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Short Communication

Ancestry informative markers: Inference of ancestry in aged bone samples using an autosomal AIM-Indel multiplex

Carola Romanini [a], Magdalena Romero [a], Mercedes Salado Puerto [a], Laura Catelli [a], Christopher Phillips [b], Rui Pereira [c], Leonor Gusmão [c,d], Carlos Vullo [a,*]

[a] Forensic DNA Laboratory, Argentinean Forensic Anthropology Team (EAAF) Independencia 644,3A, 5000 Cordoba, Argentina
[b] Forensic Genetics Unit, Institute of Legal Medicine, Faculty of Medicine, University of Santiago de Compostela, ES-15705 Santiago de Compostela, Galicia, Spain
[c] Institute of Molecular Pathology and Immunology of the University of Porto (IPATIMUP), Porto, Portugal
[d] DNA Diagnostic Laboratory (LDD), State University of Rio de Janeiro (UERJ), Rio de Janeiro, Brazil

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ABSTRACT

Ancestry informative markers (AIMs) can be useful to infer ancestry proportions of the donors of forensic evidence. The probability of success typing degraded samples, such as human skeletal remains, is strongly influenced by the DNA fragment lengths that can be amplified and the presence of PCR inhibitors. Several AIM panels are available amongst the many forensic marker sets developed for genotyping degraded DNA. Using a 46 AIM Insertion Deletion (Indel) multiplex, we analyzed human skeletal remains of post mortem time ranging from 35 to 60 years from four different continents (Sub-Saharan Africa, South and Central America, East Asia and Europe) to ascertain the genetic ancestry components. Samples belonging to non-admixed individuals could be assigned to their corresponding continental group. For the remaining samples with admixed ancestry, it was possible to estimate the proportion of co-ancestry components from the four reference population groups. The 46 AIM Indel set was informative enough to efficiently estimate the proportion of ancestry even in samples yielding partial profiles, a frequent occurrence when analyzing inhibited and/or degraded DNA extracts.

1. Introduction

The ancestry of a forensic evidence contributor, based on ancestry informative marker (AIM) analysis, can provide valuable information [1–7]. Although lineage markers from mitochondrial DNA (mtDNA) and Y chromosome give information on the maternal and paternal ancestries, respectively, they are haploid non-recombinant genetic markers that represent just a very small proportion of the genome. Therefore, the ancestry of admixed or non-admixed individuals is more comprehensively estimated when a selection of recombining autosomal genetic markers is used. It has been shown that ancestry informative single nucleotide polymorphisms (AIM-SNPs) are able to distinguish the major continental population groups relatively efficiently [5,7–10]. More recently, AIM-Indels [11,12] that share many of the characteristics seen in SNPs [2] were also found to reliably distinguish ancestry amongst the main continental populations of Africa, Europe, East Asia and America. In addition, Indels bring increased capability to detect mixed-source DNA, since they produce much more balanced peak patterns (within locus, the two alleles for a heterozygote show similar peak heights) than those of single base extension SNP tests. Of equal relevance to forensic analyses is the fact that SNP and Indel multiplexes used for identification purposes [13,14] or ancestry analysis [2–4,12,15] have been developed specifically to analyze very short amplicons to enable increased sensitivity in the genotyping of highly degraded biological material.

When dealing with challenging samples such as human skeletal remains, DNA degradation and/or the presence of PCR inhibitors from the soil may constrain the correct typing of the sample [16,17]. Typing mini-STRs or binary markers like SNPs or Indels through the PCR of short amplicon fragments (less than 200 bp) can overcome this problem in degraded DNA extracts [18,19]. The ability of the 46 AIM-Indel multiplex developed by Pereira et al. [12] to obtain reliable ancestry information, in combination with the relatively short fragment amplification strategy, can help to address the challenge of achieving useful ancestry information

* Corresponding author. Tel.: +54 351 4240434; fax: +54 351 4240418.
E-mail address: cvullo@fibercorp.com.ar (C. Vullo).

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from DNA extracts of extremely degraded samples. In order to evaluate the efficiency of the 46plex AIM-Indel set to ascertain ancestry on bones with variable degrees of DNA degradation, in the present study, DNA extracts from aged bones from routine casework were analyzed, from four different continental regions: Sub-Saharan Africa, Native America, Europe and East Asia. This report presents the results obtained when applying the AIM-Indel genotyping protocol to this skeletal material, as part of the identification process undertaken.

2. Materials and methods

2.1. DNA samples from aged bones

Bone samples included in this study were obtained from routine work with human skeletal remains for human identification purposes in different countries, as presented in Table 1. A total of 20 skeletal samples from different geographical locations were analyzed. Three remains belonged to victims of the Apartheid system in South Africa (1960–1994) of sub-Saharan African origin (AFR). The South African National Prosecuting Authority (NPA) set up the Missing Persons Task Team (MPTT) to identify victims who disappeared in the political oppression of that period and continue tracing their fate. The EAAF DNA laboratory has now been integrated into this identification project. Two East Asian origin remains (EAS) belong to victims of the Vietnam War (1959–1975). According to ante mortem information the victims were buried in 1968. In October 2012, the EAAF exhumed twelve remains that were submitted to anthropological and genetic analysis for identification purposes. Four East Timor remains (EAS) belong to the period of the Indonesian invasion of East Timor (1975–1999). Nine remains were collected from different Latin American countries to represent Native American origin (NAM): three belong to victims of the Argentinean military dictatorships (1976–1983), two are from victims of the Bolivian dictatorships (1964–1982) and four from the El Salvador War (1980–1992). Two samples from European individuals (EUR) were also analyzed; these two European origin samples were collected and identified in Argentina within the framework of the Latin American Initiative for the Identification of the "Disappeared" (LIID) conducted by EAAF [20] : both identified remains belong to a Welsh and an Austrian individual who disappeared in Argentina.

2.2. DNA extraction and AIM-Indel genotyping

The bone samples analyzed were different anatomical elements of femur, tooth or temporal bones. The post-mortem time ranged from 35 to 60 years, according to the anthropological and historical information obtained.

DNA extraction and quantification were performed according to Romanini et al. [19]. All DNA protocols were carried out in laboratories especially designed for DNA extraction from aged bone samples with facilities to minimize risk of contamination, following ISFG recommendations [21]. Plastic-ware used was DNA-free, autoclaved and UV irradiated as an additional precaution. Bone fragments were cleaned and sanded to remove outer and inner layers and then 0.5 cm disks were cut with a rotary tool in a Biosafe cabinet. All the following steps were made in sterile laminar flow cabinets. Reagent blanks accompanying the extraction procedure were processed and checked for contamination. After cleaning and sanding, samples were washed (2×) with sterile distilled water and decontaminated 2 min with 10% bleach, then washed (4×) with sterile distilled water, two times in 95% alcohol and left to dry. Bone pieces were then ground with a Spex 6750 cryogenic grinder freezer-mill (Spex Centriprep Inc.). Two grams of bone powder was decalcified with 20 ml EDTA 0.5 M pH 8 for 2 days at 4 °C with continuous rotation. Decalcified bone tissue was centrifuged and the supernatant discarded. Decalcified pellets and reagent blanks were then extracted using the QIAamp DNA Blood Maxi Kit (Qiagen) according to manufacturer's recommendations. The eluted DNA was concentrated to approximately 100 μl using Vivacon 2-100 MWCO columns

Table 1
Ancestry analysis data from STRUCTURE (cluster membership proportions) and *Snipper* Bayes analysis likelihood ratios.

Bone sample	Geographical origin	STRUCTURE cluster proportions				Snipper analysis (lowest likelihood ratio)
		AFR	EUR	EAS	NAM	
BS01	Bolivia	0.010	0.017	0.022	0.951	20,777 times more likely American than East Asian
BS02	Bolivia	0.035	0.338	0.007	0.620	148 times more likely American than European
BS03	East Timor	0.003	0.003	0.990	0.004	65,842,476,070 times more likely East Asian than American
BS04	East Timor	0.273	0.124	0.593	0.010	45,951 times more likely East Asian than European
BS05	East Timor	0.005	0.021	0.967	0.007	93,056,370 times more likely East Asian than American
BS06	East Timor	0.053	0.421	0.503	0.023	5.5 times more likely East Asian than European
BS07	El Salvador	0.012	0.305	0.010	0.673	17,413 times more likely American than European
BS08	El Salvador	0.026	0.564	0.048	0.362	44 times more likely European than American
BS09	El Salvador	0.275	0.716	0.004	0.005	6,446,150,707 times more likely European than African
BS10	El Salvador	0.025	0.579	0.009	0.387	6497 times more likely European than American
BS11	South Africa	0.963	0.004	0.020	0.013	150,334,685,781,751,083,565,056 times more likely African than East Asian
BS12	South Africa	0.980	0.006	0.006	0.008	163,899,108,611,827,657,867,264 times more likely African than European
BS13	South Africa	0.993	0.002	0.003	0.003	19,202,072,389,817,456,943,513,703,809,024 times more likely African than E Asian
BS14	Argentina	0.174	0.158	0.005	0.663	231,280 times more likely American than European
BS15	Argentina	0.007	0.279	0.025	0.689	17,489 times more likely American than European
BS16	Argentina	0.093	0.668	0.038	0.201	524,535 times more likely European than American
BS17	Argentina (Welsh)	0.006	0.963	0.010	0.021	5,238,127,897 times more likely European than East Asian
BS18	Argentina (Austrian)	0.002	0.918	0.022	0.058	469,647,983 times more likely European than East Asian
BS19	Vietnam	0.009	0.007	0.978	0.006	59,864,948,269 times more likely East Asian than American
BS20	Vietnam	0.003	0.019	0.968	0.009	4172 times more likely East Asian than European

NAM, Native America; EAS, East Asia; EUR, Europe; AFR, Africa. BS17 and BS18 from Argentina have known European origin, as indicated.