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Routine Y-STR typing in forensic casework

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Abstract

In the field of molecular diagnosis, forensic casework analysis is one of the most demanding investigations, due to its social impact. Optimization of DNA typing multiplex reactions with identical cycling conditions as those required by autosomal short tandem repeats (STR) multiplex reduces errors, and saves time and reagents. Previously, we validated a five Y-STRs set, all of them generating single band patterns. This work reports the optimization of combined multiplexes, a triplex (DYS19, DYS390 and DYS391) and a duplex (DYS392 and DYS393), that can be amplified in identical cycling conditions as those required by commercially available multiplex autosomal STR kits. In addition both Y chromosome multiplexes can be combined for co-injection on a capillary electrophoresis based automated sequencer. Statistical attributes of the haplotypes of the five Y-STR investigated were evaluated in unrelated males from different metropolitan areas of Argentina. This system was successfully used for investigating more than 350 forensic routine cases in our country.

Keywords: Y-STRs; Forensic casework; Argentina; Population database

1. Introduction

Multiplex autosomal short tandem repeats (STRs), combined with the gender determination system amelogenin represent at present the most popular approach to DNA based forensic identification. In addition to these robust autosomal markers, a number of Y chromosome specific STRs are nowadays available for patrilineage tracking. The first Y-STR described was Y27H39 known at present as DYS19 [1]. Soon after, an increasing number of Y-STRs were described. Its applicability was demonstrated in a wide range of forensic cases, such as: deficiency paternity testing [2], human remains identification in mass disasters [3,4], identification of a felon [5]. Although, over 25 Y-STR have been described [6], a basic set of nine or an extended set of eleven Y-STR were thoroughly investigated and a Y-STR haplotype reference database is online available ([7], this issue).[1] Standardization of protocols was attained through an intense cooperative inter laboratory work [8]. Different platforms were employed for typing, including radioactive incorporation, silver staining and fluorescence detection in automated sequencers. Different multiplexes were developed [9] and some of them successfully employed in forensic cases [4,10]. However at present, no commercial kits are available, limiting an extended routine forensic casework use. Inclusion of Y-STR typing in all forensic cases involving males became routine in our lab. We describe here the development of a Y-STR multiplex system, its haplotype frequency in Argentina and the forensic casework strategies employed in over 350 cases. The applicability and robustness of the five Y-STR multiplex system in routine casework was proved.

2. Material and methods

2.1. Samples

A total of 361 unrelated male individuals from different metropolitan areas including those of the most important cities of Buenos Aires, Rio Negro, Mendoza, Misiones,

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[1] http://ystr.charite.de.

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Corrientes, Chaco, Entre Rios and Santa Fe provinces were selected for a local haplotype reference database construction.

2.2. Y-STRs analysis

A set of five Y-STRs that generate single band patterns were selected for analysis. The PCR amplifications comprised the tetranucleotide STR loci DYS19, DYS390, DYS391 and DYS393 as well as the trinucleotide locus DYS392. A triplex reaction included: DYS19 (forward primer labeled with TET), DYS390 and DYS391 (both forward primers labeled with 6-FAM). A duplex reaction included DYS392 and DYS393 (both forward primers were 6-FAM labeled). The amplification reaction mix contained 1 U Amplitaq Gold (PE), 0.1 mM of each primer for DYS19 and DYS392, 0.05 mM of each primer for DYS390 and DYS391, and finally 0.01 mM of each primer for DYS393, 200 mM dNTPs, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris–HCl/pH 8.3 and 0.01% gelatin (Amplitaq Gold buffer). Cycling conditions were the same as those used by Profiler Plus/Cofiler protocols (User's Manual, PE Applied Biosystems). The five Y-STRs were amplified in a duplex and a triplex reaction, amplicons were mixed and loaded in a single injection in an ABI 310 automated sequencer (PE Applied Biosystems).

2.3. Electrophoresis conditions

1 μl of the duplex amplicon and 1 μl of the triplex amplicon were mixed with 20 μl of formamide and 0.5 μl of TAMRA 500 size standard. The electrophoresis was performed using a 47 cm capillary and POP-4 polymer, at 15 kV for 25 min at 60°C. Electropherograms were analyzed by means of matrix C. Y-STRs were scored by comparison with allelic ladders kindly supplied by P. de Knijff, Leiden University, The Netherlands.

3. Haplotype construction and statistical evaluation

Haplotype frequencies were determined for all five Y-STR, four (DYS19, DYS390, DYS391, DYS392), three (DYS19, DYS390, DYS391) and two (DYS19, DYS390) in accordance with Kayser et al. [8]. Genetic diversity was determined in accordance with Edwards et al. [11].

4. Routine forensic casework strategy

Blood from paternity tests or from reference samples in forensic cases (sexual assaults, homicides, relatives of mass disaster victims, etc.) were spotted onto FTA paper (Life Technologies, USA), air dried, and eight punches (2 mm in diameter each) were cut. Contaminant proteins and debris were extracted with DNA purification reagent (Life technologies, USA).

Swabs: DNA from vaginal, rectal or oral swabs was extracted by means of differential procedure, in all cases both epithelial and sperm fractions were analyzed in parallel with the reference samples when available. DNA from cadaveric tissues was extracted by means of either CTAB [12] or SDS/proteinase K followed by organic solvent extraction and Microcon 100 (Amicon, USA) purification.

In all cases in which males were involved simultaneous multiplexing, including Profiler Plus, Cofiler, Y-STR duplex (DYS392 and DYS393) and Y-STR triplex (DYS19, DYS390 and DYS391) amplification, was carried out.

Only in those cases in which human remains are being investigated, a two step strategy is adopted. Samples are typed by Profiler and Cofiler and after amelogenin gender determination, males are then typed by Y-STRs multiplex when patrilineage analysis is required.

5. Results and discussion

Optimized amplification reactions that allow amplification of autosomal and Y chromosome STRs under the same cycling conditions, facilitate, reduce errors and speed up the typing process when numerous samples are being investigated. Typical profiles are depicted in Fig. 1, where the electropherogram of the co-injected amplicons produced by the triplex and duplex reaction mixtures are presented. Some overlapping may include allele 17 of DYS19 and allele 21 of DYS390. Nevertheless, in order to circumvent this undesired situation and to improve the interpretation of results, DYS19 was labeled with TET (green) and DYS390 with 6-FAM (blue). In addition the overlapping alleles of both markers exhibit the lowest frequency in our population (0.013 and 0.019, respectively), no sample denotes a haplotype containing both alleles in the 361 Argentinean individuals analyzed, also no match was found in the Y-STR haplotype reference database (YHRD) currently composed of 4688 males of European descent.

The four most frequent haplotypes are detailed in Table 1, their frequencies are compared with those present in the YHRD and the cities in which the highest haplotype frequencies were detected. Table 2 was constructed in order to compare decreasing DP values of Y-STR haplotypes and autosomal STRs. The highest frequency for a five Y-STR haplotype is 0.14. This figure is smaller than that of the most frequent allele of each for the 12 autosomal markers included in Profiler Plus and Cofiler systems. In addition, it can be observed that the discriminative power for the most frequent five Y-STR haplotypes exhibited the highest value [13].

The attained statistical parameter values justify the use of the five Y-STR combination as a rapid and informative forensic DNA typing system. In addition, since these markers allow patrilinage tracking, their use must be combined with autosomal counterparts when personal identification is required.

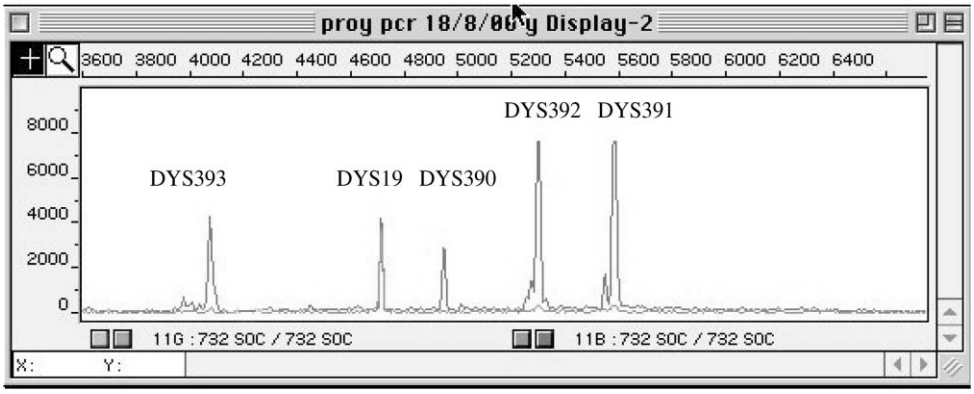

Fig. 1. Electropherogram of co-injected duplex and triplex Y-STRs amplificons. Only DYS19 is labeled with TET, the other four STRs with 6-FAM.

Table 1
Most frequent five loci Y-STR haplotypes in Argentina compared with the Y-STR haplotype reference database (YHRD) indicating the European location with highest frequencies

Haplotype	Argentina	YHRD	Highest European frequency
A (14-24-11-13-13)	0.136	0.078	Modena 0.24
B (14-24-10-13-13)	0.052	0.041	Porto 0.158
C (14-23-10-11-12)	0.033	0.016	Rome 0.061
D (13-24-10-11-13)	0.033	0.018	Innsbruck 0.081

A total of 152 (42%) different haplotypes were detected in 360 individuals, 92 haplotypes were unique in our population (25%). A clear geographical difference concerning the distribution of the three most frequent five Y-STRs haplotypes can be observed in Fig. 2. Only one particular haplotype (A = 14-24-11-13-13) was present with the highest frequency in all the five provinces considered for comparison. As can be seen in the figure a total of eight most frequent different haplotypes are detected. Differential haplotype distribution may be explained to the geographical origin of the immigrants that peopled Argentina in different migration waves since the beginning of 20th century, from distinct European areas. At present, the aboriginal component in the Argentinean population is restricted to geographically isolated areas and their demographic influence is negligible since only a 0.5% of the populations is of aboriginal descent. Hence, we can consider that the major impact of the Y-STR haplotype combination is mostly due to the European immigrants. As indicated in Table 1 the most frequent haplotypes are also detected in European populations. Detailed statistical comparison between the different areas of Argentina and those included in the YHRD is under course.

Table 2
Comparison of discriminative power (DP) and highest haplotype/allele frequency (*F*) of autosomal and Y-STRs

Y-haplotype or STRs	DP (%)	*F*
DYS19, 390, 391, 392, 393	97.29	0.14
FGA	96.99	0.15
D18S51	96.92	0.16
DYS19, 390, 391, 392	96.18	0.15
D21S11	95.47	0.26
D8S1179	94.01	0.29
DYS19, 390, 391	93.99	0.18
D13S317	93.65	0.29
D16S539	92.82	0.29
D7S820	92.05	0.27
VWA	91.38	0.30
THO1	91.08	0.25
D3S1358	90.88	0.34
D5S818	89.27	0.37
CSF1PO	87.31	0.32
DYS19, 390	87.25	0.29
TPOX	82.91	0.48

In our lab, the combined five Y-STR system has been successfully used for routine forensic casework [14]. To present this system was employed in over 350 cases, mostly rape cases, corps identification and homicide investigations.

Even in multiple rape cases and those in which subungueal perpetrator tissues are collected after homosexual assaults, investigation by these means usually provides clear and unambiguous information. In mass disaster victims identification information provided by Y-STRs allows a

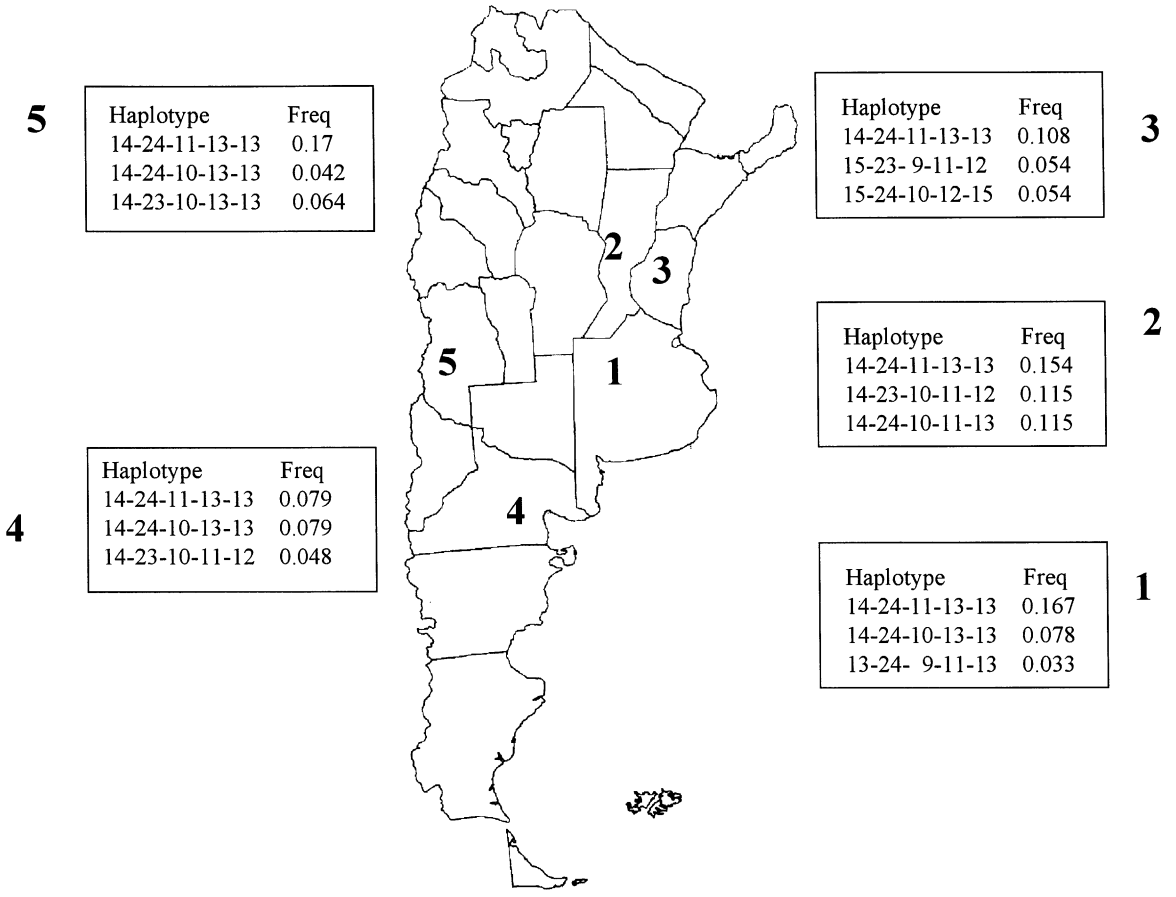

Fig. 2. The map shows five metropolitan areas of Argentina for which the most frequent five Y-STR haplotypes are summarized. References: 1, Buenos Aires ($n = 90$); 2, Santa Fe ($n = 26$); 3, Entre Rios ($n = 37$); 4, Rio Negro ($n = 63$); 5, Mendoza ($n = 47$).

rapid analysis of the paternal relatives. The sensitivity of either duplex or triplex Y-STR reactions was slightly lower than that observed in autosomal STR commercial kits. The reduced sensitivity is mostly reflected in the smaller peak area in comparison with autosomal STR profiles. In some unfrequent cases no results at all are obtained. In addition, neither mistyping nor allele drop out of the larger loci being typed (e.g. DYS391 and DYS392 in each reaction) were detected even when highly degraded DNA samples from cadaveric tissues was analyzed.

In an aircraft accident occurred in August 1999 in Buenos Aires, 13 family groups (who provided 25 blood samples) were looking for identification of a set of 13 corpses (whose genetic profiles were detected in a total of 38 fragmentary cadaveric remains), seven of them belonged to males as established by the XY amelogenin profile after Profiler and Cofiler typing.

Only three remains exhibited identical Y-STR haplotypes to their male relatives, autosomal STR typing certified their biological kinship. Another male material was unable to be compared since no male relative was available and the other three remains depicted completely different haplotypes. This discrepancy can not be addressed to false exclusions since confirmation of our data by other labs demonstrated that serious errors were committed during the initial corpse identification based on conventional forensic approaches. Those errors were committed previous to the cadaveric tissue selection to be employed in DNA based identification. Molecular information including Y-STR haplotype and autosomal STR typing, helped to detect incorrect identification based on conventional approaches and to solve the mistake by typing corpses that were erroneously returned to alleged relatives.

By increasing the number of Y-STR the haplotype frequency will decrease and the DP will accordingly be increased. Since many informative Y-STR are available, why should we restrict our system to a reduced combination of five markers? This question might be responded according to the scope of interest. Y-STR haplotype frequency will contribute for statistical calculations as a single locus (since no recombination does occur out of PAR 1 and 2 in the Y chromosome). In addition, if its typing does not require

different experimental conditions and is compatible with cycling conditions of the most popular autosomal systems and also allows to co-inject the amplicon of the five Y-STR it will reduce the time of analysis and more important, reduces the chance of sample handling errors.

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