Comparison of DNA Extraction From Blood Stain and Decomposed Muscle in STR Polymorphism Analysis

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Abstract

Forensic samples that are often degraded and limited in quality cause DNA typing analysis by conventional methods unsuitable. We performed a single tube-multiplex PCR on 9 STR loci (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, and D7S820) and the X-Y homologous gene amelogenin of DNA extracted from six week postmortem blood stain and decomposed muscle by using QIAGEN QIAamp blood or tissue procedure. An automated genetic analyzer based on fluorescent dye technology was used to detect STR allele patterns. The DNA profile of blood stain sample obtained a complete and unambiguous pattern, whereas, that of muscle DNA extracted from QIAamp tissue and Chelex plus QIAamp blood protocols showed detected STR alleles for 70 per cent and 50 per cent of all tested alleles, respectively. The degraded muscle DNA could not yield amplified products of large size STR alleles; CSF1PO, D13S317 and D7S820. However, the analysis which relied upon the PCR-based STR polymorphism analysis and automated genetic analyzer system offers an ideal strategy for forensic identification.

Key word : DNA, Decomposed Muscle

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PCR-based STR analysis has become a useful method in forensic identity testing[1-3]. The limitations of quantity, quality and available source of samples for forensic purpose has lead to complications of the investigation. Eventhough the PCR technique is a sensitive method, degraded DNA templates often yield unsatisfactory results. In this report, we present an efficiently simple method for decomposed muscle DNA extraction by QIAGEN QIAamp DNA isolation procedure combining with automated genetic analyzer for sensitive detection of amplified products. Moreover, we also demonstrate that degraded DNA prevented PCR amplification of the loci having large allele sizes.

MATERIAL AND METHOD

Samples

At the end of January 1998, a medical student disappeared from her home. Six weeks later, a man admitted being involved in events and confessed to killing her by shooting at her head and then cutting her head off. Then, he tried to destroy the corpse by cutting all the flesh from the body into small pieces and subsequently, flushing it down the toilet. Later, pieces of decomposed muscle were collected from the sewage system, sent for DNA typing and compared to the results of a blood stain on a plastic bag found in the suspected victim's car.

DNA extraction from blood stain

The blood stain on a plastic bag was cut into approximately 4 cm^2 area and sent for DNA analysis. DNA was extracted by using QIAamp blood kit (QIAGEN, Inc, Chatsworth CA). Blood cells were eluted from a target plastic by soaking for 1 hour in 200 µl of PBS buffer. DNA was extracted as described previously[4]. Briefly, the suspension was incubated at 70°C for 10 min in QIAGEN's lysis buffer AL and Proteinase K. After addition of ethanol, the lysate was applied to a spin column and centrifuged for 1 min. Subsequently, the samples were washed. After centrifugation for 1 min, 200 µl of eluted DNA was collected. Then, 20 µl of DNA was amplified *in vitro*.

DNA extraction from decomposed muscle

The decomposed muscle was separated into 2 pieces (approximately 1 cm^3 each). DNA from the first piece was extracted by Chelex followed by QIAamp blood protocol and that from the second piece was extracted solely by QIAamp tissue protocol (QIAGEN, Inc, Chatsworth CA). Chelex plus QIAamp blood protocol was modified from elsewhere[5]. Decomposed muscle was chopped into small pieces and incubated at 55°C overnight in 3 ml of White Cell Lysis Buffer [10 mM Tris-HCl (pH7.6), 10 mM Na_2EDTA, 50 mM NaCl] with the addition of 750 ml of 20 per cent Chelex, 32 ml of Proteinase K (20 mg/ml) and 62.5 ml of 10 per cent SDS. One milliliter of saturated sodium acetate was added and the tube shaken manually for 30 sec and then centrifuged at 4000Xg for 10 min. The supernatant containing DNA was transfered to a new polypropylene tube, 5 ml of 100 per cent isopropanol were added and mixed for at least 10 min to precipitate the DNA before centrifugation at 4000Xg for 10 min. The supernatant was discarded; the remaining whitish-yellow pellet of DNA was suspended in 250 µl of 70 per cent ethanol, transfered to a 1.5 ml microtube and centrifuged at 13000Xg for 1min. The pellet was dried in an incubator at 37°C, reconstituted in 250 µl of sterile distilled water and 200 µl of crude DNA extract was subsequently purified by QIAamp blood kit as described above. Twenty microliters of DNA was subsequently used for amplification *in vitro*.

QIAamp tissue kit protocol was started by cutting the muscle into small pieces and incubating the chopped muscle at 55°C overnight in 180 µl of QIAamp buffer ATL and 20 µl of Proteinase K. Then, it was followed by incubation at 70°C for 10 min in 20 µl of buffer AL. After addition of 210 µl of ethanol, the lysate was applied to a spin colum and centrifuged for 1 min. The DNA was washed twice with 500 µl of buffer AW. Finally, the DNA was eluted with 200 µl of distilled water and 20 µl of DNA was used for PCR.

Amplification of DNA samples

We co-amplified 9 STR loci: D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317, D7S820 and the segment of the X-Y homologous amelogenin gene in a single tube. The PCR amplification kit was purchased from PE Applied Biosystem (Perkin Elmer, USA). In the kit, one primer of each locus-specific primer was labeled with either the 5-FAM, JOE or NED NHS-ester dye. Amplifications were carried out in thin-walled MicroAmp tubes (Perkin Elmer) in a GeneAmp PCR System 2400 (Perkin Elmer), using the

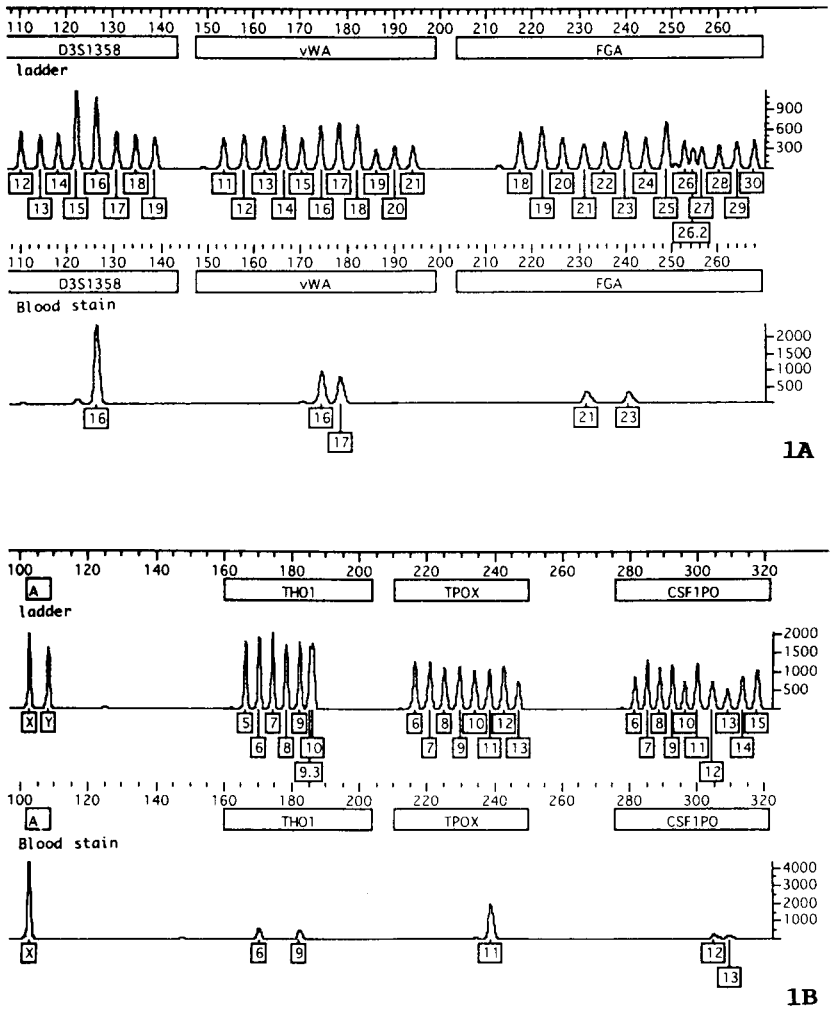

Fig. 1. DNA profile of blood stain sample. (A) the first row refers to AmpF/STR blue allelic ladder (PE Applied Biosystems) and the second row refers to STR alleles of loci D3S1358, vWA and FGA, respectively. (B) the first row refers to AmpF/STR green II allelic ladder (PE Applied Biosystems) and the second row refers to STR alleles of loci Amelogenin, THO1, TPOX and CSF1PO, respectively.

following conditions: 95°C for 13 min, 94°C for 1 min, 59°C for 1 min, 72°C for 1 min, for 28 cycles and followed by 60°C for 45 min.

The amplified products were separated by automated capillary electrophoresis (Applied Biosystem automated DNA sequencer model 310). DNA profiles were generated using Genescan and Genotyper solfware.

RESULTS

In order to identify the unknown blood stain, we compared the genotype profile of the victim with the suspected parents and the genotype showed the pattern inherited from the suspected parents (data not shown). Amplified products from blood stain showed a complete and unambiguous profile (Fig. 1 and Table 1).

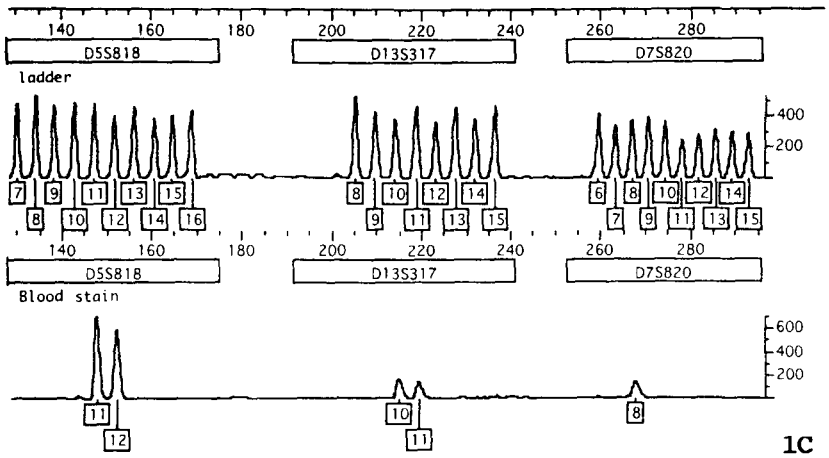

Fig. 1. DNA profile of blood stain sample. (C) the first row refers to AmpF*l*STR yollow allelic ladder (PE Applied Biosystems) and the second row refers to STR alleles loci D5S818, D13S317 and D7S820, respectively.

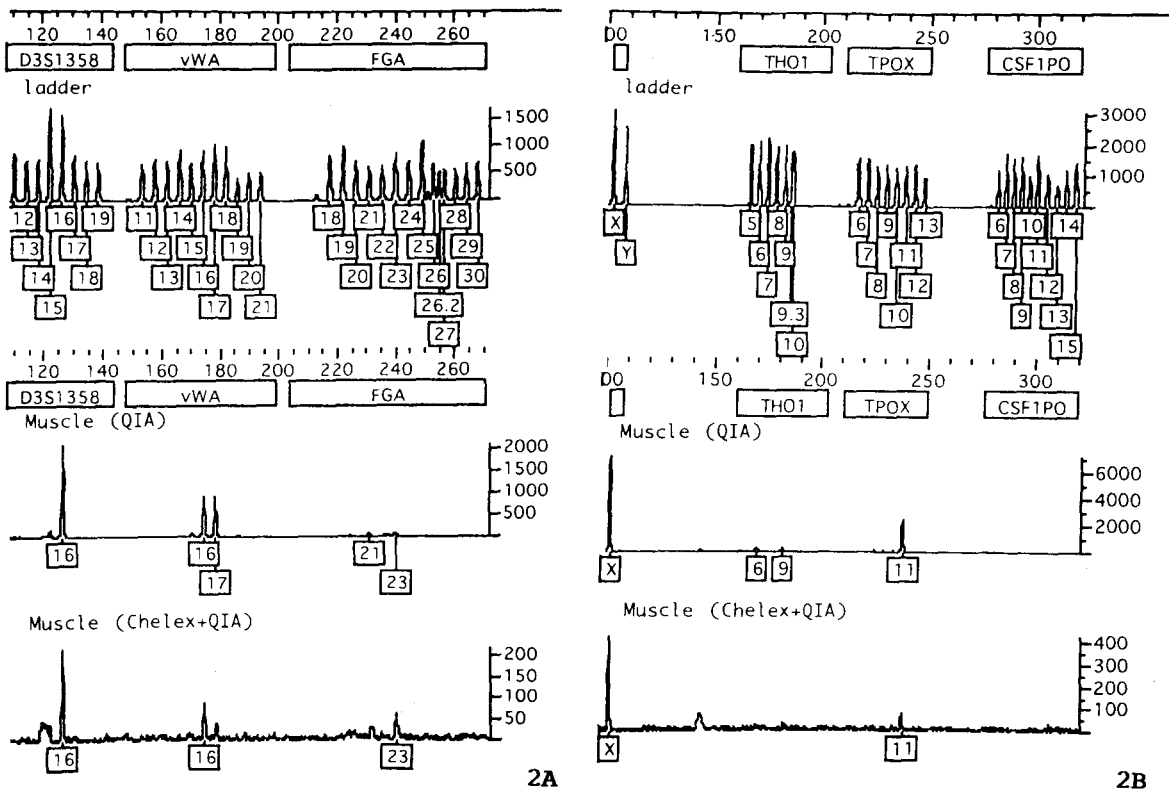

Fig. 2. DNA profile of muscle sample extracted by QIAamp protocol (QIA) or by Chelex plus QIAamp protocol (Chelex+QIA). (A) the first row refers to AmpF*l*STR blue allelic ladder (PE Applied Biosystems) and the second and third rows refer to STR alleles of loci D3S1358, vWA and FGA respectively from DNA extracted by QIAamp protocol (QIA), and by Chelex plus QIAamp protocol (Chelex+QIA). (B) the first row refers to AmpF*l*STR green II allelic ladder (PE Applied Biosystems) and the second and third rows refer to STR alleles of loci Amelogenin, THO1, TPOX and CSF1PO respectively from DNA extracted by QIAamp protocol (QIA), and by Chelex plus QIAamp protocol (Chelex+QIA).

Table 1. Genotypes of samples tested.

Sample	Genotype and size range (bp)*									
	D3S1358 (114-142)	vWA (157-197)	FGA (219-267)	Amel (107-113)	THO1 (169-189)	TPOX (218-242)	CSF1PO (281-317)	D5S818 (135-171)	D13S317 (206-234)	D7S820 (258-294)
Blood stain	16, 16	16, 17	21, 23	X, X	6, 9	11, 11	12, 13	11, 12	10, 11	8, 8
Muscle (Chelex+QIA)	16, 16	16	23	X, X	-	11, 11	-	11, 12	-	-
Muscle (QIA)	16, 16	16, 17	21, 23	X, X	6, 9	11, 11	-	11, 12	-	-

* All observed alleles differ in size by a factor of one tetranucleotide repeat unit for all loci.

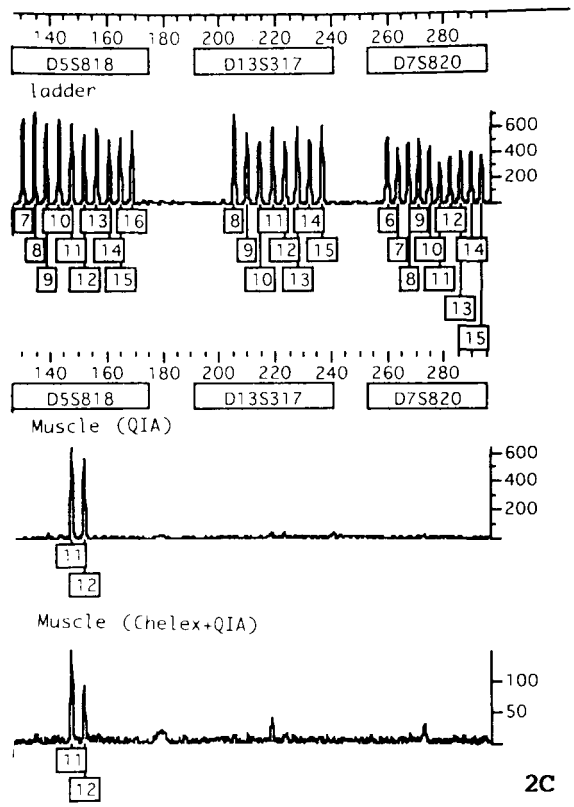

Fig. 2. DNA profile of muscle sample extracted by QIAamp protocol (QIA) or by Chelex plus QIAamp protocol (Chelex + QIA) (C) the first row refers to AmpF*l*STR yollow allelic ladder (PE Applied Biosystems) and the second and third rows refer to STR alleles loci D5S818, D13S317 and D7S820 respectively from DNA extracted by QIAamp protocol (QIA), and by Chelex plus QIAamp protocol (Chelex+QIA).

The next sample to be investigated was decomposed muscle found in the sewage system. We extracted the muscle DNA by 2 protocols: (i) Chelex plus QIAamp blood protocol and (ii) QIAamp tissue protocol. Chelex is a chelating resin that has a high affinity for polyvalent metal ions. It removes impurities from the solution and alkaline pH disrupts the cell membranes resulting in release of DNA(6). The amplified products of DNA extracted from both methods are shown in Fig. 2 and Table 1. STR alleles which could not be detected from both protocols were 3 loci, CSF1PO (size range between 281-317 bp), D13S317 (size

range between 206-234bp), and D7S820 (size range between 258-294 bp). For Chelex plus QIAamp protocol, only vWA and FGA loci were detected but THO1 allele was not detected. The percentage of detectable STR alleles in the blood stain, muscle from Chelex plus QIAamp protocol and from QIAamp tissue protocol was 100, 50 and 70 per cent respectively (Table 2).

Table 2. Percentage of STR alleles detected.

Sample	No. of STR alleles detected	%
Blood stain	20/20	100
Muscle (Chelex+QIA)	10/20	50
Muscle (QIA)	14/20	70

DISCUSSION

PCR-mediated STR analysis for routine forensic work offers several advantages over the use of DNA single locus probe profiling[7]. These include the ability to obtain results from very small samples and from those containing degraded DNA. We analyzed the STR polymorphism of DNA specimen extracted from six week postmortem blood stain and decomposed muscle. The genotype of blood stain sample was unambiguously identified, whereas, that of degraded muscle was not completely identified. The muscle DNA was isolated by two alternative methods, Chelex plus QIAamp blood protocol and QIAamp tissue protocol. The STR alleles from DNA extracted by Chelex plus QIAamp blood protocol and QIAamp tissue protocol were detected for 50 per cent and 70 per cent of all tested alleles, respectively. The detected STR alleles correlated with the results of another group who reported that the amplification for the loci with alleles of more than 200 bp would be easily inhibited, but that for loci with small size alleles was not so much affected[8]. They concluded that a minimum molecular size and amount of template DNA were needed for STR allele amplification. Eventhough an effective way of removing PCR inhibitors was to subject the DNA extract to agarose gel electrophoresis and isolate the DNA from the gel[5], we adapted using QIAGEN QIAamp protocol. Because our samples in this investigation were decomposed for six weeks prior to DNA isolation step, we could not find any DNA products from these DNA extracts in the stained agarose gel (data not shown). However, the automated capillary electrophoresis was sufficiently sensitive to detect the fluorescently tagged-PCR products. Our finding revealed that QIAamp protocol was simple and spent 2 days to obtain final results of STR polymorphism pattern. The QIAamp DNA isolation procedure is ideally suitable for preparation of samples for forensic identification both from old blood stain and decomposed tissue. In summary, the PCR-based STR analysis of DNA extraction by QIAamp procedure together with automated genetic analyzer system allowed successful DNA typing of old blood samples and decomposed tissue.

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การเปรียบเทียบการสกัดดีเอ็นเอจากคราบเลือดและกล้ามเนื้อที่เน่าเสียในการวิเคราะห์โพลิมอฟิซึมของเอสทีอาร์

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การเสื่อมสภาพของพยานวัตถุเป็นข้อจำกัดด้านคุณภาพของการวิเคราะห์ลายพิมพ์ดีเอ็นเอ ทำให้วิธีการตรวจลายพิมพ์ดีเอ็นเอวิธีดั้งเดิมนั้นไม่เหมาะสม รายงานนี้เกี่ยวกับการทำปฏิกิริยาลูกโซ่แบบ multiplex ซึ่งใช้ในการวิเคราะห์ STR จำนวน 9 ตำแหน่ง (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 และ D7S820) รวมทั้งการวิเคราะห์เพศในการวิเคราะห์ลายพิมพ์ดีเอ็นเอของตัวอย่างคราบเลือดจากผู้เสียชีวิตแล้ว 6 สัปดาห์ และตัวอย่างกล้ามเนื้อที่มีการย่อยสลายแล้วโดยวิธีการใช้ชุดน้ำยาสำเร็จรูป QIAGEN QIAamp ที่ใช้สกัดดีเอ็นเอจากเลือดและเนื้อเยื่อรวมทั้งมีการใช้เครื่องวิเคราะห์ลำดับดีเอ็นเออัตโนมัติและผลของรูปแบบดีเอ็นเอมีดังนี้ ลายพิมพ์ดีเอ็นเอของตัวอย่างคราบเลือดสามารถวิเคราะห์ STR ได้อย่างสมบูรณ์ทุกตำแหน่ง ส่วนลายพิมพ์ดีเอ็นเอของกล้ามเนื้อมี 2 แบบคือ แบบที่สกัดโดยใช้ชุดน้ำยาสำเร็จรูป QIAamp สำหรับสกัดเนื้อเยื่อ และแบบที่ใช้ชุดน้ำยาสำเร็จรูป QIAamp สำหรับสกัดเลือดโดยใช้สาร Chelex ด้วย สามารถตรวจหาลายพิมพ์ดีเอ็นเอได้ 70 และ 50 เปอร์เซ็นต์ตามลำดับ เนื่องจากการเสื่อมสลายของตัวอย่างกล้ามเนื้อมีผลกระทบต่อการเพิ่มปริมาณสารพันธุกรรมของอัลลิลย์ STR ที่มีขนาดใหญ่ซึ่งได้แก่ CSF1PO, D13S317 และ D7S820 โดยสรุปการวิเคราะห์ลายพิมพ์ดีเอ็นเอโดยใช้เทคนิคการเพิ่มปริมาณสารพันธุกรรมในหลอดทดลองสำหรับการตรวจวิเคราะห์ STR ร่วมกับการใช้เครื่องวิเคราะห์ลำดับดีเอ็นเออัตโนมัติเป็นวิธีการที่มีประสิทธิภาพและเหมาะสมในการพิสูจน์พยานวัตถุ

คำสำคัญ : ดีเอ็นเอ, เน่าสลาย, กล้ามเนื้อ

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