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Banco Nacional de Datos Genéticos and human identification of forensic cases

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Abstract. Molecular techniques based on DNA technology such as STRs typing (nuclear and Y chromosomes) and Mitochondrial DNA Sequencing are used today for human remains identification depending its success, mainly, on the type and the state of the sample. In this study, we describe the work of Banco Nacional de Datos Genéticos (Buenos Aires, Argentina) on bones, teeth and other forensic samples and evaluate the rate of success for each marker analyzed.

Keywords: Human identification; STRs; Y chromosome; Mitochondrial DNA; Forensics

1. Introduction

Human identification from forensic samples often presents difficulties due to the poor quality and quantity of DNA obtained, caused by contamination and degradation. That is the reason why new molecular technologies of DNA multiplex PCR amplification of nuclear STRs [1–5] and automated DNA Sequencing of Mitochondrial D-Loop Region [6–8] have become strong tools to be used in forensic science.

2. Materials and methods

We analyzed forensic samples from 45 cases: 121 samples including bones and teeth and 10 samples including swabs, stains of biological fluids and paraffin embedded tissue (Fig. 1). Bones and teeth were pulverized in a Freezer Mill 6 800, digested with proteinase K and SDS buffer, extracted by chloroform/phenol/isoamyl alcohol method, and then purified with Centricon 100 columns. DNA from paraffin embedded tissue was extracted with QIAmp DNA Mini kit (QIAGEN). In all cases, we used controls of extraction to avoid contamination.

Mitochondrial DNA D-Loop Region was amplified for HV1 and HV2 Regions. The symmetric PCR reaction was performed in a thermal cycler GeneAmp PCR System

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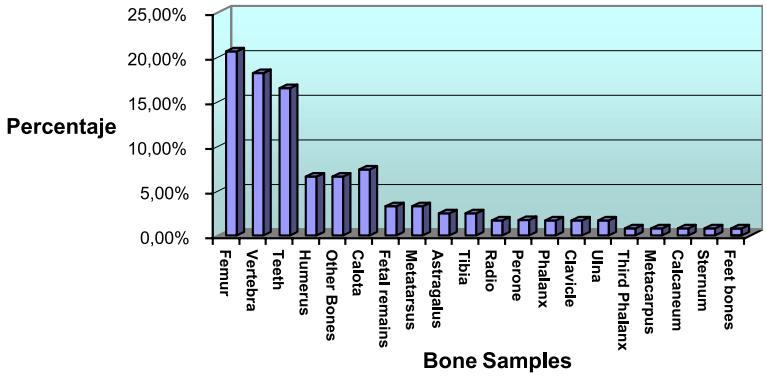

Fig. 1. Bone samples ($n = 121$).

9700® (Perkin Elmer). PCR product was run in agarose gel, dyed with ethidium bromide, visualized with UV light and photographed. It was purified using Microspin S-300 HR Columns (Amersham/Pharmacia). Sequencing reaction was performed with "Big Dye Terminator Cycle Sequencing Kit" (PE Applied Biosystems). For forensic samples, the primers used were: L-15997/H-16255; L-16099/H-16255; L-16209/H-16401 for D-Loop Region HV1 Segment and L-00030/H-00262; L-00140/H-00366, L-00241/H-00412 for D-Loop Region HV2 Segment. Templates were purified using Centrisep-Spin Columns (Princeton Separations) and subsequently analyzed in an ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems) following manufacturer's specifications.

Autosomal STRs were amplified using Amp FlSTR® Profiler Plus™ and Amp FlSTR® Cofiler™ Kits (PE Applied Biosystems) [3–5] in duplicate, with negative and positive controls. The following loci were analyzed: D3S1358, vWA, FGA, D8S1179, D21S11, D18S51, D5S318, D13S317, D7S820, D16S539, TPOX, THO1, CSF1PO and Amelogenin.

For the Y chromosome STRs analysis, we used Y-Plex™ 6 Reliagene Kit which amplified the following loci: DYS393, DYS19, DYS389 II, DYS390, DYS391, and DYS385. PCR products for all these markers were analyzed in an ABI PRISM 310

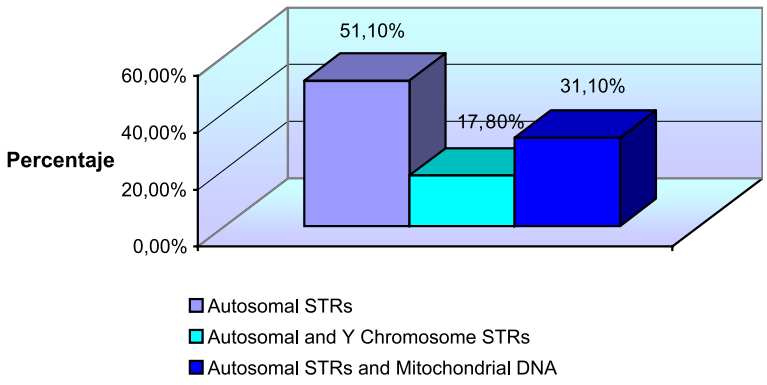

Fig. 2. Genetic markers analyzed.

Table 1

Samples	Positive results	Negative locus
Femur (25)	25 (100%)	two samples (TH01; CSF1PO; D21S11 and D18S51)
Vertebra (22)	20 (91%)	four samples (D18S51; D7S820; CSF1PO; D8S1179; D21S11 and TPOX)
Teeth (20)	20 (100%)	four samples (D18S51; D7S820; CSF1PO; D8S1179; D21S11; FGA; D16S539 and TH01)

Genetic Analyzer (PE Applied Biosystems) using Genescan® Analysis 3.1 software and Genotyper® Analysis 2.5.2 software following manufacturer's specifications.

3. Results and discussion

In all the forensic samples studied for autosomal STRs, Y chromosome STRs and Mitochondrial DNA D-Loop Region (Fig. 2), we analyzed the rate of success for each marker.

Among the 121 forensic samples including bones and teeth we analyzed for autosomal STRs: 25 (20.6%) femur samples, obtaining successful results in all of them except for two that were incompletely typed for loci CSF1PO, D21S11 and D18S51; 22 (18.2%) vertebra samples with successful results for 20 (91%) and with incomplete typing for 4 of them (20%) for loci D18S51, D7S820, D21S11, D8S1179 and CSF1PO; 20 (16.5%) teeth samples with successful results in all of them but 5 (25%) with incomplete typing for loci: D21S11, D18S51, CSF1PO, FGA, D7S820, D16S539 and D8S1179 (Table 1).

From data obtained, we conclude that incomplete typing are represented by loci of higher molecular weight (higher than 200 bp), which demonstrates the poor quality of the sample analyzed due to its state of degradation. The paternity and/or maternity probabilities calculated were always over 99.99% when all the loci were amplified. In the 33 (27.3%) samples where it was necessary Mitochondrial DNA D-Loop Region Sequencing, we obtained successful results in 27 (82%) of them for HV1 Region. In 9 of them, we obtained sequences of about 150–200 bp (20–25 years old samples) and in 18 of them we sequenced fragments longer than 200 bp. Only 8 of them could be analyzed for HV2 Region (samples with 5 years old or less).

The age of the samples and its conservation and are factors closely related which affect DNA viability.

Autosomal STRs solved all the samples analyzed in our study, but Y chromosome analysis and Mitochondrial DNA Sequencing are also important and necessary markers in cases where we only have paternal or maternal relatives, respectively.

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