Extraction of DNA from Bone using Temperature Controlled Extraction

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Background

Bones are often the final source of DNA for identification. However, DNA extraction from these samples are challenging due to the hardness and mineralisation of this tissue. Degradation of the DNA over time and external DNA contamination can also diminish the success of identification. The method used for DNA extraction can influence final yield and purity. The Exymes chemistry uses a single-tube, enzyme-controlled process for rapid DNA extraction. Cell lysis is driven by the thermophilic protease, *forensic*GEM (figure 1) during an incubation at 75°C.

Exymes chemistry is compatible with enzymatic downstream applications, such as PCR and STR. The extraction occurs within a single tube with minimal handling or liquid transfers. This reduces both the loss of DNA from purification steps and the chance to introduce DNA contamination. As the chemistry requires no post-extraction purification, we hypothesized that less bone material could be used to isolate DNA.

The *forensic*GEM chemistry has had success with bone samples, including producing a 22-locus and a 15-locus profile from two highly degraded samples from the 9/11 World Trade Center attacks[1]. The *forensic*GEM chemistry has also had success with teeth samples[2].

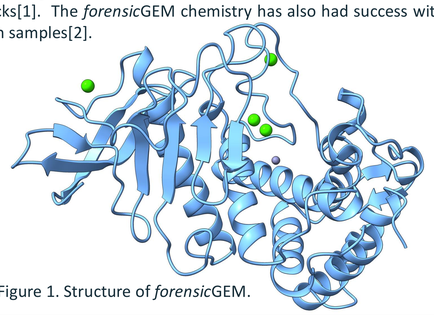


Figure 1. Structure of *forensic*GEM.

Materials and Methods

Samples

Four female femora bones from a skeletal collection.

Bone Processing

The surfaces of the bone were cleaned with a diamond saw. Bones were cut with a diamond saw and pulverized with an agate mortar and pestle. Bone powder was weighed and divided into aliquots of 50 mg, 40 mg, 30 mg, 25 mg, 20 mg, 10 mg, 5 mg, and 1 mg.

DNA Extraction

Bone powder was dispensed into thin-walled PCR tubes and 100 µl of Exymes *forensic*GEM reaction mix (79 µl DNA-free water, 10 µl 10x Orange buffer, 1 µl forensicGEM, 10 µl Histosolv) was added (figure 2).

Samples were incubated at: 52°C 5 min, 75°C 5 min, 95°C 3 min (figure 3). At completion, liquid was transferred to fresh PCR tubes.

DNA Quantitation

Human DNA quantification and degradation index was determined using the PowerQuant System (Promega Corporation, Madison, WI, USA) and QuantStudio (Thermo Fisher Scientific) following the manufacturer's protocol.

STR profiling

The Promega PowerPlex® Fusion 6C System (Promega Corporation) was used to amplify and characterize 23 autosomal STRs, 3 Y–STRs, and the Amelogenin gene according to the manufacturer's protocol in 1 ng of DNA both for positive control and samples. Fragment analysis was carried out on SeqStudio (Thermo Fisher Scientific). The fragment analysis was performed under the following parameters: 7 s injection time; 1200 V injection voltage; 1440 s run time; and 9000 V run voltage. DNA profiling was achieved through the Microsatellite Analysis software on Thermo Fisher Cloud. A threshold of 150 RFUs was used for peak detection analysis.

DNA Extraction Workflow

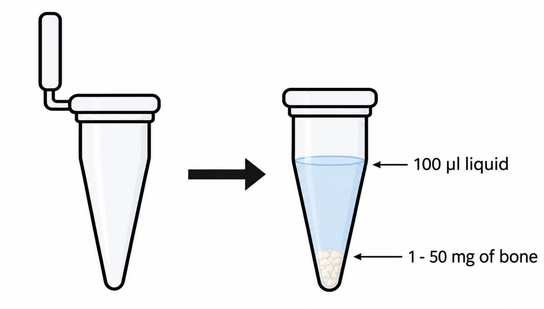


Figure 2. DNA from bone is extracted in a single tube reaction containing 1 - 50 mg of bone powder in a 100 µl extraction mix. Reaction mix contains two enzymes and PCR/STR-compatible buffer.

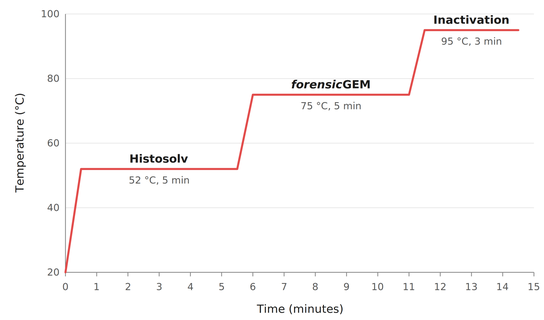


Figure 3. DNA extraction is driven by temperature modulation. Initial 52°C activates a lower temperature enzyme, then *forensic*GEM is activated at 75°C. Inactivation of the enzymes occurs during the 95°C incubation. Total extraction time is ~15 minutes.

DNA Retrieval

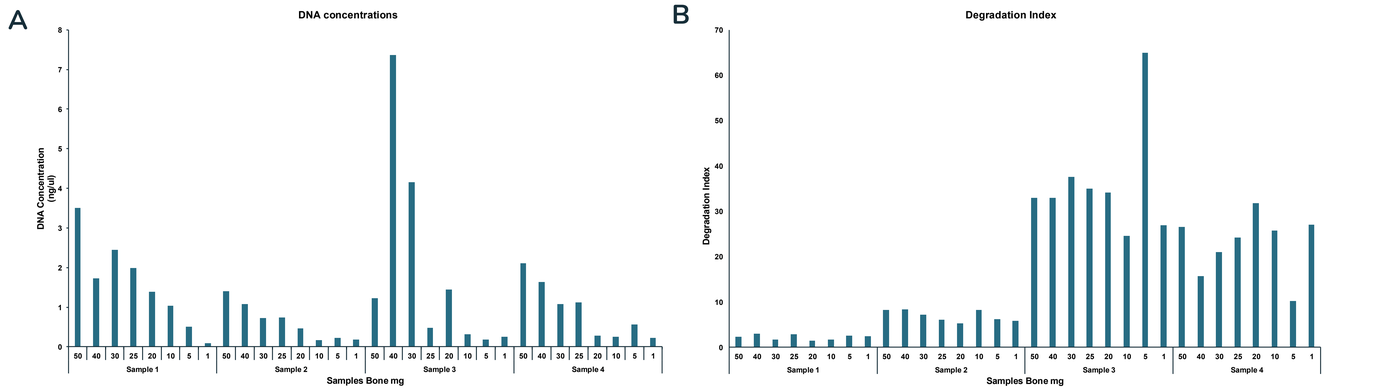


Figure 4. A) DNA concentration and B) degradation index for each femur sample using decreasing amounts of bone powder.

STR Profiles

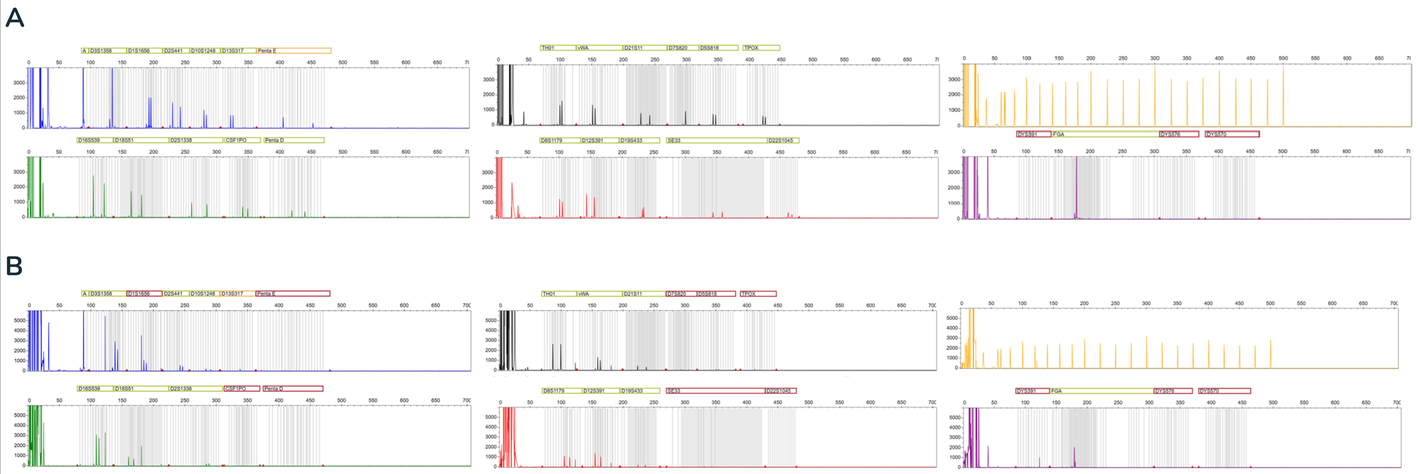


Figure 5. STR Profiles for 5 mg of bone using sample 1 (A) and sample 3 (B).

Results

DNA Retrieval

All four bone samples and starting weights yielded enough DNA to proceed to generation of an STR profile from 1 ng of DNA.

Degradation Index

The degradation ratios were beyond the established threshold in most of process samples, although it remained consistent within each sample. In sample 1, ratios were between 1.3-2.97; sample 2, between 5.17-8.26; sample 3, between 26.86-64.93; sample 4, between 10.25-31.78.

STR Profile

DNA was extracted from 1 mg, 5 mg and 25 to 50 mg ground bone of each sample was used to perform STR profiles. The number of STRs for each profile is given in Table 1. The degradation ratio rather than the DNA concentration had the greatest impact on STR number. Sample 1, with the lowest degradation ratios, gave a full DNA profile from 25 mg and 5 mg of starting material; from 1 mg bone, only 2 STRs were missing. Sample 2 had a higher degradation ratio but still produced a full STR profile from 30 mg of starting bone, with 2 STRs from 10 mg, and 6 STRs from 1 mg of starting material missing. Samples 3 and 4, with higher degradation ratios retrieved partial STR profiles from all starting amounts, decreasing the number of STRs with decreased bone input. Table 1 shows the number of STRs obtained per sample and input weight.

Table 1. STR Results for Bone Samples

Sample	Weight	Number of STRs
1	25 mg	23 + Amelogenin
1	5 mg	23 + Amelogenin
1	1 mg	21 + Amelogenin
2	30 mg	23 + Amelogenin
2	10 mg	21 + Amelogenin
2	1 mg	17 + Amelogenin
3	50 mg	16 + Amelogenin
3	5 mg	10 + Amelogenin
3	1 mg	16 + Amelogenin
4	40 mg	17 + Amelogenin
4	20 mg	13 + Amelogenin
4	1 mg	14 + Amelogenin

Conclusion

Exymes chemistry was effective in obtaining quantifiable DNA for subsequent STR analysis. However, successful retrieval of a full STR profile depends not only on the amount of DNA isolated but on the degree of sample degradation. Using less degraded samples, it was possible to obtain a full STR profile with only 5 mg of bone. Samples with a greater degradation ratio yielded fewer complete STRs as expected, however, all extracted DNA samples were able to participate in the STR profiling process. This further suggests that the Exymes chemistry is a credible method for extracting DNA from small amounts of human bone for the purposes of human identification.

References

[1] Vega, Falyn R., "Extraction of Challenging Forensic Samples Using the MicroGEM DNA Extraction Kit" (2023). *CUNY Academic Works.* https://academicworks.cuny.edu/jj_etds/282

[2] C Zapico S, Matos S, Conte J. Assessment of the efficiency of DNA isolation and profiling applying a temperature-driven method in human remains. Electrophoresis. 2024 May;45(9-10):805-813. doi: 10.1002/elps.202300273.

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