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Mitochondrial DNA analysis of ancient human teeth from a XVIth century archeological excavation

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Abstract

Our laboratories are involved in a large-scale study investigating the different hypotheses about the founding Canary Islands populations—making use of physical–anthropological, genetic and archeological approaches. The main problems associated with ancient DNA studies are low yield, high fragmentation and contamination with exogenous DNA. In this study, we tried to evaluate the viability of our laboratory set-up to accomplish mitochondrial DNA (mtDNA) isolation and analysis from ancient samples. We present preliminary results of HVI region mtDNA sequencing of several teeth from the Monastery of Los Silos (North of Tenerife Island), an archeological excavation site dating back to the XVIth century, used as a graveyard for some time. We also make some considerations about the amplification yield by comparing two different PCR strategies.

Keywords: Ancient DNA; Mitochondrial DNA; Sequencing

1. Introduction

Our laboratories are involved in a large-scale study whose purpose is to investigate the different hypotheses about the founding Canary Islands populations, making use of physical–anthropological, genetic and archaeological approaches. The study of mitochondrial DNA (mtDNA) variation represents a powerful tool for the interpretation, not only of

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phylogenetic and evolutionary studies, but also the tracing of population dynamics and the reconstruction of demographic history.

2. Objective

The main problems associated with genetic studies of ancient samples are the low DNA yield, the high fragmentation and the contamination with exogenous DNA, both external- and laboratory-induced. The main objective of this work is to evaluate the viability of our laboratory set-up to perform mitochondrial DNA isolation and analysis from ancient samples, as a preliminary step for a wider project to study the genetic structure and origin of the old Canarian populations.

3. Material and methods

To accomplish this goal, we analyzed 15 teeth from the Monastery of Los Silos (North of Tenerife Island), an archaeological site dated back to the XVIth century, used as a graveyard for some time, that was excavated in 1996.

The teeth surfaces were cleaned with cotton swabs moistened with bleach. Then they were pulverized individually by being crushed between a pair of heavy steel plates as previously described [1], and digested with proteinase K, SDS and DTT for 6 h at least. Then DNA was isolated with phenol–chloroform and Centricon-30® devices (Millipore). Amplification reactions were performed using standard reagents, including Taq Gold® (Applied Biosystems), with a profile of 36 cycles and annealing at 58 °C. PCR products were quantified in agarose gels and, after primer removal, about 30 ng product were extended using the dRhodamine Terminator Cycle Sequencing™ kit (Applied Biosystems). Extension products were cleaned with home made Sephadex-G50 columns, and analyzed with an ABI Prism 310™ Genetic Analyzer. Sequences were edited using Sequencing Analysis™ and SeqEd™ software packages (Applied Biosystems).

All the isolation and amplification procedures were made in a laminar flow hood, using autoclaved and UV treated material. Negative controls of extraction and amplification

Table 1
Primers used to generate six overlapping fragments spanning the HVI region. Sequences of primers can be found in Refs. [4,5]

Primers		Fragment size (bp)
Forward	Reverse	
L15997 (A1)	H16071 (A1R)	113
L16055 (1F)	H16139 (1R)	124
L16131 (2F)	H16218 (2R)	126
L16209 (3F)	H16303 (3R)	133
L16287 (4F)	H16379 (4R)	131
L16347 (5F)	H16401 (5R)	93

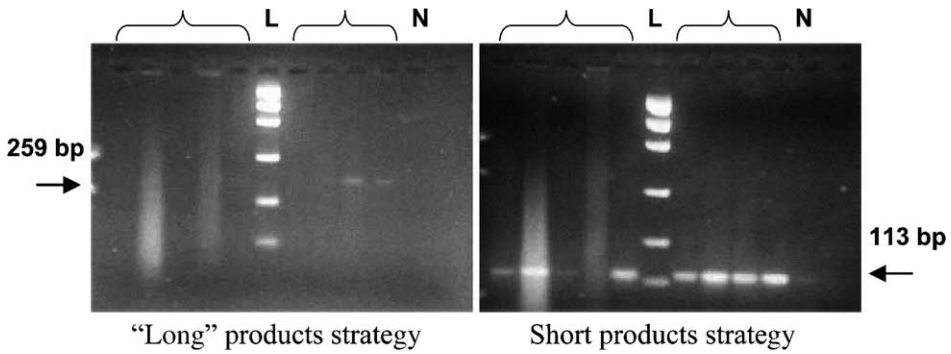

Fig. 1. Comparison of the efficiency between both amplification strategies. Brackets show samples analyzed. N = Negative control of extraction.

were always made for every pool of pieces processed. To resolve ambiguities, forward and reverse sequencing were performed for all PCR products. When more than one piece was available from one mandible, the overall process was repeated for the other piece by another operator.

4. Results and discussions

The initial strategy for the amplification of the HVI mtDNA region was based on two overlapping fragments [2]. This attempt generated specific PCR products, but not enough in quantity to perform sequencing reactions. Because ancient DNA is normally degraded to fragments 100–200 bp in length [3], a strategy based on the amplification of shorter fragments was chosen, to increase the chances of success. Table 1 shows the 12 primers used (previously described in Refs. [2,4,5]) in six separate PCR reactions, that generated six overlapping products to make possible the reconstruction of the HVI region. Fig. 1 shows a comparison of efficiency between both amplification strategies. The six different amplifications generated similar amounts of PCR products, when one sample is considered. The reconstruction of the HVI region was possible by overlapping the sequences

Table 2
Sequences obtained after reconstruction of HVI region, as discrepancies with reference sequence [6]

Sample	Discrepancies with Anderson sequence
13319	16311 C
217	No discrepancies
227	No discrepancies
232	16223 T, 16292 T
213a	16189 C, length heteroplasmy
214a	No discrepancies
215a	No discrepancies
212a	16293 G, 16304 C

from the six fragments. We obtained final results just in 50% of the samples processed (Table 2), possibly due to the humid and warm conditions of the location, that could accelerate degradation processes.

5. Conclusions

(1) The teeth pulverization method by using two heavy steel plates is fast and inexpensive, yields a powder fine enough for a successful extraction, and prevents contamination due to the laboratory personnel [1].

(2) The amplification of >200-bp mtDNA fragments generally yields insufficient product for sequencing, so the strategy using primers to amplify shorter overlapping fragments is more successful.

(3) No appreciable difference of yield was detected between intact and fractured pieces, which is in agreement with the idea that the major concentration of DNA is inside the dentine rather than inside the pulp (data not shown).

(4) Ancient DNA samples often produce noisy sequencing electropherograms that make it necessary to use special care in the sequence editing step. Attention must be paid to the basecalling method and the matrix file used.

(5) Our amplification strategy, based on six short overlapping fragments, often consumes all of the DNA sample, making it impossible to repeat the study to confirm results.

(6) Although our study was technically successful, we consider it necessary to develop a *multiplex-PCR* to analyze mtDNA in ancient samples, which would make it possible to use less sample volume and allow studies in other regions (HVII, rRNA, *Alu*I site, etc.) [4].

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