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16 Y-specific STR analysis in human remains identification

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Abstract. Degraded samples were studied in order to obtain Y-STR haplotype to provide additional data in paternal lineage identification caseworks. Multiplex reactions were used comprising the minimal Y-STR haplotype DYS19, DYS390, DYS391, DYS392, DYS393, DYS19, DYS389I/II and DYS385 belonging to the Y-STR database. The other Y-STR loci—GATA A 7.1, GATA A 7.2, GATA C4, GATA H4, DYS437, DYS438 and DYS439—were included in the Y-Chromosome Quality Control Group of the Spanish and Portuguese Group (GEPY) of the ISFG. Y-STRs results were successful in almost all the samples when applied some modifications in amplification methods.

Keywords: Y-STRs; Paternal lineage; Degraded DNA; Haplotype

1. Introduction

Forensic investigation based on DNA typing often requires the use of degraded biological material, sometimes even highly degraded, especially for determining the identity of human remains. Due to their paternal inheritance Y-STRs offers new perspectives for identification and kinship analysis, especially in forensic deficiency cases as a complement to autosomal STRs. The aim was the study of 16 Y-STR loci to perform human remains identification, including the minimal Y-STR haplotype (DYS19, DYS390, DYS391, DYS392, DYS393, DYS19, DYS389I/II and DYS385) belonging to the Y-STR database and GATA A 7.1, GATA A 7.2, GATA C4, GATA H4, DYS437, DYS438, DYS439 loci that are part of the Y-Chromosome Quality Control Group of the Spanish and Portuguese Group (GEPY) of the ISFG.

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Table 1
Y-STRs alleles in degraded samples when applied mentioned modifications in amplification methods

	Sample	Pentaplex					GEPY I				GEPY II				Triplex I			Triplex II		
		DYS 393	DYS 390	DYS 19	DYS 389I	DYS 389II	Gata A7.2	DYS 437	DYS 438	Gata C4	Gata A7.1	Gata A10	DYS 439	Gata H4	DYS 391	DYS 437	DYS 439	DYS 438	DYS 392	DYS 385
Autopsy blood stains	1	13	22	16	13	30	12	17	8	23	10	16	11	27	11	17	11	8	11	15–16
	2	13	23	14	14	30	12	15	12	23	*	14	12	29	10	15	12	12	14	11–15
	3	14	23	14	14	29	13	15	13	23	13	15	*	28	11	15	12	13	14	11–14
	4	13	24	14	14	31	12	15	12	23	11	16	13	28	10	15	13	12	9	12–15
	5	13	24	14	12	28	13	15	12	24	11	15	12	28	11	15	12	12	13	11–14
Blood stored at 4° C	6	13	24	13	13	30	12	14	10	23	9	15	12	28	10	14	12	10	11	16–18
more than 1 year	7	13	24	13	14	30	*	14	10	*	11	15	10	28	9	14	10	10	11	13–14
	8	13	*	*	*	*	*	*	*	*	*	*	*	*	11	15	12	12	9	11–14
	9	13	22	16	14	30	*	*	*	*	10	16	*	*	11	15	11	*	*	*
	10	13	24	13	14	30	13	14	10	21	16	14	10	28	9	14	10	10	11	13–14
Muscle	11	13	22	14	12	*	12	16	10	21	11	17	11	27	10	16	*	10	11	13–15
Bone	12	13	24	14	13	*	12	*	12	23	7	14	13	*	*	*	*	*	*	*
	13	14	21	15	12	29	11	16	10	21	10	13	*	27	10	16	11	10	11	13–16
	14	14	24	14	13	29	12	15	*	23	11	14	13	28	11	15	13	*	*	*
	15	*	*	*	*	*	12	*	*	21	*	14	*	*	*	*	*	*	*	*
	16	14	22	15	13	29	*	*	*	*	*	14	11	27	11	16	11	10	11	12–12
	17	14	*	*	*	*	12	*	*	22	*	14	*	*	*	*	*	*	12	*
Teeth	18	14	23	16	12	27	11	16	10	21	10	14	12	28	10	16	12	10	10	14–15
	13	14	21	15	12	29	11	16	10	21	*	*	*	*	10	16	*	*	*	*
	15	*	*	*	*	*	*	*	*	*	*	14	*	28	*	*	*	*	*	*
	19	14	23	15	13	31	12	14	10	22	*	14	12	27	10	14	12	10	12	15–15

* No results.

2. Material and methods

Samples from 19 deceased individuals—bone, teeth, muscle, blood submitted to toxicological analysis kept at 4 °C for more than 1 year, bloodstains collected during autopsy—were studied and compared with bloodstains of relatives. DNA was extracted by phenol:chloroform methods, and for comparative bloodstains chelex method was used. A pentaplex reaction was carried out according to Kayser et al. [1] and de Knijff et al. [2]. GEPY I and II multiplexes (Table 1) were performed according to Gusmão et al. [3]. Triplex I and II were carried out as described by Ballard et al. [4]. For genetic typing, ABI Prism 31OO Sequencer was used.

3. Results and discussion

The Y-STR typing in degraded biological material has major technical challenges since each sample has unique characteristics. Most of the samples required protocol modifications such as different dilution extractions, distinct DNA and Taq polymerase concentrations, to achieve Y-STRs typing. Pentaplex was the multiplex system that seems to give better results with degraded samples. GEPY I and II systems need more technical modifications than Triplex II and I.

The reason that best results were obtained in autopsy bloodstains may be attributed to the fact that autolysis mechanisms are still incipient. Otherwise, liquid blood maintained at 4 °C for more than a year did not provide the same good results as it may contain porfirinic compounds from hemoglobin, which are powerful PCR inhibitors that difficult to eliminate.

In bone and teeth samples, a haplotype with at least 10 loci was obtained. Two of them did not yield results even when we applied AmpliTaqGold® LD, reamplification and 40 cycles PCR program. In these type of samples, which were exposed to adverse environmental and soil conditions, several problems need to be overcome—such as high DNA fragmentation, high amount of DNA from other microorganisms which invade biological materials, or other source of inhibitors.

Most of the results were obtained with dilution of DNA extraction and adding more Taq, sometimes by 2- or 3-fold. Thus the problem with the studied samples seems not to be DNA quantity, but the out-of-control presence of inhibitors that interfere not only with the extraction but also with the amplification process.

Allele typing in almost all Y-STRs enables comparison between these samples and individuals with the same paternal lineage.

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