Optimized Recovery of DNA and Subsequent Short Tandem Repeat Profiling of Different Tissues Sampled from Embalmed Human Cadavers

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Abstract

Introduction: Storage of specimens sampled from human remains for pathological testing, embalming for burial purposes, and for human identification often requires formalin fixation and/or paraffin embedding. Current knowledge in molecular biology techniques and forensic DNA analysis makes it possible to optimize the extraction of amplifiable DNA from formalin-fixed tissues by improving the pre-treatment, optimizing the digestion condition of proteinase K, simplifying the extraction protocol and purifying the extracted DNA with optimized volumes of alcohol. **Aim:** This research sought to extract amplifiable DNA from thirteen brain, bone marrow and cartilage samples from four formalin embalmed human cadavers. **Materials and Methods:** Brain, cartilage and bone marrow samples were taken from four different cadavers at autopsy at the Ghana Police Hospital mortuary in Accra, Ghana sixty-two days after embalming. An optimized preparation and DNA extraction protocol was carried out on all the samples. Brain samples were also taken from a non-formalin treated fifth cadaver of known STR profile, and standard DNA extraction performed to serve as positive control. **Results:** Our optimized protocol yielded detectable quantities of DNA from the samples when quantified with the 7500 Real-Time PCR equipment. The extracted DNA also yielded full STR profiles with varying peak heights for forensic identification purposes. The measured degradation indexes of the DNA samples were greater than 1.0, with peak heights of generated STR profiles above the limits of detection of the 3500 genetic analyzer. **Conclusion:** Our current study demonstrated an optimized method of DNA extraction from tissues (brain, cartilage and bone marrow) sampled from formalin embalmed human cadavers. The optimized protocol reduced the concentration of formalin fixation residues in extracted DNA from formalin-fixed tissues, thereby improving the amplification efficiency for STR profiling. Brain, bone marrow and cartilages can be a good source of DNA from embalmed and degraded human remains, though for skeletonized human remains together with teeth and long bones.

Keywords: Formalin fixation, DNA profiling, STR analysis, optimized protocol, embalmed cadavers

Introduction

Preservation of human cadavers for pathological testing and embalming for burial purposes employ formalin fixation and/ or paraffin embedding. Genetic materials (DNA and RNA) from formalin-fixed tissues have been isolated and used for numerous purposes including genetic studies and forensic cases.[1] Most often, the quantity and quality of recovered

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DNA are dependent on the type of tissue and the DNA extraction procedure employed, as formalin fixation induces DNA damage, chemical modifications, and degradation, thereby reducing the quantity and quality of DNA available for downstream genetic analyses.[2]

Recent advances in molecular biology and forensic DNA profiling have shown that DNA extraction from formalin-fixed tissues can be optimized by improving the pretreatment, optimizing the digestion condition of proteinase K, simplifying the procedures of the DNA extraction, and purifying the extracted DNA with optimized volumes of alcohol.[3]

The success rate of genetic or short tandem repeats (STRs) analysis depends, to a large extent, on the quality and quantity of DNA extracted from biological samples.

STRs are generally highly polymorphic and their alleles are differentiated by the number of copies of the repeat sequence within each of the STR locus.[4] The greater the number of STR loci used for forensic DNA typing, the greater the discriminative value, as the probability that two individuals are chosen at random in a population possessing exactly the same number of STRs being analyzed is extremely rare.[5,6] The target STRs in forensic DNA analysis are usually the large autosomal, small autosomal, and the Y (male) STRs.

In forensic DNA casework, samples usually employed include blood, semen, saliva, epithelial cells, hair with roots, bones, and various other human tissues obtained at autopsy as well as human tissues fixed in formalin.[7,8]

Fixation of human tissues with formalin and subsequent preservation with paraffin mostly preserve biological tissues for histopathological examination and embalming human remains for medical study or in preparation for burial.[9] Extraction of genetic material from formalin-fixed and paraffin-embedded tissues serves as a rich source of DNA or RNA for forensic identification, medicolegal investigations, missing persons' identification, and relationship testing.[10,11]

Preservation of biological samples with formalin for forensic and medical purposes induces protein-formaldehyde reactions between carbonyl groups of the formaldehyde and the amine groups of proteins resulting in fixation and chemical modification of molecules, cells, and fibers within human tissues.[12-14]

During DNA extraction from embalmed human tissues, formalin acts as an enzyme inhibitor and reduces proteinase K activity.[2,15] The effectiveness of formalin fixation depends largely on its concentration in the arteries, veins, and capillaries, as the extent of vascularity of human tissues may determine the effectiveness of the formalin fixation process.[16,17] Tissues such as skin, muscle, and internal organs with high blood supply will likely be perfused with more formalin solution than those tissues with less vascularization such as cartilage, tendons, and ligaments.[18-20] A reduction in downstream STRs amplification is expected with more vascularized human tissues than less vascularized ones.[2,21]

The aim of this study is to genetically profile some formalin-fixed, relatively vascularized tissues such as the brain, bone marrow, and avascular cartilage.

We report on an optimized DNA extraction method and subsequent STR profiling of some embalmed human tissues collected from the Ghana Police Hospital Mortuary, Accra.

Materials and Methods

Collection of samples

Four cadavers (HC1, HC2, HC3, and HC4) were embalmed with formalin fluid containing 2.0 L of formaldehyde, 500.0 ml methylated spirit, 150.0 ml glycerin, and 3.0 ml of distilled water in conformity with national and international standards. The brain, cartilage, and bone marrow samples were taken from each cadaver at autopsy at the Ghana Police Hospital mortuary in Accra, Ghana, 62 days after embalming. They were then placed in 2.0 ml centrifuge tubes and stored at 20.0°C for DNA extraction.

Brain samples were taken from a nonformalin-treated fifth cadaver of known STR profile as a positive control.

Samples

Cadaver 1:
- HC1B: Brain sample from cadaver 1
- HC1C: Cartilage sample from cadaver 1
- HC1M: Bone marrow sample from cadaver 1

Cadaver 2:
- HC2B: Brain sample from cadaver 2
- HC2C: Cartilage sample from cadaver 2
- HC2M: Bone marrow sample from cadaver 2

Cadaver 3:
- HC3B: Brain sample from cadaver 3
- HC3C: Cartilage sample from cadaver 3
- HC3M: Bone marrow sample from cadaver 3

Cadaver 4:
- HC4B: Brain sample from cadaver 4
- HC4C: Cartilage sample from cadaver 4
- HC4M: Bone marrow sample from cadaver 4

Cadaver 5:
- MSKP: Nonformalin-treated male brain sample of known DNA profile (positive control).

Ethical clearance was obtained from the Committee on Human Research, Publication and Ethics of Kwame Nkrumah University of Science and Technology, Kumasi Ghana (Number: CHRPE/RC/061/13 on April 02, 2013) and informed consent was obtained from relatives of cadavers.

Preparing embalmed tissues for DNA extraction

About 100.0 mg each of the brain, cartilage, and bone marrow samples were taken from HC1, HC2, HC3, and HC4 cadavers and placed into 1.5 ml nuclease-free microtubes. The samples were then subjected to DNA extraction process as described below.

The samples were first deparaffinized with 1.0 ml of xylene PA (Merck KGaA, Darmstadt, Germany), and rehydrated

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with 2.0 ml absolute ethanol (Merck KGaA, Darmstadt, Germany). The samples were then homogenized by pulse vortexing and incubated at 56°C, for 30 min, in digital thermomixer (Eppendorf AG, Hamburg, Germany).

After incubation, the samples were centrifuged at 14,000 rpm for 5 min (Eppendorf AG, Hamburg, Germany) and the supernatant was discarded. The centrifugation process was repeated to remove excess xylene and ethanol from the mixture. The supernatant was decanted and 1.0 ml 1X phosphate-buffered saline (PBS) was added to the sediment. The mixture was incubated at room temperature for 10 min and centrifuged at 14,000 rpm for 2 min (Eppendorf AG, Hamburg, Germany). The supernatant was discarded and 600 µl of 1.0 ml 1X PBS was added for a second wash. The mixture was incubated at room temperature for 10 min and centrifuged at 14,000 rpm for 2 min (Eppendorf AG, Hamburg, Germany). The supernatant was discarded. The sediment was centrifuged again at 14,000 rpm for 5 min to remove excess PBS and the supernatant discarded.

The sediment at the bottom of the tube was washed three times, each with 1.0 ml of absolute ethanol (Merck KGaA, Darmstadt, Germany). The supernatant was discarded after each wash. The sediment was air-dried at room temperature for 45 min before DNA extraction.

DNA extraction

Genomic DNA was extracted from 13 pelleted samples from the brain, cartilage, and bone marrow from the four embalmed human cadavers and positive control using the QIAamp DNA Investigator Kit (Qiagen, Hilden, Germany) following manufacturer's instructions, for genetic testing with optimization.

Briefly, about 10.0 mg each of the brain, bone marrow, and cartilage tissue samples were transferred into 1.5 ml microcentrifuge tubes containing 200.0 µl of Buffer ATL and left to equilibrate at 15°C–25°C room temperature. About 30 µl of proteinase K was added to each tube and mixed by pulse-vortex for 30 s. The mixture was then incubated at 56°C overnight in an orbital incubator to completely lyse the samples. About 200.0 µl of Buffer AL and 15.0 µl of carrier RNA were added to the mixture; the lid was closed and mixed by pulse-vortexing for 15s to give a homogeneous solution. To the mixture, 200.0 µl of ethanol (96%–100%) was added; the lid was closed and mixed thoroughly by pulse-vortexing for 15 s. The mixture was then incubated for 5 min at room temperature. The mixture was briefly centrifuged to remove drops from the lid of the 1.5 ml tube. The lysate was carefully transferred into a 2.0 ml QIAamp MinElute column, the lid was closed, and centrifuged at 8000 rpm for 2 min. The flow-through was discarded.

The QlAamp MinElute column was carefully opened and 500.0 µl Buffer AW1 was added without getting the rim wet. The lid was closed and the mixture was centrifuged at 8000 rpm for 2 min. The flow-through was discarded.

To the QIAamp MinElute column, 700.0 µl Buffer AW2 was added and centrifuged at 8000 rpm for 2 min. The flow-through was discarded.

To the QIAamp MinElute column, 700.0 µl of ethanol (96%–100%) was added without wetting the rim. The cap was closed and the mixture was centrifuged at 8000 rpm for 2 min. The flow-through was discarded. The membrane was completely dried by centrifuging for 3 min at 14,000 rpm. The flow-through was discarded.

The QIAamp MinElute column was placed in a new 1.5 ml microcentrifuge tube, the lid was carefully opened and the pellet incubated at room temperature for 30 min.

To the column, 50.0 µl of Buffer ATE (equilibrated to room temperature) was applied to the center of the membrane.

The lid of the column was closed and incubated at room temperature for 3 min. It was then centrifuged at 14,000 rpm for 2 min to elute the DNA.

Extracted DNA samples were stored at −20°C before further analysis.

DNA quantification

DNA extracted from the 13 pelleted samples was quantified with the 7500 quantitative polymerase chain reaction (qPCR) equipment using the Quantifiler™ Trio DNA amplification kit (Applied Biosystems, Foster City, CA) following the manufacturer's instructions.

Short tandem repeat amplification and capillary electrophoresis

The extracted DNA from the 13 samples was 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, USA), following the manufacturer's instructions.

Results

Quantitative polymerase chain reaction results

The negative control template included in the DNA quantification showed amplification for the internal positive control (IPC), but there was no amplification for the other DNA targets. This meant that the quantitative PCR assay worked well and that the sample preparation procedure was devoid of contamination. There was optimum amplification of the positive control, which showed detectable DNA for human (large autosomal and small autosomal) and Y targets. Slopes of −3.442, −3.483, and −3.300 were obtained from the standard curve for the Y-target, large autosomal and small autosomal, respectively with amplification efficiency of

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99.8% [Figure 1]. The cycle threshold (CT) values for all amplified targets were <40, which indicated optimum amplification in these samples. Figure 1 shows the DNA standard plot of the three targets STRs.

Table 1: DNA concentrations and internal positive control cycle threshold values from real-time quantification

Sample name	Concentration (ng/μl)	IPC CT value	Degradation index
Cadaver 1			
HC1B	1.616	27.268	1.521
HC1C	1.294	27.354	1.732
HC1M	2.621	27.621	1.919
Cadaver 2			
HC2B	1.342	27.615	2.327
HC2C	1.921	27.412	2.125
HC2M	2.414	27.532	2.614
Cadaver 3			
HC3B	2.101	27.810	1.932
HC3C	2.414	27.645	1.817
HC3M	2.957	27.648	2.141
Cadaver 4			
HC4B	1.632	27.214	2.813
HC4C	1.826	27.652	2.916
HC4M	1.468	27.642	3.003
Positive control			
MSKP	16.214	27.462	1.090

IPC: Internal positive control, CT: Cycle threshold,

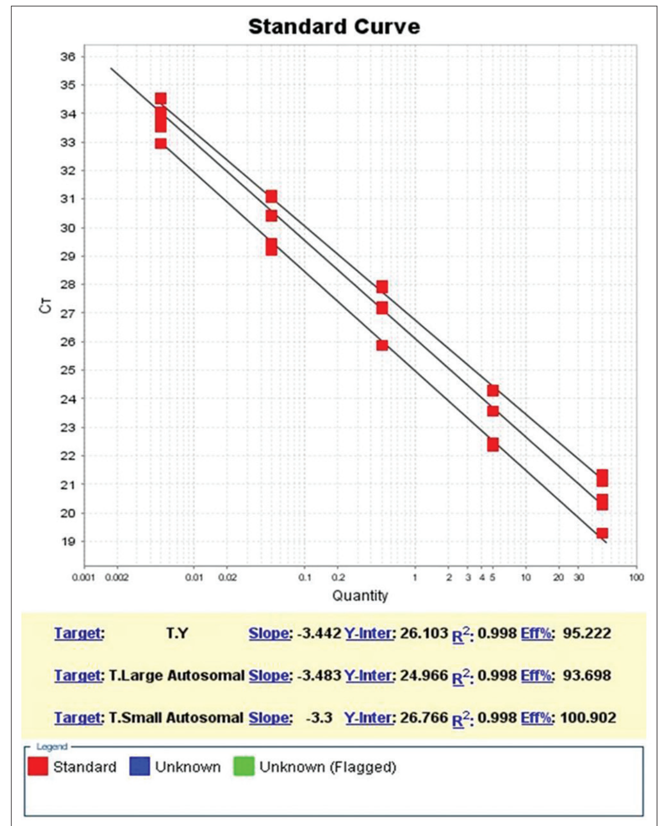

Figure 1: DNA Standard plot of the three STR targets

Table 2: Typing results of the analyzed samples

STR locus	Positive control (MSKP)	Cadaver 1 (HC1B)	Cadaver 2 (HC2C)	Cadaver 3 (HC3M)	Cadaver 4 (HC4M)
D3S1358	14, 15	14, 16	16, 17	15, 16	15, 17
vWA	16, 17	16, 18	15, 16	15, 18	15, 18
D16S539	10, 13	9, 13	11	13	9, 11
CSF1PO	7, 11	10	9, 12	8, 13	10, 12
TPOX	8, 11	9, 11	10	11, 12	9, 11
D8S1179	15	13, 14	12, 15	7, 11	14
D21S11	29, 30	28, 31.2	30, 31	28, 30	28, 33.2
D18S51	16, 18	16, 19	15, 16	16, 19	17, 22
DYS391	10	10	11	10	N/A
D2S441	11, 14	11, 14	11, 12.3	11, 14	11, 14
D19S433	11, 15.2	12, 13	16	14, 15.2	11, 15
TH01	6, 8	7, 9.3	8, 10	8, 9	7
FGA	24, 26	23, 26	18.2, 27	22, 24	22, 25
D22S1045	10, 15	11, 16	15, 17	14, 17	11, 17
D5S818	12, 13	12	11	12, 13	11, 13
D13S317	12	13	12, 13	12, 13	12, 14
D7S820	11, 12	8, 14	8, 10	8	8, 10
SE33	17	16, 23.2	18, 19	14, 17	17, 27.2
D10S1248	12, 13	10, 13	14, 15	13	12, 14
D1S1656	14, 16.3	10, 13	14, 16	11	15, 16.3
D12S391	20	17, 21	15, 18	16, 18	17,18
D2S1338	17, 24	20, 21	22, 24	20, 23	21, 22
Y INDEL	2	2	2	2	N/A
AMEL	X, Y	X, Y	X, Y	X, Y	X

STR: Short tandem repeat, MSKP: Male brain sample of known DNA profile (positive control)

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ΔRn means Rn minus the baseline. Rn means the fluorescence of the reporter dye divided by the fluorescence of a passive reference dye; thus, Rn is the reporter signal normalized to the fluorescence signal of Applied Biosystems ROX dye.

Detectable DNA concentrations were observed for the brain, bone marrow, and hyaline cartilage samples from all four cadavers as well as the positive control.

Table 1 shows the DNA concentrations and IPC CT values from real-time quantification.

Comparison of DNA degradation

Degradation indexes of more than 1 were observed for the brain, cartilage, and bone marrow samples for all four cadavers and the positive control [Table 1]. Degradation indexes of more than 1 indicated a measure of degradation in all samples. This was expected especially as the cadavers were embalmed with formalin.

Electrophoresis

Allelic ladder that was run alongside the 13 samples in the capillary electrophoresis passed; the negative control showed a null profile, and the positive control gave a full STR profile. This was an indication that the capillary electrophoresis run was good, with no contamination of the samples or reagents. Figure 2 shows the DNA ladder indicating the various alleles at the 24 STR loci.

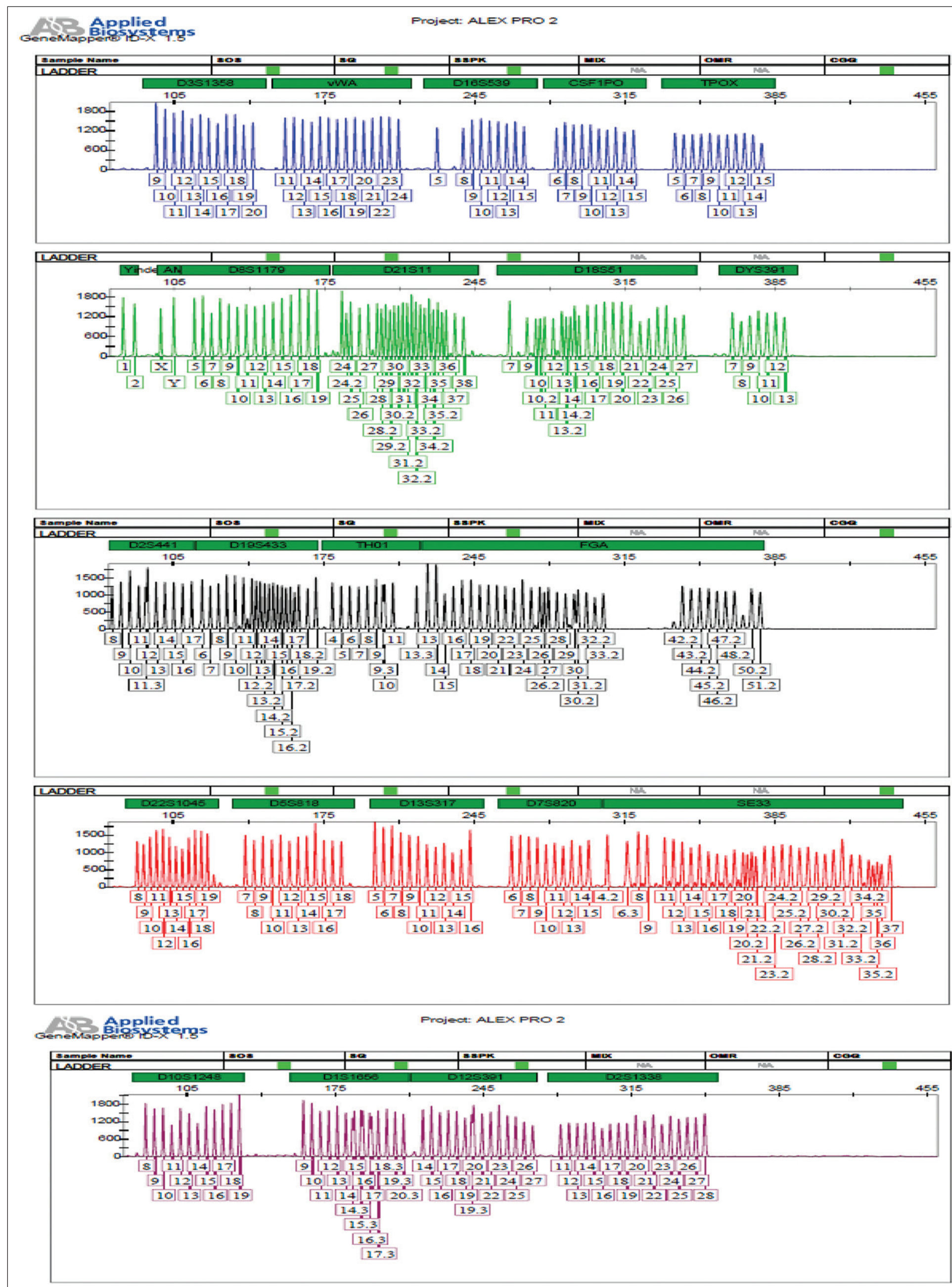

Figure 2: DNA ladder indicating the various alleles at the 24 STR loci. STR: Short tandem repeat

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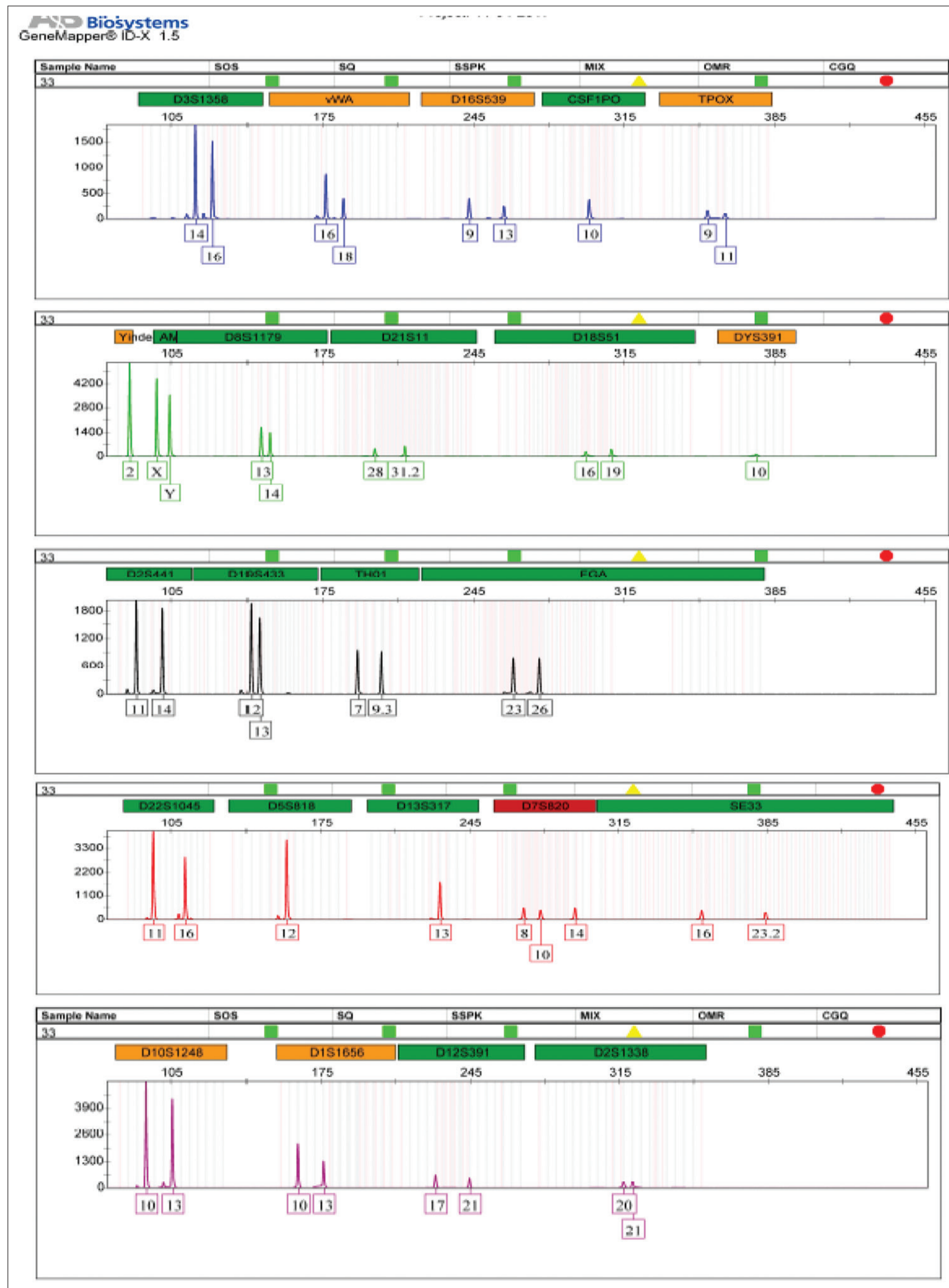

Figure 3: STR typing results of the analyzed samples from cadaver 1. STR: Short tandem repeat

Detected alleles

DNA extracted from the brain, bone marrow, and cartilage from all four cadavers as well as the positive control gave full DNA profiles as shown in Figures 3-7. Table 2 below shows detected alleles of the analyzed samples.

Figure 3 below shows the STR typing results of the analyzed samples from cadaver 1.

Figure 4 below shows the STR typing results of the analyzed samples from cadaver 2.

Figure 5 below shows the STR typing results of the analyzed samples from cadaver 3.

Figure 6 below shows the STR typing results of the analyzed samples from cadaver 4.

Figure 7 below shows the STR typing results of nonformalin-treated male brain sample of known profile (positive control).

Discussion

Embalming of human cadavers for burial and preservation of human tissues for medicoforensic testing employs formalin fixation and/or paraffin embedding, and these processes/activities generally result in DNA damage, chemical modifications, and DNA degradation.[2] This preservation

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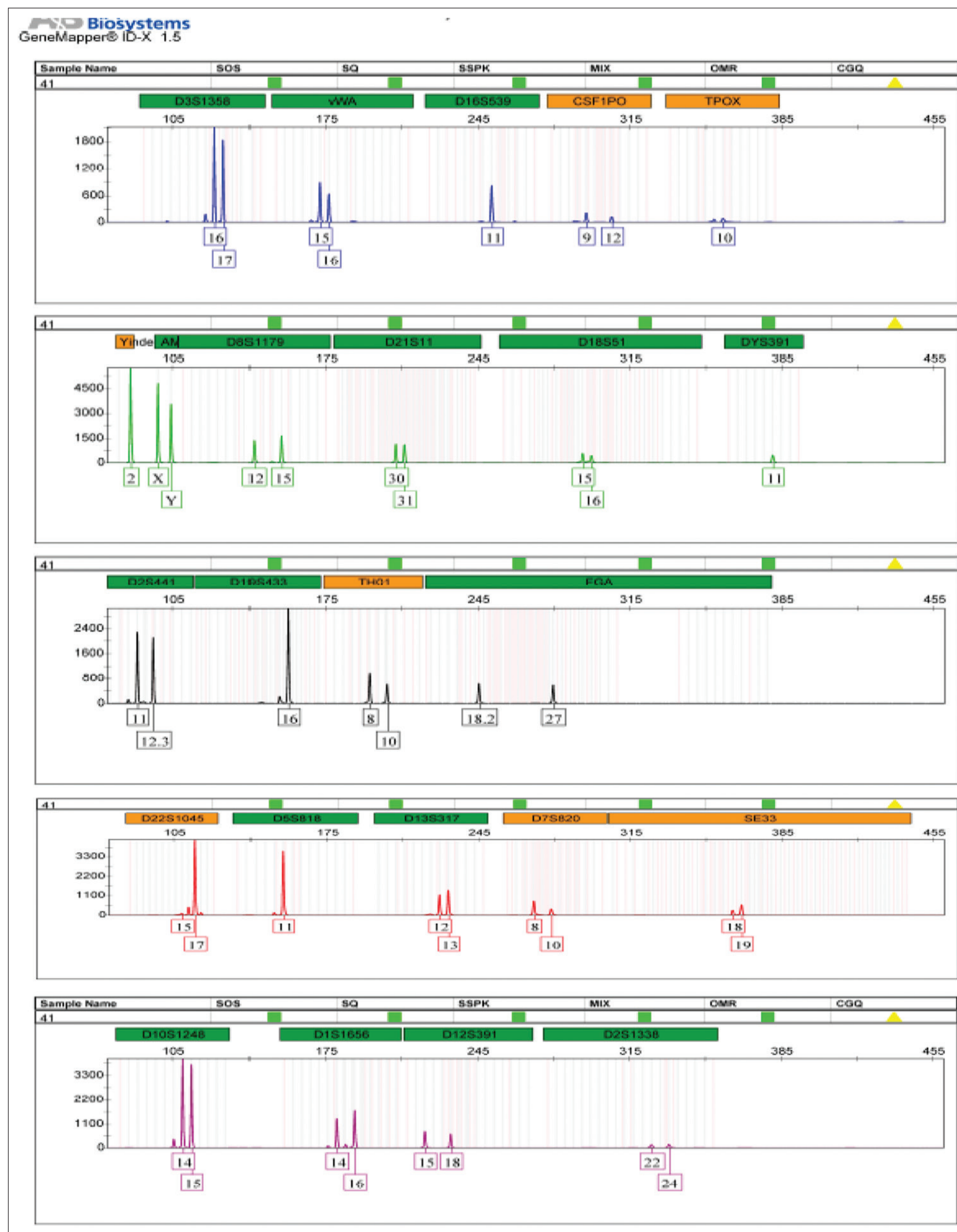

Figure 4: STR typing results of the analyzed samples from cadaver 2. STR: Short tandem repeat

method results in reduced quantity and quality of DNA available for downstream genetic analyses.

The quantity and quality of recovered DNA are mostly dependent on the type of tissue and the DNA extraction method employed, just as the success rate of STR profile analysis depends, to a large extent, also on the quality and quantity of DNA extracted from biological samples.[3,22-24]

Recent developments in molecular biology and forensic DNA profiling have shown that DNA extraction from formalin-fixed tissues can be optimized by improving the pretreatment, optimizing the digestion condition of proteinase K, simplifying the procedures of the DNA extraction, and purifying the extracted DNA with optimized volumes of alcohol.[3]

We have demonstrated in our current study an optimized method of DNA extraction from tissues (brain, cartilage, and bone marrow) sampled from formalin-embalmed human cadavers.

Tissues such as skin, muscle, and internal organs with high blood supply typically retain high volumes of formalin after treatment than those tissues with less vascularization such as cartilage, tendons, and ligaments.[18-20] This negatively impacts downstream STR amplification of more vascularized human tissues than less vascularized ones,[2,21] due to the high quantities of formalin-fixed residues in the extracted DNA.

We hypothesized that formalin is water-soluble, and reducing the concentration of formalin-fixed residues in extracted DNA from less vascularized tissues can greatly improve amplification

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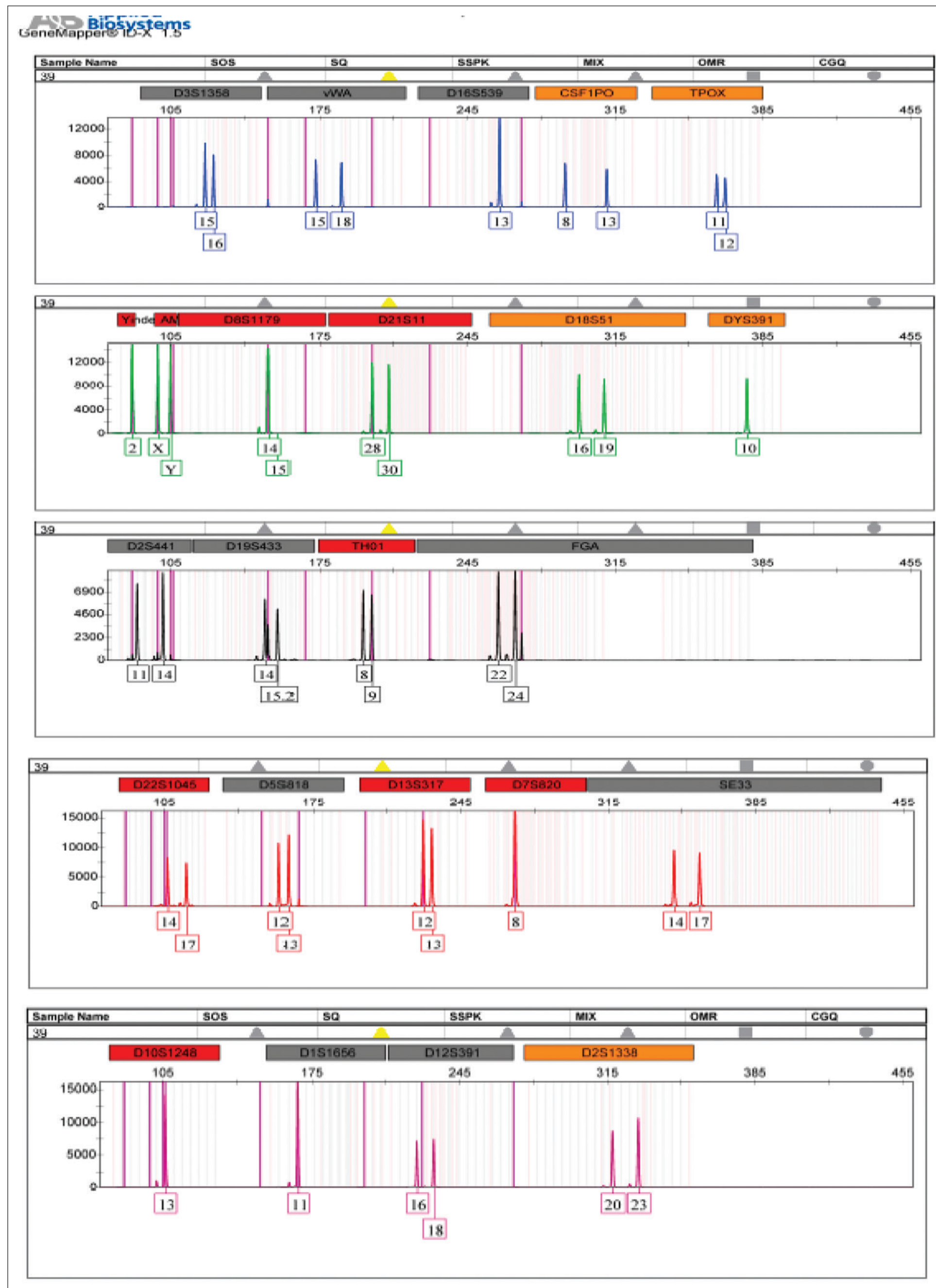

Figure 5: STR typing results of the analyzed samples from cadaver 3. STR: Short tandem repeat

efficiency and subsequent STR profiling in forensic casework. Our optimized protocol yielded detectable quantities of DNA from the samples [Table 1] when quantified with the 7500 real-time PCR equipment (Applied Biosystems, Foster City, CA).

The successful amplification of DNA extracted from these embalmed and less vascularized tissues agrees with the results from previous studies which found that the amplification efficiency of these samples was significantly improved when PBS washing step was introduced into the DNA extraction protocol.[25-27] The PBS solution may have solubilized and removed the formalin fixation residues which usually act as PCR inhibitors, thereby improving the amplification efficiency. Furthermore, the PBS solution may have possibly created an optimum buffering medium for the polymerase enzyme to increase the quality and quantity of amplified DNA during amplification.[3]

The extracted DNA also yielded full STR profiles with varying peak heights for forensic identification purposes, suggesting the effectiveness of the DNA extraction procedure. Although the measured degradation indexes of the quantified DNA samples were >1.0, the peak heights of the generated STR profiles were above the limits of detection of the genetic analyzer, suggesting that there was limited effect of the formalin on downstream amplification of the DNA samples.

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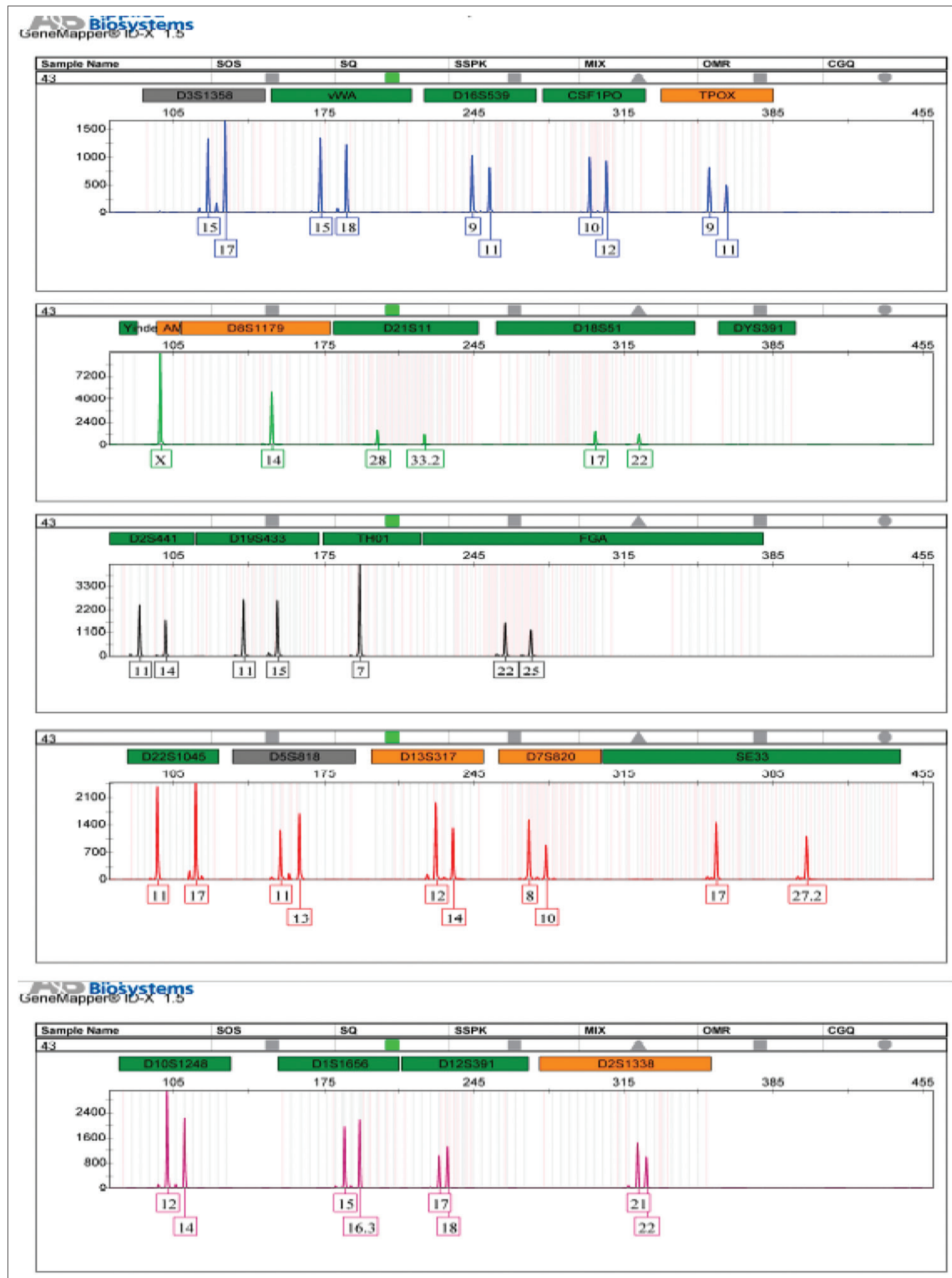

Figure 6: STR typing results of the analyzed samples from cadaver 4. STR: Short tandem repeat

The results from the current study are also consistent with the results obtained by other researchers on embalmed human cadavers[2,9] who were able to extract detectable amounts of DNA and generated full STR profiles from the bone marrow and brain. The current study also showed that although different quantities of DNA were obtained from bone marrow, brain tissues, and cartilage [Table 1], they all yielded full profiles of varying peak heights [Figures 3-7]. The obtained data indicate that the quantity of DNA used as PCR starting material typically does not solely determine the success of STR profile obtained, but most likely, varying levels of DNA degradation and chemical modifications due to the formalin fixation and presence of formalin-fixed residues further reduced DNA amplification.

Conclusion

The current research sought to extract amplifiable DNA from the brain, bone marrow, and cartilage from formalin-embalmed human cadavers.

We demonstrated in the current study that by optimizing the DNA extraction protocol, amplifiable quantities of DNA can be obtained from the brain, bone marrow, and cartilage samples from formalin-embalmed human cadavers. Our

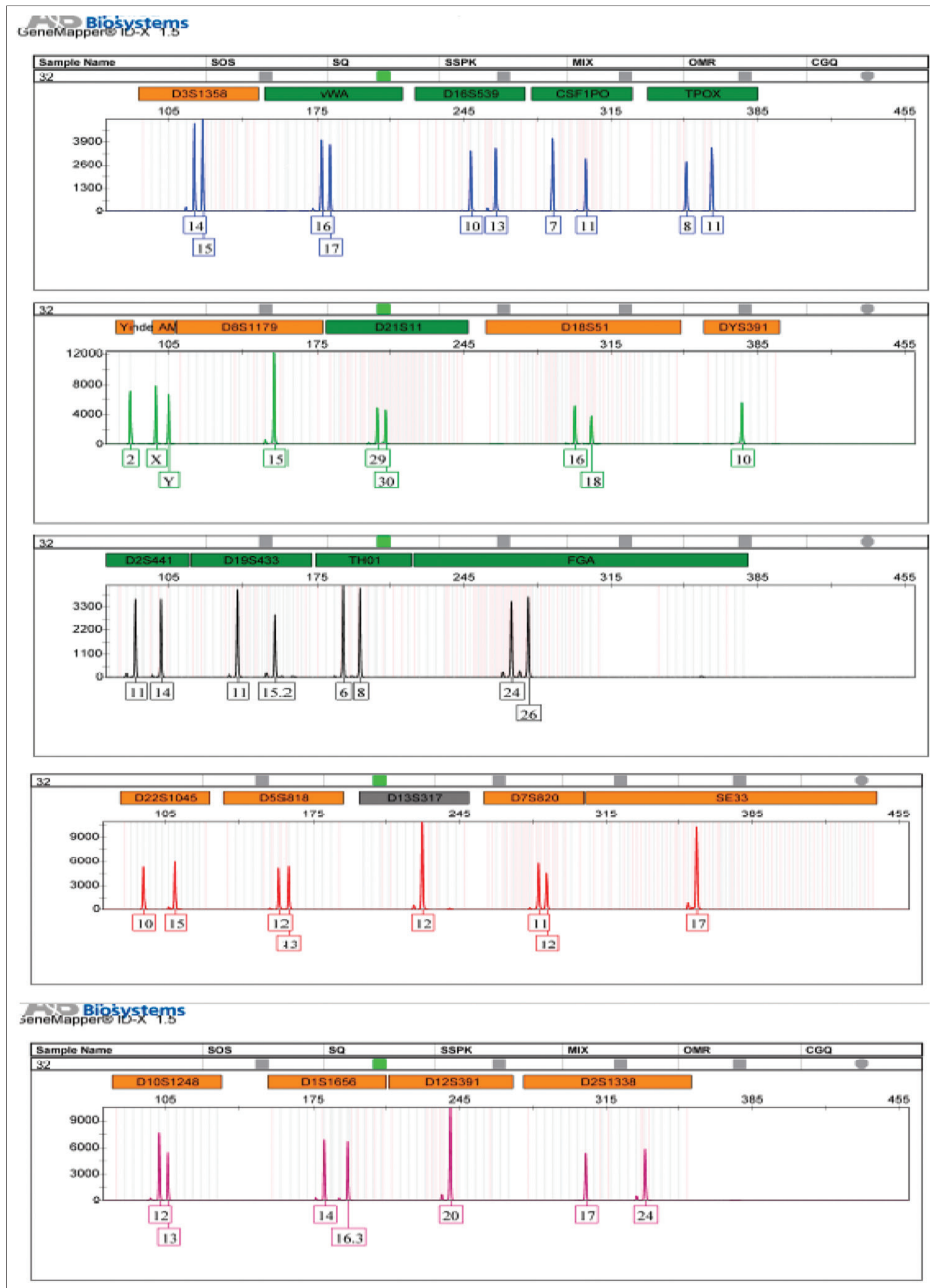

Figure 7: STR typing results of the analyzed samples from cadaver 5 (positive control). STR: Short tandem repeat

optimized protocol reduced the concentration of formalin fixation residues in extracted DNA from formalin-fixed tissues, thereby improving the amplification efficiency for STR profiling.

The extracted DNA from the various tissues from embalmed human cadavers also yielded full STR profiles with varying peak heights for forensic identification purposes.

For human identification purposes, the brain, bone marrow, and cartilage can be good sources of DNA from embalmed and degraded human remains, although for skeletonized human remains, teeth and long bones still remain the samples of choice for DNA extraction.

Research involving human participants and/or animals

Approval was obtained from the Ethics Committee of the Kwame Nkrumah University of Science and Technology, Kumasi.

Author contributions

All authors contributed equally to this research.

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Conflicts of interest

There are no conflicts of interest.

References

1. Gielda L, Rigg S. Extraction of amplifiable DNA from embalmed human cadaver tissue. BMC Res Notes 2017;10:737.
2. Wheeler A, Czado N, Gangitano D, Turnbough M, Hughes-Stamm S. Comparison of DNA yield and STR success rates from different tissues in embalmed bodies. Int J Legal Med 2017;131:61-6.
3. Gouveia GR, Ferreira SC, Ferreira JE, Siqueira SA, Pereira J. Comparison of two methods of RNA extraction from formalin-fixed paraffin-embedded tissue specimens. Biomed Res Int 2014;2014:151724.
4. Panneerchelvam S, Norazmi MN. Forensic DNA profiling and database. Malays J Med Sci 2003;10:20-6.
5. Ziętkiewicz E, Witt M, Daca P, Zebracka-Gala J, Goniewicz M, Jarząb B, *et al.* Current genetic methodologies in the identification of disaster victims and in forensic analysis. J Appl Genet 2012;53:41-60.
6. Budowle B, van Daal A. Forensically relevant SNP classes. Biotechniques 2008;44:603-8, 610.
7. Lehmann U, Kreipe H. Real-time PCR analysis of DNA and RNA extracted from formalin-fixed and paraffin-embedded biopsies. Methods 2001;25:409-18.
8. Coombs NJ, Gough AC, Primrose JN. Optimisation of DNA and RNA extraction from archival formalin-fixed tissue. Nucleic Acids Res 1999;27:e12.
9. Funabashi KS, Barcelos D, Visoná I, e Silva MS, e Sousa ML, de Franco MF, *et al.* DNA extraction and molecular analysis of non-tumoral liver, spleen, and brain from autopsy samples: The effect of formalin fixation and paraffin embedding. Pathol Res Pract 2012;208:584-91.
10. Afrifah KA, Badu-Boateng A, Antwi-Akomeah S, Motey EE, Boampong E, *et al.* Forensic identification of missing persons using DNA from surviving relatives and femur bone retrieved from salty environment. J Forensic Sci Med 2020;6:40-4.
11. Farrugia A, Keyser C, Ludes B. Efficiency evaluation of a DNA extraction and purification protocol on archival formalin-fixed and paraffin-embedded tissue. Forensic Sci Int 2010;194:e25-8.
12. Hansen J, Lesnikova I, Funder AM, Banner J. DNA and RNA analysis of blood and muscle from bodies with variable postmortem intervals. Forensic Sci Med Pathol 2014;10:322-8.
13. Lin J, Kennedy SH, Svarovsky T, Rogers J, Kemnitz JW, Xu A, *et al.* High-quality genomic DNA extraction from formalin-fixed and paraffin-embedded samples deparaffinized using mineral oil. Anal Biochem 2009;395:265-7.
14. Ludyga N, Grünwald B, Azimzadeh O, Englert S, Höfler H, Tapio S, *et al.* Nucleic acids from long-term preserved FFPE tissues are suitable for downstream analyses. Virchows Arch 2012;460:131-40.
15. Zagga AD, Tadros AA, Ismail SM, Ahmed H. PCR inhibitory effects of aldehyde fixing agents on DNA extracted from embalmed human skeletal fragments and teeth specimens. Nurs Health Sci 2013;1:33-7.
16. Hamatani K, Eguchi H, Takahashi K, Koyama K, Mukai M, Ito R, *et al.* Improved RT-PCR amplification for molecular analyses with long-term preserved formalin-fixed, paraffin-embedded tissue specimens. J Histochem Cytochem 2006;54:773-80.
17. Topp T, Müller T, Huss S, Kann PH, Weihe E, Ruchholtz S, *et al.* Embalmed and fresh frozen human bones in orthopedic cadaveric studies: Which bone is authentic and feasible? Acta Orthop 2012;83:543-7.
18. Körbler T, Grsković M, Dominis M, Antica M. A simple method for RNA isolation from formalin-fixed and paraffin-embedded lymphatic tissues. Exp Mol Pathol 2003;74:336-40.
19. Mundorff A, Davoren JM. Examination of DNA yield rates for different skeletal elements at increasing post mortem intervals. Forensic Sci Int Genet 2014;8:55-63.
20. Pooniya S, Lalwani S, Raina A, Millo T, Dogra TD. Quality and quantity of extracted deoxyribonucleic Acid (DNA) from preserved soft tissues of putrefied unidentifiable human corpse. J Lab Physicians 2014;6:31-5.
21. Antica M, Paradzik M, Novak S, Dzebro S, Dominis M. Gene expression in formalin-fixed paraffin-embedded lymph nodes. J Immunol Methods 2010;359:42-6.
22. Johnston E, Stephenson M. DNA profiling success rates from degraded skeletal remains in Guatemala. J Forensic Sci 2016;61:898-902.
23. Constantinescu CM, Barbarii LE, Iancu CB, Constantinescu A, Neagu E, Iancu D, *et al*. Challenging DNA samples solved with MiniSTR analysis. Rom J Leg Med 2012;20:51-6.
24. Welch L, Gill P, Tucker VC, Schneider PM, Parson W, Mogensen HS, *et al.* A comparison of mini-STRs versus standard STRs – Results of a collaborative European (EDNAP) exercise. Forensic Sci Int Genet 2011;5:257-8.
25. Srinivasan M, Sedmak D, Jewell S. Effect of fixatives and tissue processing on the content and integrity of nucleic acids. Am J Pathol 2002;161:1961-71.
26. Rivero ER, Neves AC, Silva-Valenzuela MG, Sousa SO, Nunes FD. Simple salting-out method for DNA extraction from formalin-fixed, paraffin-embedded tissues. Pathol Res Pract 2006;202:523-9.
27. Taguchi M, Inoue H, Motani-Saitoh H, Yajima D, Hayakawa M, Otsuka K, *et al.* DNA identification of formalin-fixed organs is affected by fixation time and type of fixatives: Using the AmpF l STR (R) Identifiler (R) PCR Amplification Kit. Med Sci Law 2012;52:12-6.