Effective Powder-free DNA Extraction Workflow for Skeletal Samples using the PrepFiler® BTA™ and AutoMate Express™ Systems

Michelle Harrel[1*], BS; Sheree Hughes-Stamm[1,2], PhD

[1]Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340
[2]School of Biomedical Sciences, University of Queensland, St Lucia, Brisbane, Australia

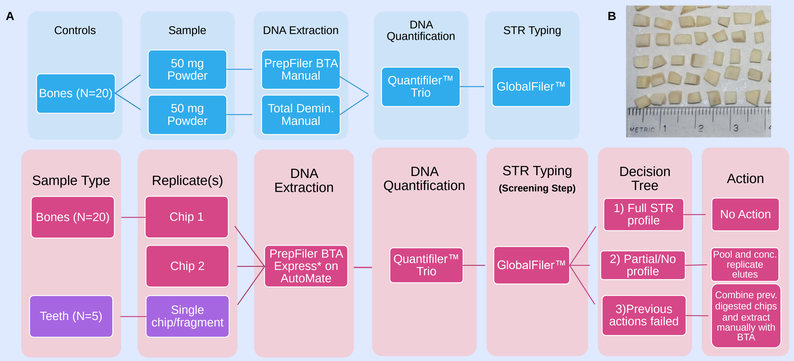
INTRODUCTION

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 regional "hub" 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). 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 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 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 while also retaining the ability to automate the process (if desired).

MATERIALS AND METHODS

Twenty bones and five tooth fragments were collected from nine sets of contemporary skeletal remains that have been environmentally challenged (fire exposure, embalming, burial, and advanced decomposition).



* Using standard incubation time and BTA buffer volume (2 hrs, 230 µL)

Figure 1 – A) Methodology flowchart for powdered (top) and chipped bone and tooth (bottom) samples; B) Examples of 50 mg bone chips

Sample Preparation and DNA Purification:

- Bone surface was sanded (Dremel) and cut into smaller pieces (3-5 mm²) or bone chips (~50 mg each) (Fig. 1B).
- 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. Aliquots of powder (50 mg) were then digested (Fig. 1A).
- Chips (50 mg) were digested in 230 µL BTA lysis buffer master mix for 2 hrs.
- Powdered bone samples (50 mg) were digested and extracted with the PrepFiler® BTA™ Forensic DNA Extraction kit (ThermoFisher Scientific) or according to a previously published total demineralization and MinElute® purification protocol (if applicable – see Fig. 1A) [1].
- Automated extractions were performed on the Automate Express™ platform (ThermoFisher Scientific) after digestion steps (Fig. 1A).
- Elution volume for all methods was 50 µL.

RESULTS

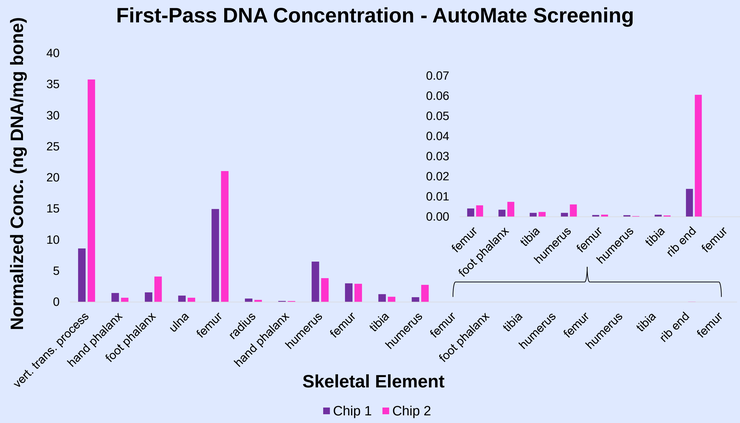


Figure 2 – DNA concentration for 20 bone chips processed in duplicate with an automated extraction. Values are normalized to the weight of the bone chip for accurate comparisons.

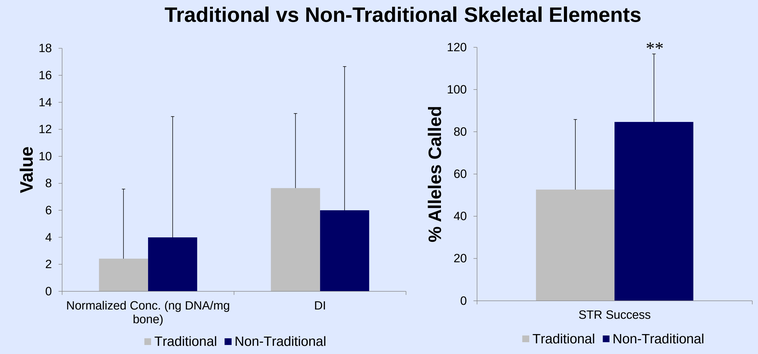


Figure 3 – Comparison of DNA extract and STR profile quality between traditional (N=24) and non-traditional (N=21) skeletal elements. Traditional elements include: humerus, femur, tibia; Non-traditional elements include: teeth, radius, ulna, rib ends, hand and foot phalanges.
**p < 0.01

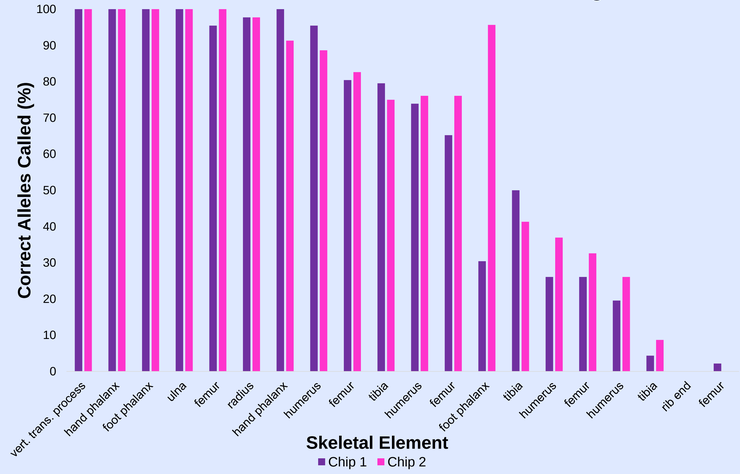


Figure 4 – Percentage of correct alleles called for 20 bone chips processed in duplicate with an automated extraction.

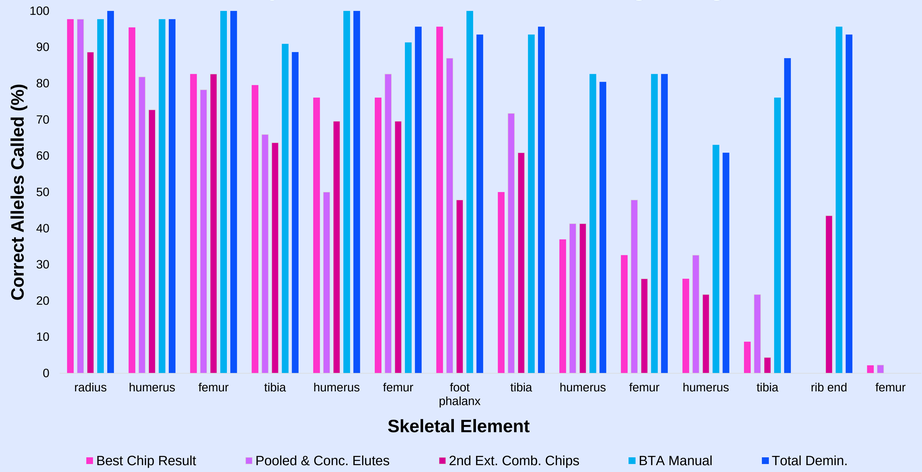


Figure 5 – Percentage of correct alleles called for 14 bone chip samples that required re-work to improve allele recovery. Secondary processes included: pooling and concentrating original elutes, combining replicate bone chips and extracting manually with PrepFiler BTA, and manually extracting from bone powder with either PrepFiler BTA or total demineralization.

DISCUSSION

DNA Yield

- DNA concentrations ranged from 0 to 35.8 ng/mg of bone for bone chips (Fig. 2) and from 0.0009 to 23.4 ng/mg of tooth fragment (data not shown) when extracted with PrepFiler® BTA™ chemistry on the AutoMate Express™.
- Powdered samples extracted manually yielded slightly more DNA on average ($p > 0.05$; data not shown).

Traditional vs Non-Traditional Skeletal Elements

- Non-traditional elements yielded slightly higher and less degraded DNA and resulted in significantly more alleles reported ($p < 0.01$) compared to traditional elements (Fig. 3). Therefore DNA can be effectively recovered from many different bony elements using this non-powdering method.

STR Success and Profile Quality

- In total, 6 out of 20 bones (Fig. 4) and 3 out of 5 teeth fragments resulted in full STR profiles.
- Pooling and concentrating replicate eluates improved allele recovery for half of re-worked samples.
- Manual extraction from bone powder improved allele recovery from 12/14 re-worked samples; 4 of those samples resulted in full STR profiles (Fig. 5).

Overall, this research has shown that full STR profiles can be quickly recovered from contemporary whole bone and tooth chips with an automated workflow. Additionally, sample quality may be screened in-house before (or in lieu of) outsourcing to specialized labs.

REFERENCES

[1] Loreille OM, Diegoli TM, Irwin JA, Coble MD, Parsons TJ. High efficiency DNA extraction from bone by total demineralization. Forensic Sci. Int. Genet. 2007;1(2):191-5.

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