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Evaluation of HumDNA Typing (Yanhuang-PCR) Kit for Usability and Reliability in Forensic Sciences and Comparison with Two Different PCR Kits (Identifiler Plus & GlobalFiler)

HumDNA Typing (Yanhuang-PCR) Kitinin Güvenilirliği ile Adli Bilimlerde Kullanılabilirliğinin Değerlendirilmesi ve İki Farklı PCR Kiti (Identifiler Plus & Globalfiler) ile Karşılaştırılması

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Abstract

Forensic science is a multidisciplinary field in which experts in different fields collaborate to shed light on crime. Forensic genetics, a discipline to distinguish crime commuters/innocents, has a great place within the scope of forensic sciences. Here, Short Tandem Repeat (STR) analyses are the most frequently conducted forensic genetic analyses applied in interval situations such as kinship determinations, parenting tests, comparison of evidence obtained from the crime scene and/or suspects, or identification of individuals. The number of these biomarkers varies in the content of the kits benefited in the analysis. To support the aim of the study, the usability of the HUMDNA Typing Yanhuang PCR Kit in the field of forensic sciences is evaluated. In this study, in addition to two different kits—Identifiler Plus and GlobalFiler—frequently preferred in the field, the HUMDNA Typing Yanhuang PCR Kit is analyzed, and all three types of kits are compared. In terms of discrimination power in forensic sciences, the variety of number of examined loci is vitally important. In addition to this comparison, three different size standards (LIZ500, LIZ600, and Salmon 500 included in the kit) are tested in the electrophoresis of samples amplified—using the HUMDNA Typing Yanhuang PCR Kit—and cleaner results are obtained with LIZ600. Besides, PCR mixtures at different ratios (1, 1/2.5, 1/5) are prepared and tested to demonstrate how helpful the kit works in eliminating insufficient sample deficiencies—a crucial issue in forensic sciences. To obtain bear fruits in conducted studies in every ratio, results should be achieved by minute quantity of DNA. Thereby, the HUMDNA Typing Yanhuang PCR Kit is supposed a beneficial kit for genetic studies carried out within forensic sciences. This study suggests that essential data—as well as possible population studies—are potentially added to the literature to be conducted in the following studies.

Keywords: Yanhuang PCR Kit, HumDNA Typing Kit, Identifiler Plus, GlobalFiler, Short Tandem Repeats (STRs), Forensic Genetics

Öz

Adli bilimler, bir suçun aydınlatılması için birçok farklı konuda uzmanın beraber çalıştığı multi-disipliner bir alandır. Birden fazla suç türünde suçlunun ve masum bir insanın ayırt edilmesinde yararlanılan bir disiplin olan adli genetiğin adli bilimler kapsamında yeri büyüktür. Kısa ardışık tekrar (STR) analizleri en sık yürütülen adli genetik analizlerden olup akrabalık tayinleri, ebeveynlik testleri, olay yerinden ve/veya şüphelilerden elde edilen delillerin karşılaştırılması ya da bireylerin kimliklendirilmesi gibi birçok farklı durumda kullanılmaktadır. Bu biyobelirteçlerin sayısı, analizlerde kullanılan kitlerin içeriğinde değişiklik gösterebilmektedir. Çalışmanın amacı doğrultusunda HUMDNA Typing Yanhuang PCR Kitinin adli bilimler alanında kullanılabilirliği değerlendirilmiştir. Bu çalışma kapsamında da alanda sıklıkla tercih edilen iki farklı kite (Identifiler Plus ve GlobalFiler) ek olarak HUMDNA Typing Yanhuang PCR Kiti kullanılmış olup bu üç farklı kit karşılaştırılmıştır. İncelenen lokus sayılarının farklı olması adli bilimlerde ayrım gücü açısından oldukça önemlidir. Bu karşılaştırmaya ek olarak HUMDNA Typing Yanhuang PCR Kiti kullanarak çoğaltılan örneklerin yürütmesinde üç farklı iç standart (LIZ500, LIZ600 ve kit dahilindeki Salmon500) test edilmiş ve LIZ600 ile daha temiz sonuç alındığı görülmüştür. Son olarak adli bilimlerde oldukça önemli bir konu olan örnek miktarı azlığındaki sıkıntıların giderilmesi için kitin ne kadar kullanışlı olduğunu görmek adına farklı oranlarda (1, 1/2.5, 1/5) PCR karışımları hazırlanmış ve test edilmiştir. Yürütülen analizlerde her oranda da başarılı sonuçlar elde edilmiş ve böylelikle çok az miktarda DNA ile dahi sonuç alınabildiği görülmüştür. Sonuç olarak HUMDNA Typing Yanhuang PCR Kitinin adli bilimler dahilinde yürütülen genetik çalışmalarında kullanışlı bir kit olduğu düşünülmektedir. Gelecekte yapılacak muhtemel mutasyon tespiti çalışmaları ile birlikte oldukça önemli verilerin literatüre kazandırılabileceğini düşünmekteyiz.

Anahtar Kelimeler: Yanhuang PCR Kit, HumDNA Typing Kit, Identifiler Plus, GlobalFiler, Kısa Ardışık Tekrarlar (STR'ler), Adli Genetik

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1. Introduction

Genetic science has many different research areas within itself, and one of them is forensic genetics. Many different analyses are carried out within the scope of forensic genetics, which serves justice, to solve the crime, ensuring that criminals receive the punishment they deserve, proving the innocence of those who the crime (1). Examples of biological evidence encountered at crime scenes and for which forensic genetics are carried out include blood, semen, saliva, urine, hair, bone, feces, and vaginal fluid. In forensic genetic analyses, it is very important to perform DNA isolation and quantification, polymerase chain reaction (PCR), analysis and data interpretation accurately and efficiently.

The discovery of the PCR method is of great importance in the development of short tandem repeat (STR) based DNA profiling. STRs are repetitive DNA sequences with a length of 2-6 base pairs, which make up approximately 3% of the human genome, and are biomarkers that provide high discriminatory power since the number of repeat units is highly variable between individuals (2). Some of the STR regions in the human genome are used in forensic analysis (3). The features of STR used in forensic sciences are strong amplification power, regular repeat units, high heterozygosity, and distinguishability of alleles. The frequency of STR regions used in forensic sciences may vary between countries (4,5). The high number of analyzed STR regions is very important in forensic science as it will increase the specificity in the identification of individuals.

Three different PCR kits were used in this study and they are Identifiler Plus PCR Kit, GlobalFiler PCR Kit, and HumDNA Typing YanHuang PCR Kit. The features of these kits, which have identical and different STR regions, are as follows;

AmpFlSTRTM IdentifilerTM Plus PCR Kit

The AB Identifiler Plus Kit (The AmpFlSTR Identifiler Plus Kit) has the same number of loci (16-locus multiplex) and discrimination power as the AB Identifiler kit. The precision and durability of the Plus kit have been improved compared to the previous kit (The AmpFlSTR Identifiler Kit). The PCR cycling conditions of the renewed Plus kit have been modified, thus increasing its sensitivity. In addition, a renewed buffer formulation was used to improve the performance of inhibited samples. Finally, an improved process for primers was used to obtain a cleaner electrophoretic background (6).

AmpFlSTRTM IdentifilerTM Plus PCR kit amplifies 15 tetranucleotide repeat regions and Amelogenin, a sex-determining biomarker, in a single PCR reaction. The loci amplified in this PCR kit are as follows; D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818 and FGA. The dyes used in this PCR system, which was developed with 5 fluorescent dye labels, are 6-FAMTM (blue), VICTM (green), NEDTM (yellow), and PETTM (red). LIZTM (orange) is preferred for marking the internal standard. The PCR Master Mix in the PCR kit contains enzymes, salts, deoxynucleotide triphosphates (dNTPs), carrier protein, and 0.04% sodium azide (6).

GlobalFiler PCR Kit

GlobalFilerTM PCR Kit is a PCR kit developed by Thermo Fisher Scientific. The loci included in the kit are as follows; D8S1179, D21S11, D7S820, CSF-1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818, FGA, D10S1248, D22S1045, D2S441, D1S1656, D12S391, SE33, DYS-391, Y- INDEL. The amount of DNA recommended by the kit manufacturer as input is 0.5ng-1.5ng human DNA. However, they stated that samples containing less than 0.5ng of human DNA could also be typed. The dyes used in this PCR system, developed with 6 fluorescent dye labels, are 6-FAMTM (blue), VICTM (green), NEDTM (yellow), TAZTM (red), and SIDTM (purple). LIZTM (orange) is preferred for marking the internal standard. Master Mix in the PCR kit contains MgCl2, dATP, dGTP, dCTP, dTTP, bovine serum albumin, 0.05% sodium azide, enzyme-containing buffer, and salt (7).

HUMDNA Typing Kit (Yan Huang-PCR)

HUMDNA TYPING (Yanhuang) kit, a PCR kit manufactured in China, allows the amplification

of 28 STR loci. STR loci included in the kit; Y-INDEL, AMEL, D3S1358, D13S317, D7S820, D16S539, SE33, D10S1248, D5S818, D21S11, TPOX, D1S1656, D6S1043, DXS6795, D19S433, D22S1045, D8S1179, Penta E, D2S441, DYS391, D12S391, D2S1338, vWA, Penta D, TH01, D18S51, CSF1PO and FGA. The kit to be investigated has been developed as a STR multiplex PCR system with 6 fluorescent dye labels that can be used in challenging forensic samples. It has been stated by the manufacturer that it is compatible with existing tool platforms and that this kit, which contains 28 loci, can be used in paternity testing, individual identification, and database creation. One of the important features of the HUMDNA Typing Yan Huang PCR Kit is its high compatibility with many locus standards (8).

In addition to its high compatibility feature, other advantages of the kit according to the manufacturer are as follows; a) 28 loci can be detected at once; b) It applies to different populations, including Asia, Europe, and Africa, and is compatible with universal populations; c) Provides high efficiency and accuracy by completing the entire PCR process in 90 minutes; d) It can be easily applied, allowing the amplification of DNA from various biological materials such as human blood stains, saliva, and hair follicles.

In addition, considering the alleles present in a mixture, exclusion power, which is evaluated as the probability of excluding a random person contributing to the mixture, is also an important parameter in forensic science (9).

The purpose of the study is to evaluate the usability of the HUMDNA Typing Yanhuang PCR Kit in the field of forensic sciences. Within the scope of this study, the HUMDNA Typing Yanhuang PCR Kit was used for the amplification of the samples, in addition to two different kits (Identifiler Plus and GlobalFiler) that are frequently used in the field. In addition to comparing these three different kits, two different internal standards (LIZ500 and LIZ600) were tested in addition to the Salmon 500 included in the kit (HUMDNA), in the analysis of samples amplified using the HUMDNA Typing Yanhuang PCR Kit. Finally, PCR mixtures at different ratios (1, 1/2.5, 1/5) were prepared and tested to see how useful the kit is in case of low sample quantity, which is a very important issue in forensic science.

2. Materials and Methods

Isolation of DNA using silica method

In the study, oral swap samples were taken from 40 unrelated volunteers, over 18 years of age, after signing the informed consent form (with approval from the IU-Cerrahpasa ethics committee, numbered E-86669574-302.14.06-240232 and dated 18.11.2021). DNA extraction was then completed using the QiaAmp mini kit and the samples were stored at -20°C until quantification.

Quantification of DNA by fluorometric method

Qubit® fluorometer (InvitrogenTM), was preferred for the quantification of DNA samples, and quantification was performed with Quant-iT™ dsDNA Assay Kit [High Sensitivity (HS)].

Polymerase Chain Reaction (PCR)

The samples taken from 40 volunteers who signed the Volunteer Consent Form were then grouped. For comparison, 30 samples were amplified with the HUMDNA YanHuang PCR kit in accordance with the kit protocol. Afterward, the same 30 samples were amplified with the GlobalFiler PCR kit and Identifiler Plus PCR kit in accordance with the kit protocols. The remaining 10 samples were used to test PCR mixtures prepared at different ratios.

Amplification of samples with HUMDNA YanHuang PCR kit

The amounts of each sample to be amplified with the HUMDNA Typing YanHuang PCR kit are as in Table 1. In order to measure the performance of this kit on different DNA amounts, samples were prepared separately for each dilution. The quantities of the full reaction and 1/2.5 reaction mixtures are taken from the kit guide. In addition, 1/5 reactions were also tested

Table 1 HUMDNA Typing YanHuang PCR kit components and their required quantities according to the reaction rate.

	Volume (µl) (1)	Volume (µl) (1/2.5)	Volume (µl) (1/5)
PCR Master Mix	12.5	5	2.5
Primer Set	5	2	1
DNA (0.1-2ng)	2.5	1	0.5
Nuclease-free Water	5	2	1
Final Reaction Volume	25	10	5

to see the kit's performance. Samples from 10 people were studied 3 times, with 10 samples for each dilution. For comparison with the other two kits, samples were prepared at the full reaction rate.

Appropriate amounts of PCR Master Mix and Primer Set mixtures were prepared for each sample to be analyzed at different dilutions. Subsequently, an appropriate amount of DNA (0.05-0.125ng/l) was added to each tube (2.5l for full reaction samples, 1l for 1/2.5 reaction, 0.5l for 1/5 reaction). For negative control, the same amount of low-TE buffer (10X Buffer with EDTA- ThermoFisher Scientific) was added instead of DNA. For the positive control, a sample with a known DNA profile was selected.

The final reaction volume was 25l, 10l, and 5 l for the full reaction, 1/2.5 reaction, and 1/5 reaction, respectively, and the missing liquid volume was completed with nuclease-free water. After a short centrifugation process, the tubes were placed in the PCR device (Veriti, 96 well thermal cyclers) and the analysis was started by adjusting the parameters shown in Table 2.

Table 2 PCR cycling conditions for the three PCR kits.

Step	Temperature (°C)			Time			Cycle Number		
	HumDNA	Identifiler Plus	GlobalFiler	HumDNA	Identifiler Plus	GlobalFiler	HumDNA	Identifiler Plus	GlobalFiler
Initial Incubation	95	95	95	5 minutes	11 minutes	1 minute			
Denaturation	95	94	94	10 seconds	20 seconds	10 seconds	28	28	29
Annealing	58	59	59	1 minute	3 minutes	90 seconds			
Extension	70			20 seconds					
Final Extension	60	60	60	30 minutes	10 minutes	10 minutes			
Final Hold	4	4	4	∞	Up to 24 hours	Up to 24 hours			

Amplification of samples with GlobalFiler PCR kit

Samples were prepared according to the manufacturer's instructions. Then, samples were placed in the PCR device (Veriti, 96 well thermal cyclers) and the analysis was started by adjusting the parameters shown in Table 2.

Amplification of samples with Identifiler Plus PCR Kit

Samples were prepared according to the manufacturer's instructions. Then, samples were placed in the PCR device (Veriti, 96 well thermal cyclers) and the analysis was started by adjusting the parameters shown in Table 2.

Before all samples were analyzed, LIZ 500 (GeneScan™500 LIZ®), LIZ 600 (GeneScan™600 LIZ®), and the Salmon 500 included in the kit were compared to see which internal standard could provide a better analysis interpretation. To make this comparison, three sets of internal standard samples were prepared with samples from 4 different people. The mixture contains 9.6l Hi-Di Formamide (ThermoFisher Scientific) and 0.4l Salmon 500 for the first set; 8.7l Hi-Di Formamide and 0.3l LIZ 500 for the second set; 9.6l Hi-Di Formamide and 0.4l LIZ600 for the third set. A total of 12 mixtures prepared for 4 different people were then distributed into PCR tubes, 9l each. In the next step, 1l DNA was added to the 9l mixture, and the mixtures were denatured for 3 minutes at 95°C in the PCR device (Veriti 96 well thermal cycler). After denaturation, the samples were kept on ice for 3 minutes and then loaded into the capillary electrophoresis device for analysis. As a result of the analysis, it was realized that better results were obtained with LIZ600 and it was decided to use LIZ600 in all samples.

Sample Analysis

Positive and negative control mixtures for each kit were prepared according to the kit protocol. Then, the PCR product was added to each tube to obtain a homogeneous mixture.

Finally, the sample plate was placed in the genetic analyzer (ABI 3500), appropriate parameters (Table 3) were set and analysis was performed. Electrophoresis of the samples was performed using a 36cm capillary and POP4 polymer (manufactured by ThermoFisher Scientific).

Data Analysis

After electrophoresis was performed on the ABI 3500 genetic analyzer device (8 capillaries), the imaging and typing of the raw data was completed using the GeneMapper 5 analysis program. For this purpose, new panel & bin sets were created. By adding the data obtained after a run, typing was made by overlapping the PCR products and the allelic ladder.

3. Results

Comparison of Salmon500/LIZ500/LIZ600

As a result of the analysis, it was realized that better results were obtained with LIZ600 and it was decided to use LIZ600 in the samples. Although Salmon 500 gives a higher peak value compared to LIZ 500 and 600 in terms of quality (peak heights), it has been observed that in samples in which Salmon 500 was used, peaks shifted 1-2 bases from where they should be in some STR loci. Although the correct profile can be reached in a short time by adjusting the parameters in the GeneMapper program, it has been observed that better results can be obtained without any corrections with LIZ 600.

PCR Results of Samples with Different Dilutions (HumDNA Typing Yanhuang PCR Kit)

One of the parameters evaluated within the scope of the study was to observe how different dilutions affect PCR results. For this purpose, full reaction, 1/2.5 reaction, and 1/5 reaction were studied with the HumDNA Typing Yanhuang PCR Kit to evaluate the performance of the kit, and 2.5l, 1l, and 0.5l DNA was added per sample in each reaction, respectively. As a result of PCR and electrophoresis, it was seen that successful results could be obtained in all three dilutions.

Comparison of GlobalFiler PCR Kit and HumDNA Typing Yanhuang PCR Kit

All 24 STR regions amplified within the GlobalFiler PCR

Table 3 Electrophoresis parameters.

Injection Time	15 sec
Injection Voltage	1.2 kV
Run Time	1210 sec
Run Temperature	60°C
Run Voltage	15kV
Matrix	G5
Dye Set	FGI_6dye

Kit (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818, FGA, D10S1248, D22S1045, D2S441, D1S1656, D12S391, SE33, DYS-391, Y-INDEL) could also be amplified with the HumDNA Typing Yanhuang PCR Kit. In addition, 4 STR regions (D6S1043, DXS6795, Penta E, Penta D), which can be amplified in the HumDNA Typing Yanhuang PCR Kit but not in the GlobalFiler PCR Kit, were also successfully amplified in our study.

Comparison of Identifiler Plus PCR Kit and HumDNA Typing Yanhuang PCR Kit

All 16 STR regions amplified within the Identifiler Plus PCR Kit (D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818 and FGA) could also be amplified with the HumDNA Typing Yanhuang PCR Kit. In addition, 12 STR regions (D10S1248, D22S1045, D2S441, D1S1656, D12S391, SE33, DYS391, Y-INDEL, D6S1043, DXS6795, Penta E, Penta D) that can be amplified in the HumDNA Typing Yanhuang PCR Kit but not in the Identifiler Plus PCR Kit, were also successfully amplified in our study.

4. Discussion

For the purpose of the study, the usability of the HUMDNA Typing Yanhuang PCR Kit in the field of forensic sciences was evaluated and compared with two different kits (Identifiler Plus and GlobalFiler) that are frequently preferred in forensic genetics laboratories. The STR locus numbers in these three kits are different, and the kit that allows the examination of the most regions is the HUMDNA Typing Yanhuang PCR kit. 16 STR regions can be reproduced in The AmpFlSTR Identifiler Plus Kit (6) and these regions are D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, Amelogenin, D5S818 and It is FGA. The GlobalFiler PCR kit (7) contains all the loci included in the Identifiler Plus PCR kit. In addition to these loci, 8 more STR loci (D10S1248, D22S1045, D2S441, D1S1656, D12S391, SE33, DYS-391, and Y-INDEL) can be amplified with GlobalFiler PCR kit. HumDNA Typing Yan Huang PCR kit (8) has the capacity to amplify the most STR loci among these three kits. It contains 4 more STR loci (D6S1043, DXS6795, Penta E, Penta D) than the GlobalFiler kit and 12 more STR loci than the Identifiler Plus kit. The importance of locus numbers on discriminatory power and exclusion power is considerable (9). As the number of analyzed loci increases, the power of discrimination between individuals also increases. In the data provided by the kit manufacturer, the cumulative discrimination power of Identifiler Plus, GlobalFiler, and HumDNA Typing Kit Yan Huang PCR kits are 1-6.65e-17, 1-1.96e-24, and 1-3.53e-29, respectively (8). The combined exclusion power of Identifiler Plus, GlobalFiler, HumDNA Typing Kit Yan Huang PCR kits is 0.999999, 0.999999999, and 0.99999999999, respectively (8). According to the data, it can be seen that the best kit in terms of the highest discrimination

power and exclusion power among the three kits is the HumDNA Typing Kit Yan Huang PCR kit, which contains 28 loci. In addition to these two parameters, locus numbers can also be very important in degraded samples. Some damaged STR loci due to degradation may not be amplified in PCR (10).

However, if the number of STR loci that can be amplified with PCR kits is high, the number of information that can be obtained from degraded samples may be increased. Therefore, the most advantageous kit is the HumDNA Typing Kit Yan Huang PCR kit. In situations that require very rapid analysis (for example, identification in mass disasters), it is an important advantage that the PCR kit used provides efficient results in a short time. 28, 29, and 28 cycles were preferred for the amplification of samples with Identifiler Plus, GlobalFiler, and HumDNA Typing Yan Huang PCR kit, respectively. Approximate PCR times calculated based on these cycle numbers and each kit's procedures are 115 minutes, 60 minutes, and 77 minutes for the Identifiler Plus, GlobalFiler, and HumDNA Typing Yan Huang PCR kit, respectively. When the kit procedures are examined, it is seen that the annealing and extension steps of the GlobalFiler and Identifiler Plus PCR kits occur in a single step, while the annealing and extension steps of the HumDNA Typing Yan Huang PCR kit occur at separate temperatures. Considering the greater STR region amplification capacity, discrimination power, and exclusion power, the time difference (17 minutes) between GlobalFiler and HumDNA Typing Yan Huang PCR kit is compensable. In addition to the time parameter, the high number of STR loci examined in mixture samples may be very important in distinguishing the samples. Therefore, the HumDNA Typing Yan Huang PCR kit is more advantageous compared to GlobalFiler and Identifiler Plus PCR kits in this respect. As an internal standard, the Identifiler Plus kit includes LIZ500, and The GlobalFiler kit includes LIZ600 (6, 7). Unlike these two frequently used internal standards, Salmon500 is included in the HumDNA Typing Yan Huang PCR kit. To see which internal standard could provide better results, all three internal standards were compared for the samples to be amplified with the HumDNA Typing Yan Huang PCR kit. As a result, it was seen that better peaks could be achieved with LIZ600. It is very important to choose an accurate internal standard to evaluate the raw data. This difference in peaks may be due to differences in the formulation of internal standards. Another parameter evaluated by the HumDNA Typing Yan Huang PCR kit is the effect of different PCR dilutions on the analysis of samples. The reason for evaluating this parameter is that in some cases in forensic science, the amount of sample to be analyzed may be very low. Considering the possibility that there may be more than one analysis that needs to be performed, careful and efficient use of the DNA is very important. Accordingly, in forensic science studies, it is a great advantage that a PCR kit provides results even when used in limited quantities. For this purpose, PCR mixtures at different dilutions (1, 1/2.5, 1/5) were prepared and tested. Full reaction and 1/2.5 reaction mixtures are the mixtures included in the kit protocol. In addition, a 1/5 reaction mixture was prepared and compared. In this comparison, the DNA dilution (0.1 ng/l) was kept constant, but PCR was performed by adding different volumes of DNA. Successful results were obtained in every mixture ratio and thus it was seen that results could be obtained even with a very small volume of DNA. This data is very valuable in the field of forensic sciences.

Therefore, it has been confirmed that the tested HumDNA kit is useful in overcoming the problem of small sample quantities. As the number of STR loci examined in forensic genetic studies increases, the power of discrimination between individuals and populations increases. Therefore, it is very important to conduct population studies. Only one population study that used HumDNA Typing Yan Huang PCR kit was found in the literature. In this population study conducted in Rwanda, Africa, genetic diversity in the population was evaluated by analyzing 24 autosomal STR loci out of 28 STR loci in the kit (11). In a possible population study to be conducted with this kit in the Turkish population, the polymorphism of the 28 loci included in the kit can be evaluated. In this way, information will be obtained about which individuals with STR loci can be distinguished more easily from the rest of the population. In this study, it was observed that the only disadvantage of using the HumDNA kit is that it is more difficult to contact and get support about the kit compared to other kit manufacturers. Apart from this problem, the overall performance of the kit was

good; thus, it can be used within the scope of forensic sciences. Consequently, the most important advantages of the kit are allowing the amplification of a large number of STR regions in a short time and providing efficient results even with small amounts of samples in low reaction mixture ratios.

5. Conclusion

For the purpose of the study, the usability of the HUMDNA Typing Yanhuang PCR Kit in the field of forensic sciences was evaluated. Within the scope of this study, the HUMDNA Typing Yanhuang PCR Kit was used in addition to two different kits (Identifiler Plus and GlobalFiler) that are frequently preferred in the field. The STR region numbers in these three kits are different, and the kit that allows the examination of the most regions is the HUMDNA Typing Yanhuang PCR kit. In addition, three different internal standards (Salmon500, LIZ500, and LIZ600) were tested in the amplification of samples using the HUMDNA Typing Yanhuang PCR Kit, and better results were observed with LIZ600. Another parameter evaluated in the study was the usability of the HUMDNA Yanhuang PCR kit in case the sample amount was low. For this purpose, PCR mixtures at different dilutions (1, 1/2.5, 1/5) were prepared and tested. Successful results were obtained in every dilution and thus it was seen that results could be obtained even with a very small amount of DNA. This data is very valuable because, in forensic science analyses, it is possible to work with much smaller amounts of samples compared to clinical research. Therefore, it has been confirmed that the tested kit is useful in cases where the sample amount is low. As a result, the HUMDNA Typing Yanhuang PCR Kit is thought to be a useful kit in genetic studies carried out within forensic sciences.

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