IDENTIFICATION BY mtDNA OF EXCHANGED HUMAN BODY REMAINS

Carla Cruz, Teresa Ribeiro, Claudia Vieira-Silva, Isabel Lucas, Helena Geada and Rosa Espinheira

1.Forensic Genetics, National Institute of Legal Medicine, Lisbon*, Portugal

2. Forensic Genetics, Faculty of Medicine, Lisbon, Portugal

INTRODUCTION

MtDNA offers some advantages over genomic DNA markers for the identification of human remains [1, 2]. Its sequence is completely determined and the high copy number increases the chance to obtain mtDNA in cases of limited quantiy or degraded autosomal DNA. The maternal mode of inheritance without recombination during the meiosis process, allows maternal lineage identification by a simple direct comparison of the mtDNA sequence of mother and child or between brothes and sisters [3].

CASE HISTORY:

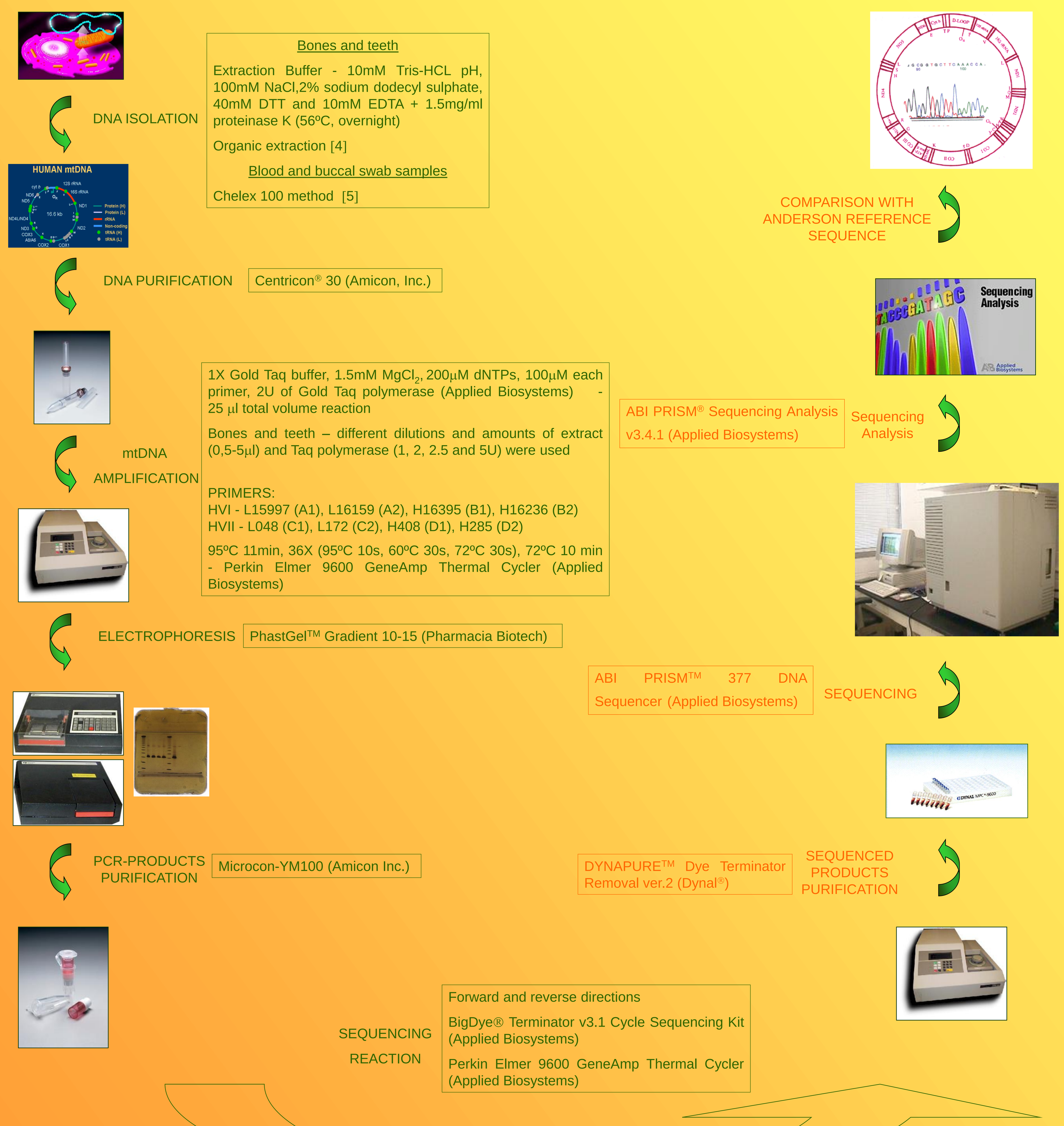
Five Portuguese citizens were killed in African. Three of them were identified and removed to Portugal, where they were buried. Two children were reported as missing. One year later, two skeletons were found in a grave 500 metres from the crime scene. Genetic typing of DNA extracted from skeletal remains was performed in order to establish their identities. One of the mothers of the two missing minors was excluded from the maternity of the skeletal remains. The hypothesis was raised of an identity exchange of the three corpses previously removed to Portugal. These were then exhumed and subjected to genetic analysis to achieve biological identification.

AIM

In this report we present the results of mtDNA sequence analysis of human body remains of five individuals and their presumptive mothers. MtDNA analysis was applied to determine the maternal relationship and, therefore, a fraternal relationship among the skeletal remains.

MATERIALS AND METHODS

Bones and teeth from five deceased individuals powdered in a 6800 freezer mill (Fisher Bioblock) and blood and buccal swab samples of four alleged mothers were subjected to the following protocol:



RESULTS

- We could obtain the mtDNA sequence in all but one sample after some changes to the protocol (Figure 1 and 2). The sample that didn't yield results was a bone of one of the corpses.
- Almost all the samples were successfully amplified with the pairs of primers A1/B1 and C1/D1. We didn't obtain results for HVII region and for HVI region from the teeth of bodies 2 and 4, respectively. Shorter overlapping fragments were amplified with the pairs of primers C1/D2, C2/D1 (for body 2), A1/B2, A2/B1 (for body 4) and the reconstruction of HVII and HVI sequences were performed.
- Forward and reverse sequencing allowed sequence confirmation. Reproducible mtDNA sequences were obtained from different samples of the same human body.
- In comparison with the HVI region of Anderson sequence, we detected 13, 2, 9, 4 and 2 nucleotide substitutions in sequences of bodies 1 to 5, respectively (table1). Regarding the HVII region, we observed 9, 7, 9, 6 and 7 substitutions, respectively (table 1). All samples presented a guanine at position 263 (HVII) instead of the adenine of Anderson sequence and six cytosines not five at position 310-314 .
- MtDNA sequence of each body matched with one of the alleged mothers sequence. The substitution points and nucleotides were the same in bodies 2 and 5 and were observed in presumptive mother 3.
- The results revealed that none of the three bodies removed subsequently and buried in Portugal had been correctly identified.

Nucleotide Position

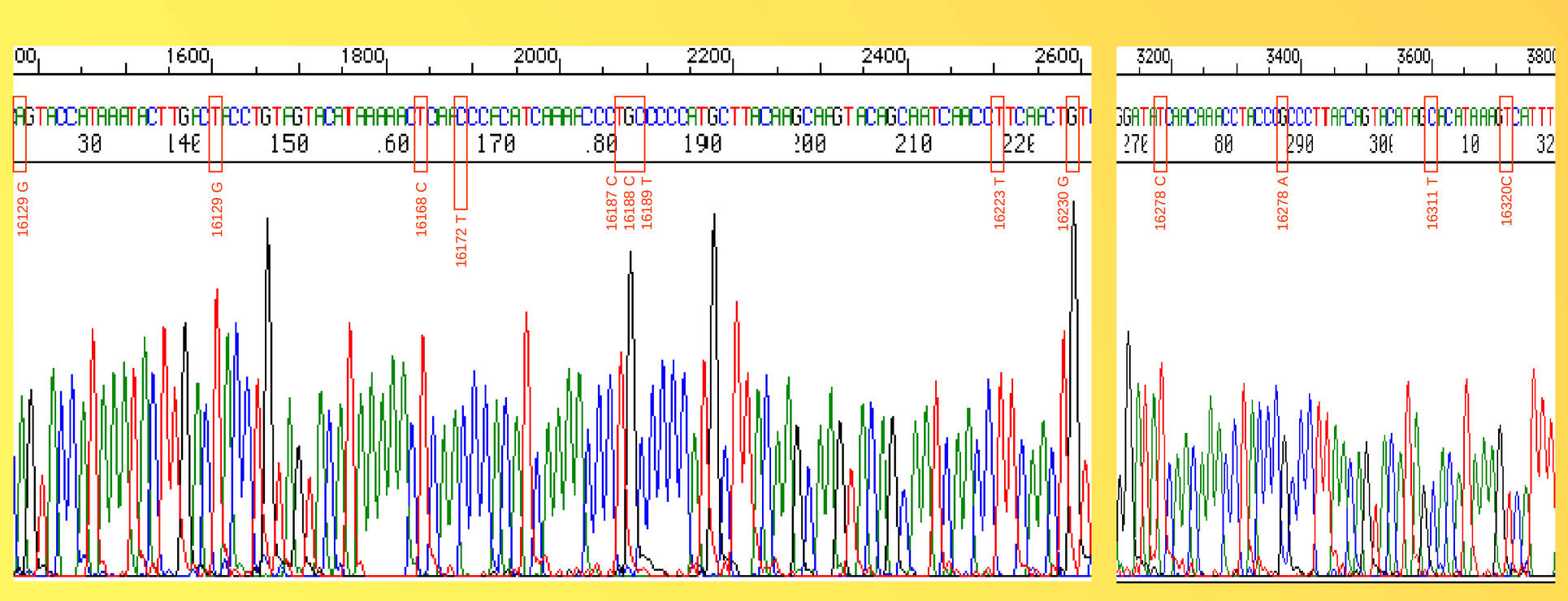
HVI

Sample origin	16069	16126	16129	16148	16168	16172	16187	16188	16189	16193	16223	16224	16230	16264	16270	16278	16293	16311	16320
Anderson	C	T	G	C	C	T	C	C	T	C	C	T	A	C	C	C	A	T	C
Body 1	.	.	A	T	T	C	T	G	C	.	T	.	G	.	.	T	G	C	T
Body 2	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.
Body 3	.	C	.	.	.	.	T	.	C	.	T	.	.	T	T	T	G	C	.
Body 4	T	C	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.
Body 5	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.
Mother 1	.	.	A	T	T	C	T	G	C	.	T	.	G	.	.	T	G	C	T
Mother 2	.	C	.	.	.	.	T	.	C	.	T	.	.	T	T	T	G	C	.
Mother 3	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	C	.
Mother 4	T	C	.	.	.	.	.	.	.	T	.	.	.	.	.	T	.	.	.

HVII

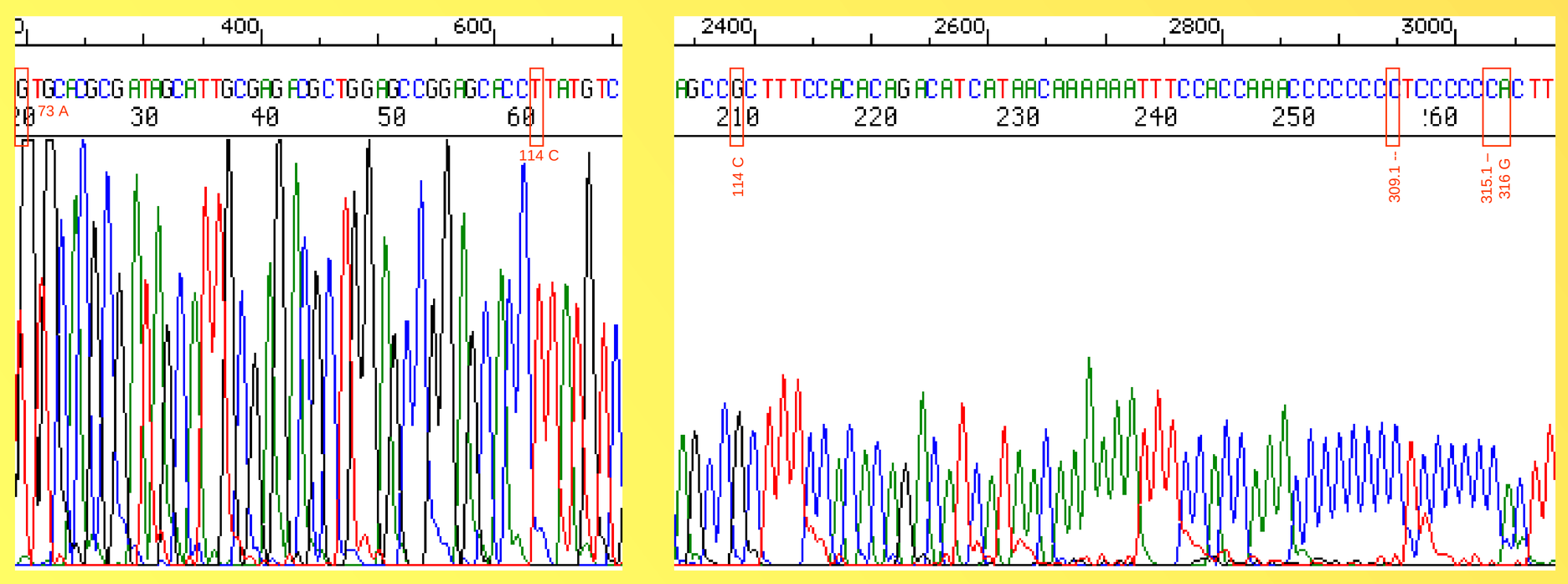
Sample origin	73	93	95	114	150	152	182	185	189	195	198	236	247	263	295	309.1	315.1	316	
Anderson	A	A	A	C	C	T	C	G	A	T	C	T	G	A	C	--	--	G	
Body 1	.	G	C	.	.	.	.	G	G	.	.	C	A	G	.	C	C	.	
Body 2	G	.	.	T	.	.	.	.	.	.	.	.	.	G	.	C	C	A	
Body 3	G	.	.	.	.	C	T	T	.	C	T	.	A	G	.	.	C	.	
Body 4	G	.	.	.	T	.	.	.	.	.		.	.	G	T	.	C	.	
Body 5	G	.	.	T	.	.	.	.	.	.	.	.	.	G	.	C	C	A	
Mother 1	.	G	C	.	.	.	.	G	G	.	.	C	A	G	.	C	C	.	
Mother 2	G	.	.	.	.	C	T	T	.	C	T	.	A	G	.	.	C	.	
Mother 3	G	.	.	T	.	.	.	.	.	.	.	.	.	G	.	C	C	A	
Mother 4	G	.	.	.	T	.	.	.	.	.	.	.	.	G	T	.	C	.	

Table 1- HVI and HVII mtDNA sequences of the five individuals and four alleged mothers compared with reference Anderson sequence. A letter in each position indicates a nucleotide substitution relative to the reference sequence and the absence means that the sequence is according reference sequence. Double dash indicates the positions where there is no nucleotide on Anderson sequence.



A) B)

Fig.1. HVI control region sequence from teeth of body 1 (PCR- 5μl of DNA dilution 1:20; 2.5U Taq polymerase) with base variations when compared to the Anderson Sequence. A) nuceotide positions 16129-16231; B) nucleotide positions 16273-16325.



A) B)

Fig.2. HVII control region sequence from bone of body 5 (PCR- 1μl of DNA dilution 1:20; 2U Taq polymerase) with base variations when compared to the Anderson Sequence. A) nuceotide positions 73-120; B) nucleotide positions 259-319.

CONCLUSIONS

- Although teeth and bones are reliable sources of DNA, it was difficult to obtain results namely from those retrieved in Africa. As we expected, these required more modifications to standard protocol. The state of the bones samples from this continent was poor, probably due to the humidity, temperature and other African environmental conditions and soil characteristics that accelerate the degradation process.
- Low amount, high fragmentation and contamination of the DNA are the main problems associated with this kind of samples [6]. In some cases, increasing the amount of DNA helped to overcome the first problem, but in some others lead to negative results, which can be explained by an increase of Taq polymerase inhibitors in the mix reaction. Regarding the degraded DNA, the application of different primers to the HVI and HVII regions allowed the amplification of two shorter overlapping fragments for each region and the reconstruction of HVI and HVII sequences. Several dilutions of DNA extract were tested to decrease the hypothetical contaminants that would act as Taq polymerase inhibitors.
- The coincidence of each of the five body sequences with one of the alleged mothers, suggest a maternal relationship. A fraternal relationship was detected between bodies 2 and 5. These share the same mtDNA sequence with mother 3. The results supported the hypothesis of exchanged human body remains. Autosomal STR loci analysis was performed and confirmed the identity of the human remains.
- MtDNA analysis was a useful tool to solve this case. Although it cannot be used to definitely identify the corpses, it provided evidence of a maternal relationship and, consequently, the exchange of human body remains.

REFERENCES:
[1] Wilson MR, DiZinno JA, Polanskey D, et al. Validation of mitochondrial DNA sequencing for forensic casework analysis. Int J Legal Med 1995;108: 68-74.
[2] Holland MM, Fisher DL, Mitchell LG, et al. Mitochondrial DNA Sequence Analysis of Human Skeletal Remains: Identification of Remains from The Vietnam War. J Forensic Sci 1993; 38 (3): 542-553.
[3] Lutz S, Weisser HJ, Heizmann J, et al. mtDNA as a tool for identification of human remains: Identification using mtDNA. Int J Legal Med 1996; 109: 205-209.
[4] Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, pp. E3-E4.
[5] Walsh PS, Metzger DA, Higuchi R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 1991; 10: 506-513.
[6] Poinar HN. The top 10 list: criteria of authenticity for DNA from ancient and forensic samples. Prog. Forensic Genet. (9) 2003; 575-579.

* bio.lisboa.inml@mail.pt