



## Case Report

# Missing Persons Identification: Genetic profiling of highly charred human remains using sixteen STR loci markers

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## Introduction

Law enforcement and Disaster Management Agencies within Governments spend substantial resources to identify human remains and dead bodies after wars, mass disasters and political unrest [1-4]. Under certain legal circumstances such as civil paternity cases and unclaimed human cadavers, biological samples may be collected from living relatives and exhumed human remains, as well as mortal remains for DNA profiling purposes [5,6]

In present-day society where socio-cultural dynamics and economic factors encourage migration in search of better living standards, relatives are always looking for missing family members.

In all these cases, DNA analysis is considered the best method of identification. Microsatellite DNA, namely Short

Tandem Repeats (STRs) loci show high variability. Degraded DNA from old skeletal remains can be amplified by PCR [7].

Biological materials that are of importance in identifying exhumed remains using DNA are bone, teeth and nail due to their ability to withstand rapid decomposition. According to Vass, et al. [8] tissues such as nails, teeth and bones are more resilient to environmental factors. DNA analysis from skeletal samples has developed rapidly since its inception in the late 1980s.

The two main objectives of forensic DNA analysis are to identify the sources of biological evidence, including associations of persons through kinship, and excluding persons mistakenly linked to some evidence.

The most widely used genetic markers for forensic DNA typing in most crime laboratories are autosomal Short Tandem



Repeat (STR) loci [4]. Commercially available STR kits, such as the SGM plus PCR amplification Kit, AmpFLSTR Identifiler PCR amplification kit (Applied Biosystems, Foster City, California) or the PowerPlex 16 system (Promega, Madison, Wisconsin) make use of a set of 10–17 STR loci to provide a high level of diversity and resolution for identity testing [9]. These kits, and STR loci, have been used widely for the identification of human remains as well as in relationship testing, such as paternity testing and family reconstructions.

We report on how a highly charred human remains were genetically identified after the victim, a female, was reported missing in 2016. Intelligence gathered by Police fourteen months later resulted in the discovery of burnt and buried skeletal remains at a location about 42Km away from her home. 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

### Sampling

The following sealed samples were submitted for forensic DNA profiling:

- i) Alleged charred human remains of missing person, as shown in Figure 1.
- ii) Buccal swabs of missing person’s alleged son.
- iii) Buccal swabs of missing person’s alleged mother.

Ethical clearance was obtained from the Ethics committee of The Kwame Nkrumah University of Science and Technology, Kumasi, and informed consent was obtained from the individual Sample providers.



Figure 1: Bone sample of charred remains of missing person.

### DNA Extraction

Genomic DNA was extracted from 100.0mg of powdered bone samples from the deceased as well as buccal swab samples from the deceased’s alleged son and mother using the QIAamp DNA Investigator Kit following Manufacturer’s instructions, for genetic testing.

### 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 manufacturer’s protocol.

### STR amplification and Capillary Electrophoresis

The extracted DNA from the samples were then amplified with the 9700 PCR machine using AmpFLSTR™ Identifiler™ Plus PCR Amplification Kit (Applied Biosystems, Foster City, CA) following 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, following manufacturer’s protocol.

### Results

The obtained results are shown in Tables 1, 2 and figure 2 respectively.

### Discussion and conclusion

The advent of DNA profiling has played a pivotal role in identifying human remains, in criminal investigations, kinship analysis and civil cases [10]. The uniqueness of the DNA molecule to an individual makes it ideal for human identification, especially as the molecule remains highly conserved in a person’s lifetime. An individual is composed of equal amounts of his or her parents’ genetic make-up, and this genetic composition can be analyzed to generate DNA profiles comparable to other DNA profiles of other close family

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

Sample Name	Concentration (ng/μl)	IPC CT Value
F (Bone)	0.248	27.139
Q (Alleged Son)	10.742	27.327
R (Alleged mother)	15.625	27.263

IPC: Internal PCR Control; CT: Cycle Threshold

Table 2: Typing results of the analyzed samples.

STR LOCUS	F (Bone)	Alleged son (Q) of missing female	Alleged mother (R) of missing female
D8S1179		12,14	11,13
D21S11	13,14	29,34	30,2,31,2
D7S820	29,30,2	10,12	12,13
CSF1PO	10,12	10,11	7,10
D3S1358	10,11	15,17	15,16
TH01	10,11	7,8	8,10
D13S317	15,16	10,11	11,13
D16S539	8	11,12	12
D2S1338	11, 12	16,26	23,26
D19S433	8,12	14,2	12,13
vWA	16,23	17	15,17
TPOX	13,14,2	9,11	9,11
D18S51	15,17	14,15	14,17
D5S818	9,11	12,13	12,13
FGA	14,19	22,24	19,24
AMEL	13	XY	XX
	19,24		
	XX	CMI = 2.088x10 <sup>4</sup>	CMI = 6.925x10 <sup>4</sup>
		PP = 99.995%,	PP = 99.999%,

CMI: Combined Maternity Index; PP: Percentage Probability

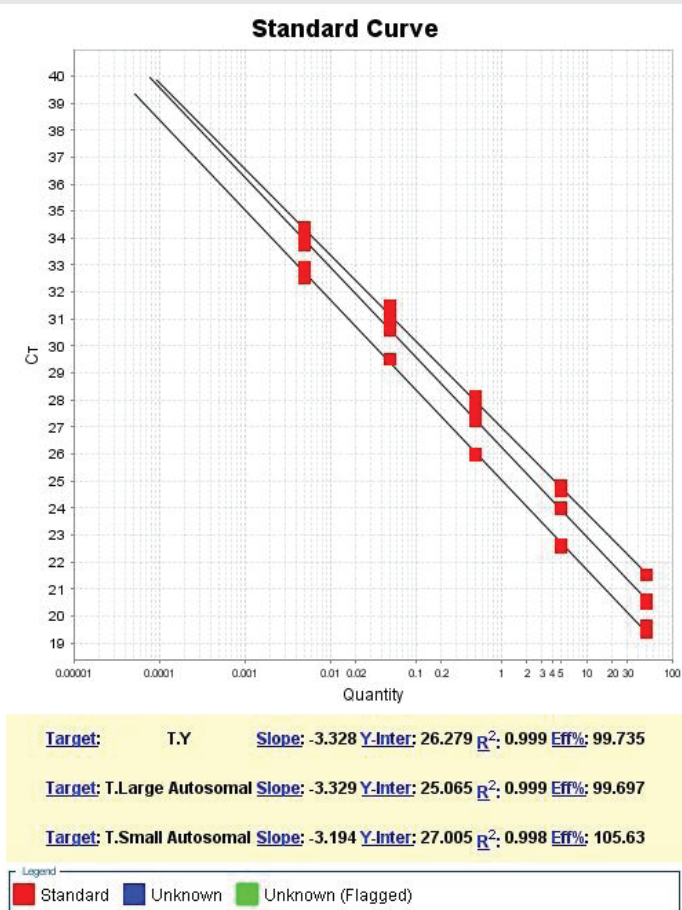


Figure 1: Bone sample of charred remains of missing person.

members. An organism's nuclear DNA molecule is a highly resilient molecule degrades slowly in hard and keratinized tissues such as fingernails, teeth and bones, and can be retrieved and profiled from minimal quantities of crime scene biological samples, such as semen, saliva, urine, bloodstains or single hair with root. These unique qualities of DNA molecule allow it to be retrieved from old crime scene biological samples even when environmental conditions are harsh [9].

Charred human bone samples recovered from crime scenes as well as samples from a variety of highly decomposed human cadavers result in a limited availability of extracted DNA for forensic analysis.

The biology (DNA) unit of the Forensic Science Laboratory at the Criminal Investigation Department of the Ghana Police Service received samples of charred human remains from police investigators after the exhibits were recovered from a crime scene. Buccal swabs of an alleged relative (son) and alleged mother of a missing person were taken at the laboratory for DNA profiling and analysis.

DNA were successfully extracted from the charred human remains and the buccal swabs taken, yielding 0.248ng/ $\mu$ l, 10.742ng/ $\mu$ l and 15.625ng/ $\mu$ l as shown in Table 1. The concentration of DNA recovered were enough to generate full DNA profiles for relationship testing. The successful recovery of DNA from charred human remains agrees with the results

of [6,11], in which the authors independently demonstrated that it was viable to recover substantial levels of endogenous DNA for forensic identification by targeting the cochlear region of the petrous bone and femur bones for cases in which there is extensive degradation to hard tissues of human skeletal remains.

Full DNA profiles were also obtained for the alleged family members (alleged mother of missing person and alleged son of missing person) and the skeletal remains for relationship testing. Assuming a prior probability of 0.5 (or 50), the applicable combined maternity Indexes were calculated to be  $6.925 \times 10^4$  for the alleged mother of missing person, and  $2.088 \times 10^4$  for the alleged son of missing person. The percentage probabilities of stated relationships are 99.999% and 99.995%, respectively, using published population data [12] and DNA View software [13]. This means the observed DNA profile was  $6.925 \times 10^4$  times more likely to occur under the scenario that the deceased is the true biological daughter of living relative (mother), as opposed to the scenario that the deceased is an unrelated person of the Sub-Saharan African population [12] to the living relative (mother). With a confidence probability of 99.999%, the conclusion is based on the calculated allele frequency of the DNA profile being rarer in unrelated individuals of the sub-Saharan African population.

Comparative genetic analysis of the charred human remains and the alleged son also yielded a combined maternity Index of  $2.088 \times 10^4$ . The percentage probability of stated relationship was 99.995%, using published population data [12] and DNA View software [13]. The observed DNA profile was  $6.925 \times 10^4$  times more likely to occur under the scenario that the deceased is the true biological mother of the living relative (son), as opposed to the scenario that the deceased is an unrelated person of the Sub-Saharan African population [12] to the living relative (son). With a calculated confidence probability of 99.995%, the conclusion is based on the calculated allele frequency of the DNA profile being rarer in unrelated individuals of the sub-Saharan African population.

The results of the genetic profiling and the relationship testing enabled the police detectives solve a cold case involving the brutal homicide of a young female, returned the remains to the family for burial and bring closure to the case to the family.

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