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Original Article

Extraction Strategy for DNA Recovery from Putrefied Teeth and Skull Bone

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Abstract

Forensic samples are commonly exposed to harsh environmental conditions which affect the degree of sample (DNA) preservation and subsequent genetic profiling. The aim of this study was to develop a better strategy for DNA extraction from hard putrefied tissues (Teeth and Skull bone).

Jaw (teeth) and the skull samples were collected from the putrefied corpses and the authors were asked to determine if the two specimens belonged to the same body. The DNA was extracted by phenol-chloroform and DNA IQ™ System Kit. The PowerPlex® 16 and the PowerPlex® Y System Kits were used for autosomal STR and Y-STR genotyping, respectively. DNA profiling found evidence in favor of DNA degradation.

Phenol-Chloroform extracted-DNA was re-extracted by using DNA IQ ™ System kit and managed to identify 13 autosomal STR loci and 13 Y-STR markers from doubly extracted DNA.

In conclusion, the combination of two DNA extraction methods (phenol-chloroform + DNA IQ™) improved the quality of DNA extracted from putrefied teeth and skull bone.

Keywords: Forensic Science, Forensic Identification, DNA Extraction, Putrefaction, Teeth, Skull Bone.

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استراتيجية استخلاص للحصول على الحمض النووي من أنسجة صلبة متحللة (الأسنان وعظام الجمجمة)

المستخلص

يمكن أن تتعرض العينات الجنائية الحيوية إلى ظروف بيئية قاسية تؤثر على جودة العينة، ما يؤثر بالتالي على صحة ودقة النتائج النهائية، وقد كان الهدف من هذه الدراسة تطوير طريقة استخلاص للحصول على الحمض النووي من أنسجة صلبة متحللة وهي الأسنان وعظام الجمجمة، حيث تم الحصول على عظام الفك (أسنان) والجمجمة من جثث متحللة. وطُلب تحديد ما إن كانت

العينات تنتمي إلى نفس الشخص.

تم استخلاص الحمض النووي بطرق مختلفة: الفينول كلوروفورم وطقم الاستخلاص DNA IQ™، وتم استخدام PowerPlex®16 وPowerPlex-Y لتحديد السمات الوراثية. وقد وُجد علامات تدل على تدهور الحمض النووي. ومن ثم تم إعادة استخلاص الحمض النووي بواسطة محلول الفينول كلوروفورم باستعمال طقم الاستخلاص DNA IQ™، وقد تم التمكن من تحديد ثلاثة عشرة موقعاً وراثياً جسدياً علامات autosomal STRs وكل مواقع Y-STRs من الحمض النووي المستخرج على نحو مضاعف. وكنتيجة نهائية فإن الجمع بين طريقتي استخلاص الحمض النووي المستخدمتين في الدراسة مكّنتا من تحسين نوعيته.

الكلمات المفتاحية: الطب الشرعي، الاستعراف الجنائي، استخلاص الحمض النووي، التحلل والتعفن، عظام الجمجمة والأسنان.

1. Introduction

DNA profiling (also called DNA fingerprinting or DNA typing) is a forensic technique used to identify individuals on the basis of unique characteristics of their DNA [1]. It is often challenging to obtain PCR amplifiable products from forensic samples because either the DNA in those samples is degraded or mixed [2].

Forensic samples are commonly exposed to harsh environmental conditions which affect the degree of sample (DNA) preservation [3]. Under these conditions, even hard tissues such as bones and teeth are not appropriate for DNA genotyping [4]. DNA extraction methods must solve problems associated with low quantities of DNA, DNA degradation and polymerase chain reaction (PCR) inhibition [5].

Aiming to circumvent these difficulties, the present study focussed on developing a better and workable strategy for DNA extraction from putrefied teeth and skull bone.

2. Materials & Methods

2.1 Samples

The present casework was conducted at the Genetic Print Unit, Habib Bourguiba Hospital, Sfax, Tunisia in 2013. The jaw (teeth) and the skull samples were collected from putrefied corpses for DNA analysis and the researchers were asked to determine if the two specimens belonged to the same body.

2.2 DNA extraction

Samples were disinfected by ethanol, washed with distilled water and then dried. After spraying, hard tissue (frozen samples) were grinded with liquid nitrogen using a mortar and pestle and then extracted by two different methods (Phenol-Chloroform and DNA IQ™ System) followed by one another:

- Extraction with phenol-chloroform (PC) was performed by the same procedure as described for blood [6]. The amount of proteinase K (PK) added was 1.5 mg. The dried DNA was eluted in 40 µL of Tris-EDTA buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0).
- Extraction using tissue and hair extraction kit in conjunction with DNA IQ™ System (Promega) [7]: 0.25 g of pulverized bone was incubated with 472 µL of bone incubation buffer along with 28 µL of PK at 56 °C for 2 hours. After incubation, the solution was transferred to another tube containing 500 µL of lysis buffer / Dithiothreitol (DTT) and 14 µL of magnetic resin, which has very high affinity for DNA. This mixture was incubated for 15 min at room temperature. The resin was washed as per the manufacturer's instructions using a magnetic rack and then dried. The DNA was finally eluted by adding 25 µL of elution buffer.

Two extraction methods, PC and DNA IQ™ Kit, were also combined. The substrate was the purified DNA extracted by PC: to 20 µL of DNA extracted by PC, we added

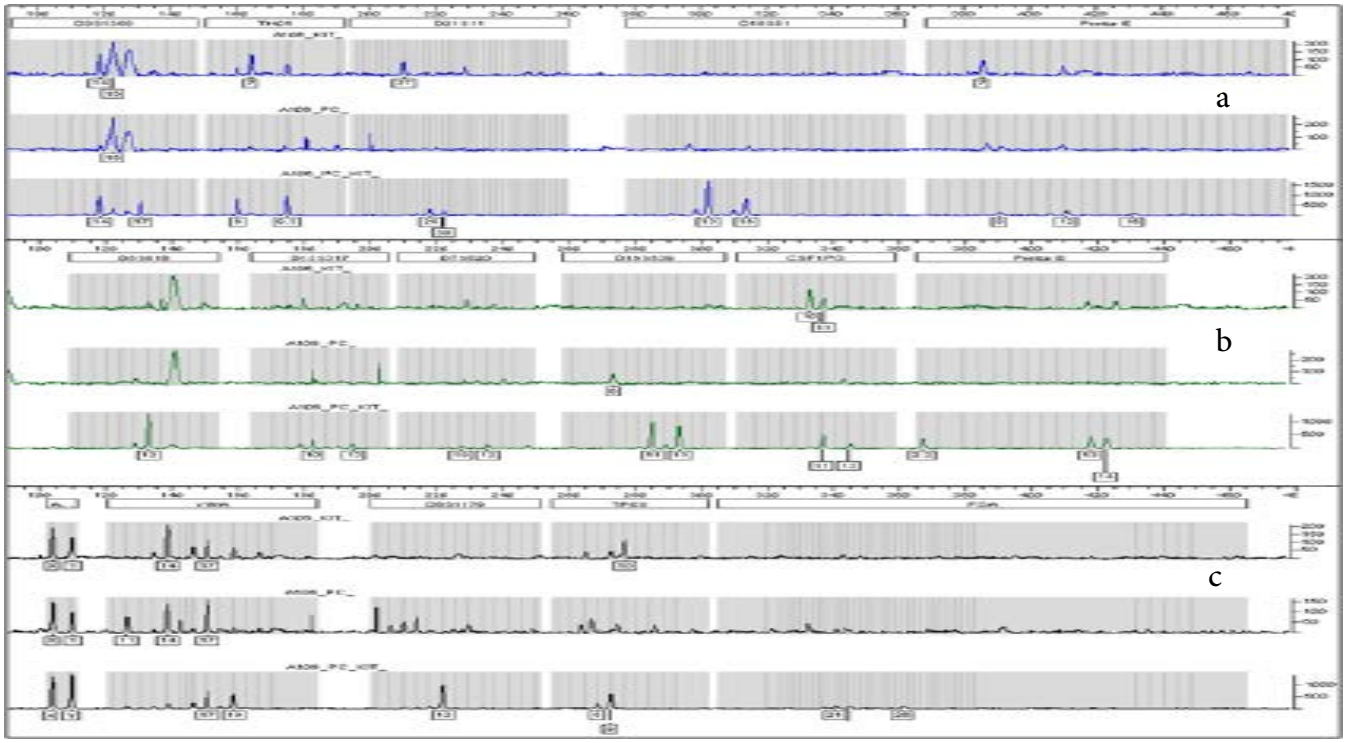

Figure 1- *Electropherograms of the DNA extracted from teeth using the PowerPlex 16™ amplification kit and three different extraction methods. a: DNA extracted using tissue and hair extraction kit in conjunction with DNA IQ™ System kit; b: DNA extracted by phenol chloroform method; c: DNA extracted by phenol chloroform method then purified by the DNA IQ™ System kit.*

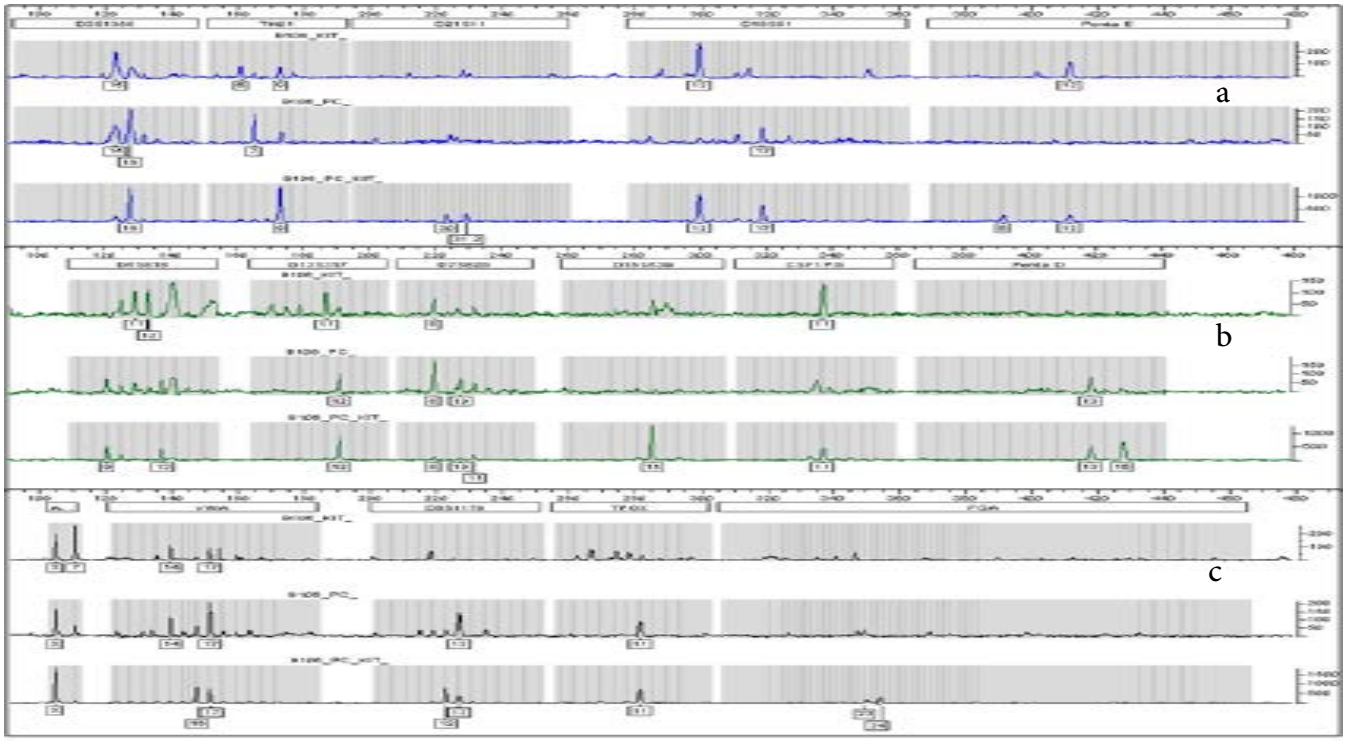

Figure 2- *Electropherograms of the DNA extracted from skull using the PowerPlex 16™ amplification kit and three different extraction methods. a: DNA extracted using tissue and hair extraction kit in conjunction with DNA IQ™ System kit; b: DNA extracted by phenol chloroform method; c: DNA extracted by phenol chloroform method then purified by the DNA IQ™ System kit.*

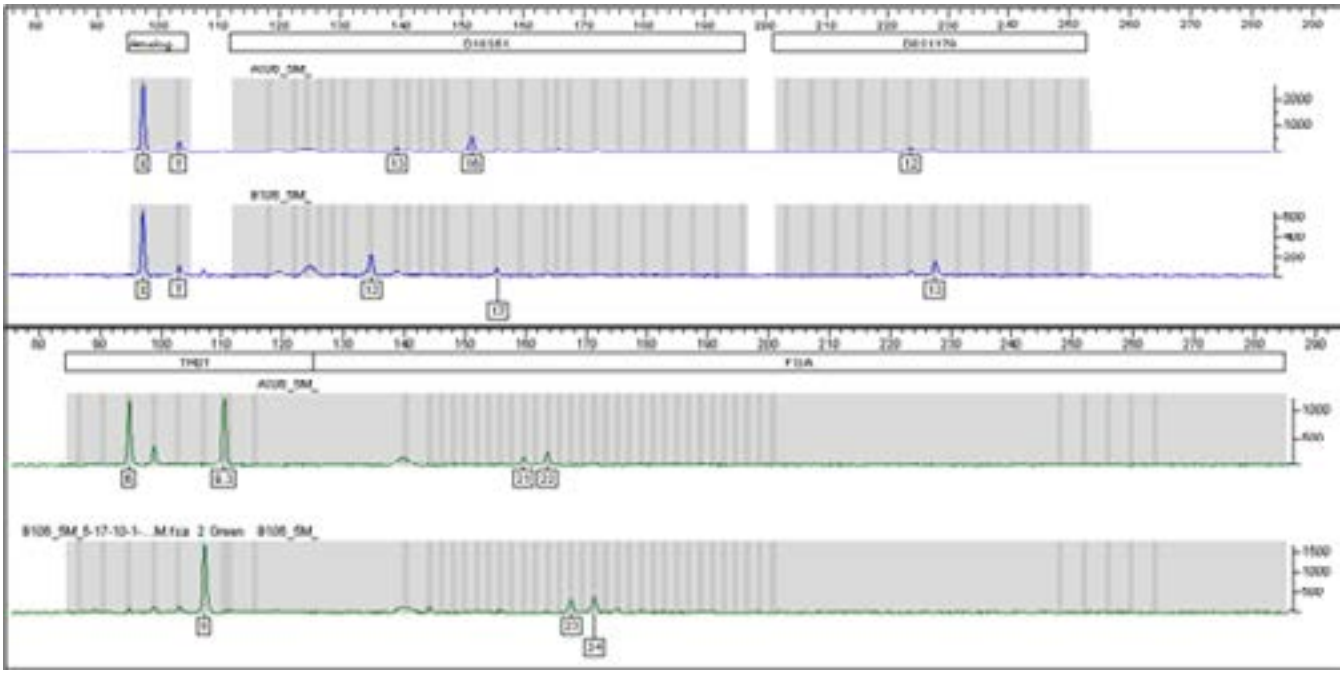

Figure 3- *Electropherograms of the doubly extracted DNAs using the PowerPlex® S5 System kit (DNA extracted by phenol chloroform method then purified by the DNA IQ™ System kit). A106 : teeth. B106 : skull.*

100 µL of lysis buffer / DTT and 7 µL of resin. After incubation for 15 minutes at room temperature, the same [7] resin washing and drying steps were followed, before eluting the DNA in 30 µL of elution buffer.

2.3 STR amplification and typing

The DNA samples were amplified using the PowerPlex® 16 HS and the PowerPlex® Y System Kits following the manufacturer's recommendations [8-9]. The amplified products were analyzed using an ABI Prism® 310 Genetic Analyzer.

Initial fragment sizing was performed by the GeneScan® Analysis Software (Applied Biosystems). Allele calling was performed by Promega's PowerTyper™ 16 and PowerTyper™ Y Macros operating within the Genotyper® software program (Applied Biosystems).

3. Results

Electropherograms using the PowerPlex® 16 kit are presented in Figure-1 (teeth) and Figure-2 (skull). In PC and DNA IQ™ extracted DNAs, genetic profiling found evidence in favor of DNA degradation: peak unbalance, allele drop-out, allele drop-in. Only the male gender could be identified.

Using the doubly extracted DNA (PC + DNA IQ™), we managed to identify respectively 13 and 14 autosomal STR markers from teeth and skull, as well as the male gender. In order to verify results, the doubly extracted DNA were amplified using the PowerPlex® S5 System kit. The electropherogram shown in Figure-3 indicates the amplification of 4 STR markers (D18S51, D8S1179, TH01 and FGA) plus amelogenin.

The identified genetic profiles led us to conclude that the jaw and the skull did not belong to the same individual.

PowerPlex® Y System kit (Promega) were also used for the amplification of 13 markers on the Y chromosome: the two samples shared the same Y haplotypes. In conclusion, the 2 samples belonged to different individuals who had the same paternal lineage.

4. Discussion

In forensic investigations, given that bones are the only potential source of genetic material in many scenarios, robust protocols for DNA extraction are required [10]. Thanks to the compact structure of bone, DNA is generally less degraded than in other tissues.

There is no correlation between the bone age and the amplification success. DNA integrity depends mainly on the environment where the bone was preserved (moisture, organic acids etc.) [11]. In fact, organic acids such as humic acid may bind DNA and limit its availability for the amplification process [12], or bind Taq DNA polymerase's active site [13]. This acid is of major concern when analyzing skeletal remains because from the surrounding soil it seeps into collagen [14] and, therefore, may also be present in the powdered bone.

Several methods are used to eliminate a possible contamination of the surface of the bone prior to DNA extraction: washing of the bone by an acid, irradiation by ultraviolet rays, washing with absolute ethanol or washing with sodium hypochlorite (bleach) [4,10,11,14]. The author chose to wash the bone by absolute ethanol. In fact, chlorine washing would damage the bone itself. It would start to break down the structure of the bone and would continue to even after it is rinsed and dried [15].

In a comparative study between different DNA extraction methods from decayed tissue, extraction by silica had shown the best performance [16]. DNA IQ™ is a solid phase extraction method using magnetic silica beads, in which DNA isolation can be performed in a single tube by simply adding and removing readymade solutions. This method is reported to be able to deal with a number of problematic forensic samples and perform better in the presence of soil inhibitors, as well as being a rapid extraction procedure [17] with a good effectiveness in terms of DNA recovery [18].

The resin which has been coated onto the magnetic silica beads has a defined DNA-binding capacity in the presence of excess DNA. Thus, the researcher can bypass the quantitation step typically necessary with other purification procedures [19].

The author used two methods for DNA extraction, namely PC and DNA IQ™ System kit (Promega) and were unable to identify the DNA profiles due to DNA degradation (Kit and PC in Figures-1 and 2). However, by combining the two techniques, the author managed to identify almost all of the alleles (PC-Kit in Figure-1 and 2). Indeed, small-sized degraded DNA fragments have an inhibitory effect on PCR amplification of forensic samples [20]. The small DNA fragments would have monopolized primers and prevented amplification of the few intact DNA fragments. Using the PC extracted DNA as a substrate for the DNA IQ™ System kit, the resin having a low affinity for the small-sized DNA fragments allowed us to recover the less degraded DNA. Moreover, DNA IQ™ System proved to be very effective for the removal of known PCR inhibitors that are routinely found in DNA extracts of compromised forensic samples [21]. Results of this study are also in agreement with previous studies which used a combined protocol for the isolation and purification of DNA from ancient bones and related sources [22, 23].

Many extraction strategies combining purification protocols provided better results in the presence of inhibitors [22-23]. The DNA IQ™ System is the most convenient due to the combination of DNA extraction and purification. PCR amplification of degraded DNA samples can be better accomplished with smaller target product sizes [24].

Using the PowerPlex® S5 System genotyping kit, the authors confirmed 5 autosomal markers; the peaks height was greater than that of the PowerPlex 16 kit amplicons. In fact, the primers used in this kit hybridized closer to

the repeated sequence. Thus, the amplified fragments are shorter (90 to 280 bp). This PowerPlex® S5 System kit system is more efficient in degraded DNA amplification than using the PowerPlex 16 kit.

5. Conclusion

In conclusion, the combination of two DNA extraction methods (PC + DNA IQ™) improved the quality of DNA extracted from putrefied teeth and skull bone.

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