Validation of a Non-Destructive DNA Recovery Method in Forensic-Age Samples

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Introduction

Responding to a need within ancient DNA studies to develop DNA extraction protocols that are as minimally invasive as possible, Bolnick et al. (2012) tested a non-destructive DNA recovery method that relies on soaking specimens in proteinase K and EDTA (proK/EDTA) to recover DNA. As with the archaeological and museum collections targeted by ancient DNA studies, forensic DNA work faces similar problems, including limited availability of sample material due to fragmentary remains, high failure rates for degraded or low copy number templates, and expectations for the return of remains. Here, we assess the applicability of this non-destructive protocol to freshly acquired human teeth as a stand-in for forensically-relevant samples, targeting mitochondrial and nuclear DNA.

Bolnick et al.'s (2012) method consists of two key steps: 1) a soaking step and 2) a DNA extraction step from Rohland & Hofreiter (2007). In addition to testing this same method on modern tooth samples, we also tested the efficacy of the proK/EDTA soaking step with an alternate DNA extraction protocol (Kemp 2012).

Materials & Methods

- **Sample Procurement** – 15 teeth from 15 anonymous donors were obtained from a local dentist. Immediately after oral extraction, the teeth were individually placed in DNA-free HPLC-grade water in DNA-free tubes and stored at 4 °C.
- **Sample Preparation** – In a dedicated pre-PCR clean room (see below), tooth samples were removed from the water and air-dried in a biohazard hood for four months. When necessary, soft tissue was removed using sterile forceps. All teeth were then decontaminated by soaking in 6% w/v sodium hypochlorite (full strength bleach) for 15 minutes (Kemp & Smith 2005), rinsed twice with DNA-free HPLC-grade water, and air dried.
- **Test of proK/EDTA soak with Rohland & Hofreiter's (2007) DNA extraction protocol** – Ten teeth were soaked in a demineralization buffer (10 mL of 0.5M EDTA plus 150uL proK) for 24 hours. The buffer solution was DNA-extracted using Rohland and Hofreiter's (2007) silica-based protocol, as recommended by Bolnick et al. (2012). The teeth were then rinsed with molecular grade water and air dried for storage.
- **Test of proK/EDTA soak followed by Kemp's (2012) DNA extraction protocol** – The remaining five teeth were soaked using the same demineralization buffer described above. After soaking for 48 hours, teeth were extracted using Kemp's (2012) protocol that integrates the Promega Wizard® PCR Preps DNA Purification System.
- **PCR amplification** – All samples were PCR-amplified using a 167 base pair (bp) mitochondrial DNA (mtDNA) primer set from hypervariable region I (HVI) (Kemp 2006). Confirmation of nuclear DNA (nuDNA) was performed by amplification of a 67 bp target from the *c-fms* proto-oncogene for the *CSF-1* receptor gene on chromosome 5 (Swango et al. 2006). Post-PCR results were visualized using GelRed™ on a 3% agarose gel.

Contamination Controls at the Molecular Anthropology Laboratories at the University of Tennessee, Knoxville (MAL-UTK)

These forensic-age samples were treated as ancient DNA samples, so that all relevant precautions for preventing and detecting exogenous contamination were followed (Kaestle & Horsburgh 2002; Knapp et al. 2012):

DNA extractions and PCR set-up took place in a restricted access clean room that is dedicated to pre-PCR forensic genetic research and is equipped with dedicated equipment, overhead UV lights, positive air pressure, and HEPA-filtered ventilation. The post-PCR laboratory is located in a separate wing of the building and is on a separate air handling system. Personnel movement between facilities is unidirectional (from pre-PCR forensic genetic laboratory to post-PCR). A decontaminated face shield, plus disposable coveralls with hood, shoe covers, and gloves were worn at all times in the forensic genetic laboratory. Workspaces and equipment were regularly decontaminated with a 10% household bleach solution and/or DNA AWAY™, and UV-irradiated between uses. Reagents and disposable tubes were certified DNA-free and/or molecular grade whenever possible. Negative (blank) controls were included at all stages of extraction and amplification.

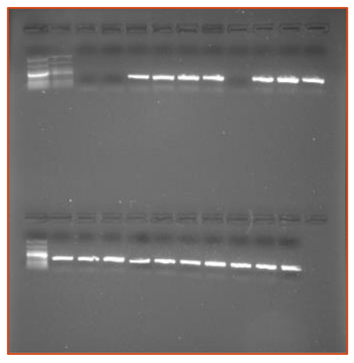
Results: PCR Amplification

MtDNA (167 bp target). All samples using the proK/EDTA soak followed by Rohland & Hofreiter's (2007) DNA extraction protocol amplified for the 167 bp target. Four out of five samples amplified using the proK/EDTA soak followed by Kemp's (2012) DNA extraction protocol (Figure 1).

NuDNA (67 bp target). All samples successfully amplified for nuDNA using the proK/EDTA soak followed by either DNA extraction protocol.

Figure 1 (right). Gel image of mtDNA PCR amplification.

- *Top row.* Lanes 1 & 2: Low molecular weight ladder; Lane 3: PCR negative control, Lane 4: extraction negative control; Lanes 5-9: samples treated with proK/EDTA soak + Kemp (2012); Lanes 10-12: samples extracted following Bolnick et al. (2012).
- *Bottom row.* Lane 1: Low molecular weight ladder, Lanes 2-10: samples extracted following Bolnick et al. (2012); Lane 11: PCR positive control.

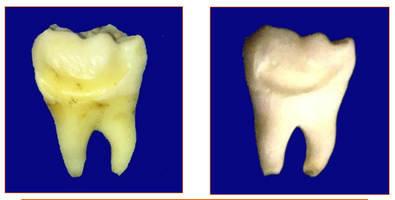


Results: Sample Integrity

We confirm that this method – the use of a proteinase K/EDTA soaking step prior to DNA extraction – is "non-destructive" both because (1) the method did not involve breaking, crushing, powdering, or otherwise disfiguring the sample during sample preparation, and (2) samples retained their structural integrity throughout the process (see example in Figure 2), with the caveat that the use of a soaking step prior to DNA extraction may nevertheless produce chemical changes in the sampled material or physical alterations that are not visible to the naked eye.

Figure 2. Photographs of a single tooth sample before (left) and after (right) DNA extraction.

In this example, the sample was treated with the pro/EDTA soak step.



Before After

Discussion

Our results indicate that Bolnick et al.'s (2012) method is appropriate for degraded samples of forensic age. In addition, our research demonstrates that alternate DNA recovery methods may be modified by adding the proK/EDTA soaking step prior to DNA extraction.

Moreover, use of Kemp's (2012) "New Method" may provide results of equal quality, with several added benefits. Unlike Rohland & Hofreiter's (2007) DNA extraction method, Kemp (2012) offers a relatively faster, less toxic, and cost-effective DNA extraction protocol. For example, the binding buffer used in Rohland & Hofreiter (2007) requires ~ 24 g of guanidine thiocyanate (GuSCN), a chemical which can be harmful if inhaled or makes skin contact. In contrast, the Kemp (2012) requires 6 M guanidine HCl, which is in solution and therefore reduces chances of inhalation as well as the production of cyanide gas which can occur if GuSCN comes in contact with sodium hypochlorite (bleach), a commonly-used decontaminant. Additionally, the Kemp DNA extraction protocol requires far fewer steps, thereby reducing contamination opportunities. Finally, the reduced amount of GuSCN – and fewer reagents in general – reduces cost per sample.

Future Directions

- Additional research will compare the performance of both protocols in amplification via two extractions of additional nuclear markers, specifically short-tandem repeats (STRs), using the Promega PowerPlex® 16 HS kit.
- Fragment analyses of these samples will also compare rates of stutter and allelic dropout between the two protocols.

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