



Population analysis from 12 microsatellite loci revealed by silver stain and assisted by computer software

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Abstract. A Colombian population was typed for 12 STRs by silver stain method. Initial routine was extended from 9 to 12 STR loci for increasing forensic values in paternity testing, new multiplex developed decreased significantly Probability of Match (PM) and increased power of exclusion. Kodak 1D Image Analysis Software was used for assigning allele out-ladders with analysis of their correct molecular sizes. © 2003 Elsevier B.V. All rights reserved.

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1. Introduction

Since STRs markers are used for forensic proposes, several studies have published population data reports in worldwide [1,2]. Actually, almost all forensic laboratories do DNA typing with STRs markers by automated methods using fluorescent platforms as ABI310, ABI377 and FMBIO I–II. Proficiency testing has shown error rates higher in silver nitrate method than high throughput methods as ABI and HITACHI but lower than other methodologies (GEP-ISFG, personal communication, 2002). In spite of high precision and great processing sample of fluorescent methods, some laboratories cannot acquire this automated routines. Some tools have been created and enhanced for pattern bands analysis [3,4]. Aim of this study was increased STR profile set and work with a useful tool for DNA profiling by silver stain method that can help to decrease error rates in this typing analysis.

2. Materials and methods

A total of 336 EDTA–blood samples from mestizo unrelated born in Colombia involve in paternity testing were analyzed. A simple salting out method was made for DNA isolating [5].

One hundred samples were typed by triplex CTT, FFv and SilverSTR III distributed by Promega. DNA typing was in agree with technical recommendations. Samples were retyped with primers described by Masibay et al. [6]. The added 226 samples were typed this way. HPRTB, D8S1179 and D5S818 were analyzed in a multiplex reaction agree with primers published in STR database (NIST). D8S1179 region was expanded in order to

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eliminate cross size with D5S818 amplicons, forward primer D8S1179 (5' tcttctt-gcccacacggcctgg3') was derived of AF216671 Genbank sequence. Cycling for HPRTB, D8S1179 and D5S818 triplex was 95 °C by 5 min, 35 cycles of 94 °C by 1 min, 57 °C by 1 min, 72 °C by 75 s and incubation at 70 °C by 30 min. Into denaturing gels, PCR products were separated. Electrophoresis was done during 60 min at 40 W and 55 °C follows by Silver Stain detection. CTT, FFv and SilverSTR III Triplex allelic ladders, K562 cell line and M1 M2 GEP-ISFG 2002 reference samples were used for allelic assignation. Genetic Data Analysis v1.0 (d16c) [7] was used and other references for forensic values [8,9].

3. Results

Allelic frequencies and HW equilibrium values were found. Analyzed population was in HW equilibrium for all markers, however, significant disequilibrium was detected in F13A01 when exact test with Fisher measure was applied but not with Chi-squared measure ($p < 0.04$ and $p < 0.2172$, respectively). Linkage disequilibrium (LD) between TPOX/D7S820 and TH01/vWA was found with significant values $p < 0.0194$ and $p < 0.0084$, respectively. Probability of Match (PM), Power of Discrimination (PD), Polymorphic Information Content (PIC) and Typical Paternity Index (TPI) for individual markers, triplex reactions and 12 STR loci were found. Lowest PM values were at increasing order for D13S317, F13A01 and D8S1179 (0.0614, 0.0659, 0.0703, respectively). Triplex information showed lower PM in HPRTB, D8S1179 and D5S818 than CTT triplex (9.66×10^{-4} and 2.12×10^{-3} , respectively). D16S539, D7S820 and D13S317 triplex showed highest PE with 0.9448. TPI increased from 792.2 (9 loci) to 5 050.3 (12 loci).

Some alleles could not be assigned by direct visualization because they were not in commercial allelic ladder, alleles 18 and 17 from F13A01, allele 4 from TH01, alleles 12, 21 and 23 from vWA, all them with allelic frequency 0.0015 (one allele). 1D Image Analysis Soft-

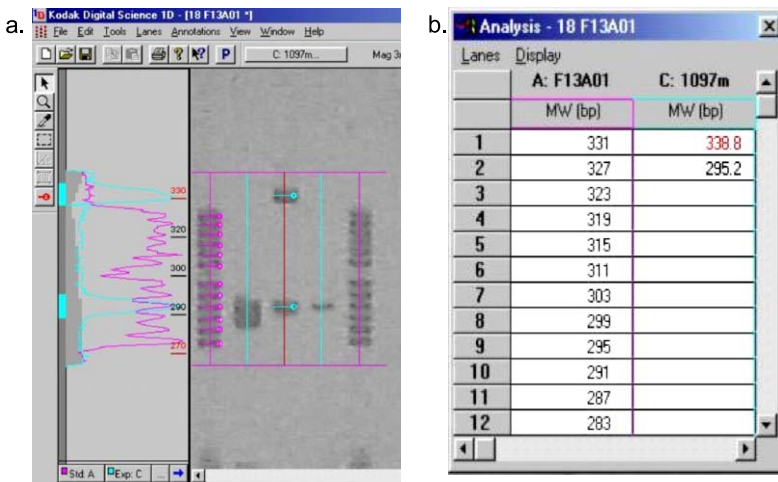


Fig. 1. Allele 18 from F13A01 analysis by 1D Image Analysis Software. (a) Band patterns are shown graphically, probably allele 18 is compared with commercial allelic ladder F13A01 (alleles 4–16) and clearly it is out-ladder range. (b) The software compared and analyzed ladder molecular sizes and determined its weight at 338.8 bp that is in agreement with the consecutive scale at base pairs (339 bp for allele 18).

ware was used for assigning corrected alleles to these samples. Fig. 1 shows allele 18 analysis by this software where molecular sizes from ladder was included into software and it did respective band pattern analysis. This allelic assignment was confirmed by ABI310 analysis in the Institute of Legal Medicine, University of Santiago of Compostela (data not shown).

4. Discussion

Population showed HW equilibrium. However LD was found between TPOX/D7S820 and TH01/vWA loci with significant p values, this fact was gotten because permutations number in exact tests. LD has been reported between FGA/D21S11, D21S11/D18S51 and D7S820/D16S539 loci with significant p values [1]. Thus, is possible to determine LD between so far genome regions. In some laboratories, STR typing silver stain method consists in nine STR profiling; however, new multiplexes can be design for increasing this routine. Additional triplex reaction showed a lower PD than CTT multiplex 0.000966 and 0.00212, respectively. Actually, polymorphic markers as D8S1179 and D5S818 cannot be found in any STR kit for silver stain detection. Enhanced silver stain routine can work 12 or more STR markers provided that laboratory decided to implement new PCR reactions. New triplex reaction increased 6.39-fold TPI. Care should be taken when a laboratory decided to increase STR profile with polymorphic markers and without many microvariants because its assignation is difficult especially when PCR products are long (FGA, D21S11 and D18S51).

Kodak 1D Image Analysis Software v 2.0.1 was useful on the assignation of alleles out-ladder, allele 18 from F13A01 assigned was confirmed by high throughput analysis (data not shown). Molecular size 338.8 bp was in agreement with its expected size 339 bp. Additional strategies here shown can help quality and performance either DNA typing routine and proficiency testing for laboratories working with silver stain detection.

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