Developing a Decision Tree for Streamlining Degraded Sample Processing Across Capillary Electrophoresis and Next-Generation Sequencing Platforms in Forensic Laboratories

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INTRODUCTION

Samples exhibiting some level of DNA degradation are often submitted to crime laboratories for analysis. Genotyping degraded DNA has traditionally been challenging due to the low amounts of intact DNA present. Increased levels of degradation can lead to drop-out of larger amplicons (1), which is problematic for traditional size-based allele calling.

Next-generation sequencing (NGS) has been proposed as a way to overcome the size-based limitations of capillary electrophoresis (CE) chemistries (2). As such, forensic laboratories face the critical decision of choosing between CE and NGS chemistries for their analytical needs. This decision has gained importance with the approval of the ForenSeq MainstAY Kit by the National DNA Index System (NDIS).

To determine the suitability of NGS chemistries over CE chemistries, quantification and degradation indices can serve as decision-points for forensic laboratories. In this study, we focused on evaluating the ForenSeq MainstAY Kit and the Investigator® 24plex QS Kit. Our evaluation involved enzymatically degraded samples and real-world challenging samples. Controlled degradation was performed using the Turbo DNA-free™ Kit to artificially degrade DNA extracts to different extents. Furthermore, thermally degraded femur samples, burned to different levels, were also examined.

The MainstAY kit demonstrated similar or improved percent recovery compared to CE for these samples. Even in cases with reduced percent recovery, the MainstAY Kit recovered more loci, providing more information than the CE method. Future studies will evaluate comparative recovery across a wider range of controlled degradation experiments and skeletal remains. This information will be used to develop a decision tree for laboratories to process degraded

MATERIALS & METHODS

Artificially Degraded Samples:

Buccal swabs from two male donors were collected and extracted using the EZ1 DNA Investigator Kit. Extracts from one donor were artificially degraded in triplicate using the Turbo DNA-free Kit (Invitrogen) with increasing amounts of DNase (0.025U, 0.035U, 0.05U, 0.1U, 0.2U). Extracts from the second donor were degraded in triplicate using the Turbo DNA-free Kit (0.2U DNase at seven time points (0, 5, 10, 15, 20, 25, and 30 min).

RESULTS & DISCUSSION

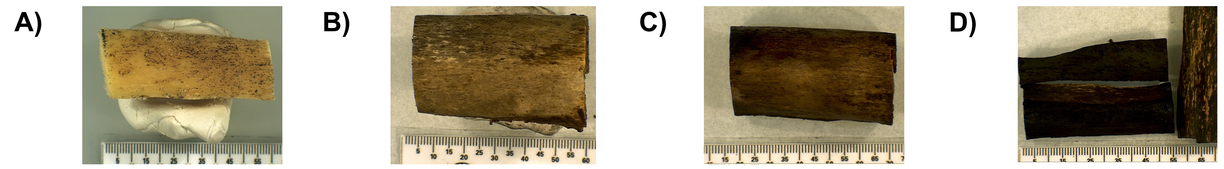

Figure 1. Representative Photos of Femur Cross-sections Thermally Degraded to Different Stages Based on Bone Color. One window cut was reserved as an **A)** Unburned control. The remaining cross-sections were thermally degraded to **B)** Burned, Light Brown; **C)** Burned, Brown; and **D)** Burned, Black color stages.

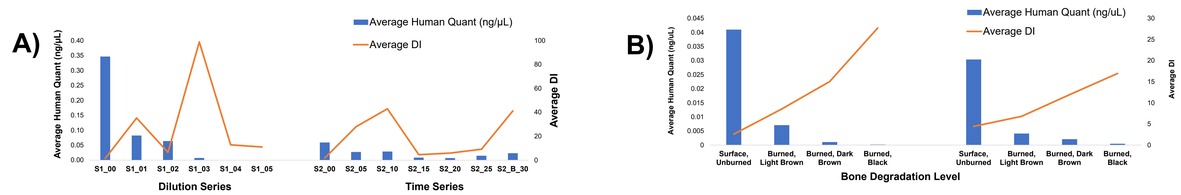

Figure 2. Comparison of the Average DNA Concentration to the Average Degradation Values of Both Artificially and Thermally Degraded Samples. Degradation values for artificially degraded samples **(A)** varied between the DNase Dilution series (*left*) and the DNase Time series (*right*). Degradation values for the thermally degraded samples **(B)** demonstrated a consistent pattern between the two burned femurs.

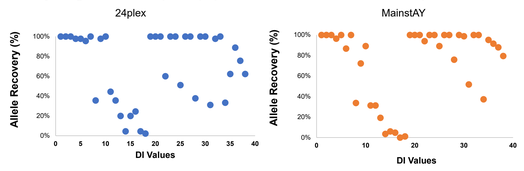

Figure 3. Comparison of Allele Recovery to DI values for Artificially Degraded DNA Samples. No pattern was observed between the DI and percentage of alleles recovered using either 24plex QS (*left*) or MainstAY (*right*).

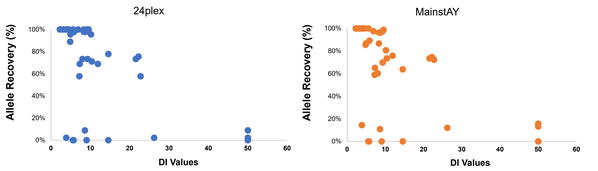

Figure 4. Comparison of Allele Recovery to DI values for Thermally Degraded DNA Samples. No pattern was observed between the DI and percentage of alleles recovered using either 24plex QS (*left*) or MainstAY (*right*).

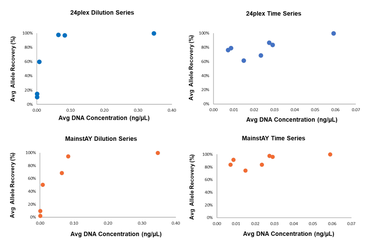

Figure 5. Comparison of Average Allele Recovery to Average DNA Concentration for Artificially Degraded Samples. A general trend of greater allele recovery with increased DNA concentration was observed for both degradation series. Furthermore, no substantial difference was seen between the patterns observed using 24plex QS (*top*) and MainstAY (*bottom*).

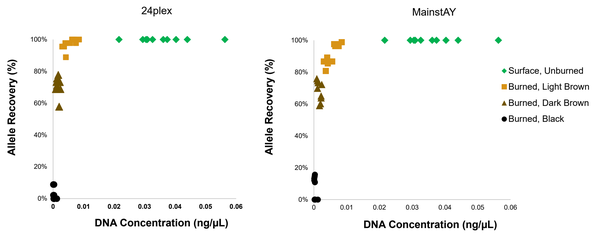

Figure 6. Comparison of Average Allele Recovery to Average DNA Concentration for Thermally Degraded Samples. A strong trend of greater allele recovery with increased DNA concentration was observed for all degraded bone samples. Furthermore, no substantial difference was seen between the patterns observed using 24plex QS (*left*) and MainstAY (*right*). As expected, more thermal degradation led to lower DNA recovery.

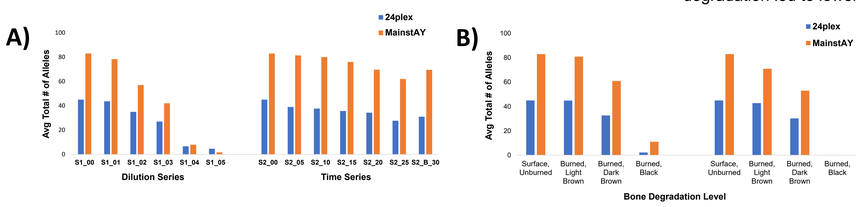

Figure 7. Comparison of Total Number of Alleles Recovered. Expected alleles recovered for 24plex and MainstAY are 47 and 83, respectively. The additional autosomal loci and Y markers in MainstAY allow for more genetic information to be recovered from both **A)** artificially degraded and **B)** thermally degraded samples.

- **Degradation (DI) values did not exhibit a predictable pattern among any of the artificially degraded samples (Fig. 2).**
- **Furthermore, DI values did not predict downstream allele recovery regardless of genotyping method (Figs. 3 and 4).**
- **A trend was observed between initial DNA concentration and downstream genotyping success (Figs. 5 and 6).**
- **Although the percentage of alleles recovered was similar between the two kits, MainstAY was able to recover a greater total number of alleles (Fig. 7).**

REFERENCES

1. Alaeddini R, Walsh SJ, Abbas A. Forensic implications of genetic analyses from degraded DNA--a review. Forensic Sci Int Genet. Netherlands: 2009 Elsevier Ireland Ltd; 2010;148-57.
2. Stephens KM, Barta R, Fleming K, Perez JC, Wu S-F, Snedecor J, et al. Developmental validation of the ForenSeq MainstAY kit, MiSeq FGx sequencing system and ForenSeq Universal Analysis Software. Forensic Science International: Genetics. 2022;61:102851.

MATERIALS & METHODS

Thermally Degraded Samples:

One femur from two donors willed to the Southeast Texas Applied Forensic Science Facility (STAFS) were selected. As unburned controls, one window cut was collected from each femur. The femurs were then cut along the diaphysis to generate cross-sections (4.16 cm – 6.0 cm) that were then placed on charcoal grills and burned to desired color (**Fig. 1**). After burning, cross-sections were washed, chipped, and powdered using a 6750 Freezer Mill (SPEX SamplePrep) with a 10-minute precool step followed by two crushing cycles (1 minute each). The powdered samples (5 replicates of 250 mg) were lysed and extracted using the EZ2 Connect Fx Extra Large-Volume Protocol. A 100 µL elution volume for each replicate was used.

DNA Quantification, Amplification, and Detection:

Extracts were quantified using Investigator Quantiplex® Pro (QIAGEN). Each extract was amplified with both the Investigator 24plex QS Kit (QIAGEN) and the ForenSeq MainstAY Kit (Verogen). 24plex QS samples were separated and detected on an ABI 3500 (Thermo Fisher Scientific). MainstAY samples were sequenced on a MiSeq FGx® (Verogen) using a MiSeq FGx Reagent Micro Kit (Verogen).

Data Analysis:

24plex QS samples were analyzed using Genemapper ID-X v1.6 (Thermo Fisher Scientific), while MainstAY samples were analyzed using UAS v2.5 (Verogen).

CONCLUSIONS

- Degradation values on their own do not accurately predict genotyping success of artificially and thermally degraded samples.
- The quantity of DNA correlates with allele recovery and needs to be evaluated in determining how to proceed with these types of challenging samples.
- MainstAY recovered a greater number of total alleles for the majority of degraded samples.
- MainstAY can lead to more probative results for challenging and degraded samples with a smaller input volume and the inclusion of Y markers.

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