

DNA EXTRACTION WITHOUT POWDERING FROM BONE AND TEETH

Hirofumi Fukushima¹, Takahito Oki¹, Hideki Asamura¹, Masao Ota¹, Kanako Nagasaki² and Yoshiya Fukuma²

¹Dept. of Legal Medicine, Shinshu University School of Medicine, Matsumoto, Japan,

²Life Science Division, Hitachi Software Engineering Co.,Ltd., Japan

The extraction and successful PCR amplification of DNA from human remains is crucial in the analysis of forensic samples. One critical factor in selecting an extraction procedure is the capacity of the process to eliminate the contamination and minimize the inhibition attributable to high-yield DNA extraction.

In general, most current DNA extraction methods for bones and teeth involve the incubation of powdered materials in an EDTA-containing extraction buffer. Surface materials are first removed from the bone or an entire tooth by the customary method of washing with neutral detergent and distilled water. A piece (about 0.2~1.0g) is then cut from each bone after washing and air-drying. Almost all extraction methods involve powdering a piece of bone or tooth before extraction. In contrast, our method involves soaking the entire tooth or bone in buffer without powdering. Following removal of these buffering materials, the samples are incubated in a lysis buffer containing proteinase K. Finally, DNA is extracted using a silica membrane (QIAamp).

We found that this method can recover adequate quantities of DNA from an entire tooth or piece of bone. The method is also fast and offers high yields, potentially eliminating both contamination and the time-consuming dialysis and concentration steps. This non-powder method provides significant advantages for successful DNA extraction, particularly for challenging specimens previously discounted from forensic DNA typing. This method has been successfully applied to numerous 60-year-old Japanese skeletal remains found buried in Russian territory since the end of World War II.

The results indicate that DNA extraction by this new method from new or degraded bone or tooth samples increases the success rate with PCR amplification, including amplification of STR and Y-STR markers and mitochondrial DNA.