Exymes

Upper Wright Valley, Ross Sea Region -Antarctica Source of boneGEM

DNA Extraction and Human Identification from Bone using PDQeX

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Introduction

Forensic identification is often achieved by extracting DNA from tissue remains. In some cases, such as when significant time has passed, the only remaining tissue is bone. While the robustness of bone preserves DNA, it also poses a challenge when extracting this DNA for profiling. In this application note, a prototype DNA extraction protocol using the PDQeX was performed by the Forensic Science Laboratory of the French Gendarmerie[1].

DNA extraction driven by a cocktail of proteases in a custom buffer compatible with most downstream applications is at the core of the PDQeX system. This chemistry uses a combination of a mesophilic and a thermophilic protease to libertate DNA from bone in under three hours. Bone tissue was collected using a trepaning tool to create bone plugs that were placed into the PDQeX cartridge for DNA extraction. This experiment compared the success of STR profiling and procedure time of the PDQeX to the AutoMate Express2

Method

Bone samples were collected from a fragment of femur from a burned body. A trepan was used to collect 2mm diameter bone plugs weighing approximately 120 mg (Figure 1). DNA was extracted from bone plugs without further processing using the PDQeX with prepGEM chemistry. This reaction mix contained 1× Orange+ buffer, and both the forensicGEM and Histosolv proteases. To a PDQeX cartridge, three bone plugs were added to the reaction mix and placed in the PDQeX2400 device. Extraction was performed using the 'Bone' program: Histosolv incubation at 52°C for 5 minutes, forensicGEM incubation at 75°C for 10 minutes, and 115°C for 5 minutes to activate the cartridge valve and inactive protease activity.

The resulting DNA sample was used at a 1:10 dilution, with 15 µl used for amplification by the GlobalFiler PCR kit (ThermoFisher). These results were compared to a typical bone DNA extraction method used on the same femur2. For the comparator method a rotary saw and grinder was used to prepare bone material.

The PrepFiler Express BTA™ Forensic DNA extraction kit (ThermoFisher) and AutoMate Express™ (ThermoFisher) were used to extract DNA from this bone material. A DNA profile was performed on amplicons generated from material obtained from both methods using a 3500XL genetic analyser (ThermoFisher) with injection at 1.2 kV / 20s.

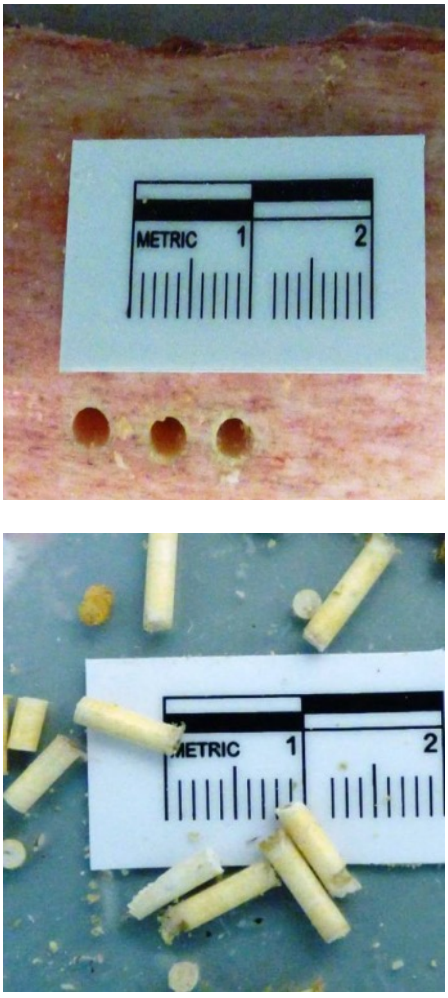

Figure 1. Bone plugs drilled from femur using a 2mm trepan.

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Results

Both the PDQeX and the AutoMate Express protocols produced full DNA profiles (figure 2). However, there was a noticeable difference in relative fluorescent intensity (rfu) between the profiles, with the PDQeX protocol giving an rfu approximately three times higher than the AutoMate Express protocol. In addition to a stronger signal, the PDQeX took less time compared to the AutoMate Express protocol. The total time for the PDQeX protocol was less than 2.5 hours whereas the AutoMate Express protocol took six hours to complete.

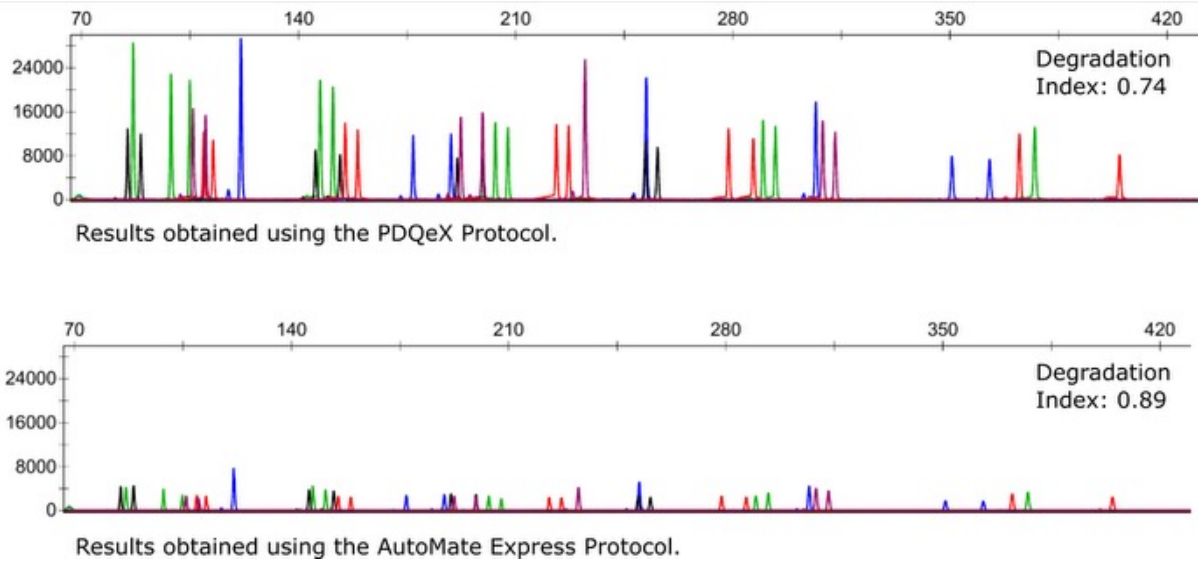

Figure 2. DNA Profiling results from PDQeX and AutoMate Express extraction protocols.

Conclusion

This experiment evaluated use of the PDQeX system for extraction of DNA from bone for forensic investigation. The results showed the profile generated from PDQeX prepared DNA was comparable to the AutoMate Express material. Significantly, the superior speed of the PDQeX along with simplified bone preparation, took less than half the time compared to the AutoMate Express protocol. Rapid sample preparation coupled with simple processing makes the PDQeX a potentially significant advance for forensic work-flows toward faster processing of critical evidence.

References

1. Hubac, S. *et al.* TREPAN AND GO: hDNA collecting and profiling from bones is not challenging anymore. (2018).

2. Liu, J. Y. *et al.* AutoMate ExpressTM Forensic DNA Extraction System for the Extraction of Genomic DNA from Biological Samples. J. Forensic Sci. 57, 1022–1030 (2012).