Human identification through DNA analysis of teeth using powder-free method – A case study

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ABSTRACT

Change is the universal law of nature, and human bodies after death cannot be an exception for a long time. In forensic science, the tissue from the hardest part of the human body is the only hope to establish the identity, and maternity/paternity of unidentified dead bodies. In this case, a foreign national on a tourist visa to one of the Himalayan states went missing when passing through a dense forest. His relatives could not trace him despite the best efforts of the search team, because of inaccessible hilly terrain. Later on, shepherds while grazing their livestock in the forest area accidentally came across the fragmented remains of a human skeleton. They informed the villagers, and then the police. Teeth collected during the autopsy and blood samples of the putative son, and wife of the missing foreign national on FTA (Flinders Technology Associates) cards were sent to DNA Division, State Forensic Science Laboratory, Junga, Shimla, Himachal Pradesh to establish the identity. DNA profiles obtained from the blood samples of the putative son, wife of missing foreign national, and teeth showed a complete, and concordant match, which established the identity of the skeleton. Moreover, the probability of paternity (>99.99%) between unidentified deceased person and the putative son also assessed the identity of the deceased. Hence, human teeth from unidentified dead bodies can establish the identity of unidentified deceased persons.

INTRODUCTION

Human identification based on teeth is needed if the possibility of achieving identification is to be maximised.[1] Forensic disciplines use teeth in establishing identity and maternity/paternity of unidentified human skeletal remains because of easy access, availability, and resistance of useful tissue to degradation even in extreme environmental conditions.[2,3] Further studies over time in this area suggest that the preservation of DNA in teeth is better than in bones.[4,5] Moreover, DNA of high quality can be isolated from teeth.[6,7] There is also less chance of contamination of DNA isolated from teeth.[8] Teeth were used as a source of DNA from mass graves resulting from wars, and armed conflicts.[9,10] Hence, teeth play an important role in forensic science. In the present case, human identity was established from the teeth of a foreign national found dead in a remote Himalayan area. According to investigating officers, the wife of the deceased reported her missing husband at the local Police station. She

made a statement that her husband went trekking in the forest and did not return. Police and search teams, despite their best efforts, could not find the person. After a few days, local shepherds, while grazing their livestock in the forest found a partially decomposed and skeletonized human body, eaten by animals. They first informed the villagers and then the police. Based on torn pieces of clothing, found at the site, the wife of the deceased recognized the clothing as her husbamd's. To confirm the identity, the teeth of the deceased person, blood samples of the putative son, and wife of the deceased on FTA cards were received in the DNA Division, State Forensic Science Laboratory, Junga, Shimla, Himachal Pradesh. By comparing the DNA profiles of the deceased person with putative son, the probability of paternity confirmed the identity of the unidentified deceased person.

MATERIAL AND METHODS

Materials

Seven intact and healthy teeth (molar and canine) of the unidentified deceased person, blood samples of putative son and deceased's wife on FTA cards were received for routine casework analysis at DNA Division, State Forensic Science Laboratory, Junga, Shimla, Himachal Pradesh and labelled as A, B, C, respectively. Since molar teeth contain optimum DNA, they are selected for DNA retrieval using the EZ1 DNA Investigator Kit from QIAGEN, Hilden, Germany. The PowerPlex® 21 PCR amplification kit was procured from Promega Corporation, Wisconsin, United States.

Methods

DNA isolation

The powder-free method was used for DNA isolation from the tooth with Qiagen EZ1 Advanced XL BioRobot.[11,12] In brief, the molar tooth was cleaned properly by scraping with a sterilized blade. The tooth was vortexed with absolute alcohol in a falcon tube and kept for two hours to remove microbial contamination. The tooth was drained of alcohol and dried at room temperature. After complete drying, the tooth was fractured with a hammer and all the pieces, with the root, were placed in an autoclaved micro vial (1.5 ml). Added to this were, buffer G2 (500 µl), proteinase K (25 µl) and lysed at 56°C for 72 hours in a NB 20 water bath (Nuve, Ankara, Turkey). The lysate containing the extracted DNA was poured into a sample tube (2 ml) and the remaining tooth pieces were discarded. Then inserted into the elution tube, was a tip holder containing filter-tip, and reagent cartridge in an EZ1® Advanced XL BioRobot (QIAGEN, Hilden, Germany) as per instructions given in the handbook. The DNA isolation was done with "Large-Volume Protocol" without adding MTL buffer, as there was sufficient lysate. The isolated DNA was stored at -20°C in a refrigerator (Celfrost, India) for further use. The DNA from FTA cards was purified as per method with slight modifications[13]. The FTA cards bearing blood samples of putative son and deceased's wife were punched with the help of Harris 1.2 mm micro punch. Punches were added in two separate micro vials containing FTA purification reagent (200 µl) and proteinase K (25 µl). The micro vials containing punches were incubated at 56°C in a NB 20 water bath (Nuve, Ankara, Turkey) for two hours, then washed twice with autoclaved distilled water and dried in a digital dry bath (Labnet International, U.S.A.). The dried FTA card punches were stored at -20°C in a refrigerator (Celfrost, India) for further use.

Pcr amplification

The isolated and purified DNA were subjected to PCR amplification with PowerPlex® 21 System kit[14]. In brief, master mix (5 µl) and primer mix (5 µl) was added in three separate PCR tubes followed by isolated DNA (15 µl) from the tooth (A) and one punch from FTA cards of the putative son (B) and deceased's wife (C). To complete reaction mixture in PCR tubes containing FTA card punches (B and C), 15 µl nuclease-free water was also added. The contents in the PCR tubes were mixed thoroughly and spun in a SPINWIN microcentrifuge (Tarsons, India). The PCR tubes were put into GeneAmp®PCR System 9700 thermocycler (Applied Biosystems, U.S.A.) and amplification was performed according to the manufacturer instructions for the PCR amplification kit. The amplified products were quantified using agarose gel electrophoresis (2%). The appropriate dilutions were made for further capillary electrophoresis.

Capillary electrophoresis
Capillary electrophoresis of amplified products was carried out with ABI 3130 Genetic Analyzer with 4-capillary using POP-4 at a current of 15 Ampere (Applied Biosystems, U.S.A.). The genotyping was carried out using GeneMapper® ID Software Version 3.2.

Biostatistical calculations
The paternity index (PI) for each marker was calculated using the following equation:

PI =X/Y

Where, X = chances of alleged father is the biological father

Y = any randomly selected person from concerned population is the biological father

The combined paternity index (CPI) was also calculated by multiplying PI values. Using CPI value, probability of paternity was calculated using following formula:
Probability of paternity = CPI/CPI + 1 x 100
These values help in inclusion or exclusion of the alleged father to be biological father of putative son.

RESULTS

The Punnett square table of autosomal STR DNA profiles generated from the PowerPlex®21 kit is shown in table 1. Amelogenin is a gender determining marker, and help in the identification of the sex of the individual. As shown in the table, amelogenin displayed "XY" alleles in the profile of the unidentified deceased person (A), which confirmed that the person was male. The electropherogram also showed amplification at all the 21 autosomal STR loci (Fig. 1). A complete DNA profile with amplification at all the 21 autosomal STR loci was also obtained from the putative son (B) with "XY" alleles at amelogenin (Fig. 2). By comparing these DNA profiles, it was observed that one of the two alleles in the genotype of the unidentified deceased person (A) showed a match with one of the non-maternal alleles found in the genotype of the putative son (shown as bold and underlined) at 20 loci except for amelogenin. This data included that unidentified deceased person as the biological father of the putative son. In addition to this, the genotype of deceased's wife (C) at amelogenin showed "XX" alleles, confirming female individual (Table 1). The electropherogram of the deceased's wife showed amplification at all the 21 autosomal STR loci (Fig. 3). One of the two alleles in the genotype of the deceased's wife also showed a match with one of the two maternal alleles in the genotype of the putative son (B) at 20 autosomal STR loci except for amelogenin (non-underlined). This data included that the deceased's wife as the biological mother of the putative son. The transfer of parental alleles (unidentified deceased person and his wife) to son followed the law of Mendelian inheritance, which assessed the identity of the deceased. Moreover, the combined paternity index (CPI) in this case was calculated to be 1,880,0922,263,36 (1.88009E+12), which resulted in the probability of paternity of >99.99%. This data also suggested that unidentified deceased person cannot be excluded as the biological father of putative son. Hence, identity of unidentified deceased person was established.
Teeth play an important role in forensic science to confirm the identity and maternity/ paternity of the unidentified dead body. The identification of such dead bodies is important for socio-legal purposes. Teeth are used for identification purposes for unidentified dead bodies. Kaur et al.[15] in a case, developed DNA profile from the teeth of an unidentified decomposed human body, who was murdered and established paternity by comparing the DNA profiles with putative children. Sweet and Sweet[16] solved a female homicide case in which the victim was completely burnt with gasoline and it became difficult to identify the person. However, the teeth of the victim tolerated the extreme temperature of gasoline and the authors were able to isolate high molecular weight DNA from the dental pulp of molar teeth. DNA profiling was done and the victim was identified. Dutra Correa et al.[17] developed full DNA profiles from the teeth of a carbonized and a skeletonized human body using the non-powder method. In another report, Xavier et al.[18] evaluated the possibility of DNA extraction from primary teeth in disaster victim identification with probative value. Recently, Dutra Correa et al.[3] performed human identification by DNA typing of healthy and restored teeth of exhumed remains. These findings suggest that teeth are less prone to degradation and contamination.[19] Further, conventional method for teeth samples has a scope for loss of DNA yield besides contamination issues. But powder-free method

involves all parts of tooth viz. enamel, dentine, pulp, cementum, and root which result in high quality DNA yield leading to complete DNA profiles for comparative analysis. Hence, teeth are a good source of DNA from decomposed human bodies for identification purposes.

Table 1. Punett square table of the analysed samples

Genetic markers	Unidentified deceased person (A)		Putative son (B)		Deceased's wife (C)	
	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2
Amelogenin	X	Y	X	Y	X	X
D3S1358	13	18	17	**18**	17	17
D1S1656	14	17.3	12	**14**	11	12
D6S1043	12	19	12	**19**	12	13
D13S317	11	14	8	**11**	8	8
Penta E	12	14	11	**12**	10	11
D16S539	11	11	**11**	12	8	12
D18S51	12	16	16	**16**	12	16
D2S1338	22	23	**23**	24	20	24
CSF1PO	11	12	11	**11**	11	12
Penta D	9	9	**9**	10	10	13
TH01	8	8	**8**	9.3	6	9.3
vWA	17	17	**17**	18	14	18
D21S11	27	33.2	**27**	30	30	31
D7S820	11	12	8	**12**	8	8
D5S818	13	13	13	**13**	12	13
TPOX	11	11	11	**11**	8	11
D8S1179	10	15	**10**	16	14	16
D12S391	18.3	23	21	**23**	18	21
D19S433	14	16	14	**16**	13	,14
FGA	22	24	**24**	25	23	25

Figure 1. Electropherogram of DNA from teeth of unidentified deceased person (A)

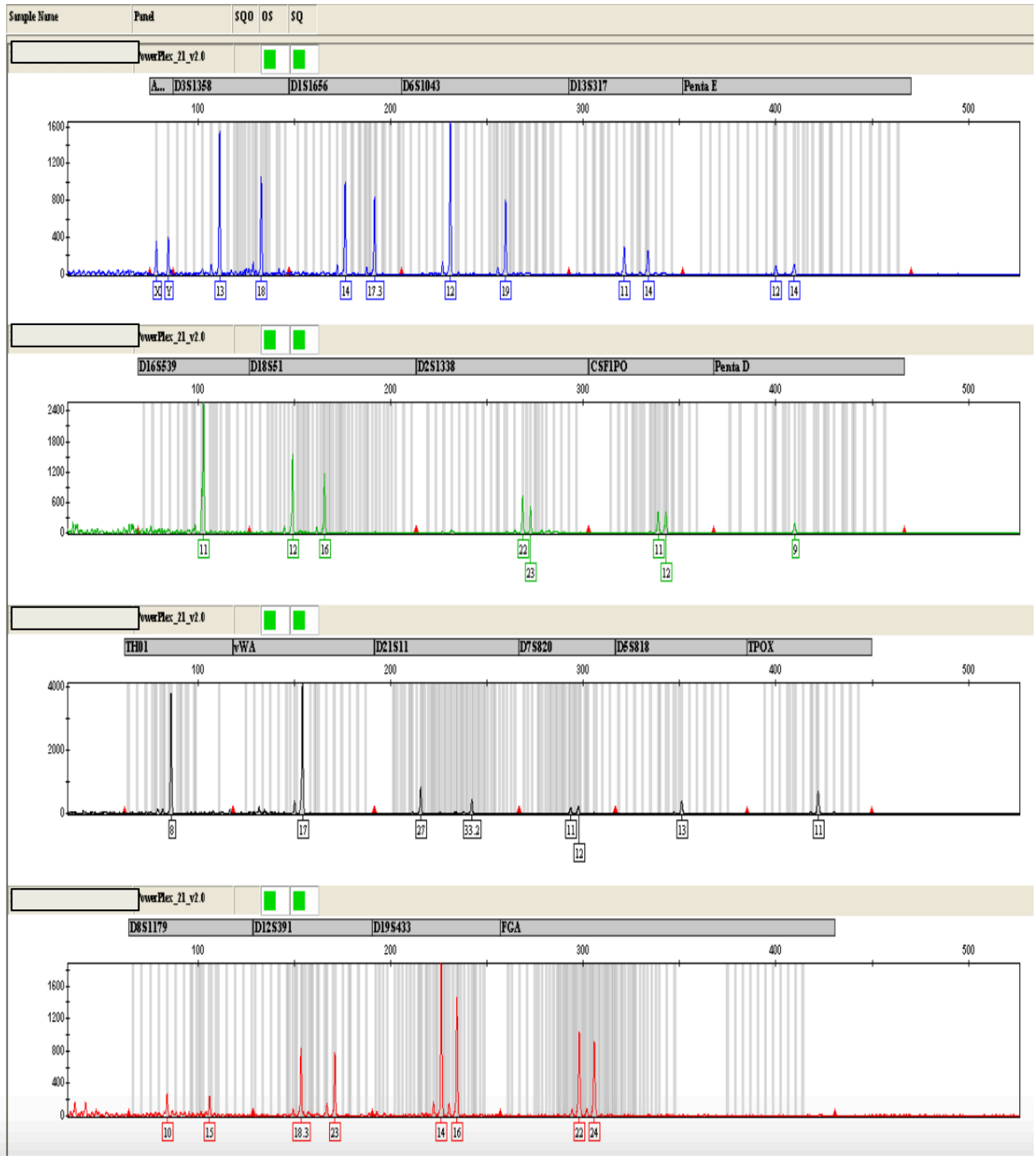


Figure 2. Electropherogram of DNA from FTA card of putative son (B)

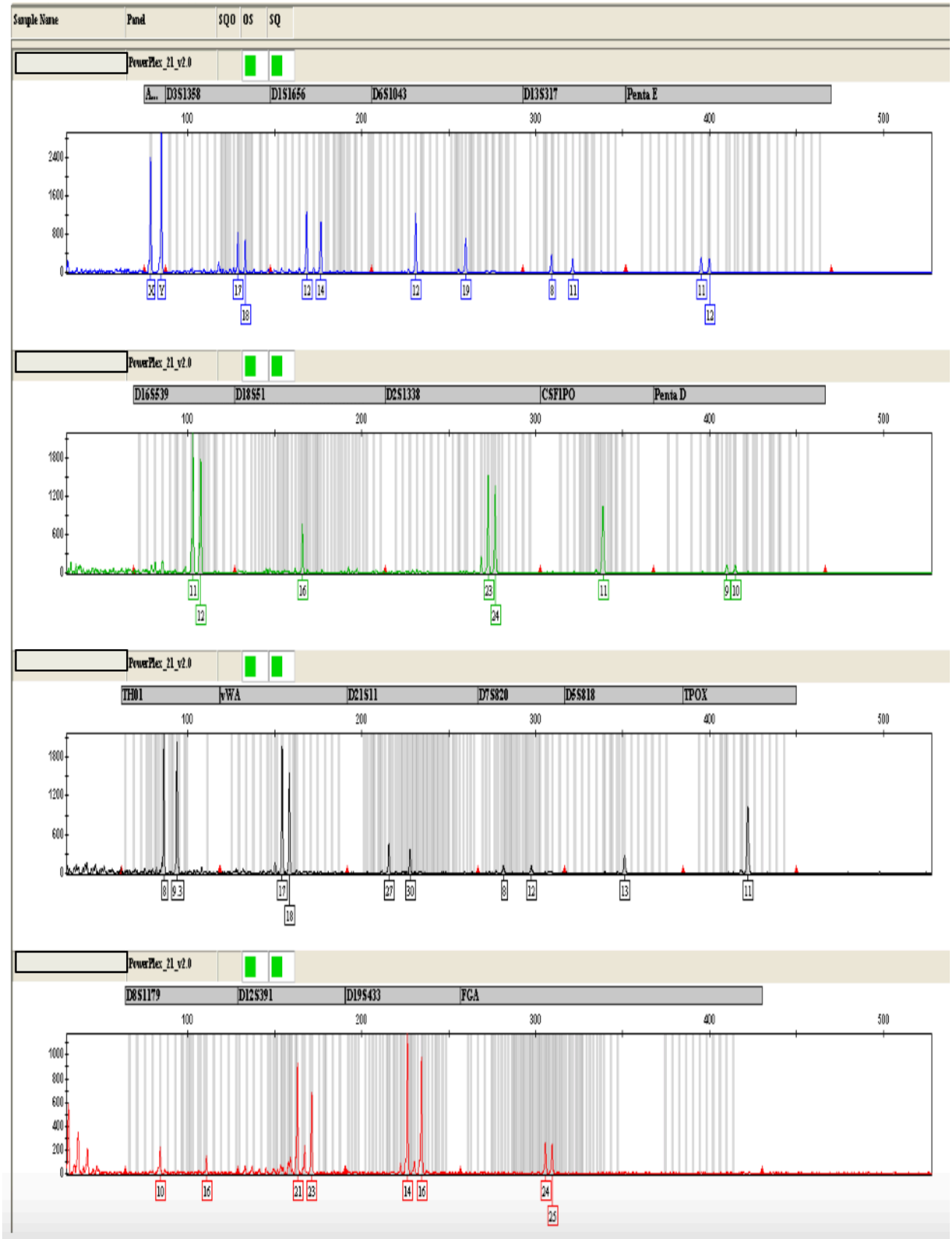
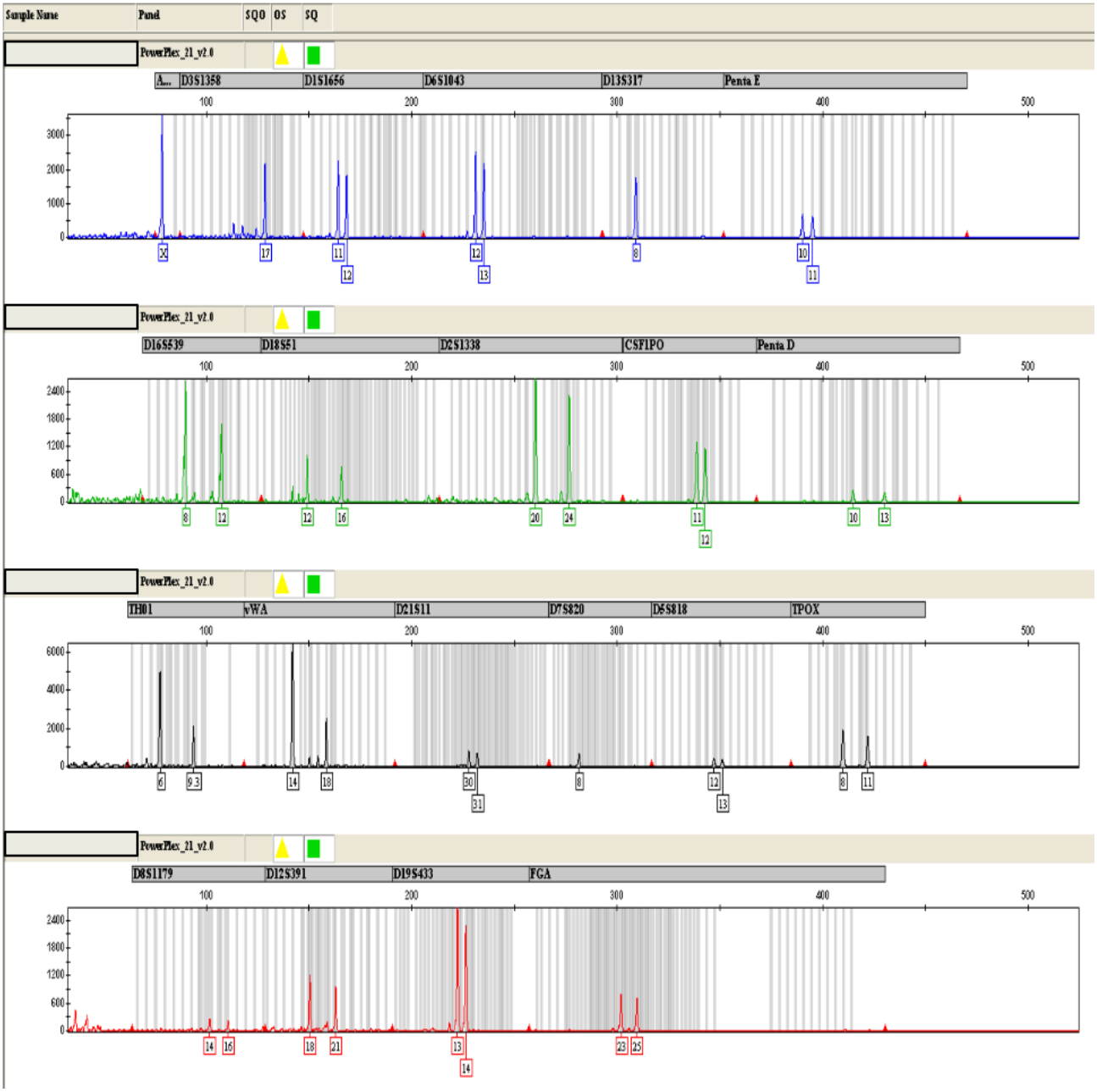


Figure 3. Electropherogram of DNA from FTA card of deceased's wife (C)



CONCLUSIONS

DNA profiling plays an important role in the identification of decomposed human bodies. In this scenario, teeth are important exhibits for DNA profiling, since teeth protect DNA from harsh environmental conditions as compared to other body parts. DNA profiles generated from teeth can be used to establish identity, maternity, and paternity. However, there are few studies available on this topic. There should be more studies on the establishment of identity from the teeth of unidentified dead bodies. In some cases, medical practitioners send broken teeth for DNA profiling after post-mortem to forensic laboratories. Such types of teeth yield degraded DNA, which results in partial DNA profiles. Full DNA profiles generated from teeth can be helpful to make DNA databases at the state and national levels.

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