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Genotyping inconsistencies and null alleles using AmpFLSTR® Identifiler® and Powerplex® 16 kits

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Abstract. Forensic genetic analyses use more and more commercially available genotyping kits such as AmpFLSTR Identifiler and Powerplex 16. Since they share many STRs, but do not use the same primers, genotyping inconsistencies can arise due to polymorphisms in the primers' annealing sequences. Indeed, if one primer does not match the target sequence, the result will be an allelic dropout and consequently one individual will be classified as homozygous with one kit and heterozygous with the other. Moreover, both can fail to anneal and then null alleles will be only detectable through mother/child or family analyses. We report: (a) the inconsistencies between the above referred kits (a primer concordance study) observed after the extensive genotyping of various population samples (mainly from Portugal and Mozambique) as well as (b) apparent opposite homozygosities in mother/child pairs using both kits. A total of 22 inconsistencies between kits was observed (for D5S818, D8S1179, D16S539, FGA and VWA). Only one mother/child incompatibility was detected out of 769 pairs when using both kits, at D5S818 (12/13), but also attributable to a one-step mutation. It is safe to conclude that the combined use of both kits practically eliminates this source of problem in kinship evaluation and databasing.

Keywords: STR; Primer concordance; Null alleles; PCR

1. Introduction

Forensic genetic analyses, namely kinship and identification, are relying more and more on commercially available genotyping kits such as the AmpFLSTR Identifiler (Applied Biosystems), which includes the STRs CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPO and VWA; and Powerplex 16 (Promega), with D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, Penta D, Penta E, TH01, TPO and VWA.

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Table 1
Observed genotype inconsistencies between AmpFLSTR Identifiler and Powerplex 16 kits

STR	Identifiler	Powerplex	Number of cases	STR	Identifiler	Powerplex	Number of cases
D5S818	11–12	12	1	D16S539	9–11	9	2
	10–12	12	3		10–11	10	2
	10–13	13	1	VWA	16	16–18	2
	12	12–13	1		16	16–19	1
	10–11	11	3		16	16–17	1
	9–11	9	1		18	16–18	1
D8S1179	14	14–16	1		18	17–18	1
FGA	25–26	26	1				

Since these two kits share many of the STRs, but do not use the same primers, it is possible that genotyping inconsistencies do arise due to polymorphisms in the primers' annealing sequences. In fact, if just one of the primers does not match the target sequence, the result will be an allelic dropout and consequently one individual will be classified as homozygous with one kit and heterozygous with the other one. Moreover, it is possible, in case of some degree of overlapping between the primers of the two kits, that both fail to anneal and, in that case, the null allele will be only detectable through mother/child or family analyses.

These issues are a major concern in forensic field, particularly for databasing [1–3]. Moreover, an extra difficulty has arisen from the fact that even different versions of the same kit from the same manufacturer can vary in primer's design [4].

In this work we report: (a) the results of the study of primer concordance between the above referred kits registering the inconsistencies observed after the extensive genotyping of various population samples (mainly from Portugal and Mozambique) as well as (b) the cases of isolated, apparent opposite homozygosity in mother/child pairs with both kits.

2. Materials and methods

Blood samples were obtained during paternity (and other kinship) analyses performed in our institute or from random blood donors. DNA was extracted by the standard Chelex method and amplified according to the manufacturers' instructions (Applied Biosystems for AmpFLSTR Identifiler and Promega for Powerplex 16). Genetic profiles were obtained using ABI 310 with the use of GeneScan software (Applied Biosystems).

Table 2
Number of meioses analyzed per STR

D2S1338	D3S1358	D5S818	D7S820	D8S1179	D13S317	D16S539	D18S51
246	1262	1262	1196	1262	1262	714	1252
D19S433	D21S11	FGA	Penta D	Penta E	TH01	TPO	VWA
248	1258	1268	714	714	1318	1316	1320

3. Results

A total of 22 inconsistencies between kits was observed, as shown in Table 1. One mother/child incompatibility was detected when using both kits, at D5S818 (m. 12/ch. 13). The total number of meioses per analyzed system is shown in Table 2.

4. Discussion

Out of the 13 STRs common to both kits, genotypic discordances have been found in five of them. They were particularly common in D5S818 (10 in 386 unrelated Portuguese) and VWA (6 individuals from the same sample). While most of false D5S818 homozygosities were detected using Identifiler, in VWA all were observed with Powerplex16. The only inconsistency in FGA was also observed in the Portuguese sample. On the contrary, for D8S1179 it was detected in the sample from Mozambique (N = 144). The case of D16S539 is much more interesting since all apparent homozygosities were detected in non-European (one individual of Cape Verdean ancestry and three in the Mozambican sample). It is also interesting to note that while the polymorphisms in the primer binding sequences for D5S818 and VWA (those that were found in Europeans) are associated with various STR alleles, those for D16S539 seem African-specific and are always in linkage with allele 11, undetected with Powerplex16.

These findings confirm the results from previous studies [1–4], showing that a non-negligible proportion of genotypic inconsistencies is expected to occur for any PCR-based system when using different primer pairs. Furthermore, the frequency of many of these sequence variants shows substantial populational diversity. This fact must be kept in mind when designing, loading and analyzing databases, as well as when performing identity or kinship investigations using results that have been obtained by different primer sets.

Concerning the single mother/child incompatibility detected when using both kits, at D5S818 the apparent exclusion can also be attributable to a one-step mutation (12>13), and it is safe to conclude that although both kits show, at least for some systems, a relatively high frequency of null alleles, the combined use of both practically eliminates this source of problem in kinship evaluation such as in paternity cases.

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