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Case Report

Genetic identification of three exhumed human remains at a hospital in Ghana: a forensic case report

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Abstract

DNA identification is very important in cases of high decomposition of dead bodies, in which the bodies cannot be identified by physical means.

To compare the results of DNA typing, it is necessary to have related subjects with which to perform comparative analyses. Such tests are normally performed by comparing DNA profiles from people known to be immediate family members of the presumptive victim, such as parents or children because they share half of their genetic material with the unidentified.

We report on how DNA analysis was used to solve a case of mixed-up bodies at a local mortuary in Ghana, West Africa. Two families and three buried human remains were in contention in this case. The first body (E9) was buried three months before exhumation. The second body (E11) was buried two and a half months before exhumation whiles the third body (E10) was buried a month before exhumation. Exhibit E5 was taken from an alleged child of the deceased, E11. Toenails of the exhumed bodies were sampled by a pathologist and used for DNA extractions using the QIAamp DNA Investigator Kit.

Profiles from relatives were generated for comparison purposes. All samples gave a quality amount of genomic DNA after quantification. DNA was amplified with a GlobalFiler PCR amplification kit. Profiles from relatives were generated for comparison purposes.

The human remains (exhibit E11) cannot be excluded as the biological father of the child (exhibit E5) because they share common alleles at all 23 genetic loci. The applicable combined paternity index was 17218125604.492 assuming a prior probability of 0.5. The probability of paternity is 99.99999999%. Based on this relationship testing, one of the bodies was successfully identified and handed over to the family for re-burial.

More Information

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Abbreviations: DNA: Deoxyribonuleic Acid; PCR: Polymerase Chain Reaction; STR: Short Tandem Repeat; Qpcr: Quantitative Polymerase Chain Reaction

Introduction

DNA identification is very important when a body has reached a point of decomposition where it cannot be identified by physical means [1,2].

To compare the results of DNA typing, it is necessary to have related subjects with which to perform comparative analyses. Such tests are normally performed by comparison with the DNA profiles from people known to be immediate family members of the presumptive victim, such as parents or children because they share half of their genetic material with the unidentified body [2,3].

Bones and nails are important in identifying decomposed bodies compared to soft tissues. Although buccal swabs and saliva samples have been demonstrated to be stable at room temperature for over one and five years respectively, DNA

from bones and nails DNA has been shown to be successful in SNP detection assays even when clippings are stored at room temperature for over 20 years [4]. This is because, during nail growth, cells differentiate into the nail plate and are filled with keratin. In this keratinization process, the cells undergo programmed cell death, which results in considerable DNA fragmentation. Once the nail root cells are keratinized (and the DNA fragmented) during nail growth, the keratin tissue probably protects the DNA from further damage because keratinous tissue makes the DNA less accessible to oxidants and does not contain water. Water in samples leads to DNA damage through hydrolytic deamination of cytosine [5].

The most widely used genetic markers for forensic DNA typing in most crime laboratories are autosomal short tandem repeat (STR) loci [6]. Commercially available STR kits, such as the SGM plus PCR Amplification Kit, AmpFℓSTR Identifiler PCR amplification kit, AmpFℓSTR Global Filer kit (Applied Biosystems, Foster City, California) or the PowerPlex 16 and PowerPlex 24 systems (Promega, Madison, Wisconsin) make use of a set of 10–24 STR loci to provide a high level of diversity and resolution for identity testing [7,8]. These kits, and STR loci, have been used widely for the identification of human remains as well as in relationship testing, such as relationship testing and family reconstructions.

We report on how one set of three different exhumed human remains stored at a local mortuary were genetically identified after a third family refused to claim a corpse handed to them by the mortuary authorities, insisting that the corpse was not that of their relative. Investigations by the mortuary authorities and the police led to the exhumation of three human remains at a local cemetery and kept at the mortuary for identification. Crime scene investigators sampled bones for forensic DNA Analysis since there was no soft tissue. Close relatives of the missing female (alleged son, and alleged mother) were invited for buccal swab sampling for comparative DNA analysis to determine the identity of the human remains.

Materials and methods

Samples

- E9: Humerus bone samples of humans remain 1
- E10: Humerus bone samples of humans remain 2
- E11: Humerus bone samples of humans remain 3

Family A

- E1: Buccal swab of an alleged child of deceased

Family B

- E5: Buccal swab of an alleged child of deceased
- E3: Buccal swab of the alleged father of deceased

DNA extractions

Genomic DNA was extracted from 100.0mg of powdered bone samples from the deceased as well as buccal swab samples from alleged relatives of exhumed human remains using the QIAamp DNA Investigator Kit (Qiagen, Hilden, Germany) following the Manufacturer's instructions, for genetic testing. Extracted samples were stored at -20 °C prior to further analysis.

DNA quantification

DNA extracted from the samples were quantified with the 7500 qPCR equipment using the Quantifiler™ Trio DNA amplification kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol.

STR amplification and capillary electrophoresis

The extracted DNA from the samples were then amplified with the 9700 PCR machine using AmpFLSTR™ GlobalFiler™ PCR Amplification Kit (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. The amplified STR targets were electrophoresed in the 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA) and the generated STR profiles were analyzed using GeneMapper IDX version 1.5 software (Applied Biosystems, Foster City, CA), following the manufacturer's protocol.

Statistical calculations

The paternity indexes were computed using allelic frequencies of the Black African race.

The letters p, q, and r in the "mother, child and Alleged Father" columns below represent allele frequencies in the co-dominant system [14].

Child	Mother	Father	Likelihood Ratio
q	pq	q	1/q
pq	p or pr	q	1/q
q	q	q	1/q
pq	p or pr	qr or pq	1/2q
pq	pq	pq	1/(p+q)
pq	pq	q	1/(p+q)
pq	pq	qr	1/(2p+2q)

Where:

p = allele in child and in mother

q = allele in child and in father

r = allele not found in the child.

Probability of Paternity = CPI/CPI + 1

Where CPI = Combined Paternity Index

A prior probability of 0.5 was assumed for all calculations.

Allele Frequencies for computation of Likelihood Ratios

and Combined Paternity Indexes were taken from published population data [13].

Results

The obtained results are shown in Tables 1, 2, 3, 4 and Figures 1 and 2 respectively.

Discussion and conclusions

DNA fingerprinting by STR loci analysis has become the ultimate method for the identification of human remains from mass disasters, armed conflicts and criminal investigations [9]. Of late, the application of improved or completely new laboratory protocols has greatly increased the success rate of nuclear DNA profiling of degraded human skeletal remains and affords forensic scientists and investigators the opportunity to accurately identify missing persons to bring closure to such cases for many families [10].

Table 1: DNA Concentrations and IPC CT values from Real-time Quantification.

Sample Name	Concentration (ng/µl)	IPC CT Value
E9 (Humerus bone samples of humans remain 1)	0.486	27.268
E10 (Humerus bone samples of humans remain 2)	0.294	27.412
E11 (Humerus bone samples of humans remain 3)	0.621	27.343
E1 (Buccal swab of an alleged child of deceased)	12.872	27.406
E3 (Buccal swab of the alleged father of deceased)	14.423	27.102
E5 (Buccal swab of an alleged child of deceased)	11.845	27.27

Table 2: Typing results of the analyzed samples.

STR locus	Human remains E9 (Humerus)	E5 (alleged child of E9)	E1 (alleged child of E9)	E3 (alleged father of E9)
D8S1179	13,14	12,13	14,16	14
D21S11	32,32.2	28,36	28,33.2	27,28
D7S820	8,10	9,10	9,10	9,10
CSF1PO	10,12	10,11	7,12	10
D3S1358	14,16	15,16	14,15	15,16
TH01	6,7	7,8	7,8	6,7
D13S317	11,13	11,12	12,13	12
D16S539	11,13	11,12	11,14	12,14
D2S1338	21	23,24	21,25	17,22
D19S433	13,15	12,13	14	11,13
vWA	15	16,20	16,19	17
TPOX	9,11	10,11	10,11	7,9
D18S51	15,19	17,18	18,19	17,19
D5S818	13	11,13	12,13	11,13
FGA	22,23	21,24	24	22,25
Y INDEL	2	-	2	2
DYS391	10	-	10	10
D2S441	12,14	11,14	11	11,14
D22S1045	17,18	10,16	11,15	14,20
SE33	20,26.2	15,28.2	20,26.2	16,29.2
D10S1248	13,15	13	13,16	13,15
D1S1656	12,15	14	11,16	14
D12S391	19,20	17,20	17,23	19,20
AMEL	XY	XX	XY	XY

Table 3: Typing results of the analyzed samples.

STR locus	Human remain E10	E3(alleged father of E10)	E5(alleged child of E10)	E1 (alleged child of E10)
D8S1179	12,15	14	12,13	14,16
D21S11	30,31	27,28	28,36	28,33.2
D7S820	8,10	9,10	9,10	9,10
CSF1PO	9,12	10	10,11	7,12
D3S1358	16,17	15,16	15,16	14,15
TH01	8,10	6,7	7,8	7,8
D13S317	12,13	12	11,12	12,13
D16S539	11	12,14	11,12	11,14
D2S1338	22,24	17,22	23,24	21,25
D19S433	16	11,13	12,13	14
vWA	15,16	17	16,20	16,19
TPOX	10	7,9	10,11	10,11
D18S51	15,16	17,19	17,18	18,19
D5S818	11	11,13	11,13	12,13
FGA	18.2,27	22,25	21,24	24
Y INDEL	2	2	-	2
DYS391	11	10	-	10
D2S441	11,12.3	11,14	11,14	11
D22S1045	17	14,20	10,16	11,15
SE33	18,19	16,29.2	15,28.2	20,26.2
D10S1248	14,15	13,15	13	13,16
D1S1656	14,16	14	14	11,16
D12S391	15,18	19,20	17,20	17,23
AMEL	XY	XY	XX	XY

Table 4: Typing results of the analyzed samples.

STR locus	Human remain E11	E3(alleged father of E11)	E5 (alleged child of E11)	E1 (alleged child of E11)	Paternity Index
D8S1179	12,14	14	12,13	14,16	4.098
D21S11	28	27,28	28,36	28,33.2	4.132
D7S820	10	9,10	9,10	9,10	2.105
CSF1PO	11	10	10,11	7,12	4.505
D3S1358	16	15,16	15,16	14,15	1.592
TH01	7	6,7	7,8	7,8	1.623
D13S317	12	12	11,12	12,13	1.376
D16S539	11	12,14	11,12	11,14	2.079
D2S1338	19,23	17,22	23,24	21,25	9.346
D19S433	12,14	11,13	12,13	14	5.000
Vwa	16	17	16,20	16,19	3.704
TPOX	8,10	7,9	10,11	10,11	5.495
D18S51	12,17	17,19	17,18	18,19	2.841
D5S818	10,13	11,13	11,13	12,13	2.101
FGA	23,24	22,25	21,24	24	2.924
Y INDEL	2	2	-	2	-
DYS391	10	10	-	10	-
D2S441	12.3,14	11,14	11,14	11	2.128
D22S1045	16	14,20	10,16	11,15	5.376
SE33	11.2,28.2	16,29.2	15,28.2	20,26.2	5.208
D10S1248	8,13	13,15	13	13,16	2.155
D1S1656	13,14	14	14	11,16	2.137
D12S391	17,20	19,20	17,20	17,23	3.185
AMEL	XY	XY	XX	XY	

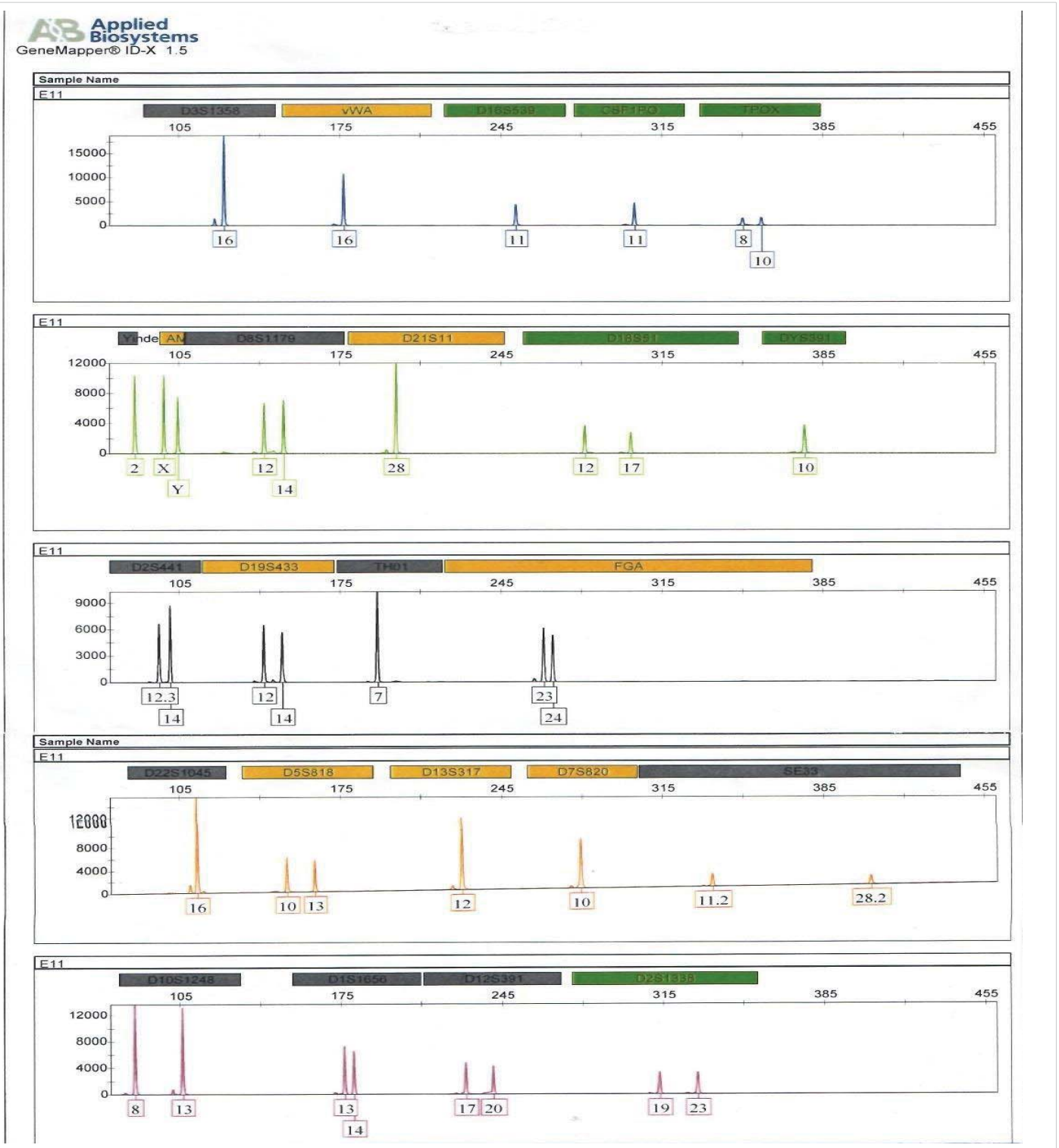

Figure 1: Electropherogram generated from short tandem repeat profiling of human remains, E11 (humerus bones).

DNA profiling is very helpful in identifying human remains and in criminal investigations. DNA molecule is very unique to an individual and remains highly conserved in the person's lifetime. Each individual's genetic fingerprint is composed of equal parts of his or her parents' DNA and can be analyzed to produce DNA profiles comparable to other DNA profiles of close relatives. DNA is a resilient molecule, degrades slowly in hard tissues, such as bones and teeth and can be recovered and analyzed from small crime scene biological samples, such as bloodstains, saliva, or single hair with roots [11].

The use of human remains sampled from the crime scene, and various taphonomic conditions usually result in small amounts of DNA being extracted for forensic analysis.

The DNA section of the Forensic Science Laboratory at the Criminal Investigation Department of the Ghana Police

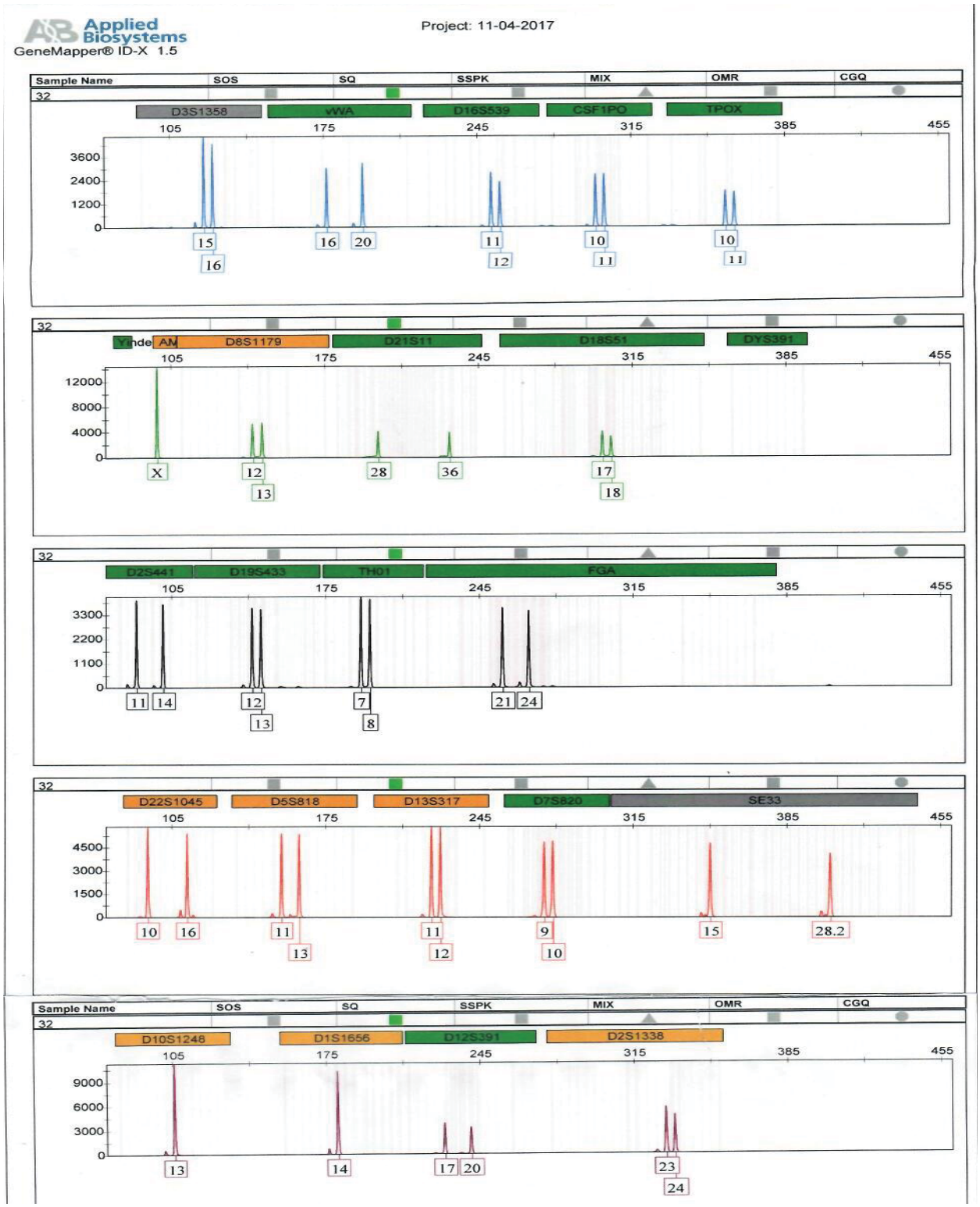

Figure 2: Shows Electropherogram generated from short tandem repeat profiling of sample of an alleged child, E5.

Service sampled human remains (humerus) from three human remains at a local hospital mortuary in the Western Region of Ghana. Buccal swabs of alleged relatives of the deceased whose remains were exhumed were also taken at the hospital for DNA profiling and comparison purposes. DNA was successfully extracted from the humerus bones and the buccal swabs taken, yielding 0.486 ng/µl, 0.294 ng/µl, and 0.621 ng/µl for human remains E9, E10 and E11 respectively as shown in table 1. Buccal swab samples of alleged relatives E1, E3, and E5 also yielded 12.872 ng/µl, 14.423 ng/µl, and 11.845 ng/µl, respectively. These values were enough to generate full DNA profiles for relationship testing.

The successful recovery of DNA from femur bone agrees

with the results of other published research that suggested the viability of obtaining higher quantities of endogenous DNA for forensic cases by sampling the petrous bone, fingernails and femur bones for cases in which there is extensive degradation to hard tissues degraded skeletal remains [3,10,12]. DNA profiles generated from the humerus of human remains E9 and E10 did not match the DNA profile from an alleged relative from family A.

Full DNA profiles were successfully generated for the human remains, E11 and alleged surviving relatives of the deceased (E5, ie, child; and E3, ie, father) for relationship testing (Figure 2). Assuming a prior probability of 0.5, the combined paternity index was calculated to be 1.047×10^8, using published population data [13] and DNA View software [14]. This means the observed profile was 1.047×10^8 times more likely to occur under the scenario that the deceased is the true biological father of surviving relative (daughter), as opposed to the scenario that the deceased is an unrelated person of the Black African population [13] to the surviving relative (daughter). With a confidence probability of 99.99999904%, the conclusion is based on the calculated frequency of the DNA profile is very rare in unrelated individuals of the Black African Race.

The DNA analysis matched members of family B to human remains E11 and brought some closure to the case for that family.

Compliance with ethical standards

Funding: Authors received no financial support from any organization.

Research involving human participants and/oranimals: Approval was obtained from the Ethics Committee of the Kwame Nkrumah University of Science and Technology, Kumasi.

Informed consent: Informed consent was obtained from the study participants.

Ethical approval: Ethical approval Committee of the Kwame Nkrumah University of Science and Technology, Kumasi.

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