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African STR data based on a newly developed tetraplex fluorescent system (CD4, F13A01, FES and MBPB)

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Abstract. The development of a fluorescent multiplex system amplifying four STRs (CD4, F13A01, FES and MBPB) was carried out in order to complement commercially available kits (Identifiler from Applied Biosystems and Powerplex 16 from Promega) mainly for application in complex parentage testing. Here, we report African population data from samples of Mozambique and the Cabinda province of Angola. Population differentiation studies were carried out and, in terms of forensic interest, some parameters are reported and compared with available commercial tetraplex kits. © 2003 Elsevier B.V. All rights reserved.

Keywords: Multiplex PCR; STRs; Mozambique; Cabinda; Population data

1. Introduction

A fluorescent multiplex system (CFFM) with the STRs CD4, F13A01, FES and MBPB, was developed to complement, in our laboratory, the routinely used commercial kits AmpFLSTR Identifiler (AB Applied Biosystems) and Powerplex 16 (Promega) which amplify a total of 17 STRs. In some difficult parentage investigations, such as those where the putative father is not accessible, the results obtained with the 17 STR set may not be satisfactory. Therefore, it is important to have other available markers for extending complex investigations (apart from Y chromosome systems or mtDNA), which can be easily and rapidly analysed in order to give an adequate resolution in the shortest period of time.

The choice of these 4 STRs resided basically on the fact that they were well studied and routinely used in singleplex reactions with silver-staining detection, before the introduction of fluorescent equipment and commercial kits in many forensic labs. The kit was

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developed for ABI fluorescent platforms and sequenced ladders were built based on the most frequent alleles observed in our previously studied Portuguese sample.

Population data for the four mentioned STRs are reported for samples located on opposite sides of the southern African continent: Mozambique on the east (Maputo area) and Angola on the west (Cabinda region). These results are compared between them and with previously published African results where data is available on all four loci [1].

In terms of forensic interest, the overall discrimination powers of this kit are also reported and compared with available data on other kits, namely Promega's FFFL kit.

2. Material and methods

Blood samples of 114 individuals from Mozambique (Maputo area) and 110 from Angola (Cabinda province) were collected. DNA was extracted with the Chelex method [2]. Primers, labels and ladders used for multiplex genotyping were the following:

CD4: 5′-GCCTGAGTGACAGAGTGAGAACC-TET [4] and 5′-TTGGAGTCG-CAAGCTGAACTAG; ladder with alleles 5, 6, 8, 10, 11, 14 and 15.
F13A01: 5-GAGGTTGCACTCCAGCCTTT-TET and 5′-ATGCCATGCAGATTA-GAAA [7]; ladder with alleles 3.2, 4, 5, 6, 7, 13, 14 and 15.
FES: 5′-GCGAAAGAATGAGACTACAT and 5′-GGGATTTCCCTATGGATTGG-FAM [8]; ladder with alleles 8, 10, 11, 12 and 13.
MBPB: 5′-GGACCTCGTGAATTACAATC-Fluorescein [9] and 5′-CTCATGTATC-CATCTATTTACC [10]; ladder with alleles 7, 9, 10, 11 and 12.

For the CD4 locus, the forward primer was modified according to the observation of a C-A substitution at the 3′ end, which would originate allele dropout [3]. The modified primer corresponds to the one described by Edwards et al. [4] without the terminal cytosine.

PCR conditions and fragment analysis procedures can be obtained from the corresponding author on demand.

The Arlequin software ver. 2.000 [5] was used for estimating allele frequencies, for testing Hardy–Weinberg equilibrium and also for population differentiation tests. Heterozygosity was calculated according to Nei [6].

3. Results and discussion

Table 1 reports genetic population data obtained for the four STRs in both African populations under study. Allele frequencies can be obtained from the corresponding author on demand.

The overall power of discrimination (PD) of the CFFM multiplex is 99.992% with a matching probability (MP) of 1/12478 individuals for the Mozambique sample, and slightly superior for the Cabinda sample with 99.995% PD with a MP of 1/19772 individuals. These values are very high when compared with Caucasian populations (e.g., 1/3846 individuals in our previously studied Portuguese sample), mainly due to the high levels of diversity observed for the CD4 locus in African samples. Compared with the commercial FFFL kit (Promega), which, like the CFFM kit, amplifies loci not contained in

Table 1
Parameters of forensic interest for CD4, F13A01, FES and MBPB loci in both African samples

Parameters	Mozambique (Maputo area) $N=114$				Angola (Cabinda province) $N=110$			
	CD4	F13A01	FES	MBPB	CD4	F13A01	FES	MBPB
Het	0.833	0.751	0.729	0.678	0.858	0.803	0.717	0.651
PD	0.951	0.911	0.881	0.846	0.962	0.938	0.878	0.825
PE	0.650	0.536	0.474	0.415	0.689	0.609	0.467	0.391
P	0.052	0.710	0.074	0.540	0.536	0.558	0.526	0.831

Het: Nei's heterozygosity; PD: Power of discrimination; PE: a priori power of exclusion; P: Hardy–Weinberg equilibrium exact test.

the Identifiler or Powerplex systems, in African-Americans, the MP is of 1/16802 individuals (Promega Technical Manual, 1999), higher than the value observed for Mozambique but lower than in Cabinda. This shows that the CFFM system is a useful alternative (as a complement to routinely used STRs) in complex parentage investigations.

Population differentiation tests were undertaken for all four loci comparing our African samples between them and with previously published data from São Tomé and Príncipe islands [1], situated on the west of Africa. There were no significant differences found between the sample from the Cabinda region (Angola) and São Tomé and Príncipe for all loci, which could be expected since both regions lie on the west side of Africa. Our Mozambique sample (on the east) showed significant differences for all loci with São Tomé and Príncipe, but with Cabinda, differences were only found in two loci, F13A01 and MBPB.

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References

[1] L. Gusmão, et al, Forensic Sci. Int. 116 (2001) 53.
[2] M.V. Lareu, et al, Forensic Sci. Int. 66 (1994) 41.
[3] G. Watanabe, et al, J. Forensic Sci. 43 (1998) 733.
[4] M.C. Edwards, et al, Nucleic Acids Res. 19 (1991) 4791.
[5] S. Schneider et al, University of Geneva, 2000.
[6] M. Nei, Molecular evolutionary genetics, Columbia Univ. Press, New York, 1987.
[7] M.H. Polymeropoulos, et al, Nucleic Acids Res. 19 (1991) 4306.
[8] M.H. Polymeropoulos, et al, Nucleic Acids Res. 19 (1991) 4018.
[9] M.H. Polymeropoulos, et al, Hum. Mol. Genet. 1 (1992) 658.
[10] L. Gusmão, et al, Int. J. Leg. Med. 108 (1996) 313.