Application Note

Quick bone protocol using EZ1® Advanced XL

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Introduction

Forensic laboratories face increasing time pressures, driving an urgent need for new developments in rapid sample processing methodologies. Here, we sought to reduce the lysis time required for bone samples from the traditional 24 hours to a more efficient 2-hour lysis using automated purification on the EZ1® Advanced XL instrument (QIAGEN® Group). This fast lysis time was supported by use of the EZ1 Advanced XL DNA Investigator® Large-Scale Bone Card. Despite the significant reduction in sample processing time, we observed no detrimental effect on DNA quality.

In combination with the fast DNA quantification and STR analysis technologies provided by the Investigator Quantiplex® Pro RGQ Kit (QIAGEN) and Investigator 24plex QS Kit, respectively, this new rapid bone protocol will allow laboratories to go from sample processing to results within the same working day.

Methods

The performance of a 2 h quick lysis protocol was tested on 7 samples from the skeletal remains of different individuals associated with missing persons cases in Russia. The results were compared to a 24 h lysis protocol to confirm that the short lysis approach did not impact the DNA concentration or the quality of subsequently generated STR profiles.

Sample processing

Samples were pulverized under liquid nitrogen in a cryogenic mill. All samples were processed using the EZ1 DNA Investigator Kit in combination with the EZ1 Advanced XL instrument and the EZ1 Advanced XL DNA Investigator Large-Scale Bone Card.

For each sample, 450 mg of powdered bone was split equally into three 2 ml sample tubes (150 mg per tube) and labeled accordingly (e.g. as bone tube 1.1, 1.2 and 1.3). It was important to not exceed this amount of bone powder in each tube.

A lysis buffer was prepared containing 225 µl of QIAGEN Buffer G2, 25 µl of QIAGEN proteinase K and 250 µl of 0.5 M EDTA pH 8.0; this buffer was added to each individual sample tube. Extraction negative controls were also included in the experiment. ▷

After mixing the bone samples with lysis buffer, the tubes were placed in a thermomixer incubator and incubated with constant motion at 56°C for 2 h. For lysis time comparison, the protocol was repeated and the samples were incubated for 24 h.

After incubation, the samples were centrifuged at 6000 rpm for 4 min to pellet any remaining debris. For each of the 7 bone samples, the resulting supernatant was transferred to 3 separate 2 ml EZ1 sample tubes, giving 3 replicate tubes containing only lysate for each sample. These were labeled accordingly (e.g. as bone tube 1, 2 or 3).

Next, 50 µl of 3 M NaOAC pH 5.0 and 1 µl of carrier RNA were added to each EZ1 sample tube. 400 µl of Buffer MTL was then added to bone tube 1, while 1120 µl of Buffer MTL was added to bone tubes 2 and 3.

The contents of bone tube 1, containing 500 µl of sample and 400 µl of Buffer MTL, were loaded into the fourth row of the EZ1 Advanced XL instrument. The contents of bone tube 2, containing 500 µl of sample and 1120 µl of Buffer MTL, were loaded into the third row. Finally, the contents of bone tube 3, containing 500 µl of sample and 1120 µl of Buffer MTL, were loaded into the EZ1 heating block position. This procedure was performed to concentrate the roughly 1.5 ml of lysis buffer per sample into 50 µl of DNA eluate.

The purification protocol for 1.5 ml of lysis buffer was selected from the Investigator Large-Scale Bone Card (protocol 2). The automated purification procedure took 32 min.

DNA quantification was performed using QIAGEN's Investigator Quantiplex Pro RGQ Kit, according to the manufacturer's instructions, on the Rotor-Gene® Q (QIAGEN Group). Samples were amplified on a GeneAmp® PCR System 9600 (Life Technologies Corporation) using the Investigator 24plex QS Kit and subjected to electrophoresis with an Applied Biosystems® 3500xL Genetic Analyzer (Life Technologies Corporation). GeneMapper® ID-X version 1.2 (Life Technologies Corporation) was used for data analysis.

Results

The DNA concentration obtained with the 2-hour lysis bone protocol was consistently higher than with the 24-hour lysis for all samples (Table 1), indicating that 2 hours is a sufficient amount of time to release the DNA contained in bone cells and bone mineral matrix. A longer incubation time appeared to have a negative effect on the DNA yield, leading to a decrease in concentration.

As shown in Figure 1, using the Investigator 24plex QS Kit following the 2-hour lysis protocol lead to an equivalent or even greater number of typed alleles compared to the 24-hour lysis protocol.

Table 1. DNA concentration (ng/µl) from 7 bone samples after a 2-hour lysis protocol or a 24-hour lysis protocol

	Incubation Time	
Sample	**2 h**	**24 h**
1	0.0347	0.0340
2	0.0174	0.0034
3	0.6017	0.3899
4	0.0911	0.0191
5	0.0014	0.0006
6	0.0896	0.0301
7	0.6052	0.4363

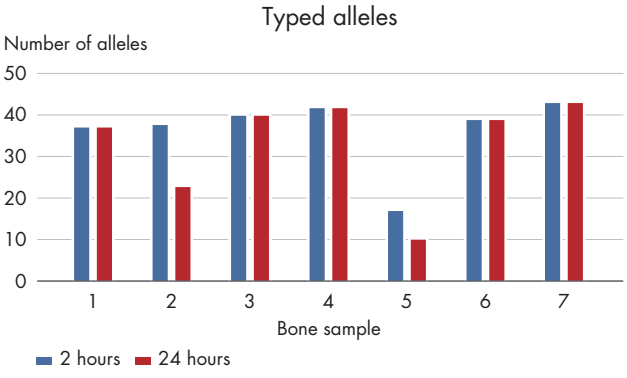

Figure 1. Number of typed alleles for each bone sample after the 2-hour lysis protocol and the 24-hour lysis protocol.

Discussion

Reducing the lysis time for bone samples from 24 hours to 2 hours would offer a significant increase in sample throughput for forensic laboratories. To replace the standard 24-hour lysis protocol, however, a rapid protocol would need to produce DNA of comparable quantity and quality. Here we find that a 2-hour lysis protocol produces comparable, if not improved, DNA yields and total callable alleles following STR analysis compared to the default 24-hour lysis method. For example, the increased DNA yields achieved for samples 2 and 5 in this study mediated an increase in the total callable alleles for these samples, allowing more alleles to be amplified above the analytical threshold.

Forensic bone samples commonly produce low DNA yields due to their naturally low DNA concentrations compared to other biological samples. These low yields are often further reduced through environmental degradation. Ensuring that the maximum possible DNA yield is achieved during sample processing is critical for successful laboratory outcomes. High yields support lower input volumes for PCR and therefore enable a greater number of amplifications across multiple assays to be performed.

Conclusion

The quick 2-hour lysis protocol described here represents a viable, and potentially preferable, option for bone extractions compared to the standard 24-hour lysis protocol.

Ordering Information

Product	Contents	Cat. no.
EZ1 DNA Investigator Kit (48)	For 48 preps: Reagent Cartridge (DNA Investigator), Disposable Filter-Tips, Disposable Tip-Holders, Sample Tubes (2 ml), Elution Tubes (1.5 ml), Buffer G2, Proteinase K, Carrier RNA	952034
EZ1 Advanced XL, System	Instrument and service agreement package: robotic workstation for automated purification of nucleic acids from up to fourteen samples using EZ1 Kits; includes installation, application training and one-year warranty on labor, travel and parts.	9001874
EZ1 Advanced XL DNA Investigator Large-Scale Bone Card	Preprogrammed card for purification of DNA using the EZ1 Advanced XL	9022497
Investigator Quantiplex Pro RGQ Kit (200)	For use on QIAGEN RotorGene Q Real-Time Systems: Quantiplex Pro RGQ Reaction Mix, Quantiplex Pro RGQ Primer Mix, Male Control DNA M1, QuantiTect Nucleic Acid Dilution Buffer	387316
Rotor-Gene Q 2plex Platform	Real-time PCR cycler with 2 channels (green, yellow), laptop computer, software, accessories: includes 1-year warranty on parts and labor, installation and training not included	9001550
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

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