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Highly efficient automated extraction of DNA from old and contemporary skeletal remains

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ABSTRACT

We optimised the automated extraction of DNA from old and contemporary skeletal remains using the AutoMate Express system and the PrepFiler BTA kit. 24 Contemporary and 25 old skeletal remains from WWII were analysed. For each skeleton, extraction using only 0.05 g of powder was performed according to the manufacturer's recommendations (no demineralisation – ND method). Since only 32% of full profiles were obtained from aged and 58% from contemporary casework skeletons, the extraction protocol was modified to acquire higher quality DNA and genomic DNA was obtained after full demineralisation (FD method). The nuclear DNA of the samples was quantified using the Investigator Quantiplex kit and STR typing was performed using the NGM kit to evaluate the performance of tested extraction methods. In the aged DNA samples, 64% of full profiles were obtained using the FD method. For the contemporary skeletal remains the performance of the ND method was closer to the FD method compared to the old skeletons, giving 58% of full profiles with the ND method and 71% of full profiles using the FD method. The extraction of DNA from only 0.05 g of bone or tooth powder using the AutoMate Express has proven highly successful in the recovery of DNA from old and contemporary skeletons, especially with the modified FD method. We believe that the results obtained will contribute to the possibilities of using automated devices for extracting DNA from skeletal remains, which would shorten the procedures for obtaining high-quality DNA from skeletons in forensic laboratories.

1. Introduction

In forensic genetic investigations, both old and contemporary skeletal remains are among the most challenging biological samples for successful STR typing. The temperature, humidity, pH and geochemical properties of the soil and the presence of microorganisms affect the preservation of the DNA in skeletal remains.[1] Hydrolytic and oxidative damage are most likely to affect DNA over time. Oxidative damage results in modified bases, while hydrolytic damage results in the deamination of bases and in depurination and depyrimidination. Both mechanisms reduce the number and size of the fragments that can be amplified by PCR.[2] Failure to amplify DNA from compromised skeletal remains samples may also result from the presence of inhibitory low molecular weight compounds that co-extract with the DNA and inhibit DNA polymerase in the PCR. Contamination with modern DNA represents another major limitation to the molecular analysis of old bones because, as a result of its higher concentration and quality, contemporary DNA amplification is favoured over that of the endogenous DNA in the sample.[2]

In many forensic cases, bones are the only potential source of genetic material. For the genetic identification of the skeletal remains of missing persons and disaster victims, it is necessary to obtain sufficient high-quality DNA.[3] The method used for DNA extraction plays a key role in the quality and quantity of DNA obtained and has a strong effect on amplification success.[4,5] The extraction methods used for obtaining the DNA need to avoid overly aggressive treatments, such as high temperatures or the use of strong detergents, to reduce the further degradation of already

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damaged and aged DNA.[6] It should be robust, effective at recovering DNA and removing inhibitors, and should also be non-toxic, cost-effective, rapid and simple. In mass disasters (air catastrophes, fires, bomb explosions, natural disasters, etc.) and unidentified human remains, it is also important to identify the victims as soon as possible and automated extraction can considerably reduce the time needed for genetic identification. Although DNA extraction from skeletal remains is very complex and time-consuming, there is the possibility of automating the DNA purification process. Bench-top automated systems that enable the isolation of DNA from sample lysate have gained in importance in recent years. The Biorobot EZ1 device (Qiagen, Hilden, Germany), iPrep (Invitrogen, Carlsbad, CA, USA), Maxwell 16 (Promega, Madison, WI, USA) and AutoMate Express (Thermo Fisher Scientific, Waltham, MA, USA) are being used in forensic laboratories. Automated devices enable fast, simple and efficient extraction procedures from most casework forensic samples. Some studies on the performance of the AutoMate Express Forensic DNA Extraction System have been published and demonstrated efficient extraction from blood, saliva, semen, contact stains, hair roots[7–13] and hair shafts.[14] The PrepFiler Express BTA™ Forensic DNA Extraction kit (Thermo Fisher Scientific) was developed for DNA extraction from bones, teeth and adhesive type samples[8] using the AutoMate Express bench-top system and some studies were performed on contemporary skeletal remains.[7] No study has been published on the automated extraction of genomic DNA from old skeletal remains using the AutoMate Express device.

This paper describes the performance of the AutoMate Express system using the PrepFiler BTA kit for the extraction of DNA from skeletal remains and the optimised method developed to acquire high quality DNA from Second World War skeletal remains. The same optimised method was also applied in the molecular genetic identification of unknown decomposed or skeletonised bodies in routine forensic casework where only bones and teeth are suitable for DNA typing. In forensic DNA analyses, contemporary human skeletal remains are mostly used for the identification of crime and disaster victims, missing persons, and determining family relationships. As shown by the latest studies, total demineralisation is the best method of DNA extraction from old bone material,[15,16] since total demineralisation significantly increases the proportion of full profiles, reflecting a correlation with better DNA quality. Loreille and co-workers[17] succeeded in gaining a sufficient quantity of DNA when they used complete demineralisation on old skeletal remains that gave no results without it. We improve the extraction method using the AutoMate Express automated purification system incorporating total demineralisation prior to genomic DNA extraction.

2. Materials and methods

The quality of the DNA extracts obtained from the AutoMate Express system was evaluated by the quantity of DNA recovered (as determined by real-time PCR), the presence of inhibitors and the quality of STR profiles obtained with the NGM amplification kit. For each bone and tooth sample, two automated extractions were performed. The first without any decalcification stage – no demineralisation method (ND method), and the second using total demineralisation – full demineralisation method (FD method). The ND method was performed following the protocol recommended by the manufacturer's user manuals[18,19] using 0.05 g of bone or tooth powder, and using the FD method the protocol was modified incorporating total demineralisation with 0.5 M ethylene diamine tetra acetic acid – EDTA (Promega). The contemporary and aged skeletal remains samples used in this study were amplified in duplicate to confirm the allele calls. All the aged bone and tooth samples from WWII had already been typed in previous studies with the purpose of genetic identification and research work[3,20–24] and their autosomal STR profiles, obtained using different autosomal STR amplification kits (Identifiler, PowerPlex 16, PowerPlex ESX 17, Investigator Essplex Plus or NGM) were used for the confirmation of the genetic profiles obtained with the NGM kit and determining the dropouts in present study.

This study covers 25 skeletons from Second World War and 24 contemporary skeletons from routine forensic casework resulting in a total of 49 skeletons. The research project was approved by the Medical Ethics Committee of the Republic of Slovenia. From the comparative study of the performance of nuclear DNA typing of skeletal remains (we typed teeth, femurs and tibiae but we didn't type any smaller elements of the hands and feet in the past) from the Slovenian mass graves of the Second World War, our laboratory discovered that teeth are most suitable for typing, followed by the femur bones and tibiae.[21,22] Similar conclusions were also reached by Miloš et al.,[25] Misner and colleagues[26] and Edson et al.[27] Recently Mundorff et al.[28,29] found that smaller elements of the hands and feet (metatarsals, metacarpals, phalanges) were very similar or even better in DNA yield as the femora and tibiae. These bones can be easily sampled with a disposable scalpel and thus reduce potential DNA contamination. Based on recent studies, the current recommendations for the preferential testing of long bones from the legs may need to be re-evaluated and the sampling strategy for laboratories typing bone samples may change in the future.[29] Since long bones and teeth were sampled from mass graves in Slovenia in the past, we used femurs, tibias and teeth (molars) from Second World War victim skeletons excavated from two mass graves where mass executions took place at the end of WWII for the present study. The Commission on Concealed Mass Graves in Slovenia has recently registered almost 600 hidden mass graves with approximately 100,000 victims from that period.[30] From the forensic casework contemporary skeletons, we used bones and teeth sent to the molecular genetic laboratory (femurs, rib, mandible, part of the skull, hip bone, sternum, mastoid processus, molars, premolar and canine).

2.1. Prevention of contamination

To prevent and detect contamination with modern DNA, skeletal remains were treated under conditions designed to minimise contamination. In order to reduce contamination from previous handling, we performed washing in bi-distilled water, detergent and ethanol[31]; radiation with UV light and removing the bone surface by drilling; and acquiring the bone or tooth material directly from the inside of the specimen.[16,32,33] Pre- and post-PCR work were carried out in separate rooms.[34–37] In the pre-PCR, it was necessary to separate the dust-producing working stages from the contamination-susceptible stages like buffer preparation and PCR setup.[6] We have separate working localities in the pre-PCR laboratory to separate each step in the bone typing procedure.[32,33] Cleaning and grinding the bones and teeth took place in a room designed exclusively for processing old skeletal remains in a closed microbiological safety cabinet MC 3 (Iskra Pio, Šentjernej, Slovenia, EU). Extractions were performed with strict precautions including protective clothing (sterile disposable coat, cap, mask, double latex gloves),[34,35] and equipment and surfaces treated with bleach and irradiated with UV light.[6,32,33,35,36,38–40] All sample manipulations were performed in laminar flow cabinets equipped with HEPA filters and UV lights,[33,35] using dedicated pipettes, disposable sterile filter tips and sterile tubes.[6,34,37] Between each sample, the entire working surface and all the tools for drilling, cutting and grinding bones were cleaned by washing with bleach – 6% sodium hypochlorite (Kemika, Zagreb, Croatia, EU) or DNA