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EFFECTIVENESS OF NUCLEAR DNA MINI PRIMER SET AT LOCI FGA, CSF1PO & D21S11 IN HIGH-TEMPERATURE DNA DEGRADATION WITH POLYMERASE CHAIN REACTION (PCR) METHOD

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ABSTRAK

Identifikasi forensik dengan pemeriksaan DNA yang dapat digunakan untuk menentukan asal usul anak; kasus paternitas; hubungan kekeluargaan; maupun identifikasi korban tak dikenal, semakin hari semakin diakui keberadaannya dalam menunjang penegakan hukum di tanah air. Hanya saja dalam perkembangannya pemeriksaan dengan menggunakan bahan DNA ini bukannya tanpa persoalan. Salah satu persoalan yang seringkali menjadi masalah yang serius bagi ahli DNA forensik maupun ahli DNA lainnya adalah kondisi DNA yang degraded atau yang dikenal dengan istilah degraded DNA. Salah satu alternatif yang ditempuh dalam degradasi DNA saat ini oleh ahli DNA forensik adalah melalui penggunaan mini primer set, yakni melalui metode pengurangan ukuran STR assays, pada pemeriksaan lokus DNA inti. Penelitian ini menggunakan mini primer CSF1PO, FGA & D21S11 pada bahan gigi molar dengan perlakuan paparan 5000C dalam waktu 20 dan 30 menit serta suhu 7500C dalam waktu 20 dan 30 menit. Hasil yang didapatkan terjadi penurunan kadar DNA gigi yang bermankna ($p<0.05$) sebagai efek paparan suhu tinggi. Visualisasi hasil PCR didapatkan hanya lokus CSF1PO yang masih terdeteksi dengan mini primer pada paparan suhu 7500C selama 30 menit (paparan maksimal penelitian ini) sehingga melalui lokus tersebut sangat potensial dalam pemeriksaan identifikasi melalui analisis DNA terutama dalam kondisi terdegradasi efek paparan suhu tinggi, serta mempercepat proses identifikasi terutama pada kejadian bencana (mass disaster) maupun kasus-kasus kriminal lainnya.

ABSTRACT

Forensic identification by DNA testing that can be used to determine the origin of the child; paternity cases; family relationships; and identification of victims is not known, is increasingly recognized in supporting law enforcement in this country. Only in its development with the use of DNA examination is not without problems. One issue that is often a serious problem for forensic DNA experts and other DNA experts is that degraded DNA or condition which is known as DNA degraded. One alternative adopted in the current degradation of DNA by DNA forensic expert is through the use of mini-primer set, namely through the STR ??method of size reduction assays, the examination of nuclear DNA loci. This study uses a mini primer CSF1PO, FGA and D21S11 on molar tooth material with exposure treatment 5000C in 20 and 30 minutes and the temperature of 7500C in 20 and 30 minutes. The results obtained significant decline of DNA levels in teeth ($p <0.05$) as the effect of high temperature exposure. Visualization of PCR results obtained are still only CSF1PO loci were detected with a mini primer on the exposure temperature of 750^0C for 30 minutes (maximum exposure of this study) so that potential through these loci in the examination of the identification through DNA analysis, especially in a degraded state effects of exposure to high temperatures, as well as speed up the process identification, especially in the event of a disaster (mass disaster) as well as other criminal cases.

Keywords: *effectiveness, DNA degradation, mini primer, high temperature*

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INTRODUCTION

Forensic identification by DNA testing that can be used to determine the origin of the child; paternity cases; family relationships; and identification of victims is not known, is increasingly recognized in supporting law enforcement in this country. This was proved by the examination he admitted that was discovered by Sir Alec Jeffrey this as evidence in courts and religious courts since 1997 (Atmaja 2005). Even for purposes of identification of victims of the Bali bombing in 2002, did, by using a DNA identification of victims of this, take a very significant role.

Only in its development with the use of DNA examination is not without problems. One issue that is often a serious problem for forensic DNA experts and other DNA experts is that degraded DNA or condition which is known as DNA degraded. One alternative adopted in the current degradation of DNA by DNA forensic expert is through the use of mini-primer set, namely through the STR method of size reduction

assays, the examination of nuclear DNA loci, as proposed by Coble and Butler (2005). This study uses the average temperature treatment in the study S.Thanakum (1999) namely 500^0C and research Sosiawan (2007) ie 750^0C and 20-minute time span is the highest time S.Thanakum research and time span of 30 minutes highest Sosiawan research. Yet until now there have been no specific studies about the successful use of 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 loci (especially loci CSF1PO, FGA, and D21S11) are potentially used for the examination of degraded DNA.

The purpose of this study was to determine the effectiveness of using mini-primer set in the core DNA loci CSF1PO, FGA and D21S11 on the alleged DNA has been degraded by using the Polymerase Chain Reaction (PCR), Explaining the value of the effective use of mini-primer set of core DNA loci CSF1PO, FGA and D21S11 on the alleged DNA has been degraded by using the Polymerase Chain Reaction (PCR), and describes the successful detection of DNA profiling through DNA amplification by PCR, using a mini-STR primers (locus CSF1PO, FGA, and D21S11). The research was useful to help in the forensic identification of mini-primer on the use of a particular locus. And can result in mapping loci that can be used as a reference in Forensic DNA profiling in forensic DNA laboratories in Indonesia.

MATERIALS AND METHODS

This type of research is experimental laboratories. The study design: Randomized post test only control group design. The samples are two of bodies molar T4 as many as 16 pieces of gear. The study was conducted at the Laboratory of Human Genetics Unair ITD. DNA extraction material is DNAzol Reagent, Solution 100% - 70% ethanol, while the material for the PCR is a PCR Mix (12.5 ul) comprising: dNTP (ATP, CTP, TTP GTP), MgCl2 and Taq polymerase, sigma DW (Nuclease free water), mini-STR primer, mini CSF1PO (Promega Primer, Gen Bank Accession X14720), 5'-ACAGTAACTGCCTTCATAGATAG-3 ', 5'-GTGTCAGACCCTGTTCTAAGTA-3', mini FGA (Promega primer, Gen Bank Accession M64982), 5 ' -AAATAAAATTAGGCATATTTACAAGC-3 ', 5'-GCTGAGTGATTTGTCTGTAATTG-3' and mini D21S11 (Promega primer, Gen Bank Accession AP000433), 5'-ATTCCCCAAGTGAATTGC-3 ', 5'-GGTAGATAGACTGGATAGACGA-3'. Material is agarose electrophoresis, Tris Boric EDTA (TBE) 0.5%, Marker 100 bp and 0.03% bromphenol blue.

The research instrument is the PCR cycle (PCR System 9700, Applied Biosystem), UV-Visible Spectro-phometer; electrophoresis; Centrifuge; Ependorf micropipette; Tips Micropipet; Transluminator; Polaroid camera; Tubes eppendorf; Microwave (Imarflex) Electronic Scales (Shimadzu). Data collection procedures carried out by extraction of DNA at a molar tooth. The first molar tooth decalcification process is done in two, and carried out DNA extraction sweat and blood spots with DNAzol Reagent. Then, DNA content and purity were measured through a UV-spectrophotometer. PCR amplification performed with primers mini FGA, and D21S11 CSF1FO (Opel 2002). Initial denaturation 95^0C for 10 'and the final extension 65^0C for 45'. Agarose gel electrophoresis used 2%.

RESULTS

Samples of dental materials (two molars) are taken from the bodies of T4, material weight measurements were taken before and after treatment, the average weight of dental materials before and after treatment as follows below:

Table 1. The results of measurements of the mean weight of dental materials before and after treatment (exposure to high temperatures).

Mean of dental material weight (gram)			
Before treatment	After treatment		
2.8	500 ^{0}C	20’	2.0
2.4		30’	1.4
2.7	750 ^{0}C	20’	1.7
2.5		30’	1.3

Table 1 shows a decrease in weight due to treatment of dental materials, from 50-60% decline before treatment. Here are the average levels of DNA from dental materials, as can be seen in Table 2.

Table 2. The mean levels of DNA isolation from dental materials

Exposure (^{0}C)		Dental DNA Level (x ± SD) ng/µl
No exposure		269.25 ± 10.25
500^0C	20’	147.32 ± 9.07[1,2,3]
	30’	128.40 ± 5.41[1,4,5]
750^0C	20’	110.37 ± 9.51[2,4,6]
	30’	72.18 ± 3.09[3,5,6]

The sign[1,2,3,4,5,6] : the same number in each line indicates significant difference ($p<0.05$)

Table 2 shows a decrease in DNA content of dental materials through exposure to high temperatures. Can be stated that the higher the temperature of the material presented on the dental material decline in DNA content of the tooth. ANOVA test results indicate there is a treatment effect of reduced levels of DNA material (the value obtained sig: 0000, significantly limit of $p < 0.05$). T test results showing a comparison of DNA content of bone material are significantly different (borderline significant if p value <0.05) between exposure to temperature: 500^0C-20 minutes: 500^0C-30 minutes; 500^0C-20 minutes: 750^0C-20 minutes; 500^0C-20 minutes: 750^0C-30 minutes; 500^0C-30 minutes: 750^0C-20 minutes; 500^0C-30 minutes: 750^0C-30 minutes; 750^0C-20 minutes: 750^0C-30 minutes.

Figure 1 to 3 is the result of agarose gel electrophoresis with 2% in the CODIS STR loci with a mini-primer PCR results on the conditions of exposure to temperatures of 20 and 500^0C for 30 minutes; 750^0C for 20 and 30 minutes. Figure 3.1 FGA locus PCR agarose gel electrophoresis visualization of 2% in a sample of dental materials. Visualization of the results of the FGA locus PCR DNA primer mini dental materials with figure 5.3, indicating exposure to 500^0C temperature for 20 minutes can still be detected in the range between 118-170 bp, while the temperature of 500^0C for 30 minutes and 750^0C for 20 and 30 minutes is not can be detected.

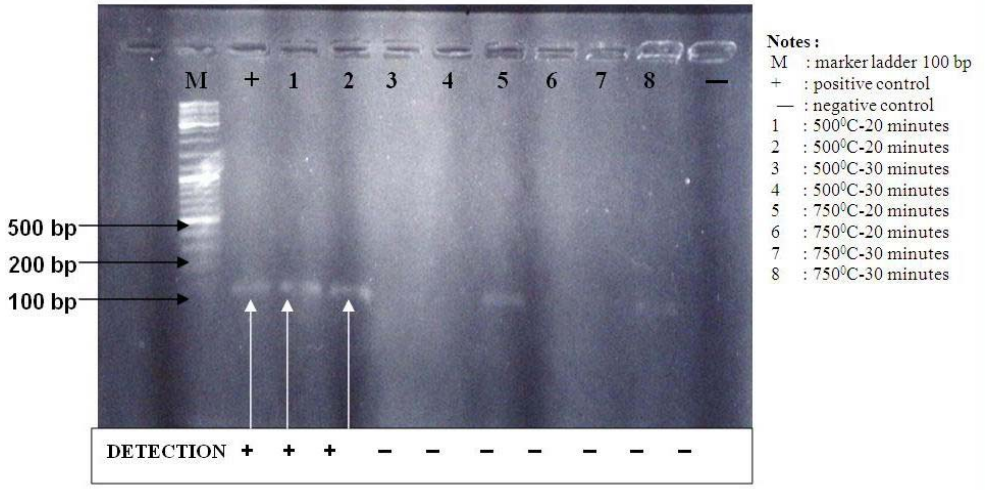

Figure 1. Visualization of the FGA locus PCR with primers to the mini dental materials.

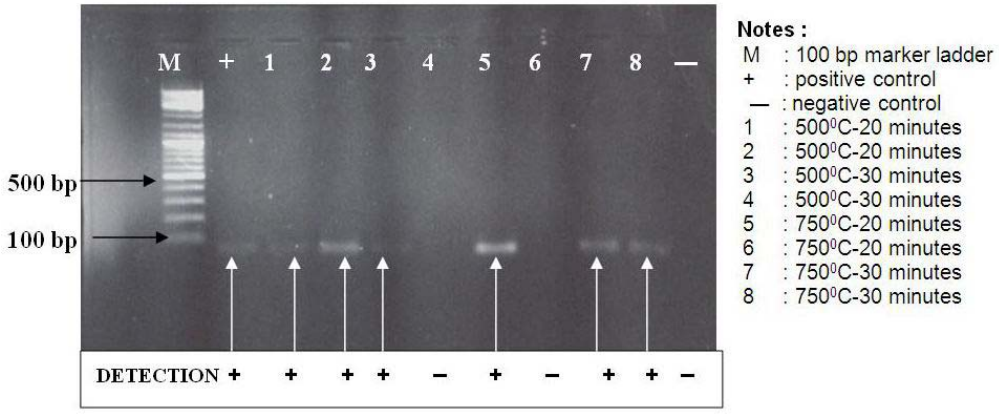

Figure 2. Visualization of the PCR loci CSF1PO with mini primer on dental materials.

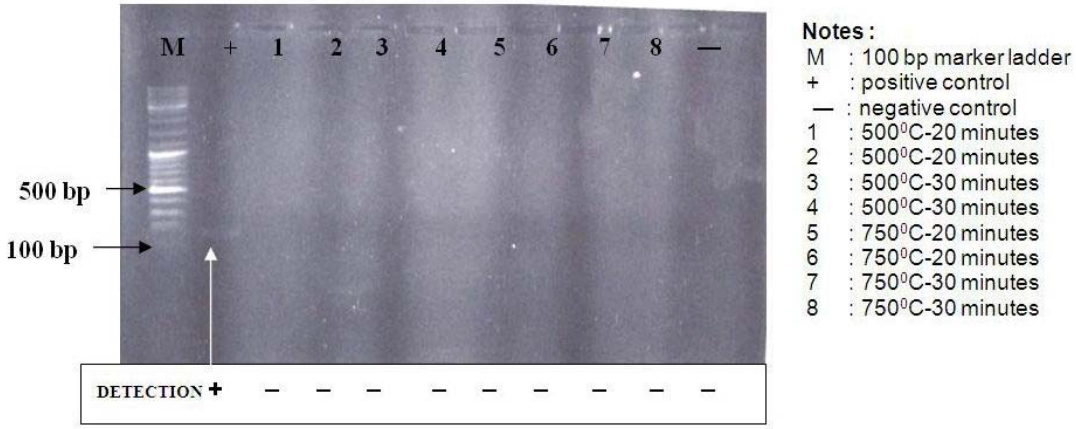

Figure 3. Visualization of the PCR loci D21S11 with a mini primer on dental materials.

Visualization of the results of PCR Figure 1, shows only the exposure to temperature 500^{0}C-20 'can be detected in the range between 118-170 bp. The following visualization of the results of PCR loci CSF1PO using agarose gel dental materials 2% in the mini primary exposure to temperatures under 500^{0}C for 20 and 30 ', and 750^{0}C for 20 and 30'. Visualization of the results of PCR Figure 2, shows the temperature exposure time of 20 and 500^{0}C for 30 min and the temperature of 750^{0}C for 20 and 30 minutes of time can still be detected in a range between 89-129 bp. The following visualization of loci D21S11 PCR results using agarose gel dental materials 2% with a mini primary exposure to temperatures under 500^{0}C and 750^{0}C respectively for 20 and 30 minutes. Figure 3 visualizes the results of PCR, indicating exposure to 500^{0}C temperature for 20 minutes exposure to temperatures up to 750^{0}C for 30 minutes cannot be detected in the range between 153-221 bp. The full results of the examination detection FGA locus, CSF1PO and D21S11 on the mini dental materials as a primary effect of high temperature exposure in Table 3.

Table 3 shows that the examination of DNA STR locus FGA dental materials through exposure to the temperature of 500^{0}C for 20 and 30 min and 750^{0}C for 20 minutes can still be detected (31.25% of the sample) while the locus CSF1PO in exposure temperature 500^{0}C for 20 minutes until 750^{0}C for 30 minutes can still be detected (56.25%). D21S11 locus on exposure to temperature 500^{0}C for 20 minutes until the exposure temperature 750^{0}C for 30 minutes cannot be detected.

Table 3. The results of DNA detection in CODIS STR effect of high temperature exposure of DNA material in a variety of dental treatment temperature and duration of exposure, the locus FGA, CSF1PO and D21S11

Exposure		FGA		CSF1PO		D21S11		Total (% rslt +)
		Detection result		Detection result		Detection result		
		+	-	+	-	+	-	
500^{0}C	20’	2	2	4	0	0	4	50 %
	30’	2	2	2	2	0	4	50 %
750^{0}C	20’	1	3	2	2	0	4	25 %
	30’	0	4	1	3	0	4	8.5 %
Total DNA + after exposure (%)		5/16 31.25%		9/16 (56.25%)		0%		

DISCUSSION

DNA content is an important factor in forensic DNA examination that is influential on the success of STR genotyping the DNA samples. Decreased levels of DNA to 1 ng potential to decrease the ability of short tandem repeat detection (STR) to 95% (Bergen et al. 2005). According Notosoehardjo (1999) and Gatut (2004) DNA content of the required minimum on forensic DNA testing of each at 50 ng and 20 ng, while Butler (2005), levels of DNA in the examination of minimal 0.5-2.5 ng STR. Besides the DNA content of the samples in a DNA-based Polymerase Chain Reaction (PCR) is also required DNA quality is sufficient. The quality of the DNA is that DNA is used in the analysis must be in a state that has not been degraded. If DNA is badly degraded primary cause cannot stick to the target DNA to be duplicated (Muladno 2002; Rudin 2002; Yuwono 2006). According Muladno (2002), adequate visualization of the results of the purity of DNA needed adequate and appropriate levels of DNA, so DNA can be used as a DNA examination is included in this case is the identification and paternity testing. DNA damage caused by abnormal exposures to such high temperatures for example, according to Watson, (1987), caused by damage to DNA is irreversible hydrogen bonds. These conditions resulted in damage to the purine-pyrimidine pairs in DNA, where the pair purine-pyrimidine is a major component in the structure of DNA.

This study used a sample from the body of a non-permanent residence status (T4). Damage to DNA samples after the death of an endogenous process has been started since the death. DNA damage can be concurrent with the decay process, namely through autolysis and bacteria. According to Hofreiter (2001), DNA damage in post mortem (bodies) as a result of the process of autolysis can be a pyrimidine modification, baseless sites, intermolecular crosslinks and low molecular weight of DNA strand breakage due. The results of this study is only a mini-primer CSF1PO loci show detectable at the exposure temperature was 750^0C for 30 minutes which is the maximum temperature in this study. This suggests that the DNA testing dental materials obtained through the detection of STR loci detection of different responses at different exposure to high temperatures has been given to the tooth samples.

Teeth also has a 'hard tissue mineral' is more complete. The mineral known mostly as hydroxyapatite. Besides, the teeth have an important secondary minerals, which contain the teeth is higher than bone, namely: calcite, limonite, pyrite and vivianite, so that the teeth have a strong resistance or protection (Hillson, 1996). The primary use of the mini is a great alternative as a replacement for the standard primer in a state of degraded DNA, which with the use of standard primer on these conditions, the success rate is low. Mini primer is the primer redesign reduces the amplicon size by shifting the primary position as close as possible to the local loop (Chung 2004; Butler 2003). According Mc.Cord (2005) mini-primer is a more attractive alternative option for the purposes of forensic analysis of degraded DNA in comparison to forensic analysis using mtDNA.

The success of this locus is supported by the detection of differences in product amplicon, GC content or guanine-cytosine bonds at each locus. According Muladno (2002), GC content has a high degree of stability against denaturation than the bond between adenine and thymine. The results of calculation of the ratio of GC content have a significant value. Of the loci studied, the ratio of GC content in the primary locus CSF1PO was as follows: 42.6%, FGA: 35.7%, D21S11: 34.1%. In addition, the presence of adenine row (consecutive adenine) is a potential target for DNA damage caused by exposure to hot temperatures. Adenine is the most easily oxidized base (K Kawai 2005). Barriers/obstacles encountered in the implementation of this research are: not homogeneous sample is obtained, this is due to the limited number of bodies with the status of T4 (non-permanent residence). Besides, it is also a period of time the primary pivot ordering.

CONCLUSION

CSF1PO loci that are detected with a mini-primer on the exposure temperature of 750^0C for 30 minutes so that potential through these loci in the examination of the identification through DNA analysis, especially in a degraded state effects of exposure to high temperatures, as well as speed up the identification process, especially in the event of a disaster (mass disaster) as well as case other criminal cases. Need to do more research on the use of mini-primer probability index both CODIS STR and mtDNA population in Indonesia, both with more samples, with exposure to different temperatures and in samples that had rotted, so that the image obtained more advantages in the use of mini-primer DNA examination profiling, which can be used more widely.

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