

DNA Isolation from bones

DNA extraction from bone using forensicGEM™

The scientific literature maintains that the most effective procedure for extracting DNA from skeletal material is a method first described by Boom et al in 1990¹, and more recently developed by Höss and Pääbo². This method used widely by both forensic³⁻⁵ and anthropology⁶ practitioners to extract DNA from bone samples. The main disadvantage of the technique is its complexity and unsuitability for automation.

ZyGEM's forensicGEM™ offers a simple, effective and automatable solution to the extraction of DNA from bone. The method is amenable to either manual extraction in PCR tubes or for automated, high-throughput extraction in microtitre dishes (Figure 1).

Extraction Method

All preparation should be performed in a clean-room or PCR hood. Plastic-ware should be UV irradiated prior to extraction for 5 minutes.

[A] Preparation

1. Decontaminate bone samples by soaking in 3% (w/v) sodium hypochlorite for 30 minutes.
2. Rinse samples with DNA/RNA free water.
3. Allow to air dry in sterile environment.
4. UV irradiate all surfaces for 30 minutes prior to handling.
5. Using an electric sanding disk, remove 1 - 2mm of the surface of the bone samples and discard.
6. Drill clean bone and collect resultant powder for extraction.

[B] Extraction

1. To 5 mg of drilled bone powder add 88 µl of DNA-free water, 10 µl of the 10 x buffer (supplied) and 2 µl forensicGEM™.
2. Incubate samples for 15 minutes at 75°C followed by 15 minutes at 95°C.
3. Store samples at 4°C until required.

1. Boom, R., et al., *Rapid and simple method for purification of nucleic acids. Journal of clinical microbiology*, 1990. 28: p. 495-503.
2. Höss, M. and S. Pääbo, *DNA extraction for pleistocene bones: a silica-based purification method. Nucleic Acids Research*, 1993. 21(16): p. 3913-3914.
3. Meyer, E., et al., *Extraction and amplification of authentic DNA from ancient human remains. Forensic science international*, 2000. 113: p. 87-90.
4. Höss, M. and S. Pääbo, *DNA extraction for pleistocene bones: a silica-based purification method. Nucleic Acids Research*, 1993. 21(16): p. 3913-3914.
5. Höss, M., et al., *DNA damage and DNA sequence retrieval from ancient tissues. Nucleic acids research*, 1996. 24(7): p. 1304-1307.
6. Matisoo-Smith, E., et al., *Ancient DNA from polynesian rats: Extraction, amplification and sequence from single small bones. Electrophoresis*, 1997. 18: p. 1543-1537.

Silica-based Method

- ➡ Add pre-extraction buffer and protK. Incubate overnight at 37°C
- ➡ Add Extraction buffer. Incubate with rotation at 60°C for 1 hour
Centrifuge
- ➡ Add extraction buffer + silica suspension
- ➡ Remove supernatant
Incubate at room temperature for 10 minutes
Centrifuge
- ➡ Discard supernatant
➡ Resuspend silica in buffer
Centrifuge
- ➡ Discard supernatant
➡ Resuspend silica in buffer
Centrifuge
- ➡ Discard supernatant
➡ Ethanol wash
- ➡ Elute
- ➡ Elute

Total time >24 Hours

- ➡ Tube opened
- Yield Loss

forensicGEM™

- ➡ Add buffer + forensicGEM, mix
Incubate 75°C
Incubate 95°C
Total time ~ 35 min