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Short communication

Validation of the Microreader™ 23sp ID system: A new STR 23-plex system for forensic application

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ABSTRACT

Microreader™ 23sp ID system is a new 23-plex STR genotyping system that amplified 21 non-CODIS STR loci (D6S477, D18S535, D19S253, D15S659, D11S2368, D20S470, D1S1656, D22-GATA198B05, D7S3048, D8S1132, D4S2366, D21S1270, D13S325, D9S925, D3S3045, D14S608, D10S1435, D12S391, D2S1338, D17S1290 and D5S2500), one CODIS STR locus (D16S539) and the amelogenin locus in one reaction. Microreader™ 23sp ID system was validated according to the guidelines of "Validation Guidelines for DNA Analysis Methods (2012)" described by the Scientific Working Group on DNA Analysis Methods (SWGDAM), including PCR-based studies, sensitivity study, precision and accuracy evaluation, stutter percentage and peak height ratio, inhibitors, species specificity and DNA mixture studies. Our results suggested that Microreader™ 23sp ID system is a useful tool for identification and parentage testing.

1. Introduction

STR has been widely used in paternity test and individual identification since it was firstly applied in a forensic case in 1991. Many commercial STR kits were developed to provide more genetic information as well as get a higher the power of discrimination (PD) and probability of exclusion (PE) value to accommodate various forensic cases. The vast majority of these kits contained 13 CODIS core loci [1–4], thus cannot maximize the distinguishability when used in combination with other STR kits to deal with missing persons cases, complex kinship cases and the presence of STR mutation cases. In addition, most of common STR loci might be polymorphic and informative in different populations while another small part performed poor, a typical example might be TPOX [5,6].

In this context, a new 23-plex system named Microreader™ 23sp ID system (Suzhou Microread Genetics, Suzhou, Jiangsu, China) has been developed. This system contained 21 non-CODIS STR loci (D6S477, D18S535, D19S253, D15S659, D11S2368, D20S470, D1S1656, D22-GATA198B05, D7S3048, D8S1132, D4S2366, D21S1270, D13S325, D9S925, D3S3045, D14S608, D10S1435, D12S391, D2S1338, D17S1290 and D5S2500), one CODIS STR locus for comparison (D16S539) and the amelogenin locus, and the arrangement of loci from small to large amplicons in each dye channel were showed in Fig. 1. Significantly, 22 loci (except the amelogenin locus) in Microreader™ 23sp ID system were chosen specifically for greater genetic variation in Chinese Han population. These 23 markers were evenly distributed across 23 chromosomes, which greatly reduced the probability of the linkage. As a result of current 5-dye technology applied, Microreader™ 23sp ID system was compatible with ABI Prism® 310, 3100 and 3100-Avant Genetic Analyzer and the Applied Biosystems® 3130, 3130xl, 3500 and 3500xl Genetic Analyzer. Furthermore, previously reported population data of Microreader™ 23sp ID system in Han population mentioned that the 23-plex system presented an extremely high combined power of discrimination (CPD) and combined probability of exclusion (CPE) value in the Chengdu Han population [7].

In this study, validation of the Microreader™ 23sp ID system was carried out in accordance with guidelines of "Validation Guidelines for DNA Analysis Methods (2012)" [8] issued by the Scientific Working Group on DNA Analysis Methods (SWGDAM)

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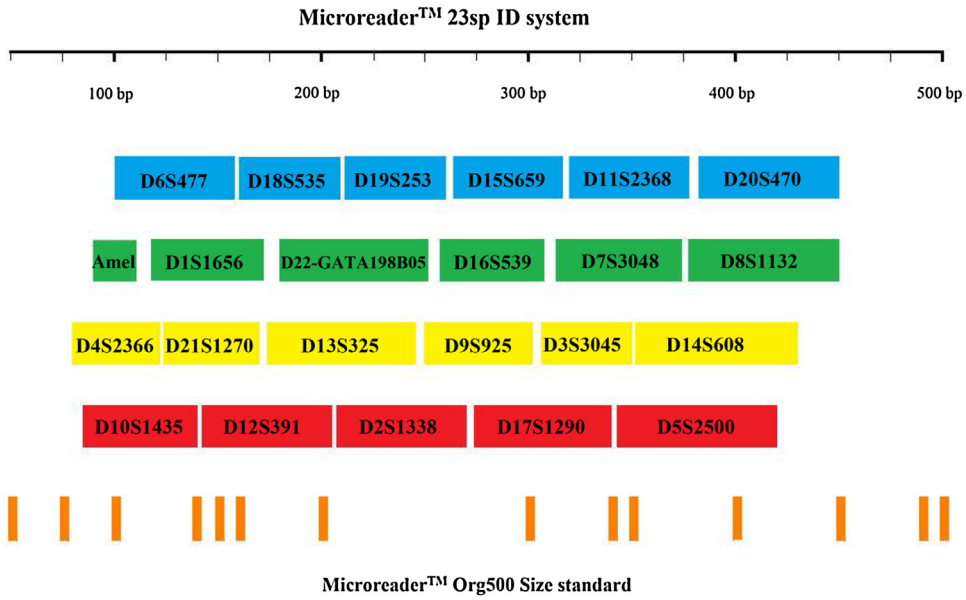

Fig. 1. The arrangement of 23 loci of the Microreader™ 23sp ID system.

and a series of tests formed by PCR-based studies, sensitivity, precision and accuracy evaluation, stutter percentage and peak height ratio, inhibitors, species specificity and DNA mixture studies were conducted. The results showed that Microreader™ 23sp ID system was a robust, sensitive and accurate tool for identification and parentage testing.

2. Materials and methods

2.1. Samples preparation

Typical control DNA 9947A and 9948 (Applied Biosystems, Foster City, CA) were applied to PCR-based studies, sensitivity study, precision evaluation, inhibitors and DNA mixture studies. Besides, 9947A was also used as positive samples.

Whole blood samples collected from 200 unrelated individuals followed informed consent were prepared for accuracy evaluation, stutter percentage and peak height ratio statistics. Human genomic DNA was extracted using salting out method [9].

Samples for specificity testing contains common animals (pig, dog, bovine, goat, cat, mouse, cavy, rabbit, chick and duck) and common microorganisms (*Staphylococcus aureus, Escherichia coli, Staphylococcus albus* and *Pseudomonas aeruginosa*). Animals DNA was extracted by salting out method [9] while microorganisms DNA was obtained with TIANamp Bacteria DNA kit (TIANGEN Biotech, Beijing, China).

DNA were quantitated by the NanoDrop1000 spectrophotometer and analyzed by NanoDrop 2.4.7c software (NanoDrop Technologies Inc, Wilmington, DE), according to the manufacturer's recommendations.

2.2. PCR amplification

Unless otherwise stated, the experimentation processes were done strictly according to manufacturer's instruction as described. The Microreader™ 23sp ID system was carried out in a 20 μL volume system, including 1 ng template DNA, 8 μL 2.5 × Buffer A, 4 μL 5 × Primer Mix and 0.4 μL Taq DNA polymerase I. PCR was performed on the Mastercycler® pro (Eppendorf, Germany), optimum parameters were as follows: preincubation at 95 °C for 11 min, then 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min; finally a 60 min extension at 60 °C, hold at 4 °C for further analysis.

2.3. Capillary electrophoresis and data analysis

PCR products were subsequently separated and detected on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). 1 μL of the PCR products or allelic ladder was combined with 9 μL of a 20:1 mixture of Hi-Di formamide (Applied Biosystems, Foster City, CA) and ORG-500 size standard (50, 75, 100, 139, 150, 160, 200, 300, 340, 350, 400, 450, 490, 500) (Suzhou Microread Genetics, Suzhou, Jiangsu, China) for electrophoresis. Samples were injected at 3 kV for 10 s and electrophoresed at 15 kV for 1000 s by a run temperature of 60 °C, with filter set E5 and POP7 polymer (Applied Biosystems, Foster City, CA). Genotyping data were analyzed by GeneMapper ID Software v3.2.1 (Applied Biosystems, Foster City, CA). Unless otherwise stated, allele peaks were interpreted with a threshold of 50 relative fluorescence units (RFU).

2.4. PCR-based studies

It was necessary to establish the parameter range of amplification conditions to evaluate the accuracy of the STR genotyping results obtained from forensic cases, here some key parameters were detected. Specifically, reaction volumes (20 μL, 10 μL and 5 μL), annealing temperature (56 °C, 58 °C, 60 °C, 62 °C and 64 °C), 5 × Primer Mix concentration ((2 μL, 4 μL and 8 μL), concentration of 2.5 × Buffer-A (0.5 ×, 1 ×, 1.5 × and 2 ×) and amount of Taq DNA polymerase I (0.2 μL, 0.4 μL, 0.8 μL and 1.6 μL) were tested in triplicate. In a variety of conditions only tested parameter changed while others keep the same with the recommended conditions. The same concentration of 9947A DNA (0.05 ng/μL) was tested for reaction volume testing. Concretely, 1 ng DNA was amplified in 20 μL reaction volume, 0.5 ng DNA in 10 μL reaction volume and 0.25 ng DNA in 5 μL reaction volume. Other experiments mentioned above were examined with 1 ng DNA in 20 μL reaction volume. Control DNA 9947A was used as DNA template.