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Comparative performance between "next generation" multiplex systems and the new European Standard Set of STR markers in the Portuguese Population

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ABSTRACT

Various multiplex STR systems have been developed by the major commercial companies in the forensic genetics field to comply with the recent establishment of the global European Standard Set (ESS) of markers. Of the various alternatives available, our laboratory decided to test the recent ESSplex Plus system (Qiagen) and the NGM kit (Life Technologies), which share the same 15 STR loci and comprise the most recently established ESS markers (D1S1656, D2S441, D10S1248, D12S391 and D22S1045). Apart from evaluating the kits' technical performances, a population and segregation study was carried out on a Portuguese sample, with the aim of introducing the ESS markers in routine forensic casework. A total of 370 individuals were sampled for this purpose, comprising 120 true trios (125 fathers, 125 mothers and 120 sons/daughters). No deviations from Hardy–Weinberg equilibrium were detected for the five new loci in the Portuguese population and no genotyping inconsistencies were observed between kits. Parameters of forensic interest revealed that, of the five ESS markers, D1S1656 was the most informative in our sample. Comparison of performances between all autosomal multiplex systems available in our laboratory (ESSplex Plus, NGM, Identifiler Plus and Powerplex 16 HS), revealed that the multiplex kits with the ESS markers generally showed better performances and, among these, the ESSplex Plus kit showed slightly higher sensitivity and a better detection of degraded DNA information.

1. Introduction

Human Short Tandem Repeat (STR) analysis by commercially available multiplex amplification kits allows genotyping of polymorphic loci relevant to forensic and population studies. The European Standard Set of STRs (ESS) comprised only TH01, VWA, FGA, D21S11, D3S1358, D8S1179 and D18S51 as the core of European database loci, selected by the European Network of Forensic Science Institutes (ENFSI) [1]. Standardization of DNA profiling throughout Europe was proposed by European Working Groups (ENFSI and EDNAP), since differences exist between countries in their choice of loci, for the purpose of constructing national forensic DNA databases. Thus, apart from the previously established seven STRs, a new European Standard Set (ESS) of five additional loci (D1S1656, D2S441, D10S1248, D12S391 and D22S1045) was proposed to be adopted by all European Member States [2]. In this way, new multiplex STR systems were developed, such as the ESSplex Plus (Qiagen) and NGM (Life Technologies) kits, among others, fulfilling the European instructions and needs for improved sensitivity and robustness, also for degraded DNA analysis [3].

The ESSplex Plus system amplifies the same 15 loci as the NGM system, plus the Amelogenin locus. In this work, these kits were used to characterize the five new ESS markers in a Portuguese sample. An evaluation of the kits' performance was also carried out and compared to the routinely used multiplex systems previously set up in our laboratory, namely the Identifiler Plus (Life Technologies) and Powerplex 16 HS (Promega) kits, in what concerns sensitivity and detection capability in degraded DNA analysis.

2. Materials and methods

2.1. Sampling strategy

Since one of the aims of this work was to carry out both population genetics analyses and segregation studies for the five

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new ESS loci, 370 DNA samples were selected, comprising 120 true trios (125 fathers, 125 mothers and 120 sons/daughters). These trios served as the basis for the segregation study, and all analyses performed for the population study included the typing of the fathers and mothers (250 unrelated individuals living in Portugal, distributed through all districts from North to South of the country). All samples derived from these individuals were involved in prior paternity investigations and were thus already genetically investigated using the well-established PCR multiplex kits Powerplex® 16 HS kit (Promega) and AmpFlSTR Identifiler® kit (Life Technologies). Written informed consent from the tested individuals was obtained at the time of sample collection.

For the performance studies, DNA samples from different sources were used: blood stains, whole blood and different types of buccal swabs were taken from laboratory staff, mimicking the types of samples normally analyzed in routine cases.

2.2. Extraction

DNA was extracted from buccal swabs and blood samples, using adapted protocols of the standard Chelex extraction procedure [4].

2.3. Quantification

Extracted DNA samples were quantified by a Real-Time PCR method (RT-PCR), using the Investigator Quantiplex kit (Qiagen), according to the manufacturer's instructions. The reactions were run on a 7500 Fast Real-Time PCR System (Life Technologies).

2.4. Amplification

The amplification reactions were performed according to the manufacturer's instructions for each multiplex system, with a reduced PCR volume of 10 μl.

2.5. Fragment detection and analysis

All the amplification products were separated and detected on an ABI Prism 3130 Genetic Analyser (Life Technologies). Genotyping was undertaken using the GeneMapper® ID Software v.3.2 by comparison to the corresponding allelic ladder supplied in each kit. All the multiplex kit panels, bins and analysis methods were obtained from the respective companies.

2.6. Population study

For population genetic studies, between 243 and 246 unrelated individuals living in Portugal were genotyped using the Investigator® ESSplex Plus Kit. These samples compose the current database in our laboratory for genetic identification and kinship analysis and were previously analyzed with Identifiler and Powerplex 16 systems.

Allele frequencies were estimated for each of the five new markers (D2S441, D22S1045, D12S391, D10S1248 and D1S1656) using GenePop (v.4.1.0) software [5]. This software also allowed for assaying the Hardy–Weinberg equilibrium through an exact test [6]. Forensic parameters such as expected and observed heterozygosity were calculated according to Nei [7], and polymorphic information content (PIC), power of discrimination (PD) and power of exclusion (PE) were estimated based on formulas accessed in [8] for PIC and in [9] for PD and PE. A comparison was also made between our sample (Portuguese population) and other European samples available in the literature, by using the Arlequin Software [10].

Moreover, a set of 120 true trios (mother, father and son/daughter) was typed in order to test Mendelian inheritance of these five new markers and detect phenomena such as mutations and silent alleles.

2.7. Sensitivity study

Serial dilutions of different types and sources of DNA were made: 6 blood samples stained on FTA type paper and total blood in EDTA tubes, and 9 swab type samples: Omni Swab (Whatman), brush and cotton swabs. After extracting and quantifying the DNA obtained from each source, serial dilutions were performed, with a total of seven different concentrations, ranging from 0.5 ng/μl to 5 pg/μl (0.5, 0.2, 0.1, 0.05, 0.02, 0.01 and 0.005 ng/μl).

All the serial dilutions were quantified again to ensure greater reliability in our procedures.

For comparison between kit performances, including evaluation of stochastic effects and artefacts, five representative dilution series were selected and were amplified with the Investigator ESSplex Plus, NGM, Identifiler Plus and Powerplex 16 HS kits.

2.8. Degradation study

For degraded DNA analysis, tests were conducted on artificially (UV-light) degraded DNA samples. We based our method in previously described procedures [11,12], taking into account the type of UV-light (UV-C) and the respective wavelength spectrum (100–280 nm). For this study, a Mini-V/PCR vertical laminar flow bench (Telstar), containing a 254 nm wavelength UV lamp with 15 W, was used.

The procedure undertaken was as follows:

I. A DNA sample was chosen, previously quantified and diluted to 0.5 ng/μl.
II. 7 tissue slides were cleaned and dried with ethanol.
III. 10 μl of the DNA sample were placed in each slide, approximately in the centre.
IV. All 7 slides were placed on a stand at an approximate distance of 10 cm from the UV-light lamp, inside the Mini-V/PCR cabinet. All slides were previously identified with the sample code and time of exposure.
V. After turning the UV light on, each slide suffered different times of UV exposure. Each slide was removed after turning the UV-light off when the respective period of exposure was finished. UV exposure time intervals were the following: 20″, 40″, 1′, 3′, 10′, 15′ and 20′.
VI. After all the samples were removed, the slides were left to dry completely. 10 μl of sterilized water were added to each dried stain and mixed thoroughly to rehydrate the samples;
VII. The 10 μl from each slide were transferred to previously identified tubes and kept at −20 °C for future PCR analysis.

3. Results and discussion

3.1. Population study

The allele frequencies for the 5 new loci were estimated for the Portuguese population (Table 1). No deviations from Hardy–Weinberg equilibrium were observed. Forensic parameters were also estimated and are depicted in Table 1. Overall it can be observed that D1S1656 is the most informative marker in our sample, and that D22S1045 shows the lowest level of polymorphism. Comparison of this data with previously published Portuguese data [13] on the STRs included in Identifiler and Powerplex 16 kits, shows that the new markers fall in a similar range of informativeness. In fact, both D1S1656 and D12S391 are