ORIGINAL ARTICLE

Amplification of vWA, FGA, and TH01 loci of DNA Samples Isolated From Ring Stored at Room Temperature

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ABSTRACT

Introduction: Acts of criminal behaviour that occur have various modes and motives. Also, criminals always try to hide or eliminate evidence at the crime scene. In most cases, police or forensic experts often find DNA on items at the crime scene. One of these items is a ring, which is an item that humans often wear. **Methods:** This study used 24 samples of rings that had been worn for 8 hours and were incubated at room temperature. All these 24 samples then were distinguished into 4 groups, in which each group was consisting of 6 samples and incubated for 0, 1, 3, and 7-days. DNA identification was then carried out using UV spectrometer for DNA quantification and DNAzol method for DNA extraction. **Results:** The mean result of DNA quantification on day 0 (control) was 1020,833 ± 0.28903 ng/µL, day 1 was 546 ± 0.093569 ng/µL, day 3 was 1066.333 ± 0.117372 ng/µL, and day 7 was 1054.083 ± 0.070733 ng/µL. PCR process used STR primers with loci vWA, FGA, and TH01 and visualization used the silver nitrate method. **Conclusion:** The final results showed that all samples could be amplified using 3 STR loci, namely vWA, FGA, and TH01.
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Keywords: vWA, FGA, TH01, DNA, ring

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INTRODUCTION

A cell is the smallest unit of living organisms, which is the basis of the constituent parts of the body. There are different proteins needed to keep the cellular system operating. Most of human beings consist of about trillion cells, 100 trillion cells to be exact, in which all of these cells originate from sole cell (zygote) formed by the fusion of a sperm and an ovum. Each of the cells is also containing the same genetic programming. There is an important chemical substance called DNA (Deoxyribonucleic Acid) inside the nucleus of our cell that is also containing coded information to replicate the cells and build proteins they need. The position of DNA in cell nucleus is called as nuclear DNA. Whereas, some of the DNA that is located in mitochondria is called as mitochondrial DNA, which is a cellular powerhouse. DNA examination methods in the medical world have various functions, one of which is human identification in forensic cases (1,2).

STR (Short Tandem Repeat) is a short synthetic nucleotide used in human identification, which is located in a non-coding region in the chromosome. These microsatellites DNA make up about 3% from the total of the human genome. More than about one million polymorphic microsatellite loci have been found ad characterized in human's DNA (3). The STR markers are distributed through the genome and also occur on the average of every 10,000 nucleotides, but not all the STR loci show inter-individual variability (4). The history of world forensic experts and the Federal Bureau of Investigation (FBI) determined the 13 of STR loci examined in human identification called STR-CODIS. The loci are TP0X, TH01, vWA, CSF1PO, FGA, D13S317, D16S539, D3S1358, D18S51, D5S818, D8S1179, D21S11 and D7S820 (5).

At a scene of a case, it is possible for the perpetrator

or victim to leave DNA traces on items that have the potential to be worn by them. The attached DNA can be used to identify who wore the items. The condition of the DNA obtained also affects the results of the examination. A study that conducted by Hanssen et al. showed that degree of degradation in samples of sperm and blood spots had an effect on the interpretation of the results of PCR (Polymerase Chain Reaction) and Sequencing (6). Another study by Yudianto et al. by using a swab examination on earphones detected using MtDNA Hypervariable Region II showed a decrease from day 1 to day 20 (7). There are so many items that have the potential to contain human DNA, such as the miswak, which is often used to clean teeth in addition to using a toothbrush. The DNA attached to the miswak has a good quantity when compared to a toothbrush (8). Therefore, studies on the quantity of DNA contained in various media (items) should be developed. Ring is among commonly worn daily accessories which potentially retain DNA traces of its user (9). Duration and how the ring is being worn may affect the yield of transferred and retained DNA (10). This study aimed to determine the quantity of DNA on ring.

MATERIALS AND METHODS

This study has been deemed ethically by the Health Research Ethical Clearance Commission of the Faculty of Dental Medicine, Universitas Airlangga under the number 337/HRECC.FODM/VII/2020. The samples were taken from the swabs on rings worn by six volunteers for eight hours. The number of samples was 24 rings which were divided into four groups with six samples in each group. Group I to IV were left at room temperature for 0, 1, 3, and 7 days, respectively. All samples were extracted using the DNAzol method (11–14). The extracted DNA was calculated in quantity using a UV spectrophotometer (15).

The results of the DNA quantification were then selected two samples from each group that has the highest and lowest quantification for amplification. Higher DNA yield is more suitable for analysis using polymerase chain reaction (PCR), while low DNA yield is often not recommended for PCR analysis due to the possibility of failed or unbalanced amplification (16,17). Thus, we selected samples with high and low DNA yield to determine whether PCR amplification would be possible to be carried out. The next step was DNA amplification using the Bio-Rad T100TM PCR machine and 3 STR primers, namely the loci of vWA with the arrangement F: 5′-CCTAGTGGATGATAAGAATAATCAGTATG-3′ and R: 5′-GGACAGATGATAAATACATAGGATGGATGG-3′, FGA with arrangement F: 5′-GCCCCATAGGTTTTGAACTCA-3′ and R: 5′-TGATTTGTCTGTAATTGCCAGC-3′, TH01 with arrangement F: 5′-CTGGGCACGTGAGGGCAGCGTCT-3′ and R: 5′-TGCCGGAAGTCCATCCTCACAGTC-3′ (18–20). The PCR results were visualized using 2% acrylamide gel and then stained using silver staining (21–23). The primary targets were 123-171 bp for vWA, 322-444 bp for FGA, and 156-195 bp for TH01.

RESULTS

Observations made on 24 ring samples showed results as shown in Table I, with varying levels of DNA. With these results the samples could still be continued for the PCR process. Table II shows the results of DNA purity with the length of storage time at room temperature. From all samples, two samples with the lowest and highest DNA purity were selected to represent each group to proceed to the PCR process. Samples E and M represent day 0, samples D and M represent day one, samples AA and Y represent day three, and samples N and E represent day seven.

Table I: Average DNA content

Treatment	Average DNA content (ng/μL) ± SD
Control	1020.833 ± 0.28903
Day 1	546 ± 0093569
Day 3	1066,333 ± 0.117372
Day 7	1054.083 ± 0.070733

Table II: Results of DNA purity of samples

Sample	DNA purity day 0	DNA purity day 1	DNA purity day 3	DNA purity day 7
N	1.12	1.40	1.29	1.29
E	1.02	1.30	1.25	1.37
M	1.17	1.80	1.30	1.30
AA	1.08	1.37	1.22	1.31
D	1.11	1.31	1.32	1.30
Y	1.09	1.36	1.56	1.32

In the visualization stage using acrylamide gel, shown in Fig. 1, the samples from day 0 to day 7 have been positively confirmed or amplified using the vWA locus with locations between (123-171 bp). In Fig. 2 using the FGA locus, all samples have been positively confirmed or amplified as well. Furthermore, in Fig. 3 using the TH01 locus, all samples were also successfully amplified.

DISCUSSION

The results of this study have showed that the average levels and also the purity varied. The levels and also the purity of DNA is influenced by several factors causing DNA to be degraded. One of these factors is endogenous, which is a factor derived from the cell. Cell damage begins with the process of autolysis and decay which is then followed by aerobic decomposition. Another factor is exogenous, which is the influence of the environment such as temperature and humidity. A study showed that samples stored at minus 20oC had higher levels and purity compared to samples stored at 4oC (24). The results of other studies have shown that there

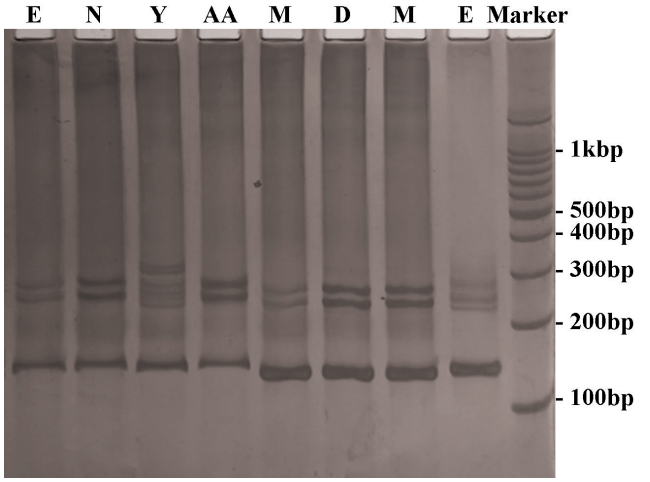

Figure 1: Results of visualization of ring DNA samples using the vWA locus (123-171 bp)

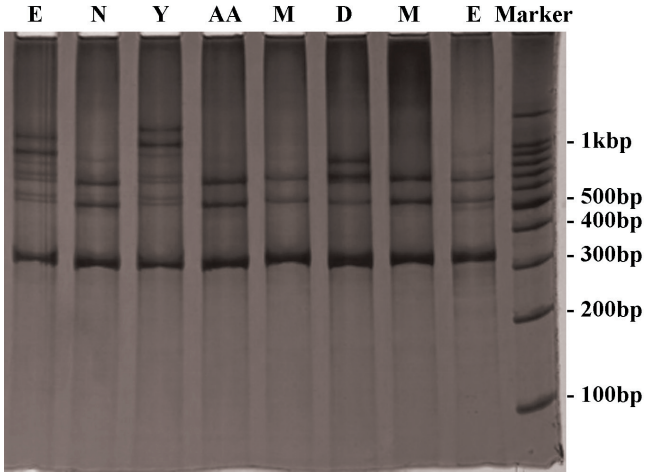

Figure 2: Results of visualization of ring DNA samples using the FGA locus (322-444 bp)

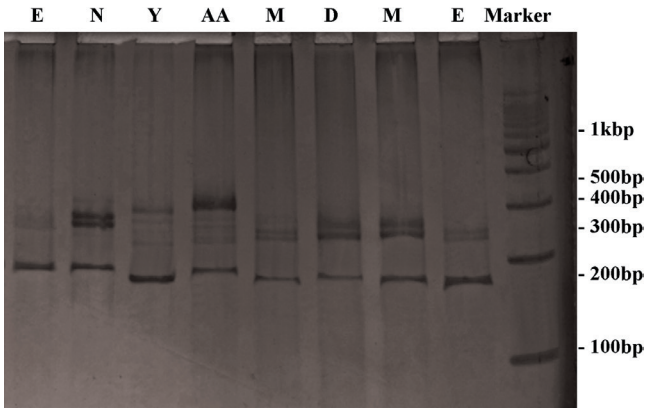

Figure 3: Results of visualization of ring DNA samples using the TH01 locus (156-195 bp)

are many factors that can influence DNA degradation such as chromatin structure, transcriptional activity and cellular location. So, DNA analysis is suggested to not only examine nuclear DNA but also to identify using mitochondrial DNA (25).

DNA degradation is also affected by hydrolytic attack on the base bonds of glycosidic sugars which can lead to base sites by depurination (26). The same mechanism can be followed by single and double-strand cleavage in an alkaline environment or by DNA-DNA cross-linking and DNA-protein in an acidic environment. Exposing the sample to UV light also induces DNA-DNA cross-linking which causes DNA to be damaged (27). Oxidation largely leads to the conversion of pyrimidines to hydantoins. Base sites, DNA fragmentation, cross-linking, and oxidative transformations mentioned above are types of damage that inhibit the PCR process (28). With DNA degradation, identification using STR markers is not optimal (6).

The amplification in this study used the STR marker because this marker is a recommendation from world forensic experts and the FBI used for human identification. This study used the vWA, FGA, and TH01 loci. vWA is included into a tetranucleotide compound that found in the intron 40 of Von Willebrand Factor gene on the short arm of the chromosome 12. This locus has TCTA repeats that interspersed with TCTG repeats. The STR multiplex kit is the only one of the three repeaters that present in the Von Willebrand Factor gene region that targeted the vWA marker. vWA has also been known in the literature as vWA and VWF. While others have not been found to be polymorphic (29). FGA is included into a repeating tetranucleotide compound that found in the third intron of the Human Alpha Fibrinogen loci on the long arm of chromosome four. The loci contain of CTTT repeats that flanked on the both sides by degenerating repeats. The size of the allele spreads for FGA was greater than for all STR core loci. Reported allele sizes range from spanning over 35 repeats, 12.2 repeats to 51.2 repeats, 2 bp of deletion from the CT loss in the region just before the core repeat motif. FGA has also been known in the literature as FIBRA or HUMFIBRA (30). TH01 is defined as a simple tetranucleotide repeat that found in intron 1 of the Tyrosine Hydroxylase gene on the short arm of chromosome 11. The locus name comes from the initials for tyrosine hydroxylase and intron one (e.g., 01). In Locus naming, sometimes in writing of TH01 is "THO1" using the letter "O" instead of "zero". TH01 has got a simple tetranucleotide concatenation with a TCAT repeat motive on the upper strand concatenation in the GenBank reference. In the literature, TH01 has also been known as and HUMTH01 and TC11 (31).

These three loci have very high discrimination in testing human DNA samples. A study by Kido showed that there is no deviation from Hardy-Weinberg equilibrium that observed in Asian, namely, Japanese, Bangladeshi and Indonesian populations for the vWA, TH01, TPOX, and F13A01 loci. The combined power of discrimination (PD) for the four loci was 0.99991 in Bangladesh, 0.9998 in Indonesia and 0.9995 in Japan (32). Other studies also showed that vWA, FGA, and TH01 loci are very effective in the Taiwanese population by obtaining very specific allele frequency results (33). The three loci was also used to calculate allele frequencies in Java and Madura (34). Genetic variation was also investigated in a population of 210 individuals in Thailand using fifteen short tandem repeats (STR) loci—D21S11, D18S51, D2S1338, D7S820, D13S317, D16S539, D19S433, D8S1179, CSF1PO, D3S1358, vWA, TPOX, TH01, and FGA5S818 (35).

CONCLUSION

From the results and discussion of the study above, it can be concluded that the storage of ring samples at room temperature for the duration of 0, 1, 3, and 7 days causes DNA degradation to occur in terms of the average levels, and results of DNA purity. The DNA amplification process using the vWA, FGA, and TH01 loci showed positive results on the target DNA fragments indicated by the presence of bands that appeared on the acrylamide gel.

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