Optimization of InnoXtract™ Extraction and Purification System for DNA extraction from skeletal samples

Forensic Technology CENTER OF EXCELLENCE

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1. Introduction

Skeletal remains are often the only evidence discovered in human identification cases. These samples can be challenging to process due to the low-template level, endogenous inhibitors, DNA fragmentation, and environmental factors [1,2]. Because DNA typing from skeletal samples is prone to multiple challenges, it is important that an efficient extraction method is utilized to obtain the quality and quantity of DNA necessary for human identification. Most skeletal extraction methods are either time consuming or require a large input sample size [1,3]. However, some partial demineralization methods, such as PrepFiler™ BTA allow for a reduced analysis time, automation, and the ability to process limited skeletal samples [4]. Further investigation of a new extraction method with these benefits is important to the forensic science community.

InnoXtract™ Extraction and Purification System (InnoGenomics, New Orleans, LA) is a DNA extraction kit marketed for low-level samples such as rootless hair shafts [5,6]. InnoXtract™ targets smaller fragments of DNA allowing for greater success with these compromised samples, suggesting that skeletal samples may also be suitable for extraction using this kit [5,6]. An optimized lysis and digestion method was necessary to successfully extract the quality and quantity of DNA needed for human identification using InnoXtract™. Overall, this optimized InnoXtract™ protocol can provide DNA analysts with a new method for DNA extraction from limited, highly inhibited, or degraded skeletal samples in an efficient and potentially automatable manner.

2. Methods

Sample Preparation and Selection:

- Skeletal samples (N = 16) were collected from 11 cadavers donated to the Southeast Texas Applied Forensic Science Facility (STAFS) at Sam Houston State University
- Sample insults: buried, fresh and older surface decomposition, embalmed, burned, and cremated remains
- Window cuts were collected from 9 femurs, 4 tibias, 2 humeri, and 1 vertebra
- Samples were then sanded, dried, and powdered using a freezer mill (SPEX 6770)
- Due to quality and quantity of samples, initial optimization was performed on a one-year-old surface decomposed femur

Lysis and Purification Method Comparison:

- Compared, in triplicate, two established extraction methods PrepFiler™ BTA (Thermo Fisher Scientific) and Intermountain Bone/Tooth Processing and Purification (Intermountain Forensics) and the coupling of the established lysis parameters with InnoXtract™ purification methods **(Table 1)**

Table 1. Pairings of Lysis and Purification Methods Tested

Lysis Parameters	*Purification Parameters*
PrepFiler™ BTA [4]	PrepFiler™ BTA [4]
PrepFiler™ BTA [4]	InnoXtract™ [6]
Intermountain Bone/Tooth Processing and Purification [7]	EZ1 – large volume protocol [8]
Intermountain Bone/Tooth Processing and Purification [7]	InnoXtract™ [6]

Optimization Study:

- **Lysis Parameter Optimization:** Compared extractions, in triplicate, on 50 mg of bone powder with varying lysis parameters **(Figure 1)**
  - Quantification: Quantifiler Trio Quantification Kit on ABI 7500
  - Amplification: Investigator® 24plex QS Amplification Kit
  - CE: ABI 3500 Genetic Analyzer

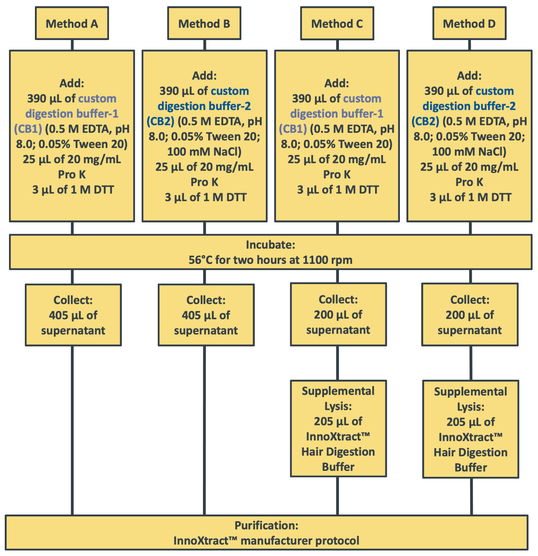

Figure 1. InnoXtract™ Optimization Parameters Schematic

- **Magnetic Bead Volume Optimization:** DNA purifications performed (replicates of five) using 10, 15, 20, 25, and 30 µL of magnetic binding beads
- **Sample Input Optimization:** DNA extractions performed (replicates of five) on 25, 40, and 50 mg of bone powder using the optimized method

Comparison to PrepFiler™ BTA Methods:

- Extractions performed (replicates of five) using the optimized InnoXtract™ method and PrepFiler™ BTA methods

Versatility Study:

- Extractions performed in triplicate on buried, surface decomposed, embalmed, burned, and cremated remains (N = 11)
- STR typing performed on select samples (N = 7) if sufficient large target DNA (> 35 pg) could be targeted for STR amplification

3. Results

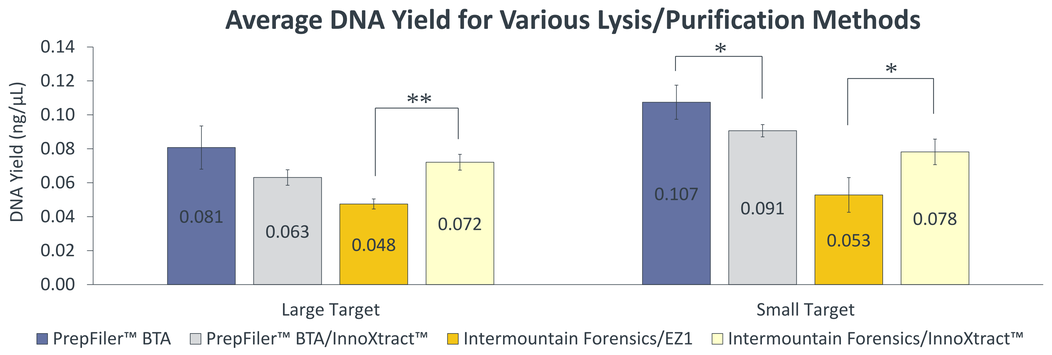

Figure 2. Average DNA Yield for Lysis methods – The coupling of InnoXtract™ purification with PrepFiler™ BTA lysis provided a higher small target yield (n.s.) compared to Intermountain Forensics lysis and InnoXtract™ purification; (Data reported as µ ± σ; * indicates a p-value < 0.05; ** indicates a p-value < 0.005).

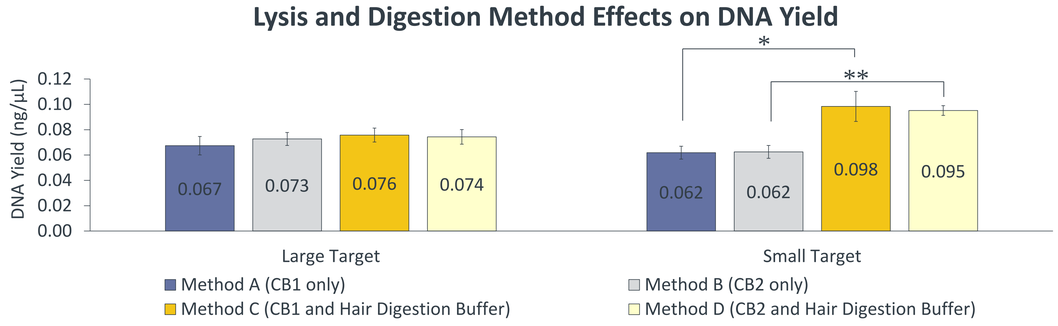

Figure 3. InnoXtract™ Lysis and Digestion Method Effects on DNA Yield – Two-part digestion resulted in a significant increase in small target DNA yield. No significant difference was seen with the addition of salt; (Data reported as µ ± σ; * indicates a p-value < 0.01, ** indicates a p-value <0.005).

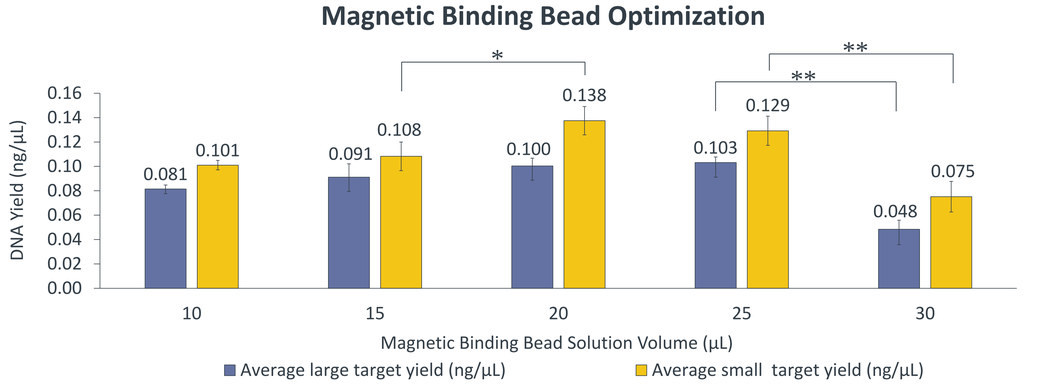

Figure 4. Magnetic Binding Bead Volume Effects – 20 and 25 µL of magnetic binding beads provided the highest DNA yields; (Data reported as µ ± σ; * indicates a p-value < 0.05, ** indicates a p-value < 0.005).

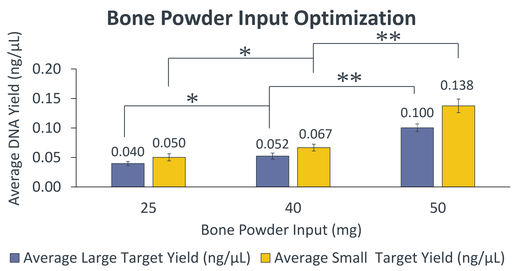

Figure 5. Bone Powder Input Effects – 50 mg of bone powder resulted in significantly higher small and large target DNA yield compared to 25 and 40 mg of bone powder; (Data reported as µ ± σ; * indicates a p-value < 0.05, ** indicates a p-value < 0.001).

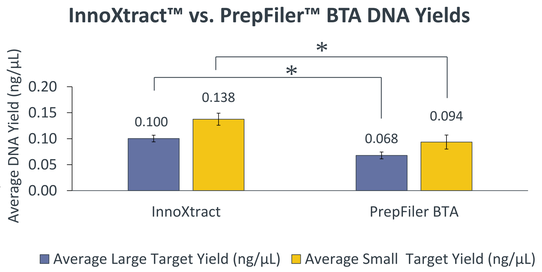

Figure 6. Extraction Method Comparison – InnoXtract™ provided significantly higher small and large target yield relative to PrepFiler™ BTA methods; (Data reported as µ ± σ; * indicates a p-value < 0.001).

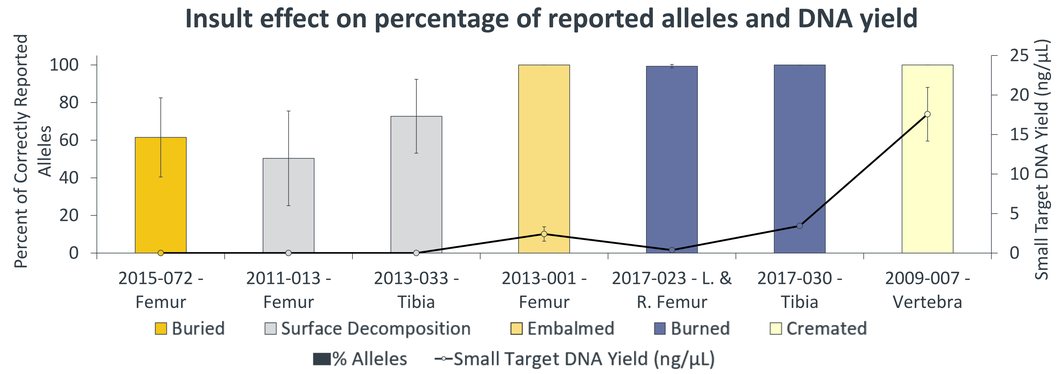

Figure 7. Average DNA Yield for Lysis methods – Despite variation in DNA yield, full profiles were recovered in four of the five insult groups. Only one full STR profile was recovered from surfaced decomposition remains and buried remains resulted in partial profiles for all replicates. **Note:** these extractions were performed before magnetic binding bead volume optimization was performed.

4. Discussion

Lysis and Purification Method Comparison:

- Although not significant, the higher recovery of small target DNA when coupling InnoXtract™ purification with PrepFiler™ BTA vs. Intermountain Forensics lysis parameters suggested designing the InnoXtract™ custom digest buffer and lysis parameters similar to PrepFiler™ BTA methods **(Figure 2)**

Optimization Study:

- Addition of a supplemental lysis using the InnoXtract™ Hair Digestion Buffer resulted in significantly higher small target yields, likely due to the Hair Digestion Buffer being designed to interact with the magnetic beads and binding solution **(Figure 3)**
- Magnetic beads are likely oversaturated when following InnoXtract™ protocol since it is designed for extraction from a single rootless hair shaft
  - Increasing the volume of magnetic beads to 20 and 25 µL allowed for higher DNA yields **(Figure 4)**
    - 20 µL was chosen as optimal volume to limit reagent use

Comparison to PrepFiler™ BTA Methods:

- InnoXtract™ methods had significantly higher small and large target yields relative to PrepFiler™ BTA **(Figure 6)**
  - Demonstrates that the optimized InnoXtract™ method is comparable to established methods

Versatility Study:

- Full profiles were recovered from 4 of the 5 insult groups and total allele recovery was 83.12% **(Figure 7)**
- 94.54% of alleles were recovered when examining STR markers that result in amplicons less that 200 bp, whereas only 76.70% of large amplicon alleles were recovered indicating the success InnoXtract™ has with capturing highly fragmented DNA

5. Conclusions

- InnoXtract™ was successfully optimized for use with skeletal samples using 50 mg of bone powder, a homebrew digestion buffer, a two-part lysis, and 20 µL of magnetic beads
- Quality and quantity of DNA yield is comparable between InnoXtract™ and PrepFiler™ BTA methods
- InnoXtract™ successfully recovered full profiles and removed inhibitors in several samples

6. Next Steps

- Perform further extractions using both InnoXtract™ and PrepFiler™ BTA methods
  - Increase number of replicates
  - Increase variability for a more comprehensive examination and comparison of method
    - Sampling location
    - Donors
    - Insult
- Analyze extracts with various downstream methods allowing for implementation of novel investigative methods
  - STR typing – Investigator® 24plex QS (QIAGEN)
  - Microarray SNP typing – Infinium Global Screening Array (Illumina Inc.)
    - Forensic genetic genealogy methods
  - MPS techniques – ForenSeq Signature Prep kit and ForenSeq Kintelligence kit (Verogen)
    - Phenotype/Ancestry predictions

References and Suggested Citation

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More Information

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