TECHNICAL NOTE

Susan A. Greenspoon,[1] Ph.D.; Paula J. Lytle,[1] M.S.; Stefanie A. Turek,[1] B.S.; Jeffrey M. Rolands,[1] B.S.; Marco A. Scarpetta,[2] Ph.D.; and Cathy D. Carr,[1] B.S.

Validation of the PowerPlex 1.1™ Loci for Use in Human Identification*

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ABSTRACT: STR typing is now the favored method of DNA analysis for the purposes of human identification in the forensic community. The Forensic Services Division of the Detroit Police Department has completed its validation of the PowerPlex 1.1™ loci (CSF1PO, TPOX, THO1, vWA, D16S539, D7S820, D13S317, and D5S818) for use in forensic casework. Detroit Metro Area Red Cross samples were typed from each of five racial/ethnic groups—the Hispanic, Caucasian, African American, Asian, and American Indian populations—and allele and genotype frequencies were calculated. A rare off-ladder variant (9.1 allele at D7S820) was identified among the database samples.

A number of validation studies were performed. DNA was extracted from different substrates and typed as expected, except for the DNA extracted from leather (signal absent from the D16S539, D7S820, D13S317, CSF1PO, and TPOX loci) and from dirt (no PCR product generated). The minor contributor in the mixture study (250 pg input DNA) was facile to discern. The Concordance study, the variety of fluids from the same individual, and NIST standards studies all produced the expected results. Finally, STR data confirmed previous DNA typing results from adjudicated casework samples.

KEYWORDS: forensic science, DNA typing, short tandem repeat, D16S539, D7S820, D13S317, D5S818, CSF1PO, TPOX, THO1, vWA, microvariant, validation

The application of multiplex STR characterization to the realm of human identity hastens the analyst's ability to discern whether the DNA profile obtained from the forensic evidence is highly likely or not to have derived from the individual or individuals in question (1–6). Also, application of specifically located STRs can further enhance our ability to pick apart the contributors to a complicated mixed forensic sample (7). Finally, STRs can be analyzed using a variety of different formats. For example, STRs may be evaluated by acrylamide gel using both silver stain and a gel imaging system capable of detecting fluorescent tags (8–12), capillary electrophoresis (13–15), Tof MALDI mass spectrometry (16–18), and microchip (19).

[1] Forensic Services Division, Detroit Police Department, Detroit, MI 48201.
[2] National Legal Laboratories, Okemos, MI 48864.

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One hundred and fifty Detroit Metro Area Red Cross samples were typed from each of four racial/ethnic groups: the Hispanic, Caucasian, African American, and Asian populations, as well as 58 American Indian samples. Allele and genotype frequencies were calculated and the allele frequencies were very similar in value to those published by Promega. We observed a rare off-ladder variant among our database samples—a 9.1 allele at the D7 locus. This allele as well as a 22 allele at vWA was sequenced and verified.

For the matrix study, DNA extracted from different substrates typed as expected, except for the DNA extracted from leather (signal absent from the D16S539, D7S820, D13S317, CSF1PO, and TPOX loci) and from dirt (no PCR product generated). From the mixture study we were able to easily discern the minor contributor (250 pg input DNA) STR type. The Concordance study, the variety of fluids from the same individual, and NIST standards studies all produced the expected results. Adjudicated casework samples which had been typed for the PM/DQA1 and D1S80 loci were retyped for the PowerPlex 1.1™ and all the data were consistent with the previous DNA typing results.

Materials and Methods

Purification of DNA

All samples were extracted using the QIAamp Tissue Kit. The extractions were performed according to the manufacturer's recommendations for dried bloodstains.

The DNA from sexual assault samples containing a mixture of sperm and epithelial (nonsperm) cells was prepared following the differential extraction procedure (20–23). After the initial differential lysis, nonsperm and sperm cell fraction DNAs were purified utilizing the QIAamp Spin columns (24).

Analysis of DNA

STR loci were typed using the PowerPlex 1.1™ (Promega) kit which allowed for DNA amplification of eight loci simultaneously. Routinely 2 ng of input DNA was used for amplification in a 480 or 9600 thermocycler (Perkin Elmer), using the manufacturer's recommended amplification parameters for the PowerPlex 1.1™ kit (25). 1.5 μL of the amplification product was mixed with 2 μL of the loading buffer, plus 0.5 μL of CXR ladder. 3.5 μL of this mixture was loaded onto a Gel-Mix 6 (Gibco BRL)

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acrylamide gel. The gels were scanned using a Hitachi FMBIO-100 fluorescence imaging system (Analysis v. 6.0.11). Gels containing the PowerPlex 1.1™ loci were scanned using a 585 nm filter for the CSF1PO, TPOX, THO1, and vWA loci; a 505 nm filter for the D16S539, D7S820, D13S317, and D5S818 loci; and a 650 nm filter for the CXR in-lane size standard. Scanning parameters used were as follows: sensitivity 80%; resolution 150 DPI horizontal, 150 DPI vertical; rate of scan 0.1024 s/line; repeats 1; autoband settings—gradient start 2.0, end 2.0, duration 0.1 mm, noise level 15, OD calculation with background, type 4; gray scale correction—low 50%, high 1%, type—range. No changes were made for these parameters during the course of this validation study.

STaRCall™ (v2.1, Hitachi) software program was utilized for all percent stutter calculations and to calculate the bp sizes of off-ladder variants as well as all standard alleles. Threshold for percent stutter was set at 15%. Percent stutter values were estimated by averaging the percent stutter calculated using STaRCall™ from at least 17 different samples with stutter at that particular locus, for each locus on six different acrylamide gels.

Sample Preparation

Dried bloodstains were prepared from blood samples provided by the American Red Cross to produce the database samples for the Caucasian, African American, Hispanic, Asian, and Native American populations in the Detroit metro area (150 samples for the first four and 58 for the latter). The blood samples were deposited on sterile gauze and air dried overnight before storage at −20°C.

Matrix, Multiple Samples/Same Source and Adjudicated Samples

Matrix study samples were prepared by depositing approximately 300 μL of liquid blood (preserved with EDTA) onto leather, polyester fabric, dyed cotton, grass, home carpet, dirt, vinyl, silk, cotton, and sterile gauze (positive control). The samples were air dried and stored at 4°C for several days prior to use. Multiple samples/same source study was performed using the semen (air dried on cotton), blood (air dried on cotton), hair and buccal scrapings (Omni Swab, FITZCO) donated by three male volunteers. Twelve adjudicated casework samples from a total of three different cases were used in the validation experiments. All 12 samples were previously typed for PM, DQA1 and D1S80 and had been stored at −70°C until use.

Mixture Study

The mixture study was performed with purified database samples where the concentrations had been determined using the Quanti-Blot kits (Perkin-Elmer) according to the manufacturer's recommendations. The total quantity of DNA placed into the amplification reactions was 2.5 ng. Two different DNA samples were mixed together reciprocally in the following ratios: 2.5 ng/0 ng, 2.25 ng/0.25 ng, 2.0 ng/0.5 ng, 1.75 ng/0.75 ng, 1.5 ng/1.0 ng, 1.25 ng/1.25 ng prior to amplification. PCR products were analyzed as described above.

Concordance Study

Ten Detroit Metro Area Caucasian database samples (samples Cau 30–39, described above) were typed for the eight PowerPlex 1.1™ loci and these same ten bloodstain samples were shipped to the Michigan State Police Crime Laboratory for DNA typing. The Michigan State Police Crime Laboratory made a reciprocal exchange of ten samples (dried bloodstains) for our laboratory to type for the PowerPlex 1.1™ loci. DNA types generated by both laboratories were in complete agreement.

NIST Samples

Standard Reference Material 2391 was purchased from NIST and components 3–12, 19 and 20 were typed for the PowerPlex 1.1™ loci. Accuracy of results was confirmed by comparison with the DNA types reported by NIST.

Results and Discussion

Our laboratory completed the DNA typing of 658 Detroit Metro Area database samples for the PowerPlex 1.1™ loci (CSF1PO, TPOX, THO1, vWA, D16S539, D7S820, D13S317, and D5S818), with 150 samples each coming from the Caucasian, African American, Hispanic, and Asian populations. An additional 58 samples were typed from the Native American population. Allele frequencies were calculated for the PowerPlex 1.1™ loci and, as expected, the allele frequency values differed substantially between racial/ethnic groups, but values were consistent with allele frequencies previously reported (26) within the racial/ethnic groups (Table 1). All database information was sent to the FBI for linkage and Hardy-Weinberg equilibrium analyses.

A simple average for the percent stutter was calculated with the use of STaRCall™ v2.1 (Hitachi) for each of the eight loci contained in the PowerPlex 1.1™ kit. The threshold for stutter was set at 15% for all gels. The average percent stutter values that we obtained, as well as the highest and lowest percent stutter calculated, were similar in value to those obtained by Promega (Table 2).

While typing the database samples for the PowerPlex 1.1™ loci we observed one uncommon microvariant, a 9.1 allele at the D7 locus of an Asian database sample (Fig. 1, panel B). Both the 9.1 D7 allele as well as a 22 vWA allele (Fig. 1, panel A) were sequenced and verified. Sequence analysis of these two off-ladder variants confirmed that the 22 allele at vWA was a true additional repeat and the 9.1 allele at D7 was a three basepair deletion in the first repeat (Ann Lins, pers. comm.). Two other laboratories have posted unpublished reports of the 9.1 microvariant at D7 (27).

The use of the 43 cm gels allowed us to resolve what appeared to be a thick 9.3 homozygote band into a 9.3, 10 heterozygote at the THO1 locus for two database samples (data not shown). The STaRCall™ program was utilized on the 32 cm gels in which the 9.3, 10 heterozygotes were originally electrophoresed, but the software was unable to resolve them and instead called them 9.3 homozygotes (data not shown). No attempt to try other means of increasing the resolving ability of the 32 cm gels was made, such as less template DNA, dilution of the PCR product, longer electrophoresis time, different instrument settings, or any combination of these. Discovery of the 9.3, 10 heterozygotes occurred during a final review of gels containing the 658 database samples prior to calculation of allele frequencies. Difficulty resolving the 9.3, 10 THO1 heterozygote using various formats has been reported by others (28).

Additional studies were performed in order to complete our validation of the PowerPlex 1.1™ loci such as the mixture. Concordance, multiple sources from the same individual, NIST standards and adjudicated casework studies. A mixture study was per-

TABLE 1—*Allele frequency comparison of Promega and the Detroit Metro Area.*

	CSF1PO	TPOX	THO1	vWA	D16S539	D7S820	D13S317	D5S818
21	…	…	…	0.0 (0.0)	…	…	…	…
20	…	…	…	0.0 (0.021)	…	…	…	…
19	…	…	…	0.063 (0.087)	…	…	…	…
18	…	…	…	0.21 (0.202)	…	…	…	…
17	…	…	…	0.29 (0.265)	…	…	…	…
16	…	…	…	0.193 (0.211)	…	…	…	…
15	0.003 (0.0)	…	…	0.12 (0.082)	0.0 (0.002)	…	0.003 (0.0)	0.007 (0.005)
14	0.017 (0.014)	…	…	0.123 (0.131)	0.033 (0.031)	0.0 (0.012	0.027 (0.057)	0.027 (0.012)
13	0.06 (0.06)	0.0 (0.0)	…	0.0 (0.0)	0.213 (0.167)	0.03 (0.057)	0.13 (0.083)	0.17 (0.19)
12	0.35 (0.330)	0.06 (0.037)	…	0.003 (0.0)	0.293 (0.269)	0.143 (0.121)	0.3 (0.307)	0.36 (0.35)
11	0.31 (0.309)	0.26 (0.284)	0.003 (0.0)	…	0.303 (0.319)	0.193 (0.195)	0.313 (0.305)	0.33 (0.369)
10	0.24 (0.251)	0.07 (0.056)	0.006 (0.005)	…	0.033 (0.079)	0.28 (0.295)	0.05 (0.052)	0.06 (0.057)
9.3	…	…	0.317 (0.331)	…	…	…	…	…
9.0	0.02 (0.033)	0.09 (0.093)	0.16 (0.155)	…	0.107 (0.107)	0.153 (0.152)	0.07 (0.052)	0.05 (0.01)
8	0.007 (0.002)	0.52 (0.528)	0.107 (0.117)	…	0.017 (0.026)	0.2 (0.155)	0.113 (0.143)	0.0 (0.002)
7	0.0 (0.0)	0.0 (0.0)	0.18 (0.148)	…	…	0.0 (0.01)	0.0 (0.0)	0.0 (0.005)
6	0.0 (0.0)	0.0 (0.002)	0.22 (0.237)	…	…	0.0 (0.002)	…	…
5	…	…	0.003 (0.007)	…	0.0 (0.0)	…	…	…

NOTE—Promega data are displayed in parentheses. Promega allele frequency data were obtained from the Promega Technical Manual, GenePrint PowerPlex™ 1.1 System 9/97.

TABLE 2—*Percent stutter calculation.*

	Detroit			Promega		
Locus	Average (%)*	Highest (%)	Lowest (%)	Average (%)	Highest (%)	Lowest (%)
CSF	4.8	8.1	2.7	4.1	9.5	0
TPOX	2.7	4.9	0.3	2.5	5.6	0
THO1	2.7	4.1	0.9	1.7	5.2	0
vWA	6.6	10.6	0.9	6.6	11.4	3.7
D16S539	5.8	10.4	0.3	5.1	8.6	1.7
D7S820	4.7	10.7	0.6	3.7	8.2	1.6
D13S37	3.6	7.6	1.0	4.8	7.5	2.8
D5S818	4.7	8.1	0.9	6.1	9.0	0

* When calculating the average percent stutter, the *n* varied from 17 (lowest) for D5 to 32 (highest) for vWA. Promega percent stutter values kindly provided by A. Linns.

formed using two purified DNA samples of known concentration. The DNAs were mixed together in amplification reactions at varying ratios as described in Materials and Methods. The total amount of DNA placed into the reaction mixtures was 2.5 ng. All eight STR loci of the minor contributor DNA were readily detectable at 250 pg of input DNA (Fig. 2, only the 585 nm scan is shown).

For the Concordance study, our laboratory performed a reciprocal swap of database samples with the Michigan State Police Crime Lab as described in Materials and Methods. All ten MSP lab samples were accurately typed for the PowerPlex 1.1™ loci by our lab and the MSP lab correctly typed all ten of our database samples (data not shown). All the DNA types obtained from multiple sources/same individual were the same (data not shown) and the NIST standards (SRM 2391) typed by our lab were confirmed to be correct (data not shown, Materials and Methods).

Six adjudicated evidentiary casework samples, as well as six reference samples from a total of three different cases were used in the validation study. The different samples typed were as follows:

Case No.	Sample Types	Previous PCR Systems
1	1 vaginal swab	PM/DQA1 and D1S80
2	1 vaginal swab, 1 panty swatch	PM/DQA1 and D1S80
3	2 swabs (bloodstained), 1 cutting from shirt (bloostained)	PM/DQA1 and D1S80

All results were consistent with the previous DNA types generated for the cases (data not shown). Moreover, all analysts in the lab were required to retype several adjudicated cases for the PowerPlex 1.1™ loci prior to its application to casework and all those results were consistent with the previous results (also PM/DQA1 and D1S80, data not shown). Finally, we demonstrated with the matrix study that no alterations in the PowerPlex 1.1™ DNA types occurred when the blood sample (single source) was deposited on a

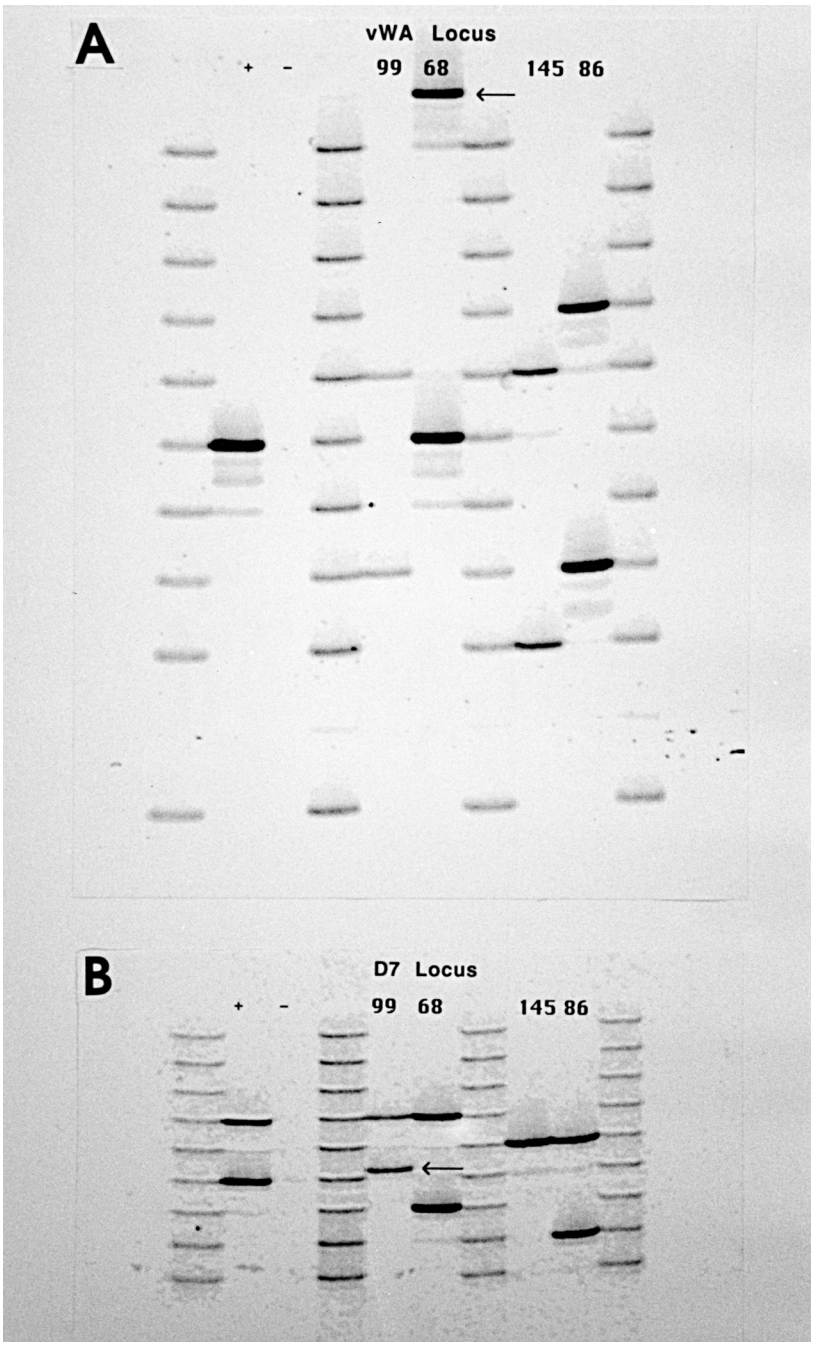

FIG. 1—*An off-ladder variant and microvariant of the PowerPlex™ 1.1 loci. STR PCR products were electrophoresed in a 6% 43 cm acrylamide gel at 50 W for approximately 2 h.* (Panel A) *The vWA locus is shown. The arrow points to the 22 allele.* (Panel B) *The D7 locus is shown. The arrow points to the 9.1 microvariant at D7. Key: + = positive control. − = negative control. Numbers above sample lanes refer to sample number. Gel images generated using an Hitachi FMBio as described in the Materials and Methods section.*

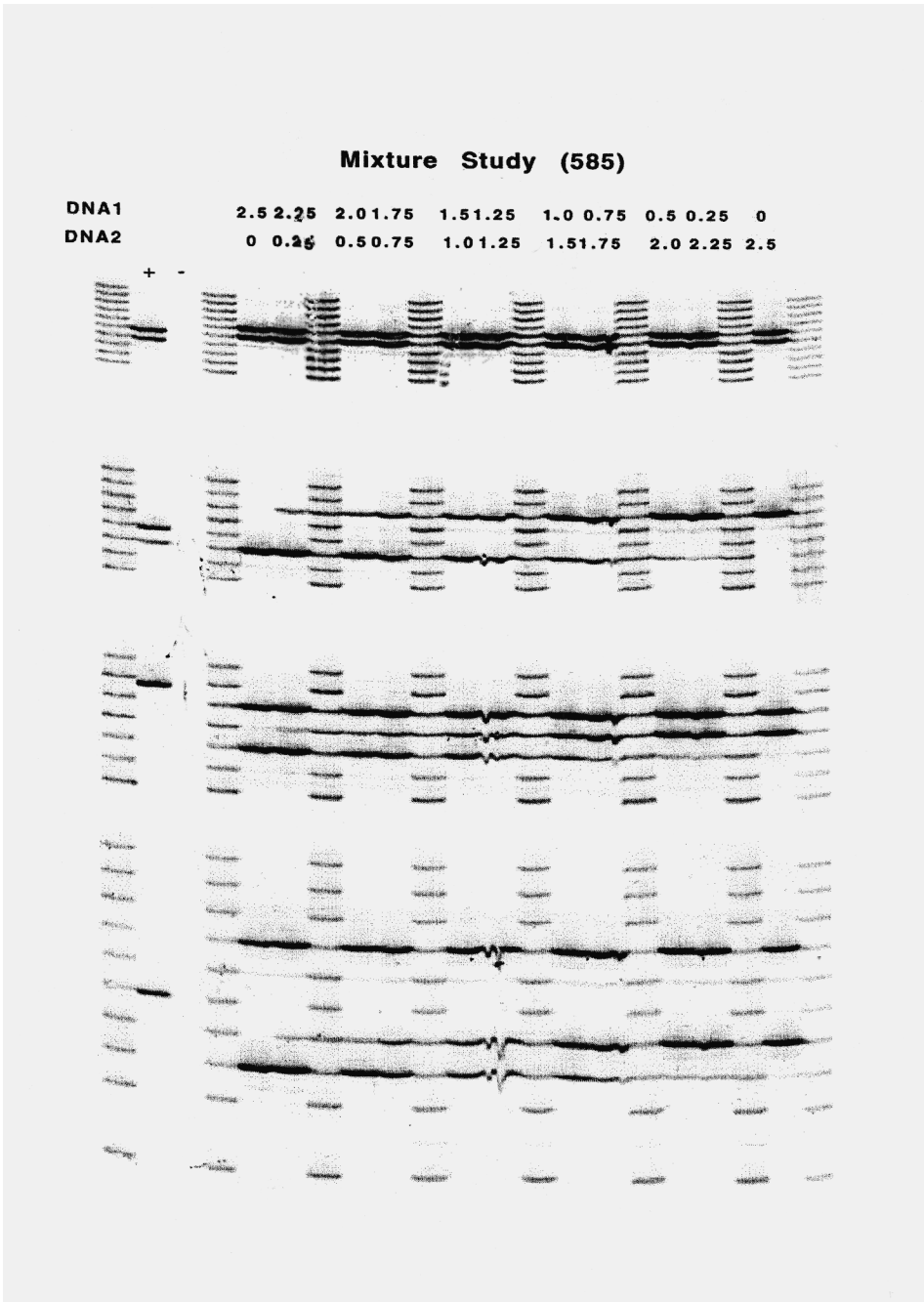

FIG. 2—*Mixture study. Purified DNA samples were quantitated then mixed together in PowerPlex1.1™ amplification reactions as described in the Materials and Methods section. Numbers above the lanes refer to nanograms of the DNA sample placed into the amplification reaction (top row is DNA1 and second row is DNA2). Key: + = positive control. − = negative control. Samples were electrophoresed in a 6% acrylamide 32 cm gel at approximately 50 W for 1 h, 20 min. Gel images generated on an Hitachi FMBio as described in the Materials and Methods section.*

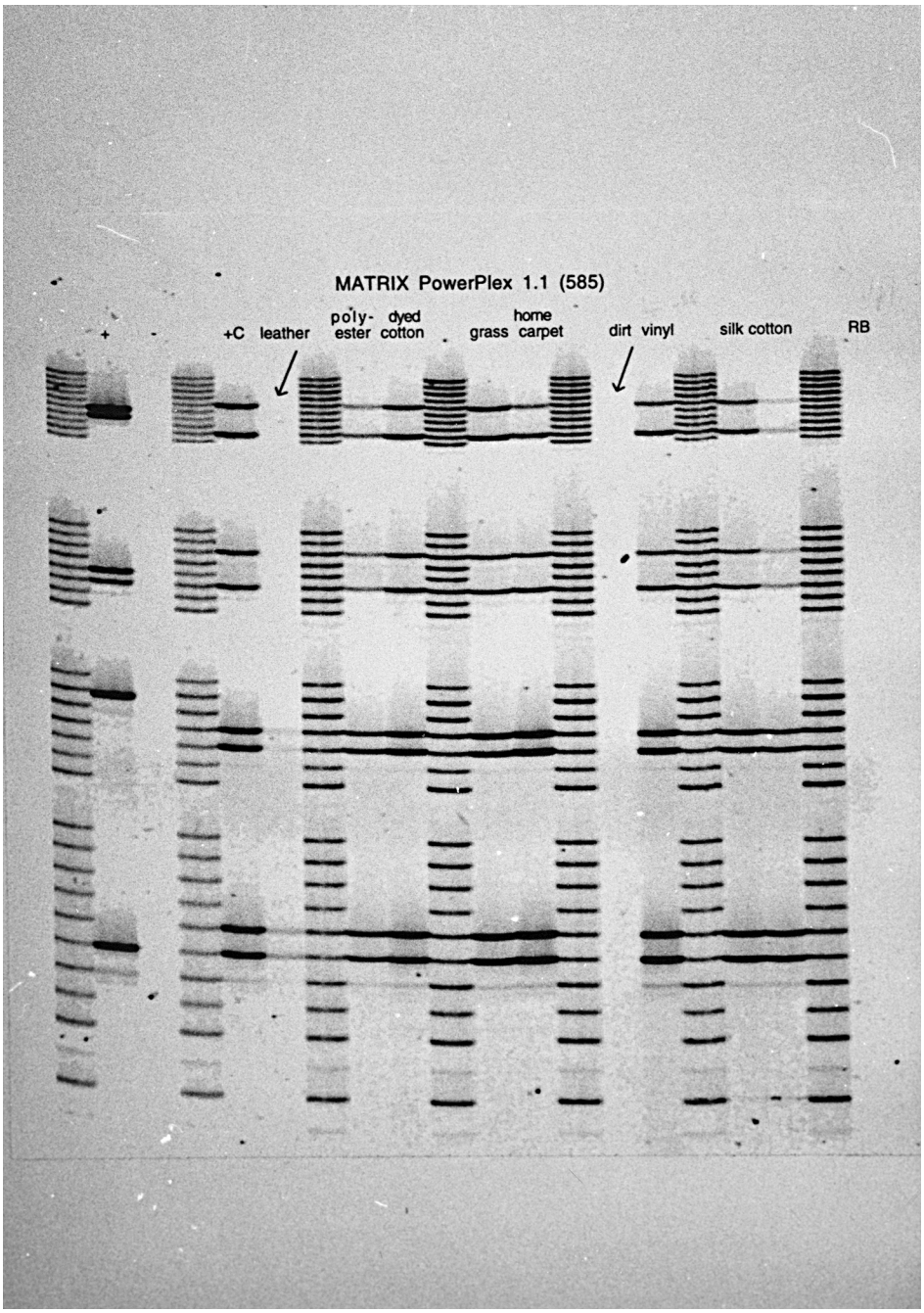

FIG. 3—*Matrix study for the PowerPlex™ 1.1 loci. Liquid blood (containing EDTA) was deposited on the variety of surfaces shown and air-dried overnight. DNA was isolated as described in the Materials and Methods section. Key: + = positive control. − = negative control. +C = substrate positive control (sterile cotton gauze). RB = reagent blank. Arrows point to samples with signal missing at all or some loci. Samples were electrophoresed in a 6% acrylamide 32 cm gel at approximately 50 W for 1 h, 20 min. Gel images generated on an Hitachi FMBio as described in the Materials and Methods section.*

variety of surfaces (Fig. 3). However, a complete loss of signal at all loci was observed in the dirt sample. A loss of signal from the larger of the loci (D16S539, D7S820, D13S317, CSF1PO, and TPOX loci) and a reduction in the signal at the remainder of the loci was observed in the leather sample.

Conclusion

The Forensic Services Division of the Detroit Police Department has successfully completed its validation of the eight PowerPlex 1.1™ loci for use in forensic human identification. The Detroit Metro Area database was completed and allele frequencies generated for each of the eight loci are comparable to those reported by Promega. The 9.1 microvariant at the D7 locus in the Asian population was readily detected by STaRCall™ as "not in range." Reamplification and electrophoresis on a long, 43 cm gel confirmed that it was a bona fide microvariant and sequence analysis conclusively identified it as such.

Additionally, we performed most of the TWGDAM suggested studies to verify that we were capable of producing accurate and re-

producible DNA typing results. DNA that were extracted from different substrates typed as expected, except for the DNA extracted from leather (signal absent from the D16S539, D7S820, D13S317, CSF1PO, and TPOX loci) and from dirt (no PCR product generated). Interestingly, it has been demonstrated that many substrates contain inhibitors of PCR, and the DNA extraction method impacts upon whether the inhibitors are removed from the preparation or co-purify with the DNA (29). All eight of the PowerPlex1.1™ loci were readily detectable from the minor contributor in the mixture study (250 pg input DNA). The Concordance study, the variety of fluids from the same individual, and NIST standards studies all produced the expected results. Finally, STR data confirmed previous PM/DQA1 and D1S80 DNA typing results from adjudicated casework samples. We have 400 samples yet to type for D18S51, D21S11, D8S1179, FGA, and D3S1358 in order to complete the validation of all 13 of the CODIS loci.

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References

1. Lins A, Micka K, Sprecher C, Taylor J, Bacher J, Rabbach D, et al. Development and population study of an eight-locus short tandem repeat (STR) multiplex system. J Forensic Sci 1998;43(6):1–13.
2. Hammond H, Jin L, Zhong Y, Caskey C, Chakraborty R. Evaluation of 13 short tandem repeat loci for use in personal identification applications. Am J Hum Genet 1994;55:175–89.
3. Anderson J, Greenhalgh M, Butler H, Kilpatrick S, Piercy R, Way K, et al. Further validation of a multiplex STR system for use in routine forensic identity testing. For Sci Int 1996;78:47–64.
4. Busque L, Desmarais D, Provost A, Schumm J, Zhong Y, Chakraborty R. Analysis of allele distribution for six short tandem repeat loci in the French Canadian population of Québec. J Forensic Sci 1997;42(6): 1147–53.
5. Budowle B, Monson K, Chakraborty R. United States population data on the multiplex short tandem short tandem repeat loci—HUMTHO1, TPOX, and CSF1PO—and the variable number tandem repeat locus D1S80. J Forensic Sci 1997;42(5):846–9.
6. Budowle B, Moretti T, Keys K, Koons B, Smerick J. Validation studies of the CTT STR multiplex system. J Forensic Sci 1997;42(4):701–7.
7. Prinz M, Boll K, Baum H, Shaler B. Multiplexing Y chromosome specific STRs and performance for mixed samples. For Sci Int 1997; 85:209–18.
8. Fregeau C, Fourney R. DNA typing with flourescently tagged short tandem repeats: a sensitive and accurate approach to human identification. Biotechniques 1993;15:100–19.
9. Lins A, Sprecher C, Puers C, Schumm J. Multiplex sets for the amplification of polymorphic short tandem repeat loci—silver stain and fluorescent detection. Biotechniques 1996;20:882–9.
10. Kimpton C, Gill P, Walton A, Urquhart A, Millican E. Automated DNA profiling employing multiplex amplification of short tandem repeat loci. PCR Methods and Appl 1993;3:13–22.
11. Micka K, Sprecher C, Lins A, Comey C, Koons B, Crouse C, et al. Validation of multiplex polymorphic STR amplification sets developed for personal identification applications. J Forensic Sci 1996;41(4):582–90.
12. Sprecher C, Puers C, Lins A, Schumm J. General approach to analysis of polymorphic short tandem repeat loci. Biotechniques 1996;20(2): 266–76.
13. Butler J, McCord B, Jung J, Allen R. Rapid analysis of the short tandem repeat HUMTHO1 by capillary electrophoresis. Biotechniques 1994;17: 1062–70.
14. Buel E, Schwartz M, LaFountain M. Capillary electrophoresis STR analysis: comparison to gel-based systems. J Forensic Sci 1998;43(1): 164–70.
15. Lazaruk K, Walsh P, Oaks F, Gilbert D, Rosenblum B, Menchen S, et al. Genotyping of forensic short tandem repeat (STR) systems based on sizing precision in a capillary electrophoresis instrument. Electrophoresis 1998;19(1):86–93.
16. Braun A, Reuter D, Darnhofer-Demar B, Tang K, Little D, Köster H. Improved analysis of microsatellites using mass spectrometry. Genomics 1997;46(1):18–23.
17. Becker C, Li J, Shaler T, Hunter J, Lin H, Monforte J. Genetic analysis of short tandem repeat loci by time-of-flight mass spectrometry. Proceedings from the Seventh International Symposium on Human Identification (Promega 1996):158–162.
18. Butler J, Li J, Shaler T, Monforte J, Becker C. Reliable genotyping of short tandem repeat loci without an allelic ladder using time-of-flight mass spectrometry. Int J Legal Med 1998;112(1):45–9.
19. Schmalzing D, Koutny L, Adourian A, Belgrader P, Matsudaira P, Ehrlich D. DNA typing in thirty seconds with a microfabricated device. PNAS 1997;94:10273–8.
20. Blake E, Mikalovich J, Higuchi R, Walsh S, Erlich H. Polymerase chain reaction (PCR) amplification and human leukocyte antigen (HLA)-DQα oligonucleotide typing on biological evidence samples: casework experience. J Forensic Sci 1992;37:700–26.
21. Gill P, Jeffries A, Werrett D. Forensic applications of DNA fingerprints. Nature 1985;318:577–9.
22. Guisti A, Baird M, Pasquale S, Balazs I, Glassberg J. Application of deoxyribonucleic acid (DNA) polymorphisms to the analysis of DNA recovered from sperm. J Forensic Sci 1986;31:409–17.
23. von Beroldingen C, Blake E, Higuchi R, Sensabaugh G, Erlich H. Applications of PCR to the analysis of biological evidence. In: PCR Technology: Principles and Applications for DNA Amplification, H. Erlich, editor, New York: Stockton Press, 1989; 209–23.
24. Greenspoon S, Scarpetta M, Drayton M, Turek S. QIAamp spin columns as a method of DNA isolation for forensic casework. J Forensic Sci 1998;43(5):1024–30.
25. Promega Technical Manual. GenePrint PowerPlex™ 1.1 System 9/97.
26. Promega Technical Manual. GenePrint PowerPlex™ 1.1 System 9/97.
27. National Institute of Standards STR website.
28. Crouse C, Rogers S, Amiott E, Gibson S, Masibay A. Analysis and interpretation of short tandem repeat microvariants and three-banded allele patterns using multiple allele detection systems. J Forensic Sci 1999;44:87–94.
29. Comey C, Budowle B. Validation studies on the analysis of the HLA DQα locus using the polymerase chain reaction. J Forensic Sci 1991; 36(6):1633–48.

Additional information and reprint requests:
Dr. Susan Greenspoon
Sr. Forensic Serologist
Serology/Trace Evidence Unit
Forensic Services Division
2600 Brust St.
Detroit, MI 48201