Megaplex STR Analysis from a Single Amplification: Validation of the PowerPlex® 16 System

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INTRODUCTION

Recent advances in forensic DNA analysis have focused on the implementation of technology (e.g., multiplex PCR, capillary electrophoresis and robotics) to increase throughput and enable laboratories to meet the ever-growing demand for testing. Most commercial STR amplification kits, including the Amp*fl*STR™ COfiler™ and Profiler Plus™, and the PowerPlex® 1 and 2 Systems[a,b], necessitate two amplification reactions to amplify all 13 CODIS (Combined DNA Index System) STR loci and Amelogenin. The PowerPlex® 16 System[a,b] is currently the only commercially available kit that can be used to co-amplify sixteen loci, including the 13 CODIS tetranucleotide STR loci, Amelogenin and two pentanucleotide STR loci (1,2). The ability to perform STR analysis from a single amplification will result in increased efficiency and sample conservation, as well as a reduction in sample handling and the potential laboratory error associated with it. Prior to use in forensic casework, the PowerPlex® 16 System, as its predecessors, must undergo testing to evaluate its performance and determine its reliability in this arena. A collaborative validation study was designed to evaluate the reproducibility, sensitivity and forensic applicability of this STR multiplex system. These data were generated as a part of that study.

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SINGLE-SOURCE CONDITIONS DETERMINATION

All PCR product separations were performed by capillary electrophoresis using an ABI PRISM® 310 Genetic Analyzer and results were analyzed using GeneScan® and Genotyper® software and the PowerTyper™ 16 Macro. Five single-source DNA templates were used to determine optimal template input, cycling parameters and injection times. DNA in concentrations of 0.25ng, 0.5ng and 1.0ng was amplified using setup and cycling parameters recommended in the PowerPlex® 16 System Technical Manual (#TMD012). In addition to the 10/22 cycling profile recommended, a 10/20 profile was also assessed and injection times of 3 and 5 seconds were evaluated.

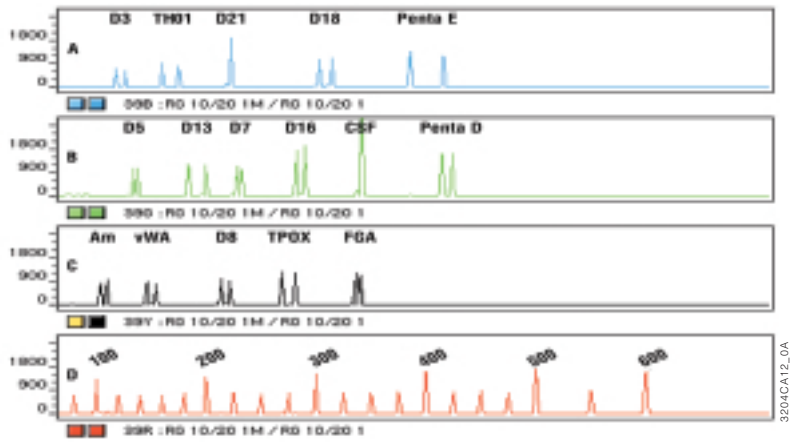

Figure 1. PowerPlex® 16 single-source determinations. DNA template (1ng) was amplified in a GeneAmp® PCR System 9700 thermal cycler using the protocol described in the PowerPlex® 16 Technical Manual with a 10/20 cycling profile. One microliter each of amplification product and Internal Lane Standard 600 were combined in 24µl deionized formamide and run on an ABI PRISM® 310 Genetic Analyzer. The following conditions were used: 1) POP4 A Module for a 1ml syringe, 2) 3-second injection at 15kV, 3) 60°C capillary temperature and 4) 30-minute run time. **Panel A:** Peaks of the fluorescein-labeled loci: D3S1358, THO1, D21S211, D18S51 and Penta E. **Panel B:** Peaks of the JOE-labeled loci: D5S818, D13S317, D7S820, D16S539, CSF1PO and Penta D. **Panel C:** Peaks of the TMR-labeled loci: Amelogenin, vWA, D8S1179, TPOX and FGA. **Panel D:** CXR-labeled fragments of the Internal Lane Standard 600. Similar results are obtained with a 5-second injection from a 0.5ng amplification (data not shown).

A collaborative validation study was designed to evaluate the reproducibility, sensitivity and forensic applicability of this STR multiplex system.

PRECISION/REPRODUCIBILITY

Once the conditions for single-source samples were determined, allelic size measurements from 10 injections (each of the amplification products of 9 samples and the PowerPlex® allelic ladder) were evaluated. Table 1 shows the results obtained from the analysis of one DNA sample. The "MAX DIFF" represents the greatest difference (in bases) seen between any two of the ten measurements per allele. In this case, the maximum difference was ≤0.17 bases for 96.7% (29/30) of the alleles sized and only 0.21 bases for the 10 allele at D16. These results are typical in that multiple allelic measurements are generally within 0.2 bases of one another. For example, in multiple measurements of the allelic ladder, the greatest difference seen between any two measurements, from 198 of 210 alleles (94.3%) evaluated, was <0.2 bases (data not shown). These data demonstrate that single-base resolution is readily achieved with the PowerPlex® 16 System.

SENSITIVITY AND STUTTER

Forensic samples may contain minimal amounts of DNA, degraded DNA or a mixture of DNA from multiple sources. The successful typing of these samples necessitates the use of a system that has high sensitivity and maximal mixture interpretation capability. The sensitivity of the PowerPlex® 16 System, as determined by the detection limits of single-source DNA or of the minor contributor of a mixture, was examined.

Table 1. Results Obtained from the Analysis of One DNA Sample.

LOCUS	ALLELE	MIN	MAX	MEAN	STD DEV	MAX DIFF
D3	14	118.21	118.31	118.257	0.0460	0.1
	16	126.47	126.59	126.531	0.0390	0.12
THO1	6	160.21	160.31	160.22	0.0316	0.1
	9.3	175.01	175.12	175.071	0.0407	0.11
D21	30	222.56	222.71	222.613	0.0558	0.15
D18	13	302.82	302.94	302.873	0.0445	0.12
	16	314.37	314.48	314.427	0.0283	0.11
Penta E	7	385.51	385.66	385.581	0.0484	0.15
	13	415.82	415.96	415.888	0.0469	0.14
D5	12	134.05	134.22	134.139	0.0538	0.17
	13	138.12	138.24	138.208	0.0449	0.12
D13	10	183.91	184.06	183.985	0.0479	0.15
	14	199.78	199.89	199.869	0.0443	0.11
D7	10	228.23	228.39	228.291	0.0524	0.16
	11	232.29	232.45	232.351	0.0475	0.16
D16	10	282.11	282.32	282.204	0.0568	0.21
	12	290.18	290.32	290.234	0.0523	0.14
CSF	12	341.19	341.32	341.243	0.0383	0.13
Penta D	12	414.13	414.26	414.197	0.0416	0.13
	14	423.66	423.78	423.754	0.0347	0.12
vWA	16	146.33	146.48	146.4	0.0650	0.15
	18	154.27	154.44	154.38	0.0558	0.17
D8	10	213.63	213.8	213.739	0.0586	0.17
	12	221.67	221.83	221.745	0.0633	0.16
TPOX	8	268.86	269	268.959	0.0409	0.14
	11	280.76	280.88	280.821	0.0398	0.12
FGA	20	336.21	336.34	336.26	0.0392	0.13
	21	340.3	340.42	340.373	0.0430	0.12

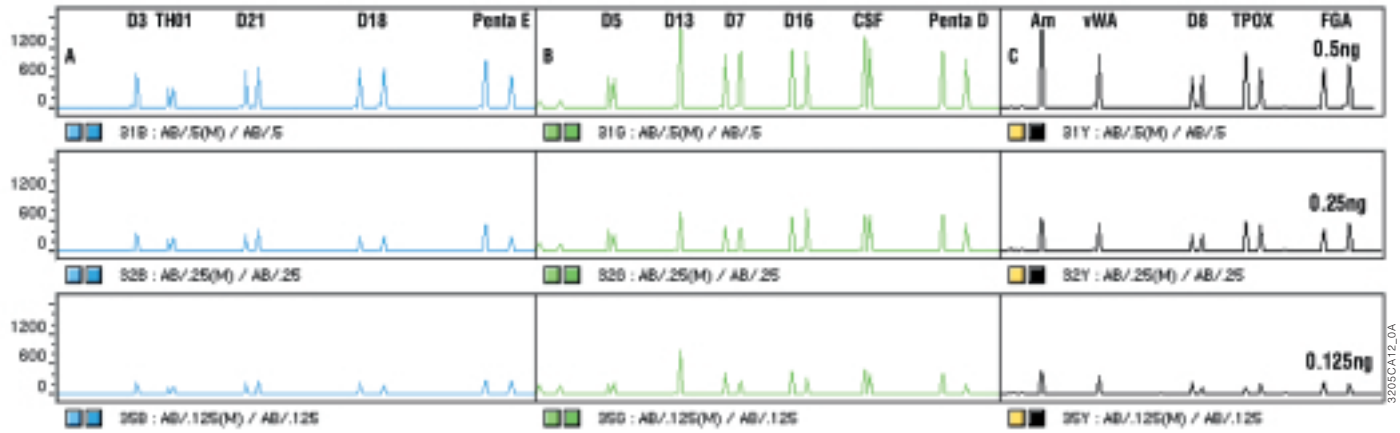

Figure 2. Sensitivity. Concentrations of 0.5, 0.25 and 0.125ng of genomic DNA (top, middle and lower electropherograms in Panels A, B, and C, respectively) were amplified in a GeneAmp® PCR System 9700 thermal cycler using the protocol described in the PowerPlex® 16 Technical Manual with a 10/20 cycling profile. One microliter each of amplification product and Internal Lane Standard 600 were combined in 24µl deionized formamide and run on an ABI PRISM® 310 Genetic Analyzer. The following conditions were used: 1) POP4 A Module for a 1ml syringe, 2) 5-second injection at 15kV, 3) 60°C capillary temperature and 4) 30-minute run time. **Panel A:** Peaks of the fluorescein-labeled loci: D3S1358, THO1, D21S211, D18S51 and Penta E. **Panel B:** Peaks of the JOE-labeled loci: D5S818, D13S317, D7S820, D16S539, CSF1PO and Penta D. **Panel C:** Peaks of the TMR-labeled loci: Amelogenin, vWA, D8S1179, TPOX and FGA.

To determine the detection limits of single-source DNA, five samples were amplified using input DNA template levels of 2.0, 1.0, 0.5, 0.25, 0.125 and 0.0625ng, respectively. Figure 2 shows the resulting electropherogram of a single sample from amplifications of 0.5, 0.25 and 0.125ng template DNA. Examining the middle electropherogram in each panel (0.25ng), no imbalance is seen at any of the 13 CODIS STR loci. An imbalance is detected in Panel A at the Penta E locus (296/547), yielding a heterozygous peak ratio of 54%. It should be noted, however, that the balance appears to be restored at the 0.125ng level (bottom electropherogram, Panel A), with a heterozygous peak ratio of 92% and that the ratio at 0.5ng (top electropherogram, Panel A) is only 71%. Heterozygous imbalance (47%–67%) is seen at several loci (Penta D, D7, D8, TPOX and D16) in the 0.125ng amplification (bottom electropherograms, Panels B and C). As expected, decreased template DNA resulted in decreased peak heights, yet at 0.125ng, only two alleles fell below 150rfu (136rfu for allele 1 at TPOX, and 141rfu for allele 1 at TH01). Subsequently, five additional DNA samples were amplified at the 0.25ng and 0.125ng levels and similarly analyzed. In general, 9 of the 10 templates generated satisfactory results at DNA input levels ≥0.25ng, with little or no stochastic effects noted and rfu values >200. Heterozygous imbalance was seen in varying degrees in the 0.125ng amplifications, though most peaks were in excess of 150rfu and all genotype results were correct. Both allelic and locus drop-out were seen in the analysis of 0.0625ng amplifications where the resulting peak heights were less than 75rfu. These data suggest that successful analysis of single-source samples can be readily achieved with as little as 0.125ng DNA; however, stochastic effects may be seen with input DNA below 0.25ng.

A similar detection limit for the PowerPlex® 16 System was determined through mixture analysis (data not shown). A single mixture, containing two DNA samples in defined ratios (1:1, 1:2, 1:4, 1:9, 1:19, 19:1, 9:1, 4:1 and 2:1), was examined. The total amount of input DNA was held constant at 1ng. In general, for the 1:1, 1:2 and 1:4 ratios, the peak heights/ratios were as expected based upon the DNA input. For example, at loci where both sources were heterozygous and no allele sharing occurred, the 1:1 ratio resulted in four peaks of approximately equal height. In this particular mixture, the minor contributor was easily detected in each of the 1:9 and 9:1 mixtures and complete profiles were obtained at several of the loci. The correct genotype was obtained for all of the alleles (75rfu cut-off). Therefore, the limit of detection for the minor contributor of a mixture of two sources in which 1ng is amplified is expected to be approximately 0.100ng or 10%.

The presence of stutter products (typically seen as minor peaks 4 bases smaller than the actual allele in tetranucleotide repeat loci), which are the same size as actual alleles, can complicate interpretation of a DNA profile from mixed samples. The presence of loci that demonstrate little or no stutter could aid in distinguishing the presence of a minor contributor from stutter artifacts. The PowerPlex® 16 System has incorporated two pentanucleotide-repeat loci (Penta E and Penta D), which have been reported to show reduced stutter products relative to the 13 CODIS STR loci (2). In this study, 10 samples (1ng) were amplified, and the peak heights of the main alleles and the corresponding stutter peaks were determined. Figure 3 shows the average and range of the stutter percentages obtained. Given the small sample size, mean stutter percentages are likely to change; however, the trend of reduced stutter rates of the pentanucleotide loci relative to most of the 13 CODIS STR loci is evident.

CASEWORK ANALYSIS

Twenty-two samples from five nonprobative cases were used to evaluate the performance of the PowerPlex® 16 System on typical forensic samples. In addition, samples from one case (two reference samples, and sperm and epithelial fractions from a vaginal swab) were also amplified using the Amp*fl*STR™ COfiler™ and Profiler Plus™ systems for a side-by-side comparison. A clean, single-source profile was obtained from the male fraction in this case; however, the female fraction was a mixed sample consistent with that of the victim and suspect. Figure 4 is a composite electropherogram consisting of paired profiles obtained from the female fraction after amplification with the PowerPlex® 16 System and either the Profiler Plus™ or COfiler™ system. In generating this figure, the scale of the electropherograms and windows was maintained so that direct comparison could be made of the Y-axes (baseline and peak heights). The relative peak heights seen in a profile within any given locus are consistent regardless of the STR amplification kit used. No trend indicating an increased sensitivity for any of the three amplification kits was apparent. The peak heights obtained at D5 and TH01 may seem to indicate an increased efficiency or sensitivity of Profiler Plus™ or COfiler™

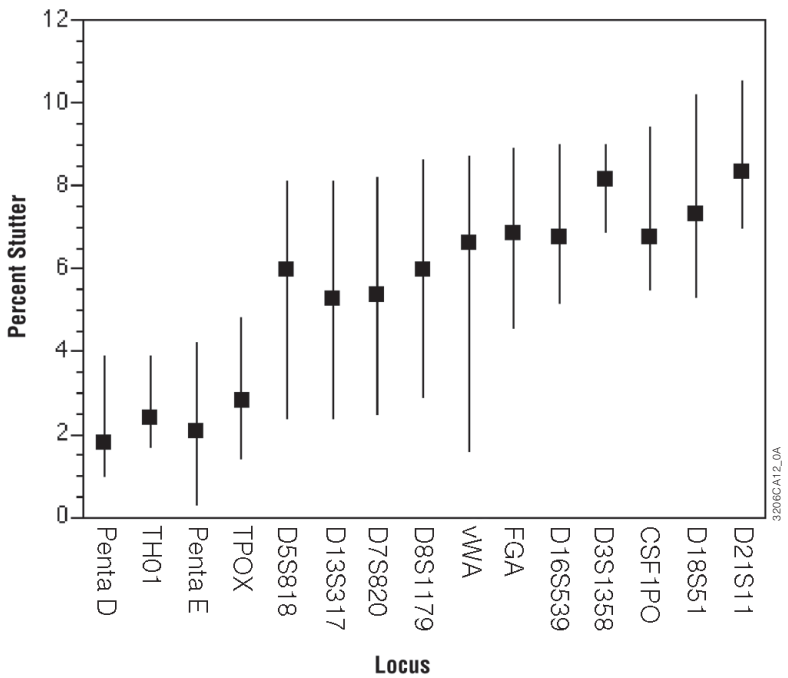

Figure 3. Percent stutter. Measurements were taken from 10 (1ng) amplifications. Boxes represent the average percent stutter of the minor N-4 or N-5 peak relative to the main allele. The bars indicate the high and low range of stutter percentage observed for all alleles at a locus.

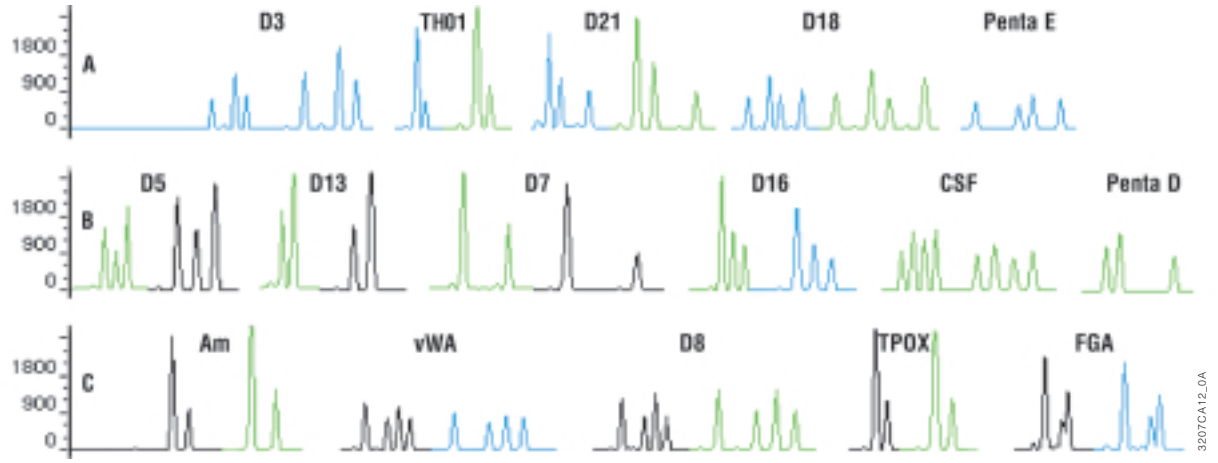

Figure 4. Nonprobative casework. DNA from the female fraction of a vaginal swab (1ng) was amplified in a GeneAmp® PCR System 9700 thermal cycler according to the manufacturer's instructions (PowerPlex® 16 Technical Manual with a 10/20 cycling profile or Amp*fl*STR™ User's Manual). PCR products were separated on an ABI PRISM® 310 Genetic Analyzer following a 3-second injection. Paired electrophoretic profiles are shown beneath each locus designation with the PowerPlex® 16 appearing to the left and the corresponding Profiler Plus™ (D3, D21, D18, D5, D13, D7, Am, vWA, D8 and FGA) or COfiler™ (D16, THO1, TPOX and CSF) profile to the right. **Panel A:** A composite electropherogram showing peaks in order of the PowerPlex® 16 fluorescein-labeled loci: D3S1358, THO1, D21S211, D18S51 and Penta E. **Panel B:** A composite electropherogram showing peaks in the order of the PowerPlex® 16 JOE-labeled loci: D5S818, D13S317, D7S820, D16S539, CSF1PO and Penta D. **Panel C:** A composite electropherogram showing peaks in the order of the PowerPlex® 16 TMR-labeled loci: Amelogenin, vWA, D8S1179, TPOX and FGA.

relative to PowerPlex® 16; however, examination of D13, D7, and D16 would indicate the converse. Satisfactory results were obtained for all twenty-two forensic samples. Conclusions and genotypes (where applicable) were consistent with previous testing (DQA1/PM or STR).

CONCLUSION

The PowerPlex® 16 System is easy to use and, with 16 loci amplified in a single reaction, the most efficient and discriminating kit currently available. Sensitivity studies demonstrated that reliable results were consistently obtained from DNA quantities as low as 0.25ng, and a limited mixture study supports a mixture detection limit of 1:10 for a 1ng amplification. Nonprobative case material was successfully amplified and genotyped with no mistyping occurrences. Additional mixture studies and nonprobative casework are underway, but all studies to date indicate that the PowerPlex®16 System will yield specific, sensitive and reproducible results.

ACKNOWLEDGMENTS

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REFERENCES

1. Sprecher, C. *et al.* (2000) The PowerPlex® 16 System. *Profiles in DNA* **4**(1), 3.

2. Bacher, J. and Schumm, J.W. (1998) Development of highly polymorphic pentanucleotide tandem repeat loci with low stutter. *Profiles in DNA* **2**(2), 3.