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Research Paper

Evaluation of 20 CODIS Core STR Loci For Touch DNA Analysis

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Abstract

The DNA profile is a combination of STR alleles loci as a result of template DNA amplification. The FBI has defined 20 core STR loci for human identification in forensic cases. The aim of this study was to evaluate the twenty CODIS core STR loci to be a reference in DNA touch examination. Nine donors with three shedder statuses, high, intermediate, and low, were holding the sample while tying two types of ropes, porous and non-porous. The touch sample is then sampled using two techniques on each rope: double swab and tape lift. DNA was extracted and quantified by real-time PCR technique to determine the concentration of DNA obtained from each treatment. Twenty-four STR loci of the DNA were amplified, and the fragments were analyzed by capillary electrophoresis. Two PCR cycles were chosen to determine the effect of adding cycles on the concentration of DNA that was not successfully amplified. The results of this study indicate that the quantity of touch DNA has a greater influence on the success rate of STR amplification. Each allele on 20 STR CODIS Loci failed to amplify from touch DNA concentration below 0.0625 ng/µl. It is shown that the longer the target allele at each locus, the easier it is for allelic drop-out to occur. However, the optimal STR locus length for DNA testing is under 200 nucleotides.

Keywords: *CODIS core STR loci; Forensic DNA; Human Identification; Touch DNA*

INTRODUCTION

Human identification based on DNA profiles began in 1985 when a genetic researcher named Alec Jeffreys discovered DNA regions that repeated closely together and differed in the number of repetitions in each individual. This area is called variable tandem repeats (VNTR) with the RFLP method, resulting in DNA profiling or DNA fingerprinting. The size of this VNTR is 10-100 nucleotides per repetition and can repeat up to 5 times more. Along with the development of research in the field of forensic DNA, shorter polymorphisms, namely short tandem repeats (STRs), have been discovered. STR has a shorter repeat sequence size of 1-4 nucleotides, making it more suitable for forensic samples that are in low quantity and quality (Butler, 2005).

The International Forensic DNA Association makes a consensus STR locus that can be used together to make it easier to identify people across nations. The FBI in 2013 determined these 13 loci in the CODIS system, and in 2017 the number of these loci was added to 20 core loci to increase the level of discrimination. The first thirteen loci are D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D21S11, CSF1PO, FGA, TH01, TPOX, vWA; following 7 additional loci added D1S1656, D2S441, D2S1338, D10S1248, D12S391, D19S433, D22S1045 (Butler and Willis, 2020).

This study will evaluate the use of these 20 CODIS core loci in touch DNA analysis. Touch DNA is DNA obtained from shed skin cells and other biological materials transferred from one individual to another or other objects. According to Locard's exchange theory, every contact leaves

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a trace. Therefore, touch DNA is a significant sample to identify victims and perpetrators in criminal cases. Touch DNA samples are generally small in amount and depend on several factors (Sessa et al., 2019). These factors are shedder status, substrate type, collection method, and extraction method (Alketbi, 2018).

DNA analysis by profiling the STR utilizes PCR technology whose success depends on the quality and quantity of DNA. Therefore, in this study, we will observe each locus in each DNA touch condition obtained, hoping to provide information on STR locus markers that can be used in each DNA touch condition.

LITERATURE REVIEW

Identification of humans based on DNA STR requires information from the entire genome. Therefore, STR markers are used for identification purposes. Human DNA profiles combine STR markers from different chromosomes or in the same chromosome but with long distances to avoid linkages between markers. Apart from that, in 1997, an institution in America, namely the Federal Bureau of Investigation (FBI) determined the STR markers used in the CODIS (Combined DNA Index System). There are 13 STR markers, namely CSF1PO, FGA, TH01, TPOX, vWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, and D21S11. Meanwhile, through the European Network of Forensic Science Institute (ENFSI), European countries established seven core STR markers, namely TH01, vWA, FGA, D8S1179, D18S51, D21S11 and D3S1358. Along with the increasing development of the use of DNA in individual identification, some researchers increased the polymorphism between individuals by adding several STR markers (Butler and Hill, 2012).

The forensic DNA examination steps combine three sciences: biology, technology, and genetics. The forensic sample comes from biological material, which is initially extracted. Then, its concentration is calculated and amplified using PCR. The technology here separates and detects the results of amplification, resulting in genotypes. Genetic science is useful for obtaining genotype comparisons and probability calculations from existing population data (Butler, 2010).

"Trace DNA" is DNA obtained from various sources of low concentration and quality, usually obtained without visual detection of a spot or body fluids. It includes DNA left on surfaces or clothing when talking or sweating, as well as "touch deposits," defined as materials left when touching or holding a substrate. The DNA obtained from this touch deposit is then referred to as touch DNA. Touch DNA is not always obtained from the first individual's hand but can also be obtained from the transfer of DNA from other individuals who came into contact with the first individual's hand (Burrill et al., 2019).

Research conducted by Van Oorschot showed that a person's DNA profile can be obtained from the swab of an object that has been touched by that person. This discovery makes it easier for law enforcers to identify perpetrators or victims related to the crime scene. However, greater caution is required in DNA sampling and interpretation of results. Based on this research, it is known that the concentration of DNA obtained from the hands has an effect on the DNA profile of the touch obtained(Goray and van Oorschot, 2015).

RESEARCH METHOD

Touch DNA Collection

In this study, we use two types of ropes (fabric and plastic), which is 20 cm long and 1 cm in diameter. Previously, the two types of rope were sterilized by spraying DNAZapTM PCR DNA Degradation Solution according to the protocol, then exposed to UV light in LAF for 30 minutes to remove the DNA attached to the rope.

Nine donors were asked to wash their hands with soap and dry them with sterile tissue. For 15 minutes, donors are asked to do other activities but are advised not to make contact/touch with

other individuals, eat with their hands, or wear gloves. For 15 minutes, researchers watched the donor's movements to control their activities. After that, each donor was asked to hold the rope with their two hands, each type of substrate done on a different day, for 5 minutes while tying/knotting. Each type on a substrate was held two times (for tapelift and double swab sampling). Each donor held and tied the rope three times in each kind of substrate to make a total of 108 samples.

Touch DNA Sampling

Touch DNA was retrieved on objects using two techniques, namely the tapelift technique and the double swab technique. The tapelift technique uses 3M Scotch® adhesive tape. The adhesive portion of the tape is affixed to the part of the rope that has been touched by the donor, especially in the knot area and in between. The double swab technique is carried out by wetting the Nylon Swab (4N6 FlOQSwabs® Crime Scene) using Nuclease Free Water, then applying pressure to the rope that has been held by the donor, followed by wiping the dry nylon swab on the same part as the first swab.

DNA Analysis

DNA extraction in all samples was performed using the PrepFiler™ BTA Forensic DNA Extraction kit according to the protocol. All extracted DNA concentrations were measured in real-time quantitative PCR using special human primers (Quantifiler™ Trio DNA Quantification kit) following the product manual. STR locus analysis was performed with the GlobalFiler™ Amplification kit with a reaction volume of 25 µl, 29 cycles. For samples that cannot be fully amplified, the LCN method was used with the addition of 5 cycles and 34 cycles on PCR. The amplification product fragments were separated and detected on an ABI 3500 Genetic Analyzer (Life Technologies) with 1 µl of PCR product added to a mixture of 8.7 µl HI-Di™ formamide and 0.3 GeneScan™ 600 LIZ® Size Standard v2.0 (Thermo Fisher Scientific). The comparison/positive control DNA profile is a buccal swab from each donor (reference sample). In contrast, the negative controls were swabs and tapelifts, both types of rope, after being sterilized and without being touched by the donor.

Data Analysis

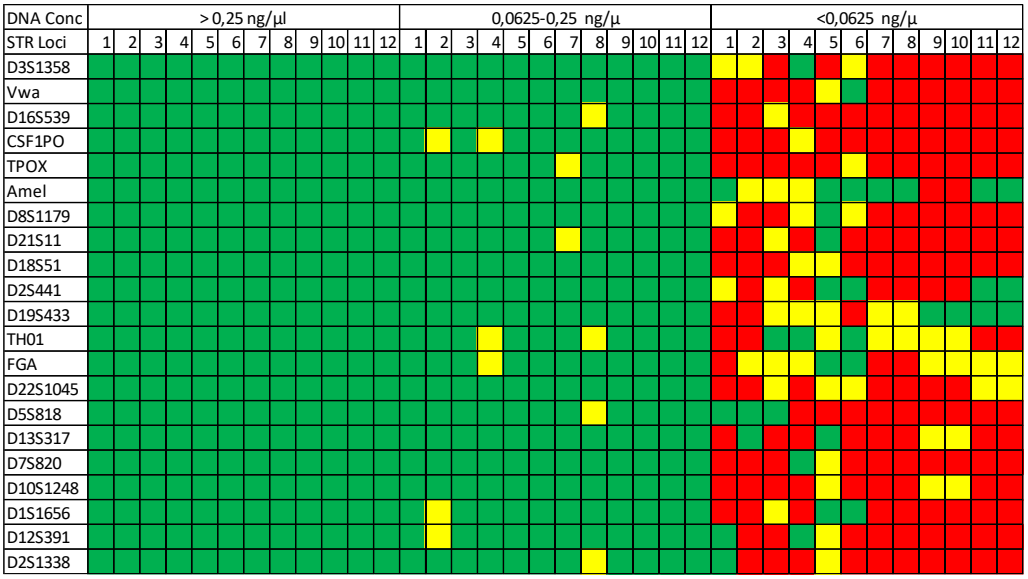
The data obtained was in the form of successful allele amplification at each of the 20 CODIS STR loci examined. If successfully amplified, each locus will have one pair of alleles (homozygous or heterozygous). Then the data were compared in each range of DNA concentrations of the touch DNA samples and analyzed descriptively.

Ethical Clearance

This research has passed the ethical clearance issued by the Faculty of Dental Medicine Health Research Ethical Clearance at Airlangga University. Certificate Number: 191/HRECC.FODM/II/2023.

FINDINGS AND DISCUSSION

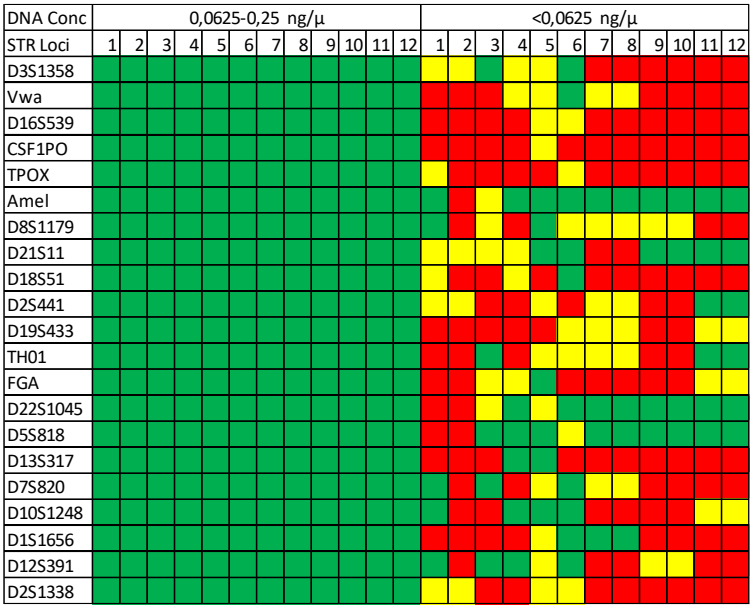
The twenty STR loci and Amelogenin markers were amplified in each DNA sample obtained. The results of the amplification can be seen in Figure 1.



Green block: Alleles at the locus are perfectly amplified. Yellow block: one of the alleles at the locus is not amplified. Red block: Both alleles at the locus are not amplified.

Figure 1. The Summarize of Allele Amplification on each 20 STR CODIS Loci.

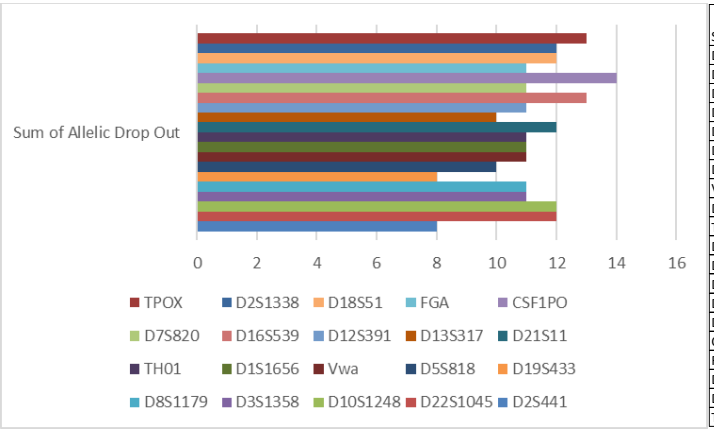
The LCN method, with the addition of amplification cycles, was carried out on DNA samples that were not completely amplified previously. The results of the LCN method can be seen in Figure 2.



Green block: Alleles at the locus are perfectly amplified. Yellow block: One of the alleles at the locus is not amplified. Red block: Both alleles at the locus are not amplified.

Figure 2. The Summarize of Allele Amplification on each 20 STR CODIS Loci with the addition of 5 cycles (LCN Method).

Failure to amplify alleles at each locus known as allelic drop out. Figure 3 shows the average number of allelic drop outs at each locus.



STR Loci	Average length (nucleotide)
D2S441	95,105
D22S1045	104,595
D10S1248	107,475
D3S1358	118,89
D8S1179	142,815
D19S433	145,085
D5S818	160,875
Vwa	182,975
D1S1656	183,655
TH01	198,675
D21S11	211,26
D13S317	221,135
D12S391	242,475
D16S539	247,53
D7S820	280,49
CSF1PO	300,72
FGA	300,995
D18S51	301,705
D2S1338	315,69
TPOX	358,275

Figure 3. The average number of allelic drop outs at each locus and the average size of the target length at each locus.

The DNA profile is a combination of a pair of alleles at each locus examined. Forensic DNA examination with the aim of obtaining individual DNA profiles uses the PCR method to reveal alleles at each locus. The PCR method's main requirements are the template DNA's concentration and quality (Butler, 2010). In this study, it can be seen in Figure 1 that the DNA concentration above 0.25 ng/µl results in a full DNA profile. Meanwhile, in the DNA concentration range of 0.0625-0.25 ng/µl, DNA profiles varied from partial to full profile. However, in the DNA concentration below 0.0625 ng/µl, no DNA profile was produced; in other words, all alleles were not amplified successfully. According to Ludeman, the optimal DNA concentration limit to produce a perfect DNA profile is 0.125 ng/µl, whereas at a concentration of 0.0125 ng/µl only three alleles can be amplified (Ludeman et al., 2018).

The LCN method was carried out at DNA concentrations below 0.1 ng/µl. Adding PCR cycles to 34 cycles is part of the LCN method (Butler, 2005). In this study (See Figure 2), the concentration of touch DNA in the range of 0.0625-0.25 ng/µl succeeded in obtaining a perfect DNA profile after an additional cycle in PCR. Meanwhile, at concentrations of touch DNA below 0.0625 ng/µl, a perfect DNA profile not obtained. From this study, we know that the touch DNA concentration limit for the LCN method is 0.0625 ng/µl.

The longer the target allele at each locus, the easier it is for allelic drop out to occur (figure 3). According to Tvedebrink, the possibility of allelic drop out in the sample will increase as the concentration of DNA decreases. It could be because the smaller the cell as the source of DNA, the easier it is for DNA to be damaged, which can affect the STR sequence (Tvedebrink et al., 2013). CSF1PO loci (300,72 nucleotide) and TPOX loci (358,75 nucleotide) showed the most allelic drop out, while D2S441 loci (95,105 nucleotides) and D19S433 showed the lowest allelic drop out (145,085 nucleotide).

CONCLUSIONS

The quantity of touch DNA has a greater influence on the success rate of STR amplification. Each allele on 20 STR CODIS Loci failed to amplify from touch DNA concentration below 0.0625 ng/µl. The longer the target allele at each locus, the easier it is for allelic drop out to occur. The optimal STR locus length for DNA testing is under 200 nucleotides.

LIMITATION & FURTHER RESEARCH

This study can be a reference to other future research to ensure the concentration of touch DNA samples to get good and valid results. For future studies, the DNA touch sample can be separated into some kind of touch material.

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