Bridging Databases for Today and Tomorrow: The PowerPlex® Fusion System

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Introduction

STR technology has become globally established as the foundation to human identification, and although STR technology is used universally, each region of the world has adopted distinct sets of loci to differentiate resident ethnic groups. As the world becomes increasingly connected, authorities have begun to see the benefit of a larger combined panel of loci for sharing across borders and improved discrimination. An STR system that includes loci commonly used throughout the world would build more inclusive databases and, thus, allow more profile information to be exchanged. Additionally, existing databases with millions of previously collected profiles would not be sacrificed but instead would be used as a foundation.

The PowerPlex® Fusion System includes the current core CODIS STR loci and the European Standard Set (ESS). This comprehensive set supports compatibility with present databases across multiple regions and encompasses an expanded set of loci to address future growth. Even with an extensive number of loci, the PowerPlex® Fusion System has the sensitivity and robustness to handle common casework samples and various direct-amplification sample types.

The 24 loci included in the PowerPlex® Fusion System meet both the 13 core CODIS (US) and 12 core European Standard Set requirements and include five currently supplemental loci (Figure 1). Because the system covers both United States and European Union requirements, profiles generated with the PowerPlex® Fusion System can be used and shared with either database. This collection of loci allows a database to transition from one requirement to the other or simply expand the amount of profile information collected. In the US, CODIS requirements will be expanding in the near future (1) (2) . Though the loci have not been finalized, all of the Section A loci and two of the three Section B loci are contained within the system.

Figure 1. The 24 loci included in the PowerPlex® Fusion System. This system allows co-amplification of Amelogenin, D3S1358, D1S1656, D2S441, D10S1248, D13S317 and Penta E labeled with fluorescein; D16S539, D18S51, D2S1338, CSF1PO and Penta D labeled with JOE; TH01, vWA, D21S11, D7S820, D5S818, TPOX and DYS391 labeled with TMR-ET; and D8S1179, D12S391, D19S433, FGA and D22S1045 labeled with CXR-ET. The CC5 Internal Lane Standard 500 (CC5 ILS 500) is labeled with CC5 dye and contains 21 DNA fragments of 60, 65, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475 and 500 bases in length.

In addition to the expanded CODIS set, the PowerPlex® Fusion System includes five loci not currently in either US or EU requirements (DYS391, Penta E, Penta D, D2S1338 and D19S433) to further the utility of the system. The DYS391 locus serves as a second gender marker in the event of a null allele at Amelogenin. The Penta D and Penta E loci are included in many databases throughout the globe and are valued due to their high discrimination across multiple ethnic groups. Lastly, D2S1338 and D19S433 are popular loci included in a number of databases. With all loci, primer sequences remain the same as those of previous products to maintain a high level of concordance with previously released systems.

The Probability of Identity, or the probability that randomly selected individuals have matching genotypes, for the PowerPlex® Fusion locus set is unprecedented and unmatched by current systems. The CODIS 13 loci, incorporated in kits such as the PowerPlex® 16 HS System, AmpF*l*STR® Identifiler® Plus Kit and PowerPlex® 18D System, are comparable in discrimination to the ESS loci included in kits such as the PowerPlex® ESI and PowerPlex® ESX Systems and AmpF*l*STR® NGM™ Kit (Table 1). The unique loci between CODIS and ESS sets were selected as the preferred Section A loci for the expansion of CODIS. This combined set of 18 loci (CODIS 19 – DYS391) offers a significant increase in discrimination over the current 13 core CODIS loci. The PowerPlex® Fusion System adds Section B loci (D22S1045, TPOX, Penta E and Penta D) for additional resolution between profiles. The Probability of Identity for the system is 1.36×10^{-28}, several orders of magnitude higher than previously released systems.

STR Typing Kits (Locus Combinations)	Total (n = 1036)	African Americans (n = 342)	U.S. Caucasians (n = 359)	U.S. Hispanics (n = 238)	U.S. Asians (n = 37)
CODIS 13	5.02×10^{-16}	1.13×10^{-15}	3.01×10^{-15}	1.39×10^{-15}	1.71×10^{-14}
AmpF/STR® Identifiler® Kit	6.17×10^{-19}	1.03×10^{-18}	6.94×10^{-18}	2.76×10^{-18}	5.30×10^{-17}
PowerPlex® 16 System	2.82×10^{-19}	6.08×10^{-19}	4.22×10^{-18}	1.29×10^{-18}	2.54×10^{-17}
PowerPlex® 18D System	3.47×10^{-22}	5.55×10^{-22}	9.76×10^{-21}	2.58×10^{-21}	7.87×10^{-20}
ESS 12	2.99×10^{-16}	9.14×10^{-16}	9.68×10^{-16}	2.64×10^{-15}	3.41×10^{-14}
PowerPlex® ESI 16 and ESX 16 Systems and AmpF/STR® NGM™ Kit	2.74×10^{-20}	6.02×10^{-20}	2.21×10^{-19}	4.03×10^{-19}	9.80×10^{-18}
PowerPlex® ESI 17 and ESX 17 Systems and AmpF/STR® NGM SElect™ Kit	1.81×10^{-22}	6.44×10^{-22}	1.74×10^{-21}	3.99×10^{-21}	1.86×10^{-19}
PowerPlex® 21 System	6.71×10^{-27}	2.08×10^{-26}	2.51×10^{-25}	7.96×10^{-26}	5.80×10^{-24}
CODIS 19 (–DYS391)	1.95×10^{-23}	1.08×10^{-22}	1.66×10^{-22}	1.93×10^{-22}	6.01×10^{-21}
AmpF/STR® GlobalFiler™ (–DYS391)	1.60×10^{-27}	5.63×10^{-27}	2.95×10^{-26}	5.10×10^{-26}	2.57×10^{-24}
PowerPlex® Fusion System (–DYS391)	1.36×10^{-28}	2.83×10^{-28}	5.25×10^{-27}	4.81×10^{-27}	2.01×10^{-25}
Data kindly provided by John Butler (NIST) using data collected by Becky Hill and analysis tools developed by Dave Duewer.					

Table 1. The Probability of Identity for Several Current STR Systems and Locus Standards. The same sample set was tested with each system.

The PowerPlex® Fusion System contains a 5X primer pair mix and 5X master mix, which allow convenient pipetting during reaction setup. Also included in the kit are the pre-amplification reagents: amplification-grade water and 2800M Control DNA. The post-amplification reagents include the PowerPlex® Fusion Allelic Ladder and CC5 ILS 500 size standard. Reactions can be performed using extracted DNA or direct-amplification materials. Storage material compatible with direct amplification include FTA® punches, swabs pretreated using the SwabSolution™ Kit (Cat.# DC8271) and nonlytic card punches pretreated using the PunchSolution™ Kit (Cat.# DC9271). Samples can be processed quickly with amplification times of less than 90 minutes. Direct amplification bypasses DNA purification and quantitation steps and affords additional time and cost savings. Detection of PowerPlex® Fusion amplification products is performed on the 3130 or 3500 series of Applied Biosystems Genetic Analyzers using current 5-dye chemistry. Implementation is simplified because the system uses previously validated instrumentation and software, as well as current laboratory expertise.

Sensitivity is a major concern due to the limited amount of DNA encountered with casework samples. Within a single amplification, the PowerPlex® Fusion System can retrieve 24-locus profile information with 100pg of DNA or less. To demonstrate, a titration of extracted DNA from 1ng to 50pg was created then amplified using 30 cycles. Full profiles were reliably observed down to 100pg, and greater than 95% of alleles were called with 50pg (Figure 2).

A second common obstacle of casework is obtaining profiles from samples containing inhibitors. The PowerPlex® Fusion System was developed to handle substantial concentrations of a variety of inhibitors. Common inhibitors such as hematin, humic acid and tannic acid were added to PowerPlex® Fusion reactions containing 500pg of extracted DNA. Full profiles were obtained in the presence of up to 500µM hematin, 100ng/µl humic acid and 100ng/µl tannic acid (Figure 3). This is a dramatic advance over the PowerPlex® 16 HS System and is equivalent to the PowerPlex® ESI 16 System.

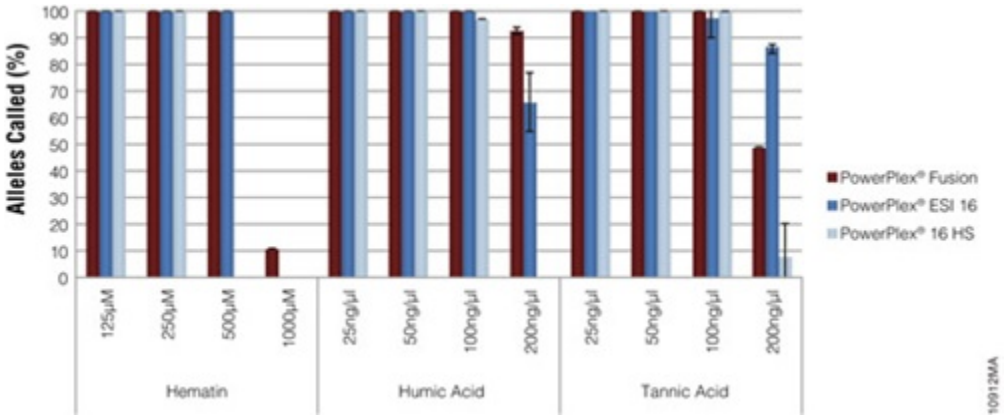

Figure 3. Percent of alleles called with increasing concentrations of inhibitors in PowerPlex® Fusion, ESI 16 and 16 HS reactions with 500pg of DNA template. Data were generated using the Applied Biosystems® 3130 Genetic Analyzer and a 3kV 5-second injection. n = 3

In contrast to casework samples, reference samples are defined single-source samples. Direct amplification offers a way to dramatically reduce total processing time of these samples by eliminating DNA purification steps. However, direct amplification can be hampered by the wide variety of solid support materials available, including FTA® cards, swabs and nonFTA cards. FTA® cards contain lytic, denaturing chemicals that make the DNA available for amplification but carry a heavy inhibitor burden. Alternatively, nonlytic materials are less expensive and less inhibitory, but deposited DNA is still encased in cellular proteins and less available for efficient amplification. Lastly, storage card punches are physically much different to process than swabs. Simple protocol modifications allow the PowerPlex® Fusion System to amplify DNA from a wide variety of solid substrates despite the challenges of lytic and nonlytic substrates (Figure 4). FTA® card punches are simply added directly to amplification reactions. The PowerPlex® Fusion 5X Master Mix overcomes inhibitors within the FTA® punches without wash steps. Swabs are incubated in SwabSolution™ Reagent, a pretreatment solution, to create an extract, then 2µl of this extract is added directly to PowerPlex® Fusion reactions, similar to adding extracted DNA. Lastly, punches from nonFTA cards are processed with PunchSolution™ Reagent, a pretreatment solution specific to punches. Punches are incubated at 70°C with PunchSolution™ Reagent to dryness and used directly in PowerPlex® Fusion reactions. To demonstrate each method, FTA® punches, swabs and nonFTA punches were processed as described in the *SwabSolution™ Kit Technical Manual* TMD037 or *PunchSolution™ Kit Technical Manual* TMD038, then added to PowerPlex® Fusion reactions. Reactions were amplified for 25 cycles, five fewer cycles than the extracted DNA protocol. Full and balanced 24-locus profiles were observed with all samples types (Figures 5, 6 and 7).

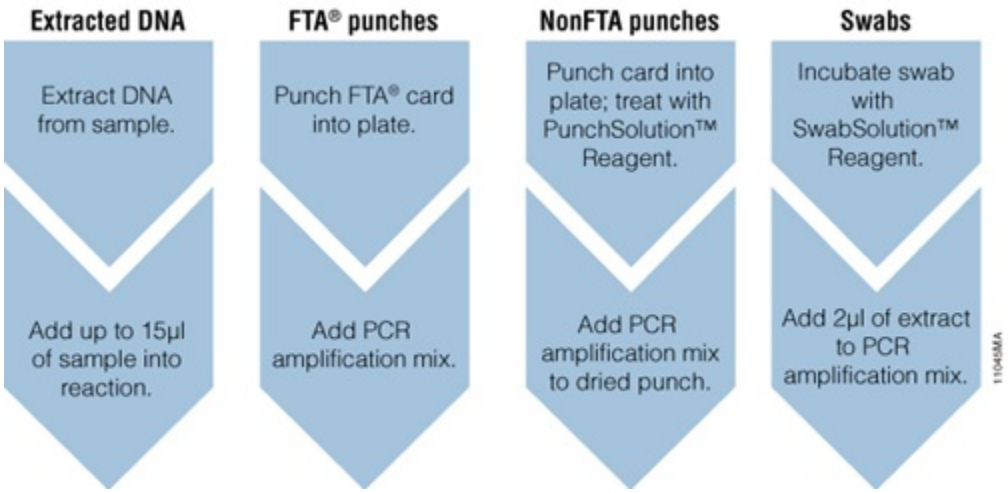

Figure 4. Direct-amplification protocol modifications based on solid support type: FTA® card, nonFTA card or swab. The protocol for extracted DNA is shown for comparison purposes.

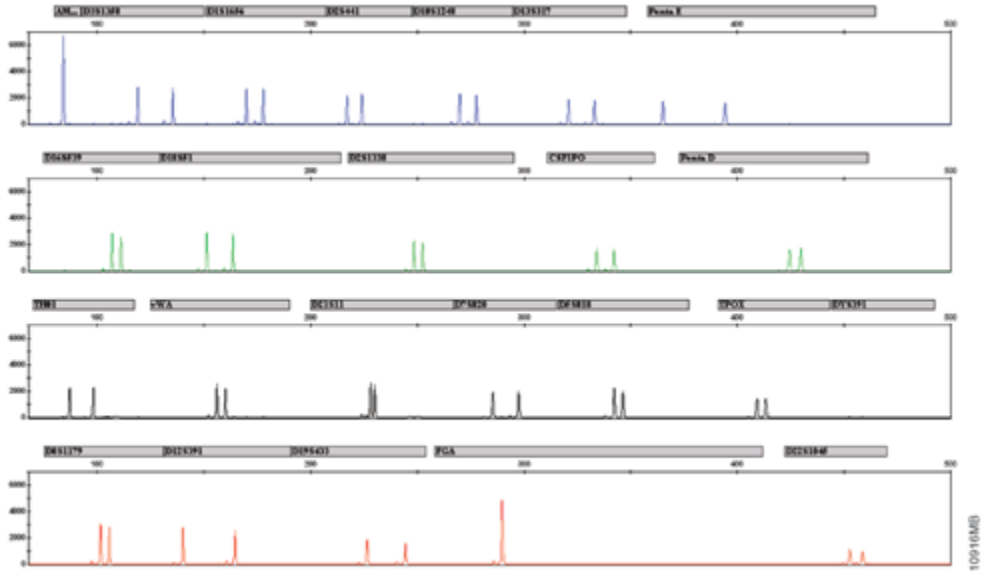

Figure 5. An electropherogram showing representative amplification results from one 1.2mm FTA® punch containing a buccal sample in a 25µl PowerPlex® Fusion reaction. Data were generated using the Applied Biosystems® 3130 Genetic Analyzer and a 3kV 5-second injection

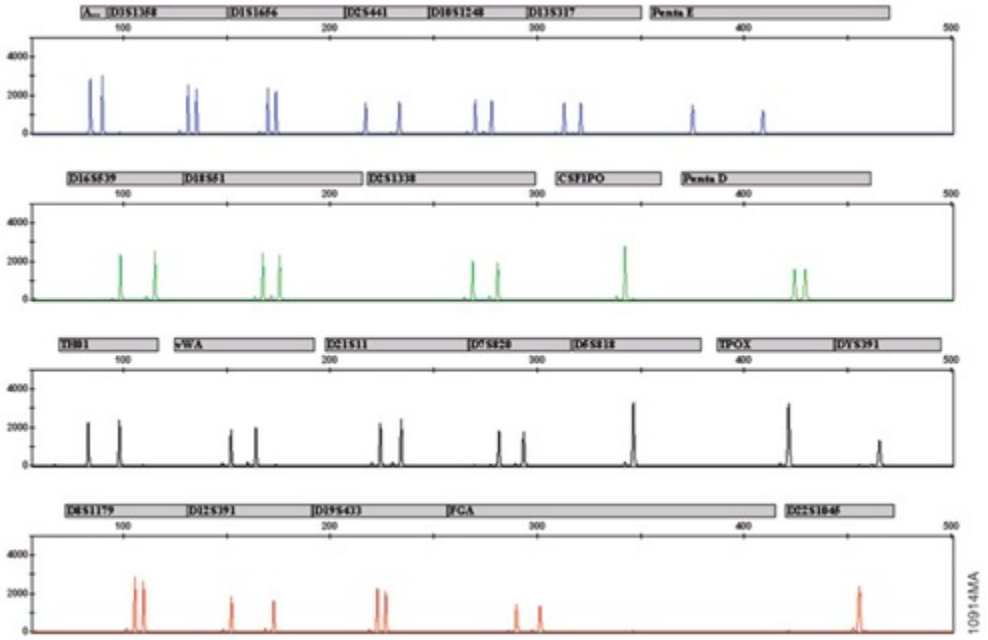

Figure 6. An electropherogram showing representative amplification results from 2µl of SwabSolution™ extract in a 25µl PowerPlex® Fusion reaction. Data were generated using the Applied Biosystems® 3130 Genetic Analyzer and a 3kV 5-second injection.

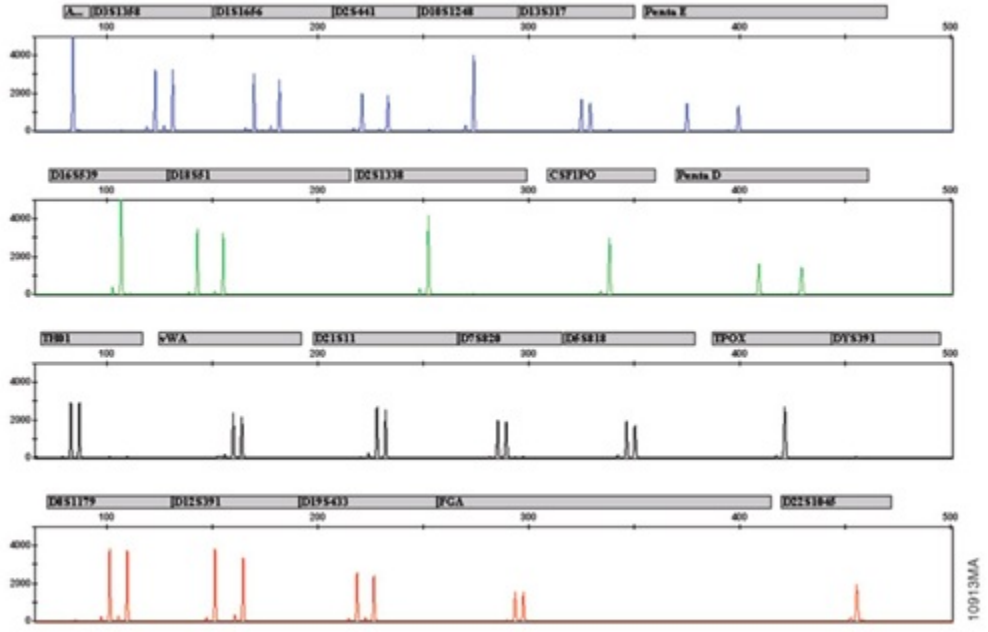

Figure 7. An electropherogram showing representative amplification results from one 1.2mm, PunchSolution™-treated S&S 903 punch containing a buccal sample in a 25µl PowerPlex® Fusion reaction. Data were generated using the Applied Biosystems® 3130 Genetic Analyzer and a 3kV 5-second injection

Conclusions

In today's world of global communication and exchange, the PowerPlex® Fusion System bridges the requirements of multiple databases, covering the current CODIS and ESS loci, and addresses future database changes by including all of the Section A loci and the majority of Section B loci for the expanded CODIS set. This collection of loci, with the addition of Penta E and Penta D, is universal and extremely discriminating. Forensic and database samples continue to complicate identification, requiring amplification systems to be sensitive and capable of handling inhibitors. Due to economic and management pressures, systems must be fast and able to handle various sample types. The PowerPlex® Fusion System successfully addresses these challenges by providing a sensitive and robust, yet flexible system for the future of human identification.

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