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TECHNICAL NOTE

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Concordance Between the AmpFℓSTR® MiniFiler™ and AmpFℓSTR® Identifiler® PCR Amplification Kits in the Kuwaiti Population

ABSTRACT: The AmpFℓSTR® MiniFiler™ polymerase chain reaction amplification kit, developed and supplied by Applied Biosystems, complements the AmpFℓSTR® Identifiler® polymerase chain reaction amplification kit (Applied Biosystems, Warrington, U.K.) by improving the success rate when profiling DNA that is degraded or contains inhibitors. Before applying the MiniFiler™ kit to casework, the profiles from 200 unrelated Kuwaitis were compared to Identifiler® profiles. Concordance was observed for 99.875% (1598 of 1600) of the compared STR loci. The two discordant profiles displayed allelic dropout: one at the D13S317 locus due to nonamplification of allele 10 in the MiniFiler™ profile, and one at the D18S51 locus due to nonamplification of allele 18 in the Identifiler® profile.

KEYWORDS: forensic science, DNA profiling, short tandem repeats, miniSTR, concordance, D13S317, D7S820, D2S1338, D21S11, D16S539, D18S51, CSF1PO, FGA, Amelogenin, Kuwaiti population

Short tandem repeat (STR) markers are routinely applied to crime scene analysis, human identification, and paternity testing (1–3). The latest generation of autosomal STR multiplexes, the Identifiler® kit and PowerPlex® 16 System, can coamplify 15 STR loci and the amelogenin locus in one reaction and have gained widespread use within the forensic community (4,5). The larger loci in both kits are, however, prone to allelic and locus dropout when the DNA is degraded and/or the DNA extract contains polymerase chain reaction (PCR) inhibitors (6,7).

To increase the success rate when analyzing degraded and/or inhibited samples, miniSTRs have been developed, with PCR primers close to the repeat region of the STR, reducing the length of the amplicons (8–11). In 2007, Applied Biosystems introduced the first commercially available miniSTR multiplex: the AmpFℓSTR® MiniFiler™ PCR amplification kit. It amplifies the amelogenin locus and eight of the larger STR loci contained within the Identifiler® kit: CSF1PO, FGA, D2S1338, D7S820, D13S317, D16S539, D18S51, and D21S11; the amplicons are reduced by up to 201 bp in the MiniFiler™ profiles as compared to the Identifiler® profiles (12).

The DNA Identification Laboratory of the State of Kuwait has applied forensic genetics to the identification of human remains. In particular, DNA profiling has been used to assist the identification of victims of the First Gulf War when 605 individuals, including 544 Kuwaitis, went missing during the Iraqi occupation 1990–1991. In 2001, a DNA database was established containing the Identifiler® profiles of the relatives of the victims. Following the overthrow of Saddam Hussein, in 2003, several mass graves were discovered, both in Kuwait and Iraq. The skeletal remains of hundreds of individuals have been recovered; the process is ongoing. To date, 233 victims have been identified with DNA analysis playing a crucial part in the identification process. In some cases, however, the skeletal samples displayed high levels of degradation and also contained potent PCR inhibitors, which has prevented successful analysis. In an attempt to increase the success rate for DNA profiling it is planned to use the MiniFiler™ kit. We, therefore, examined the concordance of the MiniFiler™ and Identifiler® kits as part of a validation of the new MiniFiler™ kit.

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Materials and Methods

Venous blood was collected from 200 unrelated Kuwaitis and preserved on FTA® paper (Whatman, Maidstone, U.K.). The individuals were relatives of missing persons who disappeared during the Iraqi occupation of Kuwait in the First Gulf War (1991): these included individuals of Persian and Arabic origin.

FTA® punched disks (1.2 mm) were washed with FTA® Purification Reagent (Whatman) and TE (10 mM Tris–HCl, 0.1 mM EDTA, pH 8.0) buffer according to the manufacturer's protocol. PCR using the MiniFiler™ (Applied Biosystems, Foster City, CA), Identifiler® (Applied Biosystems) and the PowerPlex® (Promega Corporation, Madison, WI) 16 kits followed the manufacturers' protocols with the exception that half reaction volumes were used.

After PCR amplification, 1 μL of each sample was diluted with 8.7 μL Hi-Di™ formamide (Applied Biosystems), and 0.3 μL Genescan™ -500 LIZ® internal size standard (Applied Biosystems). The diluted product was analyzed using an ABI 3130xl genetic analyzer, using the injection and run parameters recommended by the manufacturer (Applied Biosystems), POP4™ (Applied Biosystems) with a 36-cm capillary (Web Scientific, Crewe, U.K.), and v3.0 data collection software (Applied Biosystems). Genemapper® ID v3.2 software (Applied Biosystems) was used to analyze the data, using a threshold value of 50 rfu.

TABLE 1—*The short tandem repeat loci results from the two samples that showed discordance.*

Locus	MiniFiler™	Identifiler®	PowerPlex® 16
D13S317	8	8,10	8,10
D18S51	15,18	15	15,18

Both are likely to be caused by mutation in primer binding sites.

Results and Discussion

A total of 200 samples from individuals in the Kuwaiti population were evaluated with the MiniFiler™ and Identifiler® kits. Full concordance was seen in 198 out of 200 profiles. Out of 1600 STR loci profiles (eight in each individual's profile) 1598, or 99.875%, were identical. The two loci that were not concordant in the two samples are shown in Table 1. These samples were amplified with PowerPlex® 16 to confirm that in both cases the allelic dropout was an artifact (Fig. 1), most likely caused by a primer site mutation (13).

The level of concordance was very similar to that found in other studies: Drabek et al. (14) found 99.77% concordance when comparing the profiles from 12 miniSTRs to the PowerPlex® 16 kit; Hill et al. (13) found 99.7% concordance when comparing the MiniFiler™ and Identifiler® kits.

The dropout of allele 10 in the MiniFiler™ profile at the D13S317 locus has been seen before in a previous concordance study (13), where dropout of alleles 8, 9, and 10 at the D13S317 locus in the MiniFiler™ profile accounted for all the discordant profiles at this locus and for over 50% (14 out of 27) of the discordant profiles detected in the study; the allelic dropout was seen in individuals of African-American, Caucasian, Hispanic, and Asian descent (13). The dropout of allele 18 at the D18S51 locus in the Identifiler® profile has been seen before in Kuwaiti individuals and is probably caused by a relatively common primer binding site mutation, which occurs at a frequency of 1–2% within the Kuwaiti population (15). One of the discordant profiles at the D18S51 locus, seen by Hill et al. (13), was also due to an Identifiler® primer binding site mutation.

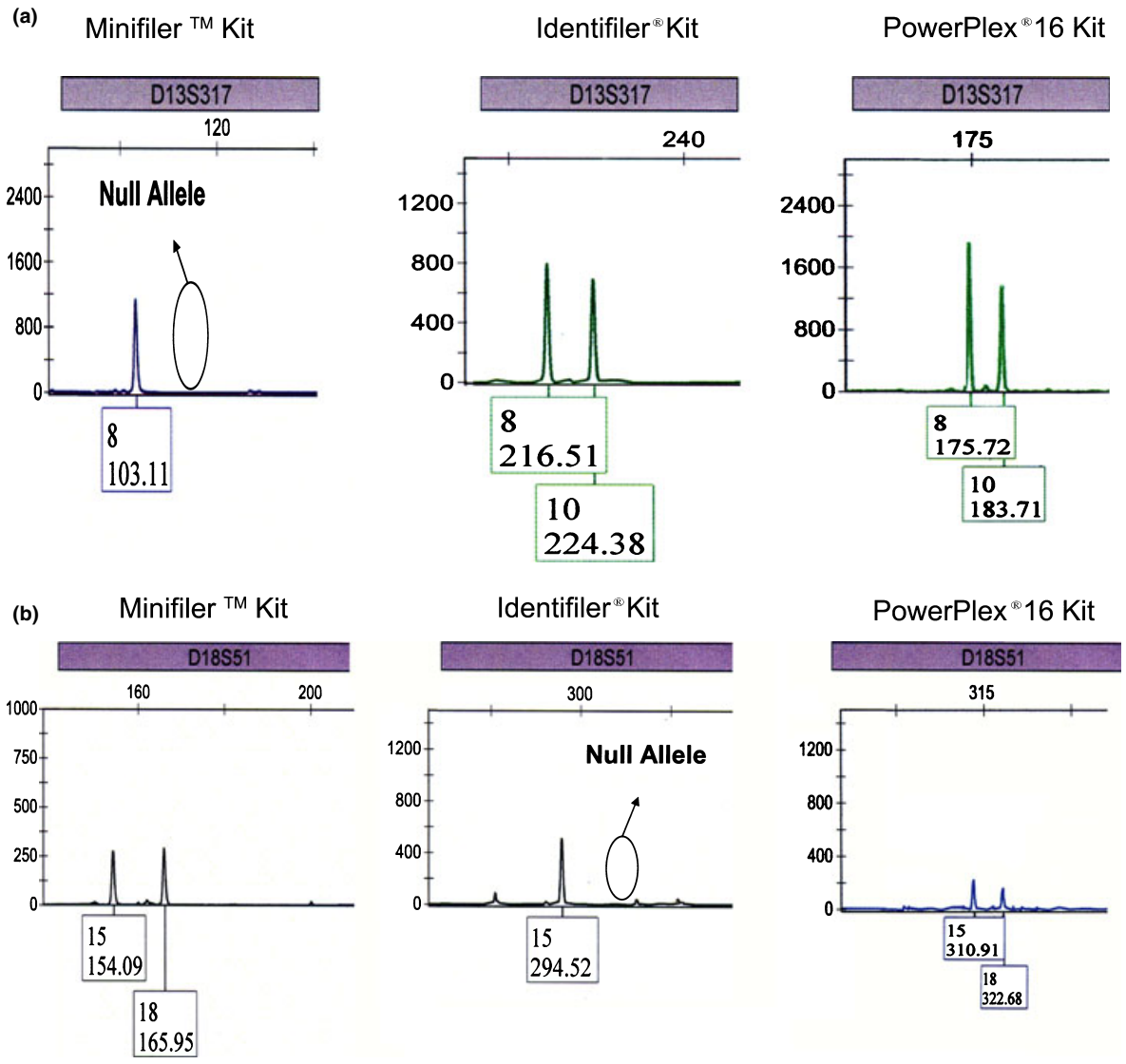

FIG. 1—*Illustration of the discordant samples: (a) dropout of allele 10 in the MiniFiler™ D13S317 profile; and (b) dropout of allele 18 in the Identifiler® D18S51 profile. The estimated sizes of the PCR products and the allele designations are shown in boxes below each peak in the electropherograms.*

The level of discordance between the MiniFiler™ and Identifiler® kits is very low in the Kuwaiti population, but has to be taken into account when comparing profiles that have been generated using different multiplex systems.

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