MAPPING PATTERN OF CORE DNA LOCI IN FORENSIC IDENTIFICATION SAMPLE EXAMINATION USING POLYMERASE CHAIN REACTION (PCR) IN LOCI CSF1PO, THO1 AND TPOX

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ABSTRACT

The identification of victims and suspects in cases of crime such as murder, rape with a murder which is increasingly also increase the quantity and quality, increasingly confirmed the existence of forensic medicine. So far the molecular identification in forensic cases with failure of conventional identification has not been much in the know. This study aims to explain the molecular forensic methods of identification in case of failure of identification by conventional methods. The environmental affects DNA content. As is known environmental factors such as humidity and temperature environment is affecting the condition of DNA that are used as materials in the field of forensic DNA identification, as in any other field of DNA examination. The environmental factors affecting DNA degraded or degraded DNA. This degradation can be fast or slow, it depends on factors that influence and timing of exposed. This study showed that all STR loci tested both on samples of blood spots and patches of sweat from day 1, day 10 and day of the 20 shows can still be detected through visualization electrophoresis polyacrilamid agarose gel. The success on these three STR loci, which in this study found the sequence from small to big success is CSF1PO, TPOX and THO1. It was found that the locus TPOX THO1 or equally likely in the success of the examination compared with the STR loci CSF1PO. This is consistent with the calculation of the ratio of GC content and TPOX THO1 have the same relative value that is 0.48, compared with CSF1PO namely 0.33.

Keywords: *STR loci, TPOX THO1, blood spots, patches of sweat*

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INTRODUCTION

Forensic medicine as part of Medical Science, lately increasingly visible role in efforts to address victims of mass disasters ever increasing number and scale as well as upholding justice and the disclosure of criminal cases in the homeland (Atma 2005). Various roles have been undertaken by the forensic medicine of Indonesia to carry out missions assigned by the state so far, as well as the identification of victims of the earthquake in Yogyakarta and Central Java in June 2006 and then, or victims of the Tsunami disaster in Aceh and the earthquake in Nias, the end of 2005, as well as identification of victims of bomb blasts in our country precisely in Legian Kuta Bali, which claimed 182 people died, which followed the identification of victims of tragedy in Situbondo tourist bus accident early October 2003, which killed 56 people on board, as well as the identification of victims of the collapse of Mandala Airlines Aircraft in Medan mid-2005, which claimed 149 lives, until the identification of Dr. Azhari adds a long row of the role of Forensic Medicine in the homeland. Not to mention the identification of victims and suspects in cases of crime such as murder, rape with a murder which is increasingly also increase the quantity and quality, further confirmed the existence of forensic medicine as a branch of medical science that deserves to be taken into account role. This means that the development of DNA analysis has and will give investigators, a tool or device that is very important in the context of the identification process.

Only DNA forensic experts are often faced with the condition of the material or specimen DNA examination, not as expected, just as the specimen is not in condition to be fresh or fresh DNA typing or known as the degraded DNA (Butler et al. 2004). This condition is especially common in cases of individuals who are buried in a relatively long time, or severe burns. So that the DNA obtained is insufficient for forensic DNA examination or DNA profiling. So far the molecular identification in forensic cases with failure of conventional identification has not been described.

On the identification of victims who have been rotting or burned, forensic identification often can not be enforced through associative identification, or other conventional identification. Thus, other forensic

identification method is required, which one of them is through DNA analysis (Kusuma 2004). This fact brings a forensic DNA expert to find ways of forensic DNA examination can help identify the victim, where the DNA contained in it were damaged or degraded DNA.

One alternative is pursued at present by forensic DNA expert is through the use of mini-primer sets, namely through the method of reducing the size of STR assays, the examination of nuclear DNA loci. Yet until now there have been no specific studies about the successful use of the mini primer sets as an alternative way for forensic DNA identification by using DNA that has been degraded or degraded DNA, especially in nuclear DNA. It is necessary to be able to determine the locus-locus of potential use for degraded DNA examination. With the mapping of locus-locus nuclear DNA, that would be known and determined locus-locus DNA that can be used in forensic DNA examination. This article examines whether the use of effective methods of forensic molecular identification in forensic examination of material that can not be identified conventionally. This study aims to explain the molecular forensic methods of identification in case of failure of identification by conventional methods. Theoretically, this study's findings are expected to be scientific information for forensic medicine in Indonesia, which deals with forensic DNA analysis on the condition of the fragmented DNA. In practical terms this study's findings assist in forensic identification of the primary use of a particular locus in order to generate mapping loci that can be used as a reference in Forensic DNA profiling in forensic DNA laboratories in Indonesia.

MATERIALS AND METHODS

This was an observational analytic study to map the locus of DNA that can still be used in the examination of degraded DNA. DNA samples were derived from blood spots and patches of sweat from volunteers. Sample size consisted of 20 pieces 10 pieces 10 pieces of bloodstains and sweat patches. Research carried Human Genetic Study Group, and the Tropical Disease Center (TDC), Airlangga University. Examination materials are patches of sweat and blood spots on the fabric. Material for DNA extraction is DNAzol Reagent, Solution 100% & 70% ethanol, and Destilated Water (Sigma). Materials for PCR: PCR Mix (12.5 ul): dNTPs (ATP, CTP, TTP GTP), MgCl 2 and Taq polymerase, Nuclease Free Water, Primary Short Tandem Repeat (STR), which includes the loci-loci: CSF1PO (Gen Bank Accession M25858): 'ACAGTAACTGC CTTCATAGATAG3' 5'GTGTCAGACCCTG TTCTAAGTA 3 ', TH01 (Gen Bank Accession D00269): 5'-CCTGTTCCTCCCTTATTTCCC-3' 5'-GGGAACACAGACTCCATGGTG-3 'and TPOX (Gen Bank Accession M68651): 5'-3-CTTAGGGAAC CCTCACTGAATG ' -GTCCTTGTCAGC GTTTATTT GC 5'-3 '

Extraction of DNA on the sweat and blood spots (DNAzol Reagent)

Fabrics that contain patches of sweat and blood after drying for 10 days and 20 days, cut into small pieces and inserted into the tube to distilled water soaked with 3-6 ml, were incubated at room temperature for 24 hours. Fluid samples were collected and centrifuge 6500 rpm 30 minutes at 4^{o}C. Supernatant discarded, and pellets obtained DNA was extracted using DNAzol. Pellets of sweat patches (or 0.1 ml of blood samples) plus 1 ml DNAzol Reagent, and then incubated 5 minutes at room temperature. Next in centrifuge 10,000 rpm for 10 minutes at 4^{o}C temperature, viscous supernatant was taken and transferred a new tube. Next on the new tubes was added 0.5 ml 100% ethanol, incubation at room temperature 1-3 minutes, centrifuge 4000 rpm for 1-2 minutes at 4^{o}C. Supernatant discarded. Pellet was washed with 0.8 to 1 ml of 75% ethanol 2 times. The tube is placed in an upright position for 0.5 to 1 minute, after it disposed of 75% ethanol by pipeting or decanting. Pellet was dried by letting the tube is open for 5-15 seconds. Pellets containing the DNA was dissolved in 25-30 ul/l Distilled water (DW), in the vortex to taste, and then stored at a temperature of -200 C. Measurement of DNA content and purity through UV-spectrophotometer. In Phase I of PCR amplification, initial denaturation performed in 96^{o}C 2 '. For 10 cycles, subsequent denaturation 94° C for one minute, annealing 64^{o}C for one minute and extension 70^{o}C for one minute thirty second. For 30 cycles denaturation 90°C for one minute, annealing 64^{o}C 1', and extension 70^{o}C for one minute thirty second.

Procedure of Polyacrylamid Composite agarose Gel Electrophoresis

Agarose gel made from 30 cc of 0.5 X TBE and agarose 0.15 grams, is heated in a microwave until clear, then cool at 50^{o}C. Then added Acrylamid Temed Bus 4.5 cc and 15 ul. Ammonium persulphate is then added 100 μL, then poured into the mold (gel bed), wait until chilled/frozen. DNA PCR results of 12.5 μL with loading buffer 2 μL inserted and run at voltage 70 volts for 2 hours.

Table 1. Concentration and purity of DNA samples

Samples	Length of Exposure	Repository Site			
		Room Temp.		Humidity	
		Level (ng/µl)	Purity	Level (ng/µl)	Purity
Blood spot	Day 1	790	1.37	450	1.25
	Day 10	850	1.27	356	1.36
	Day 20	220	1.59	210	1.21
Sweat patches	Day 1	98.38	1.23	80.9	1.23
	Day 10	60.12	1.15	59.18	1.19
	Day 20	51.38	1.46	49.46	1.02

Silver Staining Procedure Polyacrylamid composite agarose gels

Drying: (methanol 20% + 2% glycerol) in 10^{o}C distilled water for 5 minutes. Fixation: (ethanol 10% + glycerol 5% acetic acid) in 100 cc distilled water for 20 minutes. Wash/rinse with 1x distilled water quickly. Staining: 0.1% AgN03 in 100 cc during 50-80 distilled water minutes. Developing: (NaOH 1.5% + Formaldehyde 100 ul) in 100 cc distilled water, then viewed with UV light to visible.

RESULTS

Average yield of taking the measurements of DNA content by UV Spectrophotometry to study samples from blood spots and patches of sweat that will be used in the examination of Short Tandem Repeat (STR) loci CSF1PO, and TPOX THO1.DNA content of the research sample storage at room temperature and high humidity over a period of 10-20 days there is a tendency to decline (Table 1). This means that the storage of DNA content and duration are stored will affect its reduction.

Table 2. Results Visualization STR Locus

Samples	Length of exposure	Visualization of STR Locus		
		CSF1PO	TPOX	THO1
Blood spot	Day 1	+	+	+
	Day 10	+	+	+
	Day 20	+	+	+
Sweat patches	Day 1	+	+	+
	Day 10	+	+	+
	Day 20	+	+	+

Results visualization of locus-STR loci by amplification with primers CSF1PO, TPOX THO1 and this band was still detectable bands (Table 2). So here is needed again using the mini primer sets again.

Results visualization electrophoresis loci CSF1PO day to 20 with polyacrylamid-agarose gel showed that all samples of either blood spots or patches of sweat on various long exposure can still be detected (Figure 1).

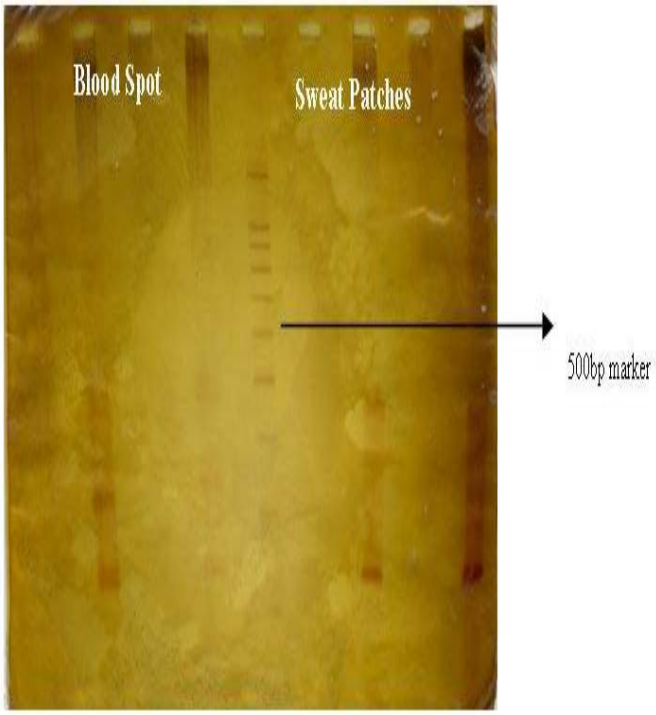

Figure 1. Visualization results of the 20-day Locus CSF1PO

Results visualization electrophoresis THO1 locus with polyacrylamid-agarose gel showed that the samples of bloodstains on various long exposure can still be detected (Figure 2). While the results of visualization electrophoresis locus TPOX day 1 with polyacrylamid-agarose gel showed that in all samples of both blood spots or patches of sweat on various long exposure can still be detected (Figure 3).

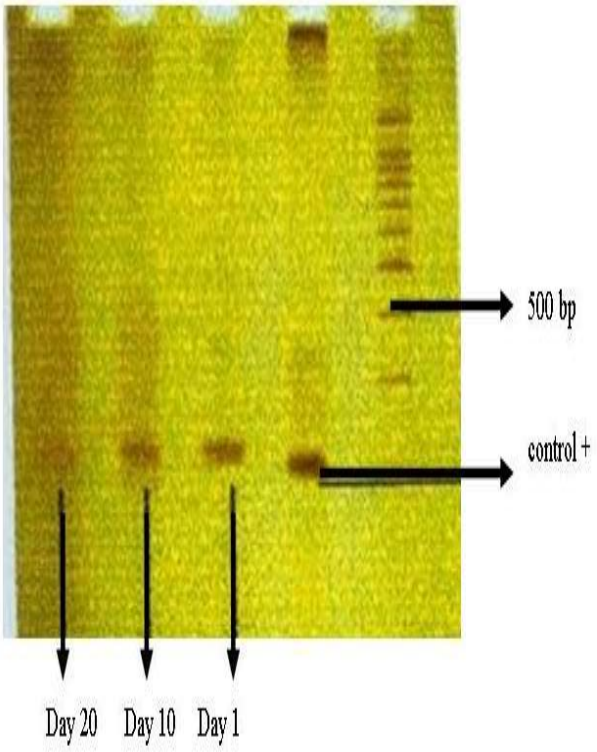

Figure 2. Results visualization THO1 locus on blood spots

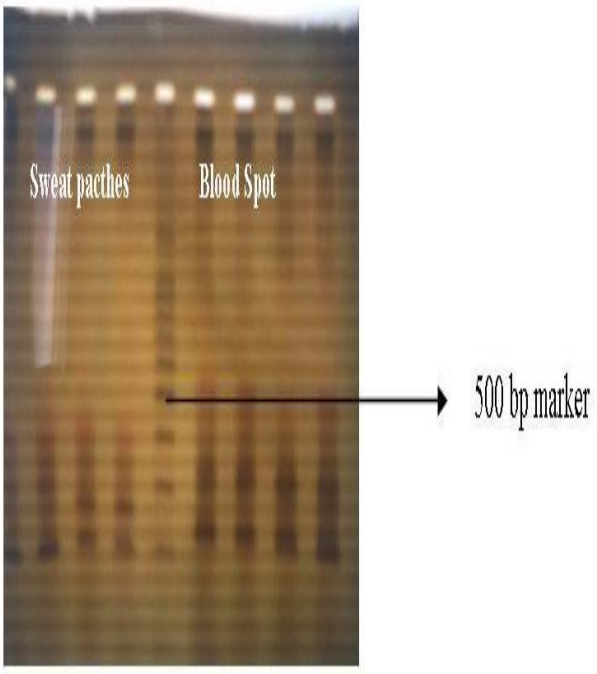

Figure 3. Results Visualization locus TPOX day 1

DISCUSSION

In principle, the personal identification of victims died in the field of forensic medicine is a series of actions benchmarking data from the examination of corpses (postmortem data) with data suspect the victim while still alive (antemortem data). The data will then be compared with data belonging to the alleged victim, where the data can be obtained from the family, medical records, medical records tooth, police data, and so forth. With compatibility between data antemortem and postmortem data, will narrow the number of suspected victims. Thus, this will further strengthen the notion that the victim is really those who have suspected all along. The discovery of DNA fingerprinting by Sir Alec J Jeffreys in the mid eighties, has led to the development of DNA technology in the field of forensic medicine in the direction of encouraging progress, the identification of victims in the field of forensics is not a peculiar problem anymore. It is given that the examination of DNA on the victim which are difficult to identify it, no longer based on physical characteristics of victims, but on the region (locus) DNA of the victim. This examination is based on the fact that human DNA turns out to be individualized and specific. This means that the composition of human DNA is unique to each individual. So it can be used to distinguish individuals from one another.

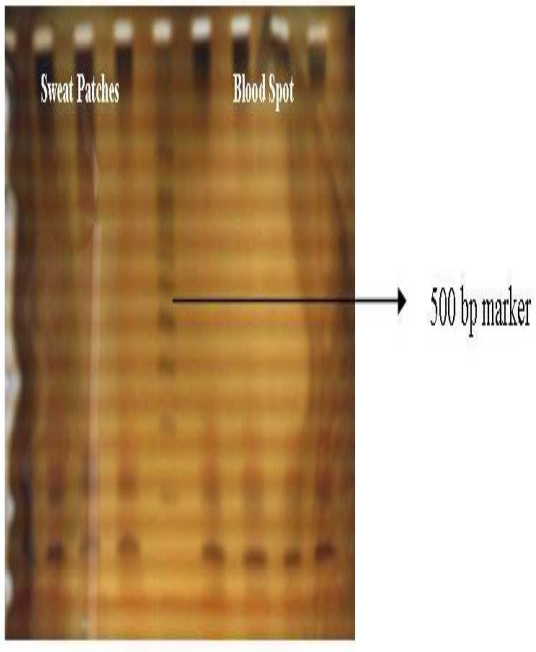

Figure 4. Visualization Results of the 20 loci TPOX day

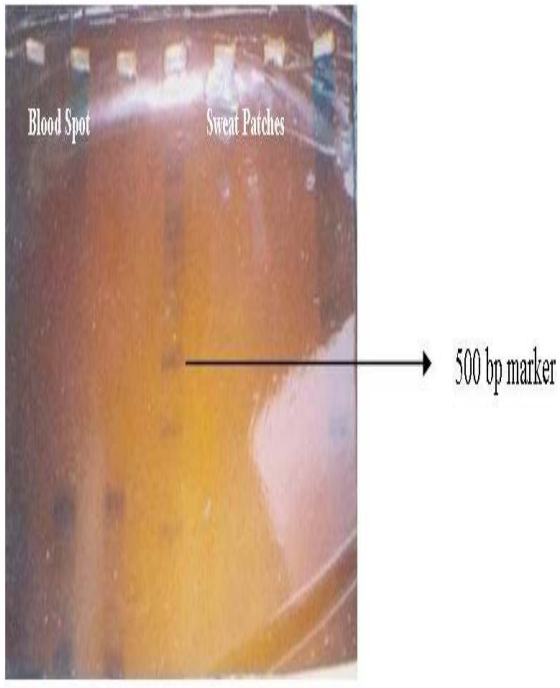

Figure 5. Visualization Results of the 20 loci CSF1PO day

Yet examination of DNA as a tool in the forensic identification process is not without weaknesses. This is based on a reality that the DNA will be damaged (damage), when exposed by abnormal exposure-exposure, which can cause DNA damage as well as chemicals, pH, temperature, and exposure to others. DNA damage, caused by exposure-exposure to abnormal high temperature is caused by damage to the DNA binding irreversible hydrogen bond. These conditions resulted in damage to purine-primidin pairs in the DNA, where the pair purine-primidin. This is a major component in the structure of DNA. DNA damage (DNA damage) caused by exposure-exposure such as X-rays, chemical agents, spontaneous instability, or by very high temperatures or extreme temperature, result in many types of damage, such as chain damage (strand) DNA (both double strand and single strand), base damage (damage to DNA bases), sugar damage, and even the occurrence of DNA-DNA crosslinks and DNA-protein crosslinks.

From the results of this study the environmental effects in terms of temperature and long exposure to these effects in this study proves the existence of influence on the measurement of DNA content contained. It is seen from the measurement of DNA content by UV Spectrophotometry showed reduced levels of sweat patches on samples stored at room temperature and places with high humidity from day 1 to day 20 there is a significant decline, it also occurs in DNA samples from blood spots. But with the decline in these levels, not an obstacle because the levels of the remaining DNA still memungkingkan for examination of DNA profiling that is at least 50 ng (Notosoehardjo 1999).

DNA content is an important factor in forensic DNA examination of the effect on the success of STR genotyping of DNA samples. Decreasing levels of up to 1 ng of DNA has the potential to decrease the detection ability of short tandem repeat (STR) to 95% (Bergen et al 2005). The number of minimum levels of DNA that can be used in DNA analysis, in principle, depends on the needs and types of tests done. On examination of forensic DNA Restriction Fragment Length based Polymorphism (RLFP) for example, it takes relatively large DNA content which is about 100ng, still 'fresh' with a view to increase the likelihood of success in the management of DNA profiling (Thomson et al 1999). While the levels required in the examination of DNA Short Tandem Repeat (STR) requires only minimal DNA concentrations ranging from 0.25 to 2 ng (Simon et al 2005). In addition to sample DNA content in the examination of DNA-based Polymerase Chain Reaction (PCR) is also required that sufficient DNA quality. The quality of the DNA in question ie that the DNA used in the analysis should be in conditions that was degraded. If DNA is badly degraded primary cause can not be attached or annealed to target DNA to be duplicated.

According Muladno (2002), theoretically to get the required purity adequate visualization of DNA that adequate and appropriate levels of DNA, so DNA can be used as a DNA examination materials included in this case is the identification and paternity testing. Because it is a good quality of DNA into fundamental prerequisite for the success of the reaction Polymerase Chain Reaction (PCR) as a whole (Yamada et al 2002). According to Chung et al (2004) states that the sensitivity of Polymerase Chain Reaction (PCR) is a function of the number of cycles and grade as well as the integrity of DNA.

This research using STR analysis, because in general samples of forensic DNA examination, 40% had suffered degradation or contamination (Notosoehardjo 1999b). So with the analysis of Short Tandem Repeat (STR) which has the core sequences less than 1 kb (kilobase) are very effective and the value of success is quite high, especially in degraded DNA will terfragmented (cut into pieces) by producing a short fragment. FBI bersinergis with 16 CODIS STR loci have been designing for a recommendation in the examination of forensic identification or paternity (Kusuma 2004). Regarding the minimum requirements

of STR loci used for the examination until now there has been no agreement. There are several laboratories require a minimum of 3 loci for paternity investigation or identification of DNA, at Airlangga University TDC do 7-8 loci (Kusuma 2004), was in Jakarta (Atma 2005) indent the nine loci plus Amelogenin locus in a paternity test. Nidom (2005), say 5 to 6 STR loci have a value ratio of 1: 100 billion. So in principle regarding the number of loci examined is that more loci are used in the examination, the better the value of accuracy.

STR analysis in this study planned to use the locus THO1, TPOX, and CSF1PO. STR loci typing methods, especially THO1 is a method that makes sense, strong and efficient so it is a useful method in forensic cases. In this study indicate that environmental influences affect the levels of DNA. As has been known environmental factors such as humidity and temperature environment is affecting the condition of DNA that are used as materials in the field of forensic DNA identification, as in any other field of DNA examination. The environmental factors affecting DNA degraded or degraded DNA. This degradation can be fast or slow, it depends on factors that influence and timing of exposure. DNA damage was divided into 2 types of damage from inside e.g. caused by reactive oxygen species (ROS) and damage from external factors, such as temperature, humidity and others.

Free radicals, often called reactive oxygen species (ROS), also can be formed through enzymatic or metabolic. Activated by oxygen free radical-forming agent, such as ionic radiation, can induce DNA lesions that cause defects, mutations and even death. DNA damage is shown by the sugar and bases are easily oxidized, causing degradation and destruction of single-strand, as well as protein cross-linking. Degradation of DNA bases will produce products such as 8-hidroksiguanin, hydroxymethyl urea, thymine, glycol, thymine and adenine open chain, as well as other saturated products.

Force - which easily oxidized groups such as aldehydes, ketones and hydroxyl or molecules that easily turn into a compound that has one of these groups seemed to be the target of reactive free radical compounds. Therefore, nucleic acids like DNA and RNA, which is known to have a lot of carbohydrates such as ribose (in RNA) and deoxyribose (in DNA), are vulnerable to free radical compounds attacks. Single-strand damage occurs because the sugar is oxidized by hydroxyl radicals. On the physiological condition of hydrogen peroxide and superoxide can not cause damage to strand, but its toxicity in vivo with the metal catalyst to form Fenton reaction.

Cross-linking of DNA by proteins is a further hydroxyl radical attack, which occurred in the DNA or protein. Although cross-linking of DNA-protein is less dangerous than the destruction of single-strand, but its existence can not be repaired and may contribute to cell death. Free radicals can also cause various changes in the DNA, such as thymine and cytosine bases hydroxyl, purine and pyrimidine core opening and breaking of chains of DNA phosphodiesterase. The damage was not so severe to be repaired by DNA repair systems. However, if severe enough, such as DNA chains were cut off in many places, the damage can not be repaired.

The results showed that all STR locus examined both on samples of blood spots and patches of sweat from day 1, day 10 and day of the 20 shows still can be detected through a visualization of agarose polyacrilamid electrophoresis gel. The success on three STR loci, which this research found that the sequence from small to big success is CSF1PO, TPOX and THO1. Meanwhile, according to Mc Cord (2003) as for the success of locus DNA sequence from small to large are TPOX, and vWA THO1. Regarding the success of these loci were detected, caused by differences in amplification products and the GC content or guanine and cytosine bonds at each locus. According to Muladno (2002) which states that the GC content or guanine cytosine bond has a high degree of stability against denaturation factor compared to the bond between Adenine and thymine. This study found that loci TPOX THO1 or equally likely in the success of the examination compared with the STR loci CSF1PO. This is consistent with the calculation of the ratio of GC content and TPOX THO1 have the same relative value that is 0.48, compared with CSF1PO namely 0.33.

CONCLUSION

There are external factors that affect the environment and the long exposure quantity and quality DNA from sweat patches and spots of blood on the clothing can be an alternative material in forensic identification. Found mapping pattern sequence is the success of the STR loci CSF1PO, TPOX and THO1. This is in accordance with the ratio of GC conten respectively. This is consistent with the calculation of the ratio of GC content and TPOX THO1 have the same relative value that is 0.48, compared with CSF1PO namely 0.33. There is the ability of STR loci (CSF1PO, THO1 & TPOX) can still detect the DNA of sweat patches and spots of blood on until day 20. For the perfection of the results of this research is necessary to do further research on the effects of treatment or environmental factors in this case in detail on the effect of moisture on the samples as raw DNA examination of the forensic identification through

forensic molecular techniques. Also on the pattern of mini-STR loci and the other in order to get the whole picture about the pattern of primary mapping standard STR and mini-STR primers both nuclear DNA and mitochondrial DNA.

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