Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsigen

Short communication

## Expediting the sampling, decalcification, and forensic DNA analysis of large elephant ivory seizures to aid investigations and prosecutions

Kyle M. Ewart<sup>a,\*</sup>, Amanda L. Lightson<sup>a</sup>, Frankie T. Sitam<sup>b</sup>, Jeffrine J. Rovie-Ryan<sup>b</sup>, Niklas Mather<sup>c</sup>, Ross McEwing<sup>a</sup>

<sup>a</sup> TRACE Wildlife Forensics Network, Edinburgh, Scotland, United Kingdom

<sup>b</sup> National Wildlife Forensic Laboratory, Department of Wildlife and National Parks (PERHILITAN), Kuala Lumpur, Malaysia

<sup>c</sup> Chalmers St, Redfern, Sydney, Australia

ARTICLE INFO

Keywords: Decalcification

Sampling

Wildlife forensics

Ivory

## $A \ B \ S \ T \ R \ A \ C \ T$

The illegal ivory trade continues to drive elephant poaching. Large ivory seizures in Africa and Asia are still commonplace. Wildlife forensics is recognised as a key enforcement tool to combat this trade. However, the time and resources required to effectively test large ivory seizures is often prohibitive. This limits or delays testing, which may impede investigations and/or prosecutions. Typically, DNA analysis of an ivory seizure involves pairing and sorting the tusks, sampling the tusks, powdering the sample, decalcification, then DNA extraction. Here, we optimize the most time-consuming components of this process: sampling and decalcification. Firstly, using simulations, we demonstrate that tusks do not need to be paired to ensure an adequate number of unique elephants are sampled in a large seizure. Secondly, we determined that directly powdering the ivory using a Dremel drill with a high-speed cutter bit, instead of cutting the ivory with a circular saw and subsequently powdering the sample in liquid nitrogen with a freezer mill, produces comparable results. Finally, we optimized a rapid 2-h decalcification protocol that produces comparable results to a standard 3-day protocol. We tested/ optimised the protocols on 33 raw and worked ivory samples, and demonstrated their utility on a case study, successfully identifying 94% of samples taken from 123 tusks. Using these new rapid protocols, the entire sampling and DNA extraction process takes less than one day and requires less-expensive equipment. We expect that the implementation of these rapid protocols will promote more consistent and timely testing of ivory seizures suitable for enforcement action.

## 1. Introduction

The illegal ivory trade continues to decimate wild elephant populations [1]. Most large-scale ivory seizures occur in Africa or Asia, many of which involve organised criminal syndicates [2,3]. Forensic testing of ivory is a key CITES recommendation, particularly for seizures  $\geq$  500 kg [4], with the aim of determining the age or origin of the ivory for the purposes of investigations and prosecutions. McEwing & Ahlers [5] suggested that the current timeframe from sampling ivory to revealing results was not fast enough to maximise investigative potential, and that building national capacity to undertake forensic testing would expedite this process.

Numerous DNA-based wildlife forensic techniques have been developed to test ivory. The purpose of these tests is to produce evidence for prosecution and/or to generate information (intelligence reports) about the dynamics of the illegal ivory trade. Generally, the first

component of an ivory identification is determining the species of the seized tusks or ivory products. However intuitively obvious the species identification may be based on morphology, the species identification needs to be robust to counter potential challenges when lookalike alternatives exist. The ivory may derive from a different species (e.g. mammoth or hippopotamus), or may be fraudulent (e.g. plastic or bone) [6,7]. Determining whether the ivory derived from African or Asian elephant is also necessary in some jurisdictions with a legal national trade (e.g. Thailand) or differing legal responses dependent upon species. Hence, species-level DNA identification is often all that is required to establish illegality in a prosecution. If the ivory derives from African elephant, two DNA-based tests are published that establish the geographic provenance of the ivory: one based on nuclear DNA microsatellite analysis [3,8] and one based on the mitochondrial d-loop region [9,10]. These geographical provenance tests are useful from an intelligence reporting perspective.

\* Corresponding author.

E-mail address: kyle.ewart@tracenetwork.org (K.M. Ewart).

https://doi.org/10.1016/j.fsigen.2019.102187

Received 9 July 2019; Received in revised form 27 September 2019; Accepted 12 October 2019 Available online 14 October 2019

1872-4973/ © 2019 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/BY-NC-ND/4.0/).





