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Research Article

Application of the iPLEX™ Gold SNP genotyping method for the analysis of Amerindian ancient DNA samples: Benefits for ancient population studies

Important developments in the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) technique have generated new perspectives regarding SNP genotyping, which are particularly promising for ancient population-based studies. The main aim of the present study was to investigate the application of a MALDI-TOF MS-based SNP genotyping technique, called iPLEX® Gold, to analyze Amerindian ancient DNA samples. The first objective was to test the sensitivity of the method, which is recommended for DNA quantities between 10 and 5 ng, for ancient biological samples containing DNA molecules that were degraded and present in minute quantities. The second objective was to detail the advantages of this technique for studies on ancient populations. Two multiplexes were designed, allowing the major Amerindian mitochondrial and Y haplogroups to be determined simultaneously. This analysis has never been described before. Results demonstrated the reliability and accuracy of the method; data were obtained for both mitochondrial and nuclear DNA using picogram (pg) quantities of nucleic acid. This technique has the advantages of both MS and minisequencing techniques; thus, it should be included in the protocols for future ancient DNA studies.

1 Introduction

Mass spectrometry (MS), initially developed for proteomics, has rapidly become widespread for the analysis of nucleic acids [1, 2]. Indeed, several SNP genotyping methods based on MS have been developed using different platforms, including electrospray ionization time-of-flight (ESI-TOF) [3, 4] or matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF). SNP genotyping by MALDI-TOF MS emerges as a very powerful and attractive approach [5, 6], due to the speed of signal acquisition, direct measurement of the intrinsic properties of molecules and, finally, the possibility of a complete automation from the sample preparation to data acquisition [7]. Therefore, numerous genotyping methods, based on diverse types of allelic discriminations, have been developed [8–10]. Among them, protocols based on a single base primer extension were targeted and incessantly improved [4, 5, 8]. Today, SNP genotyping turn-key methods like the iPLEX® Gold technology, developed by the Sequenom company, are available [11]. This method has major advantages, including those of MS, as well as the high capacity of multiplexing (up to 40 SNPs can be analyzed simultaneously) [12], and an ability to work with small-sized amplicons (around 100 bp). These advantages are very important when working on ancient or degraded DNA material.

Ancient or degraded DNA molecules present characteristics that make their analysis challenging: they are most often fragmented, present in minute quantities and co-extracted with inhibitive substances. The preparation and analysis of ancient DNA samples requires rigorous and appropriate protocols [13, 14]. Most of the studies on past human population are based on the sequencing of the first and sometimes the second hypervariable segment (HVS-1, HVS-2) of the mitochondrial DNA (mtDNA) control region. In some of these studies, sequencing is completed by the

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Abbreviations: HVS-1, mitochondrial first hypervariable segment**; mtDNA,** mitochondrial DNA**; mt-SNP,** mitochondrial DNA SNP

analysis of haplogroup-tagging SNPs of the mtDNA coding region. Until recently, this typing was done mainly by the RFLP method [15], which is a time- and sample-consuming method, or sometimes by SNaPshot® [16]. It is important to find alternative techniques, which allow greater degrees of discrimination than those achieved by the analysis of a restricted portion of the mitochondrial genome. In this framework, the recent development of high-throughput sequencing techniques [17, 18] has brought new perspectives concerning the ancient DNA problematic [19]. However, complete genome sequencing of ancient human samples remains limited to the analysis of very precious samples whose evolutionary history could be highly relevant [20]. The high cost of the complete genome sequencing approach does not allow a large number of ancient samples to be tested; thus, it cannot be used for population studies. Moreover, due to the limited quantity of endogenous DNA available in the majority of ancient samples this approach is not necessarily appropriate. An alternative to complete genome sequencing can be to target SNPs by MALDI-TOF MS. This approach may prove to be promising for ancient DNA studies since it is described as an accurate, cost-effective, fast and sensitive method, ideally suited for the analysis of short DNA fragments. The only question concerns the quantity of DNA required. Indeed, all the genotyping technologies based on MALDI-TOF MS, and in particular the iPLEX® Gold method, require starting quantities of DNA between 5 and 10 ng. Such quantities are difficult to obtain with ancient DNA extracts; thus, the aim was to determine the validity of using this method for ancient DNA.

Various aspects were addressed during this study. First, we tested the sensitivity and accuracy of the method for analyzing very low quantities of DNA, by comparing the results with those obtained by traditional methods (sequencing and STR typing). The second aspect relates to the resolution achieved in the studies on ancient DNA. For this, we tried to develop assays which allowed Y and mitochondrial phylogenies to be analyzed at the same time, with the highest possible degree of discrimination. We also wanted to evaluate the benefits of the method for ancient population studies. For this purpose, we selected ancient samples from northwest Argentina in South America. The aim was to characterize the genetic diversity of an ancient population, the Omaguacas, in order to gain a better understanding of their origin and their genetic affinities with ancient and modern populations.

2 Materials and methods

2.1 Samples

The ancient samples analyzed come from the archaeological site of Los Amarillos (LA), located in the Humahuaca Valley in northwest Argentina (Supporting Information Fig. 1). This site was occupied for several centuries, from the beginning of the 11th century until the arrival of the Europeans during the 16th century [21]. The structure of the ancient Omaguacas population in the Humahuaca valley remains relatively unknown. Archaeological evidence suggests that this valley was located in the middle of a regional circuit for the traffic of goods, from approximately the 9th century [22]. Indeed, groups coming from various regions, such as south Bolivia, the Puna region and the eastern valleys and forests (Supporting Information Fig. 1), could have contributed to the composition of this population.

Genetic analyses were carried out on teeth samples carefully taken from 23 ancient skeletons (at least two teeth from each individual), which were excavated, from 1992 onwards, within the framework of the P.I.O. (Programa de Investigacion Omaguaca) project. The sex of each individual was not morphologically determined, but the analysis of the amelogenin sex marker allowed us to determine that the sample was composed of 12 males, 6 females and 5 individuals whose sex remained unknown. All these individuals are dated between the 11th and 15th centuries, due to radiocarbon dating [21].

To validate the results and to test the assays, DNA extracts of modern Amerindian samples were included in the study: four male samples and one female sample. Each of the four male samples belongs to the Y paragroup Q1a3a∗, typical of Native American populations [23], and to a different major Amerindian mitochondrial lineage: A2, B2, C1 and D1 [24–26]. The female sample belongs to the B2 mitochondrial haplogroup.

2.2 DNA extraction and quantification

DNA extraction was preceded by a decontamination step in order to eliminate surface contamination. Each tooth was cleaned with bleach, rinsed with deionized water and, finally, irradiated under UV light for 30 min on each face. Powder was generated by grinding the tooth under liquid nitrogen with a 6870 SamplePrep Freezer Mill® (Fischer Bioblock, Illkirch, France). DNA extraction was carried out using the NucleoSpin® Extract II kit (Macherey-Nagel, Düren, Germany), following the manufacturer's protocol, except for the buffer volumes. The first step of this extraction protocol consisted of an overnight incubation at 50°C of 150–200 mg of tooth powder in a lysis buffer containing 500 µL of EDTA (0.5 µM), 50 µL of proteinase K (20 mg/mL) and 5 µL of DTT (1 M). Second, the supernatant was purified onto NucleoSpin® Extract II silica columns (Macherey-Nagel) in three steps: (i) 1 mL of binding NT buffer was added so that the DNA bound to the silica membrane; (ii) 600 µL of NT3 ethanolic buffer was added in the washing step; and (iii) purified DNA was eluted on 250 µL using the NE buffer (5 mM Tris-HCl, pH 8.5). Finally, the extracts were concentrated using Amicon® Ultra-0.5 30 KDa columns (Millipore, Billerica, USA), in a final volume of 40 µL.

Quantification of all DNA extracts was made using the Quantifiler® Human DNA Quantification Kit (Applied Biosystems, Foster City, CA, USA), on an ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Courtaboeuf, France), following the recommended protocol of the company.

Precautions were taken to avoid contamination with modern DNA, as previously described in Carnese et al. [27].

2.3 Standard STR and mtDNA typing

Fifteen autosomal STRs (D8S1179, D7S820, D3S1358, D13S317, D16S539, D2S1338, D19S433, D5S818, D21S11, CSF1PO, vWA, THO1, TPOX, D18S51 and FGA) and the sex-determining marker amelogenin were amplified using the AmpFlSTR® Identifiler™ kit (Applied Biosystems). Seventeen Y-chromosomal STR loci (DYS19, DYS385a/b, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and Y GATA H4) included in the AmpFlSTR® Y-filer™ Kit (Applied Biosystems) were analyzed for the DNA of the ancient male samples. The experimental conditions were those recommended by the manufacturer, except that 34 PCR cycles were used instead of 28 and 30 for the Identifiler and Y-filer amplification kit, respectively. The amplified autosomal and Y-chromosomal STR products were analyzed on an ABI Prism 3100 Genetic Analyzer (Applied Biosystems) using the GeneMapper software, version 3.2.1 (Applied Biosystems).

Sequencing of the mitochondrial HVS-1 portion was carried out using two sets of primers as described previously in Carnese et al. [27]. To evaluate the accuracy of the method, mitochondrial haplogroups were determined by MS genotyping and were compared to those determined from the sequencing of HVS-1.

2.4 SNP selection

In an attempt to develop an assay based on the simultaneous typing of mitochondrial and Y-chromosome SNPs in the population studied, we selected from the literature [23–26, 28, 29] a set of 24 mitochondrial and Y-chromosome markers that define the major mitochondrial and Y-chromosome Amerindian haplogroups (Fig. 1).

Concerning the mitochondrial lineages, the 13 selected SNPs permit the definition and refinement of the A2, B2, C1 and D1 haplogroups, which are the most frequently observed haplogroups in Amerindian populations [24–26]. Since the iPLEX® Gold method has not been described for low quantities of DNA, we decided to select several markers, which are characteristic of the same haplogroup, in order to optimize the probability of an interpretable result.

Regarding the Y haplogroups, the Amerindian populations present a low genetic diversity. Two major haplogroups are described: the Q haplogroup, which is the most prevalent, and the C haplogroup. We selected 11 SNPs to define these two major haplogroups and also to refine sub-haplogroups of the Q lineage, including the Q1a3a sub-haplogroup, which is considered characteristic of Native American populations [23, 28, 29]. For these markers, the nomenclature used follows that of the Y-chromosome consortium [30].

2.5 iPLEX® Gold SNP genotyping method

The genotyping analysis was performed using the MassARRAY® compact system (Sequenom, San Diego, USA) associated with the iPLEX® Gold SNP genotyping kit (Sequenom).

The manufacturer's protocol was used [11, 31], except that the quantity of DNA used in the reaction was lower
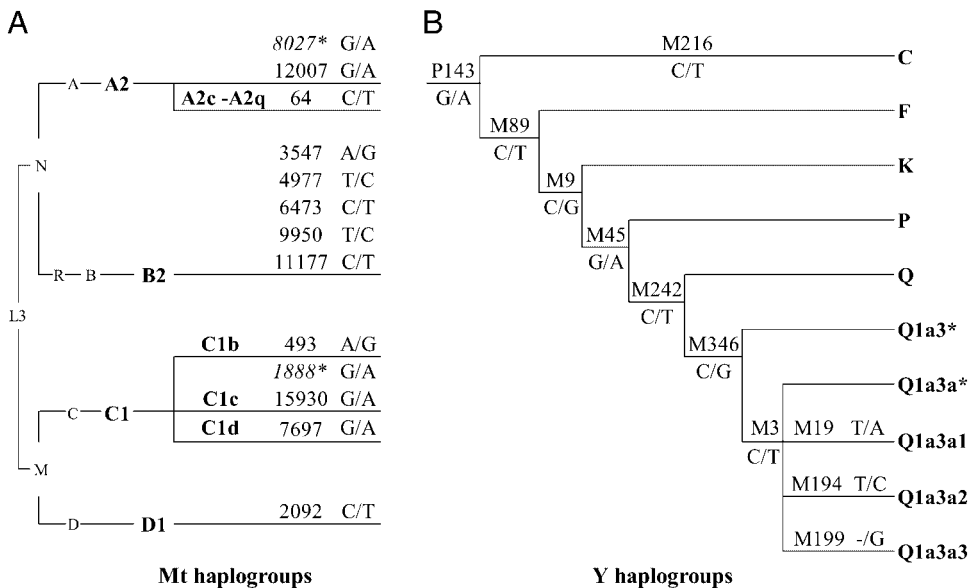
Figure 1. (A) From left to right: the characterized mitochondrial haplogroups (in bold), the positions of the selected markers on the mitochondrial DNA and the polymorphisms corresponding to these positions. The two markers in italics were rejected during the design by the software assay design. (B) Phylogenetic tree representing the 11 Y-chromosome markers selected, and the corresponding polymorphisms. The Y haplogroups characterized are indicated in bold.

than the recommended measure. With the aim of having the highest possible quantity of DNA, the volume of ancient DNA extract added in the reaction was increased from 2 to 3.4 µL. PCR amplifications were performed in a total reaction volume of 5 µL with $1 \times$ PCR buffer, $MgCl_2$ (2 mM), dNTPs (500 µM), PCR primer (0.1 µM each) and HotStar-Taq$^®$ (0.5 U). Prior to the mini-sequencing reaction, in order to digest unincorporated dNTPs, 2 µL of a solution containing SAP (0.5U) was added to the PCR reaction and incubated at 37°C for 40 min and 85°C for 5 min. The single base extension reaction was made by adding 2 µL of an iPLEX Gold extension reaction cocktail containing: $10 \times$ iPLEX buffer, $1 \times$ iPLEX termination mix, $1 \times$ iPLEX enzyme and primer mix. After a cleanup step with the SpectroCLEAN resin, extended products were arrayed onto a SpectroCHIP using the RS1000 nanodispenser. Finally, the products were detected with the MassARRAY mass spectrometer, and the data were acquired in real time with the MassARRAY$^®$ RT software. Alleles were automatically assigned by the software and associated to a reliable value.

Before genotyping valuable ancient samples, we undertook a preliminary step to test the sensitivity of the technique, consisting of a dilution range (from 10 ng to 5 pg of nuclear DNA per reaction) from modern DNA samples. We used one assay of 17 autosomal and Y-chromosomal SNPs, designed previously in the laboratory for another project (unpublished data).

To test the reproducibility of the iPLEX$^®$ method we analyzed two different extracts (obtained from two teeth), for each of the 23 ancient individuals. As recommended in the protocol, the deposits onto the SpectroCHIP were made in duplicate; we obtained four spectra for each ancient individual. From these four spectra, we determined a consensus genotype, keeping the alleles that appeared at least twice. Positive (modern Amerindian DNA) and negative controls (extraction and PCR controls, modern African DNA) were added during the genotyping process in order to detect any problems (e.g. contamination, false positive).

2.6 Primer design

The design of the amplification and extension primers was made using the MassARRAY$^®$ assay Design 4.1 software, associated with the MassARRAY$^®$ compact system. We tried to incorporate all the markers in the same assay; however, according to the software, the 24 selected SNPs were not compatible to be grouped into a single multiplex. Two mt-SNPs (mitochondrial SNPs) (A2_8027 and C1c_1888) were excluded from the assay. Finally, the software designed two multiplexes containing 11 mitochondrial and Y-chromosome SNPs each. All the amplification and extension primers used are listed in Supporting Information Table 1. Both multiplexes were applied to each sample, except the ancient female samples, for which only the second multiplex (plex 2) was analyzed.

3 Results

3.1 Quantification of the ancient DNA extracts and results of the standard STR and mitochondrial typing

The full set of quantification results, HVS-1 sequencing and STR typing obtained is reported in Supporting Information Table 2.

The quantifications carried out on the ancient DNA extracts showed concentrations ranging from 1.4 ng/µL (LA13) to values below the detection limit of the kit (1.07 pg/µL for LA1). DNA extracts of six individuals (LA2, LA5, LA6, LA11, LA19 and LA22) could not be quantified, since they were apparently too degraded.

The sequencing of HVS-1 resulted in the maternal lineages of 18 of the 23 analyzed individuals to be determined. Indeed, our sample was composed of 13 individuals belonging to the A2 lineage, three (LA9, LA16 and LA4) to the D1 lineage, one (LA17) to the B2 lineage and one (LA18) to the C1 lineage. It was not possible to determine the mitochondrial haplotypes of five samples: LA2, LA5, LA6, LA11 and LA19.

As shown in Supporting Information Table 2, we obtained relatively complete profiles with regard to autosomal and Y-chromosome STRs. For six of the ancient samples (LA1, LA2, LA5, LA6, LA11 and LA19), none of the loci, autosomal or Y-chromosome, were amplified. Partial profiles (with less than half of the loci typed) were obtained for five samples (LA7, LA10, LA12, LA22 and LA24). Regarding the remaining 12 individuals, we obtained complete or nearly complete STR profiles using the Identifiler and Y-filer kits.

Several data revealed that the DNA extracted from the ancient samples was endogenous: (i) the STR profiles of each ancient sample were unique and different from those of the operators and excavators; and (ii) the mitochondrial haplotypes obtained on sequencing of HVS-1 were concordant with the area under study.

3.2 Validation of the SNP genotyping assays

The dilution range performed prior to this study showed that the iPLEX$^®$ technique was reliable and sensitive (with a call rate greater than 85%) until quantities of 100 pg of nuclear DNA per reaction (unpublished data).

Following this sensitivity study, we chose to test the two multiplex systems designed for this analysis with modern Amerindian DNA extracts. For both multiplexes, all the selected markers were successfully amplified and the results of the SNP genotyping were concordant with the expected haplogroups of the modern individuals (Supporting Information Table 2). In addition, this first test with modern DNA underlined one problem: unexpected peaks appeared systematically with the modern female samples and the negative controls for the Y-chromosome markers CF_P143

and Q1a3a2_M194 (Supporting Information Table 2). Several hypotheses can be postulated to explain the appearance of these unexpected peaks. The first hypothesis is that of contamination. However, in the negative PCR controls (without DNA), only these two unexpected peaks appeared. If it was a contamination with exogenous DNA or a cross-contamination between samples, other peaks would have been appeared. The second hypothesis is that of non-specific primers. However, all the primers used were compared against the human DNA sequence in GenBank and appeared to be specific for the Y-chromosome. The third and most probable hypothesis is that of an artifact induced by the biochemistry of the kit [32]. For ancient female samples or ancient samples of unknown sex, the appearance of such unexpected peaks can cause doubts in the inter-pretation of results. It is therefore important to test the designed assays to locate these artifacts, in order to be able to interpret the results correctly.

3.3 SNP genotyping of ancient samples

The genotyping call rate for the mt-SNPs, calculated from all the spectra obtained for the 23 ancient individuals, was 96.4%. The results obtained for the Y markers were more differentiated. Indeed, the call rate was about 74% for the 12 ancient male samples, decreasing to 62% when the results of the ancient samples of unknown sex were added.

No discordant alleles were observed in the four different spectra obtained for each ancient specimen, which concludes that the iPLEX® Gold method is highly repro-ducible. The consensus genotypes obtained are described in Table 1. With this method, the determination of both maternal and paternal lineages was possible from the same manipulation. An example of spectra obtained is shown in Fig. 2, illustrating the differences between a male and a female spectrum.

As we can see in Table 1, the determination of both mitochondrial and Y haplogroups was possible for each quantified extract. The non-quantified extracts presented rela-tively complete spectra; ranging from two markers out of 11 (LA5) to a complete spectra for some individuals (LA2, LA6 and LA22). Mitochondrial haplogroups determined from the SNPs genotyping were concordant with the expected haplogroups deduced from the HVS-1 sequencing (Supporting Information Table 2), showing the reliability of the method. In addition, SNP genotyping has allowed the refinement of some haplogroups (A2a-A2b for LA7, and C1b for LA18), and the determination of the maternal lineages for two samples (A2 for LA2 and LA6) whose HVS-1 portion could not be sequenced.

3.4 Genetic variation observed in the Los Amarillos ancient samples

We observed in this ancient population a high prevalence of the mitochondrial haplogroup A2 (15 individuals of the 23 studied). It is interesting to note that the specimen LA7 presents the allele C for the A2_64 marker (Table 1) and thus, it belongs to a different maternal lineage from those of the other 14 samples belonging to the A2 haplogroup.

The diversity of the Y lineages is limited. Indeed, of the 12 male samples, 8 belong to the Q1a3a* paragroup, 2 belong to Q1a3* paragroup, and the remaining 2 probably also belong to the Q1a3a* paragroup, but 1 marker (Q1a3a1_M19) is lacking to confirm this.

4 Discussion

4.1 Behavior of the iPLEX® technology with ancient samples

The SNP genotyping of ancient Amerindian samples, for which DNA is fragmented, chemically modified and present in a low copy number, was an interesting means to test the sensibility of the iPLEX® method. The results obtained show the possibility of genotyping SNPs by MALDI-TOF MS from ancient biological samples contain-ing few molecules of degraded DNA. Concerning mtDNA, we have not reached the detection limit for alleles. Thus, the use of Sequenom technology allows information on ancient degraded samples to be collected [33]. However, for the reliable detection of nuclear DNA, more than 100 pg of DNA per reaction is required to obtain complete genotypes.

The two multiplexes that we developed allowed the discrimination of the major Amerindian maternal and paternal lineages at the same time, and thus, permitted the quantities of DNA extract used (3.4 µL) to be optimized. The quantity of DNA used in analyses is often a limiting factor in many ancient population studies; thus, the possibility to determine mitochondrial and Y haplogroups with one assay is an interesting advancement and perspective. We can plan, in the future, to increase the multiplexing degree of the assays (especially for mtDNA) and to integrate new markers, allowing the branches of the Amerindian phylogeny to be detailed further.

With regard to our study sample, this method allowed the genetic diversity of the Los Amarillos site to be characterized. The diversity of maternal lineages described in our sample is different from that commonly observed in ancient Andean populations, which are usually characterized by a strong dominance of the B2 haplogroup [34–36]. The paternal lineages, however, correspond to the expected diversity. Indeed, the dominance of the Q1a3a* paragroup has been reported in other Amerindian populations [37, 38]. Never-theless, the reduced sample size of 23 individuals could introduce a bias in the study, modifying the real genetic diversity of the Omaguacas. Indeed, more samples are required to better understand the genetic affinities of the Omaguacas.

Table 1. Consensus results obtained for the 23 ancient samples of the Los Amarillos site. The markers in grey correspond to the Y-chromosome SNPs

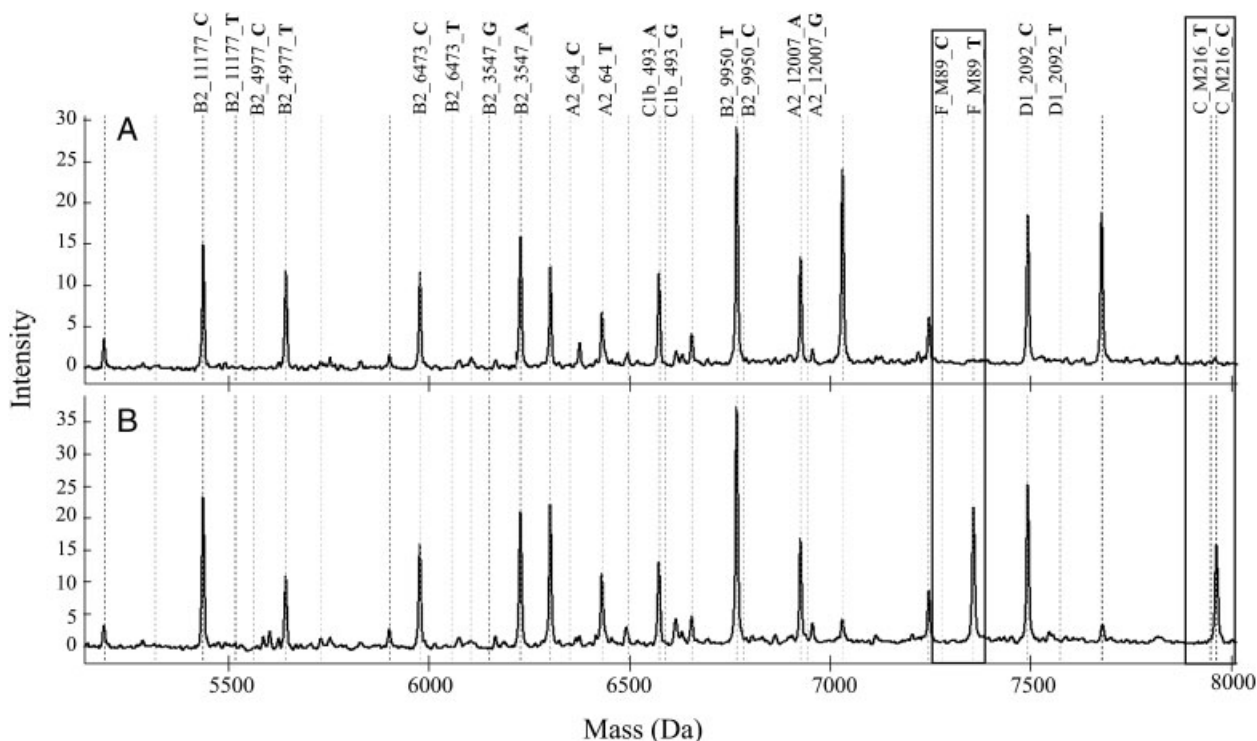|	PLEX_1												PLEX_2										Haplogroups	
	CF_P143	K_M9	P_M45	Q_M242	Q1a3_M346	Q1a3_M3	Q1a3a1_M19	Q1a3a2_M194	Q1a3a3_M199	C1c_15930	C1d_7697	A2_12007	A2_64	B2_3547	B2_4977	B2_6473	B2_9950	B2_11177	C1b_493	D1_2092	F_M89	C_M216	mt	Y
Ancient male samples																								
LA3	A	G	A	T	G	T	T		DEL	G	G	G	C	A	T	C	T	C	A	T	T		D1	Q1a3a*
LA4	A	G	A	T	G	T	T		DEL	G	G	A	T	A	T	C	T	C	A	C	T	C	A2	Q1a3a*
LA7	A	G	A		G	T			DEL	G	G	A	C	A	T	C	T	C	A	C	T		A2a-A2b	Q1a3a
LA9	A	G	A	T	G	T	T		DEL	G	G	G	C	A	T	C	T	C	A	T	T		D1	Q1a3a*
LA10	A	G	A		G	T			DEL	G	G	A	T	A	T	C	T	C	A	C	T		A2	Q1a3a*
LA12	A	G	A	T	G	T	T		DEL	G	G	A	T	A	T	C	T	C	A	C	T		A2	Q1a3a*
LA16	A	G	A	T	G	T			DEL	G	G	G	C	A	T	C	T	C	A	T	T		D1	Q1a3a*
LA17	A	G	A		G	C				G	G	G	C	G	C	T	C	T	A	C	T		B2	Q1a3*
LA20	A	G	A	T	G	T	T		DEL	G	G	A	T	A	T	C	T	C	A	C	T		A2	Q1a3a*
LA21	A	G	A	T	G	T	T		DEL	G	G	A	T	A	T	C	T	C	A	C	T		A2	Q1a3a*
LA22	A	G	A		G	C			DEL	G	G	A	T	A	T	C	T	C	A	C	T		A2	Q1a3*
LA24	A	G	A		G	T			DEL	G	G	A	T	A	T	C	T	C	A	C	T		A2	Q1a3a
Ancient female samples																								
LA1												A	T	A	T	C	T	C	A	C			A2	
LA8												A	T	A	T	C	T	C	A	C			A2	
LA13												A	T	A	T	C	T	C	A	C			A2	
LA14												A	T	A	T	C	T	C	A	C			A2	
LA18												G	C	G	C	G	C	G	G	C			C1b	
LA23												A	T	A	T	C	T	C	A	C			A2	
Ancient samples of unknown sex																								
LA2	A									G	G	A	T	A	T	C	T	C	A	C			A2	/
LA5	A									G	G												/	/
LA6										G	G	A		A	T	C	T	C	A	C			A2	/
LA11										G	G	A	T	A	T	C	T	C	A	C			/	/
LA19	A											A	T	A	T	C	T	C	A	C			/	/

Figure 2. Example of the spectra obtained for the ancient female sample LA2 (A), and the ancient male sample LA4 (B), showing the peaks corresponding to the alleles of the ''plex 2.'' Both bordered markers are Y-chromosome SNP.

4.2 Benefits for ancient population studies

This study allowed us to emphasize several advantages of the iPLEX® method for ancient population studies. The first advantage of the iPLEX® technique is that it is a turn-key method. During analysis, we noted that it was a very easy and fast method to implement in the laboratory. Indeed, it allowed a quick set-up of assays and generation of results. In addition, the mt-SNP genotyping by MALDI-TOF MS provided the possibility, in a fast and effective way and by using small quantities of DNA extract: (i) to confirm the results obtained from the sequencing of the HV1 segment; (ii) to deepen the mitochondrial phylogenies determined from the HVS-1; (iii) to obtain information from very degraded samples even when the classical techniques (sequencing, STR analysis) did not work; and (iv) to authenticate the results obtained from ancient DNA extracts, by comparing different markers.

Concerning the Y SNPs, the results were more variable. However, it is also a very promising technique that allows a rapid definition of the Y-chromosome haplogroups. For ancient DNA studies, Y haplogroups are rarely analyzed, and, thus, we can imagine that such a technique could be used to explore the history of paternal lineages in ancient populations.

To conclude, the iPLEX® method is very promising for population-based studies, and in particular for ancient populations. Indeed, this technique is a good alternative to new technologies such as complete genome sequencing, since it optimizes the ancient sample resource and results in a greater degree of discrimination than that obtained by techniques currently routinely used in ancient DNA studies.

The authors have declared no conflict of interest.

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