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Establishment of maternity by allelic comparison from the muscle tissue attached with femur bone of unidentified dead body

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Abstract

Femur bone is one of the common exhibit received in the forensic laboratories for DNA profiling from mass disasters, drowning, terrorist attacks, wars, explosion, forest fires, etc. In these cases, visual identification of deceased child's is difficult. However, identification of such dead bodies is important for socio-legal purposes. Femur is largest bone of human thigh and DNA profile generated from this bone can play important role in the identification of victims. In this study, DNA profiles were generated from muscle tissue attached on femur bone of a deceased child and blood sample on FTA (Flinders Technology Associates) card of the putative mother. DNA from muscle tissue was isolated using magnetic bead based method with Qiagen EZ1 Advanced XL BioRobot, whereas, FTA card was purified using novel purification buffer. The DNA was subjected to Multiplex PCR amplification using GlobalFiler™ PCR Amplification Kit (ThermoFisher Scientific, U.S.A.). Capillary electrophoresis were done with 3500 XL Genetic Analyzer (Applied Biosystems, U.S.A.) and data were analyzed using GeneMapper™ ID-X Software v 1.6. The autosomal DNA analysis confirmed that deceased child was the biological son of putative mother.

Keywords: DNA, Femur, FTA, Maternity, Mother.

Introduction

Unidentified dead bodies are recovered from cases such as mass disasters[1,2], armed conflicts[3], historical characters[4], disaster victim identification[5], terrorist bomb attacks[6], etc. The body of victims gets so much damaged in these cases that their identification cannot be established with dactyloscopy, facial recognition, odontology, etc.[7]. The skeletal remains are the only source of evidence for DNA profiling from such types of cases[8,9]. Among skeletal remains, bone and dental materials are the best exhibits for identification of victims because preservation of DNA is better in these sources[10]. Bones are a good source of DNA from forensic, anthropological and archaeological perspectives[11]. The success of DNA profiling from bones depends on the level of DNA damage, the amount of DNA recovered and presence/absence of inhibitors[12]. However, there are numerous challenges on extraction of DNA from bones because of the structure and chemical composition of bone[13]. In addition to this, aged bone samples are difficult for DNA profiling[14].

Among different bones, femur is one of the most common exhibit received in forensic laboratories for DNA profiling as DNA remain better preserved in these bones. Sometimes, fresh tissue remains attached to femur bones, which can act as a good source for DNA profiling. In this case, maternity was established from DNA isolated from muscle tissue of deceased's femur bones.

In this case, a child went to his relative's house to spend holidays. He, along with friends, went to the river for swimming and got drowned. Despite of efforts, his life could not be saved. Later on, his body was recovered in putrified condition. On the basis of his clothes, his relatives identified the child. After post-mortem, medical officers sent femur bone of deceased child to the DNA Division, State Forensic Science Laboratory, Junga, Himachal Pradesh (Figure-1a,b,c) as a routine case work. In order to establish maternity, the blood sample of the putative mother on FTA card was also received in the forensic laboratory. The DNA profiles were generated and maternity was established by comparing their DNA profiles.

Materials and methods

Materials: Femur bone of the deceased child and blood sample of putative mother on FTA card were labelled as A and B, respectively. EZ1 DNA Investigator Kit was purchased from QIAGEN India Pvt. Ltd. - New Delhi, India.

Methods: The DNA isolation from muscle tissue attached on femur bone of the deceased child was done by magnetic bead based method with slight modifications[15]. Muscle tissue was chosen because it was not degraded and can save time for DNA extraction as compared to powder method from femur bone. In brief, muscle tissue was added in a autocleaved microvial (1.5 ml) and washed once with autoclaved distilled water. To this

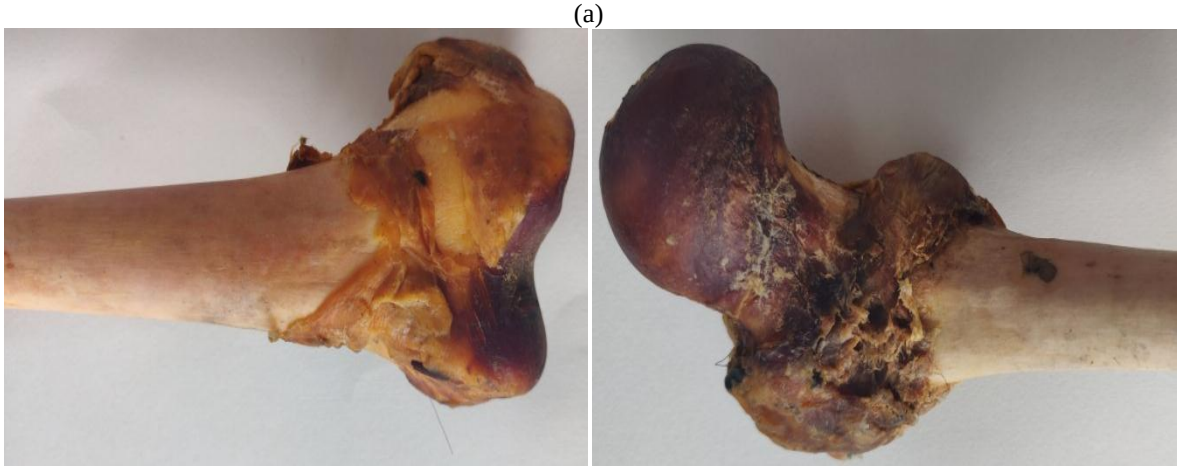
microvial, buffer G2 (350µl) and proteinase (15µl) K was added and lysed in a NB 20 water bath (Nuve, Ankara, Turkey) at 56°C for 48h. After lysis, the lysate was poured into sample tube for DNA extraction. Elution tube, tip holder containing filter-tip and reagent cartridge were inserted in EZ1® Advanced XL BioRobot (QIAGEN, Hilden, Germany). The "Large-Volume Protocol" was set for DNA isolation. The isolated DNA was stored at -20°C in a refrigerator (Celfrost, India) for further use. The isolated DNA was also quantified using agarose gel electrophoresis (0.8%) at 200V (Bio-Rad, Hercules, California, United States) for PCR amplification.

The DNA from FTA card bearing blood sample of putative mother was purified as per method given by Sahajpal et al.[16] with slight modifications. FTA card was punched with the help of Harris 1.2 mm micro punch. The punches were added in a microvial along with novel FTA purification reagent (100µl) and proteinase K (15µl). The punches were incubated at 56°C in a NB 20 water bath (Nuve, Ankara, Turkey) for 30 minutes and then washed with autoclaved water and dried in a digital dry bath (Labnet International, U.S.A.) for 30 minutes. The FTA card punches were stored at -20°C in a refrigerator (Celfrost, India) for further use.

PCR amplification: The amplification of DNA was done as per protocol given in GlobalFiler™ PCR Amplification Kit[17]. In brief, master mix (7.5µl) and primer set (2.5µl) was added in two separate labelled PCR tubes. The contents were mixed thoroughly and approximately 1.0ng of DNA from muscle tissue of femur bone and one punch of dried FTA card of putative mother was added in these tubes. The contents were mixed by tapping and spun in SPINWIN microcentrifuge (Tarsons, India). The amplification was done with Veriti™ 96-Well Thermal Cycler (Applied Biosystems, U.S.A.). Control DNA 007 was used as positive control as per kit manual, whereas nuclease free water was used as negative control. The following protocol was set for PCR amplification: 95°C for 1 minute, 94°C for 10 seconds, 59°C for 90 seconds, 60°C for 10 minutes for 29 cycles, then 4°C hold. The amplified products were stored at 4°C in a refrigerator.

Capillary electrophoresis and genotyping: Capillary electrophoresis of PCR products were done with ABI 3500 XL Genetic Analyzer (Applied Biosystems, U.S.A.) with following run conditions: Run module: HID36_POP4, Injection conditions: 1.2 kV/24 seconds, Run conditions: 13 kV/1550 seconds, POP-4 and genotyping was carried out using GeneMapper ™ID-X Software v 1.6.

(a)



(b) (c)

Figure-1: Femur bone of unidentified deceased child (a) bone with muscle tissue attached at both ends (b) distal end of femur with reddish muscle tissue (c) head of femur with dark reddish muscle tissue.

Results and discussion

The genotypes of the muscle tissue of femur bone of deceased child (A) and blood sample of the putative mother on FTA card (B) are given in Table-1. GlobalFiler™ PCR Amplification Kit is 24 loci kit in which Y indel, Amelogenin and DYS391 are sex determining markers. Y indel and DYS391 are male specific markers. As shown in the table, Y indel marker in muscle tissue of the femur bone of deceased child (A) showed 2 allele, whereas Amelogenin and DYS391 markers showed XY and 10 alleles, respectively, which confirmed the deceased child was male. Clean DNA profiles were obtained from both samples (A and B). The electropherograms of sample A and sample B are given in Figure-2 and Figure-3, respectively. The genotype of muscle tissue of the deceased child (A) showed match with one of two alleles in the genotype of blood sample of the putative mother on FTA card (B) at 21 loci (underline), which followed the law of Mendelian Inheritance. This data confirmed that deceased child was the biological son of putative mother. The positive control showed alleles as given in the kit manual, whereas no amplification was observed in negative control, which confirmed that there was no deviation in control samples. Similar to this study, Abuidrees et al[18] isolated DNA from clavicle and femur bones of an aborted buried son. The DNA profiling from bones, the suspect and female, who delivered the baby was done and suspect was excluded being alleged father. However, female suspect was found to be biological mother of aborted son. Jeffrey et al[19] isolated trace amounts of degraded human DNA from shaft of the femur of skeletal remains of Dr Josef Mengele, which were exhumed in Brazil in 1985. Comparison of femur DNA was done with Josef Mengele's son and his wife, which confirmed the paternity of Mengele's son. In another study, Siriboonpiputtana et al.[20] performed DNA profiling from femur bones from different samples and obtained complete profiles in most of cases. Zgonjanin et al.[13] did DNA analysis of fifty five femur bones from human skeletal remains and obtained good results. Johnston and Stephenson[21] performed Short Tandem Repeat (STR) profiling on bones and teeth from degraded 2595 skeletal remains of eleven cases in Guatemala. The best DNA profiling results were obtained from femur bones (36.2%), whereas for teeth, it was 33.7%. These data confirmed that femur bones are a good source of DNA from degraded human body. However, contamination free DNA extraction from these bones is a laborious work. The muscle tissue attached to them can act as an alternative to this problem.

Table-1: The genotypes of muscle tissue of the femur bone of deceased child and blood sample on FTA card of putative mother.

Locus	Positive control (Control DNA 007)		Negative control	Muscle tissue of femur bone of deceased child (A)		Blood sample of putative mother on FTA card (B)	
	Allele 1	Allele 2	Alleles	Allele 1	Allele 2	Allele 1	Allele 2
D3S1358	15	16	-	15	17	15	17
vWA	14	16	-	15	17	15	17
D16S539	9	10	-	8	9	9	14
CSF1PO	11	12	-	11	12	12	12
TPOX	8	8	-	8	8	8	10
Y indel	2		-	2		-	
Amelogenin	X	Y	-	X	Y	X	X
D8S1179	12	13	-	13	14	13	15
D21S11	28	31	-	28	32.2	27	32.2
D18S51	12	15	-	14	15	15	18
DYS391	11		-	10		-	
D2S441	14	15	-	11	11	10	11
D19S433	14	15	-	13	15	13	13
TH01	7	9.3	-	6	9	6	8
FGA	24	26	-	23	24	21	23
D22S1045	11	16	-	15	15	11	15
D5S818	11	11	-	11	13	12	13
D13S317	11	11	-	7	11	11	11
D7S820	7	12	-	8	10	9	10
SE33	17	25.2	-	23	28.2	21.2	23
D10S1248	12	15	-	15	16	15	15
D1S1656	13	16	-	10	15.3	15.3	16
D12S391	18	19	-	18	20	18	20
D2S1338	20	23	-	18	23	19	23

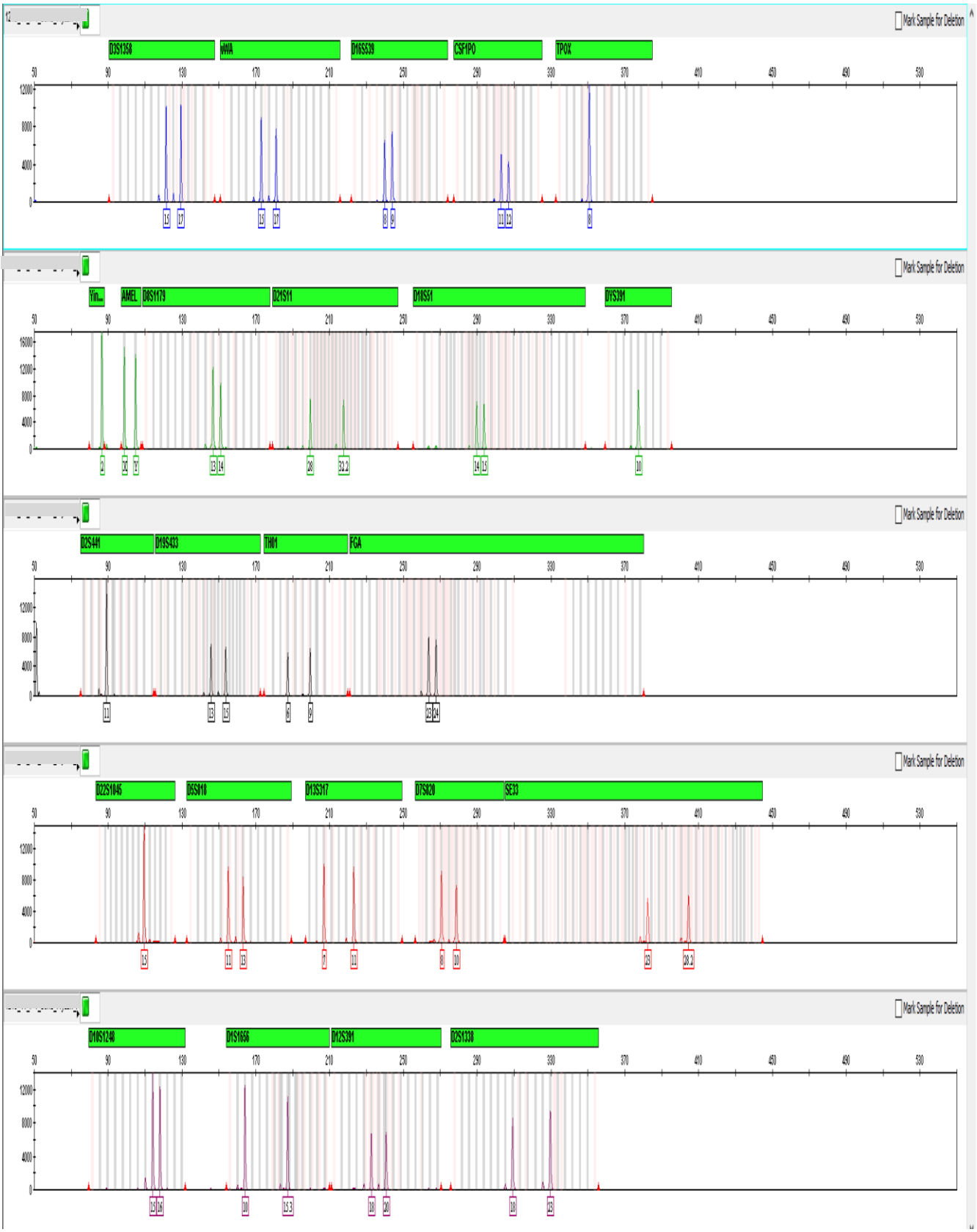


Figure-2: Electropherogram of DNA isolated from muscle tissue of femur bone of the deceased child (A).

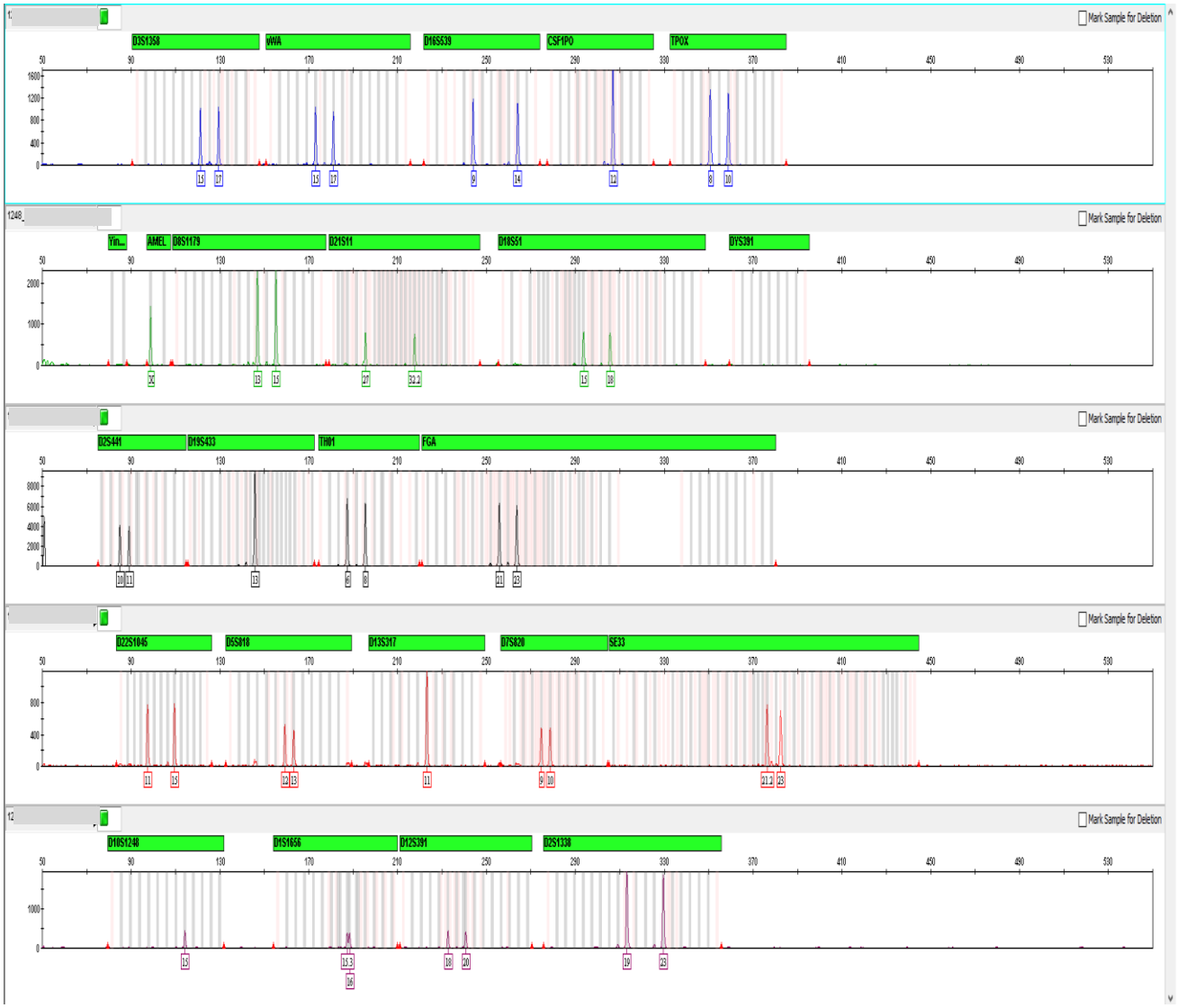


Figure-3: Electropherogram of DNA isolated from blood sample on FTA card of the putative mother (B).

Discussion: This study established maternity from muscle tissue of femur bone of the deceased child and blood on FTA card of putative mother. Femur bones are a good source of DNA profiling in case of unidentified dead bodies found in mass disasters, terrorist attack, etc. However, it is very time consuming to extract DNA from femur bones and there are more chances of contamination. Hence, if muscle tissue is attached to femur bones, it can serve as good source of DNA.

Conclusion

DNA profiling from degraded human remains is a challenging task. Forensic laboratories obtain degraded human exhibits such as bones (femur, humerus, tibia, fibula), teeth, etc. from plane crashes, fire accidents, terrosist attacks, disasters. These exhibits are either obtained for identification purpose or establishment of paternity/maternity. However, femur bone is considered an excellent source of DNA from degraded samples. To extract contamination free DNA from these bones is time consuming. Forensic laboratories should establish efficient procedures to get pure DNA from femur bone with less chances of contamination. Inhibitors, also play a role in obtaining partial DNA profiles from these bones, hence PCR inhibitor removal should also be included in DNA profiling. Clean DNA profiles obtained from femur bones can be used to generate DNA databases.

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References

1. Holland, M.M., Cave, C.A., Holland, C.A. and Bille, T.W. (2003). Development of a quality, high throughput DNA

analysis procedure for skeletal samples to assist with the identification of victims from the world trade center attacks. *Croat. Med. J.*, 44(3), 264-272.

2. Budowle, B., Bieber, F.R. and Eisenberg, A.J. (2005). Forensic aspects of mass disasters: strategic considerations for DNA-based human identification. *Leg. Med.*, 7(4), 230-243.

3. Marjanović, D., Metjahić, N.H., Čakar, J., Džehverović, M., Dogan, S., Ferić, E., Džijan, S., Škaro, V., Projić, P., Madžar, T., Rod, E. and Primorac, D. (2015). Identification of human remains from the Second World War mass graves uncovered in Bosnia and Herzegovina. *Croat. Med. J.*, 56(3), 257-262.

4. Ambers, A., Gill-King, H., Dirkmaat, D., Benjamin, R., King, J. and Budowle, B. (2014). Autosomal and Y-STR analysis of degraded DNA from the 120-year-old skeletal remains of Ezekiel Harper. *Forensic. Sci. Int. Genet.*, 9, 33-41.

5. Prinz, M., Carracedo, A., Mayr, W.R., Morling, N., Parsons, T.J., Sajantila, A., Scheithauer, R., Schmitterh, H., and Schneider, P.M. (2007). DNA Commission of the International Society for Forensic Genetics (ISFG): Recommendations regarding the role of forensic genetics for disaster victim identification (DVI). *Forensic. Sci. Int. Genet.*, 1(1), 3-12.

6. Delannoy, Y., Delabarde, T., Plu. I., Legrand, L., Taccoen, M., Tracqui, A. and Ludes, B. (2019). Terrorist explosive belt attacks: specific patterns of bone traumas. *Int. J. Legal. Med.*, 133, 565-569.

7. Westen, A.A., Gerretsen, R.R.R. and Maat, G.J.R. (2008). Femur, rib, and tooth sample collection for DNA analysis in disaster victim identification (DVI). *Forensic. Sci. Med. Pathol.*, 4, 15-21.

8. Hagelberg, E., Sykes, B. and Hedges, R. (1989). Ancient bone DNA amplified. *Nat.*, 342(6249), 485.

9. Hagelberg, E., Gray, I.C. and Jeffreys, A.J. (1991). Identification of the skeletal remains of a murder victim by DNA analysis. *Nat.*, 352(6334), 427-429.

10. Duijs, F.E. and Sijen, T. (2020). A rapid and efficient method for DNA extraction from bone powder. *Forensic. Sci. Int. Rep.*, 2, 100099.

11. Ambers, A., Votrubova, J., Vanek, D., Sajantila, A. and Budowle, B. (2018). Improved Y-STR typing for disaster victim identification, missing childs investigations, and historical human skeletal remains. *Int. J. Legal. Med.*, 132(6), 1545-1553.

12. Putkonen, M.T., Palo, J.U., Cano, J.M., Hedman, M. and Sajantila, A. (2010). Factors affecting the STR amplification success in poorly preserved bone samples. *Investig. Genet.*, 1, 9.

13. Zgonjanin, D., Antov, M., Alghafrid, R., Petković, S., Vuković, R., Stojiljković, G. and Toljića, D. (2017). DNA analysis from human skeletal remains in forensic casework. *Forensic. Sci. Int. Genet. Suppl. Ser.*, 6, e342-e345.

14. Vanek, D., Saskova, L. and Koch, H. (2009). Kinship and Y-chromosome analysis of 7th century human remains: novel DNA extraction and typing procedure for ancient material. *Croat. Med. J.*, 50(3), 286-295.

15. QIAGEN (2021). EZ1 DNA Investigator Handbook. https://www.qiagen.com/ca/resources/download.aspx?id=46064856-1b88-4b27-a825-d3f616e06c08&lang=en.17 January2021.

16. Sahajpal, V., Rajput, S., Sharma, T., Sharma, A. and Thakar, M.K. (2019). Development and evaluation of a novel DNA purification buffer and protocol for blood samples on FTA cards. *Forensic. Sci. Int. Rep.*, 1, 100014.

17. Thermofisher (2021). GlobalFiler™ and GlobalFiler™ IQC PCR Amplification Kits. https://assets.thermofisher.com/TFS-Assets/LSG/manuals/4477604.pdf. 17 January 2021.

18. Abuidrees, A.S., Alhamad, N.A. and Alsaadany, K. (2016). A suitable method for DNA extraction from bones for forensic applications: A case study. *Arab. J. Forensic. Sci. Forensic. Med.*, 1(3), 346-352.

19. Jeffreys, A.J., Allen, M.J., Hagelberg, E. and Sonnberg, A. (1992). Identification of the skeletal remains of Josef Mengele by DNA analysis. *Forensic. Sci. Int.*, 56(1), 65-76.

20. Siriboonpiputtana, T., Rinthachai, T. and Shotivaranona, J. (2018). Forensic genetic analysis of bone remain samples. *Forensic. Sci. Int.*, 284, 167-175.

21. Johnston, E. and Stephenson, M. (2016). DNA profiling success rates from degraded skeletal remains in Guatemala. *J. Forensic. Sci.*, 61(4), 898-902.