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DNA and bone structure preservation in medieval human skeletons

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Abstract

Morphological and ultrastructural data from archaeological human bones are scarce, particularly data that have been correlated with information on the preservation of molecules such as DNA. Here we examine the bone structure of macroscopically well-preserved medieval human skeletons by transmission electron microscopy and immunohistochemistry, and the quantity and quality of DNA extracted from these skeletons. DNA technology has been increasingly used for analyzing physical evidence in archaeological forensics; however, the isolation of ancient DNA is difficult since it is highly degraded, extraction yields are low and the co-extraction of PCR inhibitors is a problem. We adapted and optimized a method that is frequently used for isolating DNA from modern samples, Chelex® 100 (Bio-Rad) extraction, for isolating DNA from archaeological human bones and teeth. The isolated DNA was analysed by real-time PCR using primers targeting the sex determining region on the Y chromosome (SRY) and STR typing using the AmpFlSTR® Identifiler PCR Amplification kit. Our results clearly show the preservation of bone matrix in medieval bones and the presence of intact osteocytes with well preserved encapsulated nuclei. In addition, we show how effective Chelex® 100 is for isolating ancient DNA from archaeological bones and teeth. This optimized method is suitable for STR typing using kits aimed specifically at degraded and difficult DNA templates since amplicons of up to 250 bp were successfully amplified.

Keywords: ancient DNA; archaeological bone; archaeological teeth; Chelex; DNA typing; electron microscopy

1 Introduction

Literature on archaeological human bone morphology and ultrastructure is scarce, particularly data correlating bone structure with DNA preservation. The survival of ancient DNA is of particular interest since DNA technology has been increasingly used in archaeological forensics, for example, for the identification of diseases [1, 2], identification of migration patterns [3, 4] and patrilineage determination [5].

The isolation of amplifiable DNA does not always reflect bone taphonomy and, occasionally, DNA can be isolated from bones that appear to be morphologically degraded [6]. However, analysis at a microscopic level can provide an indication as to the preservation of amplifiable DNA; the isolation of amplifiable DNA has been correlated with integrity of the microscopic structure, particularly lamellae [7]. In addition, more compact appearance of bone in scanning electron micrographs is one of the factors that indicates that amplifiable DNA can be isolated [8]. Other factors include high collagen content, low racemization values of aspartic acid, leucine and glutamic acid, low infrared splitting factor and small size of crystallite [8].

The long-term persistence of DNA in skeletons has been associated with the fact that it can bind to hydroxyapatite, the main mineral component of bone tissue, which makes it more resistant to decay and less susceptible to degradation by enzymes [9]. In addition, DNA has been shown to bind to collagen forming a DNA-collagen complex [10], and can be extracted from archaeological bone powder in both the hydroxyapatite and collagen fractions [11]. The differential survival of DNA in ancient specimens is generally

unpredictable but depends on both the burial environment and how well they were retrieved and preserved.

DNA extraction from archaeological bones and teeth is a challenge and new analytical processes, particularly DNA extraction methods, are required. Methods chosen for extracting ancient DNA must focus on preserving the integrity of the remaining DNA during extraction, whilst ensuring sufficient yield and that it is free of PCR inhibitors [12]. Similar methods can be used for forensic samples and archaeological samples since they share similar characteristics [13]. In forensics, one frequently used method for isolating DNA is extraction using Chelex® 100 (Bio-Rad). The efficiency of the Chelex® 100 extraction method has been described for DNA extraction from various modern forensic samples, including blood, semen, buccal swabs and hair [14]. Previous research has described a protocol using Chelex® 100 for extracting DNA from fresh bone samples [15]. We have adapted and optimized the extraction protocol using Chelex® 100 for isolating ancient DNA from archaeological bones and teeth with the intention of preserving the integrity of the DNA, and ensuring the yield is sufficient and the product is free of PCR inhibitors. Subsequent autosomal STR DNA typing using the AmpFℓSTR® Identifiler® PCR Amplification Kit gave us an indication as to how well-preserved the isolated DNA was. Our results demonstrate that Chelex® 100 is effective for isolating ancient DNA from archaeological bones and teeth.

It is also important to know how well the bones have been preserved at a microscopic level since this has been shown to reflect the integrity of the isolated DNA [7, 8]. This

study therefore looked to provide information on the bone structure of medieval human bones. The morphology and ultrastructure of the archaeological human bones was analyzed by transmission electron microscopy and immunohistochemistry. The data were then correlated with the preservation of amplifiable DNA. Our results clearly show the preservation of bone matrix in medieval bones and the presence of apparently intact osteocytes.

2 Material and methods

2.1 Samples

The medieval skeletons used in this study were curated at the University of Lincoln, UK. Sixty-eight partial or complete skeletal remains were excavated in the last decade from a site on the south side of Monk's Road, Lincoln. The site was determined to be the extramural graveyard of the defunct parish of St Peter at Welles (*ad fontem*). The age of the site was estimated to between 1150 AD and 1400 AD. The appropriate Coroners licence was obtained to excavate the skeletons. Various types of bone were chosen to be analysed; femur (adult, adolescent and juvenile), humerus (adult) and ulna (adult and adolescent). Two archaeological teeth were also analysed. The study of these archaeological human skeletons was approved by the University of Lincoln ethics committee in the UK and CEP/UNIFESP in Brazil (CAAE: 07934412.2.0000.5505).

2.2 Transmission Electron Microscopy (TEM)

Transverse bone sections (75 μm thick) were obtained using a Leica SP1600 Saw Microtome (Leica Biosystems, Nussloch, Germany). The bone sections were immersed

in cacodylate-buffered 1% osmium tetroxide at pH 7.2 for 2 hours. Subsequently, the samples were washed in distilled water and immersed in 2% aqueous uranyl acetate for 2 hours. After washing, the bone slices were dehydrated in graded concentrations of ethanol, treated with propylene oxide and then embedded in Araldite. Semi-thin sections (600-800 nm) were obtained using an ultramicrotome (Leica UCT) in order to ascertain the presence of material, and then ultra-thin sections (70-85 nm) were collected onto grids and stained in alcoholic 2% uranyl acetate and in lead citrate solution, and examined using a ZEISS EM900 electron microscope (Department of Morphology and Genetics, UNIFESP).

2.3 Imunohistochemistry

Transverse bone sections (60 μm thick) were obtained using a Leica SP1600 Saw Microtome (Leica Biosystems, Nussloch, Germany), which can slice hard materials such as bone without any previous treatment that could lead to changes in bone composition and structure. The bone slices were hydrated in PBS buffer for 1 hour at 4°C and then fixed in 2% buffered paraformaldehyde for 30 min followed by washing in PBS and antigen recovery (10 min incubation in 10 mM sodium citrate pH 6 at 100°C). The bone slices were then washed, incubated in 10% hydrogen peroxide for 30 min, washed, and unspecific protein binding sites were blocked with 5% fetal bovine serum (FBS). The slices were then incubated with rabbit anti-osteocalcin (30044, Santa Cruz, Santa Cruz, CA) and mouse monoclonal anti-collagen III (Millipore MAB3392) overnight at 4°C. Bone sections were washed and then processed using the Universal Dako LSAB®+ Kit, Peroxidase (LSAB+ Kit, HRP) and Dako Liquid DAB+ Substrate Chromogen System

(Dako, Glostrup, Denmark). Finally, bone sections were incubated sequentially in 30% ethanol, 50% ethanol, 70% ethanol, 90% ethanol, 100% ethanol, ethanol:xylol (1:1), xylol, and then mounted on glass slides in Permount (Thermo Fisher Scientific Inc., Waltham, MA) and sealed with nail polish. Negative control immunostainings were performed with omission of the primary antibodies, in the presence of FBS, overnight at 4°C.

2.4 DNA extraction

2.4.1 Optimized Chelex® 100 DNA extraction method

The bones and teeth were initially cleaned in water and allowed to dry. In addition, bone samples were mechanically cleaned with sandpaper. Subsequently, the bone and tooth samples were exposed to sodium hypochlorite for 15 minutes at room temperature [16]. Bone powder and tooth powder were collected using a drill (Draper) with drill bits that had been previously exposed to sodium hypochlorite and autoclaved. The bone powder and tooth powder were stored at -20ºC until they were processed.

Bone or tooth powder (100 mg) was suspended in 1 ml 10% Chelex® 100 (Bio-Rad), and the protocol described by Willard *et al.* [15] was adapted and optimised for the archaeological samples in order to improve DNA yield and integrity. Essential modifications to the method were that the initial incubation period was increased to 3 hours at 56ºC and the boiling period to 20 minutes. The resulting solution (containing DNA) was subjected to phenol/chloroform purification (to remove PCR inhibitors) using conventional methods (adding equal volume of phenol:chloroform:isoamyl alcohol

(25:24:1), centrifuging at 15,000 g for 5 minutes and collecting the aqueous layer) followed by isopropanol precipitation [17]. Sodium acetate (500 mM final concentration) was added to the samples prior to the isopropanol. The recovered DNA was stored at -20ºC. A Chelex® 100 extraction reagent blank control was processed at the same time as the samples. Positive modern DNA controls were processed in a different location and at a different time to the ancient DNA samples, as discussed below.

2.4.2 Digestion buffer DNA extraction method

Bone powder was obtained as described above and DNA was isolated using the digestion buffer DNA extraction method as described by Foran *et al.*[18], which is ideal for recovering residual DNA. Briefly, 100 mg bone powder was suspended in 1 ml digestion buffer (20 mM Tris, 50 mM EDTA, 0.1% SDS, pH 7.5) and 15 μl of proteinase K (20 mg/ml) was added. The samples were incubated overnight at room temperature and then at 56°C for 3 hours. This initial incubation step was slightly modified since it was observed that this resulted in better DNA yield and integrity. The supernatant (containing DNA) was subjected to phenol:chloroform:isoamyl alcohol purification and isopropanol/sodium acetate precipitation, as described above. The isolated DNA was stored at -20ºC. No more than two DNA extractions were carried out at the same time, and a digestion buffer extraction reagent blank control was processed at the same time as the samples.

2.4.3 DNA extraction from control samples

DNA was isolated from cheek cells from a volunteer (male) and the investigator carrying out the STR (Short Tandem Repeat) typing assays (female) using a standard teaching protocol [19], and stored at -20ºC. The female DNA was used in the STR-typing assays as a quality control and the male DNA in the real-time PCR amplification assays as a positive control. DNA extraction from these samples was carried out in a different location on a different day to the extraction of DNA from the archaeological samples.

2.5 DNA quantification

Isolated DNA was quantified using a NanoDrop® ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA).

2.6 Autosomal STR DNA typing

The quality of the ancient DNA recovered was evaluated using the AmpFℓSTR® Identifiler® PCR Amplification Kit (Applied Biosystems, Warrington, UK) according to the manufacturer's protocol. This kit includes the following markers: D8S1179, D3S1358, TH01, D19S433, vWA, TPOX, D5S818, D21S11, D7S820, CSF1PO, D13S317, D16S539, D2S1338, D18S51, FGA and the gender identification locus Amelogenin. A control DNA sample, extracted from cheek cells from the investigator carrying out the STR typing, was also typed for quality control. The PCR products were analysed using an Applied Biosystems 310 Genetic Analyser (Applied Biosystems) and the profiles determined using the GeneMapper® ID V3.2 Software (Applied Biosystems). Alleles were assigned according to the International Society of Forensic Genetics (ISFG) guidelines for forensic STR [20].

2.7 Real-time PCR

The sex of archaeological human bone and teeth samples (adult, adolescent and juvenile femur; adult humerus; adult and adolescent ulna; and adult teeth) was identified by amplification of the sex determining region on the Y chromosome (SRY) [21, 22]. A sequence of 93-bp was amplified using the primers described by Esteve Codina *et al.* (without the initial GTTT sequence) [23]. DNA extracted from male cheek cells was amplified as a positive control.

The DNA was amplified in a total volume of 20 μl comprising DNA (40 ng), primers (3 μM), Syber Green PCR Master Mix (Applied Biosystems) in MicroAmp® Optical 96-Well Reaction Plates (Applied Biosystems). Real-time PCR amplifications were carried out in a 7500 Real-Time PCR system (Applied Biosystems) and the thermal cycling conditions were: 95°C for 10 min, 40 cycles of 95°C for 15 sec, 58°C for 1 min and 72°C for 30 sec. Amplified-product specificity was analysed by means of dissociation curves.

2.8 Quality Control

The laboratory has previously passed a quality assurance exercise (YHRD) test on 20 May 2010 [24].

3 Results

3.1 Electron microscopy

Transmission Electron Microscopy was carried out in archaeological bone samples.

Collagen fibers were observed compounding an apparently well-organized bone matrix,

demonstrating how well preserved the bones are (arrows in Figure 1 B and C).

Interestingly, an apparently intact osteocyte was observed inside a lacuna, surrounded by

bone matrix (Figure 1 A, B and C). In figure 1 B and C, an electron opaque cytoplasmic

portion of the osteocyte can be seen, with its cytoplasmic process traversing the interior

of a canaliculus. Apparently degenerated osteocytes were also observed (Figure 1 D and

E). In Figure 1D, the osteocyte is inside a large lacuna presenting disperse and flocculent

material (asterisks). This material can be seen in more detail in figure 1F, and seems to be

composed of collagen fibers and other molecules such as proteoglycans that resulted from

some form of degradation of the peri-lacuna bone matrix material, which is shown in

figure 1D as an electron opaque region surrounding the lacuna (arrows in figure 1D).
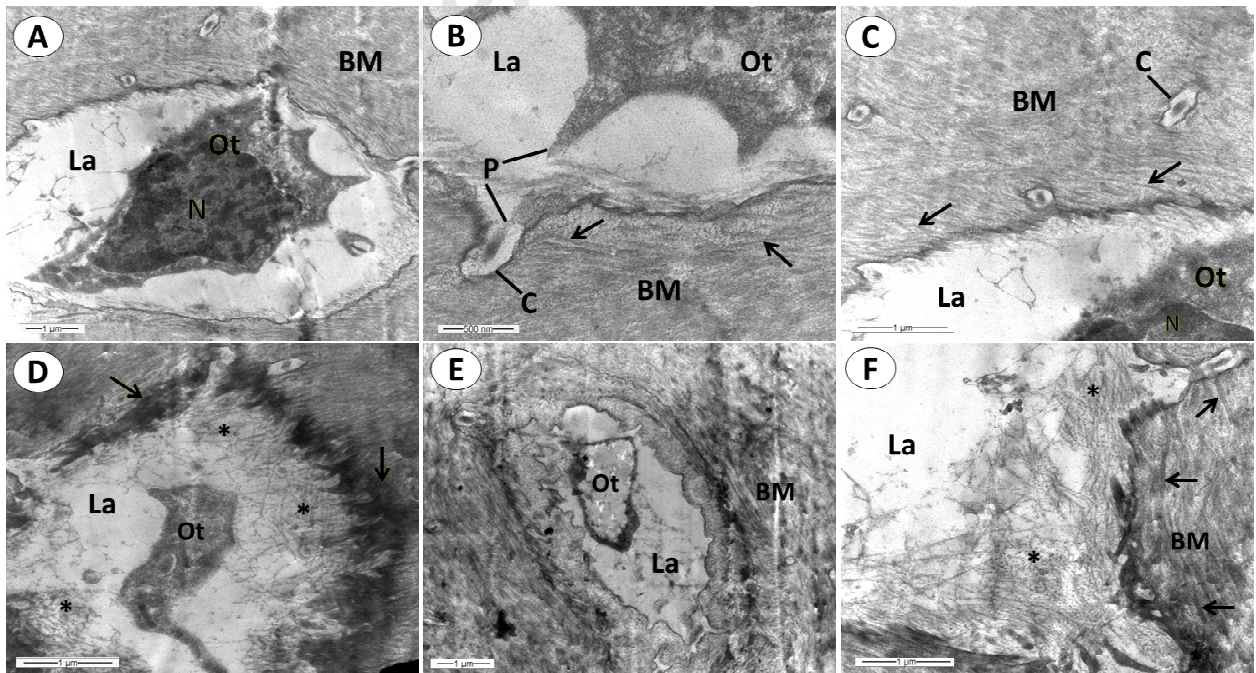
Figure 1 Electron micrographs of adolescent femur slices. **A** shows an apparently intact osteocyte (Ot) inside a lacuna (La), surrounded by bone matrix (BM). **B** and **C** represent amplified regions of **A**, showing an electron opaque cytoplasmic portion of the osteocyte (Ot), with its cytoplasmic process (P) traversing the interior of a canaliculus (C), and collagen fibers (black arrows). **D** and **E** show apparently degenerative osteocytes (Ot). In **D** the osteocyte is inside a large lacuna (La) presenting disperse and flocculent material (asterisks), which is seen in more detail in image **F.** This material seems to result from degradation of the peri-lacuna bone matrix material, the electron opaque region surrounding the lacuna (La) (arrows).

3.2 Imunohistochemistry

We analyzed the location of osteocalcin and collagen III in archaeological human bone slices. Due to the potential fragility and already compromised structure of the archaeological bones, care was taken to obtain bone slices with no previous decalcification. Osteocalcin and collagen III were immunostained using rabbit anti-osteocalcin (30044, Santa Cruz, Santa Cruz, CA) and mouse monoclonal anti-collagen III (Millipore MAB3392), respectively. A similar type of immunostaining pattern was observed for collagen III and the non-collagenous protein osteocalcin; evenly distributed throughout the bone matrix (Figure 2). The osteons are well preserved and remnants of the lacuno-canalicular network can also be observed (asterisk in figure 2).
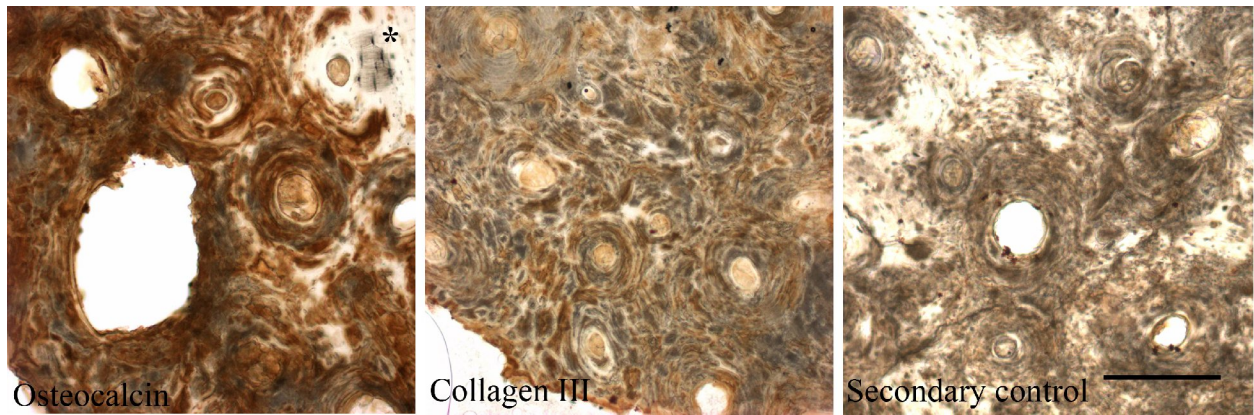
Figure 2 Immunolocalization of osteocalcin and collagen III in archaeological human bone slices. Archaeological human femur slices (60 μm thick) were labeled for osteocalcin and collagen III, and developed using DAB (brown). Remnants of the lacuno-canalicular network can be observed (asterisk). Scale bar: 200 μm.

3.3 Optimisation of the DNA extraction method

In the present study we optimised a method for extracting ancient DNA from archaeological bones and teeth using Chelex® 100 (Bio-Rad). The established method for fresh bone [15] was adapted and optimised for archaeological bones by incubating bone powder in a 10% rather than 5% Chelex® 100 suspension, and for an extended incubation period of three hours (under vertical rotation) rather than 30 minutes to ensure that sufficient DNA was released from the bone powder. Quantification of DNA isolated with incubation periods of one, three and five hours showed that the three- and five-hour periods resulted in a similar amount of DNA but more than the one-hour period. The subsequent step of boiling the DNA sample with Chelex® 100 was increased from the established 10 minutes to 20 minutes. We included the steps of phenol:chloroform purification and isopropanol precipitation following the Chelex® 100 extraction to ensure

any PCR inhibitors were removed and to finally concentrate the DNA. The ancient DNA

recovered using this method was suitable for STR typing and real-time PCR

amplification as described below.

3.4 DNA quality

The quality of the ancient DNA was evaluated by STR typing using the AmpFlSTR®

Identifiler PCR Amplification kit (Applied Biosystems). Ancient DNA extracted from

archaeological human bones using Chelex® 100 followed by phenol:chloroform:isoamyl

alcohol purification and isopropanol/sodium acetate precipitation was successfully

amplified for STR markers producing amplicons of up to 250 bp (Figure 3). The

electropherogram peaks were informative and a partial profile was obtained. DNA

recovered from archaeological skeletons using this optimized protocol would be suitable

for STR typing using kits aimed specifically at degraded and difficult DNA templates,

such as Applied Biosystems' AmpFℓSTR® MiniFiler™ PCR Amplification kit.
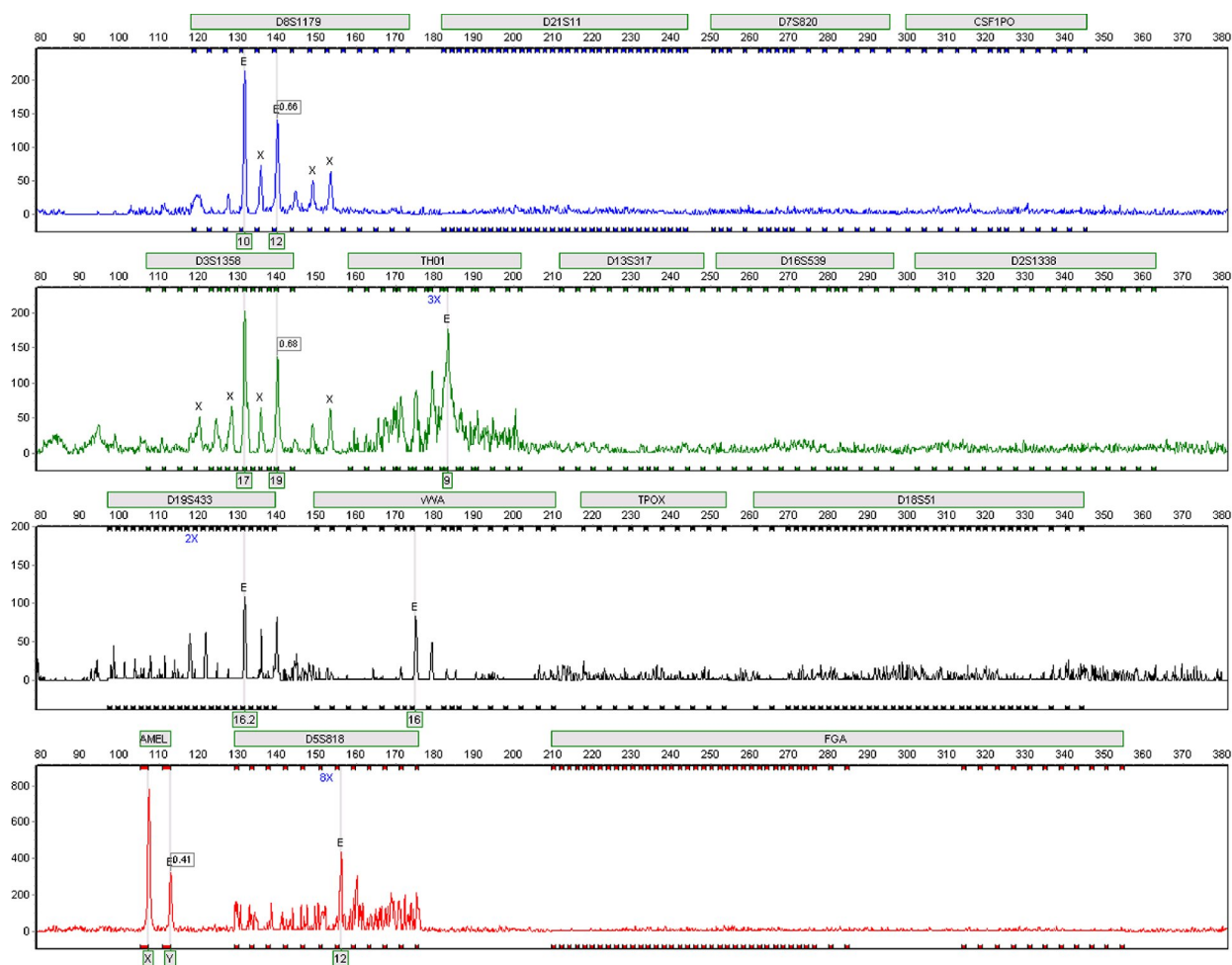
Figure 3 AmpFℓSTR® Identifiler® electropherogram for DNA extracted from archaeological human adult femur using Chelex® 100 (Bio-Rad) followed by phenol/chloroform purification and isopropanol precipitation.

Ancient DNA isolated from archaeological human bones using only Chelex® 100 (without phenol:chloroform:isoamyl alcohol purification and isopropanol/sodium acetate precipitation) resulted in unsuccessful ancient DNA amplification (Results not shown). This could be due to isolated DNA that is not concentrated enough for STR typing and/or the presence of PCR inhibitors.

DNA extracted from cheek cells from the investigator carrying out the STR typing was also STR typed for quality control purposes and the electropherogram obtained reveals a profile different to that obtained for the archaeological human bone samples (Figure 4).
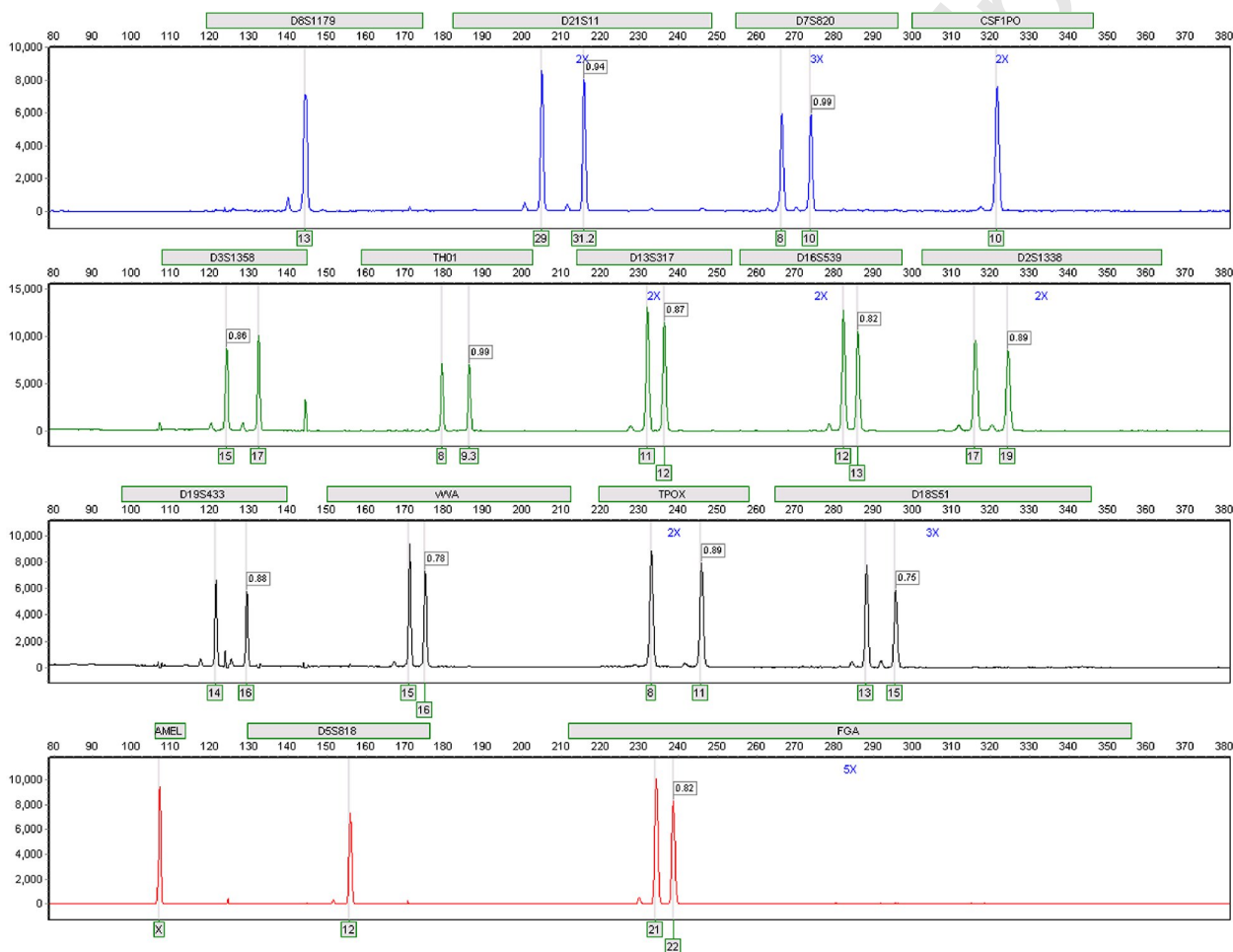
Figure 4 mpFℓSTR® Identifiler® electropherogram for DNA extracted from modern cheek cells.

3.5 PCR amplification for gender identification

DNA was extracted from a further eight archaeological human bone samples (adult, adolescent and juvenile femur; adult humerus (two bones); adult radius; and adult and

adolescent ulna) and two archaeological human teeth samples using the optimised Chelex method (Chelex® 100 followed by phenol:chloroform:isoamyl alcohol purification and isopropanol/sodium acetate precipitation). The DNA samples were amplified by real-time PCR for the sex determining region on the Y chromosome using the primers described by Esteve Codina *et al*. (without the initial GTTT sequence) [23]. The real-time PCR amplifications were initiated using 100 times more ancient DNA than would normally be used in the case of modern DNA so as to ensure sufficient template molecules. Research has stressed the need for starting with sufficient DNA for preventing PCR amplification errors [25]. DNA isolated from seven samples (juvenile femur, two adult humeri, adult and adolescent ulna, and two teeth) showed amplification of the target SRY sequence (93 bp), starting at cycles 24 to 30 (five samples, all triplicates, shown in Figure 5). DNA serving as a positive control was extracted from male cheek cells and showed positive amplification starting at cycle 22.
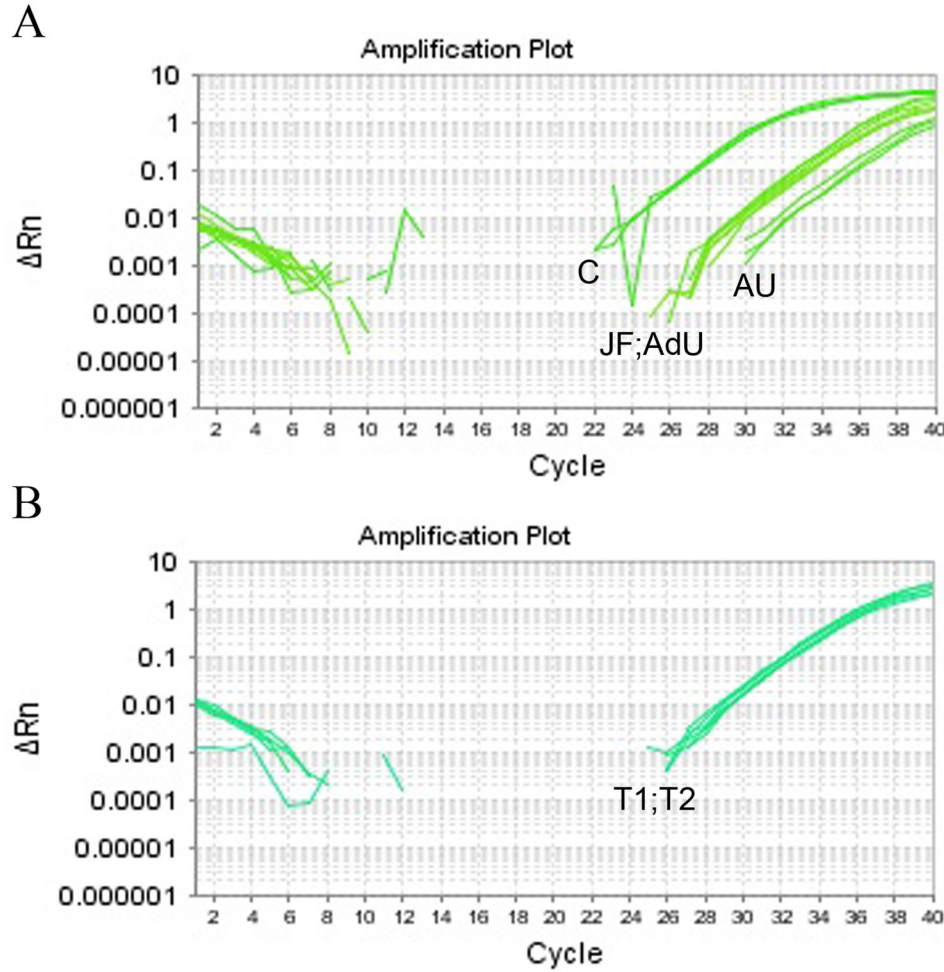
Figure 5 Real-time PCR amplification of the SRY region of DNA extracted from archaeological human bones (A) and teeth (B) using the optimised Chelex 100 method. DNA extracted from male cheek cells was used as the positive control. C: control; JF: juvenile femur; AdU: adolescent ulna; AU: adult ulna; T1: tooth one; T2: tooth two.

Amplified-product specificity was analysed by means of dissociation curves and only one peak was observed per sample indicating that only one product had been amplified (Figure 6).
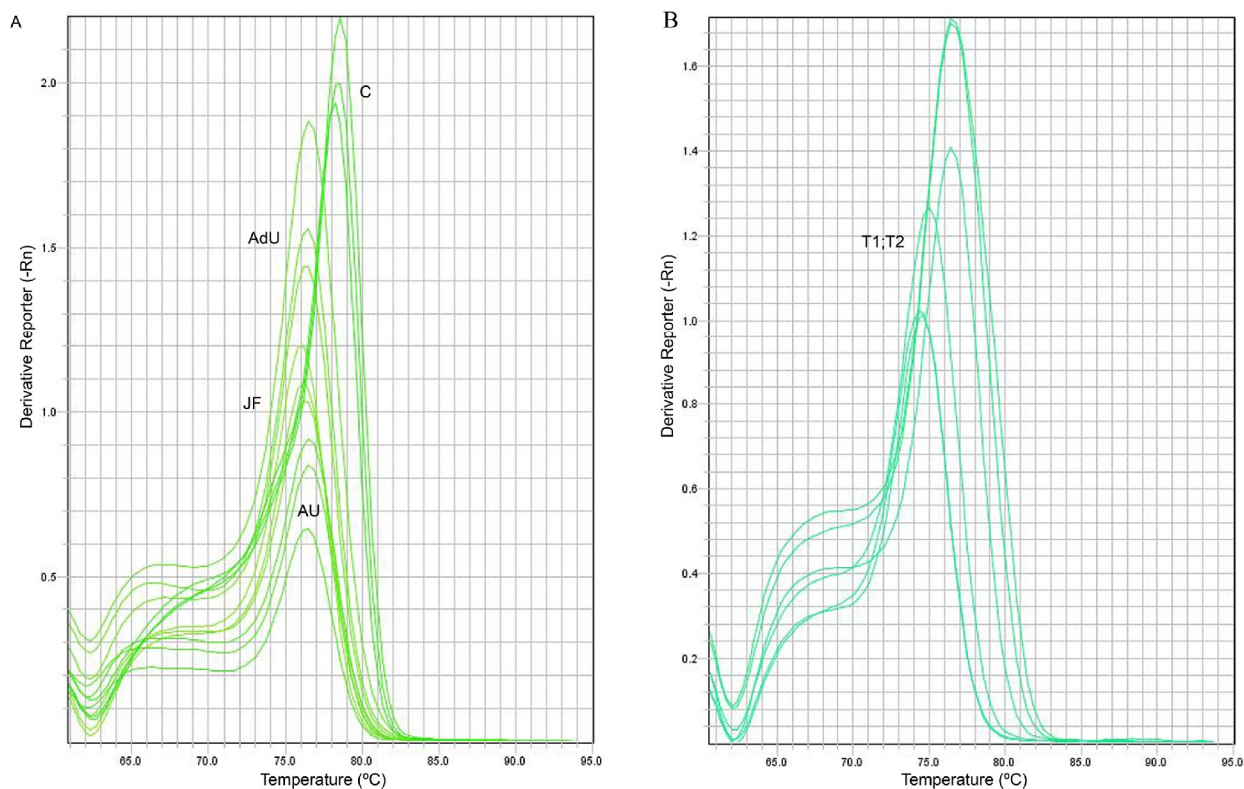
Figure 6 Melt curves of the real-time PCR amplification of DNA extracted from archaeological human bones (A) and teeth (B) using the optimised Chelex 100 method. DNA extracted from male cheek cells was used as the positive control. C: control; JF: juvenile femur; AdU: adolescent ulna; AU: adult ulna; T1: tooth one; T2: tooth two.

3.6 Comparison of the optimised Chelex® 100 DNA extraction method with a digestion buffer DNA extraction method

The isolation of ancient DNA using the optimised Chelex® 100 DNA extraction method was compared to isolation using another method frequently used in forensics consisting of proteinase K digestion followed by phenol:chloroform extraction. Larger quantities of ancient DNA were obtained using the digestion buffer DNA extraction method in comparison to the optimised Chelex® 100 DNA extraction method (Table 1). However,

on the basis of the quality of the isolated ancient DNA, the optimised Chelex® 100 DNA

extraction method proved to be a more efficient method since longer amplicons were

obtained when STR typing using the AmpFlSTR® Identifiler PCR Amplification kit.

Table 1 Ancient DNA isolated from archaeological human bone powder

Sample	DNA extraction method	Total DNA (ng)
Archaeological human adult femur	Chelex® 100	1204
	Chelex® 100 + phenol:chloroform:isoamyl alcohol	1577
	Digestion buffer + phenol:chloroform:isoamyl alcohol	6132
Archaeological human adult humerus	Chelex® 100	1417
	Chelex® 100 + phenol:chloroform:isoamyl alcohol	2721
	Digestion buffer + phenol:chloroform:isoamyl alcohol	3293
Modern cheek cells	Chelex® 100	2971
Chelex® 100 extraction reagent blank control	Chelex® 100 + phenol:chloroform:isoamyl alcohol	0
Digestion buffer extraction reagent blank control	Digestion buffer + phenol:chloroform:isoamyl alcohol	0

As mentioned above, ancient DNA extracted from archaeological human bones using the

optimised Chelex® 100 method produced amplicons of up to 250 bp (Figure 3), whereas

ancient DNA isolated using the digestion buffer method provided electropherograms

without informative peaks, due to artefacts such as additional peaks or elevated stutters,

or due to the absence of peaks (Figure 7).
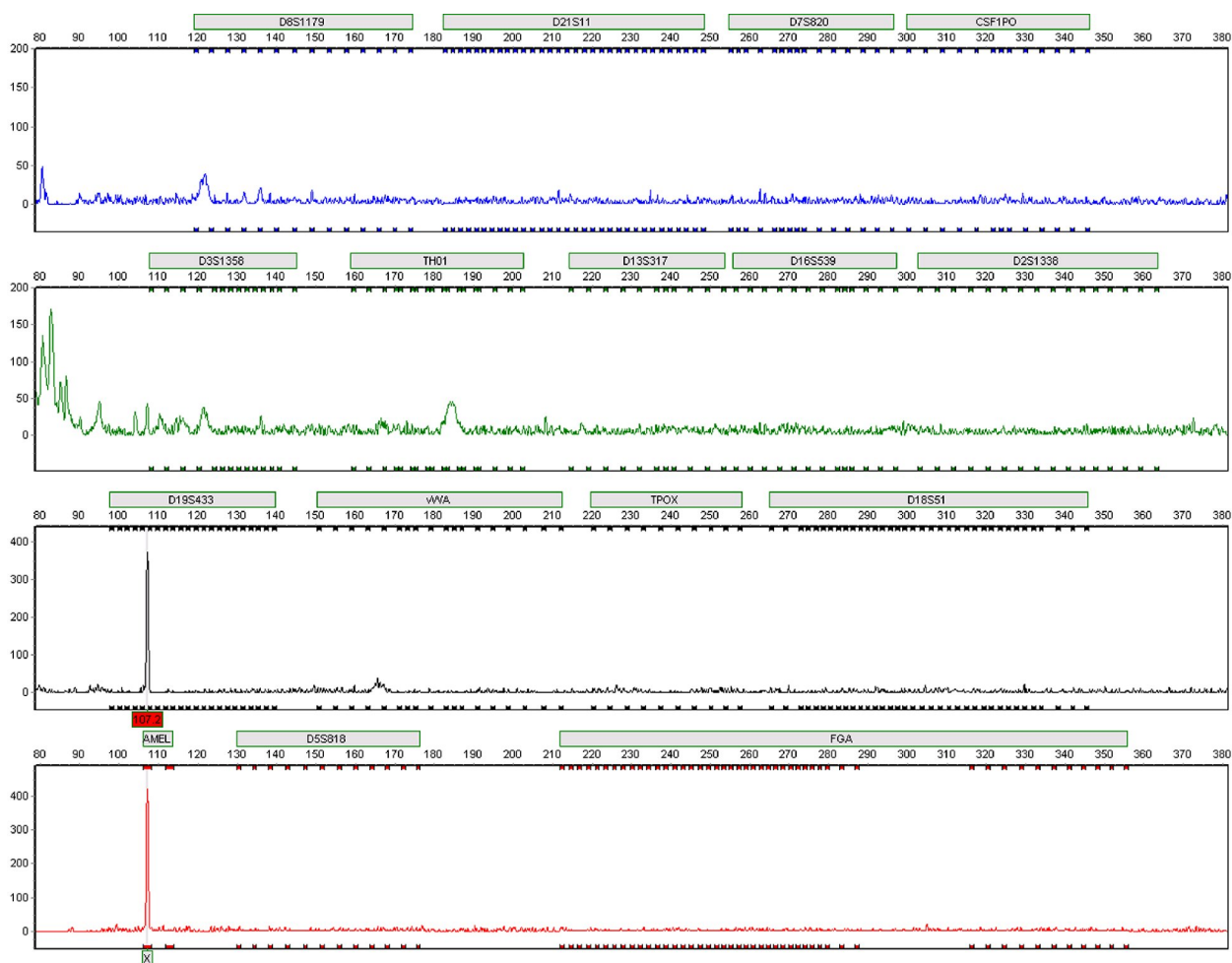
Figure 7 AmpFℓSTR® Identifiler® electropherogram for DNA extracted from archaeological human adult femur using digestion buffer followed by phenol/chloroform purification and isopropanol precipitation.

4 Discussion

DNA recovered from ancient materials does not always produce PCR amplification products due to DNA template damage [26], and the extraction method used is of critical importance for isolating amplifiable DNA. An effective method for isolating DNA frequently used in forensics is extraction using Chelex® 100 (Bio-Rad), so we optimized this method for isolating ancient DNA from archaeological bones and teeth, seeking to

preserve the integrity of the DNA, and ensure sufficient yield and purity for real-time PCR amplification and STR typing. This method proved to be a more efficient method for isolating ancient DNA than another method frequently used in forensics consisting of proteinase K digestion followed by phenol:chloroform extraction, which can result in extracts that inhibit *Taq* polymerase [25].

Since boiling forensic DNA samples with Chelex® 100 has been shown to be critical for protecting the DNA from degradation [14], we increased this incubation period in Chelex® 100 for archaeological bone and tooth samples to ensure the quality of the DNA. In order to remove PCR inhibitors from the archaeological samples, steps of phenol:chloroform purification and isopropanol precipitation were included, which have been shown to remove PCR inhibitors from ancient bone extracts [17]. These steps also served to concentrate the DNA ensuring sufficient quantity for PCR amplification.

Chelex® 100 extraction is a very effective method for isolating DNA from forensic bone samples; it has, for example, been used for successfully isolating amplifiable DNA from bones that have been heated during a prolonged period [27]. Our results clearly show that Chelex® 100 is also effective for isolating ancient DNA from archaeological bones and teeth, and the isolated DNA is suitable for STR typing and real-time PCR amplification. Other methods have been compared and optimized for extracting ancient DNA from archaeological skeletons; however, the PCR products analyzed were in the range of 100 bp and the amplification of larger products was not evaluated [28]. We were able to verify what amplicon size range could be obtained for ancient DNA isolated from

archaeological bones and teeth using the optimized Chelex® 100 extraction method by carrying out STR typing using the AmpFlSTR® Identifiler PCR Amplification kit. Amplicons of up to 250 bp were obtained, providing a partial STR profile using this kit. This size would be ideal for STR typing using kits aimed specifically at degraded and difficult DNA templates, such as the AmpFLSTR® MiniFiler[TM] PCR Amplification Kit (Applied Biosystems®).

Relatively well-preserved DNA has been identified within the protective environment of intergrown crystal aggregates within fossil bones [29]. Furthermore, the preservation of DNA in archaeological bones has been correlated with various factors including nearly perfect micromorphology, with only small areas of localized demineralization [30], lamellae integrity [7], a more compact appearance of bone in scanning electron micrographs and high collagen content [8]. We therefore analyzed the medieval human bone samples by transmission electron microscopy, which is a powerful tool that has been used to study the ultrastructural characteristics of both archaeological and fossilized bones [31, 32]. Our findings show intact osteons and an apparently well-organized bone matrix, containing molecules such as collagen and osteocalcin, in medieval human bones, demonstrating how well preserved the bones are.

Interestingly, in the transmission electron microscopy analysis we observed an apparently intact osteocyte inside a lacuna, surrounded by bone matrix. Usually, only the lacuno-canalicular network is observed in archaeological bones, such as in the immunohistochemistry images in the present study. Mineralized osteocytes have been

previously described in fossilized bone dating to the Cretaceous and Jurassic periods [32, 33] and 5 million years BP [31]. These cells have been shown to mineralize *in vivo*, as a form of *in vivo* death, and are a potential source of preserved DNA [31].

5 Conclusions

In conclusion, this paper provides morphological and ultrastructural information on medieval human bones, and describes an optimized method using Chelex® 100 for isolating ancient DNA from archaeological bones and teeth. The isolated DNA can be used for sex determination and DNA profiling, and this optimised Chelex 100 method is efficient, simple and reproducible.

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Table 1 Ancient DNA isolated from archaeological human bone powder

Sample	DNA extraction method	Total DNA (ng)
Archaeological human adult femur	Chelex® 100	1204
	Chelex® 100 + phenol:chloroform:isoamyl alcohol	1577
	Digestion buffer + phenol:chloroform:isoamyl alcohol	6132
Archaeological human adult humerus	Chelex® 100	1417
	Chelex® 100 + phenol:chloroform:isoamyl alcohol	2721
	Digestion buffer + phenol:chloroform:isoamyl alcohol	3293
Modern cheek cells	Chelex® 100	2971
Chelex® 100 extraction reagent blank control	Chelex® 100 + phenol:chloroform:isoamyl alcohol	0
Digestion buffer extraction reagent blank control	Digestion buffer + phenol:chloroform:isoamyl alcohol	0

- Electron microscopy reveals the extracellular matrix is preserved in medieval bones

- Medieval bone extracellular matrix proteins may be analyzed by immunohistochemistry

- An efficient method is provided for isolating ancient DNA for forensic analysis

- This adapted Chelex®100 method isolates high yields of archaeological skeleton DNA

- Ancient DNA isolated using Chelex®100 is suitable for STR typing and real-time PCR

Figure 1

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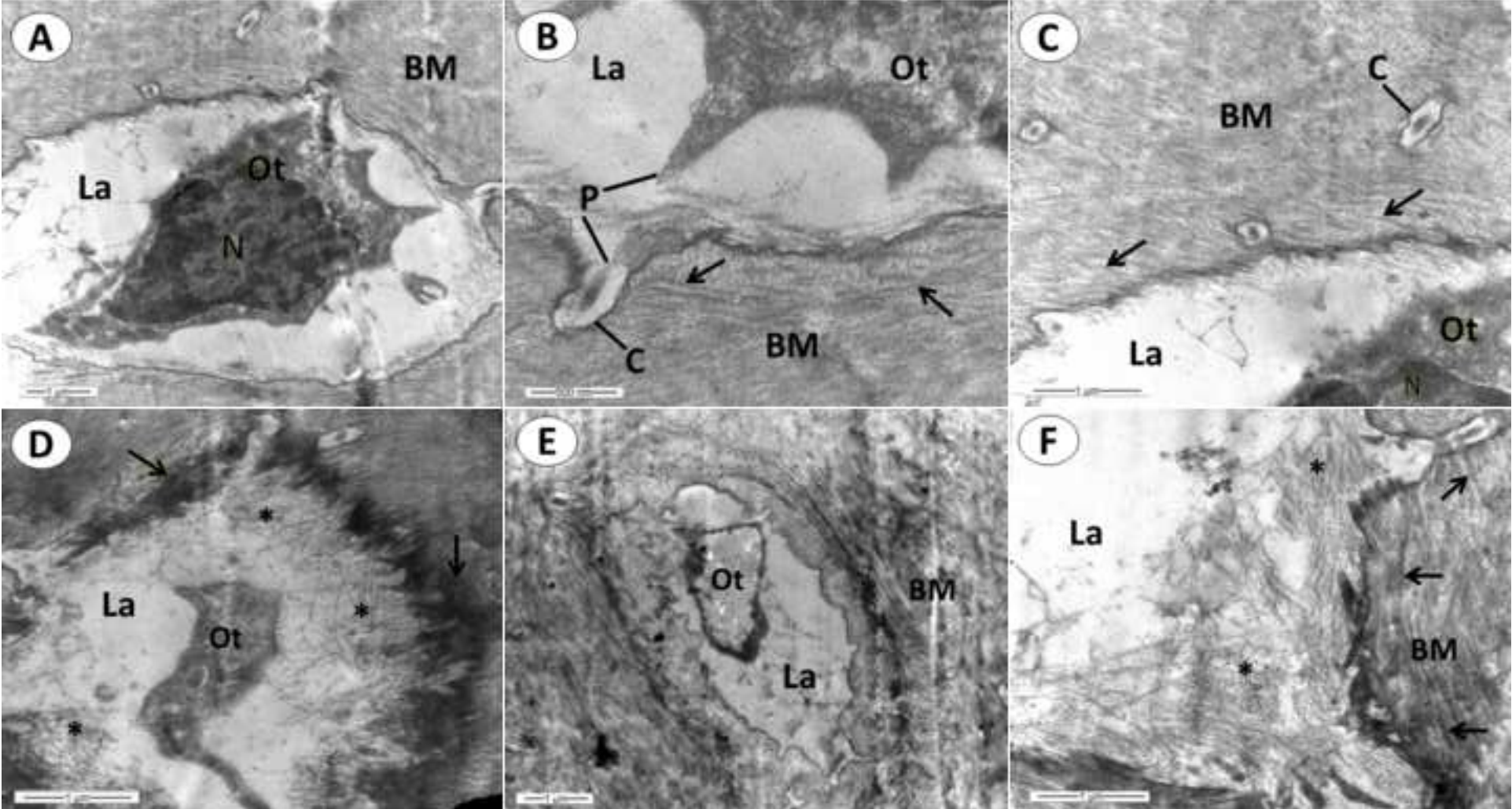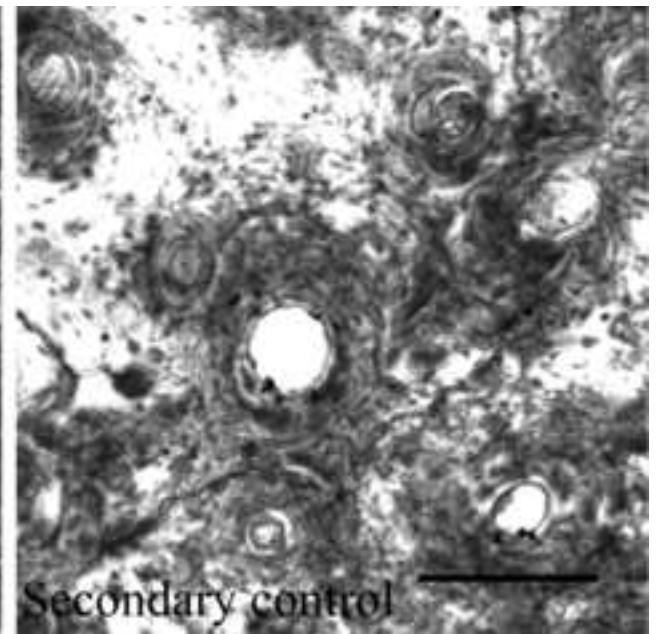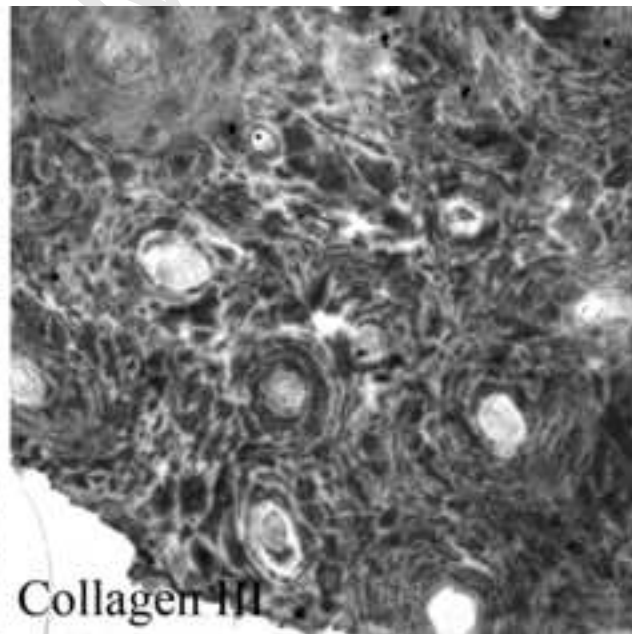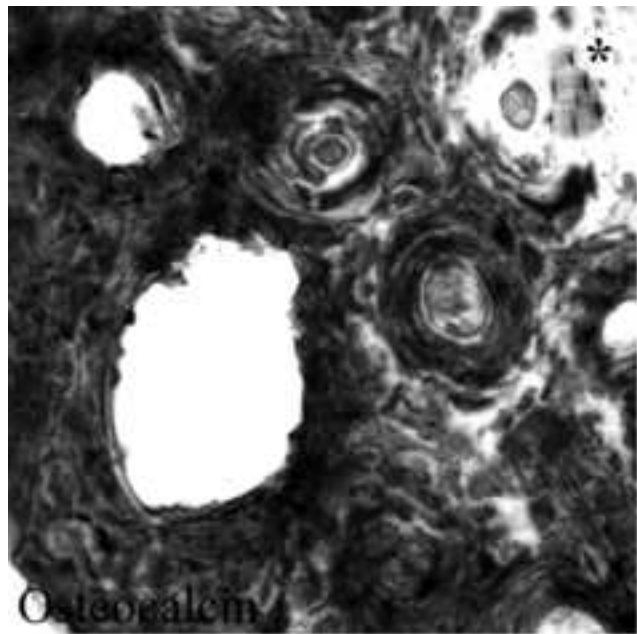
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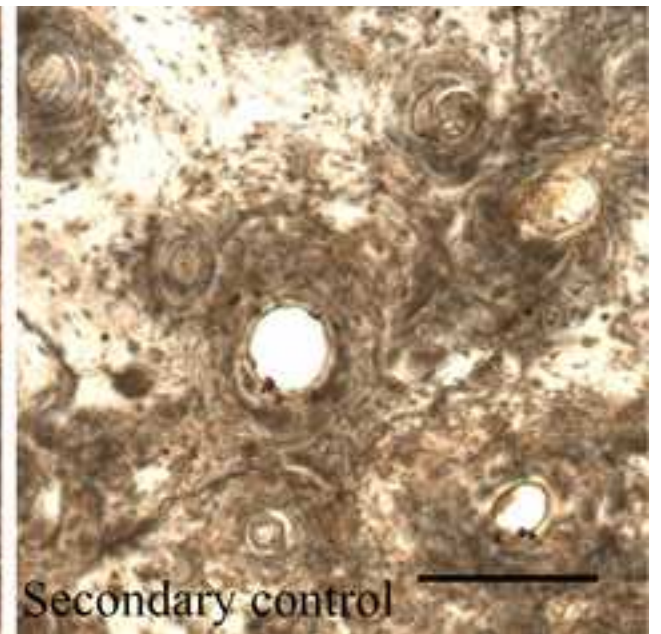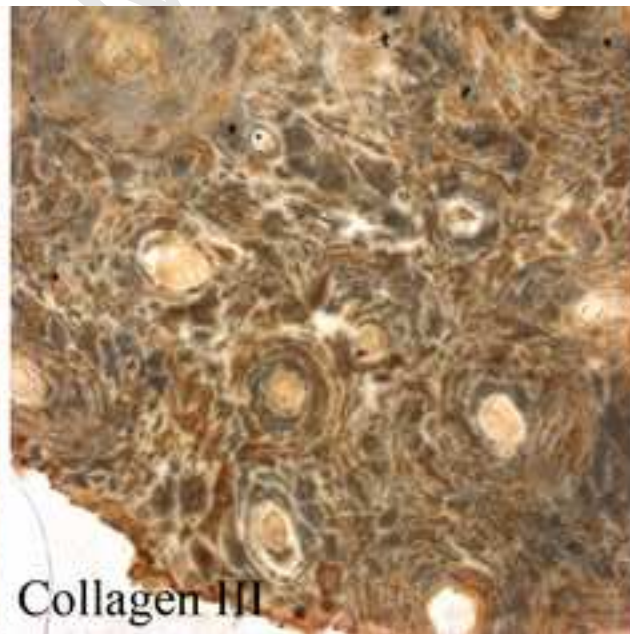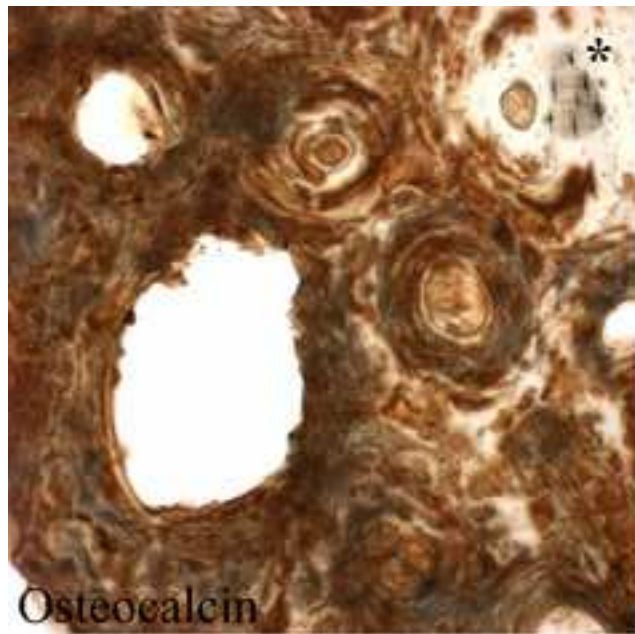
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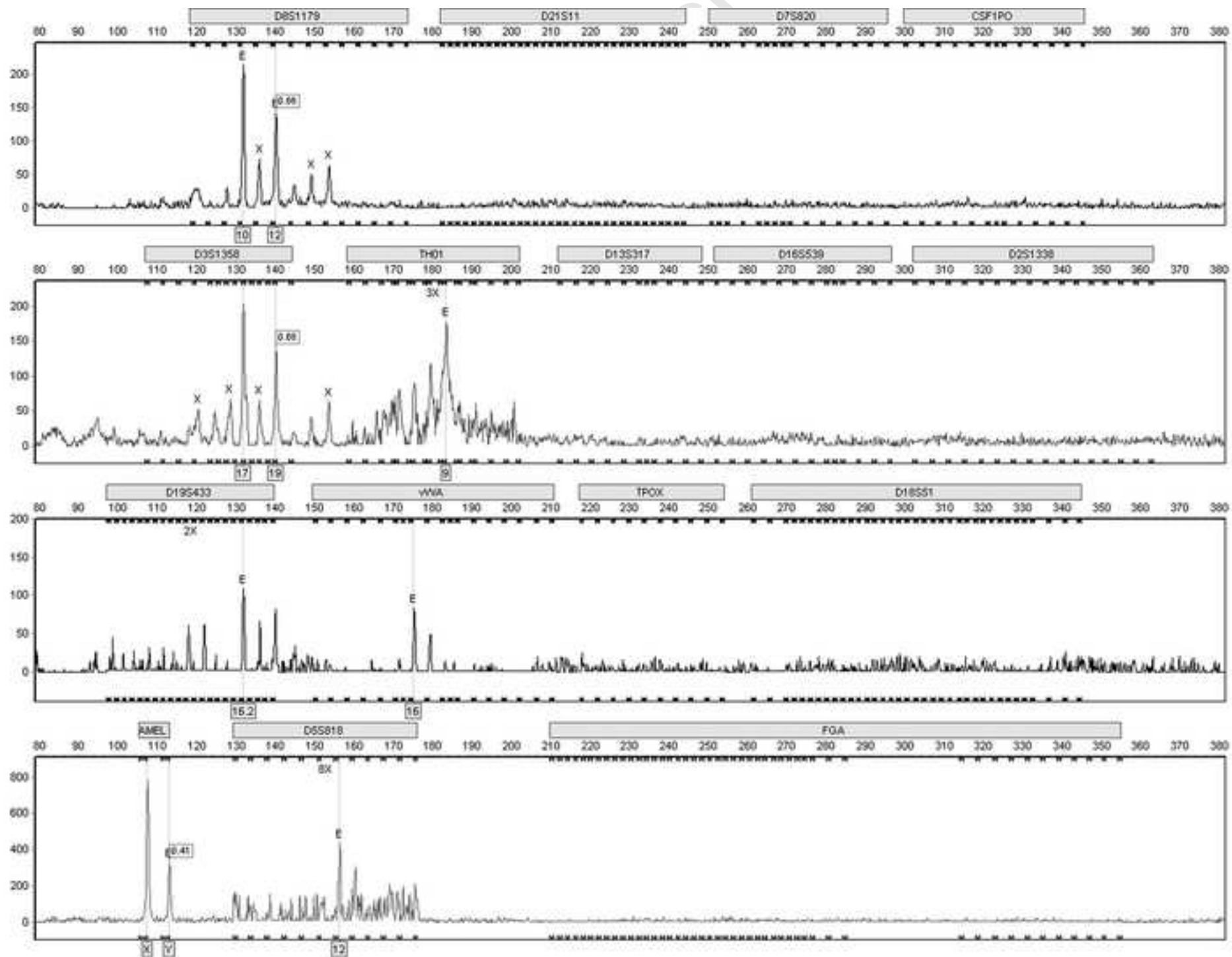
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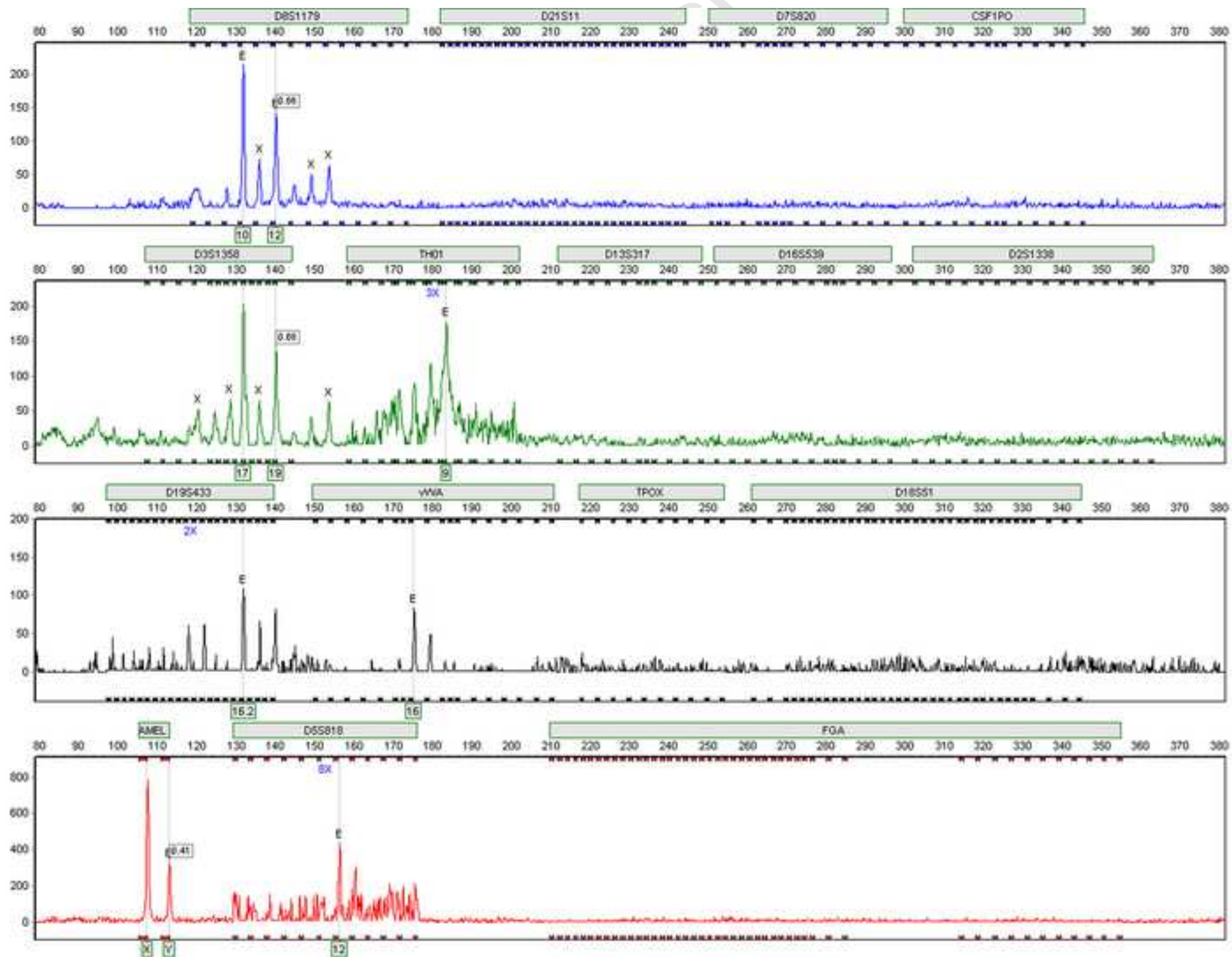
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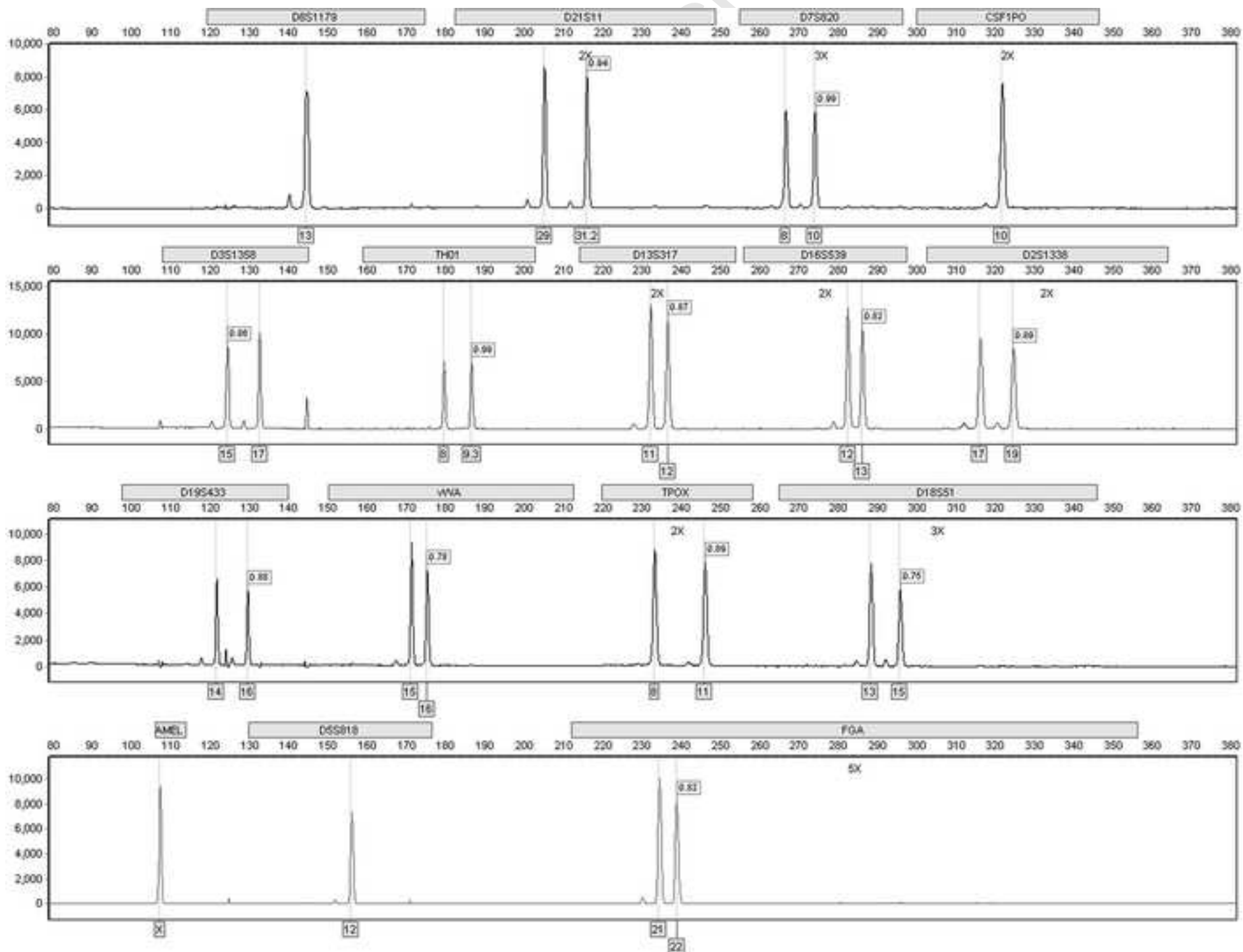
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Figure 5 black and white for printing

Figure 5 colour for web only

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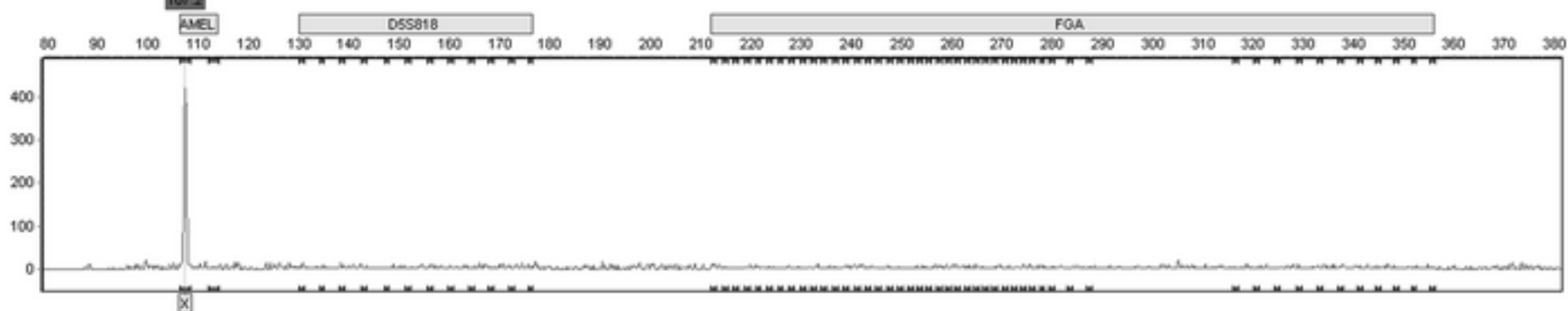
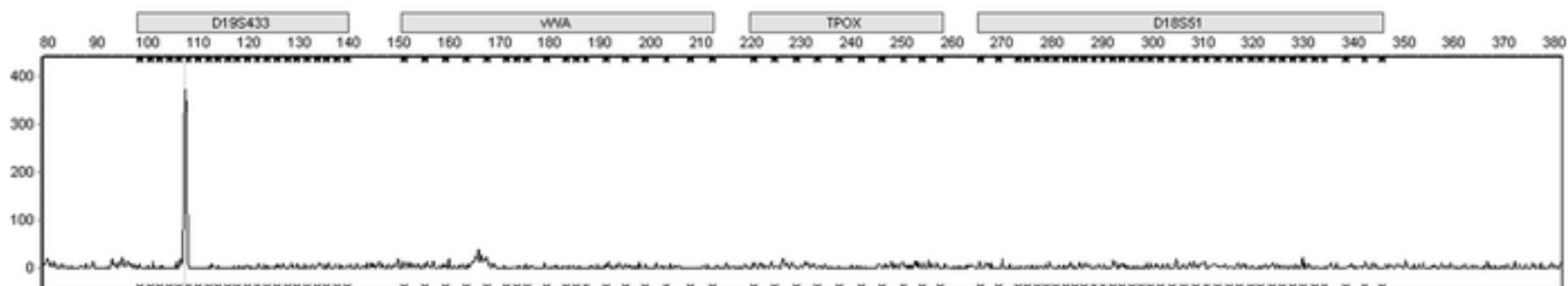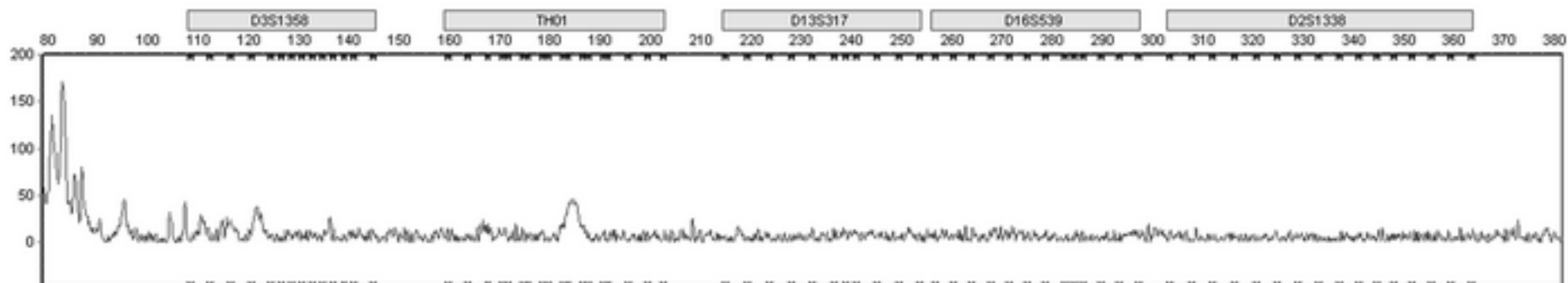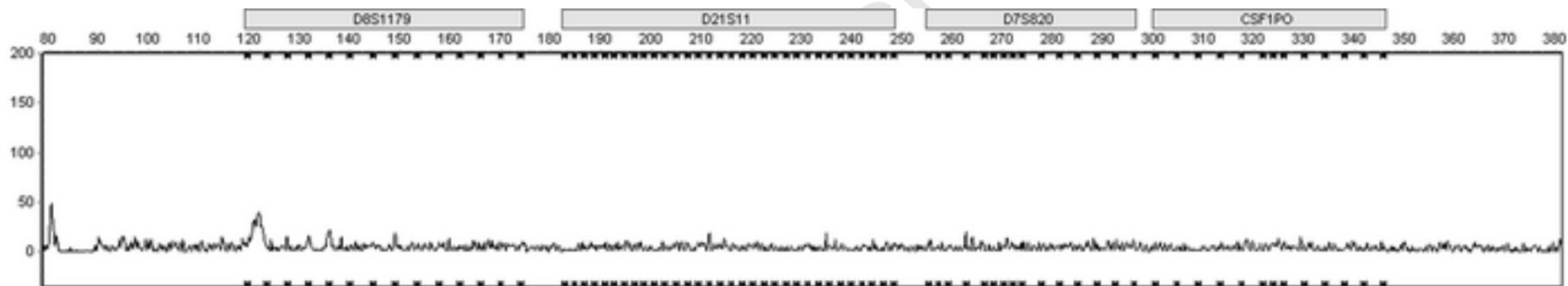Figure 6 colour for web only
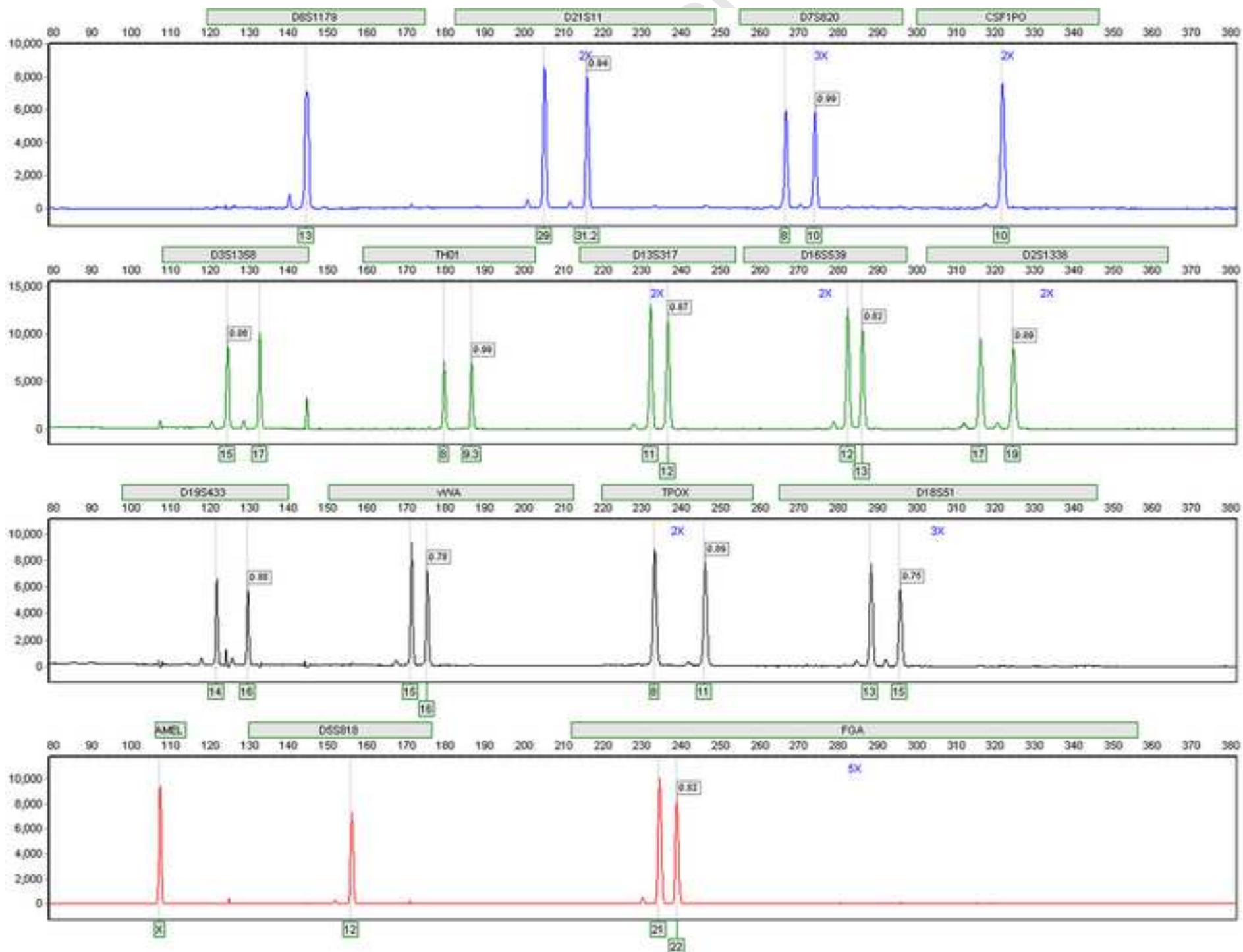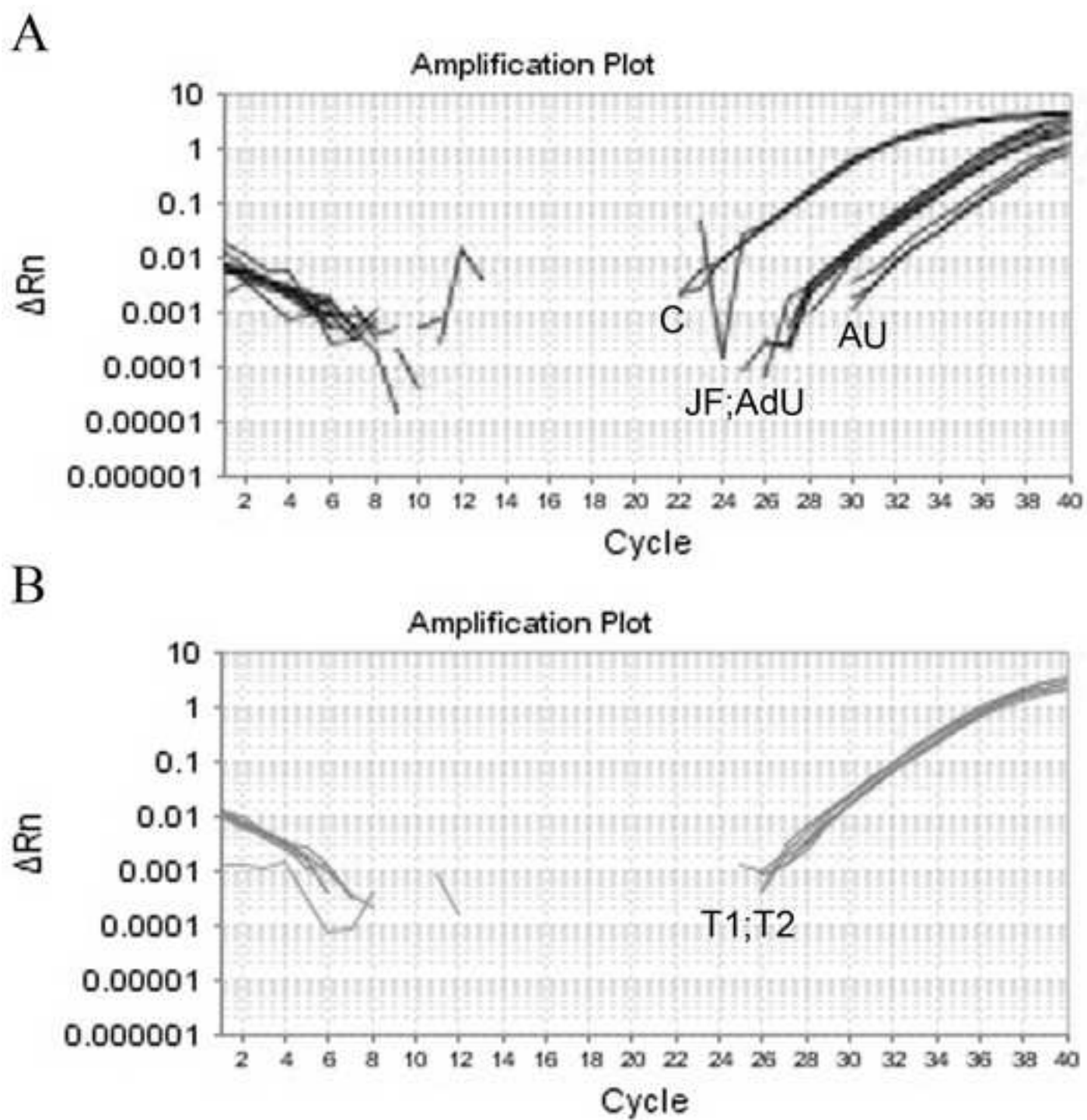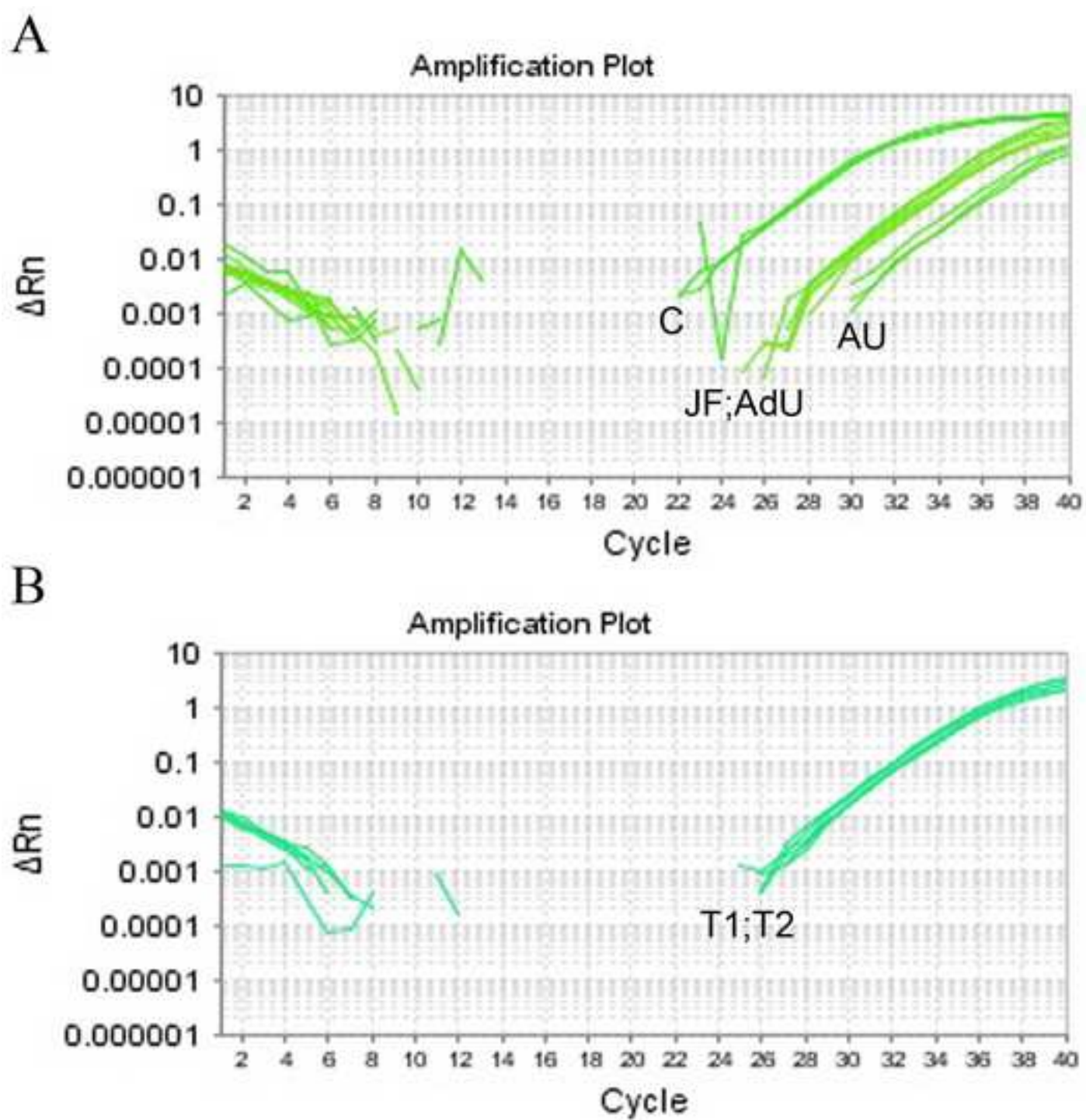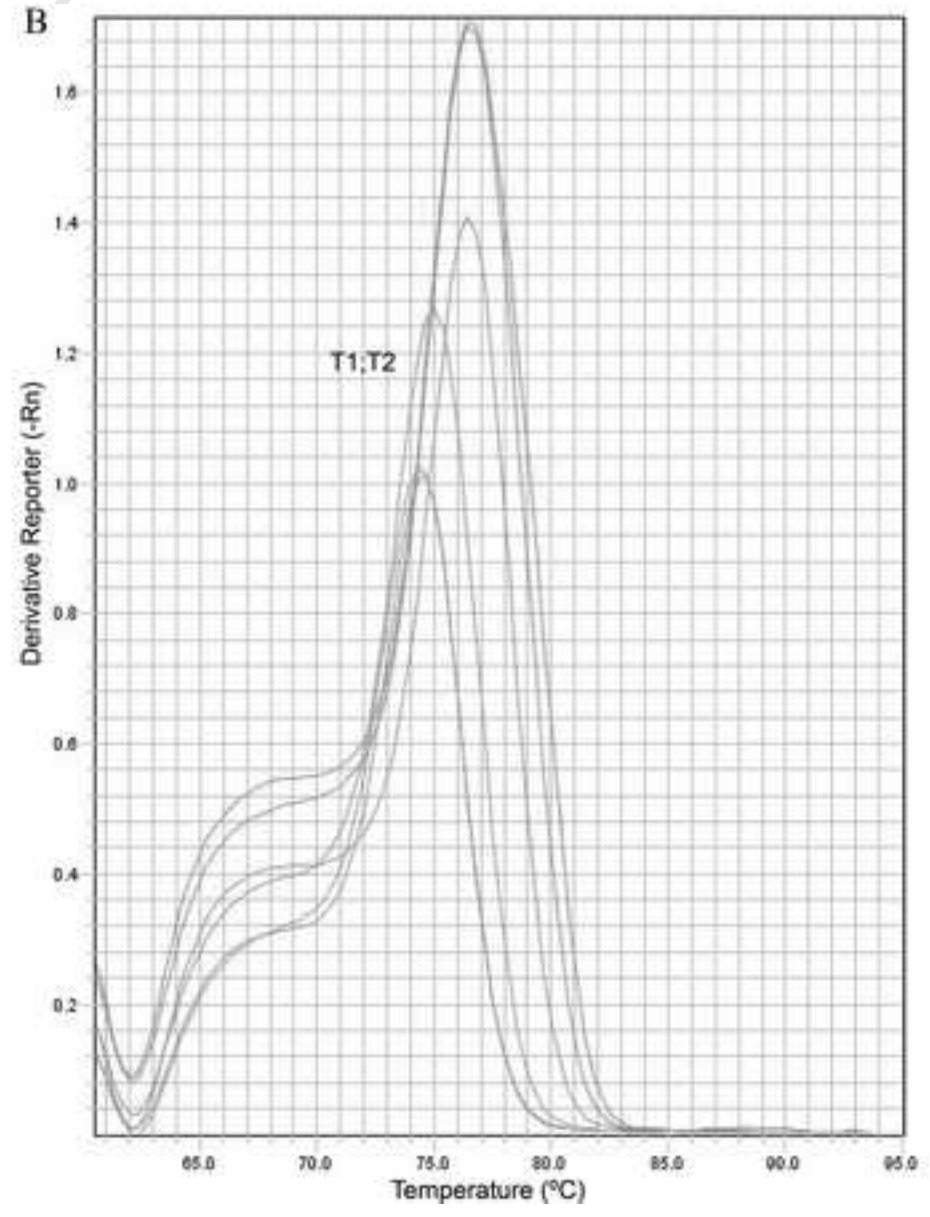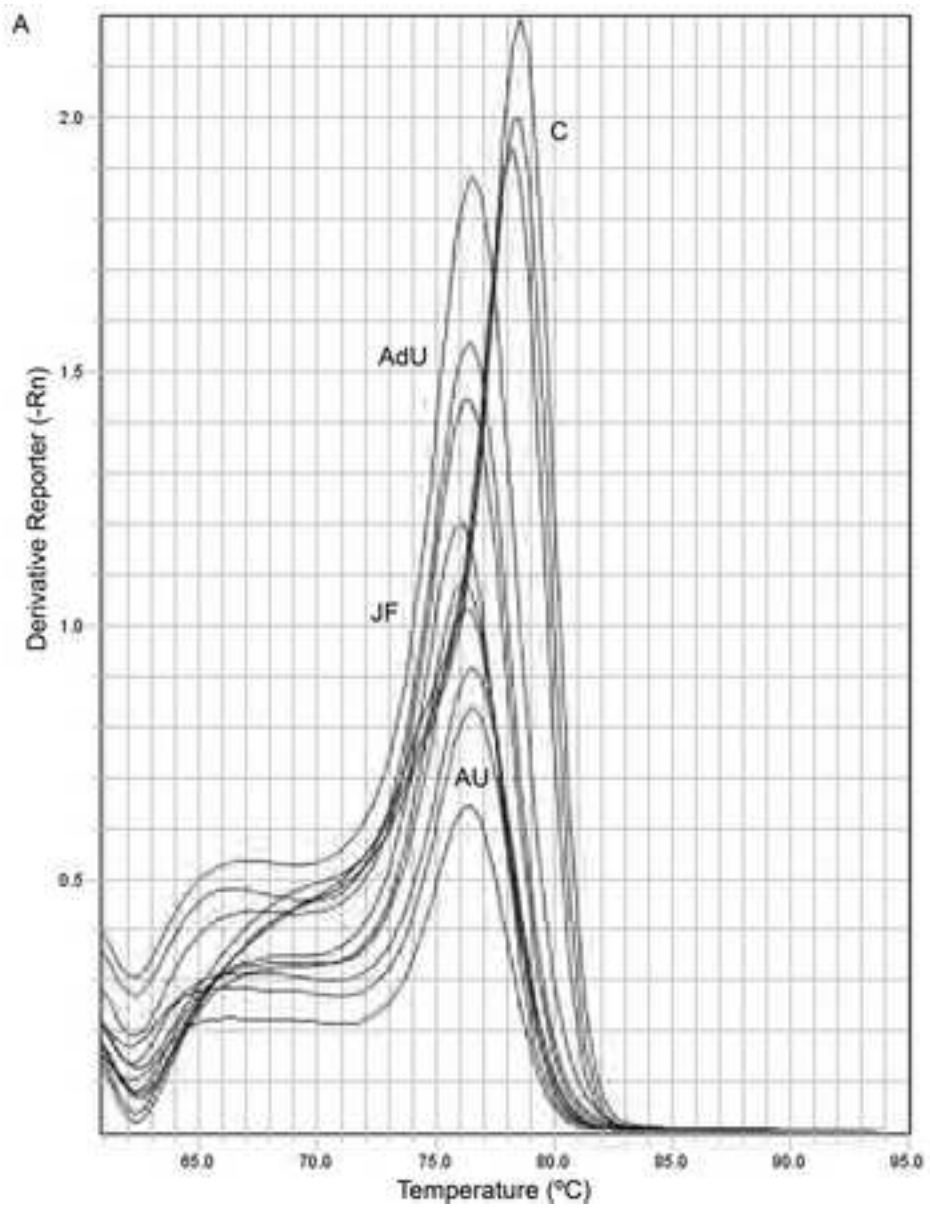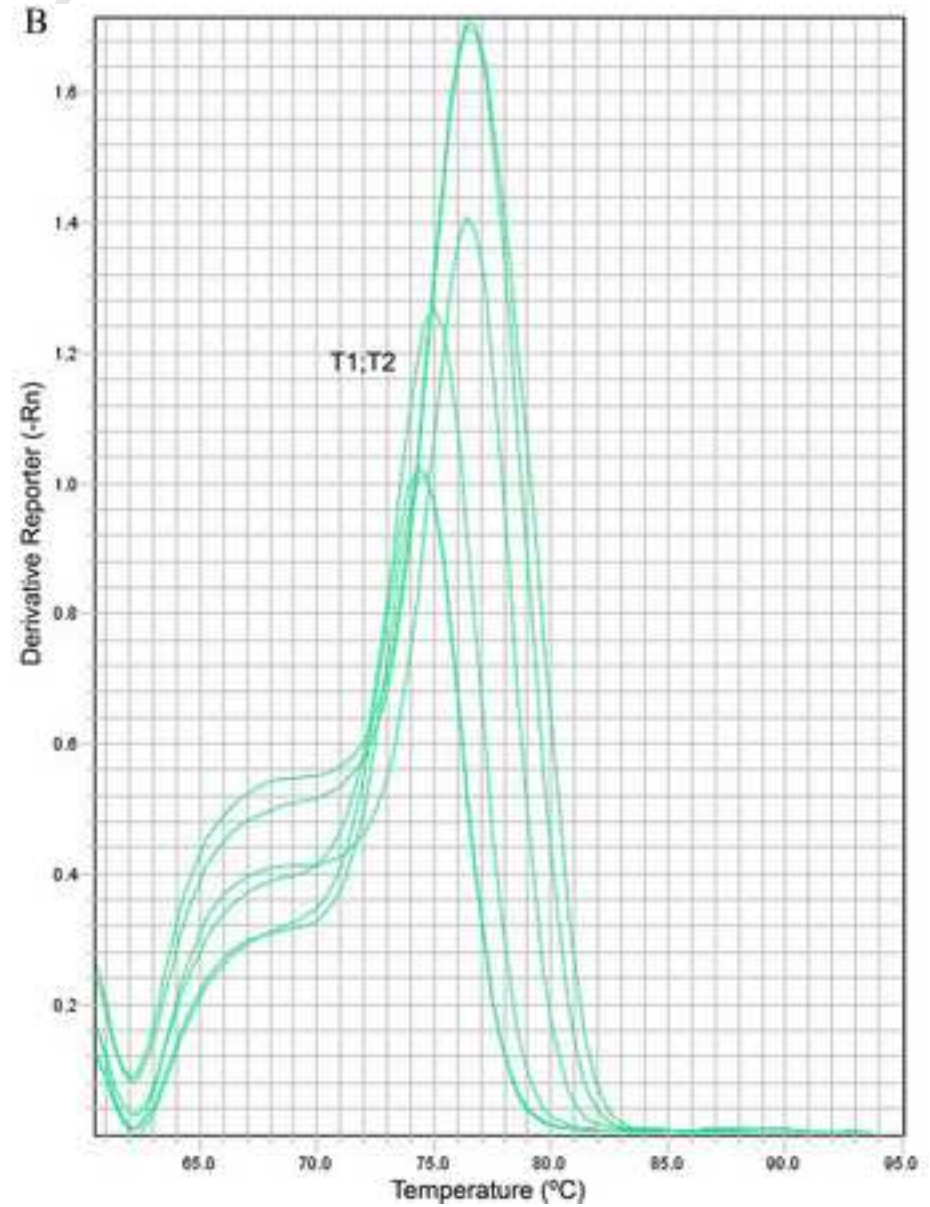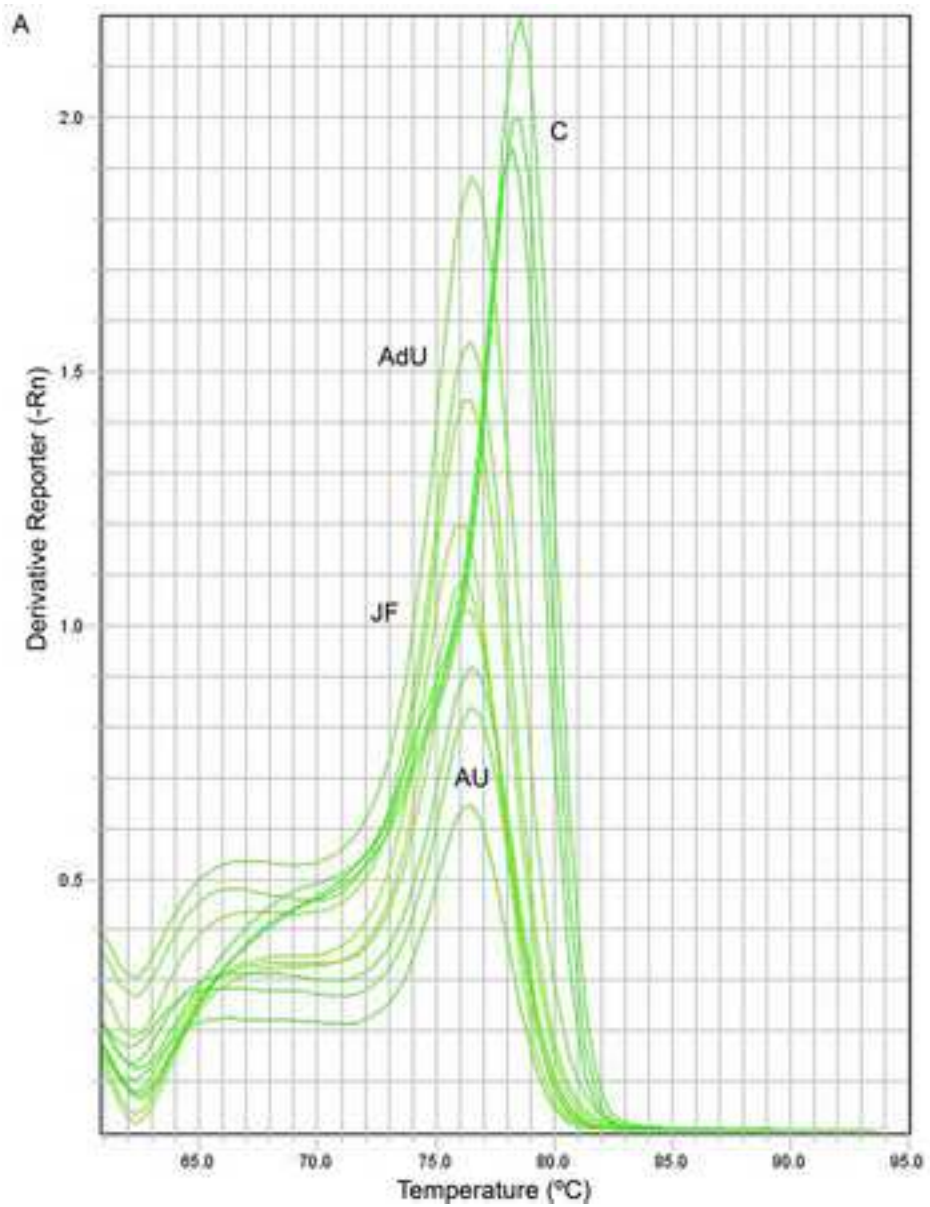
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Figure 7 colour for web only