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Isolation of DNA from small amounts of elephant ivory: Sampling the cementum with total demineralization extraction

Running Title: Improved isolation of DNA from elephant ivory

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Highlights

- A newly optimized extraction protocol for the retrieval of DNA from elephant ivory is described.
- We used complete demineralization and extraction of the outer cementum layer, if available.
- Low quality samples are concentrated and subjected to additional PCR amplification, as needed.
- Methods yielded 81% recovery of ten or more microsatellite loci needed to assign origin.

Abstract

Genotyping ivory samples can determine the geographic origin of poached ivory as well as the legality of ivory being sold in ivory markets. We conducted a series of experiments to determine where the DNA is most concentrated in ivory samples and how best to increase DNA yield from groups of samples likely to vary in DNA concentration. We examined variation in DNA amplification success from: the layer(s) of the tusk (cementum and/or dentine) being extracted, demineralization temperature and time, and the concentration of eluates. Since demineralization of the pulverized sample produces a pellet and supernatant, we also assessed DNA amplification success from the pellet, the supernatant, their combination, as well as variation in the respective amounts used for extraction. Our results show that the outer cementum layer of the tusk contains the highest concentration of DNA and should be separated and used exclusively as the source material of ivory processed for extraction, when available. Utilizing the combined demineralized lysate improves extraction efficiency, as does increasing demineralization time to 3 or more days, conducted at 4°C. The most significant improvements occurred for low template DNA ivory samples followed by medium quality samples. Amplification success of high quality samples was not affected by these changes. Application of this optimized method to 3,068 ivory samples resulted in 81.2% of samples being confirmed for both alleles at a minimum of 10 out of 16 microsatellite loci, which is our threshold for inclusion in DNA assignment analyses.

Keywords: DNA Extraction, Elephant Ivory, Poaching, Low-template DNA, Demineralization

Introduction:

The illegal ivory trade has had a devastating impact on African elephant populations, with the number of poached elephants rapidly increasing since 2006 [1-3]. DNA analyses of large ivory seizures

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