VALIDATION OF PROMEGA'S POWERPLEX® 16 FOR PATERNITY TESTING

Wayne L. Hoffman, Ph.D., Jill Cramer, B.S., Euy Kyun Shin, Ph.D., Ronald C. Barwick, Ph.D., and Robert C. Giles, Ph.D.
GeneScreen (Dallas) 2600 Stemmons Freeway, Suite 133, Dallas, TX 75207

INTRODUCTION

Over the past five years, the use of PCR-based, short tandem repeats (STR) as genetic markers for human identification has become routine. A significant number of paternity testing laboratories in the United States have implemented one or more multiplex STR systems for human parentage determination. These laboratories have used a variety of methods (radioactivity, silver staining and fluorescence) and a variety of instrumentation to identify amplified STR products. This manuscript will report the results of a concordance study involving Promega's new, single amplification, PowerPlex® 16 multiplex kit. In this study, DNA samples from 10 paternity cases were amplified using the PowerPlex® 16 kit and analyzed on ABI PRISM™ instrumentation. The identical samples were also amplified using both the PowerPlex® 1.1 and the PowerPlex® 2.1 multiplex kits and analyzed on Hitachi FMBIO® instrumentation. Subsequent studies demonstrated a lack of agreement between PowerPlex® 1.1 and PowerPlex® 16 in many cases where parent/child pairs were single-banded mismatches in the D13 system due to a "null" allele in PowerPlex® 1.1. Both phenotypic maternal single exclusions and phenotypic paternal single exclusions in PowerPlex® 1.1 were genotypic inclusions in PowerPlex® 16. In addition, this manuscript will discuss amplification parameters using the PowerPlex® 16 kit and will consider the potential impact of the PowerPlex® 16 kit on case-flow management, turnaround time and data analysis.

VALIDATION STUDIES

The PowerPlex® 16 kit has major advantages over existing technology. Sixteen STR systems are co-amplified in one reaction and the resolving power of these sixteen STR systems is greater than the combination of any two kits currently on the market. For example, when compared with the combination of the PowerPlex® 1.1 and the PowerPlex® 2.1 multiplex kits, the PowerPlex® 16 kit adds the Penta D and the amelogenin STR systems. When compared with the combination of the Profiler Plus™ and the COfiler™ multiplex kits, the PowerPlex® 16 kit adds the Penta D and the Penta E STR systems. The addition of these STR systems increases the Combined Paternity Index and the Power of Exclusion of the PowerPlex® 16 kit over the combination of these other kits. Table 1 compares the Combined Paternity Index and the Power of Exclusion of the various combinations of these STR multiplexes in Caucasians.

Before any data were collected, reference DNA samples provided by the Promega Corporation were used to standardize amplification conditions and to validate instrumentation. Optimum results suggested by Promega included: peak heights between 200 and 5000 RFU, minimal stutter and clean amplification in all systems. In our laboratory, we chose 1 ng of DNA template in 25 µl (total volume) of mix and a 10/20 amplification/temperature cycling on our Perkin Elmer 9700 thermocycler to obtain optimum results with our ABI PRISM™ 377 instrument. The optimum conditions were then used to amplify 350 CODIS samples from the Wisconsin Crime Laboratory. When the PowerPlex® 16 results of the 350 CODIS samples were compared with

the results obtained on the same samples using the combination of the Profiler Plus™ and the COfiler™ multiplex kits, there was complete concordance. In addition, when 10 paternity cases were amplified using the PowerPlex® 16 multiplex kit and the results were compared with the amplification of the same samples using a combination of the PowerPlex® 1.1 and the PowerPlex® 2.1 multiplex kits, there was also complete concordance.

VARIATIONS IN THE D13 SYSTEM

Although there was a complete concordance between PowerPlex® 16 and the other multiplex kits when the CODIS samples and the paternity cases were analyzed, subsequent studies of unusual paternity cases demonstrated differences between PowerPlex® 1.1 and PowerPlex® 16 in the D13 system. When "null" alleles were studied, both apparent homozygous maternal exclusions and apparent homozygous single paternal exclusions in PowerPlex® 1.1 were genotypic inclusions in PowerPlex® 16. This difference in the amplified product is clearly seen in Figures 1 and 2. Figure 1 shows the D13 system of a paternity case amplified using the PowerPlex® 1.1 multiplex kit and analyzed on Hitachi FMBIO® instrumentation. When the mother's phenotype (12) was compared with the child's phenotype (9) there was an apparent maternal exclusion in the D13 system. This apparent maternal exclusion in the D13 system disappeared when the same paternity case was amplified using the PowerPlex® 16 multiplex kit and analyzed on an ABI PRISM™ 377 instrument. Figure 2 demonstrates that the mother's genotype was 11,12 and the child's genotype was 9,11. Therefore, the primer in the PowerPlex® 1.1 multiplex kit did not allow amplification of the shared 11 repeat allele.

Sixteen other mother/child pairs that gave apparent homozygous maternal exclusions in the D13 system of PowerPlex® 1.1 were also amplified using the PowerPlex® 16 multiplex kit. All mother/child combinations that produced apparent homozygous maternal exclusions when amplified using the PowerPlex® 1.1 multiplex kit were heterozygous when amplified using the PowerPlex® 16 multiplex kit. The results of ten of these mother/child combinations are given in Table 2. Note the various combinations of "null" alleles in the D13 system that were not detected in the amplified product when the PowerPlex® 1.1 multiplex kit was used, but were detected in the amplified product when those same samples were amplified using the PowerPlex® 16 multiplex kit. It was assumed that the redesigned primer of the D13 system in PowerPlex® 16 allowed the amplification of those "null" alleles. Although the African American race was the most prevalent race in the seventeen cases studied with maternal exclusions attributed to "null" alleles in the D13 system of PowerPlex® 1.1, two of the seventeen maternal exclusion cases involved Caucasian mother/child combinations. Therefore, "null" alleles in the D13 system of PowerPlex® 1.1 were not limited to a single race.

Identical results were obtained when eight paternity cases with possible "null" single paternal exclusions in the D13 system of PowerPlex® 1.1 were amplified using the PowerPlex® 16 multiplex kit. The D13 "null" alleles that were not detected when the samples were amplified using the PowerPlex®M 1.1 kit were detected when the samples were amplified using the PowerPlex® 16 kit. As a result, the alleged fathers that were excluded in the D13 system of PowerPlex® 1.1 were not excluded in the D13 system of PowerPlex® 16.

POWERPLEX® 16 AND PATERNITY TESTING

The PowerPlex® 16 multiplex kit offers several advantages to the paternity laboratory. As was evident in Table 1, the PowerPlex® 16 multiplex kit has a higher Power of Exclusion than the combination of either the PowerPlex® 1.1 and the PowerPlex® 2.1 multiples kits or the combination of the Profiler Plus™ and the COfiler™ multiplex kits. The PowerPlex® 16 multiplex kit is also more robust than either the PowerPlex® 1.1 or the PowerPlex® 2.1 multiplex kits, because lower DNA template concentrations are required to obtain optimum amplification. Since these advantages are achieved with a single amplification, the PowerPlex® 16 multiplex kit is a major advance for the paternity laboratory. It is envisioned that the use of the PowerPlex® 16 multiplex kit in our laboratory will lower our current seven-day turn-around-time by decreasing the need for additional testing in a number of our cases.

Acknowledgements: We would like to thank Dr. Rick Staub for his assistance in generating the data in Table 1. We would also like to thank the Promega Corporation for providing the PowerPlex® 16 multiplex kits used in this study.

Figure and Table Legends

Table 1. A comparison of the Paternity Index (PI) and the Power of Exclusion (POE) of several commercially available STR multiplex kits and combinations of these kits. Calculations courtesy of Dr. Rick Staub.

Comparative Power of Systems

Multiplex	Avg. Cauc. PI	Avg. Cauc. POE
PP 1.1	321	.9968853
PP 2.1	13,193	.9999242
PP 1.1 + 2.1	555,556	.9999982
COfiler	90	.9888596
Profiler	8,511	.9998825
COf + Prof	119,048	.9999916
PP 16	1,666,667	.9999994

Figure 1. A phenotypic maternal exclusion in the D13 system of PowerPlex®1.1. The mother (M) and child's (C) DNA did not share a matching allele when the template DNA of this paternity case was amplified using the PowerPlex®1.1 multiplex kit and analyzed on Hitachi FMBIO® instrumentation. The unlabeled lane contained a mixture of the amplified child's DNA and the amplified alleged father's (AF) DNA.

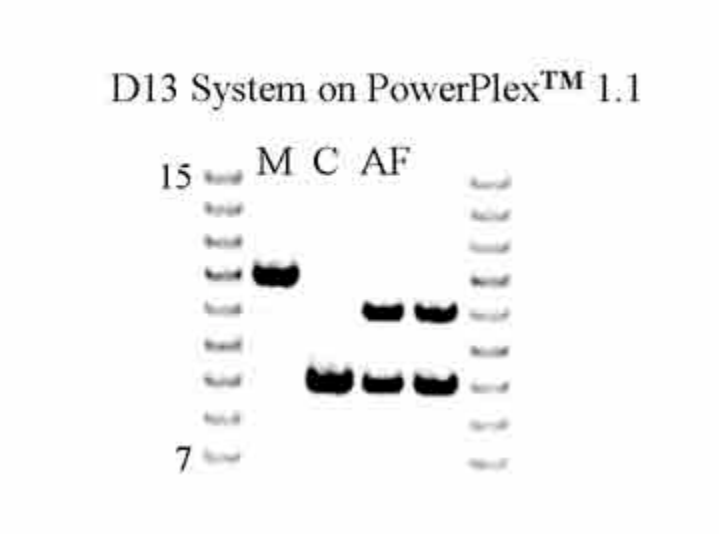

Figure 2. A genotypic maternal inclusion in the D13 system of PowerPlex®16. Template DNA from the same mother and child shown in Fig. 1 was amplified using the PowerPlex®16 multiplex kit and analyzed on an ABI PRISM™ 377 instrument. Note that the mother shares an 11 allele with the child in the D13 system of PowerPlex®16.

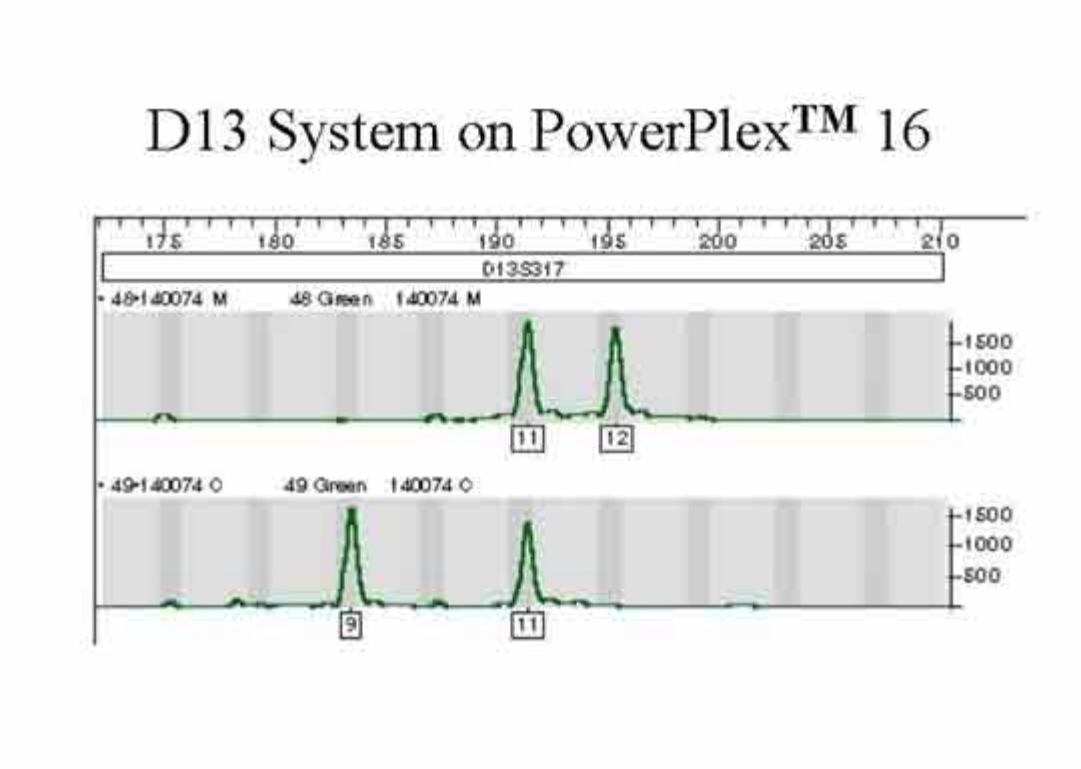

Table 2. Identification of D13 "null" alleles. This table lists the phenotype of ten maternal exclusions that were identified when template DNA was amplified using the PowerPlex®1.1 multiplex kit. This table also lists the genotypes of these same mother/child combinations obtained when template DNA was amplified using the PowerPlex®16 multiplex kit. The shared "null" alleles are indicated by bold numbering.

D13 "Null" Alleles on PP™ 1.1

# of cases	PP™ 1.1		PP™ 16	
	M	Ch	M	Ch
2	11	12	**9**,11	**9**,12
2	11	12	**10**,11	**10**,12
3	11	13	**9**,11	**9**,13
1	13	14	**10**,13	**10**,14
1	12	9	**11**,12	9,**11**
1	10	8	**10**,10	8,**10**