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Unveiling the Truth: Solving Murder Mysteries with DNA Profiles from Charred Bones

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Abstract

Murder is a heinous crime, and identifying burned bodies poses a significant challenge for forensic experts. Charred bone fragments contain precious DNA cells that can help establish the identity of the deceased. In two separate murder cases, we received charred bones from burned bodies, and successfully recovered DNA from these fragments. This DNA profiling enabled us to conclusively identify the deceased individuals and solve the murder mysteries.

Keywords: Charred bone; Sampling of DNA; Polymerase Chain Reaction (PCR); DNA profiling; Identification; Murder

Introduction

DNA technology is widely used in forensic investigations, including paternity testing, identification in sexual offense cases, and the identification of deceased individuals in murder cases [1]. Short Tandem Repeat (STR) markers, which exhibit high polymorphism, are commonly employed in DNA profiling [2]. DNA profiling from blood, hair, and saliva using STR-based analysis provides crucial evidence in a crime's investigation [3-5]. Generally common flame temperature of wood and petroleum products is around 2000°C [6]. Extracting DNA from charred bone fragments is particularly challenging due to exposure to high temperatures during burning. However, even a small number of cells present in the bones can be sufficient for DNA profiling.

Here we discuss two different cases of homicide in which bodies of the victims completely burnt. In first case, victim of the crime was murdered and cut body into two parts, one is head portion and other is body without head. First part i.e. head part was exposed to fire and burnt bone pieces of skull was buried under the earth to conceal the identity. These blackish charred bone pieces of head portion recovered from the crime scene (Figure 1). In second case, victim of the crime was murdered and kept body in car. Exposed body with car to fire to hide identity and to persuade as an accident. Burnt piece of femur bone was recovered from the burnt car on the crime scene (Figure 2).

Material and Methods

i. PrepFiler Express BTA™ Forensic DNA Extraction kits.

ii. PrepFiler Express™ Forensic DNA Extraction kit.

iii. AmpFlSTR™ Identifiler™ PCR Amplification Kit.

iv. GeneScan™ 600 LIZ™ Size Standard v2.0.

v. Hi-Di™ Formamide.

vi. Quantifiler®Duo DNA Quantification Kit.

Instruments

i. AutoMate Express™ Forensic DNA Extraction System Catalogue number: 4441763

ii. Applied Biosystems7500 Real-Time PCR System Catalogue number: 4366604

iii. Veriti™ 96-Well Thermal Cycler Catalogue number: 4375786

iv. Applied Biosystems 3500 Genetic Analyzer Catalogue number: 4406017

Samples of Burned bone pieces:

Case-1 (Figure 1)

Figure 1: Charred bone pieces of head portion.

Case-2 (Figure 2)

Figure 2: Burnt piece of femur bone.

Extraction of DNA

First, after careful inspection, a hard, slightly yellowish-brown portion of the charred bones was chosen. The charred bones surface was initially cleaned with an alcohol swab before a few of their harder sections were removed for extraction. Burned, charred bones were utilized to extract DNA. 220 µL of PrepFiler Lysis Buffer, 7 µL of Proteinase K, and 3 µL of 1M DTT were added to the Lysate tube to perform the extraction, and the mixture was then incubated for 18 hours at 56 °C and 1100 rpm in a Thermo shaker. Transfer the material into the LySep column/tube assembly after cell lysis, and centrifuge for two minutes at 10,000 rpm. The collected lysate was placed for automated extraction run using the PrepFiler Express BTA instrument protocol on the Automate Express Forensic DNA extraction system. At 4 °C, the isolated, pure DNA was kept [7]. Claimants control blood samples were also used to extract DNA using PrepFiler Express Forensic DNA Extraction Kits.

Quantification

According to the manufacturer's recommended instructions, extracted DNA was previously quantified using the Quantifiler Duo DNA Quantification Kit on an Applied Biosystems 7500 Real-Time PCR System (Foster City, CA).

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i. Bone piece-1: 0.1 ng

ii. Bone piece-2: 0.15 ng

PCR based STR Analysis

According to the manufacturer's recommended protocols, the quantified DNA was processed for STR (Short Tandem Repeat) profiling using the AmpFlSTR® Identifiler PCR Amplification Kit and the Veriti 96-Well Thermal Cycler. The Taq Gold Polymerase enzyme, PCR Reaction Mix, and Primer Set are all included in this kit. The amplification required a total of 1 ng of DNA. According to the quantification, the DNA volume was increased to 10 µL [8].

On the 3500 Genetic Analyzer system from Applied Biosystems, capillary electrophoresis was carried out by adding 1 µL /sample of PCR product to the mixture of Hi-Di Formamide (8.5 µL /sample) and GeneScan 600 LIZ Size Standard v2.0 (0.5 µL /sample). Gene Mapper® ID-X was used to analyse the material after electrophoresis was complete. Based on their sizes, several DNA molecule fragments were divided. By comparing DNA profiles with reference samples, DNA profiles were interpreted [9].

Results

The DNA extracted from the burned bone fragments was successfully typed at 15 STR loci, including the gender-specific Amelogenin locus (Table 1). In the first case, the DNA profile from the charred bone fragments matched the set of paternal alleles found in the claimant (the deceased person's child) at all 15 STR loci, confirming the identity of the deceased (Table 2). In the second case, the DNA profiles from the charred femur bone matched the obligatory paternal and maternal alleles found in the putative father and mother's DNA profiles, establishing them as the biological parents of the deceased.

Table 1

STR Locus	Genotype		
	Sternum bone Unknown male	Partly burnt bone pieces	Blood Deceased Son
D8S1179	12,12	12,12	11,12
D21S11	28,29	28,29	29,31.2
D7S820	11,11	11,11	10,11
CSF1PO	11,11	11,11	11,13
D3S1358	15,16	15,16	16,17
THO1	7,9	7,9	7,8
D13S317	9,12	9,12	11,12
D16S539	12,12	12,12	8,12
D2S1338	24,25	24,25	19,25
D19S433	13,14.2	13,14.2	13,16.2
vWA	17,19	17,19	15,19
TPOX	11,11	11,11	11,11
D18S51	14,20	14,20	14,20
AMELOGENIN	X,Y	X,Y	X,Y
D5S818	11,12	11,12	9,12
FGA	20,22	20,22	22,25

Table 2

STR Locus	Genotype		
	Blood Putative Mother	Burned femur bone	Blood Putative Father
D8S1179	11,14	14,15	13,15
D21S11	30,32.2	32.2,33.2	28,33.2
D7S820	10,10	10,12	11,12
CSF1PO	10,12	10,11	11,12
D3S1358	15,16	15,16	15,16
THO1	6,9.3	6,6	6,6
D13S317	11,11	11,12	8,12

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D16S539	9,11	9,13	12,13
D2S1338	18,19	19,19	19,23
D19S433	14,14	13,14	13,14
vWA	16,16	16,18	17,18
TPOX	9,11	11,11	8,11
D18S51	15,15	14,15	13,14
AMELOGENIN	X,X	X,X	X,Y
D5S818	11,12	12,13	11,13
FGA	22,24	21,22	21,22

Conclusion

i. DNA profiling from charred bone fragments has enabled the identification of the deceased individuals in these murder cases. Despite attempts to conceal the victims, identities by burning their bodies, our successful recovery and analysis of DNA from the charred bone fragments proved crucial in solving the murder mysteries. The application of PrepFiler Express BTA Forensic DNA Extraction kits and careful sampling techniques has demonstrated the efficacy of DNA profiling from charred bones.

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