TECHNICAL NOTE

J Forensic Sci, July 2007, Vol. 52, No. 4
doi: 10.1111/j.1556-4029.2007.00491.x
Available online at: www.blackwell-synergy.com

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Concordance Study Between the AmpFℓSTR® MiniFiler™ PCR Amplification Kit and Conventional STR Typing Kits*

ABSTRACT: The AmpFℓSTR® MiniFiler™ polymerase chain reaction amplification kit developed by Applied Biosystems enables size reduction on eight of the larger STR loci amplified in the Identifiler® kit, which will aid recovery of information from highly degraded DNA samples. The MiniFiler™ Kit amplifies CSF1PO, FGA, D2S1338, D7S820, D13S317, D16S539, D18S51, and D21S11 as well as the sex-typing locus amelogenin. A total of 1308 samples were evaluated with both the MiniFiler™ and Identifiler® STR kits: 449 African American, 445 Caucasian, 207 Hispanic, and 207 Asian individuals. Full concordance between Identifiler and MiniFiler Kits was observed in 99.7% (10,437 out of 10,464) STR allele calls compared. The 27 differences seen are listed in Table 1 and encompass the loci D13S317 (n = 14) and D16S539 (n = 10) as well as D18S51 (n = 1), D7S820 (n = 1), and CSF1PO (n = 1). Genotyping discrepancies between the Identifiler and MiniFiler kits were confirmed by reamplification of the samples and further testing using the PowerPlex® 16 kit in many cases. DNA sequence analysis was also performed in order to understand the nature of the genetic variations causing the allele dropout or apparent repeat unit shift.

KEYWORDS: forensic science, DNA profiling, short tandem repeats, DNA typing, miniSTR, concordance, CSF1PO, FGA, D7S820, D13S317, D16S539, D18S51, D21S11, D2S1338, amelogenin, U.S. Caucasian, African American, Hispanic, Asian

Short tandem repeat (STR) markers are the primary means used today for human identity and forensic DNA testing (1). STRs are highly polymorphic and capable of generating typing results from very little material through multiplex amplification using the polymerase chain reaction (PCR). However, with highly degraded DNA specimens a loss of signal is typically observed with larger-sized STR products, either due to PCR inhibitors present in the forensic evidence or fragmented DNA molecules.

Size reduction of STR markers, and thus improved success rates with degraded or inhibited DNA samples, may be accomplished by moving PCR primers in as close as possible to the STR repeat region (2–4). A major advantage of these smaller STRs, or "miniSTRs," is that database compatibility may be maintained with convicted offender samples processed using commercial STR megaplexes. Concordance studies examining current assays compared to new ones on the same DNA samples are necessary in order to identify potential allele dropout situations due to primer binding site mutations (1,4–6). This report contains a summary of concordance results obtained on over 1300 samples run with a new miniSTR assay.

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*Sources of Support: Portions of this work was funded by the National Institute of Justice (NIJ) through interagency agreement (2003-IJ-R-029) with the National Institute of Standards and Technology (NIST) Office of Law Enforcement Standards.

Received 2 Dec. 2006; and in revised form 18 Mar. 2007; accepted 18 Mar. 2007; published 4 June 2007.

Materials and Methods

Anonymous liquid blood samples with self-identified ethnicities were purchased from Interstate Blood Bank (Memphis, TN) and Millennium Biotech, Inc. (Ft. Lauderdale, FL) and extracted, quantified, and typed with the Applied Biosystems AmpFℓSTR® Identifiler® PCR Amplification Kit (Applied Biosystems, Foster City, CA) as previously described (7). Additional anonymous samples were obtained as father/son pairs from DNA Diagnostics Center (Fairfield, OH) in the form of buccal swabs. The swabs were manually extracted using the DNA IQ™ system (Promega Corporation, Madison, WI) and quantified with an Alu-based quantitative PCR assay (8).

Beta-test materials (primer sequences are the same in the manufactured product) of the AmpFℓSTR® MiniFiler™ PCR Amplification Kit (Applied Biosystems) were used in this study. This miniSTR kit permits size reduction on eight of the larger STR loci amplified in the Identifiler® kit, including CSF1PO, FGA, D2S1338, D7S820, D13S317, D16S539, D18S51, D21S11 as well as amelogenin (9). All miniSTR assays were run in accordance with the manufacturer's recommendation. The PCR reaction contains 10 μL AmpFℓSTR® MiniFiler™ Master Mix, 5 μL AmpFℓSTR® MiniFiler™ Primer Set, 1 μL of DNA template (0.5 ng/μL), and 9 μL of 10 mmol/L Tris 0.1 mmol/L EDTA (TE^{-4}). Thermal cycling was performed in a GeneAmp® PCR System 9700 (Applied Biosystems) operating in the 9600 emulation mode with the following cycling parameters: an 11-min incubation at 95°C; followed by 30 cycles of 20 sec at 94°C, 2 min at 59°C, and 1 min at 72°C; and concluded with a 45-min incubation at 60°C. A final hold at 4°C was added until samples were removed.

Additional tests with the Identifiler® and PowerPlex® (Promega) 16 kits followed manufacturer recommended conditions with the exception of half reaction volumes being used.

Following PCR amplification, 1 μL of each sample was diluted in 8.7 μL Hi-Di™ formamide (Applied Biosystems) and 0.3 μL GeneScan™-500 LIZ® internal size standard (Applied Biosystems) and analyzed with an ABI PRISM® 3130*xl* Genetic Analyzer (Applied Biosystems) using Data Collection v3.0, POP-4™ or POP-6™ polymer (Applied Biosystems), and a 36-cm array. All genotyping was performed with GeneMapper® *ID* v3.2 software (Applied Biosystems) using manufacturer provided allelic ladders and bins and panels. Allele comparisons for concordance purposes were made with in-house Perl scripts written at Applied Biosystems and Excel macros created at NIST.

DNA sequencing of the discordant alleles was performed by first amplifying the target sequences for 28 cycles of PCR with the locus-specific primers. The PCR products were cloned using the TOPO TA cloning® kit for Sequencing (Invitrogen, Carlsbad, CA) and sequenced with the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) using the M13 forward and reverse primers following the recommendations of the manufacturers. The sequencing reactions were carried out using ~200 ng of plasmid DNA purified with a QIAprep Spin Miniprep kit (Qiagen, Valencia, CA). Unincorporated dye terminators were removed using the DyeEx 2.0 Spin kit (Qiagen). Samples were electrophoresed on the ABI PRISM® 3130*xl* Genetic Analyzer using Performance Optimized Polymer (POP-4™ polymer) on a 36-cm capillary array. The sequences were analyzed using the DNA Sequencing Analysis software v5.2 (Applied Biosystems).

Results and Discussion

A total of 1308 samples were evaluated with both the MiniFiler™ and Identifiler® STR kits: 449 African American, 445 Caucasian, 207 Hispanic, and 207 Asian individuals. Full concordance between Identifiler® and MiniFiler™ kits was observed in 99.7% (10,437 out of 10,464) STR allele calls compared. The 27 differences seen are listed in Table 1 and encompass the loci D13S317 (n = 14) and D16S539 (n = 10) as well as D18S51 (n = 1), D7S820 (n = 1), and CSF1PO (n = 1). The other three STR loci, D2S1338, FGA, and D21S11, and the sex determining locus amelogenin were fully concordant at all samples examined in this study.

Three of the null alleles detected in this study (Table 1) were from children of fathers also possessing the mutation impacting the primer-binding site. Sample no. 15 is the child of sample no. 14 (receiving the D13S317 null allele 10), sample no. 20 is the child of sample no. 21 (receiving the D16S539 null allele 11), and sample no. 22 is the child of sample no. 23 (receiving the D16S539 null allele 11). Thus, our data demonstrate Mendelian inheritance of the primer binding site mutation for these D13S317 and D16S539 null alleles.

Genotyping discrepancies between the Identifiler® and MiniFiler™ kits were confirmed by reamplification of the samples and further testing using the PowerPlex® 16 kit in 17 cases. DNA sequence analysis was also performed in order to understand the nature of the genetic variations causing the allele dropout or apparent repeat unit shift (Table 1).

Insertions or deletions in the flanking region outside of the MiniFiler™ kit primer binding sites give rise to differences in allele calls

TABLE 1—*Summary of 27 discordant STR profiling results observed in this study between the Identifiler® and MiniFiler™ kits for 449 different AA, 445 C, 207 H, and 207 A samples.*

	Locus	Ethnicity	Source	MiniFiler	Identifiler	PP16	Genetic Variation
1	CSF1PO	H	IBB	11,11	11, "**11.1**"	11,11	One base insertion in Identifiler amplicon outside of MiniFiler and PP16 primers
2	D7S820	AA	IBB	8,11	8,"**9.3**"	8,11	5 base deletion in Identifiler amplicon outside of MiniFiler and PP16 primers
3	D13S317	H	IBB	**11**,11	9,11	9,11	4 base deletion in the reverse MiniFiler primer binding region
4	D13S317	H	IBB	**13**,13	9,13	9,13	*(same as sample no. 3)*
5	D13S317	H	IBB	**14**,14	9,14	9,14	*(same as sample no. 3)*
6	D13S317	AA	IBB	**11**,11	9,11	9,11	*(same as sample no. 3)*
7	D13S317	AA	IBB	**12**,12	8,12	8,12	*(same as sample no. 3)*
8	D13S317	AA	IBB	**11**,11	8,11	8,11	*(same as sample no. 3)*
9	D13S317	AA	IBB	**13**,13	10,13	10,13	*(same as sample no. 3)*
10	D13S317	AA	IBB	**11**,11	9,11	9,11	*(same as sample no. 3)*
11	D13S317	AA	IBB	**12**,12	9,12	9,12	*(same as sample no. 3)*
12	D13S317	AA	DDC	**10**,10	9,10		
13	D13S317	C	IBB	**12**,12	9,12	9,12	*(same as sample no. 3)*
14	D13S317	C	DDC	**11**,11	10,11		
15	D13S317	C	DDC	8,**8**	8,10		
16	D13S317	A	DDC	**12**,12	10,12		
17	D16S539	AA	DDC	9,**9**	9,11		
18	D16S539	AA	IBB	**12**,12	11,12	11,12	A/G SNP in MiniFiler primer binding site
19	D16S539	AA	MLN	**11**,11	9,11	9,11	*(same as sample no. 18)*
20	D16S539	AA	DDC	**14**,14	11,14	11,14	*(same as sample no. 18)*
21	D16S539	AA	DDC	9,**9**	9,11	9,11	*(same as sample no. 18)*
22	D16S539	AA	DDC	**13**,13	11,13		
23	D16S539	AA	DDC	**12**,12	11,12		
24	D16S539	AA	DDC	**12**,12	11,12		
25	D16S539	AA	DDC	9,**9**	9,12		
26	D16S539	A	ABI	**11**,11	10,11		G/A SNP in MiniFiler primer binding site
27	D18S51	H	IBB	13,15	**15**,15	13,15	Allele 13 C/T SNP in Identifiler primer binding site

Sample sources include IBB, MLN, DDC, and ABI. With only three exceptions (see samples no. 1, 2, 27), PowerPlex® 16 (PP16) results agree with the Identifiler® results for these samples. DNA sequencing was performed to ascertain the genetic variation responsible for the discordance of the impacted allele (shown in bold font). Note that sample *no.* 15 is the child of sample no. 14, sample no. 20 is the child of sample no. 21, and sample no. 22 is the child of sample no. 23.

AA, African American; C, Caucasian; H, Hispanic; A, Asian; MLN, Millennium; IBB, Interstate Blood Bank; DDC, DNA Diagnostic Center; ABI, Applied Biosystems.

between the Identifiler® kit and the MiniFiler™ kit for CSF1PO (Table 1, sample no. 1) and D7S820 (Table 1, sample no. 2). Likewise, an Identifiler® kit primer-binding site mutation in D18S51 can cause allele dropout (Table 1, sample no. 27). The PowerPlex® 16 kit was run on a subset of our samples and found to exhibit 14 discordant calls (10 for D13S317 and 4 for D16S539) relative to the MiniFiler™ kit (see Table 1 and Fig. 1). By way of comparison, for the samples examined, there were a total of four discordant results between the PowerPlex® 16 and Identifiler® kits (Fig. 1).

Comparisons were also made to previous miniSTR primer sets described by Butler et al. (4) and the concordance reported by Drabek et al. (5). As illustrated in Fig. 2, the reverse primer for D13S317 in the MiniFiler™ kit is in a different place relative to the primer reported in Butler et al. (4) causing a different amplification outcome. Data from this study are available at http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm.

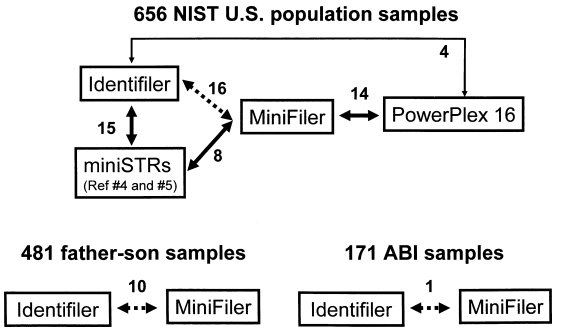

FIG. 1—*A schematic representation of the various comparisons conducted in this study. The number of discordant genotypes is shown for each comparison, which is illustrated by the double-headed arrow between the Identifiler®, MiniFiler™, and PowerPlex® 16 kits. The 27 discordant calls noted in this work are a composite of the dashed arrow comparisons.*

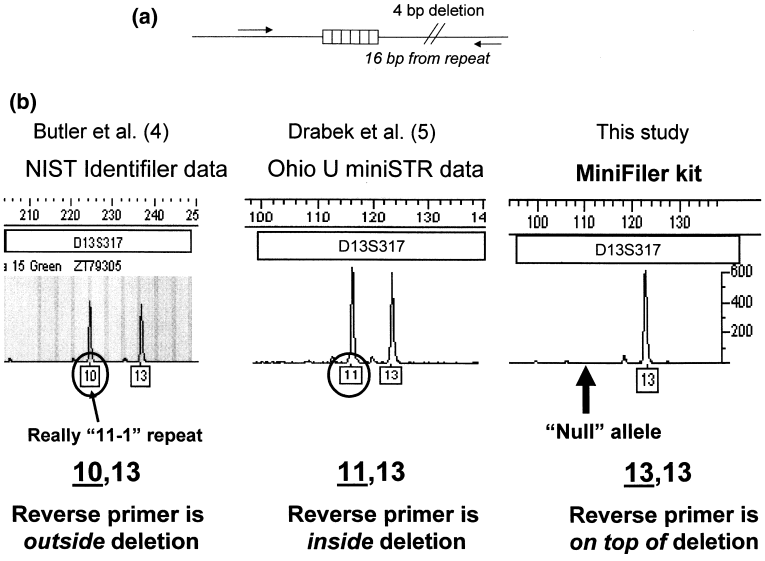

FIG. 2—*(a) Illustration of 4 bp deletion found near the D13S317 repeat region. (b) Different primer sets produce different genotyping results on the same DNA sample due to the relative positions of the reverse primer compared to the 4 bp deletion. A repeat shift in the Identifiler® kit "10" allele is observed with the Drabek et al. (5) result on the same sample, whereas the allele dropped out in this study due to the MiniFiler™ kit primer being on top of the deletion.*

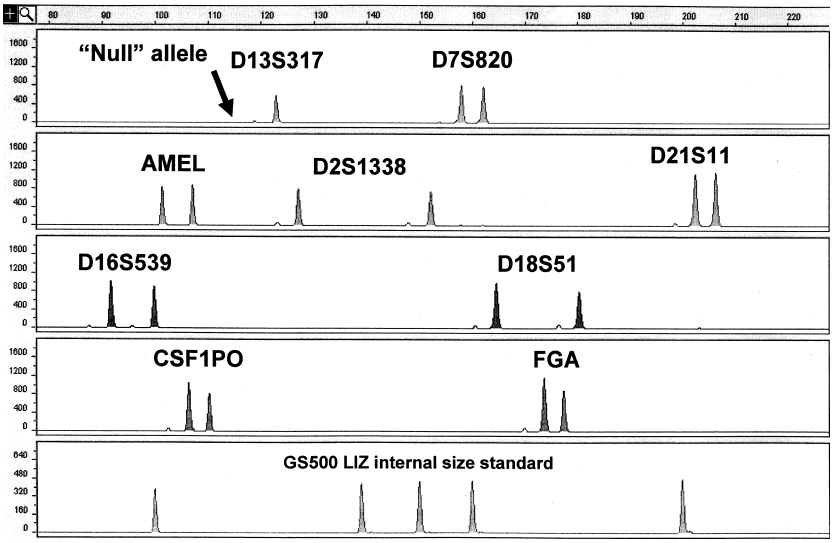

FIG. 3—*Genotyping results using MiniFiler™ kit from the same sample shown in Fig. 2 with the arrow indicating the allele dropout.*

With a multiplex amplification that produces well-balanced PCR product yields across loci, it is possible to detect allele dropout at a locus by noting when an apparent "homozygous" allele is similar in peak height to the two alleles present in a neighboring heterozygous locus (Fig. 3). A section of the NIST STRBase website has been established to collect information on allele dropout "observed" between different STR testing systems such as Mini Filer™ and Identifiler® or Identifiler® and PowerPlex® 16 kits (see http://www.cstl.nist.gov/biotech/strbase/NullAlleles.htm). Laboratories observing these null alleles are invited to submit data so that up-to-date null allele frequencies can be made available to the community.

Acknowledgments

We acknowledge the efforts of Jan Redman, Richard Schoske, Peter Vallone, and Amy Decker in preparing many of the DNA samples used at NIST as well as Tom Reid from DNA Diagnostics Center for providing the father/son samples. Madison Jordan assisted with sequencing some of the DNA samples at Applied Biosystems.

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