A Faster, Easier, and More Effective Bone Processing Method for DNA Analysis

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FSF Emerging Forensic Scientist Award
Poster Presentation

INTRODUCTION

In missing persons cases, fire fatalities, mass disasters, and some forensic casework, skeletal samples are commonly used for human identification (HID) purposes. Bone and tooth samples are not routinely processed by all forensic laboratories, as the laboratory may not have the resources required such as bone grinding equipment, adequate lab facilities, or experienced analysts. Alternatively, specialized DNA analyses (e.g. mitochondrial analysis) may also be required. Due to the more complicated nature of these samples, skeletal remains may be sent to alternative laboratories for processing.

Traditional DNA extraction protocols involve the powdering of bone followed by a lengthy digestion (e.g. total demineralization) and DNA purification (e.g. organic or silica-based) (1). While many laboratories that process skeletal remains prefer to process bone samples manually using their own in-house protocols, several commercial DNA extraction kits are available (2,3) to standardize the process and improve sample throughput. However, these kits still require bone to be ground into a fine powder. This study explored the efficacy of a commercial DNA extraction kit and an automated platform to purify DNA from small bone fragments in order to eliminate the need to crush the bone into a powder. This option has the potential to save time, reduce the risk of contamination, conserve evidence, and more effectively triage samples by local laboratories while also retaining the ability to automate the process if desired.

MATERIALS AND METHODS

Sample Preparation and DNA Purification:

- Bone and tooth fragments were collected from nine sets of contemporary skeletal remains that have been environmentally challenged (fire exposure, embalming, burial, and advanced decomposition).
- Bone surface was sanded (Dremel) and cut into small pieces (3-5 mm²) or individual bone chips (~50 or 150 mg each).
- All bone cuttings were cleaned with a series of 5 min washes (10% bleach, dH_2O, 100% ethanol) and dried overnight.
- The 3-5 mm² pieces were powdered in a 6700 SPEX liquid nitrogen freezer mill.
- For the pilot study, aliquots of powder (50 or 150 mg) and bone chips (50 or 150 mg) from a single source of bone were digested in 230 µL PrepFiler® BTA lysis buffer master mix for 2, 4, or 16 hrs (Fig. 1) with 5 replicates per variable combination.
- For the skeletal survey, DNA was extracted from 20 bones (50 mg chips) using the PrepFiler® BTA™ kit (Thermo Fisher Scientific) with a 2 hr digestion and processed on the Automate Express™ platform. Powdered bone (50 mg) was also processed manually.

DNA Quantification and STR Analysis:

- DNA extracts were quantified with the Quantifiler® Trio DNA Quantification kit and amplified with the GlobalFiler® PCR Amplification Kit (Thermo Fisher Scientific).
- Data analysis was performed using either Statistica or Excel.

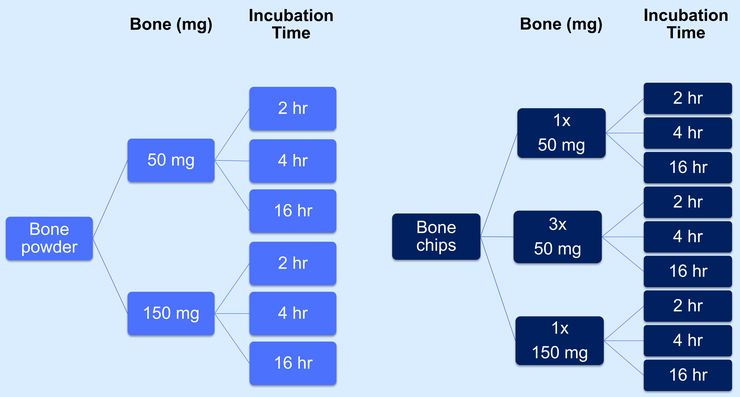

Figure 1 – Pilot study methodology flowchart for powdered bone (left) and chipped bone (right) samples.

RESULTS

PILOT STUDY

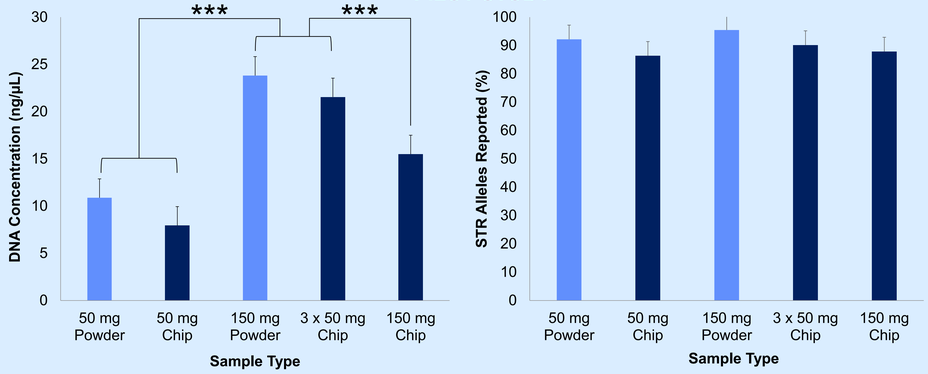

Figure 2 – Comparison of average DNA concentration (left) and STR profile completeness (right) based on sample type and mass used for extraction (n=15). Error bars denote 0.95 confidence interval. *** $p < 0.001$

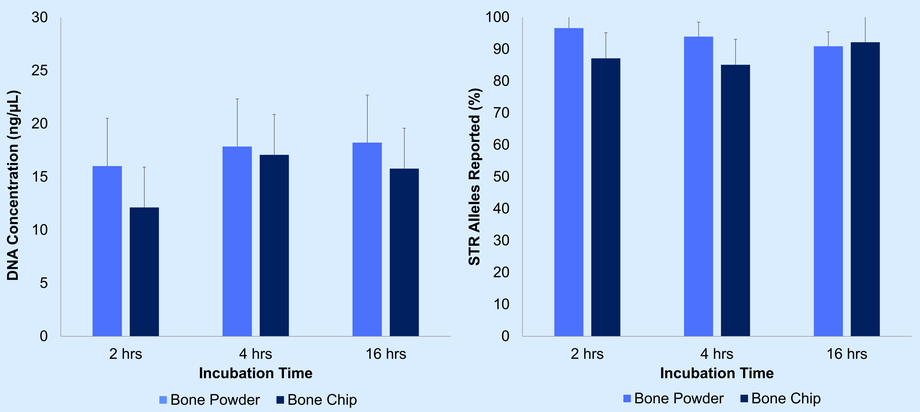

Figure 3 – Comparison of average DNA concentration (left) and STR profile completeness (right) based on incubation time for powder (n=10) and chip (n=15) samples. Error bars denote 0.95 confidence interval.

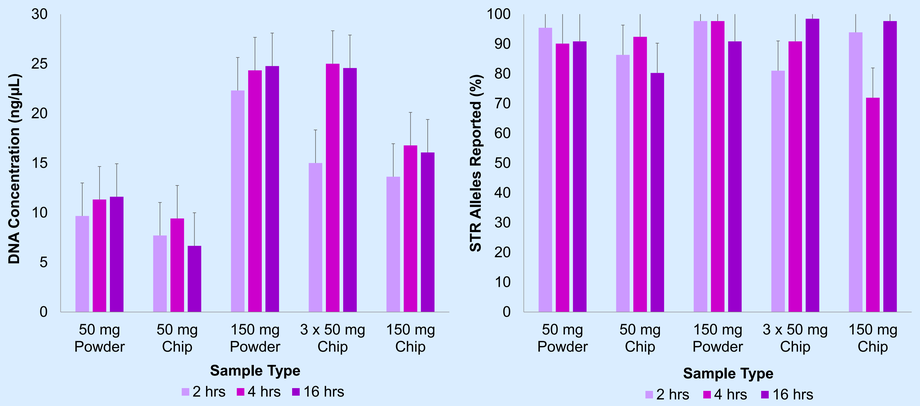

Figure 4 – Comparison of average DNA concentration (left) and STR profile completeness (right) based on sample type/mass and incubation time (n=5). Error bars denote 0.95 confidence interval.

SKELETAL SURVEY

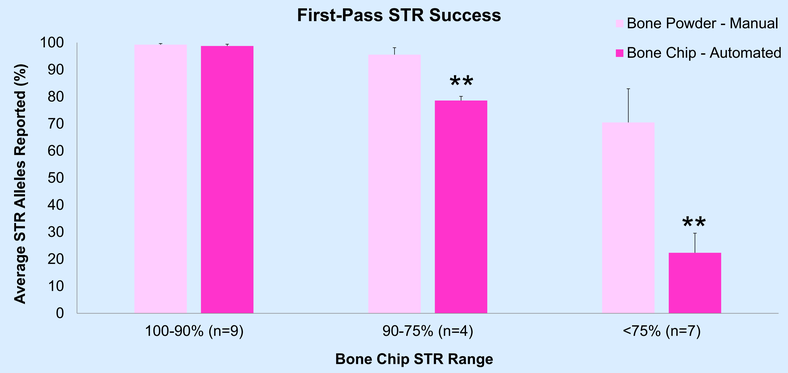

Figure 5 – Average percentage of correct alleles reported for 20 bones processed manually (50 mg bone powder) or on the AutoMate (50 mg bone chip) with PrepFiler® BTA™ chemistry based on bone chip performance. Error bars denote standard error of the mean (SEM). ** $p < 0.01$

DISCUSSION

Pilot Study

- Average DNA concentrations ranged from ~8 ng/µL (50 mg bone chip) to 24 ng/µL (150 mg bone powder) and the average percent of alleles called ranged from 86% (50 mg bone chips) to 95% (150 mg bone powder) (Fig. 2).
- As expected, the 150 mg samples produced significantly more DNA than the 50 mg samples ($p < 0.001$; Fig. 2).
- Both 50 mg of bone powder and 50 mg bone chips yielded comparable DNA concentrations. Likewise, 150 mg of bone powder and three 50 mg bone chips produced similar DNA concentrations ($p > 0.05$; Fig. 2).
- Incubation time (2, 4, or 16 hrs) had no significant effect on DNA concentration or reportable alleles (Fig. 3) for powdered or whole bone chips ($p > 0.05$).
- No significant difference was observed in STR success based on the size/number of bone chips or incubation time (Fig. 2-4).

Therefore, as none of the alternate variables tested in this study generated significantly better results than the manufacturer's existing protocol, one 50 mg whole bone chip and a 2 hour incubation was selected for the skeletal survey.

Skeletal Survey

- DNA concentrations ranged from 0 to 35.8 ng/mg of bone for bone chips when extracted with PrepFiler® BTA™ chemistry on the AutoMate Express™.
- Powdered samples extracted manually yielded slightly more DNA on average but this result was not statistically significant ($p > 0.05$; data not shown).
- In total, 9 of the 20 bone chips (Fig. 5) resulted in full (or near complete) STR profiles using this automated screening approach, which was comparable to powdering for those samples.
- When bone chip samples yielded DNA in low amounts (<0.007 ng/µL) and/or was degraded (DI>7), powdering generated more complete profiles (>10% more alleles; $p < 0.01$).
- Bones traditionally sourced for DNA extraction (e.g. femur, tibia, humerus) did not consistently produce better results than other skeletal elements.

Overall, this research has shown that complete (or near complete) STR profiles can be quickly recovered from whole bone chips from environmentally challenged skeletal remains using an automated workflow without the need to crush the tissue into a powder. This approach may also provide useful in screening skeletonized remains for STR success from various bony elements in-house before, or in lieu of, outsourcing to specialized labs.

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ACKNOWLEDGEMENTS

- This project was funded in part by a Forensic Sciences Foundation Lucas Grant.
- The use of human subjects for this research was made possible by the Applied Anatomical Research Center, located in the Center for Biological Sciences.
- We also thank Thermo Fisher Scientific for providing some of the reagents and support required for this research.