Results

In Table 1, the results are presented. DNA quantification showed good integrity and a high concentration of DNA in the tooth from Case 1. The tooth from Case 2 showed lower DNA concentration and a difference in the quantity of small and large DNA fragments, indicating degradation. Both DNA profiles were complete, although Case 2 profile confirmed that the DNA in that tooth was degraded, by observing the decreasing heights of the peaks (Figures 1-4). Nonetheless, Case 1 and Case 2 samples showed concordant profiles when compared to the electropherograms obtained from the previously typed muscle and femur samples.

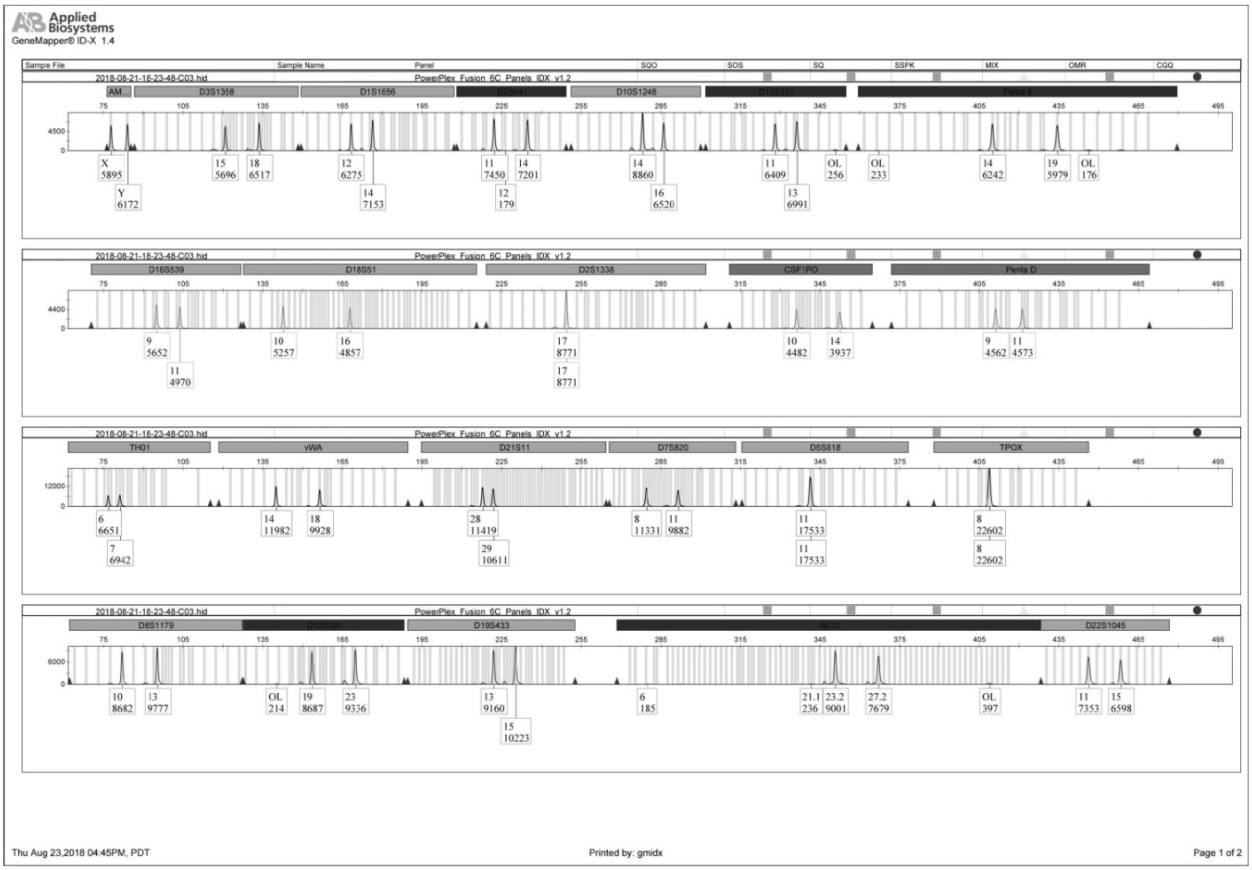

Figure 1: Electropherogram of tooth sample from Case 1 (Part 1).

Sample	Tooth	Quantification (ng/μL)			Degradation Index*	DNA Markers	Degraded Profile
		Small	Large	Y			
1	Molar	53.5	62.5	47.6	0.85	27/27	No
2	Molar	1.56	0.65	0.96	2.4	27/27	Yes

Table 1: Summary of the DNA quantification results (*Calculated dividing the small target by the large target).

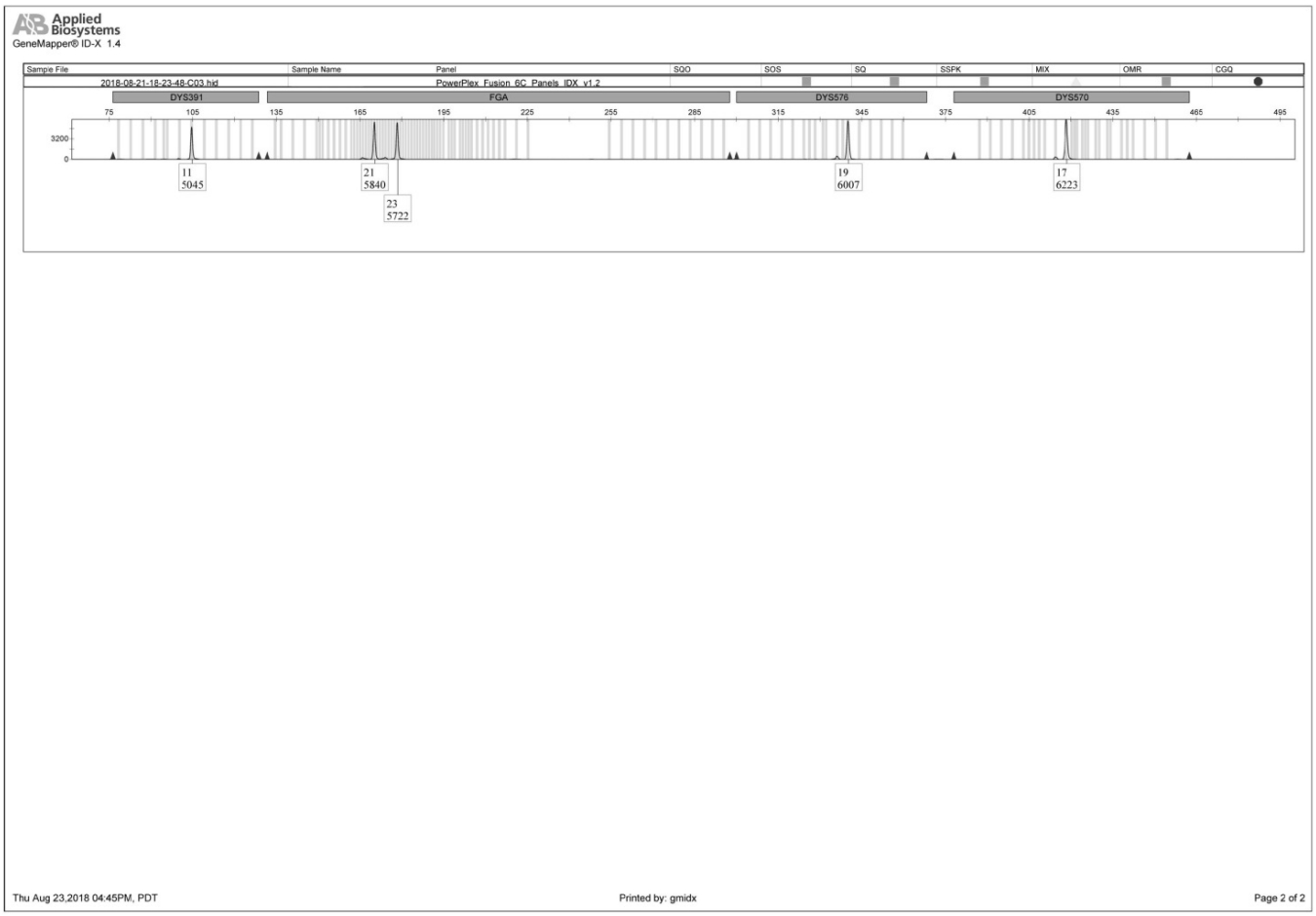

Figure 2: Electropherogram of tooth sample from Case 1 (Part 2).

Discussion

The present study reports the attainment of full DNA profiles analysing 27 markers in the teeth of a carbonized and a skeletonized human body. The approach was minimally destructive and yielded results in 2 working days. Case 1 tooth yielded as much DNA as the muscle sample previously analysed. Even though the body was carbonized, the results were expected since well-preserved structures were observed during autopsy. On the other hand, the cadaver in Case 2 was skeletonized, thus, the DNA was expected to be degraded and in low concentration as shown by the analyses.

In a case report of two human bodies that were burned inside a house [22] were able to recover a full DNA profile from a muscle sample of one of the victims, while they could not get a DNA profile from a tooth of the other body. The authors analysed 16 genetic markers, while, in this study, we analysed the same 16 genetic markers plus another 11. In the same sense [19] analysed the DNA of the tips of the roots of teeth from two human corpses, one was found under water and the other was buried. The researchers analysed 17 genetic markers and were not able to retrieve a full profile from any of the two cases. In this study, we have also analysed the same 17 markers plus other 10.

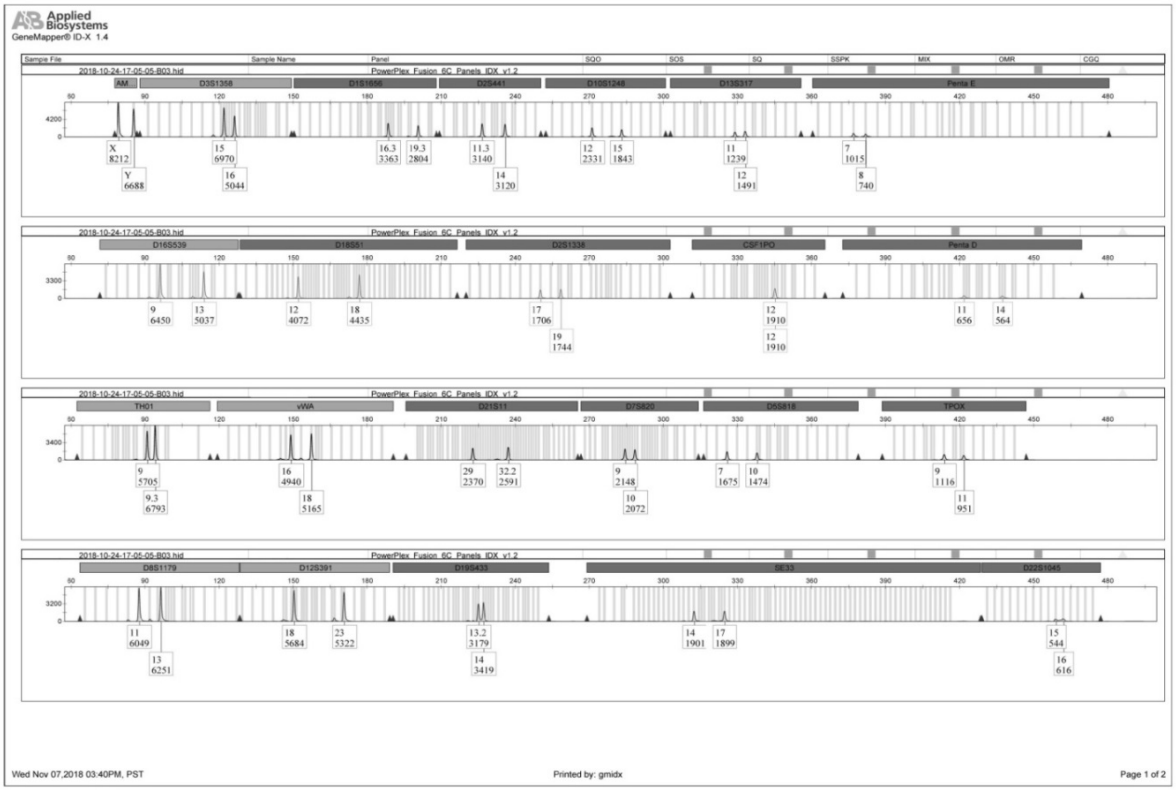

Figure 3: Electropherogram of tooth sample from Case 2 (Part 1).

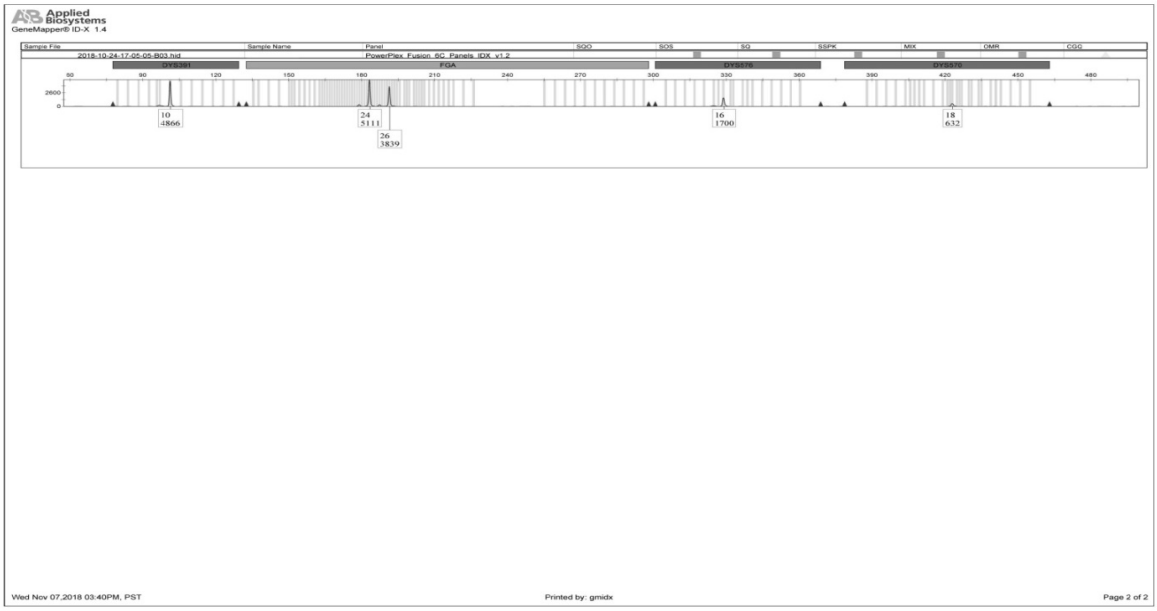

Figure 4: Electropherogram of tooth sample from Case 2 (Part 2).

According to this and another report [21], it appears that, after decalcification, demineralized portions of teeth can have their DNA extracted using protocols designed for biological tissue, since only the collagenous matrix is left. This could be advantageous considering that

protocols for undecalcified bones and teeth usually require working with powdered sample and much larger volumes of reagents during DNA extraction [1-3,6,7]. Besides, covering the enamel with parafilm blocks calcium extraction, preserving crown morphology and allowing deeper decalcification of the roots, which are the main target of the technique [22].

Conclusion

Given the small number of samples included in this study, this protocol may not yield good results in all teeth and in all cases. Thus, another validated protocol should be followed in those circumstances. Furthermore, we cannot draw any conclusions as to whether this protocol may be more efficient than the ones mentioned in the discussion because a systematic comparison between them was not performed. Nonetheless, this pilot study showed that the presented protocol, specifically targeting tooth cementum, allowed reliable DNA typing of a burnt and a skeletonized human body.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Research Involving Human Participants

The authors declare that this study was conducted in accordance with the 1948 Declaration of Helsinki.

Informed Consent

Not applicable.

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