Application Note

DNA extraction from archaeological skeletal remains using an automated protocol on the EZ2® Connect Fx instrument

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Introduction

One of the greatest challenges that scientists face when processing skeletal samples is obtaining reliable information. Degraded skeletal remains, especially if archaeological in origin, contain a meagre amount zof DNA. In addition, external factors that contribute to degradation of skeletal elements directly affect the preservation of DNA. Degraded DNA is more prone to contamination and the presence of PCR inhibitors.

Although a conventional organic phenol-chloroform method can efficiently extract DNA from different skeletal samples, this method has numerous disadvantages. These include use of toxic and aggressive reagents, long extraction time, potential loss of DNA due to transferring sample between tubes, continual contact of technicians with samples potentially leading to cross-contamination, contamination with recent DNA, and to the possibility of switching DNA samples (1, 2).

An alternative automated method of DNA extraction based on silica-coated magnetic bead technology offers a solution to these obstacles. The EZ2 Connect Fx instrument from QIAGEN is equipped with preinstalled protocols for DNA purification and there are protocols that provide for samples with a limited amount of DNA or low-template DNA samples. The only manual steps involve preparing samples for pretreatment then loading consumables, reagent cartridges and samples into the instrument. Here we present the results of our study aimed to evaluate the efficiency of DNA extraction from archaeological and recent skeletal remains using the EZ2 Connect Fx automated system with EZ1&2® DNA Investigator® Kit.

Materials and methods

Samples

Altogether 16 skeletal samples were processed. Nine were well-preserved archaeological teeth samples obtained from different necropoli dating to the period of medieval Bosnia (Figure 1).

- A1: Medieval royal city of Bobovac
- A2, A7, A8: Different medieval cemeteries in the Travnik area in central Bosnia and Herzegovina
- A3, A9: Localities in the south of Bosnia and Herzegovina
- A4, A5, A6: From the Tuzla area in southeastern Bosnia and Herzegovina

Seven recent samples were femoral bone fragments from subjects of unknown age at time of death (Figure 2).

Bone surfaces were cleaned using a grinding stone attached to a Dremel® rotary tool to remove potential contaminants, remnant soft tissue and soil traces. All samples were washed according to standard protocol (5% Na-hypochlorite 3x, distilled water 3x and absolute ethanol 3x) then air dried for five to seven days. Teeth samples were ground to a fine dental powder using a sterilized IKA® Tube Mill. A Dremel rotary tool equipped with a new grinding stone was used to grind the recent bone samples. To prevent contamination, all grinding was performed using sterilized grinding equipment in a sterilized negative pressure hood dedicated to the work with skeletal samples.

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Figure 1.
Archaeological teeth samples collected from different medieval Bosnian necropoli. (A) Sample A2 from Glavica-Han Bila necropolis in the Travnik area; (B) Sample A8 from Klisa-Guča Gora necropolis in the Travnik area; (C) Sample A3 from Čelebići near Konjic (south B&H); (D) Sample A6 obtained from a necropolis in the Tuzla area.

Figure 2.
One of the recent femoral bone samples used in this study (sample R1).

DNA extraction and quantification

Approximately 400 mg of dental/bone powder per sample was pretreated by disruption and lysis. Samples were prepared for pretreatment in a sterilized hood using sterilized laboratory tools and equipment. The lysis mix per sample included reagents supplied in the EZ1&2 DNA Investigator Kit and followed the "Bone Extra Large Volume Protocol" (3): 950 µl Buffer G2, 100 µl Proteinase K. A volume of 1050 µl 0.5 M EDTA was added to each sample followed by incubation at 56°C for 4 hours. After lysis, DNA extraction/purification of supernatants was continued according to the same protocol on the EZ2 Connect Fx instrument with an elution volume of 20 µl. DNA extracts were quantified using the Qubit® dsDNA HS Assay Kit as per the manufacturer's recommendations on the Qubit Fluorometer.

PCR amplification and post-amplification

DNA amplification employed the Investigator 24plex QS Kit with the QIAamplifier® 96 according to the manufacturer's recommendations (4). A DNA extract volume of 10–15 µl was added to Master Mix up to a volume of 25 µl per reaction. Amplified fragments were subjected to electrophoresis with Applied Biosystems® 3500 Genetic Analyzer and further analyzed with GeneMapper™ ID-X 1.6 Software with an analytical threshold of 50 RFU. PCR negative controls (NTCs) were included in the analysis to check for possible contamination of the reagents and inadvertent transfer between samples. All PCR and postamplification activities were performed in separate laboratory sections using sterilized laboratory tools dedicated to PCR and postamplification work, respectively.

Results and discussion

Concentration of extracted DNA

The concentration of DNA extracted from archaeological dental samples varied among samples from ≥0.010 ng/µl to 28.80 ng/µl. Two out of the nine archaeological samples (A1 and A6) showed an extremely low or nondetectable concentration of DNA (Table 1). Similarly, the concentration of DNA recovered from the recent femoral bone samples varied among samples, with a range from a low value of 0.010 ng/µl to 80.20 ng/µl (Table 2).

DNA amplification

As expected, the archaeological samples yielded partial STR profiles. More than 55% of amplified alleles were detected in 6 out of 9 processed archaeological samples (Table 1). Correlation between concentration of DNA and amplified alleles was observed only in the case of sample A7. All other archaeological samples, especially A2, A6 and A9, showed a poor correlation between quantified DNA concentration and the number of amplified alleles.

All seven recent femoral bone samples provided almost full STR profiles with ≥19 observed in these samples (Table 2). Discrepancy between results of DNA quantification and amplification was also observed in recent skeletal samples, except sample R3. An accurate PCR-based quantification method with information on DNA degradation and the presence of inhibitors might be more appropriate than fluorometry for the processing of bone samples.

No evidence of allelic peaks above the threshold of 50 RFU was observed in negative controls, confirming the absence of contamination events that would compromise the integrity of the results. Presence of Quality Sensors QS1 and QS2 enabled verification of a successful PCR reaction in every sample and negative control well.

Table 1. DNA concentration and number of amplified alleles in archaeological samples (maximum alleles=22)

Sample code	DNA concentration (ng/µl)	Amplified alleles	Percentage of amplified alleles (%)	Quality Sensors
A1	≤0.010	4	18	Present
A2	5.70	9	0	Present
A3	9.92	12	56	Present
A4	7.78	19	86	Present
A5	5.10	13	59	Present
A6	Not detected	17	77	Present
A7	28.80	20	90	Present
A8	14.70	13	59	Present
A9	24.0	6	27	Present
NTCs	–	–	–	Present

Table 2. DNA concentration and number of amplified alleles in recent samples (maximum alleles=22)

Sample code	DNA concentration (ng/µl)	Amplified alleles	Percentage of amplified alleles (%)	Quality Sensors
R1	11.30	20	90	Present
R2	0.010	21	95	Present
R3	80.20	21	95	Present
R4	5.96	19	86	Present
R5	5.18	21	95	Present
R6	9.38	21	95	Present
R7	12.60	20	90	Present
NTCs	–	–	–	Present

Conclusion

This study suggests that automated DNA extraction using the EZ1&2 DNA Investigator Kit and EZ2 Connect Fx instrument can be considered an effective and valuable technique in the analysis of skeletal remains, especially of recent or relatively well-preserved skeletal samples. The study also reconfirmed the reliability of the Investigator 24plex QS Kit for STR typing of challenging forensic samples.

Summary

Our results indicate that an automated magnetic bead DNA extraction method can deal with challenges that come with the analysis of forensic samples such as skeletal remains. Compared to a manual approach of DNA extraction from skeletal remains, e.g., the organic phenol-chloroform method (5), automated DNA extraction using the EZ1&2 DNA Investigator Kit provides:

- Extraction with less toxic and harmful reagents
- Simultaneous DNA extraction from a larger number of samples
- Faster protocol that reduces hands-on time
- Protection against sample switching, contamination and cross-contamination

Ordering Information

Product	Contents	Cat. no.
EZ2 Connect Fx System	Benchtop instrument for automated isolation of nucleic acids from up to 24 samples in parallel, using sealed prefilled cartridges; includes 2x EZ2 Connect racks (EZ2 Connect Fx Tip Rack and the EZ2 Connect Fx Tip Rack – Flip Cap Tubes), EZ2 Connect Fx Cartridge Rack and 1-year warranty on parts and labor.	9003220
EZ1&2 DNA Investigator Kit (48)	For 48 preps: Reagent Cartridges (DNA Investigator), Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 ml), Elution Tubes (1.5 ml), Buffer G2, Proteinase K, Carrier RNA	952034
7 mL Large-Volume Tubes (48)	Two bags of 24 large-volume tubes (7 mL)	951954
Investigator 24plex QS Kit (100)	Primer Mix, Fast Reaction Mix including Taq DNA Polymerase, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO), and nuclease-free water	382415
Investigator 24plex QS Kit (400)	Primer mix, Fast Reaction Mix including Taq DNA Polymerase, Control DNA, allelic ladder 24plex, DNA size standard 24plex (BTO), and nuclease-free water	382417

Learn more about EZ2 Connect Fx for your lab. Visit **qiagen.com/EZ2ConnectFx**

References:
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4. *Investigator 24plex QS Handbook*. February 2021.
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