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Concordance and population studies along with stutter and peak height ratio analysis for the PowerPlex® ESX 17 and ESI 17 Systems

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ABSTRACT

The PowerPlex® ESX 17 and ESI 17 Systems for short tandem repeat (STR) amplification were developed by the Promega Corporation to meet the European Network of Forensic Science Institutes (ENFSI) and the European DNA Profiling (EDNAP) Group recommendations for increasing the number of STR loci included in the European Standard Set (ESS). The PowerPlex ESX 17 and ESI 17 Systems utilize different PCR primer combinations to co-amplify the following 17 loci: D1S1656, D2S441, D2S1338, D3S1358, D8S1179, D10S1248, D12S391, D16S539, D18S51, D19S433, D21S11, D22S1045, FGA, TH01, vWA, SE33, and the sex-typing locus amelogenin. A total of 1443 U.S. population samples were evaluated with pre-commercialization versions of both kits. Stutter and heterozygote peak height ratios have been used to characterize kit performance. Typing results have been used to estimate the match probabilities provided by the chosen loci as well as in concordance studies. Full concordance between the typing results for the two kits was observed in 99.994% (49,055 out of 49,062) STR allele calls compared. All genotyping discrepancies were confirmed by DNA sequence analysis. As a result of these comparisons, a second forward primer for the D22S1045 locus has been added to the PowerPlex ESX 17 System to address a primer binding site mutation and the D1S1656 locus reverse primer in the PowerPlex ESI 17 System was modified to eliminate an amplification-efficiency reducing primer dimer.

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1. Introduction

As many national DNA databases are growing at a rapid rate, concern for the potential of false matches with a large number of comparisons being made within and between databases has prompted the desire to add additional loci [1–3]. This is particularly important in Europe where cross-border criminal investigations need to be able to compare DNA data between countries [3,4]. The original European Standard Set (ESS) includes only seven loci: D3S1358, D8S1179, D18S51, D21S11, FGA, TH01, and vWA [3]. In 2006, the European Network of Forensic Science Institutes (ENFSI) and the European DNA Profiling (EDNAP) groups published recommendations to extend the ESS loci [5,6] by adopting three miniSTR loci [7]: D2S441, D10S1248, and D22S1045, as well as two additional polymorphic loci [8]: D1S1656 and D12S391.

The recently released PowerPlex® European Systems from the Promega Corporation (Madison, WI) were created to include these

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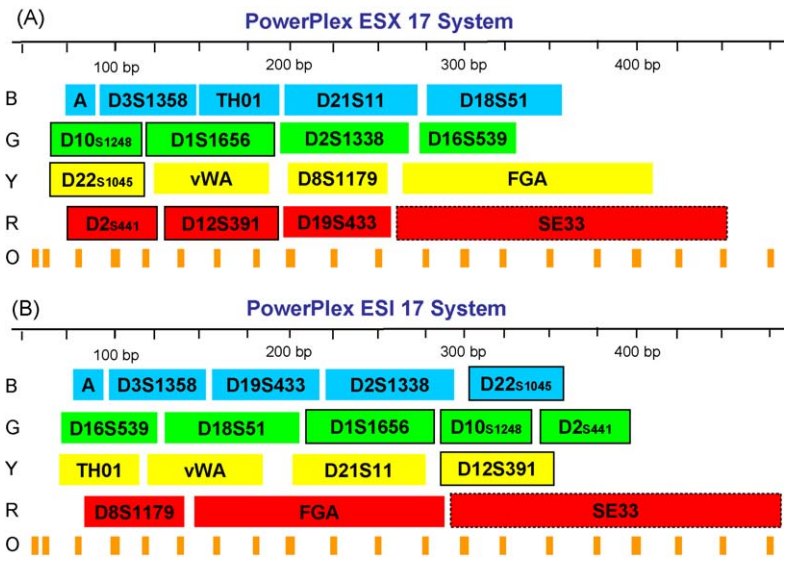

Fig. 1. Schematic of PCR product size ranges and dye color configurations for the STR loci present in (A) PP-ESX17 and (B) PP-ESI17. The "A" in the blue channel is amelogenin. B = blue channel, G = green channel, Y = yellow channel, R = red channel, O = orange channel. The ILS 500 is represented in the orange channel. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.).

extra loci and meet the ENFSI and EDNAP 2006 requests [9]. The PowerPlex ESX 17 (PP-ESX17) and PowerPlex ESI 17 (PP-ESI17) Systems allow co-amplification of 17 STR loci with different size range and dye label configurations as shown in Fig. 1 [10,11]. The PowerPlex ESX 16 (PP-ESX16) and PowerPlex ESI 16 (PP-ESI16) Systems are the same as the PP-ESX17 and -ESI17 Systems with the exception that they do not co-amplify the SE33 locus.

While the PP-ESX17 and PP-ESI17 Systems amplify the same STR loci, different polymerase chain reaction (PCR) primer sequences are utilized for 13 of the 16 STR loci (including SE33) enabling cross-checking for potential primer binding site mutations when the same DNA samples are examined with both kits [12,13]. In order to assess the frequency of allele dropout due to primer binding site mutations, concordance studies were performed between the two new kits as well as other widely used kits [14,15]. In addition, heterozygote peak height ratios and stutter percentages were evaluated as part of characterizing kit performance [16]. Population variation for the 16 STR loci in U.S. Caucasian, African American, Hispanic, and Asian groups are also reported [17].

2. Materials and methods[1]

2.1. DNA samples

Anonymous liquid blood samples with self-identified ethnicities were purchased from Interstate Blood Bank (Memphis, TN) and Millennium Biotech, Inc. (Ft. Lauderdale, FL) and extracted, quantified, and previously typed with the Identifiler® kit (Applied Biosystems, Foster City, CA) [18], the PowerPlex® 16 (PP16) System (Promega, Madison, WI) (data not published), MiniFiler® kit (Applied Biosystems) [19], and the three in-house assays: NIST-23plex, -NC01 and -NC02 [20]. A set of father and son samples previously used for mutation rate studies were also evaluated [21]. The 10 genomic components of Standard Reference Material (SRM) 2391b PCR-based DNA Profiling Standard [22], K562 (Promega), and ABI 007 (Applied Biosystems) were tested for concordance to certified materials and common positive controls. A total of 1461 samples were evaluated in this study with 1455 providing complete genotyping data for concordance testing and 1443 used for peak height ratio and stutter calculations (Table 1).

2.2. PCR amplification and detection

Prototype versions of the PP-ESX17 and PP-ESI17 Systems were used in this study. With the exception of two minor primer changes in D22S1045 and D1S1656, the evaluated materials have the same PCR primers as the now released commercial kits. An additional D22S1045 forward primer was added to correct for a primer binding site mutation in the PP-ESX17 System. The D1S1656 reverse primer in the PP-ESI17 System was changed to avoid generating a primer dimer with the D21S11 labeled primer that reduced amplification at both loci (D21S11 and D1S1656).

These STR kits allow a single amplification of 17 loci in a five-color detection platform using four channels for the PCR products and the fifth channel for the size standard (Fig. 1). All STR assays were run in accordance with manufacturer's recommendations. Each PCR reaction contained 5 μL PP-ESX or PP-ESI 5X Master Mix, 2.5 μL PP-ESX17 or PP-ESI17 10X Primer Set, 16.5 μL diH_2O, and 1 μL of DNA template (0.5–1 ng/μL) for a total reaction volume of 25 μL. Thermal cycling was performed in a GeneAmp PCR System

Table 1
Samples examined in this study.

Self-identified ethnicity	Number of samples		
	POP[a]	F/S[b]	RM[c]
Caucasian	261	199	12[d]
African American	255	190	
Hispanic	139	197	
Asian	2	200	
Total samples	657	786	12
	1443[e]		12
	1455[f]		

[a] U.S. population samples [18]
[b] U.S. father/son samples [21]
[c] SRM 2391b [22], K562, ABI 007.
[d] The ethnicity is unknown for these samples.
[e] These samples were used for peak height ratio (PHR), stutter percentage calculations and population variation studies.
[f] These samples were used for concordance testing.

[1] Certain commercial equipment, instruments and materials are identified in order to specify experimental procedures as completely as possible. In no case does such identification imply a recommendation or endorsement by the National Institute of Standards and Technology nor does it imply that any of the materials, instruments or equipment identified are necessarily the best available for the purpose.