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Received June 20, 2019
Revised August 31, 2019
Accepted September 26, 2019

Research Article

Developmental validation of the Microreader™ 20A ID system

The Microreader™ 20A ID system is designed for forensic applications such as personal identification, parentage testing, and research. It includes 13 combined DNA index system (CODIS) short tandem repeat (STR) loci (CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11), three expanded CODIS STR loci (D12S391, D19S433, and D2S1338), three non-CODIS STR loci (D6S1043, Penta D, and Penta E), and the amelogenin locus in one reaction with a six-dye fluorescent (FAM, HEX, TAMAR, ROX, PUR, and QD550) analysis system. In this study, the Microreader™ 20A ID system was validated according to the Scientific Working Group on DNA Analysis Methods validation guidelines for forensic DNA Analysis methods and Chinese national standard, including PCR-based studies, sensitivity study, precision, and accuracy evaluation, stutter calculation, inhibitor tests, species specificity, and DNA mixture studies. Our results suggest that the Microreader™ 20A ID system is a useful tool for personal identification and parentage testing.

Keywords:
Capillary electrophoresis / Forensic science / Microreader™ 20A ID system / Short tandem repeat (STR) / Validation
DOI 10.1002/elps.201900221

Additional supporting information may be found online in the Supporting Information section at the end of the article.

1 Introduction

The rapid development of DNA detection methods in recent years has promoted the advancement of forensic genetics, especially the utilization of massively parallel sequencing methodology. This has facilitated the analysis of a large number of genetic markers in a single multiplex [1–3]. However, most laboratories only have CE platforms. Therefore, STR loci are widely used in routine biological evidence detection [4–6]. Data sharing and comparison are becoming a concern, as different regions usually choose different loci for their own population. The Microreader™ 20A ID system (www.microread.com, Beijing Microread Genetics, Beijing, China) is a kit designed for forensic DNA casework with superior performance and economics. Based on 13 CODIS core loci, the 6 loci of Penta E, Penta D, D2S1338, D19S433, D12S391, and D6S1043 were added to the Microreader™ 20A ID system for good database compatibility.

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Abbreviations: **CODIS**, combined DNA index system; **OL**, Off ladder; **RFU**, relative fluorescence units

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Color online: See article online to view Figs. 1–6 in color.

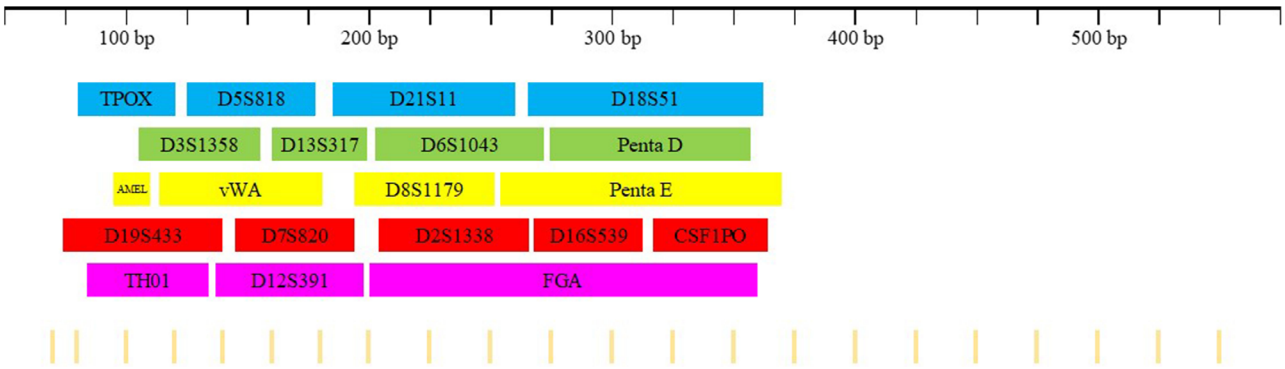

Figure 1. Arrangement of 20 loci of the Microreader™ 20A ID system.

One limitation of STR markers is that most of these markers may have an amplicon size greater than 200 base pairs (bp). However, the Microreader™ 20A ID system focuses on minimizing the length of the amplicon and can obtain the typing of ten mini STRs (<200 bp), which is more helpful in improving the success rate of typing degradation samples [7–9]. Second, the design of the primer has been significantly improved during the development process. Primers and new fluorescence dyes are combined to improve the balance between different dye groups, which helps analysts to analyze data more easily. The arrangement of all loci in each dye channel is shown in Fig. 1. To ensure the performance of the Microreader™ 20A ID system, a series of validation experiments was carried out in this study according to the Chinese national standard "Criterion of forensic science human fluorescence STR multiplex amplification reagent" (GB/T 37226-2018) and the "Validation Guidelines for DNA Analysis Methods (2016)" described by the Scientific Working Group on DNA Analysis Methods (SWGDAM) (https://docs.wixstatic.com/ugd/4344b0_813b241e8944497e99b9c45b163b76bd.pdf) [10, 11]. The results demonstrated that the Microreader™ 20A ID system is a sensitive, accurate, and high-performance tool for forensic cases.

2 Materials and methods

2.1 DNA samples

PCR-based studies, sensitivity study, precision and accuracy evaluation, and inhibitor tests were carried out on control DNA F312 (2 ng/μL) (Beijing Microread Genetics, Beijing, China). Both control DNA F312 and control DNA M308 (2 ng/μL) (Beijing Microread Genetics, Beijing, China) were used in the mixture studies.

Whole human blood samples from 200 unrelated individuals were obtained under the supervision of the appropriate review boards and with informed consent. DNA was extracted using the BioTeke DNA kit (BioTeke, China), according to the manufacturer's protocol.

Samples of common animal species (chicken, duck, cow, sheep, pig, fish, dog, cat, rabbit, and mouse) were prepared for the specificity studies of the system. Animal DNA was extracted using the salting out method [12]. Further, common micro-organisms (such as *Escherichia coli* and *Mycobacterium tuberculosis*) were obtained with the TIANamp Bacteria DNA kit (TIANGEN Biotech, Beijing, China) for specificity testing. All DNA samples were quantified with the NanoDrop™ 1000 spectrophotometer (Thermo Fisher Scientific).

2.2 DNA amplification

The standard condition amplification reactions of the Microreader™ 20A ID system were 25 μL in total volume, consisting of 10 μL of 2.5× Master Mix I, 5 μL of 5× Primer Mix, and 1 ng template DNA. PCR was performed in the GeneAmp 9700 PCR System (Applied Biosystems). Standard thermal cycling parameters were as follows: enzyme activation at 95°C for 5 min; 29 cycles of denaturation at 94°C for 30 s, annealing at 59°C for 60 s, and extension at 72°C for 60 s; and finally a 60-min extension at 60°C, and maintained at 4°C for further analysis.

2.3 CE and data analysis

The amplification products were detected on the ABI 3500 Genetic Analyzer (Applied biosystems, Foster City, CA) using run modules and J6 6-dye variable binning modules, as described in the Microreader™ 20A ID System User Guide (http://www.microread.com/upload/201908/26/201908261529008465.pdf). Samples were prepared for CE by adding 1 μL of the PCR products (or Microreader™ 20A Allelic Ladder) to 9 μL of a 25:1 mixture of Hi-Di formamide (Applied Biosystems, Foster City, CA) and QD550 size standard (http://www.microread.com/upload/201908/26/201908261527082527.pdf) (Beijing Microread Genetics, Beijing, China). Samples were injected at 3 kV for 10 s and electrophoresed at 15 kV for 1210 s in the Performance Optimized Polymer-4 (POP-4 polymer) (Applied Biosystems,

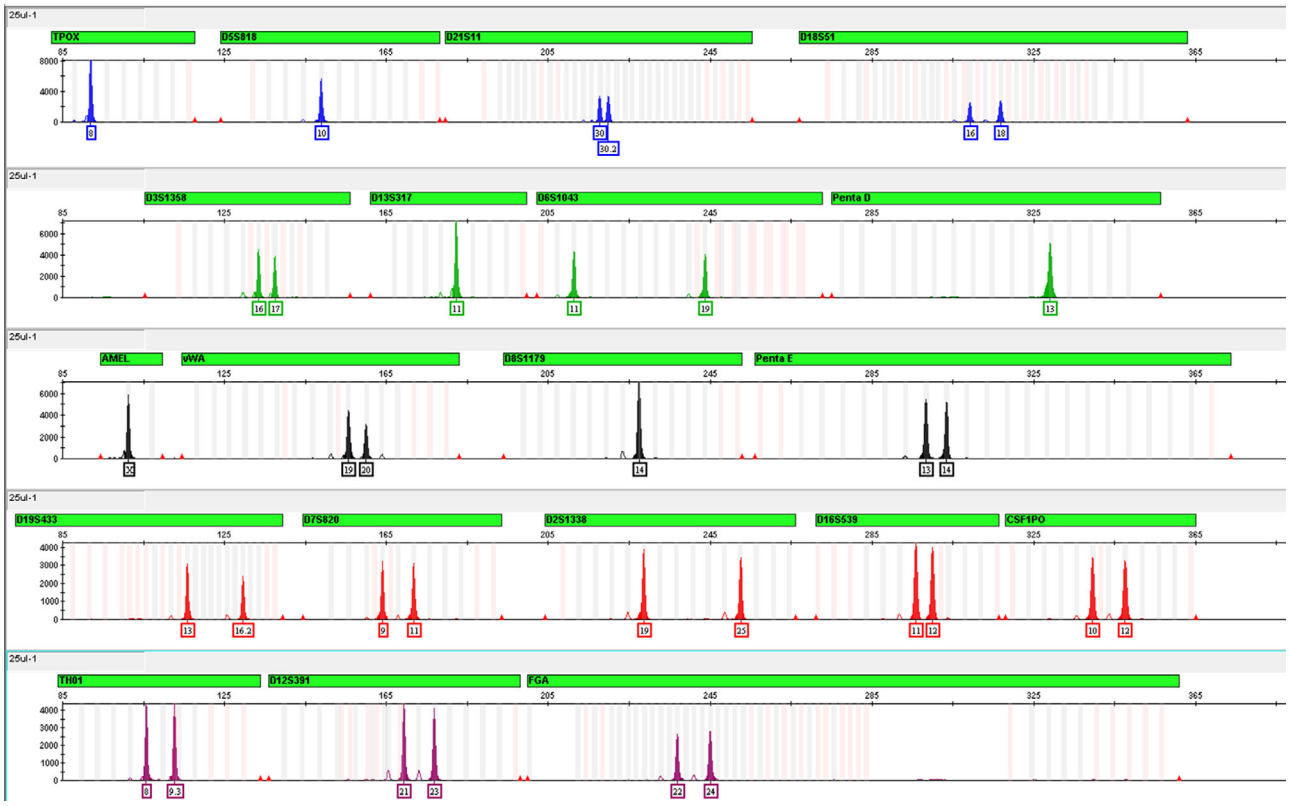

Figure 2. Control DNA F312 profile under standard thermal cycling conditions.

Foster City, CA). Unless otherwise noted, after data collection, the genotyping data were analyzed using the GeneMapper™ ID Software v3.2.1 (Applied Biosystems, Foster City, CA), with the threshold for allele peak setting at 50 relative fluorescence units (RFU). Representative profiles were generated from 1 ng of Control DNA F312 amplified using the Microreader™ 20A ID system under standard thermal cycling conditions and electrophoresed on the ABI 3500 Genetic Analyzer (Figure 2). The electropherogram of the Control DNA M308 amplified under standard conditions was shown in the Supporting Information Fig. S1.

2.4 PCR-based studies

In PCR-based studies, we tested the reaction volumes (25, 12.5, and 6.25 µL), cycling number (27, 29, 31), denaturation temperature (92, 94, and 96°C), annealing temperature (55, 57, 59, 61, and 63°C), extension temperature (70, 72, and 74°C), concentration of 5× Primer Mix (0.5×, 0.75×, 1×, 1.25×, and 1.5×), concentration of 2.5× Master Mix I (0.5×, 0.75×, 1×, 1.25×, and 1.5×), and final extension time (55, 60, and 65 min) of the Microreader™ 20A ID system. Control DNA F312 was evaluated in triplicate for each component at each concentration. Under a variety of conditions, only the test parameters were changed, whereas the other parameters were consistent with the recommended standard conditions. The concentration of the control DNA F312 in the final system also remained unchanged.

2.5 Sensitivity study

To evaluate the sensitivity and working range of the Microreader™ 20A ID system, serial dilutions of control DNA F312 were amplified in triplicate with quantities of 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015625 ng. Amplification was carried out following the manufacturer's recommended conditions. The amplified products were detected on the 3500 instrument and analyzed with the GeneMapper™ ID Software v3.2.1. The full profile percentage and mean peak height were determined for each template amount.

2.6 Species specificity

DNA samples of several common animal species (chicken, duck, cow, sheep, pig, fish, dog, cat, rabbit, and mouse) and two common microbial species (Escherichia coli and Mycobacterium tuberculosis) were tested for cross-reactivity. To evaluate the species specificity of the Microreader™ 20A ID system for human DNA, each blood sample was amplified using a standard PCR protocol. The corresponding products were analyzed in triplicate.

2.7 Precision study and sizing accuracy

The size precision of the Microreader™ 20A ID system was evaluated on the 3500 Genetic Analyzer by carrying out the capillary electrophoresis of the 24 allelic ladder samples. The mean variability of size was determined by calculating the standard deviation for each allele. The average base pair size and standard deviation (SD) were calculated for each allele in the allelic ladder using QD550 LIZ size standard and GeneMapper™ ID software.

Sizing accuracy is the difference of the size of the allele compared to the corresponding allelic ladder. In this study, 50 samples were genotyped under standard conditions to assess the sizing accuracy of the Microreader™ 20A ID system.

2.8 Mixture studies

Mixture studies were performed to assess the Microreader™ 20A ID system's reliability for the detection of mixtures. In this validation, mixture studies were performed with mixed DNA samples at known ratios. Female/male mixtures were prepared using control DNA of F312 and M308 with mixture ratios of 19:1, 9:1, 4:1, 3:1, 1:1, 1:3, 1:4, 1:9, and 1:19. A 25 µL reaction volume of each mixture, which contained 1 ng of the total template DNA in triplicate, was tested.

2.9 Stutter percentage

In the stutter percentage study, 200 samples were amplified and analyzed on a 3500 Genetic Analyzer to calculate the stutter of each locus. All samples were collected from 200 unrelated healthy individuals of the Sichuan Han population with informed consent. The stutter percentage was calculated by dividing the stutter peak height ($n \pm 1$ repeat unit) by the associated allele peak height [13]. Herein, the analytical threshold of the minimum stutter peak height was set to 20 RFU.

2.10 Stability studies

Three types of forensic inhibitors were tested in the stability studies, including hematin, humic acid, and EDTA. Stock solutions of high concentration were prepared by dissolving hematin (Sigma, St. Louis, MO, USA) in 0.1 N NaOH and humic acid (Sigma, St. Louis, MO, USA) in water. The quantity of control DNA F312 was maintained constant at 1 ng, whereas the amplification reactions contained different concentrations of inhibitors. The concentrations of humic acid were 200, 300, 400, and 500 ng/µL; hematin were 250, 500, 750, and 1000 µmol/L; and EDTA were 0.25, 0.5, 0.75, and 1 mmol/L. Each test was performed three times.

2.11 Concordance study

In the concordance study, control DNA F312 and M308 and 50 samples from volunteer donors were amplified under standard conditions with the Microreader™ 20A ID system and the AGCU Expressmarker22 STR Kit (AGCU ScienTech, Jiangsu, China) [14].

3 Results and discussion

3.1 PCR-based studies

In the PCR-based studies, we tested the reaction volumes, cycling number, denaturation temperature, annealing temperature, reaction components, extension temperature, and final extension time of the Microreader™ 20A ID system.

The reduction of reaction volume is conducive to the small amount of DNA template in forensic cases. The reaction volumes of 25, 12.5, and 6.25 µL with control DNA F312 were tested in this study, in which each component ratio remained the same as the recommendations. The complete profiles were obtained for all ranges of the reaction volume, and the genotyping results of different reaction volumes were consistent. In addition, it was observed that the reaction volume had little effect on the average peak height or heterozygous peak height ratio and error bars show the SDs between all replicates (Fig. 3). The results suggested that the Microreader™ 20A ID system could also obtain correct genotyping results when the reaction volume decreased.

In the cycling number studies, 1 ng of control DNA F312 was amplified with the manufacturers' recommendations at 27, 29, and 31 cycles. The full profiles were reliably generated in the tests using these several cycles. The average peak heights were 2195, 3192, and 17067 RFU, and the SDs were 487, 664, 3693, respectively (Fig. 3). The recommended optimal cycling number is 29 for this system. As expected, an increase in cycle number increased the overall peak height, and some degree of detector saturation owing to the excessive peak height was observed at 31 cycles (http://tools.thermofisher.com/content/sfs/manuals/4474504.pdf). The results show that when the amount of DNA template is less, we can increase the number of cycles to obtain better results.

In the tests of denaturation temperature, three values of temperatures were selected: 92, 94, and 96°C. The standard recommended denaturation temperature was 94°C. Complete profiles were obtained at each temperature. The results indicate that a temperature increase or decrease of the enzyme denaturation by 2°C has a slight effect on the average peak height or the heterozygous peak height ratio (Fig. 3).

In the annealing temperature studies, temperatures of 55, 57, 59, 61, and 63°C were tested, and the average peak heights were 5840, 5897, 5033, 7176, and 4228 RFU, respectively (Fig. 3). The complete profiles were generated and no allele drop-out or drop-in was observed when the annealing temperature ranged from 55 to 61°C. When the annealing temperature was increased to 63°C, the balance between the various loci decreased, and even some spurious alleles of the FGA locus dropped in. The temperature recommended by the instructions is 59°C, and the results show that the graph at 59°C exhibited a suitable balance and average peak

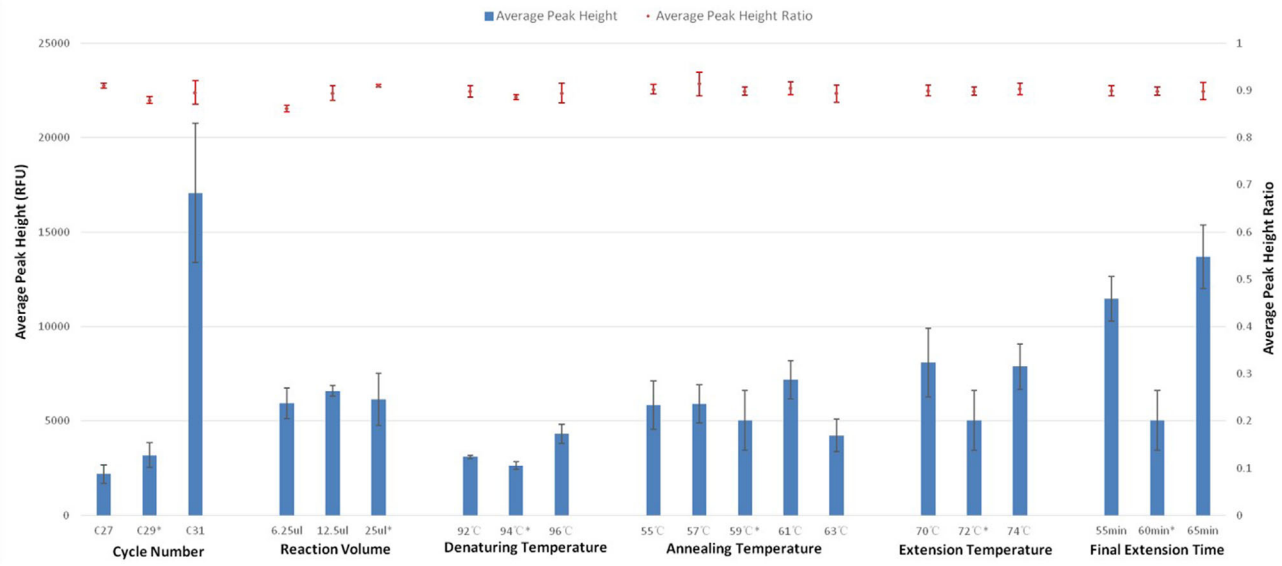

Figure 3. PCR-based study results using control DNA F312. Asterisks indicate standard conditions. Error bars show the SDs between all replicates.

height (Supporting Information Fig. S2). The results confirmed that the Microreader™ 20A ID system is well adapted to different annealing temperatures, which increases the possibility of successful amplification with inaccurate annealing temperatures.

In the studies of reaction components, the concentration of Primer Mix and Master Mix I were tested. We amplified 1 ng of control DNA F312 with 0.5×, 0.75×, 1×, 1.25×, 1.5× of 5× Primer Mix and 2.5× Master Mix I to verify the effect of different concentrations of primers and mix on the results. As a result, correct genotyping results were obtained from different concentrations of the 5× Primer Mix with no allele dropped out, and the overall peak height increased with the increase of primer concentration (Supporting Information Fig. S3). For the PCR Master Mix I, full profiles were obtained except at the 0.5× concentration, and the peak heights ascended with increasing concentration. Some loci such as D21S11, D18S51, D8S1179, FGA dropped out and off-ladder peaks were common at D13S317 when the concentrations of 2.5× Master Mix I decreased to half (0.5×) (Supporting Information Fig. S4). These studies suggest that different concentrations of the 5× Primer Mix may not have much impact on the genotyping results, but the concentration of 2.5× Master Mix I should preferably be consistent with the manufacturer's instructions. The Microreader™ 2.5× Master Mix I includes important components such as Taq DNA polymerase, magnesium ion and so on. Magnesium is an essential activator of Taq DNA polymerase, so magnesium concentration is important for PCR reactions. Therefore, the concentration of Master Mix I is quite important for the entire PCR reaction, even more important than the primer concentration. This result is similar to the other studies that have been reported [15–17].

In the extension temperature studies, temperatures of 70, 72, and 74°C were tested. Complete profiles were generated when the extension temperature ranged from 70 to 74°C. The results show that the extension temperature above or below the optimum temperature of 2°C does not affect the ability of the Microreader™ 20A ID system to generate a complete profile but may affect the average peak height or the heterozygous peak height ratio (Fig. 3).

In the tests of final extension time, 1 ng of control DNA F312 was amplified with the final extension time 55, 60 (recommended), and 65 min. Taq DNA polymerase has the ability to add an additional non-template adenosine nucleotide at the 3' end of the DNA strands during thermal cycling. This addition results in a PCR product that is one nucleotide longer than the predicted amplicon, commonly referred to as the "+ A" form. In STR multiplexing, a final extension step after amplification is typically included to ensure that nucleotides are added on all amplification products to maintain a uniform electrophoretic size. In these studies, full profiles were obtained except for the case in which the final extension time was 65 min. Incomplete +A addition effects were observed at the Penta D locus with the final extension time of 65 min in our experiments, indicating that the final extension time need not necessarily be too long. Therefore, a suitable final extension time is required to adenylate all amplified fragments. For the Microreader™ 20A ID system, the final extension time should be consistent with the manufacturer's instructions.

3.2 Sensitivity study

The sensitivity of the Microreader™ 20A ID system was tested with serial dilution of control DNA F312 at the following template amounts: 1, 0.5, 0.25, 0.125, 0.0625, 0.03125, and 0.015625 ng. Each template amount was amplified three times. Full profiles were obtained using the peak height analysis threshold of 50 RFU with the concentration of F312 from

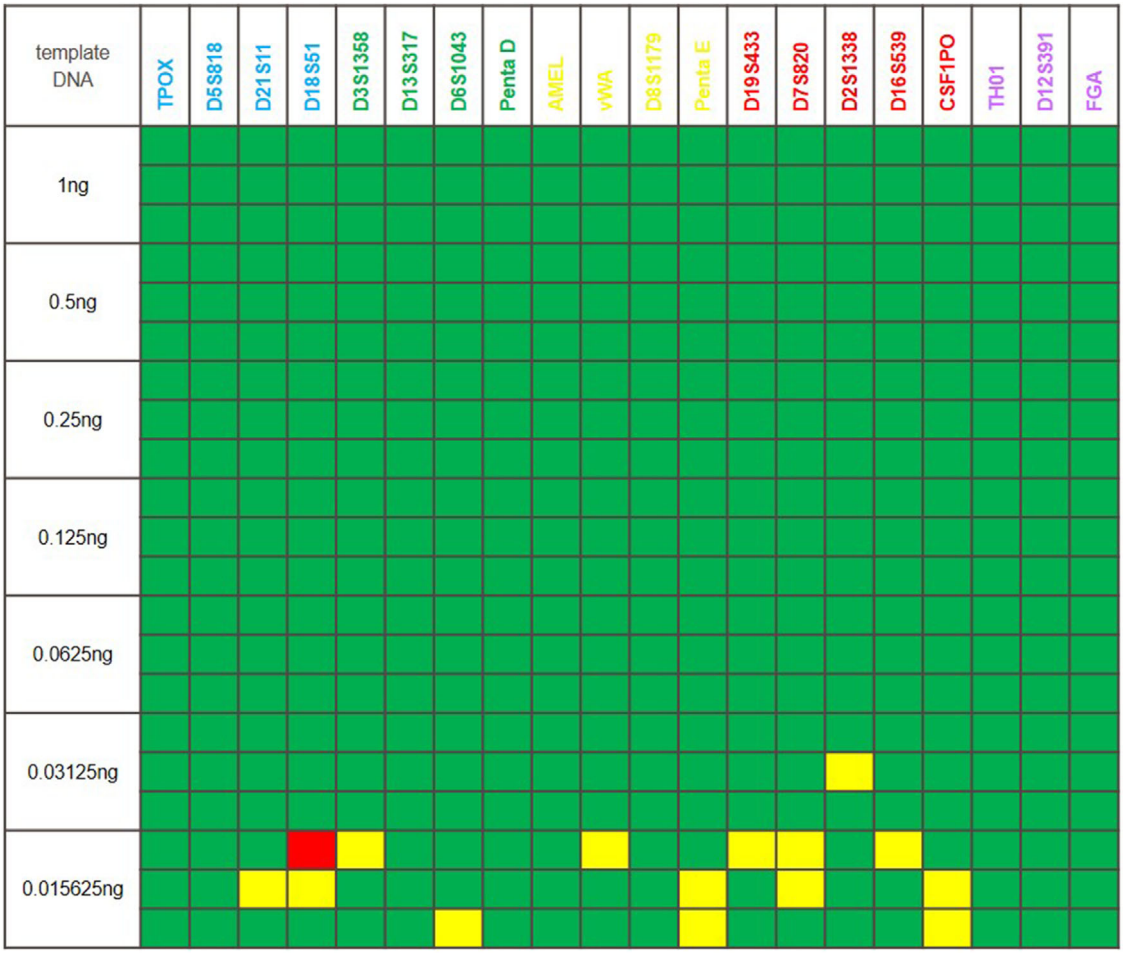

Figure 4. Results of a sensitivity study using control DNA F312 ranging from 1 ng to 0.015625 ng with a 50 RFU threshold for allele calls. Green boxes indicate no allelic drop-out, red boxes indicate no alleles recovered, and yellow boxes indicate that only one of the two expected heterozygote alleles was called.

1 ng down to 0.0625 ng (Fig. 4). As the template DNA reduced gradually from 1 to 0.0625 ng, the average peak height detected moved from 5479 to 327 RFU. When the template DNA F312 decreased to 0.03125 ng, partial profiles were observed and an average 99% of the loci were detected with an average peak height of 230 RFU. For 0.015625 ng of F312 DNA, an average 85% of the loci were detected with an average peak height of 148 RFU. Therefore, the Microreader™ 20A ID system can obtain reliable profiles at the threshold of 50 RFU above the DNA concentration of 0.0625 ng. We then increased the threshold to 100 RFU for analysis. When the allele call has a 100 RFU threshold, a sample input greater than 0.0625 ng will always produce a complete STR profile (Supporting Information Figure S5).

3.3 Species specificity

To evaluate the species specificity of the Microreader™ 20A ID system, several types of non-human genomic DNA samples were tested. DNA from several animals and microbial species, which were closely related with human activity, was amplified to test its cross-reactivity. "off ladder" peaks at the amelogenin locus were observed from the cow, pig, sheep, dog, cat with sizes ranging from 96.98 to 101.08 bp; these peaks were regular but will not affect genotyping. Other peaks above 100 RFU were detected from the chicken, with a 159.34 and 274.10 bp "outside marker range" peak (height 383 and 156 RFU, respectively), and a 213.09 bp "off ladder" peak (height 219 RFU) at the D6S1043 locus with HEX dye. Fortunately, they were all located outside of the bin sets, and definitely had no material effect on the genotyping results. In addition, a 436.71 bp peak (height 3265 RFU) with HEX dye was observed, which was out of panels (Supporting Information Fig. S6).

3.4 Precision study and sizing accuracy

Sizing precision is critical for accurate and reliable genotyping. To evaluate the size precision of the Microreader™ 20A ID system, 24 allelic ladder samples were detected on the

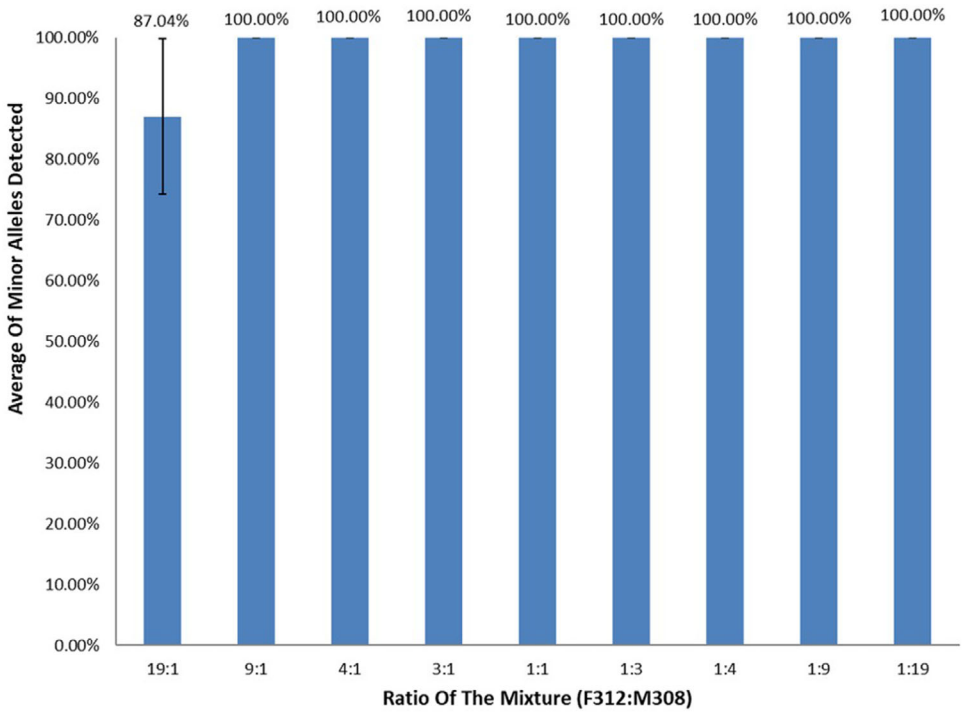

Figure 5. Total of 1 ng DNA template mixed with F312 and M308 at various ratios (19:1, 9:1, 4:1, 3:1, 1:1, 1:3, 1:4, 1:9, and 1:19) was tested in triplicate.

3500 Genetic Analyzer. The average base pair size and standard deviation were calculated for every allele. The lowest standard deviation observed was 0.010533 (vWA), the largest was 0.07397 (FGA), which was well below the target specification of 0.15 bp, indicating that the detection system had good performance precision. As expected, the SDs increased slightly as the fragment size increased (Supporting Information Fig. S7).

To assess the sizing accuracy, the size differences between the sample alleles and the allelic ladder alleles were studied with 50 random male samples. The results showed that all sample alleles (a total of 1806 alleles from 50 samples) were sized within ± 0.5 bp, which indicated that this system can reliably determine the genotypes.

3.5 Mixture studies

In the mixture studies, 1 ng DNA template mixed with a control DNA of F312 and M308 at various ratios (19:1, 9:1, 4:1, 3:1, 1:1, 1:3, 1:4, 1:9, and 1:19) was tested in triplicate. The detected percentages of minor alleles were calculated from 18 loci for each ratio of F312 and M308, based on the fact that F312 and M308 shared the same genotypes at two loci. The genotypes of the minor contributor alleles could be accurately detected in all proportions using the peak height analysis threshold of 50 RFU. We then increased the threshold to 100 RFU for analysis. When the male component M308 is the main component, the genotypes of the minor contributor alleles could be accurately detected at all the ratios (1:3, 1:4, 1:9, and 1:19); however, the minor alleles could not be completely detected with F312 and M308 in the ratio of 19:1. For the mixture ratios of 19:1, the unique minor profile was called for an average of 87.04% (Fig. 5). These results demonstrated that the Microreader™ 20A ID system can meet the requirement of the analysis for mix samples and the Chinese national standard for the human fluorescent STR multiplex PCR reagent. In the results of the mixture study, when the mixing ratio was 19:1 or 1:19, the amount of DNA of the minor component in the reaction system was only 0.05 ng. This template amount is already lower than the sensitivity of the system, so it is normal to have an imbalance. Whether it is for female/male, or male/female, or even male/male, it may happen that the detected proportions of minor components are inconsistent. Similar imbalances have emerged in other studies [18–20].

3.6 Stutter percentage

Stutter peaks caused by strand slippage during PCR amplification typically appear as one repeat unit shorter or longer than the main allele peak [13]. Owing to confusion with the minor contributor, a high percentage of stutter may make the analysis of the mixture more difficult [21]. In this study, the stutter percentage was calculated by dividing the stutter peak height by the associated allele peak height with 200 samples. The results of the average stutter percentage and SD for each locus are listed in Table 1 and the average stutter plus three standard deviations were used to set the stutter filter threshold. The lowest average percentage of stutter was observed at locus Penta D (2.16%) and the highest was D12S391

Table 1. Average stutter percentages, ranges, SDs, and average +3SD values for the Microreader™ 20A ID system. Stutter percentages were calculated for the 20 STR loci from 200 samples

Locus	No. of observations (*n*)	Minimum	Maximum	Average	SD	Recommended filter (average + 3SD)
TPOX	271	1.39%	7.14%	3.40%	1.40%	7.61%
D5S818	286	0.85%	12.10%	7.15%	2.00%	13.16%
D21S11	316	0.68%	15.13%	9.17%	2.05%	15.33%
D18S51	309	4.79%	14.30%	8.21%	2.04%	14.32%
D3S1358	260	1.25%	17.41%	11.07%	2.27%	17.87%
D13S317	301	0.55%	10.82%	4.90%	2.26%	11.68%
D6S1043	336	4.32%	12.50%	8.12%	1.56%	12.79%
Penta D	252	0.75%	4.14%	2.16%	0.67%	4.16%
vWA	292	2.59%	14.37%	7.67%	2.48%	15.10%
D8S1179	299	1.55%	12.06%	7.46%	1.67%	12.46%
Penta E	315	1.45%	11.91%	5.84%	2.13%	12.23%
D19S433	306	0.86%	13.60%	8.78%	1.72%	13.95%
D7S820	290	2.34%	11.33%	6.47%	1.87%	12.09%
D2S1338	320	1.22%	15.77%	10.47%	1.85%	16.02%
D16S539	297	1.08%	11.21%	6.76%	1.99%	12.71%
CSF1PO	287	1.92%	12.12%	7.37%	1.65%	12.33%
TH01	275	0.64%	6.00%	3.20%	0.98%	6.15%
D12S391	327	4.32%	18.74%	11.44%	2.58%	19.19%
FGA	322	0.52%	14.76%	9.27%	2.13%	15.65%

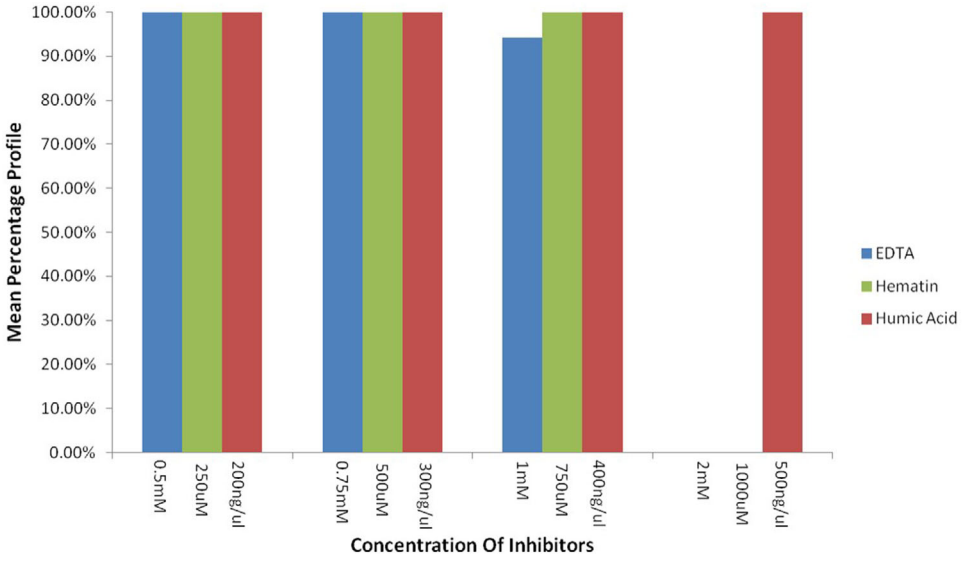

Figure 6. The final concentrations of each inhibitor in the PCR reaction system and the corresponding percentages of the alleles called.

(11.44%). The recommended stutter filters would be useful when analyzing mixed samples.

3.7 Stability studies

Three common forensic inhibitors (hematin, humic acid, and EDTA) were tested with the Microreader™ 20A ID system. Humic acid is an inhibitor that reduces the efficiency of PCR amplification by binding to DNA, whereas hematin is thought to curb the function of Taq polymerase, as is EDTA [22]. With the concentration of hematin up to 750 µM, that of humic acid up to 500 ng/µL and that of EDTA up to 0.75 mM, complete profiles were obtained. For EDTA and hematin, the allelic peak heights gradually decreased as the concentration of the inhibitor increased. As EDTA was increased to 1 mM, partial profiles were observed owing to locus drop out (Fig. 6). When the concentration of hematin was increased to 1000 µM, complete dropout occurred, and the same was true for the 2 mM of EDTA. However, for humic acid, complete profiles could be obtained even at concentrations up to 500 ng/µL, and the peak height did not decrease significantly as the concentration increased. In conclusion, the Microreader™ 20A ID system was capable of

amplifying template DNA even in the presence of inhibitors such as hematin and humic acid.

3.8 Concordance study

A concordance study was conducted to determine if the profiles were reliable and suitable for comparison between different kits provided by different companies using the same DNA samples.

Therefore, the Microreader™ 20A ID system and the AGCU Expressmarker22 STR Kit were tested in parallel to amplify the controls DNA F312 and M308 and 50 case samples. Full profiles were obtained for all DNA samples and the same loci of the same samples had concordant genotypes (data not shown).

4 Concluding remarks

Increasingly, with the development of forensic science in recent years, different commercial STR kits are frequently being presented, and the different kits have their own advantages. The Microreader™ 20A ID system is capable of obtaining the DNA genotype from degraded biological samples as a result of the presence of 10 mini-STRs. The developmental validation studies presented in this paper tested the Microreader™ 20A ID system following the Scientific Working Group on DNA Analysis Methods guidelines and Chinese criteria. The results demonstrated that the system is robust with respect to changes in annealing temperature, sensitive, and accurately types mixed samples, especially exposure to PCR inhibitors.

In conclusion, the data presented in this study clearly demonstrate the reliability, efficacy, and suitability of the Microreader™ 20A ID system for analyzing both forensic casework and database samples.

This project was conducted by our whole team. We thank to all those who contributed to this project. This work was supported by grants from the Applied Basic Research Programs of Science and Technology Department Foundation of Sichuan Province of China (2017JY0233).

The authors have declared no conflict of interest.

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