The PowerPlex™ 16 System

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INTRODUCTION

Countries around the world have begun the process of collecting and typing DNA from convicted offenders, whose genotypes are being entered into centralized searchable databases. These databases will eventually include the DNA profiles of millions of individuals and will make it possible to link suspects to crime scenes through STR typing. Several countries have started to select standard sets of loci for use in these databases. In the United States, the FBI has established the Combined DNA Indexing System (CODIS) and identified thirteen core STR loci (Table 1) that should be typed prior to searching or submitting samples (1–3). In Europe, ENFSI (European Network of Forensic Science Institutes) has selected seven loci (www.enfsi.org/home.php3), and Interpol has established a set of four loci as a pan-European standard (www.193.123.144.14/interpol-pr/index2.htm). In Latin America, GITAD (Grupo Iberoamericano de Trabajo en Analisis de DNA) has selected six STR loci for inclusion in their database system (4).

Previous commercially available systems, including the *GenePrint*® PowerPlex™ 1 and 2 combination[a,b], were able to identify all 13 CODIS STR loci but required two amplification reactions. In May 2000, Promega released the PowerPlex™ 16 System[a,b], the first commercial system that can be used to amplify all 13 CODIS STR loci, the ENFSI selected loci, the Interpol loci and the GITAD loci in a single reaction. In addition, the PowerPlex™ 16 System contains two low-stutter, highly polymorphic pentanucleotide repeat loci, Penta D and Penta E (5).

The PowerPlex™ 16 System's one-tube amplification and single-lane analysis format was designed to meet global needs for DNA databasing.

Table 1. The PowerPlex™ 16 System and STR Loci Selected for Databasing Standards.

PowerPlex™ 16	CODIS	Interpol	ENFSI	GITAD
Penta E	CSF1PO	FGA	FGA	CSF1PO
D18S51	FGA	D21S11	D21S11	TH01
D21S11	TH01	TH01	TH01	TPOX
TH01	TPOX	vWA	vWA	D16S539
D3S1358	vWA		D8S1179	D7S820
FGA	D3S1358		D18S51	D13S317
TPOX	D5S818		D3S1358	
D8S1179	D7S820			
vWA	D8S1179			
Amelogenin	D13S317			
Penta D	D16S539			
CSF1PO	D18S51			
D16S539	D21S11			
D7S820				
D13S317				
D5S818				

The PowerPlex™ 16 System supports typing of the STR loci selected by CODIS, Interpol, GITAD and ENSFI.

THE POWERPLEX™ 16 SYSTEM

The PowerPlex™ 16 System allows for the coamplification and three-color detection of 16 loci (Table 1). In the PowerPlex™ 16 System, one of the two primers for Penta E, D18S51, D21S11, TH01 and D3S1358 is labeled with fluorescein (FL), one primer specific for FGA, TPOX, D8S1179, vWA and Amelogenin is labeled with carboxy-tetramethylrhodamine (TMR) and one primer specific for Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818 is labeled with 6-carboxy-4´,5´-dichloro-2´,7´-dimethoxy-fluorescein (JOE). The incorporation of these three dyes allows analysis of all 16 loci after a single amplification reaction. Samples amplified with the PowerPlex™ 16 System may be analyzed on the ABI PRISM® 310 Genetic Analyzer or the ABI PRISM® 377 DNA Sequencer.

To assist in sizing analysis, the PowerPlex™ 16 System also includes the Internal Lane Standard 600 (ILS 600). The ILS 600 contains 22 DNA fragments ranging in length from 60 to 600 bases. Each fragment is labeled with carboxy-X-rhodamine and may be detected separately as a fourth color using the ABI PRISM® 310 Genetic Analyzer or the ABI PRISM® 377 DNA Sequencer. The design of this marker provides each fragment as a subset of the sequence of the next larger fragment. Thus, the relative migration of each fragment is dependent only on fragment length and is not disrupted by sequence variation.

Proper matrix generation is crucial for the evaluation of multicolored systems on the ABI PRISM® 310 and 377 instruments. The Matrix FL-JOE-TMR-CXR is required for matrix standardization. More information about this matrix is available at www.promega.com/geneticidentity/.

THE POWERPLEX™ 16 SYSTEM—DEVELOPMENT

In the summer of 1999, a survey was sent to customers in the United States and Europe to determine their requirements for a three-color, single amplification STR multiplex system designed for use on the ABI PRISM® 310 or 377 instruments. The survey contained questions about range of input DNA, sensitivity requirements and peak height expectations.

Test kits were then developed using the survey results as a guide. Alpha testing of the PowerPlex™ 16 System was performed in five U.S. and six European laboratories over a three-month period from November 1999 to January 2000. In the U.S., the results from a standard set of DNA templates were evaluated for instrument variability of the ABI PRISM® 310 Genetic Analyzer. Results from three of these laboratories showed peak heights comparable to those seen on instruments at Promega. The results from two laboratories showed slightly lower peak heights.

In both laboratories, increased injection time had no effect on peak height. However, in one laboratory, tests performed on a second instrument showed improved peak height. The second laboratory saw a doubling in peak height when fresh formamide was used.

Based on comments from these alpha testers, Promega made several minor adjust-

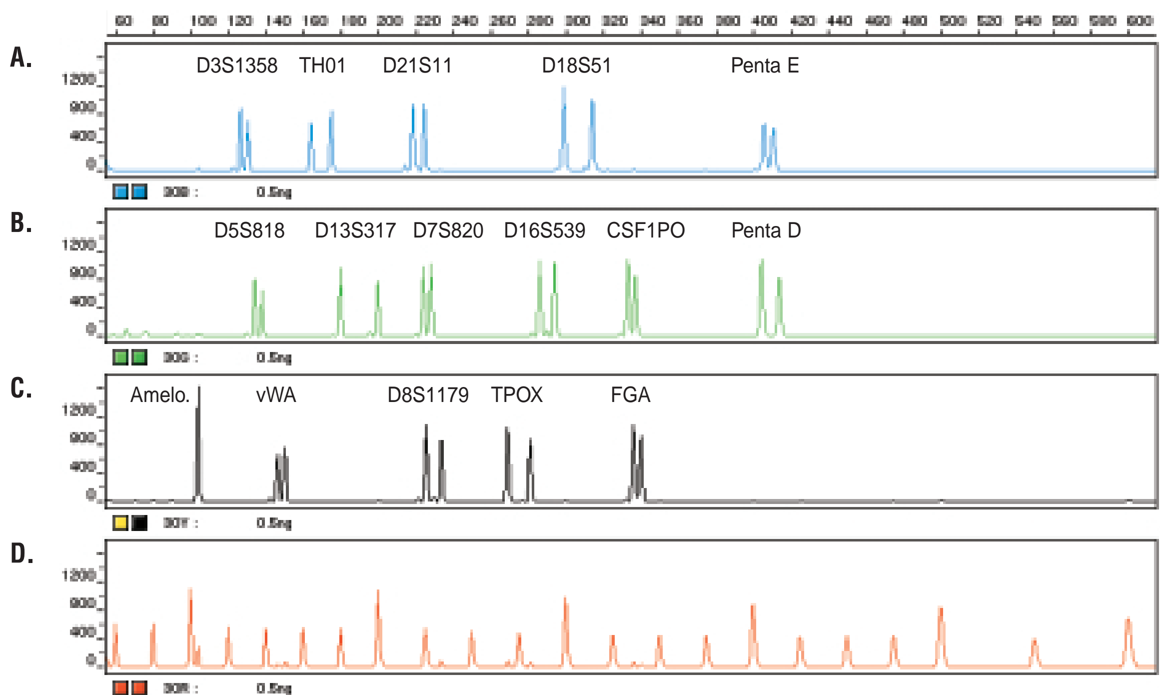

Figure 1. The PowerPlex™ 16 System. A single DNA template (0.5ng) was amplified using the PowerPlex™ 16 System. The amplification products were mixed with the Internal Lane Standard 600 and run on an ABI PRISM® 310 Genetic Analyzer using a 3-second injection time. The results were analyzed using GeneScan® analysis software. **Panel A:** An electropherogram showing the peaks of the fluorescein-labeled loci, D3S1358, TH01, D21S11, D18S51 and Penta E. **Panel B:** An electropherogram showing the peaks of the JOE-labeled loci, D5S818, D13S317, D7S820, D16S539, CSF1PO and Penta D. **Panel C:** An electropherogram showing the peaks of the TMR-labeled loci, Amelogenin, vWA, D8S1179, TPOX and FGA. **Panel D:** An electropherogram showing the fragments of the Internal Lane Standard 600.

ments to the final system. The Technical Manual was updated to include a protocol for amplification using the GeneAmp® PCR System 2400 Thermal Cycler, and more details were added to the analysis section. The system now has greater template volume flexibility and better balance with regard to the D8S1179 and D3S1358 loci. In addition, the primers used for alpha testing were used as the standard lot against which the inventory primer lots were compared, ensuring that results obtained with the PowerPlex™ 16 System will not be significantly different than those seen with the alpha test kits.

THE POWERPLEX™ 16 SYSTEM—PERFORMANCE

Figure 1 shows the results from amplification of 0.5ng of DNA template with the PowerPlex™ 16 System. The loci labeled with fluorescein (blue) are shown in Panel A, loci labeled with JOE (green) are shown in Panel B and loci labeled with TMR (black) are shown in Panel C. Panel D shows the carboxy-X-rhodamine (red) labeled Internal Lane Standard 600.

The PowerPlex™ 16 system is highly sensitive. Results obtained from amplifications using 0.2ng, 0.5ng and 1ng of template DNA are shown in Figure 2. Note that, while the relative peak intensities decrease when less template DNA is used, balance is maintained between loci. Promega uses 1ng of template DNA to set the specifications for allele balance.

To facilitate data analysis, the PowerTyper™ 16 Macro was developed for use with ABI Genotyper® software. The PowerTyper™ 16 Macro can be used with Genotyper® versions 2.0 or 2.5 and is available (free of charge) from Promega. After data from the GeneScan® analysis software is imported into the PowerTyper™ 16 Macro, the macro then uses the fluorescein-, JOE- and TMR-labeled allelic ladders to calculate offsets for each allele and assigns allele designations to sample alleles. Figure 3 shows the PowerTyper™ 16 Macro output for the five fluorescein-labeled allelic ladders, and the results for an individual sample.

THE POWERPLEX™ 16 SYSTEM—POWER OF DISCRIMINATION

The fifteen STR loci amplified with the PowerPlex™ 16 System provide powerful discrimination. Population statistics for these loci and their various multiplex combinations are being developed as part of a collaboration with The Bode Technology

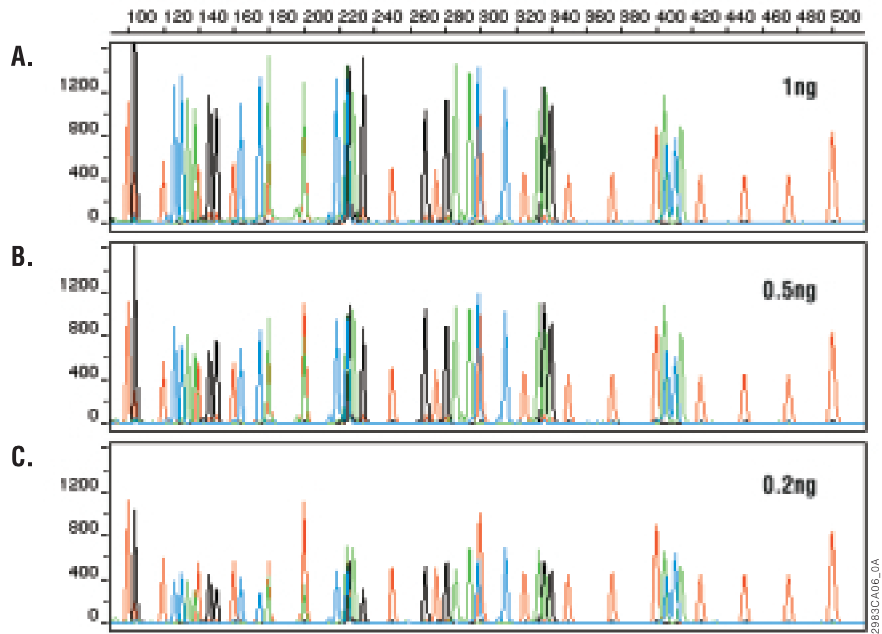

Figure 2. Sensitivity of the PowerPlex™ 16 System. Decreasing amounts of template were typed using the PowerPlex™ 16 System. **Panel A:** 1ng starting template DNA. **Panel B:** 0.5ng of starting template DNA. **Panel C:** 0.2ng starting template DNA.

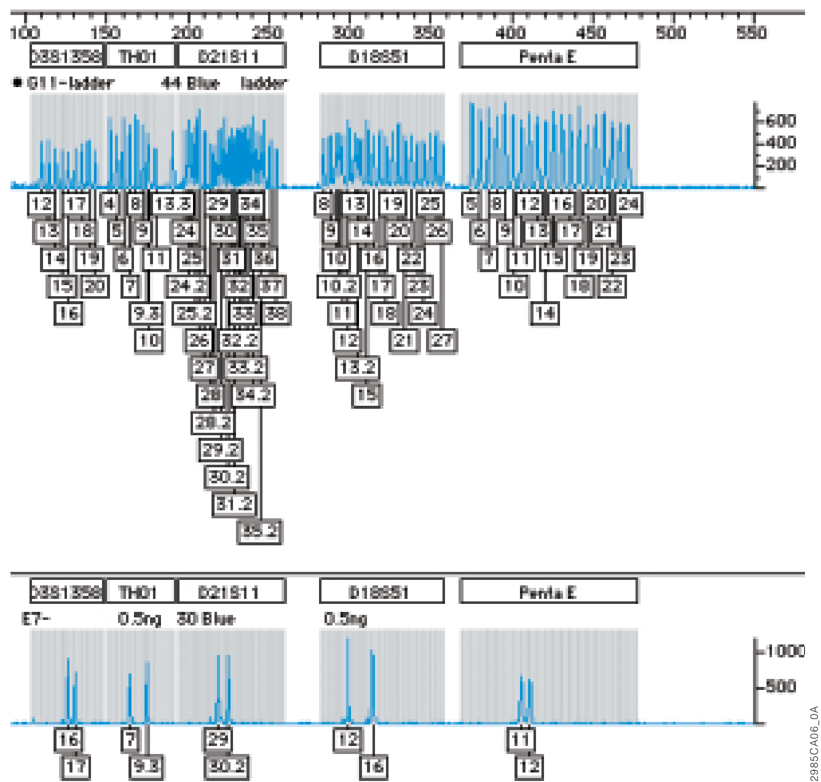

Figure 3. PowerTyper™ 16 Macro output. Example of the fluorescein output from the PowerTyper™ 16 Macro showing the allelic ladder and a sample result.

Group (Springfield, VA). Generation of these data includes analysis of over two hundred individuals from each of the three major racial and ethnic groups in the United States. Preliminary data has been used to generate the population statistics provided in Tables 2–4. This information is also provided in the Technical Manual and available online at www.promega.com/geneticidentity/.

Table 2 shows the matching probability (5) from the preliminary data for the PowerPlex™ 1.2 and 16 Systems in various populations. The matching probability of the PowerPlex™ 16 System ranges from 1 in 1.83×10^{17} for Caucasian-Americans to 1 in 1.42×10^{18} for African-Americans.

A measure of discrimination often used in paternity analyses is the paternity index (PI), a means for presenting the genetic odds in favor of paternity given the genotypes of the mother, child and alleged father (6). The typical Paternity Indices for the PowerPlex™ 1.2 and 16 Systems are shown in Table 3. The PowerPlex™ 16 System provides typical paternity indices exceeding 1,000,000 in each population group.

An alternative calculation used in paternity analyses is the power of exclusion (7). This value, calculated for the PowerPlex™ 16 System, exceeds 0.999998 in all populations tested (Table 4).

Table 2. Matching Probabilities of the PowerPlex™ 1.2 and 16 Systems in Various Populations.

STR System	African-American	Caucasian-American	Hispanic American	Asian-American
PowerPlex™ 1.2 System (8 STR loci)	1 in 2.74×10^8	1 in 1.14×10^8	1 in 1.45×10^8	1 in 1.32×10^8
PowerPlex™ 16 System (15 STR loci)	1 in 1.42×10^{18}	1 in 1.83×10^{17}	1 in 2.94×10^{17}	1 in 3.74×10^{17}

Table 3. Typical Paternity Indices of the PowerPlex™ 1.2 and 16 Systems in Various Populations.

STR System	African-American	Caucasian-American	Hispanic American	Asian-American
PowerPlex™ 1.2 System	498	260	319	471
PowerPlex™ 16 System	2,510,000	1,520,000	5,220,000	4,110,000

Table 4. Power of Exclusion of the PowerPlex™ 1.2 and 16 Systems in Various Populations.

STR System	African-American	Caucasian-American	Hispanic American	Asian-American
PowerPlex™ 1.2 System	0.9982125	0.9968853	0.9973337	0.9981793
PowerPlex™ 16 System	0.9999996	0.9999994	0.9999983	0.9999998

THE POWERPLEX™ 16 SYSTEM—THE FUTURE

Promega plans to validate the PowerPlex™ 16 System for CODIS. The validation for casework will be done following SWGDAM guidelines (8). Forensic laboratories that will participate in these validation studies have been identified. Once completed, Promega intends to submit the collaboration results for publication.

CONCLUSIONS

The globalization of DNA databasing will require a system that supports the typing of the core loci for CODIS as well as those standards set by Interpol, ENFSI and GITAD. With its one-tube amplification and one-lane analysis, the PowerPlex™ 16 System has been designed to meet the needs of forensic and paternity testing laboratories throughout the world.

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[a,b] Please refer to the patent and disclaimer statements on page 2.