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TECHNICAL NOTE

Assessment of application value of 19 autosomal short tandem repeat loci of GoldenEye™ 20A kit in forensic paternity testing

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Abstract This study was carried out to assess the application value of 19 autosomal short tandem repeat (STR) loci of GoldenEye™ 20A kit, in which 13 combined DNA index system core STR loci and PentaE, PentaD, D2S1338, D19S433, D12S391, and D6S1043 of six STR loci could be used in forensic paternity testing in Chinese population. We amplified the genomic DNA from blood samples on FTA paper of 289 paternity testing cases by using the GoldenEye™ 20A kit. The amplified products were detected by capillary electrophoresis, and then the genotypes of 20 genetic markers including 19 STR loci as well as Amelogenin for sex determination were analyzed by GeneMapper v3.2 and GeneMarker HID Software. The results of genotypes were compared to the three commonly used commercial kits including AmpF∕STR Identifiler™, PowerPlex™16, and AmpF∕STR Sinofiler™ kits. Compared to the three other common commercial kits, the GoldenEye™ 20A kit had higher value of combined paternity index in certainty of paternity or non-exclusion paternity cases, and more numbers of STR loci were excluded in exclusionary paternity cases. Our data in this study showed that the GoldenEye™ 20A kit has a higher application value in forensic paternity testing and will be of help for kinship analysis.

Keywords Forensic genetics · GoldenEye™ 20A kit · Short tandem repeat (STR) · Paternity testing · Assessment

Introduction

In the practice of forensic science, commercial autosomal short tandem repeat (STR) genotyping kits are commonly used in ordinary paternity testing, individual identification cases, and DNA database [1]. For instance, the AmpF∕STR Identifiler™ and Sinofiler™ (Applied Biosystems, Foster City, CA) [2] kits, which are exclusively developed for Chinese population, and the PowerPlex™16 kit (Promega, Madison, WI) [3] were able to type 15 autosomal STR loci in one PCR and had high discrimination power. However, in some complex paternity testing or deficiency cases or cases with mutation [4–6], one of the three kits was used separately, and it could not provide enough discrimination power. Therefore, the three common commercial kits should be combined to complement conventional analysis and get more genetic information and discrimination power. But when all three commercial kits are simultaneously used for typing, some problem would be faced and considered, e.g., the increased cost. To solve this problem, a new amplification system of GoldenEye™ 20A kit was developed by Beijing PeopleSpot Inc. in China and has been validated by the Forensic Medical Identification Centre of Beijing Public Security Bureau [7] according to the Chinese National Standards and Scientific Working Group on DNA Analysis Methods guidelines. This amplification system contains a total of 20 genetic markers including 13 combined DNA index system core STR loci and PentaE, PentaD, D2S1338, D19S433, D12S391, and D6S1043 of six STR loci as well as Amelogenin for sex determination by fluorescent multiplex amplification simultaneously. In

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other words, the GoldenEye™ 20A kit including all autosomal STR loci of three other common commercial kits aforementioned has demonstrated good detecting performance and is an ideal tool for DNA identification with potential application, such as personal identification and paternity testing.

The purpose of this study presented here was to assess the application value of the GoldenEye™ 20A kit in paternity testing by comparing it with three other commonly used international commercial kits.

Materials and methods

Samples and DNA extraction

With informed consent following the protocols approved by the Institutional Ethics Committee, a total of 289 routine paternity testing cases (including 516 unrelated individuals) of Chinese Han population were randomly selected from the Forensic Center of Beijing Public Security Bureau, and their blood samples were collected and stored at FTA paper. Blood genomic DNA was extracted through magnetic bead method [8].

PCR amplification and genotyping

Target DNA was amplified simultaneously following the user's manual recommendations of the GoldenEye™ 20A, AmpF/STR Identifiler™, PowerPlex™16, and AmpF/STR Sinofiler™ kits. The amplified products were detected in the process of a capillary electrophoresis using ABI-3130 Genetic Analyzer (Applied Biosystems, Foster City, CA), and alleles were genotyped by GeneMapper V3.2 (Applied Biosystems). Genotyping and data analysis were analyzed synchronously by GeneMarker HID.

Statistics analysis

The combined paternity index (CPI) of paternity testing cases was analyzed according to the references [9, 10]. This paper strictly follows the guidelines and the International Society for Forensic Genetics (ISFG) recommendations concerning STR nomenclature and quality for publication of population data proposed [11, 12].

Results and discussion

With the establishment and development of fluorescent multiplex amplification, the number of STR genetic markers which can be amplified simultaneously is increasing. Thus, these methods have greatly improved DNA genetic information contents and have a great effect in human identification [13]. An amplification system of the GoldenEye™ 20A kit has been proved to reach international technology level and could be useful for individual identification in routine cases and for forensic science database. Therefore, we would further access the application value of 19 autosomal STR loci of the GoldenEye™ 20A kit in paternity testing in this study by comparing with other three common international commercial kits.

Five-colored fluorescent testing technology of the GoldenEye™ 20A kit could detect 20 genetic markers including Amelogenin gene and 19 STR loci simultaneously (Fig. 1), and 19 of the autosomal STR genetic markers of the GoldenEye™ 20A kit were genotyped for testing the selected 289 paternity testing cases. According to the ISFG guidelines and the Chinese national standards recommended for paternity testing [14–16], 211 trios cases had certainty of paternity (CPI >10,000), 62 duos cases showed non-exclusionary paternity (CPI >10,000), and 16 cases showed exclusionary paternity (CPI <1/1,000.). A total 392 of exclusionary paternity cases including 196 trios and 196 duos combined randomly father and non-biological child (with the mother known) in cases of non-exclusionary paternity were subjected to subsequent statistical analysis.

According the standard for certainty of paternity, 15 STR loci (power of exclusion (PE) of each locus ≥0.5714) in trios cases and 18 STR loci (PE of each locus ≥0.411) in duos cases were tested at least, and the cumulative power of exclusion (CPE) was over 0.999 9, respectively [17]; furthermore, no locus was found against Mendel's laws of inheritance between the alleged father and children. The parenthood was used for validation (for trios) or non-exclusionary (for duos) if the value of CPI exceeded 10,000 [18]. In our study, 19 autosomal STR genetic loci were simultaneously detected by the GoldenEye™ 20A amplification kit, and the number of genetic markers was above the standard of paternity testing. The CPE of 19 autosomal STR loci with the GoldenEye™ 20A kit was 0.999999996 which was significantly higher than the system performance of paternity testing analyzing technology system. Although PE of TPOX and TH01 tested in our study was below 0.411 (Table 1S), CPI could be 7.98E+16 in trios cases with certainty of paternity and 3.87E+11 in duos cases. The CPI of 19 autosomal STR loci with the GoldenEye™ 20A kit in trios and duos cases with certainty of paternity was above 10,000 (Table 1), which was consistent with the standard of certainty of paternity (CPI >10,000) [19, 20]. When there was an isolated Mendelian inconsistency with a single mutational step between parent(s)/child and the CPI was >10,000,000, a mutation might occur, and the inconsistency of the CPI was not taken into consideration [21]. Among paternity cases of trios tested, locus vWA in one case was against Mendelian genetics and showed one step mutation (one repeat gain),

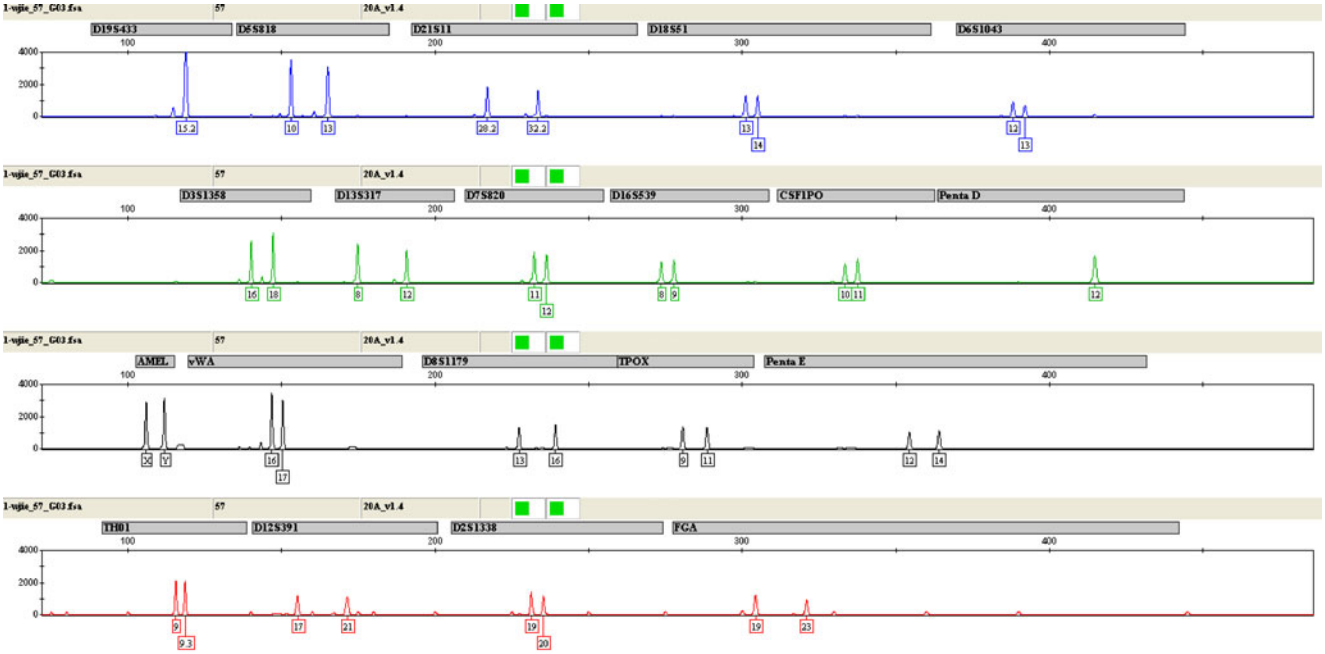

Fig. 1 Map of genotying of 19 STR loci with the GoldenEyeTM 20A kit

while the CPI was 5.39E+09 (>10,000,000) by using the GoldenEye™ 20A amplification kit. Therefore, this variation on locus vWA could be regarded as a mutation. If three common commercial kits were used separately for certainty of paternity, the CPI of 15 STR loci was less than that of 19 autosomal STR loci with the GoldenEye™ 20A kit (Table 1). In duos cases with non-exclusionary paternity, the CPI of one case by using AmpF∕STR Identifiler™ kit and PowerPlex™16 kit were 4.49E+03 and 5.67E+03, respectively, both were below 10,000. Furthermore, in the same cases, the CPI calculated separately with three other common commercial kits were lower than that of the GoldenEye™ 20A kit. Given the above, our data indicated that 19 autosomal STR loci with the GoldenEye™ 20A kit had a higher application value in paternity testing cases.

Among exclusionary paternity cases analyzed by using 19 autosomal STR loci with the GoldenEye™ 20A kit, 49~150 cases of trios and 13~126 cases of duos with only one locus excluded were observed; and the rate of exclusion was 25 % (TH01)~76.53 % (D2S1338), 6.63 % (TH01)~64.29 % (PentaE), respectively (Table 2S). Our study showed that the rate of exclusion of the 19 STR genetic loci tested in exclusionary paternity cases of trios was higher than that of duos cases. The rate of exclusion of locus TH01 and TPOX was lower in Chinese population, which might be associated with lower polymorphism and mutation rate in Chinese Han population [18]. The average number of excluded STR loci in exclusionary paternity cases of trios with the GoldenEye™ 20A kit and three other common kits were 11.3, 8.9, 9.5, and 8.8, respectively (Table 2), and the average numbers of excluded loci in the cases of duos were 8.2, 5.8, 6.8, and 6.2, respectively (Table 2). Our data in this study indicated that more numbers of STR loci were excluded in exclusionary paternity cases by using the GoldenEye™ 20A amplification kit than those of the three other common commercial kits.

Table 1 Comparison of CPI between the GoldenEye™ 20A kit and three other common kits with application in cases of certainty of paternity

Kits	CPI of trios cases	CPI of duos cases
GoldenEye™ 20A	1.59E+06~7.98E+16	8.02E+04~3.87E+11
AmpF∕STR Identifiler™	2.39E+04~7.22E+13	4.49E+03~2.19E+09
AmpF∕STR Sinofiler™	1.20E+05~2.97E+15	6.60E+04~2.70E+10
PowerPlex™16	2.62E+04~2.47E+14	5.67E+03~4.46E+09

Table 2 Comparison of average numbers of excluded STR genetic markers by the GoldenEye™ 20A kit and the other three kits in exclusionary paternity cases

Kits	Average numbers of excluded STR genetic markers	
	Trios	Duos
GoldenEye™ 20A	11.3	8.2
AmpF∕STR Identifiler™	8.9	5.8
AmpF∕STR Sinofiler™	9.5	6.8
PowerPlex™16	8.8	6.2

The greater the number of genetic markers examined, the greater the strength of the genetic evidence and, hence, the better final results and conclusions for paternity cases. According to the ISFG guidelines [14], the Chinese national standard for exclusionary paternity should test at least 15 of STR in trios cases with four or more paradoxical STR genetic loci and at least 18 of STR in duos cases with three or more paradoxical STR loci [16]; the parenthood was considered to be excluded if the value of CPI was below 1/1,000 [14]. The total numbers of excluded STR loci in trios and duos exclusionary paternity cases by using the GoldenEye™ 20A kit could reach the standard for exclusionary paternity. However, there was one case with three STR loci excluded by using AmpF∕STR Sinofiler™ and PowerPlex™16 kits, respectively, though the average numbers of STR loci excluded were above five. Our data suggested that accurate conclusion could be drawn for paternity testing with more STR genetic markers by using the GoldenEye™ 20A kit.

In summary, in the comparison of the19 autosomal STR loci of the GoldenEye™ 20A kit and three other common commercial kits, the former had higher value of CPI in certainty of paternity or non-exclusion paternity cases, and more number of STR loci was excluded in exclusionary paternity cases. Our data in this study show that the GoldenEye™ 20A kit has a higher application value in forensic paternity testing and will be of help for kinship analysis.

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Conflict of interest The authors declare that they have no conflict of interest.

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