

## Identification of old and contemporary human skeletal remains using an automated extraction method

In many forensic cases, bones are the only potential source of genetic material. Analyses of bone samples, especially old skeletal remains, is a challenging task due to the low amounts of DNA achieved and the additionally degraded aged DNA. For the genetic identification of the skeletal remains of missing persons and disaster victims, it is necessary to obtain sufficient high-quality DNA. The aim of this research was to optimise the protocol for the automated extraction of DNA from old and contemporary skeletal remains for sub-sequential reliable and reproducible high quality genetic identification DNA typing. The method used for DNA extraction plays a key role in the quality and quantity of DNA obtained and has a strong effect on success of genetic identification.



Fig. 1. The Second World War mass grave Kržeti from Slovenia with three excavated skeletons (photo Marko Pečovnik).

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 damaged and aged DNA. It should be robust, effective at recovering DNA and removing inhibitors, and should also be non-toxic, cost-effective, rapid and simple. This paper describes the performance of the automated extraction system AutoMate Express for the extraction of DNA from skeletal remains and the optimised method developed to acquire high quality DNA from Second World War (WWII) skeletal remains and contemporary decomposed or skeletonised bodies in routine forensic casework where only bones and teeth are suitable for DNA typing. An AutoMate Express system doesn't use any aggressive organic solvents like phenol or chloroform. It is automated and takes only 30 min to complete. Additionally, for the prevention of contamination, no manual pipetting is needed.

The quality of the DNA extracts obtained from the AutoMate Express system was evaluated by the quantity of DNA recovered, the presence of inhibitors and the quality of genetic profiles obtained with commercial 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 using no demineralisation and full demineralisation automated extraction method. Full demineralisation method demonstrated more successful than no demineralisation method on WWII and contemporary skeletal extracts. Full demineralisation method gave 64% of full profiles in old and 71% in contemporary DNA samples, and no demineralisation method gave 32% of full profiles in aged and 58% in contemporary DNA samples. According to our results, an AutoMate Express forensic DNA extraction system provides robust and reliable results and can be used for the efficient extraction of genomic DNA from old and contemporary skeletal remains of various ages in forensic practice. We improved the DNA recovery by total demineralization, prior to lysis and further automated purification. We believe that the results obtained will contribute to the possibilities of using automated devices for the extraction of DNA from skeletal remains, which would shorten the procedures for obtaining high-quality DNA from skeletons in forensic laboratories compared to different manual extractions. Moreover, using a commercial kit for automated bone and tooth DNA extraction will allow the standardization of tests and results in forensic laboratories, increase the throughput of bone and tooth samples and minimize the potential for human error for sample mixing.

***Irena Zupani? Pajni?***

*Assistant Professor,*

*Institute of Forensic Medicine, Faculty of Medicine,  
University of Ljubljana, Ljubljana, Slovenia*

## **Publication**

[Highly efficient automated extraction of DNA from old and contemporary skeletal remains.](#)

Zupani? Pajni? I, Debska M, Gornjak Pogorelc B, Vodopivec Mohor?i? K, Balažic J, Zupanc T, Štefani? B, Geršak K.

*J Forensic Leg Med. 2016 Jan*