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

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Highlights

1. A new 23-plex STR system contained 21 non-CODIS STR loci was validated.

2. Applicable for 10 ul even 5 ul reaction volumes.

3. Owned wide range of annealing temperatures with 58 ℃ to 62 ℃.

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.

Keywords

Forensic science; Validation; Microreader™ 23sp ID system; Short tandem repeat (STR)

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) 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 seconds, 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 seconds and electrophoresed at 15 kV for 1000 seconds 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.

2.5. Sensitivity study

In order to evaluate the sensitivity and working range of this 23-plex system, a serial dilutions of control DNA 9947A was amplified in triplicate with quantities of 1 ng, 0.5 ng, 0.25 ng, 0.125 ng, 0.0625 ng and 0.031 ng. Amplification and detection processes were performed following the manufacturer's recommended conditions. Percentage profile and mean peak height were determined for each template amount.

2.6. Precision study and sizing accuracy

Precision was measured by running the capillary electrophoresis of the 16 allelic ladder samples on the 3130 Genetic Analyzer.

For sizing accuracy, 50 samples were genotyped under standard conditions and the allele size was compared to the corresponding allelic ladder.

2.7. Stutter percentage

Percentage of the stutter peaks were determined by calculating the stutter peak height as a percentage of the main allele height. 200 samples were used for the stutter ratio calculation. Herein the analytical threshold of minimum stutter peak height was set to 10 RFU.

2.8. Mixture studies

To assess the ability of reliable detection of mixtures with Microreader™ 23sp ID system, a mixed female/male DNA sample (control DNA of 9947A and 9948) were examined at various ratios (19:1, 9:1, 4:1, 2:1 and 1:1) with 1 ng of total DNA template, and the test was performed three times to ensure the accuracy.

2.9. Stability studies

Two kinds of chemicals associated with common contaminants of casework samples, hematin and humic acid, were used in stability studies. 1 ng control DNA 9947A was amplified in accordance with the manufacturers' recommendations while amplification reactions contained different concentrations of hematin (Shanghai Aladdin Biochemical Technology, Shanghai, China) and humic acid (Shanghai Aladdin Biochemical Technology, Shanghai, China). The concentrations of humic acid were 10, 20, 30, 40, and 50 ng/μL and hematin were 10, 20, 30, 40, and 50 μM, respectively. Each test was performed three times.

2.10. Species specificity

DNA samples of several common animal species (pig, dog, bovine, goat, cat, mouse, cavy, rabbit, duck and chicken) and four common microbial species (*Staphylococcus aureus, Escherichia coli, Staphylococcus albus* and *Pseudomonas aeruginosa*) were prepared for species specificity tests. 10 ng of each non-human genomic DNA were analyzed in triplicate using the Microreader™ 23sp ID system.

3. Results and discussion

3.1. PCR based studies

3.1.1. Reaction volume testing

The recommended reaction volume for this system was 20 μL. In this study, control DNA 9947A were tested in different reaction volumes (20 μL, 10 μL and 5 μL) in which each component ratio remained the same with the manufacturers' recommendations. Complete profiles were obtained in all ranges of reaction volume. The genotyping results showed a high degree of consistency with 20 μL reaction volume when the reaction volume reduced to 10 μL and 5 μL, meanwhile，we observed no allele dropped out and consistent peak morphology (Fig. S1). Our studies suggested that the Microreader™ 23sp ID system could also obtain correct genotyping results when reduced to halved or quartered standard reaction volume. Since sometimes technician may choose smaller

reaction volumes to deal with Low Copy Number (LCN) DNA samples [10], Microreader™ 23sp ID system was able to meet requirements.

3.1.2. Annealing temperature studies

Complete profiles generated and no allele drop-out observed when annealing temperature ranged from 56 °C to 62°C. Alleles of D4S2366 locus dropped out when annealing temperature turned to 64 °C. The alleles' peak heights slightly decreased as annealing temperature increased from 56 °C to 62 °C, then drastically reduced as temperature come up to 64°C. In addition, N-1 peaks at D15S659 (fourth locus in blue), D21S1270 (second locus in yellow) and D13S325 (third locus in yellow) appeared at 62 °C to 56 °C. Here it's important to note that even at recommended annealing temperature 60 °C, N-1 peaks appeared at D15S659, D21S1270 and D13S325, with 16.52%, 16.33% and 20.18% average percentages of N-1 peaks to the main allele peaks, respectively. We need to consider these factors when interpreting data, especially for the casework samples. These N-1 peaks tend to rise gradually as annealing temperature decreased from 62 °C to 58 °C with low peaks in general, but when the temperature dipped 56 °C, N-1 peaks increased sharply and could significantly affect the detected results (Fig. S2). The profiles at 58 °C and 62 °C worked almost equally well on peak heights and peak height balance with the recommended 60 °C. So 58 °C to 62 °C all could be the appropriate annealing temperature. Results confirmed Microreader™ 23sp ID system was adapting shockingly well to differential annealing temperatures, which can reduce the possibility of unsuccessful amplification due to inaccurate temperature.

3.1.3. Concentration of PCR component studies

Concentrations of primers may influence specificity and sensitivity of the STR analysis. In this study, correct genotyping results obtained from different concentrations of 5× Primer Mix (2 μL, 4 μL and 8 μL), no allele dropped out. Profiles of 2 μL 5× Primer Mix concentration were observed average 16.67% (7 of total 42 alleles) peak heights between 50 RFU and 200 RFU, far below the other two groups. Both 4 μL of 5× Primer Mix concentration and 8 μL of 5× Primer Mix concentration could provide sensitive and balanced amplification results. Besides, N-1 peaks at loci of D15S659 and D13S325 increased a little from 1× to 2× final primer pair concentration, which had not much effect on result analysis (Fig. S3). This testing suggested even double concentrations of Primer Mix would probably not have much impact on genotyping results.

The peak heights gradually raised with the 2.5× Buffer-A concentrations increased and full profiles were detected as the concentrations ranged from 8 μL to 16 μL. However, D4S2366 and D13S325 dropped out as well as unexpected allele appeared at D6S477 and amelogenin when the concentrations of 2.5× Buffer-A decreased to half (0.5×). Besides, bleed-through occurred and pull-up peaks can be detected under double 2.5× Buffer-A concentrations (Fig. S4). So, the concentrations of 2.5× Buffer-A should keep the same with manufacturer's instruction.

Full profiles were produced from different amount of Taq DNA polymerase I (0.2 μL, 0.4 L, 0.8 μL and 1.6 μL). Curiously, the peak heights decreased obviously whether amount of Taq DNA polymerase I reduced to half or increased to quadruple (Fig. S5). Unexpected peaks appeared between 70 to 94 bases in all 4 dye channels with extremely high peak height at 4× (16 ul) Taq concentration (figures not presented here). It suggested that nonspecific amplication was took place at 4× Taq concentration and consumed vast majority of PCR components, ultimately caused peaks heights decreased at high Taq concentration. Therefore, 0.4 μL and 0.8 μL might be the suitable amount of Taq DNA polymerase I for amplification. Too much or too little amount of Taq DNA polymerase I would not be conducive to detection.

3.2. Sensitivity study

Different amounts of DNA samples would be gained from forensic actual cases, some of which were beyond the scope of the recommended quantity. Sensitivity study can determine the upper and lower limits of sample DNA quantities to provide a basis for explaining the genotyping results of LCN DNA. Thus a series of amount of control DNA 9947A was detected in triplicate with quantities of 1 ng, 0.5 ng, 0.25 ng, 0.125 ng, 0.0625 ng and 0.031 ng.

Full profiles (42 alleles) was obtained using 50 RFU peak heights analysis threshold with the concentration of 9947A above 0.25 ng. As template DNA reduced gradually from 1 ng to 0.0625 ng, the average peak height was detected from 2291 RFU to 59 RFU. When the template DNA down to 0.125 ng, partial profiles were observed and average 46.03% of the loci were detected with the average peak height at 81 RFU. Whereas, complete and accurate genotypes were visible with lower analysis threshold and well above background while the peak height of average 52.91% alleles were lower than 50 RFU, suggested that sensitivity of the Microreader™ 23sp ID system could be improved with more cyclic numbers in PCR or longer injected time in capillary electrophoresis. At the amount of 0.0625 ng DNA input, average 4.76% of the loci were detected (Fig. 2). Hence, the sensitivity of this

system was determined to be 0.25 ng in 20 μL PCR reaction. It should be noted that the average peak height tend to nonlinearly decrease with the decrease of DNA input, and this limitation would affect mixture interpretation.

3.3. Precision and accuracy

Sizing precision study can be of importance for accurate and reliable genotyping. 16 allelic ladder samples were detected on the 3130 Genetic Analyzer. Standard deviation and the average size for each allele from was calculated and showed to be from 0 (D17S1290) to 0.0679 (D5S2500) (Fig. 3), demonstrated the system had good precision. Surprisingly, all 16 allelic ladder samples shared a same size (300.35 bp) with allele 13 at D17S1290 precisely.

The sizing accuracy was detected by calculating the size differences of all the STR alleles from 50 random samples compared to the corresponding allelic ladder. All sample alleles (total of 1992 alleles from 50 samples) were sized within ±0.5 bp of a corresponding allele, suggested that there was no risk of genotyping error due to insufficient sizing accuracy.

3.4. Stutter percentage and peak height ratio studies

Stutter peaks that result from strand slippage during PCR amplification, often manifest as one repeat unit shorter or longer than the main allele peak [11]. A high percentage of stutter may make it more difficult for analyzing mixtures due to confusion with the minor contributor [12]. Stutter percentage and its relative allele peak heights for each STR were obtained from the analysis of 200 individuals with Microreader™ 23sp ID system. The results of stutter percentage and standard deviations were listed in Table 1. The lowest average percentage of stutter was observed at locus D4S2366 (3.78%), and the highest were D13S325 and D1S1656 (both for 13.05%). Compared with the recommended stutter filter values, D15S659, D22-GATA198B05 and D1S1656 had the stutter peaks exceeded the labeled line. The recommended stutter filters would be useful when met mixed samples, and may be a nice addition to existing STR profiling capabilities.

The peak height ratio (PHR) levels mirrored the balance of heterozygotes. A good balance in all analyzed markers met the requirements of correct and reliable genotyping. In this study, the calculation of the PHR was performed by analyzing 200 individuals obtaining an average PHR of 85.88%. It is notable that locus of D20S470 was observed PHR averages below 80% (79.91%) and showed 20.53% PHR lower than 70% (Table 2), this situation may be caused by relatively big fragment size of D20S470, which ranged from 378 bp to 460 bp. Due to the low PHR values observed at several loci, users should at least repeat three times when deal with LCN (low-copy number) DNA to avoid missing alleles. In general, the Microreader™ 23sp ID system provided a decent balance of heterozygous alleles.

3.5. Mixture studies

Samples mixed by more than one individual appeared frequently in forensic casework. So it is vital to perform mixture studies for mixture interpretation. A total of 1 ng DNA template mixed by 9947A and 9948 at various ratios (19:1, 9:1, 4:1, 2:1 and 1:1) was tested in triplicate. As the proportion of minor contributor DNA 9948 were reduced, a concomitant drop in peak height was observed for minor alleles (Fig. S6). The detected percentages of minor alleles were calculated from 18 loci for each ratio of 9947A and 9948, because 9947A and 9948 had shared the same genotypes of 5 loci (Fig. 4). All of the minor alleles were detected for ratios of 1:1, 2:1 and 4:1. When at 9:1 ratio of 9947A and 9948, several alleles (average 11.11%) of minor contributor DNA 9948 dropped out and some alleles were confused with stutter peaks. Things got much worse at 19:1 ratio with more minor alleles (average 39.5%) dropped out. These results demonstrated the Microreader™ 23sp ID system was able to deal with a DNA mixture confined to the ratio of 4:1.

3.6. Stability studies

Humic acid was reported to affect the template availability for amplification by binding to DNA while hematin was thought to curb Taq polymerase function [13]. The ability of the new system to obtain results from the effect of two inhibitors was evaluated. Full profile was observed with humic acid concentration up to 10 ng/μL and hematin up to 30 μM. The allelic peak heights gradually reduced with the increase in inhibitor concentration while the appearance of large amplicons prior dropped out were observed with a certain amount of humic acid and hematin (Fig. S7).

3.7. Species specificity

DNA from several animals (pig, dog, bovine, goat, cat, mouse, cavy, rabbit, duck and chicken) and microbial species (*Staphylococcus aureus, Escherichia coli, Staphylococcus albus* and *Pseudomonas aeruginosa*) which were closely related with human activity was amplified to test its cross-reactivity. Significative peaks above 50 RFU were detected from cavy, chicken and Escherichia coli. For cavy, an allele of "16" (peak height 250 RFU) at D6S477 locus with size 135.83 bp and an "OL" peak (peak height 406 RFU) at D13S325 with size 230.64 bp were detected. For chicken and *Escherichia coli*, an "OL" peak (peak height 445 RFU) at D10S1435 locus with size 124.74 bp and an "OL" peak (peak height 775 RFU) at D11S2368 locus with size 335.76 bp were detected respectively (Fig. S8). In addition, "OL" peaks at the amelogenin locus were observed from pig, dog, cat and bovine with size ranged from 100.60 to 101.19 bp, these peaks were regular and won't affect genotyping [14]. Three intense dye blobs between 75 and 105 bases were observed in Fig. S8, in fact these dye blobs were appeared at every genotyping profiles (these dye blobs didn't presented in other figures because these figures all started after dye blobs). Fortunately these dye blobs were all out of panels and definitely had no material effect on genotyping results. The species specificity results suggested we should be cautious for samples possibly combined with DNA from cavy, chicken and *Escherichia coli*.

4. Conclusion

With the development of forensic science in recent years, complicated forensic cases such as complex kinship testing and STR mutation presented more and more frequently. We have to depend on higher distinguishability provided by commercial STR kits to deal with those challenges. In this situation, one STR kit was often insufficient and always need to be used in combination with other STR kits to get a higher distinguishability. However, many commercial STR kits contained the same 13 CODIS core loci and cannot give full play to every locus' value. The Microreader™ 23sp ID system was developed to meet the demands for these situation. This system 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), a CODIS STR locus (D16S539) and the amelogenin locus in one reaction with a 5-dye fluorescent analysis system. Previously reported data demonstrated that the Microreader™ 23sp ID system showed a huge power of discrimination and an extremely high combined paternity exclusion probability value. Validation of the Microreader™ 23sp ID system was carried out based on guidelines issued by SWGDAM and results indicated that the 23-plex system was robust, sensitive and accurate. In addition, this is a newly developed kit, further work for mutation rates and comparisons to other STR kits need to be done in the future. For all these merits, the Microreader™ 23sp ID system was suitable for personal identification and parentage test, especially when used in conjunction with other common commercial STR kits, this system could be a useful tool for complex kinship cases and the presence of STR mutation.

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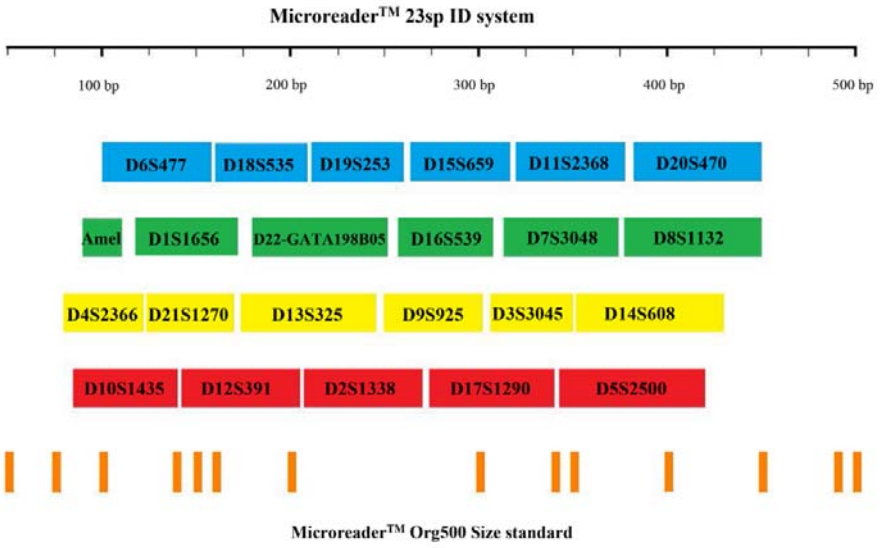

Fig. 1. The arrangement of 23 loci of the Microreader™ 23sp ID system.

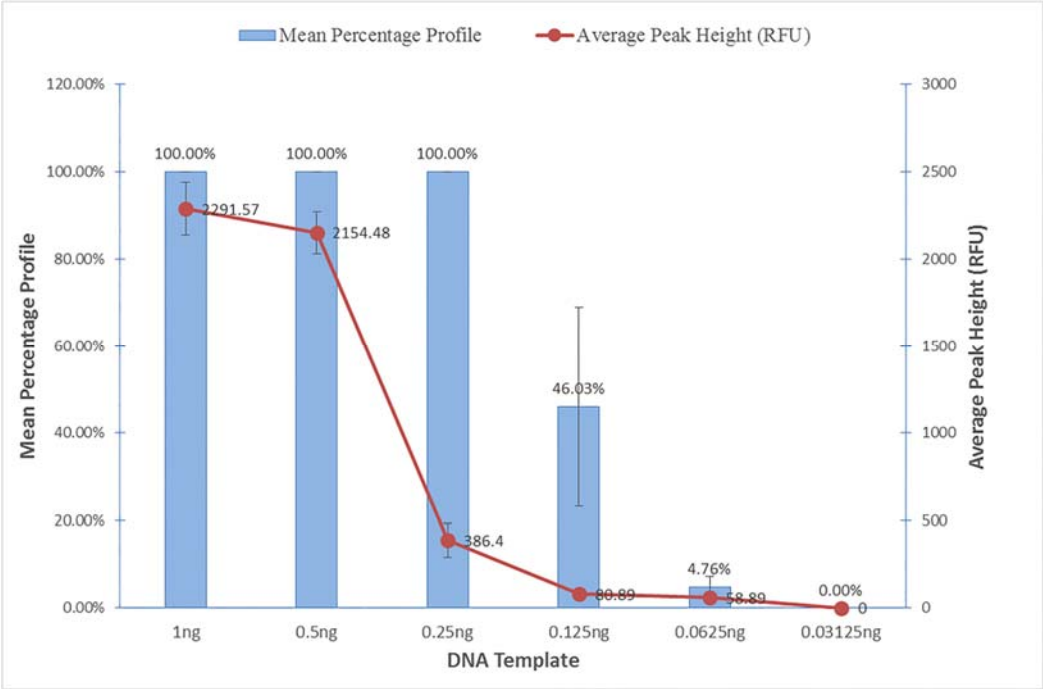

Fig. 2. Sensitivity testing of template DNA ranged from 1 ng to 0.03125 ng.

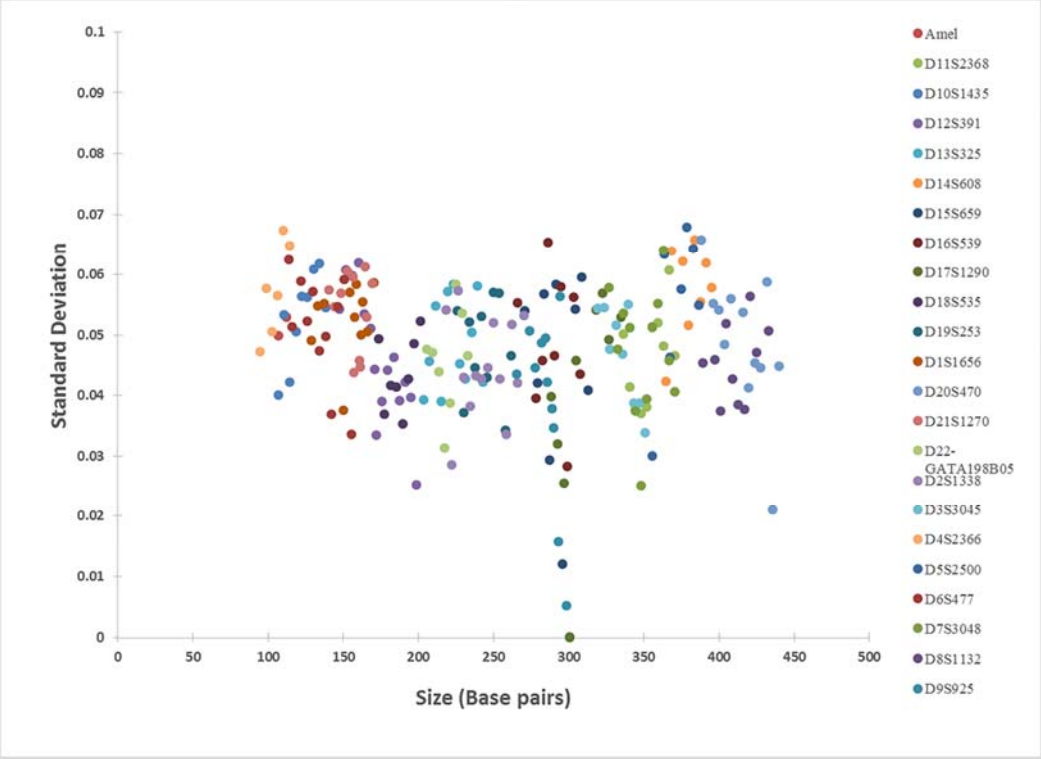

Fig. 3. Sizing variation of all allelic ladders performed on 3130 Genetic Analyzer (n = 16).

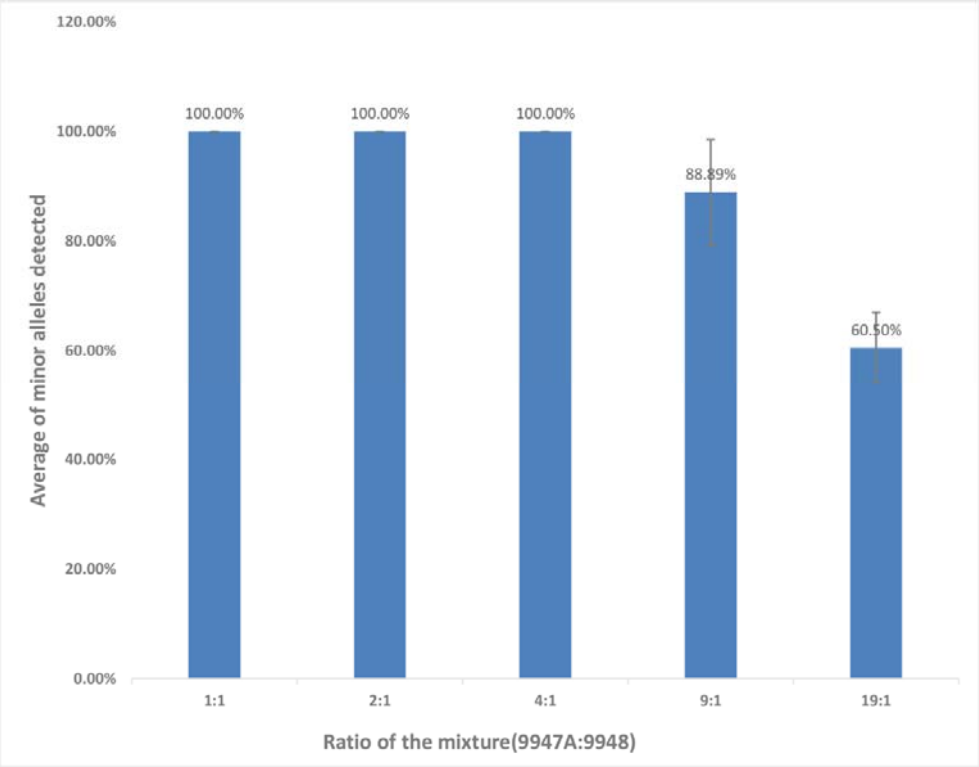

Fig. 4. A total of 1 ng DNA template mixed by 9947A and 9948 at various ratios (19:1, 9:1, 4:1, 2:1 and 1:1) was tested in triplicate. As 9947A and 9948 had shared the same genotype of 5 loci, the detected percentages of minor alleles were calculated from the rest of 18 loci.

Table 1. Stutter percentage were calculated for the 22 STR loci from 200 samples.

Locus	Minimum	Maximum	Average	SD [a]	Recommended filter [b]
D6S477	2.26%	12.47%	8.59%	1.32%	12.55%
D18S535	1.52%	13.27%	8.14%	1.91%	13.88%
D19S253	1.46%	14.72%	8.47%	2.28%	15.31%
D15S659*	1.54%	14.79%	9.46%	1.53%	14.04%
D11S2368	1.54%	18.41%	11.16%	2.47%	18.57%
D20S470	3.28%	18.99%	10.59%	2.86%	19.16%
D1S1656*	1.42%	22.63%	13.05%	2.93%	21.83%
D22-GATA198B05*	1.53%	19.38%	11.89%	2.35%	18.93%
D16S539	1.52%	12.36%	7.55%	1.64%	12.47%
D7S3048	1.19%	17.57%	11.51%	2.03%	17.60%
D8S1132	5.43%	16.49%	10.95%	2.01%	16.99%
D4S2366	0.52%	7.47%	3.78%	1.56%	8.45%
D21S1270	0.93%	9.72%	5.37%	1.71%	10.51%
D13S325	1.62%	19.06%	13.05%	2.03%	19.15%
D9S925	1.28%	11.10%	7.77%	1.29%	11.64%
D3S3045	1.32%	9.03%	4.83%	1.61%	9.65%
D14S608	1.41%	10.36%	5.56%	1.71%	10.69%
D10S1435	1.49%	13.62%	9.46%	1.75%	14.72%
D12S391	2.69%	18.03%	12.31%	2.00%	18.32%
D2S1338	3.83%	18.52%	11.84%	2.43%	19.13%
D17S1290	2.86%	13.13%	7.70%	1.87%	13.33%
D5S2500	1.34%	8.72%	5.64%	1.29%	9.52%

[a] SD: standard deviation.

[b] Recommended filter = average value + 3SD.

* Three loci had the stutter peaks exceeded the labeled line.

Table 2. Peak height ratio values were computed of 23 STR loci.

Locus	Number of observations	Minimum	Maximum	Average	SD [a]	PHR<70%
D6S477	159	64.11%	99.93%	87.32%	8.94%	3.77%
D18S535	165	66.87%	99.96%	86.99%	7.92%	1.82%
D19S253	156	64.89%	99.88%	83.85%	8.53%	5.77%
D15S659	167	61.77%	99.90%	86.73%	8.65%	3.59%
D11S2368	174	61.84%	99.53%	85.69%	8.96%	5.75%
D20S470	151	58.54%	99.82%	79.91%	10.98%	20.53%
Amel	110	62.17%	99.83%	87.51%	9.29%	5.45%
D1S1656	166	63.32%	99.95%	87.87%	8.36%	3.01%
D22-GATA198B05	159	63.80%	99.95%	85.37%	9.18%	5.03%
D16S539	148	61.25%	99.81%	86.07%	9.59%	8.11%
D7S3048	173	58.48%	99.88%	84.61%	9.21%	8.09%
D8S1132	158	54.08%	99.79%	84.88%	9.56%	8.86%
D4S2366	152	62.74%	100.00%	86.55%	9.15%	5.92%
D21S1270	167	64.47%	99.93%	85.11%	8.70%	4.79%
D13S325	159	61.10%	99.58%	86.00%	9.34%	6.92%
D9S925	153	60.14%	99.77%	86.26%	9.60%	5.23%
D3S3045	161	62.47%	99.66%	87.02%	9.25%	5.59%
D14S608	167	59.36%	99.91%	86.23%	9.48%	6.59%
D10S1435	146	59.22%	100.00%	86.90%	9.11%	6.16%
D12S391	170	64.66%	99.69%	85.11%	8.98%	7.65%
D2S1338	169	61.28%	99.92%	83.87%	10.12%	10.65%
D17S1290	166	64.83%	99.73%	87.06%	8.47%	3.01%
D5S2500	166	66.23%	100.00%	88.33%	8.74%	3.01%

[a] SD: standard deviation.