Streamlining Skeletal Remains Processing in Forensic Laboratories: Developing a Decision Tree for Capillary Electrophoresis and Next Generation Sequencing Platforms

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INTRODUCTION

Samples exhibiting varying levels of DNA degradation are often submitted to crime laboratories for analysis. Genotyping degraded DNA has traditionally posed challenges due to the low amounts of intact DNA available. Increased degradation can result in the drop-out of larger amplicons (1), which is problematic for traditional size-based allele calling. In cases of skeletal remains, particularly where samples are highly degraded or aged, alternative approaches such as mitochondrial DNA (mtDNA) testing using specialized kits or genetic genealogy techniques like Kintelligence may be more suitable for analysis. These methods are often essential when identifying distant or maternal-line relatives to help establish the identity of unidentified human remains.

Next-generation sequencing (NGS) offers an alternative to overcome the size limitations of capillary electrophoresis (CE), particularly for severely degraded samples. Forensic laboratories must choose between CE, NGS, or genetic genealogy depending on the level of degradation and available relatives.

This study evaluates the performance of the ForenSeq MainstAY Kit, mtDNA Whole Genome Kit, Kintelligence, and the Investigator 24plex QS Kit in analyzing challenging skeletal remains. The goal is to develop a decision framework to streamline the choice between CE and NGS for processing degraded remains.

MATERIALS & METHODS

Thermal Degradation & DNA Extraction:

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**). Following thermal degradation, 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.

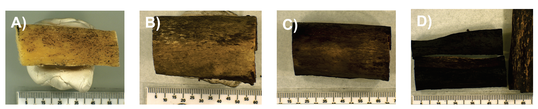

Figure 1. Representative Photos of Femur Cross-sections Thermally Degraded to Different Stages Based on Bone Color. One window cut was reserved as an **A)** Unburned control. The remaining cross-sections were thermally degraded to **B)** Light Brown; **C)** Brown; and **D)** Black.

RESULTS & DISCUSSION

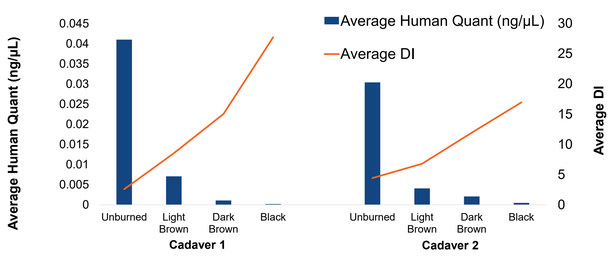

Figure 2. Average DNA Concentration to the Average Degradation Values of Thermally Degraded Samples. Both cadavers displayed the same pattern of increasing DI values and decreasing DNA concentration with more thermal degradation.

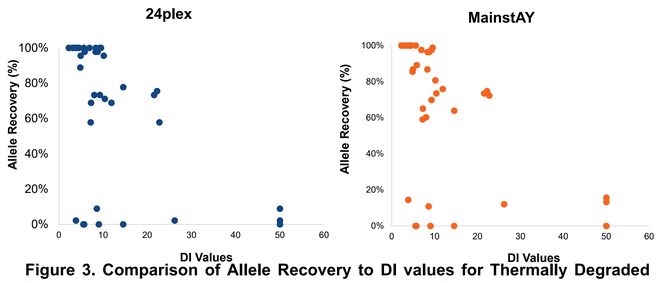

Figure 3. Comparison of Allele Recovery to DI values for Thermally Degraded DNA Samples. No pattern was observed between the DI and percentage of alleles recovered using either 24plex QS (*left*) or MainstAY (*right*).

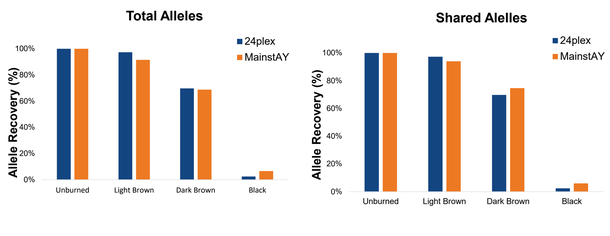

Figure 4. Comparison of Total Number of Alleles Recovered *(left)* and shared alleles *(right)* between the two chemistries. Expected alleles recovered for 24plex and MainstAY are 47 and 83, respectively. The recovery between the two methods was similar.

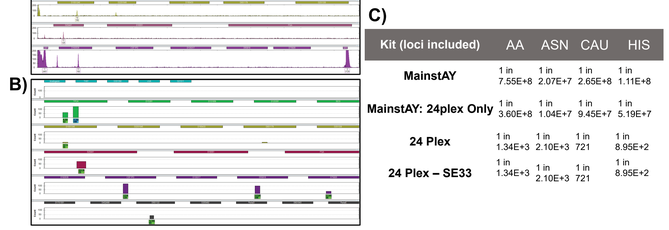

Figure 5. Electropherogram comparison of black bone samples using 24plex (A) and MainstAY (B). Although recovery was similar for both methods, due to isoalleles frequencies, **(C)** MainstAY yielded stronger likelihood ratios based on ground truth alleles.

Cadaver 1					
Sample	Insult	Human Quant (ng/µL)	DI	Reads	Haplogroup
Light Brown – R2	Burned, Light Brown	0.0085	9.52	528,419	T1a1
Light Brown – R3	Burned, Light Brown	0.0075	8.75	375,023	T1a1
Light Brown – R4	Burned, Light Brown	0.0068	6.9	361,969	T1a1
Light Brown – R5	Burned, Light Brown	0.0062	9.28	419,914	T1a1
Dark Brown – R1	Burned, Dark Brown	0.0009	11.89	471,284	T1a1
Dark Brown – R2	Burned, Dark Brown	0.0011	22.2	562,881	T1a1
Dark Brown – R3	Burned, Dark Brown	0.0013	10.38	443,203	T1a1
Dark Brown – R4	Burned, Dark Brown	0.0013	21.53	448,070	T1a1
Black – R1	Burned, Black	0.0001	Und.	433,401	T1a1
Black – R2	Burned, Black	0.0003	Und.	374,173	T1a1
Black – R3	Burned, Black	0.0002	3.85	385,297	T1a1
Black – R5	Burned, Black	0.0003	8.59	317,394	T1a1

Table 1. ForenSeq mtDNA whole genome results for cadaver 1. All samples produced concordant haplogroup calls, with most read counts approaching or exceeding the 400,000-read threshold.

Cadaver 2					
Sample	Insult	Human Quant (ng/µL)	DI	Reads	Haplogroup
Light Brown – R2	Burned, Light Brown	0.0056	8.3	348,100	T2b6b
Light Brown – R3	Burned, Light Brown	0.0042	4.84	267,780	T2b6b
Light Brown – R4	Burned, Light Brown	0.004	5.84	359,041	T2b6b
Light Brown – R5	Burned, Light Brown	0.0036	10.15	508,327	T2b6b
Dark Brown – R1	Burned, Dark Brown	0.0022	7.27	451,900	T2b6b
Dark Brown – R2	Burned, Dark Brown	0.0024	22.73	242,487	T2b6b
Dark Brown – R3	Burned, Dark Brown	0.002	7.99	421,906	T2b6b
Dark Brown – R4	Burned, Dark Brown	0.0022	14.55	446,700	T2b6b
Black – R2	Burned, Black	0.0003	14.53	1,908	-
Black – R3	Burned, Black	0.0002	Und.	1,285	-
Black – R4	Burned, Black	0.0012	5.53	2,343	-
Black – R5	Burned, Black	0.0004	5.71	1,735	-

Table 2. ForenSeq mtDNA whole genome results for cadaver 2. All light brown and dark brown samples produced concordant haplogroup calls. Black samples yielded substantially lower read counts compared to other samples.

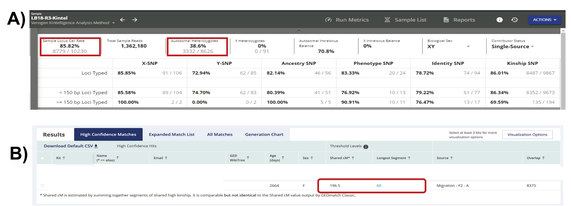

Figure 6. Summary of a light brown bone sample processed with Kintelligence. (**A**) SNP call rate and heterozygosity rate (31.6%–54.6%) indicated the sample was suitable for GEDmatch Pro upload. (**B**) GEDmatch Pro results were concordant with a fifth-degree relative identified from the reference sample search.

- DI values did not predict downstream allele recovery regardless of genotyping method (**Fig. 3**).
- Although the percentage of alleles recovered was similar between the two kits, MainstAY was able to recover a greater total number of alleles with greater statistical power (**Figs. 5 and 6**).
- Samples with similar nuclear quants did not yield comparable mtDNA results, and QIAxcel was more predictive of success (**Tables 1 and 2**).
- Preliminary results indicate that Kintelligence is a viable option for challenging skeletal remains (**Fig. 6**).

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.

MATERIALS & METHODS

DNA Quantification & QIAxcel:

Extracts were quantified using Investigator Quantiplex® Pro (QIAGEN). All extracts were screened using the QIAxcel DNA High Sensitivity Kit on the QIAxcel Connect System.

STR Typing:

Each extract was amplified with both the Investigator 24plex QS Kit (QIAGEN) and the ForenSeq MainstAY Kit (QIAGEN). 24plex QS samples were separated and detected on an ABI 3500 (Thermo Fisher Scientific).

mtDNA Whole Genome Kit:

A subset of DNA extracts (reference, light brown, dark brown, and black bones) from each cadaver was processed using the ForenSeq mtDNA Whole Genome Kit following manufacturer's recommendations. Libraries were manually normalized and pooled using Qubit.

ForenSeq Kintelligence Kit:

A subset of DNA extracts (reference, light brown, and dark brown bone) from each cadaver was processed using the ForenSeq Kintelligence Kit following manufacturer's recommendations.

Data Analysis:

24plex QS samples were analyzed using ArmedXpert™ (NicheVision Forensics, LLC), while MainstAY, mtDNA Whole Genome Kit, and Kintelligence samples were analyzed using UAS v2.5 (QIAGEN). RMPs were calculated using ArmedXpert™ for 24plex and MixtureAce™ for MainstAY based on ground truth data. GEDmatch PRO reports were exported from the UAS and uploaded as laboratory validation kits before being examined using the One-to-Many Kinship tool.

CONCLUSIONS

- Degradation values on their own do not accurately predict genotyping success of thermally degraded samples
- MainstAY can lead to more probative results for challenging and degraded samples with a smaller input volume with added statistical power of sequencing
- mtDNA and Kintelligence may be a viable option for highly degraded skeletal remains

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