Examination of the EZ2 Connect Fx for Extra-Large Volume DNA Extraction and Purification from Skeletal Samples

Jennifer Snedeker, BS; Sheree Hughes, PhD; Rachel Houston, PhD

Department of Forensic Science, Sam Houston State University, Huntsville, TX 77340

INTRODUCTION

Skeletal samples play a crucial role in identifying human remains, as DNA can be well-preserved within the tough hydroxyapatite matrix of bone. However, extracting DNA from these compromised remains poses challenges due to their low-template, degraded nature and the potential presence of PCR inhibitors [1]. Considerable research has focused on refining DNA extraction and purification methods from these complex skeletal samples, resulting in the development of custom methods with varying parameters for sample input and incubation time. The chosen method often depends on sample quality, quantity, or laboratory needs, requiring the validation of multiple methods.

This study explores the effectiveness of the Extra-Large Volume Protocol provided by the EZ1&2® DNA Investigator® Kit (QIAGEN) for DNA extraction from challenging skeletal samples on the EZ2 Connect Fx. The protocol offers flexibility in both bone powder input (100 – 800 mg) and incubation time (2-24 hrs) within a single method [2]. To evaluate its success, three variations of the protocol were tested and compared against a similar method, using 10 challenging skeletal samples.

MATERIALS & METHODS

- **Samples:** 10 skeletal remains
  - Femurs (n = 6), tarsals (n = 3), metatarsal (n = 1)
  - Surface exposed (n = 4), burned (n = 4), and buried (n = 2) remains
- **Extraction Methods:**

	Extra-Large Volume Protocol	Comparison Method

"Total" Demineralization Method	• 250 mg bone powder
	  • Additional extract performed using maximal sample input (800 mg) for Sample J
	• 24-hour incubation
	• Purify on EZ2® Connect Fx
		Adaptation of Loreille Method [1]
		• 250 mg bone powder
		  • No comparison for 800 mg of Sample J
		• 24-hour incubation
		• Purify using MinElute PCR Purification Kit (QIAGEN)

"Partial" Demineralization Method	• 50 mg bone powder – below recommended input • 2-hour incubation • Purify on EZ2® Connect Fx	**PrepFiler™ BTA Method** [3] (ThermoFisher Scientific) • 50 mg bone powder • 2-hour incubation • Purify on AutoMate™ Express (ThermoFisher Scientific)

- **Quantification:** Investigator® Quantiplex® Pro (QIAGEN)
- **Traditional STR Typing:** Investigator® 24plex QS (QIAGEN)

RESULTS & DISCUSSION

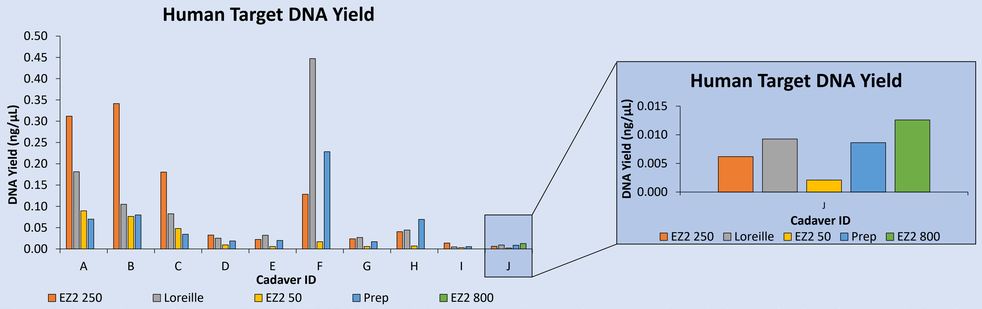

Figure 1: Comparison of Human Target DNA Yield – DNA yield was consistent when comparing similar methods. When examining the variations in the Extra-Large Volume method, the extracts with a longer incubation or larger sample input had a higher DNA yield.

- No statistically significant difference in DNA yield was identified when comparing similar methods **(Figure 1)**.
- The Extra-Large Volume Protocol had a statistically significantly higher DNA yield when implementing the 24-hour incubation on 250 mg of bone powder compared to the 2-hour incubation on 50 mg of bone powder. Similarly, increasing the bone powder input to 800 mg led to a higher DNA yield in Sample J **(Figure 1)**.

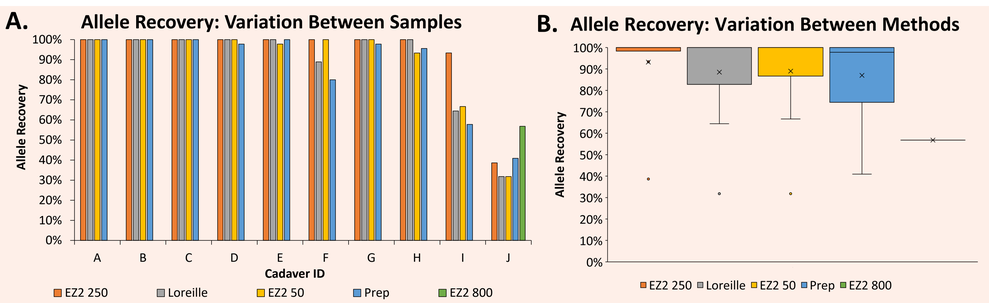

Figure 2: Comparison of Allele Recovery – No significant difference was identified in allele recovery across all four methods. **Figure 2A** highlights the variation in allele recovery based on the sample. **Figure 2B** indicates that the Extra-Large Volume Protocol was most consistent with a 24-hour incubation when 250 mg of bone powder was utilized.

- When comparing the four methods, no statistically significant difference in allele recovery was identified **(Figure 2A)**.
- However, implementing the Extra-Large Volume Protocol with a 24-hour incubation and 250 mg of bone powder resulted in the most consistent allele recovery **(Figure 2B)**.
- Allele recovery improved by as much as 25% when increasing the sample input to the maximum (800 mg) for sample J **(Figure 2A)**.

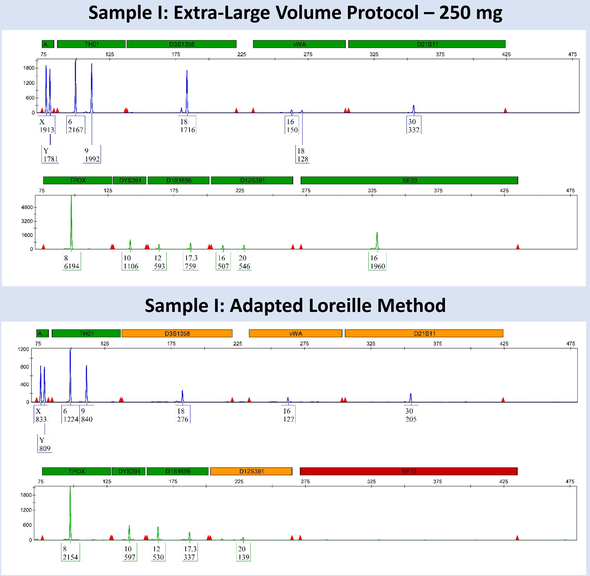

Figure 3: Example Electropherograms – The Extra-Large Volume Protocol (top) consistently provided improved allele call rates for donors that exhibited dropout compared to the Adapted Loreille Method (bottom). *Indicated by the green vs. yellow and red loci markers.*

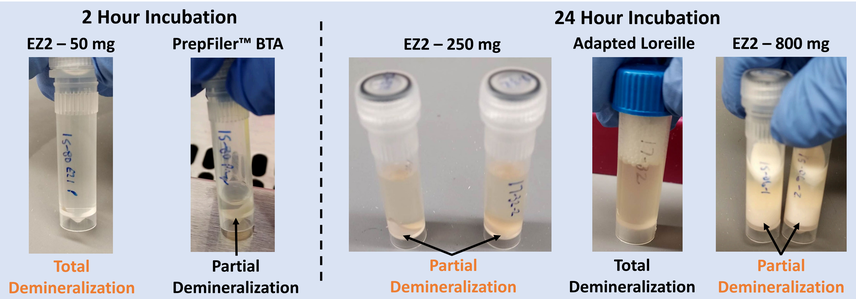

Figure 4: Level of Demineralization – Despite only being a 2-hour incubation, the 50 mg of bone powder completely dissolved when following the Extra-Large Volume Protocol. Contrarily, the 250 mg and 800 mg samples did not result in complete dissolution when a 24-hour incubation was implemented. *Arrows indicate undissolved bone powder. Orange text indicates unexpected level of demineralization.*

- Although there is no significant difference in overall allele call rates between the four methods, the Extra-Large Volume Protocol demonstrated improved allele recovery for more challenging samples (specifically buried remains) compared to the Adapted Loreille Method **(Figure 3).**
- Total demineralization did not occur with 250 or 800 mg of bone powder and a 24-hour incubation using the Extra-Large Volume Protocol **(Figure 4)**. Following the recommendations of Loreille et al. approx. 130 mg of bone powder will result in the proper ratio of bone powder to lysis solution for total demineralization (1 gram bone powder : 15 mL 0.5M EDTA) [1].

CONCLUSIONS

- The Extra-Large Volume Protocol provided by the EZ1&2® DNA Investigator® Kit is an alternative DNA extraction and purification method for challenging skeletal remains.
- DNA yield and overall profile quality was similar to other established methods while providing a more consistent result.
- The Extra-Large Volume Protocol provides flexibility in both sample input and incubation time allowing for analyst discretion depending on the sample type:
  - **Limited time and/or limited sample available:** 50 mg at 2 hours
  - **Challenging sample:** 250 mg at 24 hours
  - **Highly challenging; but readily available sample:** 800 mg at 24 hours
- The Extra-Large Volume Protocol allows individual labs to implement a method that works best for their needs.
- Automatic purification on the EZ2® Connect Fx limits analyst hands-on time.
- Mainly incorporates components from a commercially available kit. All extra reagents are readily available in most labs.

REFERENCES

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3. PrepFiler Express™ and PrepFiler Express BTA™ Forensic DNA Extraction Kits User Guide, Appl. Biosyst. Rev. B (2010).

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