A Comparison of Sample Pretreatment and DNA Extraction Methods From Human Rib Bones

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INTRODUCTION

- Bones may be the only DNA source remaining for human identification [1,2,3]
- Bones preserve DNA longer than tissues due to their mineral structure, but also require specialized extraction methods to remove minerals and PCR inhibitors [4,5]
- Many bone DNA extraction methods utilize decontamination, pulverization, and demineralization steps prior to extraction [6]
- This study serves to compare DNA recovery of three bone pulverization methods and three bone DNA extraction methods.

MATERIALS & METHODS

Rib bones were collected and macerated by the King County Medical Examiner's Office (Seattle, WA). 6 sections of rib bones from 3 individuals were used.

Pretreatment

- Initial cleaning: Rib bones were cleaned with sterile water followed by 70% ethanol, then swabbed, extracted, quantified, amplified, and profiled for reference profiles.
- Sanding and Cutting: Bones were sanded and cut using a Dremel MultiPro Model 395 Type 5 Variable Speed rotary tool (Dremel, Racine, WI, USA) with #431 60-grit sanding band (Dremel) and #426 reinforced cut-off wheel (Dremel), respectively.
- Environmental Exposure: Bone chips were placed in weigh boats outside the WSP Crime Lab (Cheney, WA) for 11 days (June 12, 2025-June 23, 2025).

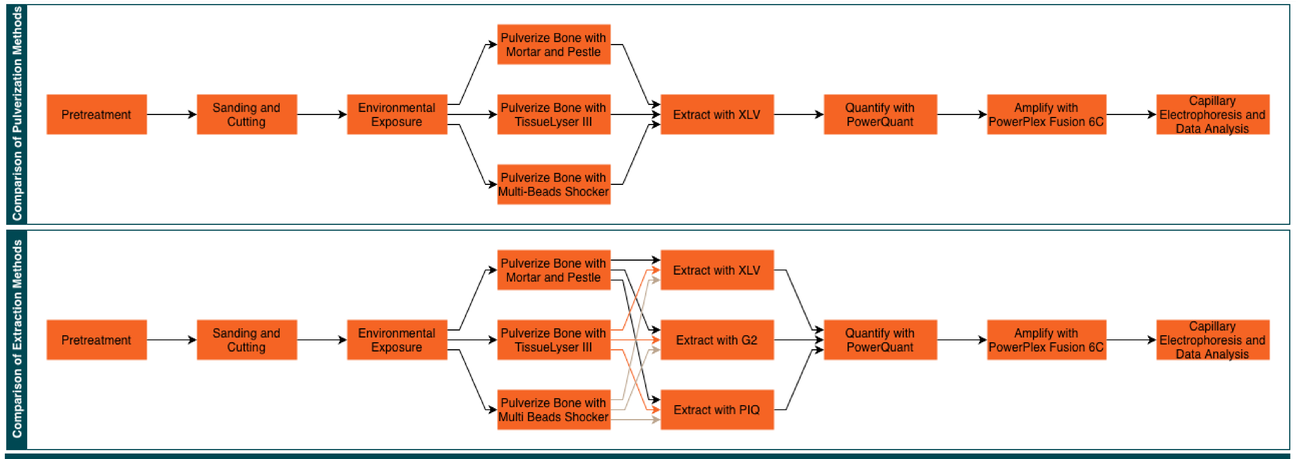
Pulverization Methods

Instrumentation	Parameters
Mortar and Pestle	room temperature, 10 minutes (until granular)
TissueLyser III (Qiagen)	stainless steel grinding jars and ball bearings, 30 Hz, 1 minute, after freezing in -20°C freezer for 1 hour
Multi-Beads Shocker (Yamato Scientific America)	room temperature, tungsten carbide rod, 3000 rpm, 10 seconds

Extraction Methods

Abbreviation	Name
G2	DNA Investigator Kit (Qiagen) following the WSP Pretreatment Protocol
PIQ	Bone DNA Extraction Kit and manual DNA IQ Chemistry (Promega)
XLV	Pretreatment Protocol of Bone or Teeth for Extraction with the Bone Extra Large Volume Protocol (Qiagen)

MATERIALS & METHODS



Quantification

- PowerQuant Kit (Promega) with an Applied Biosystems 7500 Real-Time PCR System (Thermo Fisher Scientific).

Amplification

- PowerPlex Fusion 6C System Kit (Promega) on a GeneAmp PCR System 9700 thermal cycler (Thermo Fisher Scientific) and a ProFlex PCR System thermal cycler (Thermo Fisher Scientific).

Separation and Detection

- Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific) and GeneMapper ID-X software (Thermo Fisher Scientific).

Data Analysis

- One-way repeated measures ANOVA of autosomal DNA concentrations (ng DNA/mg bone powder)

RESULTS & DISCUSSION

Comparison of Pulverization Methods

- No significant difference in DNA recovery between pulverization methods ($F(2,4)=0.573$, $p>0.05$)
- The TissueLyser III (Qiagen) recovered more DNA on average (0.176 ± 0.173 ng DNA/mg bone powder)
- Some amplified loci exhibited partial or total dropout, consistent with aged bone samples
- All profiles were single source and consistent with reference profiles

Comparison of Extraction Methods

- No significant difference in DNA recovery between extraction methods ($F(2,4)=3.098$, $p>0.05$)
- Bone DNA Extraction Kit and manual DNA IQ Chemistry (Promega) recovered more DNA on average (0.106 ± 0.060 ng DNA/mg bone powder)
- Some amplified loci exhibited partial or total dropout, consistent with aged bone samples
- All profiles were single source and consistent with reference profiles

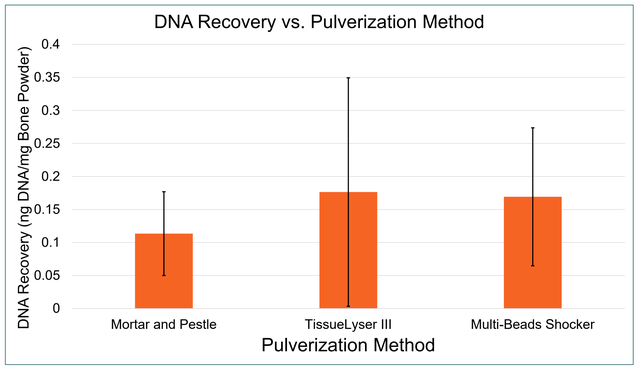


Figure 1: DNA recovery vs pulverization method

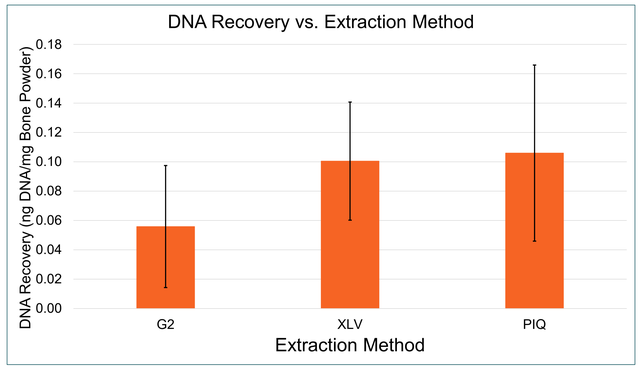


Figure 2: DNA recovery vs extraction method

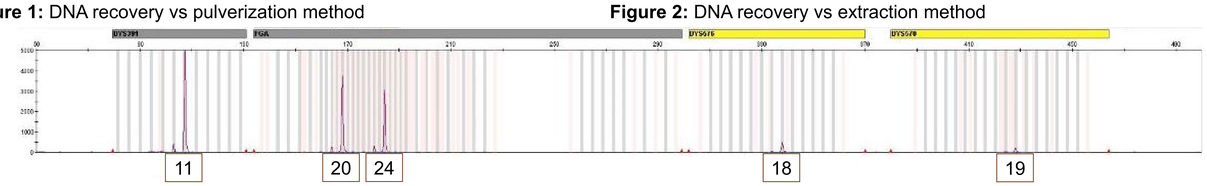


Figure 3: Profile of a bone pulverized with TissueLyser III and extracted with the Bone Extra Large Volume Protocol (Qiagen)

CONCLUSIONS

- No significant difference in DNA recovery (ng DNA/mg bone powder) between extraction methods
- Trends indicated that the Bone DNA Extraction Kit and manual DNA IQ Chemistry (Promega) and the TissueLyser III (Qiagen) generally resulted in the highest average recovery of DNA, respectively, although the difference was not significant.
- The Pretreatment Protocol of Bone or Teeth for Extraction with the Bone Extra Large Volume Protocol is automatable and faster than manual methods.
- While the mortar and pestle performed similarly to the TissueLyser III and Multi-Beads Shocker, the samples used were softer bone tissues and still required ten minutes of pulverization by hand. Other bone sample types will require more time and effort to pulverize with mortar and pestle.
- Full or nearly full profiles were developed for all genotyped bones and no contamination was detected
- The sample size was limited due to availability of human bones, limiting the conclusions that could be derived.

ACKNOWLEDGEMENTS

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