J Forensic Sci, Jan. 2004, Vol. 49, No. 1
Paper ID JFS2003031_491
Available online at: www.astm.org

TECHNICAL NOTE

Susan A. Greenspoon,[1] Ph.D.; Jeffrey D. Ban,[1] M.S.; Lourdes Pablo,[1] M.S.; Cecelia A. Crouse,[2] Ph.D.; Frank G. Kist,[3] B.S.; Chris S. Tomsey,[3] M.S.; Alex L. Glessner,[3] B.S.; Lisa R. Mihalacki,[4] B.S.; Teresa M. Long,[5] M.S.; Bruce J. Heidebrecht,[5] B.S.; Cathryn A. Braunstein,[5] M.S.; David A. Freeman,[6] Ph.D.; Carl Soberalski,[7] M.S.; Nathan Bruesehoff,[7] B.S.; Ashima S. Amin,[8] M.S.; Elizabeth K. Douglas,[8] M.S.; and James W. Schumm,[8] Ph.D.

Validation and Implementation of the PowerPlex® 16 BIO System STR Multiplex for Forensic Casework

ABSTRACT: The PowerPlex® 16 BIO multiplex short tandem repeat (STR) system contains the 13 CODIS loci (FGA, TPOX, D8S1179, vWA, D18S51, D21S11, TH01, D3S1358, CSF1PO, D16S539, D7S820, D13S317, and D5S818), plus two pentanucleotide repeat loci (Penta D and Penta E) and the sex-identifying locus, Amelogenin. The PowerPlex® 16 BIO System is optimized for use with the Hitachi FMBIO® gel imaging systems. A consortium of seven independent laboratories collaborated to perform the studies defined by the FBI standards for performing a developmental validation, including the evaluation of sample concordance, percent stutter determination, nonprobative casework, precision, sensitivity, mixture determination, effect of substrates, the impact of environmental insults, and species specificity. All samples tested for concordance were consistent except for one sample from the Virginia Division of Forensic Science database that displayed discordance at D13S317, a locus whose primer sequence was altered. Stutter values were comparable to those of other STR multiplex systems, the precision was comparable to other multiplexes analyzed by gel electrophoresis, the DNA profiles were unchanged by the substrate upon which the blood samples were placed, and the nonprobative casework samples re-typed for the PowerPlex® 16 BIO System were consistent with previous typing results. When greater than 0.125 ng of DNA was placed into the PowerPlex® 16 BIO System amplification reaction, a full profile was generated by all laboratories. The mixture study results were comparable to those reported for other multiplex systems, the environmental study demonstrated a loss of larger molecular weight loci when samples were incubated at elevated temperatures for a prolonged period of time, and the only notable cross species hybridization was observed with primate DNA samples. This extensive validation work performed demonstrates that the PowerPlex® 16 BIO System provides STR data of a quality comparable with other PowerPlex® STR multiplex kits as well as other widely used STR multiplexes and is thus suitable for evidentiary casework analysis as well as database sample profiling.

KEYWORDS: forensic science, PowerPlex® 16 BIO System, STR, gel electrophoresis, validation study

Validation studies are routinely performed in forensic laboratories prior to the application of any new technique for use with evidentiary samples or when a substantial modification to an existing technique has been made. The PowerPlex® 16 BIO multiplex short tandem repeat (STR) system contains the 13 Combined DNA Index System (CODIS) loci (FGA, TPOX, D8S1179, vWA, D18S51, D21S11, TH01, D3S1358, CSF1PO, D16S539, D7S820, D13S317, and D5S818), plus two pentanucleotide repeat loci (Penta D and Penta E) and the sex-identifying locus, Amelogenin. Validation of the PowerPlex® 16 STR multiplex has previously been reported (1,2); however, the PowerPlex® 16 STR multiplex kit is specifically optimized for analysis on the ABI 310, 373, or 377 systems. The PowerPlex® 16 BIO System is optimized for use with the Hitachi FMBIO® flat bed laser imaging systems. Although the same primer pairs are used for both PowerPlex® 16 and PowerPlex® 16 BIO STR multiplex systems, the fluorescent dyes are different for the Amelogenin, vWA, D8S1179, TPOX, and FGA loci (TMR and Rhodamine Red™-X, respectively) as well as for the Internal Lane Standard 600 (CXR and Texas Red®-X, respectively) (3,4). Variations in the dyes used, as well as color separation protocols, make it necessary to perform validation experiments prior to the implementation of the PowerPlex®16 BIO STR multiplex for use with forensic casework or convicted offender database sample analysis.

A consortium of seven independent forensic laboratories collaborated for the experiments reported in this study. The participating laboratories included: The Bode Technology Group (TBTG), Indiana State Police (ISP), Maryland State Police Crime Laboratory (MSP), North Carolina State Bureau of Investigation (NCSBI), Palm Beach County Sheriff's Office (PBSO), Pennsylvania State Police (PSP), and the Virginia Division of Forensic Science (VDFS).

[1] Virginia Division of Forensic Science, Richmond, VA.
[2] Palm Beach County Sheriff's Office, West Palm Beach, FL.
[3] Pennsylvania State Police DNA Laboratory, Greensburg, PA.
[4] Pennsylvania State Police DNA Laboratory, Bethlehem, PA.
[5] Maryland State Police Crime Laboratory, MD.
[6] North Carolina State Bureau of Investigation, NC.
[7] Indiana State Police, IN.
[8] The Bode Technology Group, Springfield, MD.

Received 8 Feb. 2003; and in revised form 10 Aug. 2003 and 11 Sept. 2003; accepted 13 Sept. 2003; published 17 Dec. 2003.

Although each participant in the consortium may have completed additional validation studies, contributors performed particular developmental validation experiments according to the Federal Bureau of Investigation (FBI) Standards as part of the body of validation work performed and reported by the consortium. The Promega Corporation disseminated DNA samples to members of the group for the mixture studies and some of the sensitivity studies so that all laboratories could work with the same DNA samples, thereby making some of the results directly comparable.

Materials and Methods

Most of the participating laboratories (VDFS, PBSO, NCSBI, ISP, and PSP) purified the DNA samples using a standard organic extraction (5); however, the Dykes salt precipitation procedure (6) was utilized by TBTG.

Quantitation of DNA samples by members of the consortium was performed using the following procedures: QuantiBlot® (Applied BioSystems), using either chemi-luminescent detection (VDFS, ISP, MSP), colorimetric detection (PSP), or CCDBIO (PBSO). TBTG used a spectrophotometric analysis. Quantitation of DNA for the species study (NCSBI) employed the use of 1% agarose yield gels stained with DAPI (4′,6-diamidino-2-phenylindole).

All DNA samples were amplified according to the manufacturer's recommendations (3) using either an Applied BioSystems 480 (ISP, MSP), 9600 (VDFS, PBSO, PSP), or 9700 (TBTG) thermocycler, or both the 9600 and 9700 (NCSBI). Typically 0.5 to 1 ng of DNA was used in the amplification reactions unless otherwise specified.

Prior to polyacrylamide gel electrophoresis, some laboratories opted to resolve a portion of the amplified DNA in an agarose gel to assess the extent of amplified product (VDFS [3% NuSieve® 3:1 agarose gel], MSP [2% ABI: agarose gel]). Once amplified, the PCR reactions were electrophoresed in either 5% Long Ranger® (Cambrex; [NCSBI, PSP, TBTG]) or 6% PAGE PLUS™ (Ameresco; [PBSO, VDFS, ISP, MSP]) polyacrylamide gels. The loading buffer utilized came with the PowerPlex® 16 BIO kit, and the DNA denaturing conditions utilized were those specified by the kit manufacturer. The STR gels were pre-run at the same wattage as for electrophoresis for approximately 15 min (TBTG), 20 min (VDFS, NCSBI), 30 min (MSP), 45 min (PBSO, PSP), and 1 h (ISP). Conditions for electrophoresis were as follows:

5% Long Ranger®	– 60 W for 1 h, 40 min (PSP) – 55 W for 1 h, 50 min to 2 h (TBTG) – 60 W for 1 h, 30 min (NCSBI)
6% PAGE PLUS™	– 60 W for 2 h (VDFS, PBSO) – 50 W for 2 h to 2 h, 15 min (ISP) – 45°C (~35 to 40 W) for 3 h (MSP)

Detection of PCR product was performed using the Hitachi FMBIO® II Fluorescent Imaging System, a flat bed laser-scanning instrument, and the FMBIO® Analysis software program. The color separation process was performed generally as described in the Promega PowerPlex® 16 BIO Technical User's Manual (3). Variations among individuals performing the color separation can occur since the color separation process will yield different results depending on the whether a matrix fragment or a sample fragment is selected for the separation process. Also, the intensity and thickness of the DNA fragments chosen for the color separation, as well as the background of the gel, can affect the color separation. Moreover, the matrix table may be manually altered to perform fine adjustments to the color separation. Every laboratory must define its own color separation strategy. Allele sizing and designation, as well as determinations for optical density values, were accomplished using the STaRCall™ genotyping software.

Concordance Study

Virginia Division of Forensic Science

Blood samples were collected from three major populations groups, Caucasian ($N = 20$), Black ($N = 20$) and Hispanic ($N = 20$). These blood samples were previously typed for the PowerPlex® 1.1 (CSF1PO, TPOX, TH01, vWA, D16S539, D7S820, D13S317 and D5S818 loci) and 2.1 Systems (FGA, D8S1179, D18S51, D21S11, D3S1358, Penta E, TH01, TPOX and vWA loci).

Palm Beach Sheriff's Office

Three hundred and one PBSO population samples, consisting of 101 Caucasion, 100 Black, and 100 Hispanic samples, were amplified with the PowerPlex® 16 BIO System and compared to previous STR results obtained from the PowerPlex® 1.1 and 2.1 Systems.

The Bode Technology Group

Four separate American populations (Caucasian, Asian, Hispanic, and African) were typed for the PowerPlex® 16 BIO System. Out of 653 samples attempted for PowerPlex® 16 BIO System typing, 160 were African American, 158 were Asian American, 188 were Caucasian American, and 147 were Hispanic American. Allele calls were generated and compared to previous population results on the same sample material generated either by Promega Corporation or from previous work done by TBTG with the PowerPlex® 2.1 System. When discordant calls for the PowerPlex® 1.1 or PowerPlex® 2.1 Systems versus the PowerPlex® 16 BIO System results were identified, the samples were re-amplified and re-electrophoresed to obtain a definitive result.

Stutter Determination

Virginia Division of Forensic Science

Nineteen gels, containing 251 database samples, were examined for the presence of stutter bands. Alleles separated by only one repeat unit from each other were not used for this study since stutter could potentially enhance the optical density (OD) value for the smaller of the two alleles. For each locus, the optical density of a stutter band was divided by the optical density of the primary (product length) allele. The average of these values was then calculated to obtain a mean stutter cutoff percentage value. Standard deviation (σ) was calculated and three times the standard deviation (3σ) was added to the mean stutter cutoff percentage value obtained at every locus. Thus, DNA fragments that migrate at the stutter position, but which have an OD value greater than the stutter threshold, will be designated as a true PCR product by the STaRCall™ software and not a PCR artifact. The mean stutter cutoff values were compared with previously derived values (by VDFS) for the same loci (except for Penta D) using the PowerPlex® 1.1 and 2.1 Systems. The mean stutter cutoff values were also applied to 26 samples from six nonprobative cases that were previously typed for the PowerPlex® 1.1 and 2.1 System loci by VDFS, then retyped using the PowerPlex® 16 BIO System. The stutter values obtained for the nonprobative casework samples were higher at D18S51 and D21S11 loci than the maximum expected values for these loci. Therefore, the stutter thresholds for D18S51 and D21S11 were raised to reflect what was observed with the nonprobative casework samples.

Nonprobative Casework

Palm Beach Sheriff's Office

Twelve nonprobative cases were analyzed using the PowerPlex® 16 BIO System, which constituted over 60 samples as well as the original controls. The original case evidence DNA typing data were derived from the HLA DQA1, PolyMarker, PowerPlex® 1.1, and/or PowerPlex® 2.1 Systems.

Virginia Division of Forensic Science

Previously purified and quantitated DNA samples from eight nonprobative cases were typed with the PowerPlex® 16 BIO System. All samples were originally typed with the PowerPlex® 1.1 and 2.1 systems. Two samples from one of the eight cases were electrophoresed an additional 3 h and scanned again after initial gel imaging to determine and/or confirm the presence of a microvariant at the Penta D locus.

Precision

Virginia Division of Forensic Science

Two hundred twenty-one PowerPlex® 16 BIO System allelic ladders were used to establish the statewide precision of the FMBIO II Fluorescent Imaging Systems used in the VDFS four laboratories. Twenty-one gels were analyzed with each containing 10 to 15 PowerPlex® 16 BIO System allelic ladders per gel in conjunction with an internal lane standard in every lane. After sizing of all the alleles, the greatest nucleotide base difference for each allele was determined on each gel for every locus. The greatest base difference was then adopted as the mean value for that locus and three times the derived standard deviation (3σ) applied to each locus, thereby defining the precision of a given locus with a 99% confidence interval. The standard deviation was calculated using all of the sizing data for each allele.

Palm Beach Sheriff's Office

Sixty PowerPlex® 16 BIO System allelic ladders along with internal lane standard were analyzed on four polyacrylamide gels with approximately 15 lanes per gel. Nucleotide base ranges were identified through allele sizing, the standard deviations calculated, and three standard deviations for each locus applied.

Sensitivity Study

Virginia Division of Forensic Science

The ranges of DNA quantities placed into the manufacturer's recommended 25-μL amplification reactions were as follows: 2, 1, 0.75, 0.5, 0.25, 0.125, 0.0625, and 0.03125 ng. DNA from three individuals was used. One DNA sample was isolated by VDFS according to laboratory procedure using organic extraction and quantified using the Quantiblot. The other two were genomic DNA samples isolated and quantitated (using spectrophotometer OD_{260} value) by Promega Corporation.

Pennsylvania State Police

Three DNA samples taken from former proficiency tests (GM9947A and two blood stains) were amplified at 5, 2, 1, 0.5, 0.25, 0.125, and 0.0625 ng of input DNA template for a total of 21 samples. An additional two DNA samples (blood stains) from a different proficiency test were also tested at the following template quantities: 2, 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 ng.

Palm Beach Sheriff's Office

The following four DNA samples were tested: GM9947A and three nonprobative, organically extracted casework samples. The following template concentrations were tested: 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, and 0.03 ng.

Maryland State Police

Both 0.8 μL (manufacturer's recommended amount of 4 units) and 1.0 μL of Ampli-Taq Gold™ DNA Polymerase (five units) were tested for all sensitivity study samples. Amplifications of GM9947A were made using the following amounts of input DNA: 10, 5.0, 2.0, 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 ng. PCR products were electrophoresed in an agarose gel prior to loading onto the acrylamide gel for DNA typing. The 1.0 ng template DNA sample was deemed the standard by which all of the other samples would be adjusted for the acrylamide gel loading volumes. Those samples showing up faintly on the agarose gel had all 6 μL of the sample loading cocktail placed onto the acrylamide gel to maximize PCR fragment detection. The amount of DNA amplified, the post-amplification dilutions made, and the volumes used for PAGE were identical for the corresponding dilution samples in the dilution series for both amounts of Ampli-Taq Gold™ tested, and thus the results could be directly compared.

Mixture Study

Laboratories reporting mixture study results utilized two genomic DNA samples, labeled "male" and "female," each provided at a concentration of 0.4 ng/μL by Promega Corporation. The following ratios (male:female) of the two DNA samples were placed into amplification reactions at a final total DNA quantity of 1 ng (VDFS, PBSO), 0.8 ng (ISP), and 0.5 ng (PSP) in a 25-μL reaction volume: 1:0, 19:1, 9:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:9, 1:19, and 0:1. PowerPlex® 16 BIO System amplifications were performed according to the manufacturer's recommendations.

Substrate Study

Virginia Division of Forensic Science

Blood was drawn from two volunteers and deposited on a variety of different surfaces. Additionally, various substances were deposited on clean cotton material prior to the deposition of blood to determine if any of the substances would cause inhibition of the PCR process. The samples were allowed to dry before DNA extraction. Approximately 200 μL of blood was deposited onto each substrate. The different substrates were: synthetic canvas, denim, carpet, black underwear, hosiery, and a sanitary bag (with plastic liner). The possible inhibitory substances placed onto cotton were: motor oil, hand cream, hand soap, contraceptive foam, and dirt.

Once the samples dried, a 5-mm^2 portion of the bloodstain was removed from each sample and extracted using the DNA IQ™ System (Promega Corporation, Madison, WI) on the BioMek® 2000 robot as described (7).

Pennsylvania State Police

A blood sample from a single source was placed on the following nine different substrates: dirty tire, denim, leaf, leather, wood, shoe,

glass, oily rag, and rusty metal and allowed to dry. A portion of the bloodstain on the substrate, approximately 1 cm^2, was removed for extraction and the entire cutting placed into the extraction buffer. Samples extracted using the organic extraction method followed microcon concentration. Some additional samples were extracted using the Chelex method.

Environmental Study

Pennsylvania State Police

Blood samples from the same source were deposited on Schleicher & Schuell (S&S) filter paper and left at five different temperatures and/or conditions (outdoors, 80°C, 50°C, 4°C, and room temperature) for six different periods of time (3 days, 6 days, 12 days, 25 days, 48 days, and 85 days). Thirty samples were prepared.

Virginia Division of Forensic Science

Blood samples collected from three volunteers of the VDFS were applied to blood stain cards (Whatman), dried, and subjected to the following environmental conditions for one day, one week, one month, and three months: room temperature, moist at room temperature, 37°C, 56°C, 80°C, and exposed to sunlight at room temperature (taped to a window). Samples were extracted using the BioMek® 2000 robot and the DNA IQ™ System (7). If DNA samples were very dilute or undetectable by QuantiBlot™ analysis, a maximum volume of 10 μL, according to the VDFS procedure, was placed into the amplification reaction.

Nonhuman DNA Study

Indiana State Police

Previously extracted and quantitated DNA samples from a variety of different animal species were amplified and typed using the PowerPlex® 16 BIO System in a 25-μL reaction volume. The following animal, yeast, and bacterial DNA samples were tested: chicken, deer, cow, parrot, dog, fly, horse, cat, monkey (unspecified), pig, rabbit, rat, *Saccharomyces cerevisiae* (yeast), *Escherichia coli* (bacteria). The following amounts of DNA were placed into the PowerPlex® 16 BIO System amplification reaction: deer, parrot, horse, cat, monkey, pig, rabbit, chicken, fly, cow, dog, and rat (500 ng), *Escherichia coli* (2.5 μg), and *Saccharomyces cerevisiae* (1 ng).

North Carolina Bureau of Investigation

DNA was extracted from a variety of animal samples as well as bacteria and fungi. One to two nanograms of DNA from each sample were amplified in a 25-μL PowerPlex® 16 BIO System reaction mixture per manufacturer's recommendations at least twice and the PCR products analyzed as described above. DNA from the bacterial and fungal sources was obtained from the sources listed below:

- North Carolina State University (*Bacillus cereus, Bacillus subtilis, Bacillus megaterium, Micrococcus luteus, Staphylococcus epidermidis, E. coli, Enterobacter aerogenes, Klebsiella pneumoniae, Proteus vulgaris, Pseudomonas fluorescens, Serratia marcescens, Staphylococcus capitis, Staphylococcus hominis*)
- University of North Carolina at Chapel Hill (*Chlamydia trachomatis, Neisseria gonorrhoeae*)
- AMSCO Scientific (*Enterococcus faecium, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella cholerae-suis, Streptococcus sanguis, Candida albicans,* Serratia *marcescens, and Aspergillus niger*)
- North Carolina Department of Health (*β-hemolytic strep* {Group G})

DNA was extracted from the following animals: horse, partridge, mouse, rabbit, dog, chicken, white-tail deer, Himalayan brown bear, African green monkey, Fascicularis monkey, lowland gorilla, crested cockatoo, Mallard duck, wild turkey, sheep, pig, cow, lemur, prosimian bushbaby, Rhesus monkey, stumptail monkey, and rat.

Results and Discussion

Concordance Study

Of the 653 DNA samples that had been typed previously at the PowerPlex® 1.1 and 2.1 loci that TBTG attempted to type at the PowerPlex® 16 BIO System loci, 601 samples provided a full 16 locus profile and two provided only a 13 locus (CODIS loci) profile. The remaining samples produced a result at less than the 13 CODIS loci. Insufficient DNA available was the reason full 16 locus PowerPlex® 16 BIO profiles were not produced for all samples. The previous PowerPlex® 1.1 and 2.1 typing data provided full profiles for both systems. However, all DNA typing data obtained were concordant with previously obtained results (data not shown).

The VDFS typed 60 single-source DNA samples for the PowerPlex® 16 BIO System loci, previously typed at the PowerPlex® 1.1 and 2.1 loci, as well as the Penta D monoplex, and one example of discordance was identified. The Hispanic American database sample had been previously typed with the PowerPlex® 1.1 System as an 11 homozygote at the D13S317 locus. Retyping with the PowerPlex® 16 BIO System produced a 10,11 genotype at the D13S317 locus. The discordance was independently confirmed by TBTG using the PowerPlex® 1.1 and 16 BIO Systems as well as the AmpF*l*STR® Profiler Plus® system (2,8,9; data not shown). This observed discordance was most likely due to the alteration of the D13S317 primers contained in the PowerPlex® 16 BIO System from those in the PowerPlex® 1.1 System.

The PBSO obtained PowerPlex® 16 BIO System typing results for 301 DNA samples that had previously been typed using the PowerPlex® 1.1 and 2.1 Systems. Three samples displayed a DNA typing discrepancy. These were determined to be microvariants at the FGA locus; thus, the discrepancies were not examples of discordance, but were instead examples of more precise fragment sizing. Two had previously been typed as an 18 with the PowerPlex® 2.1 System and were retyped with the PowerPlex® 16 BIO System as 18.2. The other discordant sample at FGA typed originally as a 20 with PowerPlex® 2.1, but typed with the PowerPlex® 16 BIO System as a 20.2 (data not shown). The increased precision was likely the result of electrophoresing the PowerPlex®16 BIO System typing gels for a longer period of time relative to the PowerPlex® 2.1 typing gels, thereby increasing the resolution (Cecelia Crouse, personal communication). All three microvariant results were verified by the Florida Department of Law Enforcement Orlando DNA unit using the ABI PRISM® 310 Genetic Analyzer and AmpF*l*STR® Profiler Plus® typing system.

Stutter Determination

Stutter products produced during the STR amplification method by *Taq* DNA polymerase are presumably due to DNA strand slippage and displacement (10). The maximum expected stutter value was applied to nonprobative samples from six different cases typed previously for the PowerPlex® 1.1 and 2.1 System loci, then retyped for the PowerPlex® 16 BIO System loci. The stutter values obtained for the nonprobative casework samples were higher at the D18S51 and D21S11 loci than the maximum expected values for these loci. Therefore, the stutter thresholds for D18S51 and D21S11 were raised to reflect what was observed with the nonprobative casework samples. Penta E and Penta D displayed no detectable stutter; however, a minimum default threshold value of 2% was assigned to each locus. For the remaining STR loci in the PowerPlex® 16 BIO System, the stutter threshold values fell below those previously obtained for the PowerPlex® 1.1 and 2.1 systems (personal observations). As a conservative approach, the stutter threshold values obtained for the PowerPlex® 1.1 and 2.1 systems were applied (Table 1).

Nonprobative Casework

Twelve non-probative cases, over 60 samples, were analyzed by the PBSO laboratory using the PowerPlex® 16 BIO System. The nonprobative case sample specimens included sexual assault samples and blood specimens. The original case evidence data were derived from the AmpliType® HLA DQA1 and PolyMarker, PowerPlex® 1.1, and/or PowerPlex® 2.1 Systems. No discrepancies were observed regarding the original inclusions and exclusions and the results obtained (data not shown). The PowerPlex® 16 BIO System amplifications frequently provided additional allelic information due to the Penta D STR locus and sensitivity of the PowerPlex® 16 BIO System. This was especially evident in mixture DNA profiles in which the minor component elicited more allele data.

Previously purified and quantitated DNA samples from eight nonprobative cases were typed by the VDFS laboratory with the PowerPlex® 16 BIO System. All case samples were originally typed at the PowerPlex® 1.1 and 2.1 System loci. All DNA typing results obtained with the PowerPlex® 16 BIO System were consistent with previously obtained results (data not shown). However, some variation was observed in the detection of minor alleles in mixtures. Additional allelic information was sometimes produced with the PowerPlex® 16 BIO System reactions for mixed samples and other times allelic information in the mixtures previously detected in the PowerPlex® 1.1 and 2.1 reactions was not observed in the PowerPlex® 16 BIO System reactions. This is most likely due to allelic dropout caused by stochastic effects on the minor DNA contributor since repeated amplification of the same mixed DNA sample using the PowerPlex® 16 BIO System can show variation in the pattern detected from low level contributors of mixed samples (personal observations). Despite this disparity in the ability to detect the minor alleles at various loci in some of the mixed samples, all conclusions based on the DNA typing results were completely consistent with previous findings.

TABLE 1—*Stutter values for the PowerPlex® 16 BIO System loci.*

Locus	*N*	Average Stutter (%)*	σ	3σ	Stutter + 3σ	Stutter Threshold Applied (%)
FGA	108	5	0.033	0.099	5.099	9
TPOX	56	4	0.020	0.06	4.06	8
D8S1179	220	6	0.023	0.069	6.069	8
vWA	232	14	0.107	0.321	14.321	14
Penta E	0					2
D18S51	102	7	0.046	0.138	7.138	13
D21S11	182	9	0.051	0.153	9.153	12
TH01	49	3	0.024	0.072	3.072	5
D3S1358	184	9	0.041	0.123	9.123	10
Penta D	0					2
CSF1PO	61	5	0.032	0.096	5.096	11
D16S539	110	6	0.029	0.087	6.087	12
D7S820	127	7	0.064	0.192	7.192	9
D13S317	203	6	0.024	0.072	6.072	9
D5S818	157	7	0.041	0.123	7.123	11

* = Values have been approximated to the next integer.

Precision

Precision studies are a necessary component of the panel of validation studies performed by a laboratory prior to the implementation of any new DNA typing technology. The precision studies performed by members of the consortium measured the Hitachi FMBIO® II instrument's ability to accurately assign allele designations for the PowerPlex® 16 BIO System loci.

The PBSO laboratory scanned a total of four polyacrylamide gels with approximately 15 lanes per gel of the PowerPlex® BIO System allelic ladders and internal lane standards. The ranges in size (nucleotide bases) were measured, with three standard deviations (σ) determined for each locus. The results indicated that the STaRCall™ software can accurately designate base sizes within a one-base (plus/minus) range for the PowerPlex® 16 BIO System loci (data not shown).

The VDFS laboratory analyzed a total of 221 PowerPlex® 16 BIO System allelic ladders, using all of the FMBIO® II systems in the four laboratories. Statewide, precision data obtained plus three standard deviations of the mean fell within a window of less than ±1 base from the known measured value (the paste value). The largest standard deviations from the mean were observed at the larger loci such as FGA, Penta E, D18S51, Penta D, CSF1PO and D16S539, thus a sizing window of ±1.0 base is utilized. A sizing window of ±0.8 base is achieved for D21S11 and TH01, a sizing window of ±0.6 to 0.65 base for the TPOX, D8S1179, vWA, D3S1358, D7S820, and D13S317 loci, and, finally, a sizing window of ±0.40 base for the D5S818 locus (data not shown). Precision for the Amelogenin locus was not determined.

Sensitivity Study

The VDFS utilized three different DNA samples for the sensitivity study, two female and one male sample. Locus dropout was observed in the two female DNA samples when only 0.125 ng of input DNA was used, but the male DNA sample still provided a full PowerPlex® 16 BIO System profile at 0.125 ng of input DNA (Fig. 1). Therefore, 0.25 ng of input DNA was sufficient to produce a full PowerPlex® 16 BIO System profile with all three pristine samples tested. For optimum results, the VDFS laboratory recommends the use of 0.5 to 0.75 ng of input DNA.

The PBSO laboratory used four DNA samples (GM9947A and three nonprobative case samples) at the following template amounts: 8, 4, 2, 1, 0.5, 0.25, 0.12, 0.06, and 0.03 ng. Signal loss at some loci (Amelogenin, TH01, D16S539, and D7S820) was observed with 0.25 ng of template DNA. Therefore, 0.5 ng of template DNA was deemed the necessary quantity for consistent, successful

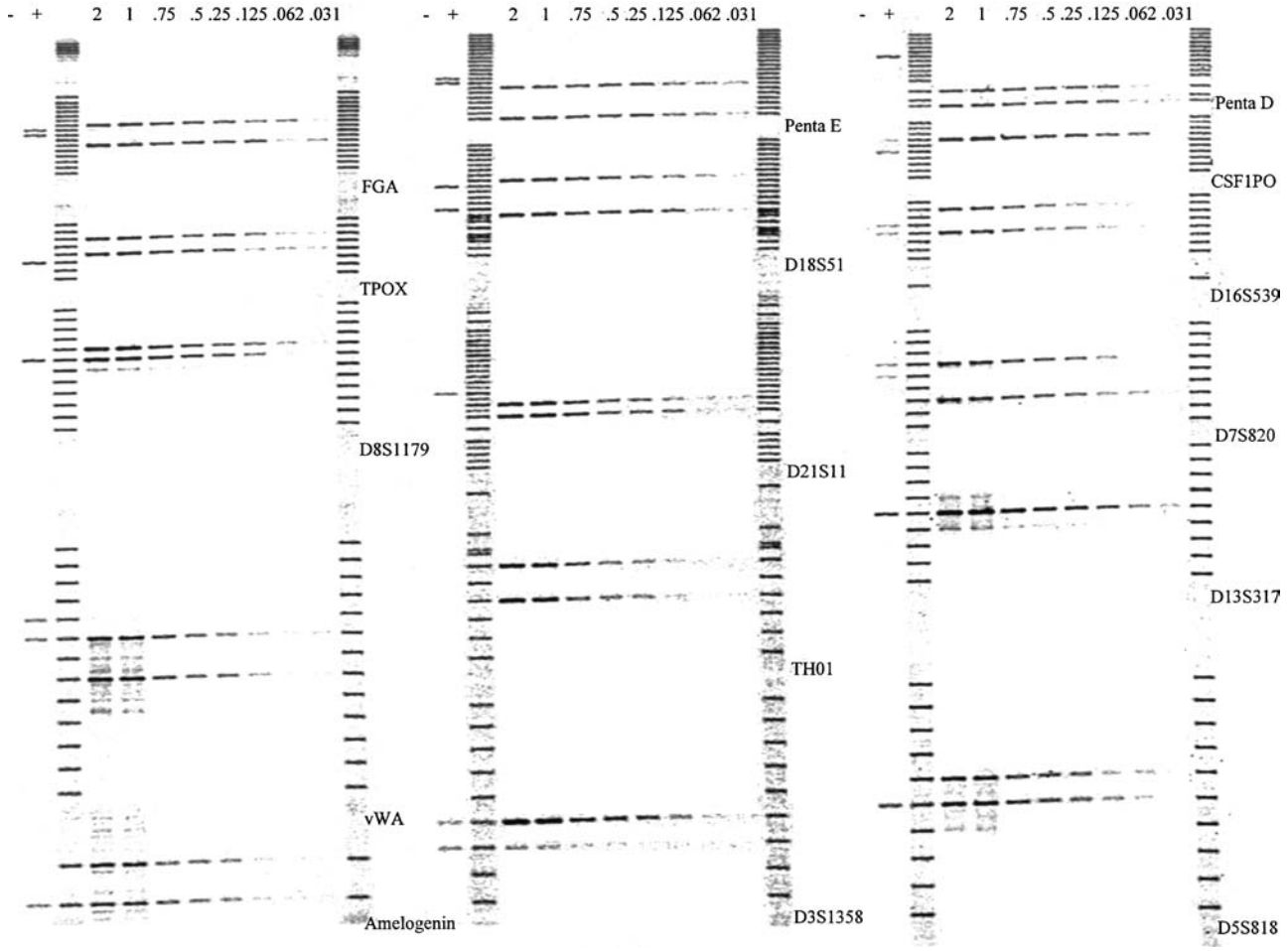

FIG. 1—*Sensitivity of the PowerPlex® 16 BIO System. Hitachi FMBIO gel images are depicted containing the dilution series for the male DNA sample. Channel 1 (Rhodamine Red™-X) is shown in the left panel, Channel 3 (Fluorescein) is shown in the center panel, Channel 4 (JOE) in the right panel, and Channel 2 (Texas Red®-X), containing the ILS 600, is not shown. The loci are indicated just below their corresponding allelic ladders. Nanogram quantities of DNA placed into each amplification reaction are indicated above each well. + = positive control (GM9947A), − = negative control.*

amplification of all the PowerPlex® 16 BIO System loci (data not shown).

The PSP laboratory amplified dilutions from three samples using 5, 2, 1, 0.5, 0.25, 0.125, and 0.0625 ng of template DNA and from two samples using 2, 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 ng of template DNA. The PCR products from the 5 and 2-ng template DNA samples demonstrated artifacts at several loci, which consisted of N + 4 base, N + 1 base, N − 1 base, N − 2 base, N − 3 base, N − 5 base, N − 6 base, and N − 8 base (data not shown). The N − 1 base, N − 2 base, N − 3 base, N − 5 base, and N − 6 base artifacts were observed primarily at the smaller loci, D8S1179, vWA, and D5S818, but also at D21S11. An N − 8 base artifact occurred at D7S820 locus using 2.0 and 1.0 ng of template DNA. An N − 2 base artifact was observed at TH01 when 5 and 2 ng of template DNA were used and also for two of the samples at 1.0 and 0.5 ng. The 1.0-ng samples produced intense stutter at the vWA locus, although the bands at the other loci were rather prominent. Locus dropout was first observed for three of the 0.0625-ng samples at vWA and Amelogenin and for one other sample at TH01 and D16S539 (data not shown). One of the DNA samples diluted down to 0.03125 ng showed dropout only at that dilution at the D8S1179, Penta E, TH01, and D16S539 loci. The target quantity of template DNA was established at 0.5 ng.

The MSP laboratory amplified 10.0, 5.0, 2.0, 1.5, 1.0, 0.8, 0.6, 0.4, 0.2, and 0.1 ng of 9947A DNA using both four units (0.8 μL, the PowerPlex® 16 BIO System manufacturer's recommended amount) and five units (1.0 μL) of Ampli-Taq Gold™ DNA Polymerase. All amplifications using four units of Ampli-Taq Gold™ and greater than 0.1 ng of template DNA produced the full DNA profiles. Only the 0.1 ng of template DNA sample demonstrated any locus dropout and this was limited to the D7S820, vWA, and Amelogenin loci. Since the amplification reactions using greater than 1.0 ng of template DNA were diluted prior to loading onto the acrylamide gel, amplification artifacts were sufficiently reduced (data not shown). The types of PCR artifacts observed when excessive amounts of DNA (10, 5, and 2 ng) were placed into the amplification reaction were N − 1 base, N − 2 base, N − 3 base, N − 5 base, N − 6 base, smearing that covered the entire sample lane, interlocus bands, an apparent Y allele in the GM9947A cell line DNA (an X,X DNA sample), and bands below the Amelogenin locus. The optimum range of template DNA for the amplification reaction decided upon was 0.8 to 1.0 ng.

The MSP laboratory test of the same series of template DNA amounts using 1.0 μL (five units) of Ampli-Taq Gold™ displayed slightly different results. Similarly, all template amounts greater than 0.1 ng provided full PowerPlex® 16 BIO System loci profiles; however, at 0.1 ng of template DNA, a greater number of loci failed to produce measurable product. Signal was lost at the TH01, D7S820, FGA, D3S1358, and vWA loci (data not shown).

Therefore, the sensitivity using the 0.8-μL volume (four units) of Ampli-Taq Gold™ was greater than that using a volume of 1.0 μL (five units) and was therefore deemed the recommended volume to be used for all PowerPlex® 16 BIO System amplifications.

Overall, the laboratories generally obtained a full PowerPlex® 16 BIO System profile when greater than 0.125 ng of input DNA was used. This is comparable to what has been reported for other STR multiplex systems (2,12–14). While the measured sensitivity of locus dropout varied among the laboratories (the PSP reported it at 0.0625 ng of input DNA, while the PBSO reported it at 0.25 ng of input DNA), it is most likely due to variations in laboratory procedures as well as differences in the sensitivity of the gel imaging systems rather than the performance of the PowerPlex® 16 BIO System.

Mixture Study

The presence of three or more alleles at a locus is generally considered an indication that a sample is a mixture. Mixture interpretation is aided by information generated regarding peak height ratios at heterozygous loci; however, when the DNA concentration of one or more of the contributors is very low, typically below 100 pg, then stochastic effects will prevail upon the PCR products produced from alleles at heterozygous loci and thus peak height ratios will not necessarily be 59% or greater (11,12). Peak height ratios were not measured as part of this mixture study since known DNA samples were utilized and the mixture proportions were also known. The mixture studies reported by four members of the laboratory consortium employed the same DNA samples (one male and one female sample), isolated and mixed in the following ratios by Promega Corporation (M:F, provided by Cindy Sprecher): 1:0, 19:1, 9:1, 4:1, 2:1, 1:1, 1:2, 1:4, 1:9, 1:19, 0:1. The summarized data from all the laboratories reporting their results for this study is displayed in Table 2. The data were very consistent among the laboratories. Laboratories reported observing no detection of the minor contributor for some loci once the 4:1 dilution was reached (13/16 loci typed for two labs, 15/16 typed for one lab, and a full profile for the other lab). Also, at the 1:4 dilution, one lab reported 13/16 loci typed for the minor contributor, one reported 10/16 loci typed, and two reported 15/16 typed. Only one laboratory reported the loss of the minor contributor at one locus (15/16 detectable) at the 1:2 dilution. While the ability to detect the minor contributor of the mixtures at various dilutions may be slightly less robust than other reported STR multiplexes (13–16), a greater number of loci are typed from a single amplification reaction than with the above referenced STR multiplexes. Conversely, when compared with the performance reported for the PowerPlex® 16 System analyzed by capillary electrophoresis, the mixture results are very consistent (2).

Substrate Study

A substrate study was designed to determine if substances contained within the substrate itself, for example a dye, or placed upon the substrate, such as dirt, could affect the performance of the PowerPlex® 16 BIO System. The VDFS and PSP report their substrate study findings here. Both laboratories placed liquid blood from two individuals (VDFS) or one individual (PSP) onto the substrates and allowed it to dry before removing a sample for DNA extraction. The following substrates were tested by either VDFS or PSP or both: dirty tire, denim, leaf, leather, shoe, wood, glass, oily rag, rusty metal, hand soap on cotton, carpet, black underwear, contraceptive foam on cotton, dirt on cotton, synthetic canvas, sanitary bag (with plastic liner), and hosiery. No incorrect DNA profiles were obtained by either laboratory, and all samples the PSP laboratory analyzed provided full PowerPlex® 16 BIO System profiles (data not shown). Partial profiles were obtained by VDFS from the sanitary bag sample for one of the blood donors and from the motor oil, hand cream, and the contraceptive foam samples for one or the other, but not both blood donors. Locus dropout from the hosiery sample was observed for one of the blood donors. This might be due to an interference of these substances with the extraction method employed at that time, as the DNA yields were lower than with control samples (personal observations, data not shown).

Environmental Study

An environmental study tests the fidelity of the DNA typing system when biological samples have been subjected to adverse conditions, a situation that frequently occurs with evidentiary samples. Liquid blood samples from three employees at the VDFS were placed onto Whatman blood stain cards and subjected to different environmental conditions described in Materials and Methods. As expected, all samples at the different conditions provided full PowerPlex® 16 BIO profiles at one day and one week (data not shown). Samples that were incubated dry at room temperature, or at 37°C, and exposed to sunlight also provided full PowerPlex® 16 BIO profiles at all incubation time periods (data not shown). However, at one month, the 56 and the 80°C samples displayed

TABLE 2—*Mixture study results using the Promega Corporation DNA samples.*

	Mixture Ratio (M:F)	1:0	19:1	9:1	4:1	2:1	1:1	1:2	1:4	1:9	1:19	0:1
VDFS	1.0 ng total DNA											
	Male loci	16/16	16/16	16/16	16/16	16/16	16/16	16/16	13/16	7/16	4/16	0/16
	Female loci	0/16	3/16	5/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16
PBSO	1.0 ng total DNA											
	Male loci	16/16	16/16	16/16	16/16	16/16	16/16	15/16	10/16	5/16	6/16	0/16
	Female loci	0/16	4/16	7/16	13/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16
ISP	0.8 ng total DNA											
	Male loci	16/16	16/16	16/16	16/16	16/16	16/16	16/16	15/16	7/16	4/16	0/16
	Female loci	0/16	2/16	6/16	13/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16
PSP	0.5 ng total DNA											
	Male loci	16/16	16/16	16/16	16/16	16/16	16/16	16/16	15/16	10/16	0/16	0/16
	Female loci	0/16	2/16	6/16	15/16	16/16	16/16	16/16	16/16	16/16	16/16	16/16

NOTE: 16/16 indicates all of the PowerPlex® 16 BIO loci amplified and were correctly typed. All numbers less than 16 indicate the number of loci that were successfully typed.

TABLE 3—*Environmental study for the PowerPlex® 16 BIO System.*

Environmental Condition	FGA	TPOX	D8S1179	vWA	Amel	Penta E	D18S51	D21S11	TH01	D3S1358	Penta D	CSF1PO	D16S539	D7S820	D13S317	D5S818
1 month 56°C	+	+	+	+	+	+/−	+	+	+	+	+/−	+	+	+	+	+
1 month 56°C	+	−	+	+	+	−	+	+	+	+	−	+	+	+	+	+
1 month 56°C	+	−	+	+	+	+/−	+	+	+	+	−	+	+	+	+	+
1 month 80°C	+	−	+	+	+	−	+	+	+	+	+/−	+	+	+	+	+
1 month 80°C	+	−	+	+	+	−	+	+	+	+	−	−	+	+	+	+
1 month 80°C	−	−	+	+	+	−	−	−	+	+	−	−	−	+	+/−	+
3 month, moist	+	+	+	−	+	+	+	+	−	+/−	+	+	+	+	+	+
3 month, moist	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+
3 month, moist	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3 month 56°C	−	−	+	+	+	−	−	−	+	+	−	−	−	−	+	+
3 month 56°C	−	−	+/−	+	+	−	−	−	+	+	−	−	−	−	+	+
3 month 56°C	−	−	+/−	+	+	−	−	−	−	+	−	−	−	−	+	+
3 month 80°C	−	+	+	+	+	−	+/−	−	+	+	−	+/−	+/−	−	+	+
3 month 80°C	−	−	−	−	+/−	−	−	−	−	−	−	−	−	−	−	−
3 month 80°C	−	−	−	−	+/−	−	−	−	+/−	+	−	−	+/−	−	+/−	+/−
48 days 80°C	−	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+
85 days 80°C	−	−	+	+	+	−	+	+	+	+	−	−	+/−	+	+	+

Key: + = complete DNA type, +/− = allelic drop-out, − = no DNA type. Each line in the table represents an individual sample.

allelic dropout and locus dropout at some loci (Table 3). At three months, a more substantial loss of DNA typing information was observed. Samples kept at 56 and 80°C displayed both allelic and locus dropout at many of the loci. The loss of DNA typing information from the 56 and 80°C samples followed a predictable pattern in that information was lost at the largest loci first, which would be consistent with progressive degradation of the DNA. What was less predictable was the observation that samples kept moist at room temperature for three months displayed a loss of DNA typing data at vWA, TH01, and D3S1358, which are some of the smallest loci.

Liquid blood from a single individual was placed onto S&S filter paper by the PSP laboratory and allowed to sit at the following conditions for 3 days, 6 days, 12 days, 25 days, 48 days, and 85 days: outside, 80°C, 50°C, 4°C, and room temperature. Locus dropout was observed only in those samples that were incubated at 80°C for 48 days and 85 days (see Table 3). As was observed with the VDFS data, those samples kept at an elevated temperature for a prolonged period of time displayed a loss of DNA typing information, primarily at the largest loci.

Nonhuman DNA Study

Although the primers for the PowerPlex® 16 BIO System are complementary to human DNA, cross-hybridization with other species is a possibility, particularly with other primates. Therefore, DNA samples from a variety of other species listed in the Materials and Methods section were tested for their ability to amplify at the PowerPlex® 16 BIO System loci. No PCR amplification products were observed from any of the microbial species following PowerPlex® 16 BIO System amplification and STR typing gel electrophoresis (data not shown), and many of the non-primate samples also displayed no products of the amplification reaction. However, a band below the Amelogenin X specific fragment was observed for Himalayan brown bear, dog, pig, horse, and cow (data not shown). Cross hybridization and amplification with several mammalian species has previously been reported for the Amelogenin locus (17). The findings reported in the previous study are in agreement with the findings reported in this study. All of the primates tested, except for the lemur, did display PCR fragment patterns (Fig. 2). These fragments apparently represent primate counterparts that are similar in size to the loci in humans. It is not known whether these fragments are species specific or constitute variability present within individuals of each species.

In addition to the nonhuman DNA study performed by the NCSBI, the ISP laboratory also performed PowerPlex® 16 BIO System amplification on a variety of nonhuman DNA samples listed in the Materials and Methods section. Fragments were detected for the dog, cow, and pig samples, positioned below the Amelogenin ladder X allele. Amplification of the chicken DNA sample generated PCR products similar in size to the human Penta D PCR products. All other animal and microbial DNA samples failed to generate any PowerPlex® 16 BIO System amplification products (data not shown).

Neither of the laboratories observed a full, 16-locus profile from the primate DNA samples tested that is characteristic of a human PowerPlex® 16 BIO System profile.

Conclusion

Samples that had been typed with other STR multiplex systems provided concordant typing results when retyped using the PowerPlex® 16 BIO System. Out of numerous samples tested by three of the participating laboratories, only one sample was truly discordant. The discordance at D13S317 was likely due to an improvement in the primer sequence to accommodate a rare nucleotide mutation in the D13S317 primer binding sequence of some samples.

Stutter values obtained for the PowerPlex® 16 BIO System were comparable to those of other STR multiplex systems. Likewise, all nonprobative case samples retyped with the PowerPlex® 16 BIO System were in complete agreement with the previous findings by both the PBSO and VDFS laboratories.

The precision studies reported by both the PBSO and VDFS laboratories demonstrate that the precision is within one base for the PowerPlex® 16 BIO System when analyzed using the Hitachi FMBIO® gel imaging instruments. One would reasonably expect to occasionally observe imprecision (by one base) in the allele designations for some microvariants. This has been observed, but rarely and primarily only with the larger molecular weight loci where band compression occurs (e.g., the FGA locus, personal observations). Several mechanisms are available to ensure that the vast majority of the time, the correct microvariant allele designation, i.e., a 22.1 versus a 22.2, will be assigned. For example, all PowerPlex®

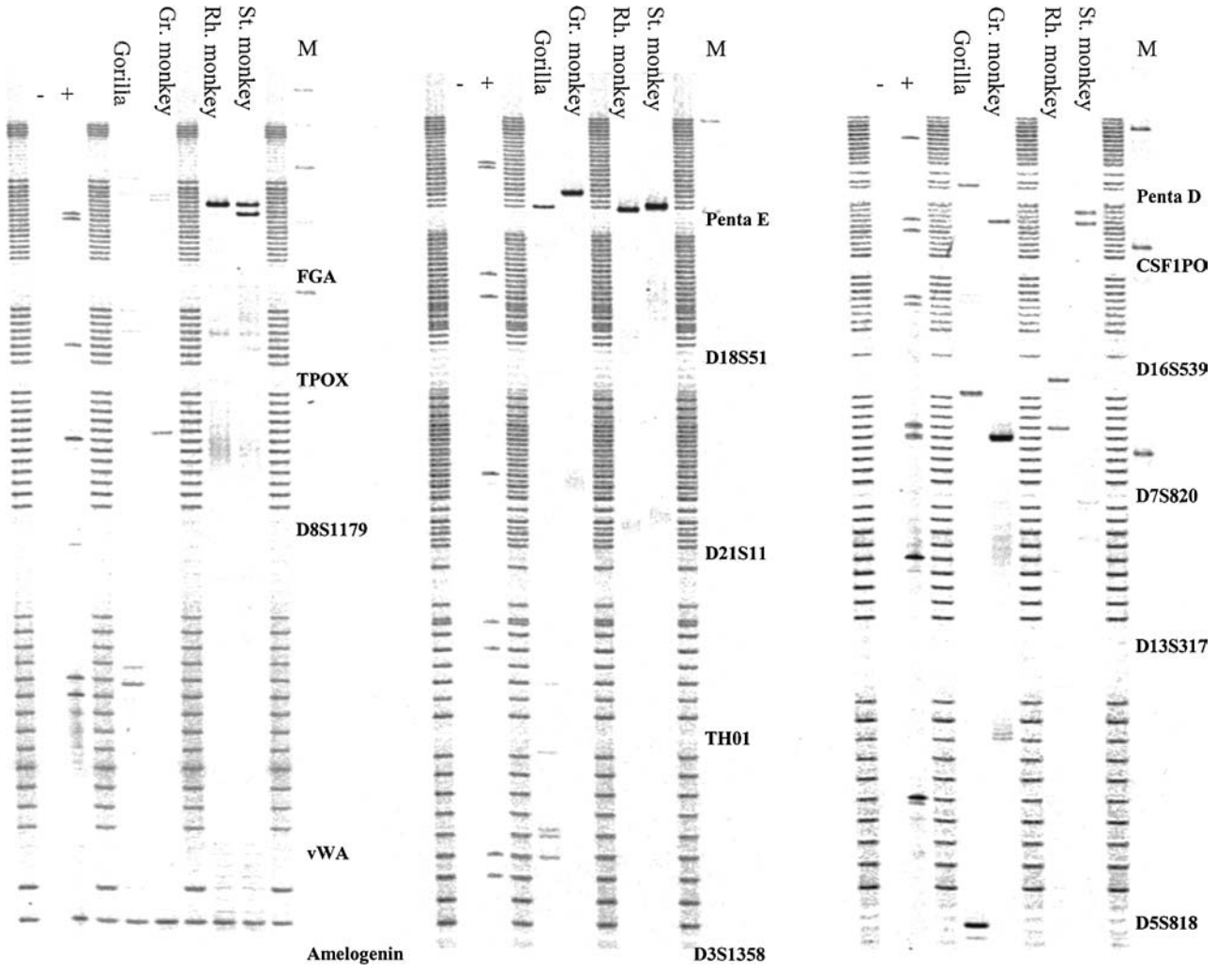

FIG. 2—*Species specificity of the PowerPlex® 16 BIO System. Hitachi FMBIO gel images are depicted containing PowerPlex® 16 BIO System amplification products from nonhuman primate DNA samples. Channel 1 (Rhodamine Red™-X) is shown in the left panel, Channel 3 (Fluorescein) is shown in the center panel, Channel 4 (JOE) in the right panel, and Channel 2 (Texas Red®-X), containing the ILS 600, is not shown. The loci are indicated just below its allelic ladder. + = positive control (GM9947A), − = negative control, M = matrix.*

16 BIO System STR data should be visually inspected in conjunction with performing the STaRCall® sizing and allele typing. When a microvariant is observed at a higher molecular weight locus, with no corresponding allele in the allelic ladder, the gel may be electrophoresed for a much longer period of time. This will advance the larger loci toward the bottom part of the gel, where the greatest separation occurs and more accurate sizing may be obtained upon rescanning and sizing of the gel.

All but one of the laboratories participating in this study reported a full PowerPlex® 16 BIO System profile for pristine samples when greater than 0.125 ng of DNA was placed into the amplification reaction. All laboratories set a recommended value of input DNA at a level higher than the lower limit of detection in their laboratory since evidentiary samples are frequently compromised.

The mixture study employed mixed DNA samples prepared by Promega Corporation and disseminated among the participants. The results were very comparable between laboratories with most laboratories able to generate a complete or nearly complete PowerPlex® 16 BIO System profile for the minor contributor at a 1:4 dilution.

The PowerPlex® 16 BIO System DNA profiles were unchanged by the substrate upon which a blood sample had been placed. There was, however, some difficulty generating a complete PowerPlex® 16 BIO profile for certain substrates, primarily due to the extraction method employed.

The environmental study produced similar results from both laboratories reporting their findings. Samples left at elevated temperatures (56 and 80°C) for a prolonged period of time (one or three months) displayed a loss of the larger loci upon DNA typing with the PowerPlex® 16 BIO System. What was somewhat surprising was the result from VDFS for the three-month incubation of samples left at room temperature in a moist environment. Those affected samples displayed a loss of some of the smallest loci: vWA, TH01, and D3S1358.

The only notable cross species hybridization of the PowerPlex® 16 BIO primers was with primate DNA samples; namely, the lowland gorilla, the green monkey, the Rhesus monkey, and the stumptail monkey. PCR products for chicken DNA in the region of the Penta D locus were also observed. The PCR fragments produced in the region of the Amelogenin locus for the other species, Himalayan brown bear, dog, pig, horse, and cow, migrate faster than the X allele of the Amelogenin locus.

The seven laboratories participating in this validation study have demonstrated that the PowerPlex® 16 BIO System is concordant with previous results and displays low stutter values. It is precise

enough to distinguish one base difference in most cases, to accurately produce STR data consistent with the previously reported results for nonprobative samples, and is comparably sensitive to other multiplexes. Mixture studies demonstrated that the minor contributor is detectable at ratios similar to what has been reported for similar mixtures analyzed with other STR multiplexes. The PowerPlex® 16 BIO System is accurate regardless of what substrate the sample has been placed upon or what the environmental conditions may be, and is human specific except for Amelogenin, where some cross-hybridization with other primates may be observed.

References

1. Budowle B, Sprecher CJ. Concordance study on population database samples using the PowerPlex™ 16 kit and AmpF/STR® Profiler Plus™ kit and AmpF/STR® Cofiler™ kit. J Forensic Sci 2001;46(3):637–41.
2. Krenke BE, Tereba A, Anderson SJ, Buel E, Culhane S, Finis CJ, et al. Validation of a 16-locus fluorescent multiplex system. J Forensic Sci 2002;47(4):773–85.
3. Promega Corporation. PowerPlex® 16 BIO System Technical Manual No. D016, 9/2001.
4. Finis CJ. Megaplex STR analysis from a single amplification: validation of the PowerPlex® 16 system. Profiles in DNA 2001;4(2):3–6.
5. Maniatis T, Fritsch EF, Sambrook J. Molecular cloning: a laboratory manual. Vol. 2. New York: Cold Spring Harbor Laboratories Press, 1989.
6. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16(3):1215.
7. Greenspoon SA, Ban JD. Robotic extraction of mock sexual assault samples using the BioMek®2000 and the DNA IQ™ System. Profiles in DNA 2002;5(1):3–5.
8. Budowle B, Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM. Population data on the thirteen CODIS core short tandem repeat loci in African Americans, U.S. Caucasians, Hispanics, Bahamians, Jamaicans, [PubMed] and Trinidadians. J Forensic Sci 1999;44(6):1277–86.
9. Masibay A, Mozer TJ, Sprecher CJ. Promega Corporation reveals primer sequences in its testing kits. J Forensic Sci 2000;45(6):1360–2. [PubMed]
10. Walsh PS, Fildes NJ, Reynolds R. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. Nucleic Acids Res 1996;24(14):2807–12. [PubMed]
11. Moretti TR, Baumstart AL, Defenbaugh DA, Keys KM, Smerick JB, Budowle B. Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. J Forensic Sci 2001;46(3):647–60. [PubMed]
12. Gill P. Application of low copy number DNA profiling. Croatian Med J 2001;42(3):229–32.
13. Levedakou EN, Freeman DA, Budzynski MJ, Early BE, Damaso RC, Pollard AM, et al. Characterization and validation studies of PowerPlex™ 2.1, a nine-locus short tandem repeat (STR) multiplex system and Penta D monoplex. J Forensic Sci 2002;47(4):757–72. [PubMed]
14. Micka KA, Amiott EA, Hockenberry TL, Sprecher CJ, Lins AM, Rabbach DR, et al. TWGDAM validation of a nine-locus and a four-locus fluorescent STR multiplex system. J Forensic Sci 1999;44(6):1243–57. [PubMed]
15. Frank WE, Llewellyn BE, Fish PA, Riech AK, Marcacci TL, Gandor DW, et al. Validation of the AmpF/STR™ Profiler Plus PCR amplification kit for use in forensic casework. J Forensic Sci 2001;46(3):642–6. [PubMed]
16. LaFountain MJ, Schwartz MB, Svete PA, Walkinshaw MA, Buel E. TWGDAM validation of the AmpF/STR Profiler Plus and AmpF/STR COfiler STR multiplex systems using capillary electrophoresis. J Forensic Sci 2001;46(5):1191–8. [PubMed]
17. Crouse, CA, Schumm J. Investigation of species specificity using nine PCR-based human STR systems. J Forensic Sci 1995;40(6):952–6. [PubMed]

Additional information and reprint requests:
Susan A. Greenspoon, Ph.D.
Virginia Division of Forensic Science
700 N. Fifth St.
Richmond, VA 23219
(804) 786-0456
E-mail: sgreenspoon@dfs.state.va.us