

Benchmarks

Preparation of Bone Samples for DNA Extraction: A Nuts and Bolts Approach

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Interest in the analysis of DNA from ancient bone material (3) has increased greatly since it was first demonstrated with the amplification of mitochondrial DNA sequences from 5500-year-old human remains (1). Although the methods involved are not technically demanding, they have remained the preserve of specialist laboratories, primarily because of problems associated with the avoidance of contamination. Contamination can occur at any stage in the analysis of ancient bone DNA, but it is particularly prevalent during sample preparation when the bone material needs to be aseptically ground into a powder fine enough for the extraction of DNA. As most ancient and forensic samples are irreplaceable, anti-contamination procedures are given the highest priority. Traditional "low-tech" solutions, such as hammering frozen bone sealed in plastic freezer bags, have proven unreliable; shards of bone tend to pierce through the bags, and more often than not, the end result is tiny chunks of bone rather than the finely powdered product required for optimal DNA extraction. Freezer mills represent a solution in which sterile technique and end product adhere to the highest standards, but the expense and maintenance of these machines frequently puts this technology out of reach of many investigators. Here, we describe an aseptic method for grinding bone fragments, which is quick and cost-effective and uses common and easily acquired materials.

The threaded ends of two stainless steel bolts were ground flat to obtain an even grinding face. The two bolts were then assembled with one stainless steel nut (all size M17) and baked in a dry oven at 400°C for 4 h to destroy any contaminating DNA. After cooling to room temperature, one bolt was removed from the nut, bone fragments were placed inside the nut, and the first bolt was then replaced. Using a pair of pliers, the entire apparatus was then placed in liquid nitrogen for 30 s. After

freezing, the apparatus was fixed into a bench-top vise to immobilize the nut and one bolt, and a spanner was used to tighten the other bolt and to grind the material contained in the nut. Since occasionally some bone splinters remained uncrushed, it was necessary to loosen one of the bolts, to rap the apparatus on the bench to reposition the bone material and to retighten the bolts 3–4 times. This ensures that all of the bone is evenly pulverized. In instances where the bone sample was not completely dry, an inert abrasive agent was included with the bone sample (in our case, sterilized glass fragments) to ensure even grinding. One of the bolts was then carefully removed from the nut, and the resulting bone powder was pushed out by screwing the remaining bolt further into the nut. Loss of bone powder due to nut removal was negligible as the powder was compacted into a tight pellet. The pelleted bone powder was decalcified by dropping it into a pre-weighed 1.5-mL microcentrifuge tube containing 0.5 mL of 0.5 M EDTA (pH 8.0), vortex mixing and incubating overnight at 45°C. Weighing the 1.5-mL microcentrifuge tube before the addition of bone powder allows for accurate measurement of the amount of sample used. The pellet readily dispersed when added to the EDTA solution.

DNA may be extracted from the decalcified bone solution in a variety of ways, including the proteinase K/Centricon method of Hagelberg et al. (1) and the silica/guanidinium thiocyanate method of Höss and Pääbo (2). One major advantage of this bone preparation method is that a large number of 1 nut/2 bolt assemblies can be prepared cheaply in advance and stored indefinitely without risk of contamination. Theoretically, it should be possible to clean and sterilize a nut-and-bolt assembly for reuse with other samples. However, considering the low cost of the nuts and bolts required for the apparatus and the importance of avoiding contamination, we would not recommend reuse of the assemblies.

REFERENCES

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