

FOR THE RECORD

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Concordance Study Between Miniplex Assays and a Commercial STR Typing Kit*

POPULATIONS: U.S. Caucasian, African American, Hispanic, and Asian

KEYWORDS: forensic science, DNA profiling, short tandem repeats, DNA typing, STR, Miniplex, miniSTR, concordance, CSF1PO, FGA, TH01, TPOX, vWA, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11

TABLE 1—Summary of 15 discordant STR profiling results observed in this study between the Identifiler kit and our Miniplex assays for 12 different African American (AA) and 3 Hispanic (H) samples. PowerPlex 16 (PP16) results all agree with the Identifiler results for these 15 samples. Single allele shifts of 1 repeat in the D13S317 heterozygotes are likely due to a 4 base pair deletion in the flanking region outside of the Miniplex primer binding site (see Ref 2). Allele dropout at D5S818 (see Ref 2), D13S317, and vWA (see Ref 3) are likely due to primer binding site mutations at specific alleles.

	Locus	Origin	Miniplex	Identifiler	PP16	Likely Cause
1	D13S317	AA	11, 13	10, 13	10, 13	deletion outside of allele 11
2	D13S317	H	14, 14	8, 14	8, 14	allele 8 primer binding site mutation
3	D13S317	AA	10, 11	9, 11	9, 11	deletion outside of allele 10
4	D13S317	H	10, 11	9, 11	9, 11	deletion outside of allele 10
5	D13S317	H	10, 14	9, 14	9, 14	deletion outside of allele 10
6	D5S818	AA	11, 11	11, 12	11, 12	allele 12 primer binding site mutation
7	vWA	AA	16, 16	12, 16	12, 16	allele 12 primer binding site mutation
8	vWA	AA	18, 18	13, 18	13, 18	allele 13 primer binding site mutation
9	vWA	AA	15, 15	14, 15	14, 15	allele 14 primer binding site mutation
10	vWA	AA	15, 15	14, 15	14, 15	allele 14 primer binding site mutation
11	vWA	AA	17, 17	14, 17	14, 17	allele 14 primer binding site mutation
12	vWA	AA	17, 17	14, 17	14, 17	allele 14 primer binding site mutation
13	vWA	AA	19, 19	14, 19	14, 19	allele 14 primer binding site mutation
14	vWA	AA	19, 19	14, 19	14, 19	allele 14 primer binding site mutation
15	vWA	AA	19, 19	14, 19	14, 19	allele 14 primer binding site mutation

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 AmpF ℓ STR[®] Identifiler[™] kit (Applied Biosystems, Foster City, CA) as previously described (1).

Miniplex short tandem repeat (miniSTR) assays described by Butler et al. (2) were used except that smaller volume reactions

were performed and run on an ABI Prism 310 Genetic Analyzer. These new primer sets reduce the size of amplified products by up to 200 base pairs in comparison to commercial STR typing kits (2). A total of 12 STR loci were compared between the single amplification Identifiler kit and the three separate Miniplex sets: “Big Mini” (CSF1PO, FGA, TH01, TPOX, D7S820, and D21S11), Miniplex 2 (D5S818, D8S1179, and D16S539), and Miniplex 4 (vWA, D13S317, and D18S51). For the “Big Mini” assay, 5- μ L volumes with 2 ng of input DNA and 28 PCR cycles were used while Miniplex 2 and Miniplex 4 utilized 5- μ L volumes, 2 ng of DNA template, and 26 PCR cycles. PCR amplification was carried out on a GeneAmp[®] 9700 (Applied Biosystems) as previously published (2).

A total of 532 samples were evaluated by both methods: 208 Caucasian, 212 African American, 110 Hispanic, and 2 Asian individuals. Full concordance was observed in 99.77% (6369 out of 6384) STR allele calls compared. The 15 differences seen are listed in Table 1 and encompass the three loci vWA ($n = 9$), D13S317

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($n = 5$), and D5S818 ($n = 1$). The other 9 STR loci, CSF1PO, FGA, TH01, TPOX, D7S820, D8S1179, D16S539, D18S51, and D21S11, were fully concordant at all samples examined in this study. Discrepancies between Identifiler kit and Miniplex assay primer sets were confirmed by re-amplification of the samples and further testing using the PowerPlex[®] 16 kit (Promega Corporation, Madison, WI) following manufacturer protocols.

Possible reasons for D5S818 allele dropouts and D13S317 allele shifts with the Miniplex primer sets were discussed previously (2). The vWA allele dropouts with miniSTR primers may be due to a polymorphism in the reverse primer that has been previously reported by Lazaruk et al. (3). This C-to-T transition was observed in a sufficient number of African American samples to cause Applied Biosystems to add a degenerate primer for vWA in their AmpF ℓ STR kits (3). Thus, the addition of a degenerate vWA primer for future Miniplex systems might also be able to correct for this or other polymorphic nucleotides. We plan to conduct sequencing studies in the future to identify the exact nature of the observed vWA allele dropout. We also intend to run more Asian samples with these miniSTR assays to ensure that no common polymorphisms fall in the Miniplex primer binding sites. The dataset for this study is available at <http://www.cstl.nist.gov/biotech/strbase/NISTpop.htm>.

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References

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