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Allelic frequencies and forensic parameters for miniSTRs D10S1248, D14S1434 and D22S1045 (NC01) in a sample from Central Andean Colombian region

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ABSTRACT

In this work NC01 (Non CODIS) triplex system (D10S1248, D14S1434 and D22S1045) was chosen for standardization and validation. Allelic ladders were constructed and validated by sequencing. Genotypes for 450 Central Andean Colombian from 6 regions were analysed, population and forensic parameters were estimated, no significant departures from H–W equilibrium were detected and loci were in linkage equilibrium. No signs of homology were detected when NC01 was evaluated on 9 new world primate species. Forensic utility is demonstrated with 25 forensic cases typed with NC01 miniSTRs Triplex.

1. Introduction

Human genetic identification relays mostly on STRs analysis, however routinely used markers (e.g. commercial kits for CODIS system) can present amplification problems such as partial or absent genetic profiles when used on forensic samples, due to their characteristics (mixtures, degradation and/or presence of inhibitors) [1–2]. MiniSTRs have proven to be a valuable tool for forensic DNA analysis, given a higher amplification rate in difficult samples. [2]. Recommendations for European laboratories by the European DNA Profiling Group (EDNAP) and the European Network of Forensic Science Institutes (ENFSI) in 2006, suggested NC01 inclusion to increase robustness and sensibility of routinely performed forensic analysis, and especially for those cases with difficult samples (e.g. mass disasters) or complex filiations analysis [3]. Population parameters and allelic frequencies for NC01 in several localities have been addressed by various authors and others have restated NC01 and miniSTRs usefulness for difficult samples treatment [2,4]. Because of the miniSTRs advantages and the need for these molecular tools in our country, a sample from Colombian Central Andean region where genotyped using NC01 triplex system. Forensic validation with difficult samples, tests for specificity and sensibility were also carried out.

2. Materials and methods

450 Blood reference samples belonging to six sub regions within Central Andean Colombian region, from non-related individuals (Informed consent signed) spotted in FTA® Cards (Whatman) were kindly provided by INMLCF (Instituto Nacional de Medicina Legal y Ciencias Forenses). DNA was extracted by Chelex-100™ (20%) method. Each sample was genotyped according to conditions reported elsewhere [2]. Cross amplification was tested using template DNA from domestic animals (*Gallus gallus, Bos taurus y Equus caballus*) and new world primates species (*Ateles chamek, Saimiri sciureus, Saguinus leucopus, Cebus xanthosternos, Saguinus labiatus, Phitecia sp., Aotus griseimembra, Cebus albifrons, Cebus robustus*). Allelic separation was achieved by CE, using genetic analyzers ABI 310 and ABI 3130. GeneScan V3.7 and Genemapper V3.1 software were used for allele assignment. Samples from 2008 GHEP's quality control were typed for NC01. Allelic ladder was constructed and validated by sequencing [5]. Allelic frequencies were obtained by direct counting; exact test provided by Genetic Data Analysis (GDA) software [6] was used to estimate deviations from Hardy–Weinberg equilibrium, PowerStats Software (Promega Corp.) [7] was used for forensic

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parameters estimation. Population structure was estimated by F-statistics and AMOVA from Arlequin software V.3.1 [8]. For forensic utility evaluation 8 forensic cases and 17 DNA bone samples that rendered incomplete profiles or no results at all when using AmpFSTR® Identifiler™ were genotyped with NC01 at conditions described above, except that amount of template DNA was increased to 2.5 μl (variable concentration in each sample) and 32 cycles were used in a final reaction volume of 12.5 μl [2].

3. Results and discussion

Triplex NC01 markers showed high specificity, no cross amplification against the new world primate's samples. However locus D14S1434 showed amplification of allele 13 when applied to birds, allele 14 was also present when bovine or equine DNA were tested (Data not shown). Cross-species amplification must be considered when analysing forensic cases where an involvement of such species can not be ruled out. In fact this result could be explained if it is considered that D14S1434 forward primer was designed overlapping GACA repeating units over 11 nucleotides (40.74%) from 27 primer length [2] and this repeated motif could allow cross-specific amplification.

Allele's distribution was in concordance with those reported in previous studies [2], no new alleles were found. Allelic frequencies, population and forensic parameters are shown in table S1. No departures from Hardy–Weinberg equilibrium were detected. Fst values were indicative of low population differentiation (0.001881–0.003901) across sub regions, thus indicate that more than 99% of total diversity can be attributable to within population's variation [9]. We suggest considering these regions as a single population for routine use in forensic analysis, these results were consistent with previous studies and they stressed that populations surveyed in this study belong to the so-called Central Andean region of Colombia [10]. Forensic parameters for D10S1248 and D14S1434 locus are located in the middle range between CODIS markers (Table S1). However D22S1045 locus that is include in some new kits for human identification, is ranked even below the less informative CODIS system marker (TPOX) for Colombian Andean region [10]. D22S1045 allele frequency distribution in this study indicates that both frequency of alleles 15 and 16 together are higher than 0.80. Vullo et al., in Argentina reported similar frequency distribution for this marker [11], 77.6 % grouped in alleles 15 and 16. This is consistent with frequencies distribution for U.S. Hispanic populations reported by Coble and Butler in 2005 [2] where 76.43% of frequencies were also concentrated in these alleles, in contrast they differ from frequencies distribution for Caucasians and African Americans whose frequencies are distributed more evenly between alleles 15, 16 and 17. This finding suggests that it would be most useful to include D14S1434 instead of D22S1045 when it comes to Hispano-American human identification kits. From 8 analysed forensic cases using NC01 triplex, 50% of them showed profiles that improved statistical strength of the analysis in different forensic cases such as sexual offenses, and complex biological samples affiliations for instance from paraffin-embedded tissues. Skeletal remains samples typed with NC01 triplex allowed obtaining full profiles in 9 of 17 tested cases (52.94%).

4. Conclusion

Usefulness of NC01 miniSTRs on forensic cases was evidenced in spite of D22S1045 limitations. Routinely application of NC01 markers to forensic samples can redirect the outcome of difficult cases since additional information to accept or reject hypothesis could be provided.

Ethical standards

Experiments comply with the current Colombian Laws.

Conflict of interest

None.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.fsigss.2015.09.033.

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