16 Y-Specific STR Analysis in Human Remains Identification

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Introduction

❖ Forensic investigation often requires use of degraded biological material, specially for determining the identity of human remains.

❖ Y-STRs, offers new perspectives for identification and kinship analysis specially on forensic deficiency cases [1] in complement of autosomal STRs. The non recombining portion of the Y-chromosome is of value providing additional data in paternal lineage identification.

❖ The aim was the study of 16 Y-STR loci to perform human remains identification - the minimal Y-STR haplotype - DYS19, DYS390, DYS391, DYS392, DYS393, DYS19, DYS389I/II,DYS385 – and – GATA A 7.1(DYS460), GATA A 7.2 (DYS461), 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.

Material and Methods

❖ Samples from 19 deceased individuals - bone, teeth, muscle, blood submitted to toxicological analysis, blood stains collected during autopsy - were studied and compared with bloodstains of relatives.
❖ DNA was extracted by Phenol:Chloroform methods[2]. For the comparative bloodstains chelex method was used [3].

❖ DYS393, DYS390, DYS19, DYS389I/II were carried out in a pentaplex reaction, according to Kayser et al [4], and de Knijff et al [5].
❖ GEPY I (GATA A 7.2 (DYS461), GATA C4, DYS437, DYS438) and GEPY II (GATA A 7.1(DYS460), GATA H4, DYS439) were performed using the primers described by Ayub et al. 2000[6], White et al. 1999[7], PCR conditions were according to Gusmão et al.[9].
❖ DYS391, DYS437 and DYS439 were amplified on a PE 9600 in a 25 µl triplex reaction mix (1X Gold Buffer (AB), 2mM $MgCl_2$(AB), 200 µM dNTPs (AB), 0.5 U Taq Gold (AB) , 0.25 µM, 0.4 µM, 0.25µM of each primer respectively)
PCR profile: 7 min 95°C pre-incubation; 8 cycles (1 min 94°C denaturation, 1 min 60°C annealing, 1 min 72°C elongation); 30 cycles (1 min 94°C denaturation, 1 min 56°C annealing, 1 min 72°C elongation); 60 min final elongation at 72° C.

❖ DYS392, DYS385 and DYS438 were amplified on a PE 9600 in a 25 µl triplex reaction mix (1X Gold Buffer (AB), 2mM $MgCl_2$(AB), 200 µM dNTPs (AB), 0.5 U Taq Gold (AB), 0.5 µM, 0.2 µM, 0.3 µM of each primer respectively)
PCR profile: 7 min 95°C pre-incubation; 8 cycles (1 min 94°C denaturation, 1 min 60°C annealing, 1 min 72°C elongation); 30 cycles (1 min 94°C denaturation, 1 min 56°C annealing, 1 min 72°C elongation); 5 min final elongation at 72° C.

❖ Different dilutions extractions, DNA amounts (1.0-2.5µl) and Taq concentrations (0.5-3U) were applied to deceased individual samples.

❖ Genetic typing on ABI 31OO automatic sequencer Perkin Elmer Applied Biosystems was used with the Genescan 2.1 analysis software. Allele designations were based on comparison with reference samples.

RESULTS

		PENTAPLEX					GEPY I				GEPY II				TRIPLEX I			TRIPLEX II		
	Sample	DYS 393	DYS 390	DYS 19	DYS 389I	DYS 389 II	GATA A 7.2	DYS 437	DYS 438	GATA C4	GATA A 7.1	GATA A 10	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	12	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 more than 1 year	6	13	24	13	13	30	12	14	10	23	9	15	12	28	10	14	12	10	11	16-18
	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
Bones	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

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

❖ Not all Y-STRs yielded products with amplification conditions. Most of the samples required modifications such as different dilutions extractions, distinct DNA and Taq polymerase concentrations, to achieve Y-STRs typing.

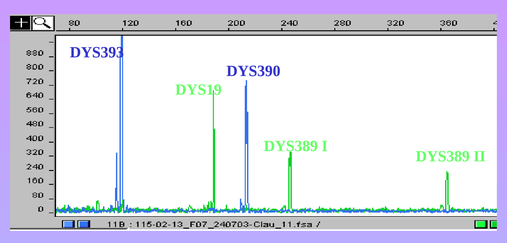
❖ Pentaplex was the multiplex system that seems to give better results with degraded samples. GEPY I and GEPY II systems need more technical modifications than Triplex II and I

❖ Concerning the different kind of samples, bloodstains collected during autopsy revealed to be the most informative. Blood stored at 4ºC more than 1 year were unsuccessful in most of the reaction mixes. Even in pentaplex we had to use 1 µl of dilution extraction and sample 8 only resulted in one pentaplex Y-STR - DYS393 - when Taq was 3U.

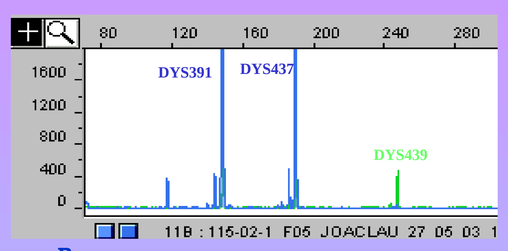
❖ In muscle sample and bone 12 was not possible to obtain results in DYS389II, even with the increment of Taq.

❖ Bone and teeth samples 15 and 17, didn't yielded results, in almost all the loci, when distinct modifications were used. In pentaplex even when applied AmpliTaqGold® LD (AB), reamplification and 40 cycles PCR program failed to obtain results.

❖ Allele typing in almost all Y-STRs make possible the comparison between this samples and the same paternal lineage individuals.



A



B

Fig 1 – Autopsy bloodstain Y-STRs results with some modifications in amplification methods. Dilution extraction in Pentaplex (A); dilution extraction and 1 U Taq in Triplex (B). Genetic comparison with his brother on table II

Sample	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 392	DYS 385
Blood stain	13	24	14	12	28	13	15	12	24	11	15	12	28	11	13	11-14
Brother	13	24	14	12	28	13	15	12	24	11	15	12	28	11	13	11-14

Table II - Y-STRs typing results from one of the studied degraded sample and his brother

Conclusions

❖ Y-STR typing in degraded biological material has major technical challenges since each sample has unique characteristics.

❖ The best results were obtained in autopsy bloodstains may be due to the fact that the autolysis mechanisms are still incipient. Otherwise blood kept at 4º C for more than a year didn't provide the same good results. Liquid blood samples from deceased individuals may contain porfirinic compounds from hemoglobin, powerful PCR inhibitors difficult to eliminate[9].

❖ In bone and teeth samples the source of inhibitors may be a high amount of DNA from other micro-organisms such as bacteria and fungi which invade bone[9] and probably this kind of contaminants can explain some of the results obtained in bones and teeth samples submitted to prolonged humidity, temperature environmental conditions and also adverse soil characteristics.

❖ 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 because most of the results were obtained with dilution of DNA extraction and adding more Taq, sometimes 2 or 3 fold.

❖ With this samples it seems that the success or failure depends on each sample itself and it should be treated individually. The number of distinct factors connected to each sample is difficult to calculate, so it is necessary to make a few attempts in order to have as much results as possible.

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