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Amplification of D18S51, D8S1179, and FES loci of DNA Samples Isolated from Guitar String Stored at Room Temperature

***Abdul Hadi Furqoni*[1,2], *Anindita Riesti Retno Arimurti*[3], *Meri Meri*[4], *Ni Luh Ayu Megasari*[5,6*], *Indah Nuraini Masjkur*[6], *Qurrota A'yunil Huda*[6], *Intan Sari Nuraini*[7], *Luluk Hermawati*[2]**

[1] Center for Biomedical Research, Research Organization for Health, National Research and Innovation Agency (BRIN), Cibinong Science Center, Cibinong - Bogor, West Java, Indonesia.
[2] Faculty of Medicine, Universitas Islam Negeri Syarif HIdayatullah Jakarta, Banten, Indonesia.
[3] Diploma Medical Laboratory Technology, Faculty of Health Science, Universitas Muhammadiyah Surabaya, Surabaya, Indonesia.
[4] Universitas Bakti Tunas Husada, Tasikmalaya, West Java, Indonesia.
[5] Postgraduate School, Universitas Airlangga, Surabaya, Indonesia.
[6] Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.
[7] Student of Forensic Science Master Program, Postgraduate School, Universitas Airlangga, Surabaya, Indonesia.
*Corresponding Author

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Abstract

The act of criminality is an unlawful behavior that constitutes a criminal offense. Criminals use various ways to hide evidence at the crime scene. Forensic experts often find DNA on items found at the crime scene, one of the types of evidence is a guitar. Guitar is one of the musical instruments often played by humans that can be used as a comparison. Methods: This study used 18 guitar string samples that had been used for five minutes and incubated at room temperature. The 18 samples were then divided into three groups, in which each group consisted of six samples and incubated for one, five, and 10 days. DNA identification was then carried out using a UV spectrometer for DNA quantification and the DNAzol method for DNA extraction. Results: The mean result of DNA quantification on day 1 was 152.57 ± 48.02 ng/µL, day 5 was 138.66 ± 47.66 ng/µL, and day 10 was 87.09 ± 9.07 ng/µL. Polymerase chain reaction was carried out using three STR primers loci, namely D18S51 (290 - 366 bp), D8S1179 (203 - 224 bp), and FES (222 - 250 bp), followed by visualization using the silver nitrate method. The final results showed that all samples could be amplified using the D18S51, D8S1179, and FES STR loci.

Keywords: D18S51, D8S1179, FES, STR Loci, Guitar String, Crime

Introduction

Forensic identification is a step that is carried out to assist investigators in identifying individuals who are victims in criminal cases that have caused death, because this is an attempt to respect human rights. Therefore, it can be interpreted that the identification process is a very crucial stage that must be carried out before proceeding to the next stage, namely the examination of the human remains (autopsy)[1].

Personal identification is very essential in the investigation of both criminal and civil cases because any mistake can be fatal in the judicial process. Identification can be done in three ways: visual

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(relatives or acquaintances seeing the human remains); in detailed (e.g., ante-mortem data that matched with the information that was collected during the autopsy and other situational information); and scientific or objective (e.g., the examination of teeth, fingerprints, or deoxyribonucleic acid (DNA))[2].

DNA is the smallest genetic structure present in all forms of life, from microorganisms to higher-level organisms such as humans, animals and plants. Each type of tissue has a different level of DNA concentration, which depends on the structure and composition of its cells. Usually, tissues that have many nucleus cells and few connective tissues tend to have high DNA concentration. Therefore the selection of DNA samples for forensic analysis is very diverse[3].

The method used in forensic DNA analysis is to examine the region of DNA repetition that have a base sequence of less than 1 kb. These regions are known as microsatellites or also called Short Tandem Repeats (STR). Short Tandem Repeats are microsatellite sequences that have repetition units between 2 and 7 base pairs in length. STR analysis is a molecular biology technique commonly used to compare the number of allele repetition at a particular locus in DNA between two or more samples. To determine the STR length, the Polymerase Chain Reaction (PCR) method is used in which is based on the length of the PCR product. Short Tandem Repeats, as the most commonly used genetic marker, is distributed widely in the human genome, and has been widely used in DNA profiling, especially in terms of individual identification and paternity testing for decades[4].

DNA identification is an attempt to compare the DNA profile of evidence with a comparison sample, with the aim of determining whether or not the DNA profile of the evidence matches with the comparison sample. The DNA identification process relies on specific inheritance patterns found in nuclear DNA and mitochondrial[5].

In DNA identification involving somatic chromosomes, the commonly used comparators are the parents (father and mother). While in DNA identification involving the Y chromosome, the comparator is usually the biological father, paternal grandfather, or paternal brother and so forth. In the examination of mitochondrial DNA, the comparator used is a family member in one lineage derived from the mother[6].

The most commonly used DNA profiling technique is Short Tandem Repeat (STR) because of its convenience, requiring only a minimum of 13 loci as reference. The FBI recommends the following thirteen loci, mainly known as Combined DNA Index System (CODIS) Core STR loci, as references: TH01, TPOX, CSF1P0, vWA, FGA, D3S1358, D5S818, D7S820, D13S317, D16S539, D8S1179, D18S51, D21S11, and added with amelogenin markers that are useful for analyzing gender[7].

Touch DNA is defined as the epithelial cells left on an object held or used by the suspect[8–11]. Touch DNA can be used for profiling analysis, and can be obtained from biological samples. Several studies have demonstrated successful DNA profiling using blood, buccal swab, hair, semen, saliva, teeth and bones samples [11–13]. In addition to biological samples, DNA can also be isolated from items found at the crime scene, such as clothes and gloves[14], masks[15], and rings[8]. Other common items, such as household objects might also provide samples for DNA profiling[16]. Musical instruments are among popularly bought and owned household items. Aside of violin and piano, guitar was reported as one of the most purchased musical instruments[17]. However, no previous study describing profiling analysis using DNA isolated from musical instrument, including guitar. As an instrument commonly owned and played, valuable touch DNA sample might be obtained from guitar.

Indonesian Supreme Court has previously reported guitar as one of the crime pieces of evidence[18]. However, examination of DNA swabs derived from guitar (guitar strings) using the STR method has not been carried out in Indonesia. This study aims to analyze DNA derived from guitar string swabs using the STR method as treated with different lengths of storage time at room temperature. The loci used in the analysis of this study include loci D18S51 D8S1179, FES. The three loci were used because they have high discrimination in the Indonesian population[19].

Materials and Methods

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This research has passed the ethical test with number 337/HRECC.FODM/VII/2020. Samples were taken from guitar strings played by three volunteers for five minutes. The total number of samples were 18 guitar strings, which were divided into three groups consisted of six strings each. Group I consisted of the first and second strings, group II of the third and fourth strings, and group III of the fifth and sixth string (Fig 1). Groups I to III were then left at room temperature for one, five, and 10 days, respectively. Room temperature was maintained around 20^{o}C[20]. using air conditioner, due to higher and fluctuating ambient temperature in Indonesia. Wet swabs samples were then collected from each strings, followed by DNA extraction using the DNAzol method[21]. The extracted DNA was quantified using a UV-visible spectrophotometer Evolution One (Thermo Fisher Scientific, United States of America)[22].

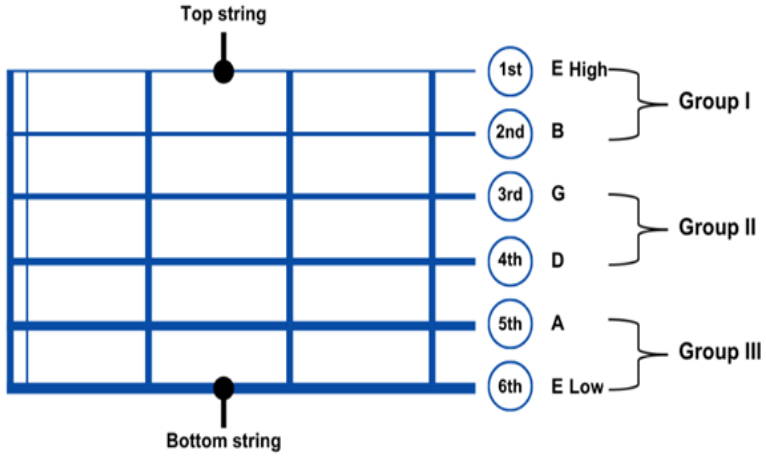

Figure 1. Illustration of the guitar strings position

After DNA quantification, two samples from each group with the highest and lowest concentration and purity were chosen for DNA amplification using polymerase chain reaction (PCR). Higher DNA yields and purity generally more suitable for PCR analysis, while low DNA yields and purity might cause amplification failure. Therefore, we selected samples with high and low DNA concentration and purity to determine whether PCR amplification could be performed in different sample condition[23]. The PCR reactions were carried out using GoTaq® Green Master Mix (Promega, United States of America) in T100™ Thermal Cycler (Bio-Rad Laboratories, United States of America). Primers used in the PCR reactions were from Promega (United States of America), and described in Table 1. PCR products were visualized using a 2% acrylamide gel run on 100V for 45 minutes, and then colored with silver staining[24].

Table 1. PCR primers[25]

Primer	Sequence	Amplicon length (base pairs (bp))
D18S51 loci	Forward Primer: 5'-AGCTTATGCAAGGACTGGAC-3' Reverse Primer: 5'-TCTTCCAGAAAGCAGGGTGT-3'	290-366
D8S1179 loci	Forward Primer: 5'-TCTGGGGCAGTAGGGAACAG-3' Reverse Primer: 5'-GGGGGCTGACTAGCAAGAGA-3'	203-224
FES loci	Forward Primer: 5'-T7-GCTTGTTAATTCATGTAGGGAAGGC-3' Reverse Primer: 5'-GTAGTCCCAGCTACTTGGCTACTC-3'	222-250

Results and Discussion

Our results showed that samples collected from group I (stored for one day) has the highest average DNA concentration and purity, and group III (stored for 10 days) has the lowest average DNA concentration and purity (Table 2 and Table 3).

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Table 2. DNA concentration of samples from each group

Treatment Group	Average DNA Concentration (ng/µl) ± SD
Group I	152.57 ± 48,02
Group II	138.66 ± 47,66
Group III	87.09 ± 9,07

Table 3. DNA purity of samples from each group

Treatment Group	Average DNA Purity (ng/µl) ± SD
Group I	1.58 ± 0.11
Group II	1.42 ± 0.12
Group III	1.21 ± 0.08

In the visualization process using an acrylamide gel, seen in Figure 1, it is evident that all selected samples from all groups have successfully shown positive amplification of locus D18S51, despite having different DNA concentration and purity. Likewise, in Figs. 2 and 3, using the D8S1179 and FES loci, all selected samples have also successfully amplified.

This study showed variations in the average DNA concentration and purity of samples collected from guitar string stored at room temperature. The concentration and purity of DNA are affected by several factors that contribute to DNA degradation. One of these factors is endogenous factors, which originate within the cell itself. The process of cell damage begins with autolysis and then undergoes decay followed by aerobic decomposition. The other is exogenous factors, which include environmental influences such as temperature and humidity[26–28]. Abdel Hady et al.[29] reported that samples stored at minus 20°C have higher levels and purity compared to samples stored at 4°C. Other studies have also shown that there are many factors that can affect DNA degradation, such as chromatin structure, transcriptional activity and cellular location. Therefore, it is recommended to perform DNA analysis not only by examining nuclear DNA but also by identifying using mitochondrial DNA[30].

Methods used for DNA extraction can also affect the quality of DNA obtained. The DNAZol methods is suitable for the isolation wide range of DNA molecules, including genomic DNA and DNA fragments down to 0.1 kb in length which are mainly targeted in DNA profiling. Isolated DNA is ready for molecular biology applications, without needing additional purification. Samples stored in DNAzol also shown stable DNA concentration and purity, despite being stored up to six months in room temperature[31].

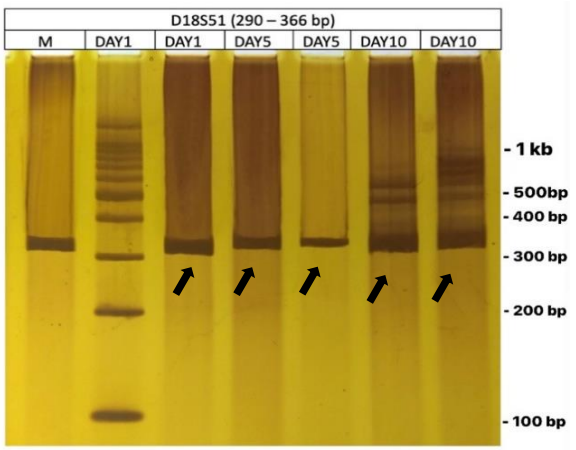

Figure 1. Visualization results of guitar string DNA using locus D18S51 (290-336 bp)

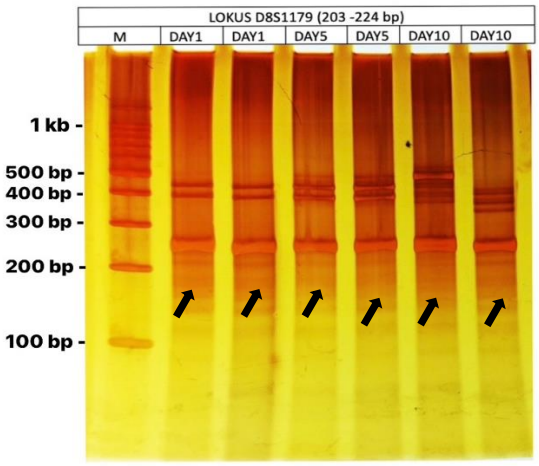

Figure 2. Visualization results of guitar string DNA using locus D8S1179 (203-224 bp)

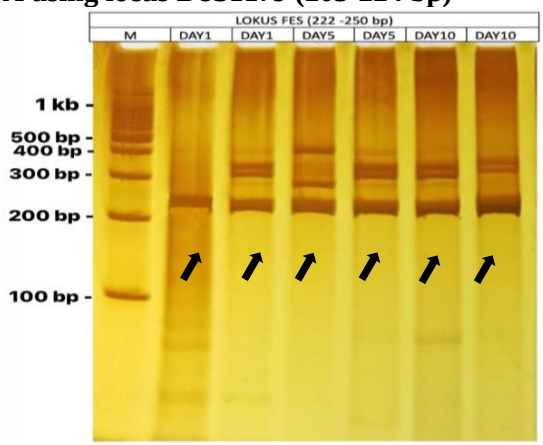

Figure 3. Visualization results of guitar string DNA using locus FES (222-250 bp)

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The degradation of DNA due to long storage periods can be caused by a number of factors, Hydrolysis is one of the factors of DNA degradation where hydrolytic reactions occur in the glycosidic sugar bonds of DNA which can lead to depurination, which is the loss of one nitrogen base from the DNA sequence over time[32]. Environmental influences also affect the process of DNA degradation where environmental factors such as temperature, humidity, and pH can affect DNA stability. For example, high temperatures or acidic or alkaline environmental conditions can accelerate DNA degradation. Exposure to ultraviolet (UV) light can damage the structure of DNA and cause cross-linking between adjacent DNA molecules. Oxidation also converts DNA bases, especially pyrimidines, into different forms such as hydantoin, which can disrupt DNA stability. All these factors can contribute to DNA degradation over time, and such damage can hinder DNA analysis such as PCR and STR analysis. Therefore, it is necessary to consider these factors in the storage and analysis of DNA samples[33].

Locus D18S51 is one of the loci in the chromosome 18. The locus has a specific DNA sequence which is useful in forensic DNA analysis, and often used for STR analysis to produce an individual DNA profile. This profile is useful for uniquely identifying individuals, as the length and sequence of DNA at locus D18S51 can vary between individuals, thus being used to distinguish one individual from another[34].

Locus D8S1179 is a specific locus on the human chromosome 8 used in forensic DNA analysis. In the context of forensic analysis, locus D8S1179 is one of the STR loci that is often tested to generate a DNA profile of an individual. STRs are sections of DNA where short sequences of multiple base pairs repeat in a sequence, and these lengths and sequences can vary between individuals. Therefore, locus D8S1179 is used to distinguish individuals in forensic identification by examining variations in the length and sequence of STRs at this locus[35]. The FES locus is a specific location on the genome that refers to a human gene known as "FES" or "Feline Sarcoma Oncogene". The locus is located in the chromosome 15. This gene is involved in the regulation of cell growth and development, and has been linked to various aspects of biology such as cancer and cell proliferation. In addition, certain loci are also used in forensic DNA analysis. In that context, the FES locus is one of the loci or sites tested to generate individual DNA profiles for forensic analysis[36].

These three loci have a high level of discrimination in personal identification using DNA. According to the study conducted by Arimurti et al.[37] showed that locus D18S51 and locus D8S1179 can be used for DNA identification of earphone swabs by STR method. According to the study conducted by Yudianto et al.[38] mentioned that the locus FES can be used to identify allele sharing patterns between siblings in the Madura tribe that are inherited by their parents.

Conclusion

From the results and discussion of the study above, it can be concluded that storage of guitar string samples at room temperature for 1, 5 and 10 days causes DNA degradation in terms of average levels and DNA purity results. The DNA isolated from guitar strings can be amplified using D18S51, D8S1179, and FES loci as detection targets; therefore, may be beneficial for forensic identification.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- No animal studies are present in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia.

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Authors' Contribution Statement

A.H.F and N.L.A.M contributed to the conception of study. A.R.R.A, I.N.M., Q.A.H., I.S.N., A.P., and R.F. performed sample collection and experiment. A.H.F. and A.R.R.A. analyzed the data. A.H.F. and N.L.A.M. wrote the manuscript. The final version of the manuscript has been reviewed and approved by all authors.

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تضخيم مواقع D18S51وD8S1179 وFES لعينات الحمض النووي المعزولة من أوتار الجيتار المخزنة في درجة حرارة الغرفة

عبد الهادي فرقوني [1،2]، أنينديتا ريستي ريتنو أريمورتي [3]، ميري ميري [4]، ني لوه أيو ميجاساري [5،6]، إنداه نوريني مسجد كور[6]، قروتا عيون الهدى[6]، إنتان ساري نوريني[7]، لولوك هرماواتي [2]

[1]مركز البحوث الطبية الحيوية، منظمة أبحاث الصحة، الوكالة الوطنية للبحث والابتكار، مركز سيبينونج للعلوم ,سيبينونج، بوجور، جاوة الغربية، إندونيسيا.
[2]كلية الطب، جامعة شريف هداية الله الإسلامية الحكومية جاكرتا، بانتن، إندونيسيا.
[3]دبلوم في تكنولوجيا المختبرات الطبية، كلية العلوم الصحية، الجامعة المحمدية في سورابايا، سورابايا، إندونيسيا.
[4]جامعة باكتي توناس هوسادا، تاسيكمالايا، جاوة الغربية، إندونيسيا.
[5]مدرسة الدراسات العليا، جامعة إيرلانجا، سورابايا، إندونيسيا.
[6]معهد أمراض المناطق المدارية، جامعة إيرلانجا، سورابايا، إندونيسيا.
[7]طالبة في برنامج الماجستير في علوم الطب الشرعي، كلية الدراسات العليا، جامعة إيرلانجا، سورابايا، إندونيسيا.

الخلاصة

الفعل الاجرامي هو سلوك غير قانوني وقد يكون جريمة جنائية ، ويستخدم المجرمون طرقا مختلفة لاخفاء الادلة في مسرح الجريمة لكن غالبا ما يجد خبراء الطب الشرعي اثار الحمض النووي على العناصر الموجودة في مسرح الجريمة, ومن هذه الادلة هو الجيتار. والجيتار من الالات الموسيقية التي يعزف عليها البشروالتي يمكن استخدامها للمقارنة . استخدمت الطرق في هذه الدراسة 18 عينة من أوتار الجيتار التي تم استخدامها لمدة خمس دقائق وحضنت في درجة حرارة الغرفة .تم بعد ذلك تقسيم العينات الـ 18 إلى ثلاث مجموعات، حيث تتكون كل مجموعة من ست عينات وتم تحضينها لمدة يوم وخمسة و 10أيام. تم بعد ذلك قياس الحمض النووي باستخدام مطياف الاشعة فوق البنفسجية للحمض النووي المستخلص بطريقة DNAzol . و كان معدل تقدير الحمض النووي في بعد الحضن لليوم الاول (48.02 ± 152.57 نانوغرام/ميكروليتر) واليوم 5 كانت (± 138.66 47.66نانوغرام/ميكروليتر)، واليوم 10 كانت (كانت 9.07 ± 87.09 نانوغرام/ميكروليتر) . وتم الكشف عن كفاءة الحمض النووي المستخلص للمقارنة بتضخيم 3 مواقع هي D18S51 و FES وD8S1179 بتنقية تفاعل البلمرة المتسلسل، حيث ظهرت الحزم ذوات الاحجام (290-366) و(250 – 222) و(224 – 203) زوج قاعدي, على التوالي. ونستنتج امكانية استخدام اوتار الجيتار كمصدر لعزل الحمض النووي بعد وقوع الجريمة بعدة ايام وبدرجة حرارة الغرفة.

الكلمات المفتاحية:D18S51، D8S1179، FES، STR Loci ، أوتار الجيتار ، الجريمة.