

## Complex paternity investigations: The need for more genetic information

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**Abstract.** The use of extra mono- or multiplex fluorescent amplification STR systems has become an important way of supplementing occasional insufficient genetic information, provided by routinely employed similar systems, namely in cases of complex parentage testing. In this work we discussed the supplementary use of the commercial amplification kit FFFL (Promega) in the solution of a complex parentage investigation. We also reported allelic frequencies and some statistical parameters with forensic interest, relative to FFFL's loci, for the Northern Portuguese population. © 2006 Published by Elsevier B.V.

*Keywords:* Complex parentage testing; Population study; FFFL loci; Northern Portugal

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### 1. Introduction

In the past few years our laboratory has witnessed an increase in the amount and complexity of difficult parentage investigations, mainly defective in putative fathers' genetic data. In some of these investigations, the routinely used commercial amplification kits Identifiler™ and Powerplex®16 [1], even combined with the complementary commercial kit Powerplex® SE33 [1], were no longer always satisfactory. Therefore, there was an urgent need of extra easily and rapidly analysable markers. Bearing that in mind, our laboratory resorted to the GenePrint® FFFL kit [2], which allows for the co-amplification of four more STR-loci (F13A01, FES, F13B and LPL). Here we described a parentage investigation case to whose solution the FFFL's data were crucial and reported relevant Northern Portuguese population's genetic data.

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## 2. Material and methods

Blood samples and oral swabs were obtained from 199 unrelated resident individuals in Northern Portugal, aiming the FFFL's population study, and from individuals involved in a parentage investigation case: Two children (CH1 and CH2), their undoubted mother, no presumptive father. Question: Were CH1 and CH2 full-sibs or half-sibs?

DNA extraction, PCR amplification, capillary electrophoresis and phenotyping were performed according to [1,2].

The statistical evaluation and interpretation of the above-mentioned parentage investigation case were accomplished manually and using Familias-v.1.7 [3] software. Two hypotheses  $H_i$  ( $i=1, 2$ ) were compared. **H1**: CH1 and CH2 are full-sibs; **H2**: CH1 and CH2 are half-sibs. The Likelihood Ratio (LR) was calculated. Posterior probability of H1,  $P(H1|D)$ , was computed using Bayes's Theorem. The changes in LR and  $P(H1|D)$  values, with increasing genetic information, were recorded (Table 1). The critical values to decide for H1 as "practically proven" were those in Hummel's chart ( $LR > 399$ ) [10].

Concerning the Northern Portugal population's genetic study, estimation of FFFL's loci allele frequencies, heterozigotes excess and deficiency testing as well as Hardy-Weinberg equilibrium assessment [4], were all executed with Genepop-version 3.4 software [5]. Testing of pair-wise genotypic disequilibrium among Powerplex® 16's, Identifiler's and FFFL's loci (SE33 not included), was also executed with Genepop. Some forensically relevant statistical parameters were calculated and documented in Table 2.

## 3. Results

### 3.1. Parentage investigation case

LR and  $P(H1|D)$  values are given in Table 1.

### 3.2. Population study

Allele frequencies and parameters of forensic interest are presented in Table 2.

## 4. Discussion

### 4.1. Parentage investigation case

An overall increase in LR and  $P(H1|D)$  was detected as the amount of studied loci raised (Table 1). However, the global values were lowered, staying below the critical decision values, when SE33 was added to the analysis, since this system yielded a LR value of 0.5. Nevertheless, the use of The FFFL kit helped bring back the global values above the critical limit and higher than before. Hence, in this case, the utilization of the FFFL kit revealed itself of the utmost importance to allow for a decision to be made.

Table 1  
Variation of the LR and  $P(H1|D)$  values with the variable amount of genetic information

	Kits employed			
	A	A+B	A+B+C	A+B+C+D
LR	196	632	319	770
$P(H1 D)^a$	0.9949	0.9984	0.9970	0.9987

A=Powerplex 16; B=AmpFLSTR Identifiler; C=Powerplex ES Monoplex System (SE33);D=FFFL; ° LR= $[P(D|H1)/P(D|H2)]$ , D=phenotypes' data.

<sup>a</sup> Assumes discrete uniform a priori distribution for  $H_i$  ( $i=1, 2$ ).

Table 2

Allele frequencies and parameters of forensic interest for the FFFL loci in the northern Portuguese population

Loci	Alleles															
	3.2	4	5	6	7	8	9	10	11	12	13	14	15	16		
LPL	—	—	—	—	0.003	—	0.030	0.357	0.344	0.198	0.065	0.003	—	—		
F13B	—	—	—	0.106	0.020	0.254	0.206	0.410	—	0.005	—	—	—	—		
FES	—	—	—	—	0.003	0.013	0.005	0.357	0.364	0.204	0.053	0.003	—	—		
F13A1	0.086	0.043	0.205	0.323	0.295	0.010	—	—	—	0.003	—	0.005	0.013	0.018		

  

Loci	Parameters of forensic interest					
	He	Hob	Pex	PD	PIC	P
LPL	0.712	0.714	0.458	0.858	0.657	0.430
F13B	0.716	0.603	0.470	0.861	0.666	0.012
FES	0.698	0.719	0.437	0.846	0.640	0.104
F13A1	0.759	0.742	0.538	0.898	0.719	0.608

He=Nei's heterozygosity [6]; Hob=Observed heterozygosity; Pex=Probability of exclusion [7]; PD=Discrimination power [9]; PIC=Polymorphic information content [8]; P=p-value of Hardy-Weinberg equilibrium exact-test [4].

#### 4.2. Population study

A bias toward heterozygotes deficit was found to globally exist among the FFFL's loci ( $p=0.07$ ) and was most significant in the F13B locus ( $p=0.004$ ;  $p>0.4$  for the remaining loci). Departure from Hardy-Weinberg expected proportions was also observed for the F13B locus ( $p=0.01$ ;  $p\geq 0.1$  for other loci). No significant departure for any of the pair-wise genotypic disequilibrium tests was detected (Bonferroni correction; overall  $\alpha=0.05$ ; all  $\alpha_i>0.00024$ ). The combined Pex of Identifiler's, Powerplex 16's and SE33's loci increased from 99.9999994% to 99.99999995% after conjugation with FFFL's loci's values. The global PD for that same first set of loci was 99.99999999999999999999995% and ended up as 99.999999999999999999999998% after conjunction with the FFFL's values. Therefore, FFFL loci are a useful tool for complementing the genetic information already provided by routinely used amplification kits, namely in complex parentage investigations, but also in forensic casework carried out amongst the Northern Portuguese population.

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