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Case Report

A Suitable Method for DNA Extraction from Bones for Forensic Applications: A Case Study

Aqeela S. Abuidrees*, Noora A. Alhamad, Kamal Alsaadany

Forensic Science Evidence, Public Prosecution, Manama, Bahrain

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Abstract

Human identification techniques are constantly developing. Before the discovery of DNA, anthropology accompanied with odontology was the most applicable technique for human identification. With the new era of molecular biology and the revolution of DNA and PCR techniques, DNA profiling has become the core of the human forensic identification process. Different types of samples can be exploited in forensic DNA analysis. In some extreme cases, bone samples are the only accessible samples of DNA due to the bad conditions of putrefaction or degradation of other biological materials and tissues. Therefore, an appropriate method should be determined to yield a full and clean profile.

A case study is presented here in order to identify human remains and conclude the most appropriate method of DNA extraction from human remains. In addition, this study looks at the best part of the skeletal remains to be considered in the extraction of DNA for the purposes of identification. A suspect admitted that he buried his aborted son six months ago. The remains were recovered and DNA analysis was performed in order to determine any genetic link of the remains to the suspect and the female who delivered the baby.

Two extraction methods were compared, the standard organic (phenol:chloroform:isoamyl alcohol) and automated extraction using magnetic beads coated with silica (Qiagen EZ1 Advanced XL). Two bone parts, femur and clavicle, were also compared in terms of DNA yield. The efficiency of the two methods of DNA extraction from bones is illustrated quantitatively and qualitatively. Paternity testing was performed and the suspect was excluded from being the alleged father.

Keywords: Forensic Sciences, DNA Typing, DNA Extraction, Bones

* Corresponding author: Aqeela Abuidrees
Email: Aqeela.s@ppb.gov.bh

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طريقة مناسبة لاستخلاص الحمض النووي من العظام لاستخدامه في التطبيقات الجنائية: دراسة حالة

المستخلص

تتطور تقنيات تحديد الهوية البشرية بشكل ثابت. فقبل اكتشاف البصمة الوراثية كان علم الأنثروبولوجي (علم الانسان) وطب الاسنان من أكثر التطبيقات المستخدمة لتحقيق الهوية البشرية. مع الحقبة الجديدة للأحياء الجزيئية والثورة التي حدثت في تقنية تحليل الحمض النووي وكذلك تفاعل البلمرة المتسلسل، أصبح تحديد السمات الوراثية أساس عملية تحديد الهوية البشرية الجنائي.

تواجه هذا المجال عدة تحديات، من أهمها نوع العينات التي يمكن أن تتوفر للاستعراف وحالتها. حيث انه في الحالات القصوى من التحلل

والتعفن لا يمكن الحصول إلا على العظام كعينة مرجعية لاستظهار الحمض النووي، لذا فأنه من المهم التوصل إلى طريقة مثلى للتعامل مع عينات العظام واستخلاصها للحصول على أفضل النتائج الممكنة. وعليه بنيت دراسة المقارنة على هذه القضية.

تتمثل القضية باعتراف المتهم بدفن ابنه الغير شرعي والذي تم إجهاضه قبل ستة أشهر، وبناءاً على ذلك فقد قام الفريق الجنائي باستظهار الرفات للتعرف على الجنين واثبات نسبه من عدمه للمتهم والمتهمة بالقضية. تمت مقارنة طريقتين لاستخلاص الحمض النووي من العظام: العضوية باستخدام الفينول- كلوروفورم وكحول الايزوبروبانول و المؤتمتة باستخدام حبيبات المغناطيس المغطاة بالسيليكا (Qiagen EZ1 Advanced XL) .

هذه الدراسة تثبت مدى فعالية جهاز (Qiagen EZ1 Advanced XL) في عملية الاستخلاص، وتبين فحوصات البنوة المتبعة لاستبعاد المتهم من كونه الأب البيولوجي للجنين المجهض وذلك حسب المعايير .

الكلمات المفتاحية: علوم الأدلة الجنائية، تنميط الحمض النووي، استخلاص الحمض النووي من العظام.

1. Introduction

Bone samples in some cases are the only biological evidence available for analysis and identification of deceased victims. Anthropological approaches were widely used before the advent of DNA technology and its application to identify human remains. However, those approaches were limited in identifying sex or age in specific cases such as prepubescent or severely damaged remains; therefore, genetic identification is more beneficial.

Degradation of human remains is a serious problem when dealing with such samples that have been exposed to stringent environmental conditions: if the body was buried, humus acids from soil can interfere with the extraction process and inhibit the amplification of the extracted DNA [1].

In order to achieve the best possible results, many techniques have been tested and published [1-3]. The organic method using phenol-chloroform-isoamyl alcohol (PCI) described by Hochmeister and Budowle [2] is one of the most efficient methods in reducing inhibitors within the extracted samples. On the other hand, the toxicity of its reagents has reduced its routine application in forensic laboratories. New techniques were then developed: the silica-based methods have been used for many years due to their easiness, efficiency to reduce inhibitors, and the ability of automation [3].

Two extraction methods are compared and reported in this case report: the classical organic PCI (25:24:1) and automated extraction using magnetic beads coated with silica (Qiagen EZ1 Advanced XL®). Clavicle and femur bones were also compared in terms of DNA yield.

2. Case Report

A 19-year-old male admitted that he had relations with a 17-year-old female, and that they aborted their 4-month old fetus, which they buried in a cemetery 6-months prior to his confession. The investigations was begin to identify the remains and determine the biological relation between the buried fetus and the two suspects. The forensic team recovered the fetal remains wrapped in a white scarf

Figure 1- *The recovery of the aborted buried fetus from the cemetery. The process is sequenced from (A) to (D).*

Figure 2- *The recovered bones showing a full human skeleton. The circle demonstrates the femur with 3 cm length (an indication of an age of 3-4 months).*

and buried 25 cm below the ground (Figure-1A-1D). The forensic pathologist collected the fetal remains and sent them to the forensic laboratory for the purpose of identification.

3. Sample Analysis

3.1 Anthropological Analysis

The remains recovered from the cemetery (Figure-2) were identified as full human skeletal bones, without any fractures, tissues, or internal organs attached. On forensic examination, the remains probabily belonged to a 3-4 month old fetus with a femur length of approximately 3 cm. The sex of the fetus was undetermined. Therefore, the samples were sent to the DNA laboratory for further identification.

3.2 DNA Analysis

3.2.1 DNA Extraction

The collected bone samples were manually cleaned with a brush, washed with 10% bleach followed by distilled water, dried, and then grinded with liquid nitrogen using Cryomill (Retsch™). Two extraction methods were assessed with 500 mg of bone powder from both the clavicle and femur, using manual organic (PCI; 25:24:1) method and automated magnetic beads coated with silica (EZ1 Advanced AL).

The classical organic (PCI; 25:24:1) method of DNA extraction was performed as described by Jakubowska and Hochmeister [1-2]. After decalcification for 5 days, the powder was digested overnight. The digested DNA sample was extracted using PCI and then concentrated with Amicon® centrifugal filters (Merck Millipore). The automated extraction was done using the EZ1 Advanced XL (Qiagen®) with DNA investigator kit according to the manufacturer's guidelines. During extraction, appropriate controls and decontamination precautions were assured to monitor and prevent any possible contamination.

3.2.2 DNA Quantification and Amplification

Both quantity and quality of DNA were assessed. Quantification was tested using a 7500 Real-Time PCR system with a Quantifiler® Human DNA Quantification kit (Applied Biosystems®) for all the extracted samples. Internal Positive controls (ICP) were used to detect the PCR inhibitors. The quality was estimated initially using an AmpFlSTR® Identifiler® plus PCR Amplification Kit (Applied Biosystems®). One ng of each DNA extract was used to amplify 15 autosomal STR loci. When partial profiles were obtained under the standard conditions, the number of cycles was increased to 32 depending on the clarity of the baseline, and profiles were then re-assessed. One microliter of amplified products was diluted in 8.7 μL Hi-Di formamide and 0.3 μL Gene ScanTM 500 LIZ internal size standard (Applied Biosystems®) and then detected using a 3130 XL Genetic Analyzer with 36 cm capillary and POP-4TM (Applied Biosystems®) with an allelic ladder in each run. Data were collected and analyzed using GeneMapper® ID software (version 3.2).

Once the profile of the fetal remains was fully determined, an AmpFlSTR® Yfiler® PCR Amplification Kit (Applied Biosystems ®) and Investigator Argus X-12 (Qiagen®) were used in order to confirm the genetic relation of the remains to both suspects. Appropriate controls and decontamination precau-

Table 1- *The Real-Time PCR output of the extracted bone samples using organic and EZ1 DNA extraction technique*

	Inhibition		DNA yield (ng/μL)	
	Organic	**EZ1**	**Organic**	**EZ1**
Clavicle	IPC (28 ± 3)	IPC (28 ± 3)	0.557	0.663
Femur	IPC (28 ± 3)	IPC (28 ± 3)	0.014	0.065

tions were assured.

3.3 Paternity Testing

Profiles were further assessed for combined paternity index (CPI) and probability of paternity (POP) using an in-house excel worksheet, involving the Bahraini allele frequencies for 15 autosomal STR loci [4].

4. Results and Discussion

Efficiency of extraction methods was determined relying on the DNA yields measured by Real-Time PCR assay. The clavicle bone samples extracted with EZ1 resulted in a higher yield compared to the organic method and the femur samples with both methods. No serious inhibition was observed with both methods (IPC = 28 ± 3), (Table-1).

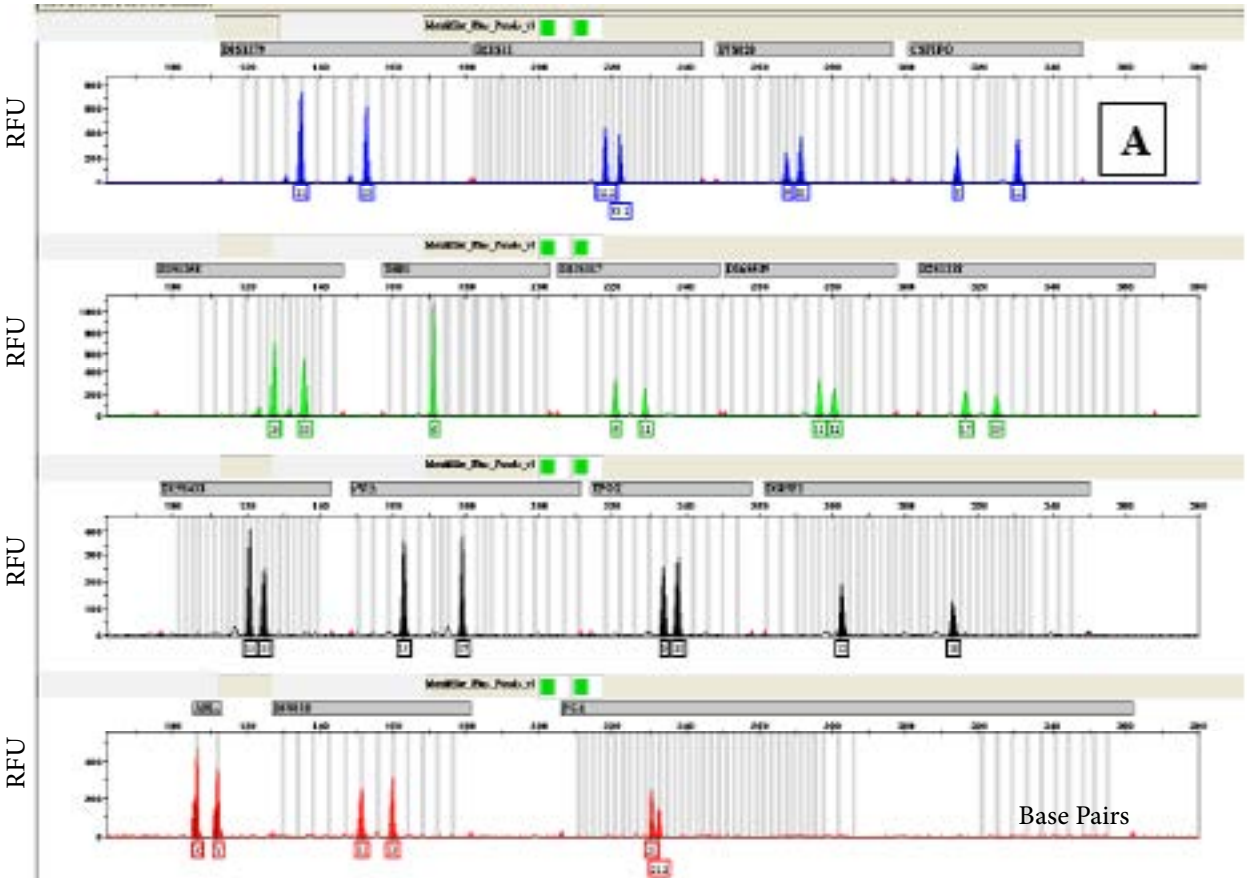

Figure 3A- *Electropherogram showing the Identifiler Plus STR profile for the clavicle bone extracted using EZ1 Advanced XL with improved peak heights.*

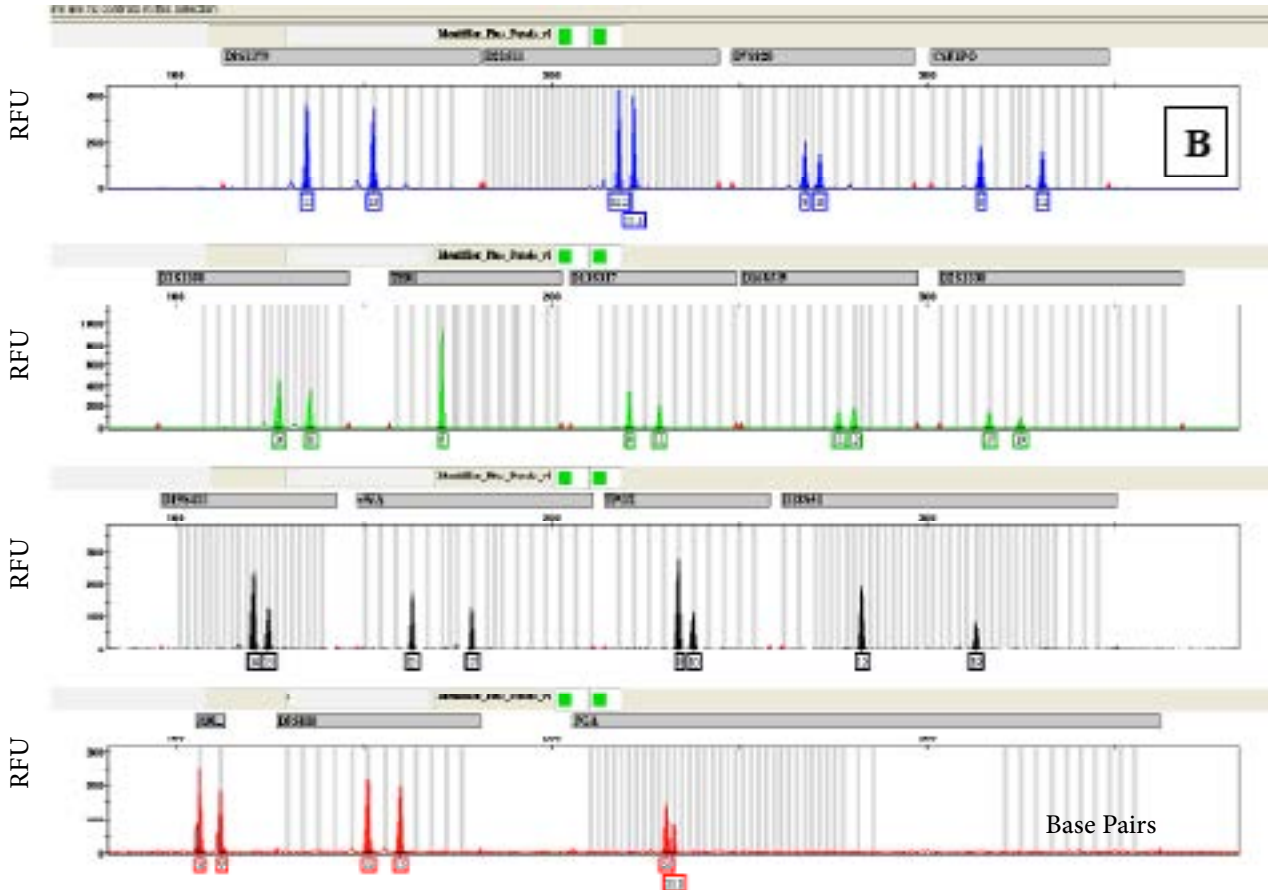

Figure 3B- *Electropherogram showing the Identifiler Plus STR profile for the clavicle bone extracted using organic extraction with imbalanced peaks.*

However, the STR profile obtained with EZ1 advanced XL extraction technology was full, with minimum baseline noise, no allelic drop-outs, and more balanced profile compared to the organic method (threshold of 50 rfu), (Figure-3A-3D).

The sex of the fetus was determined as male. The male fetus was then tested for its genetic relation to both suspects. The female suspect was confirmed as the mother of the fetus with a 99.999% probability factor (CPI= 6.58×10^{5}), while the male suspect was excluded as the biological father (CPI= 3.99×10^{-7}). Further testing with Y STRs (17 loci) and X STRs (12 loci) confirmed the absence of any genetic link between the male suspect and the fetal remains.

In the present study, the automation of DNA analysis skipped the bone extraction steps, this reduced the total extraction time [5] and resulted in a clean and full DNA profile in most of the cases. More modifications can be applied to further improve the results, such as increasing the number of cycles up to 35. In addition, the selection of bone samples can enhance the results; in this case, the femur needed more decalcification time than the clavicle and, therefore, resulted in allelic drop-outs. Many factors can affect the quality and quantity of the DNA extracted from bones, such as the mineralization levels, the pre-treatment of the bone before the extraction step and the real environmental conditions under which the remains are recovered. The mineralization levels might depend on the type and/or age of the bone. Further studies are needed to support and confirm our findings.

5. Conclusion

In most of the forensic cases involving identification of human remains, especially in determining the sex of highly decomposed bones, DNA analysis is more

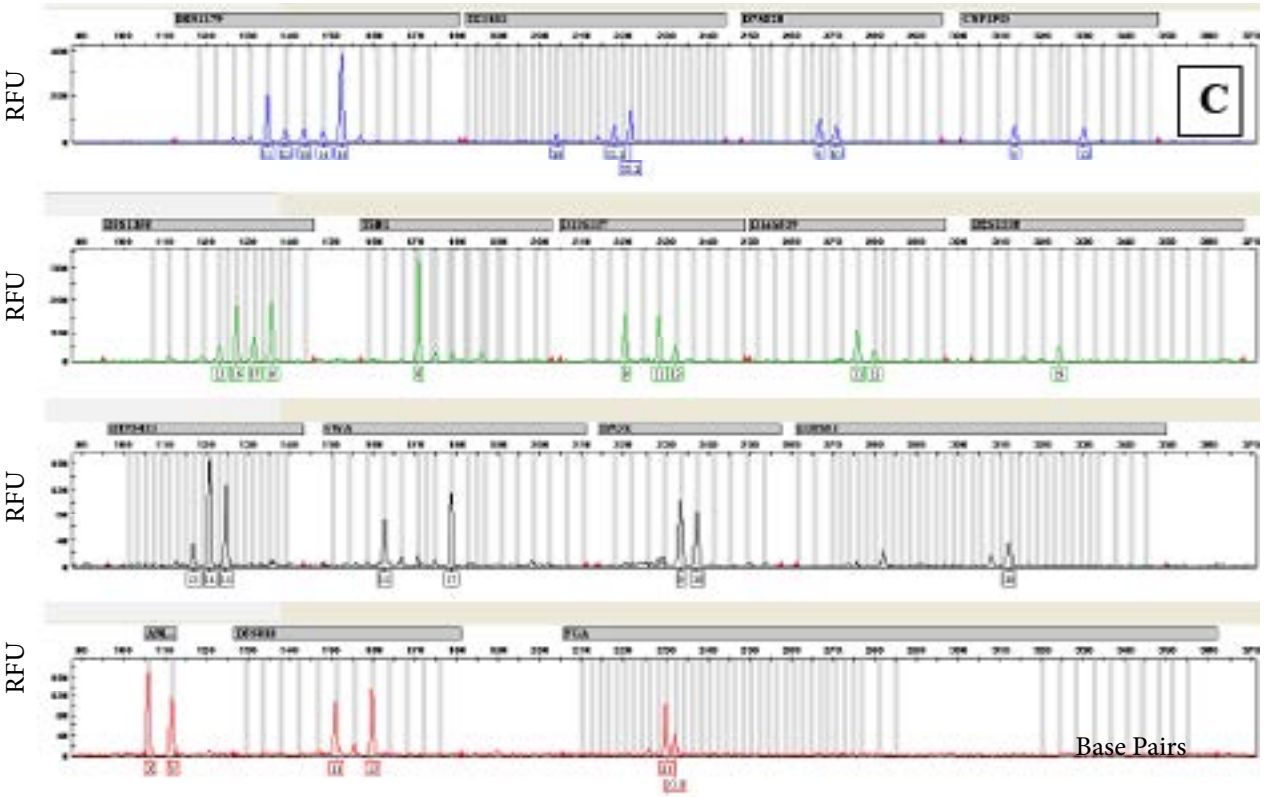

Figure 3C- *Electropherogram showing the Identifiler Plus STR profile for the femur bone extracted with EZ1 Advanced XL with some drop outs and drop in, high baseline noise, and reduced peak heights.*

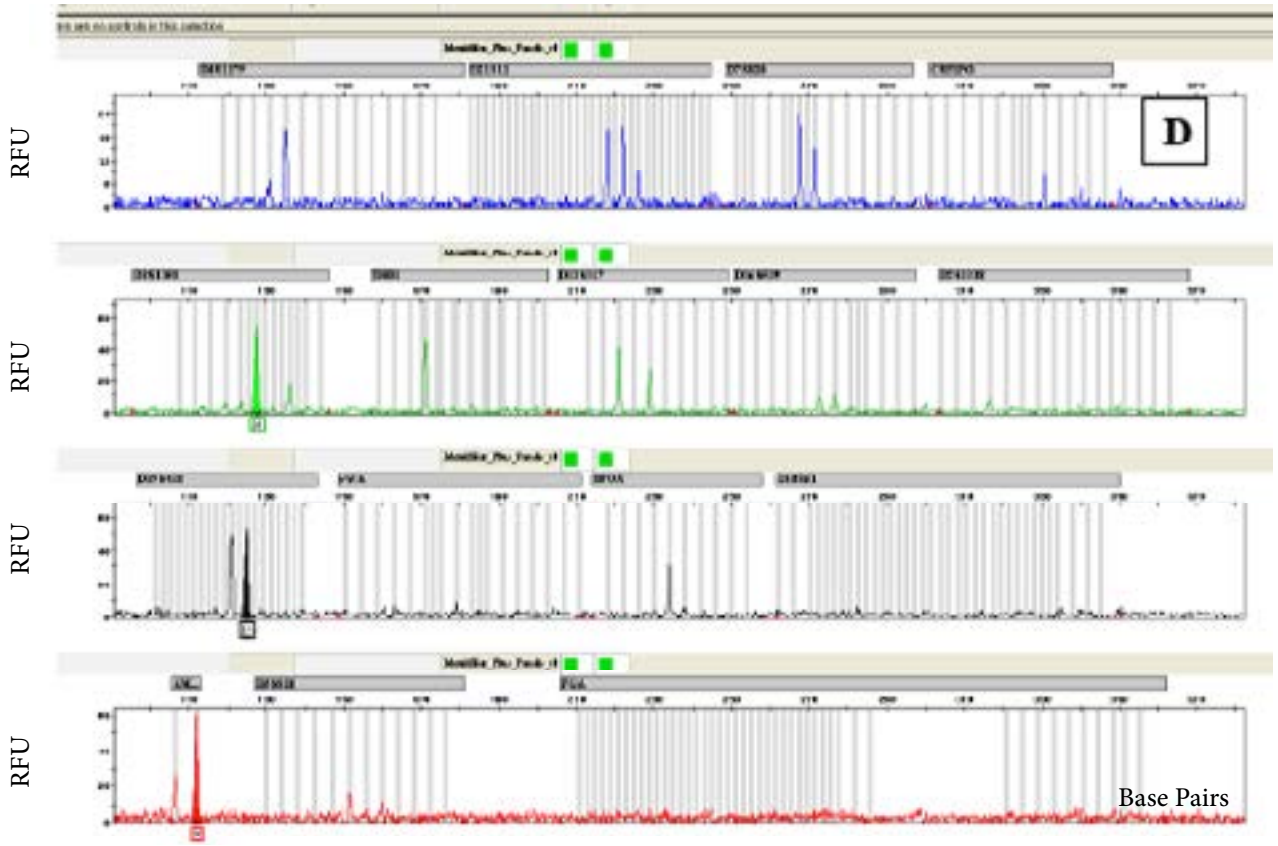

Figure 3D- *Electropherogram showing the Identifiler Plus STR profile for the femur bone extracted using organic method (phenol: chloroform:isoamyl alcohol) with low signals and locus drop outs.*

reliable than an anthropological approach. The automation of the extraction step can yield better results, producing cleaner extracts in a shorter time. Also, extraction efficiency is affected with the type of sample processed and the part of the bone recovered.

Acknowledgement

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