Investigation of downstream processing methods for challenging skeletal samples

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INTRODUCTION

In many human identification (HID) cases only skeletal remains are recovered and may require complex DNA typing methods. The gold standard in DNA typing involves the analysis of STR markers; however, low-template, inhibited, and fragmented skeletal samples often result in partial profiles, limiting definitive identifications [1, 2]. Additionally, case-specific insults (burning, burying, embalming, decomposition, cremation) may further hinder DNA recovery; therefore, more advanced methods may be required to provide improved identifications and additional investigative leads. Implementation of novel investigative methods, such as forensic genetic genealogy (FGG) or ancestry and phenotype predictions can be accomplished using microarray SNP genotyping or Next Generation Sequencing (NGS). However, these methods are not typically implemented on challenging skeletal remains.

To examine the success of these novel methods, as well as determining the best downstream analytical process for these samples, several DNA extracts from skeletal remains were analyzed using traditional STR genotyping methods (Investigator® 24plex QS Kit [3]), microarray SNP genotyping (Infinium™ Global Screening Array Platform [4]), and NGS methods (ForenSeq® Signature Prep Kit, Primer Mix B [5]; ForenSeq® Kintelligence Kit [6]). Based on these findings, analysts can implement a more streamlined process by assessing the samples on a case-by-case basis dependent on the extracts DNA quantity and quality.

MATERIALS & METHODS

Sample Collection:

- Skeletal samples (N = 62) were collected from the Southeast Texas Applied Forensic Science (STAFS) Facility at Sam Houston State University.
  - Sample insults: buried, decomposed, embalmed, burned, and cremated remains

DNA Extraction and Quantification:

- Samples were extracted following two methods:
  - PrepFiler™ BTA protocol [7]
  - In house, optimized InnoXtract™ method for skeletal samples [8]
- Quantification: Quantifiler™ Trio

Downstream Processing Analyses:

- STR Genotyping – performed on 60 extracts (30 InnoXtract™ and 30 PrepFiler™ BTA) using Investigator® 24plex QS kit
- NGS Methods – performed on 60 extracts (30 InnoXtract™ and 30 PrepFiler™ BTA) using the ForenSeq® Signature Prep Kit, Primer Mix B on the MiSeq FGx® Platform
  - Reprocessed 10 challenging extracts using the Enhanced PCR Buffer
- SNP Genotyping – two methods performed by Signature Science, LLC
  - Infinium™ Global Screening Array Platform – performed on 48 extracts (24 InnoXtract™ and 24 PrepFiler™ BTA)
  - ForenSeq® Kintelligence Kit – performed on 9 extracts (InnoXtract™ and PrepFiler™ BTA)

RESULTS

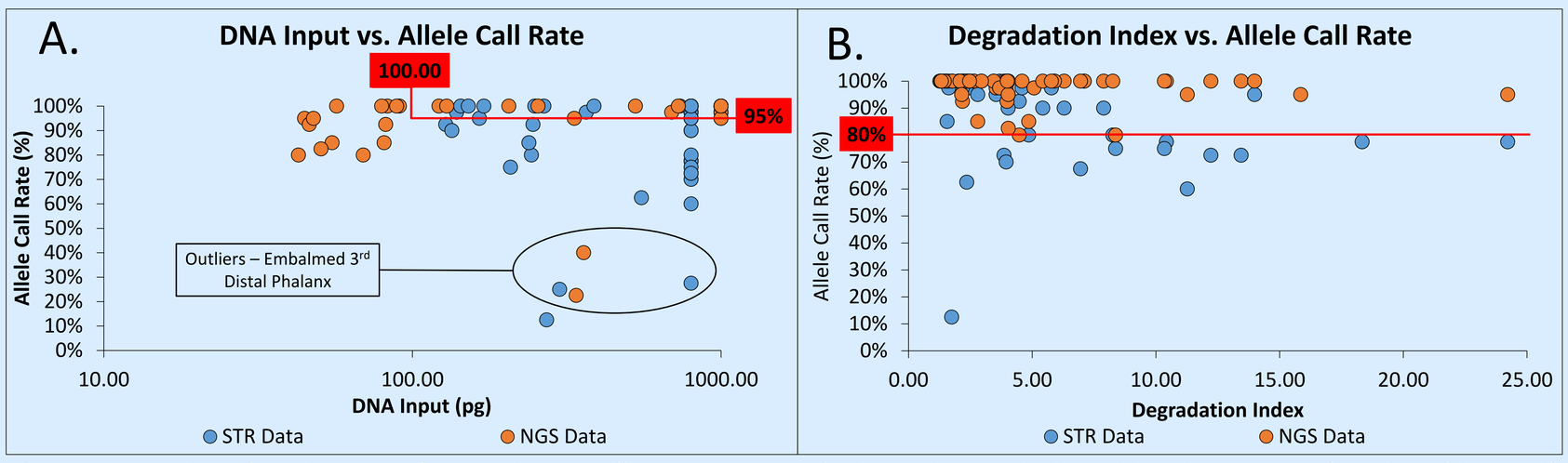

Figure 1: STR vs. NGS – A.) DNA input and **B.)** degradation index effect on allele call rate for the 20 CODIS core loci in traditional STR genotyping (Investigator® 24plex QS) and NGS (ForenSeq® Signature Prep, Primer Mix B) methods

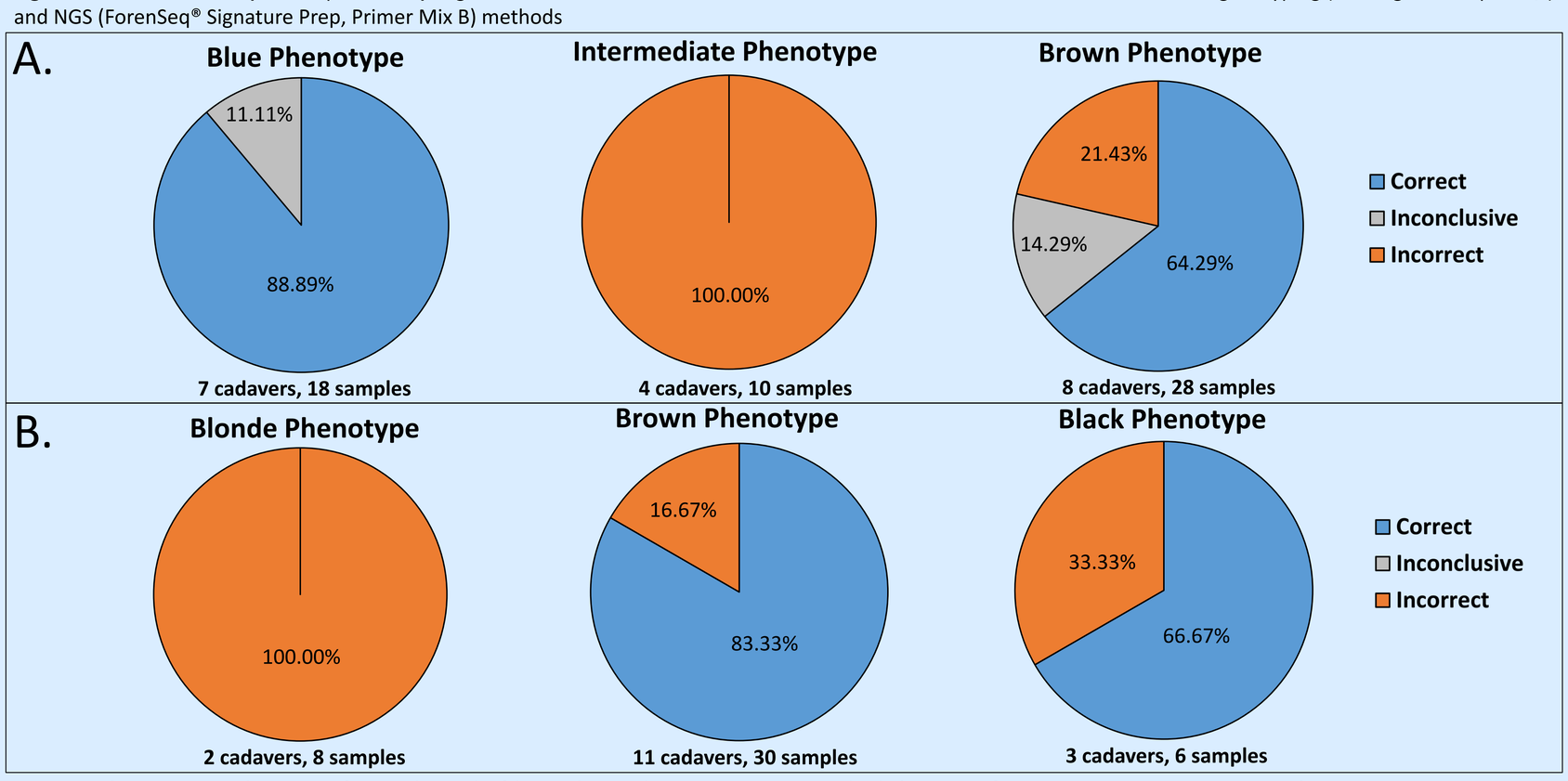

Figure 2: Phenotypic Prediction – A.) Eye color and **B.)** hair color predictions using NGS methods (ForenSeq® Signature Prep Kit; Primer Mix B)

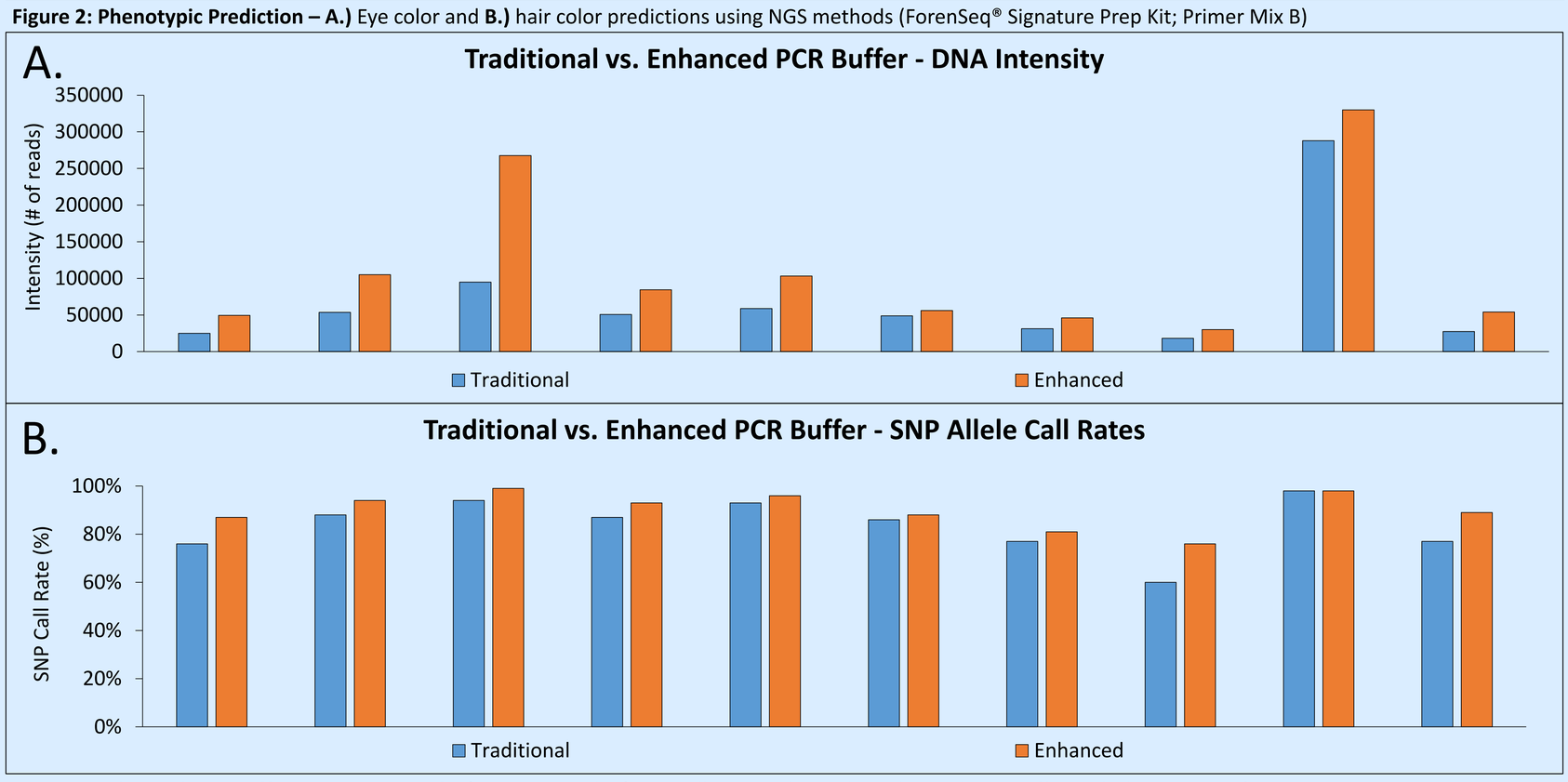

Figure 3: Enhanced PCR Buffer – comparison of **A.)** Intensity and **B.)** SNP call rates utilizing the Enhanced PCR Buffer with the ForenSeq® Signature Prep Kit, Primer Mix B on challenging skeletal remains

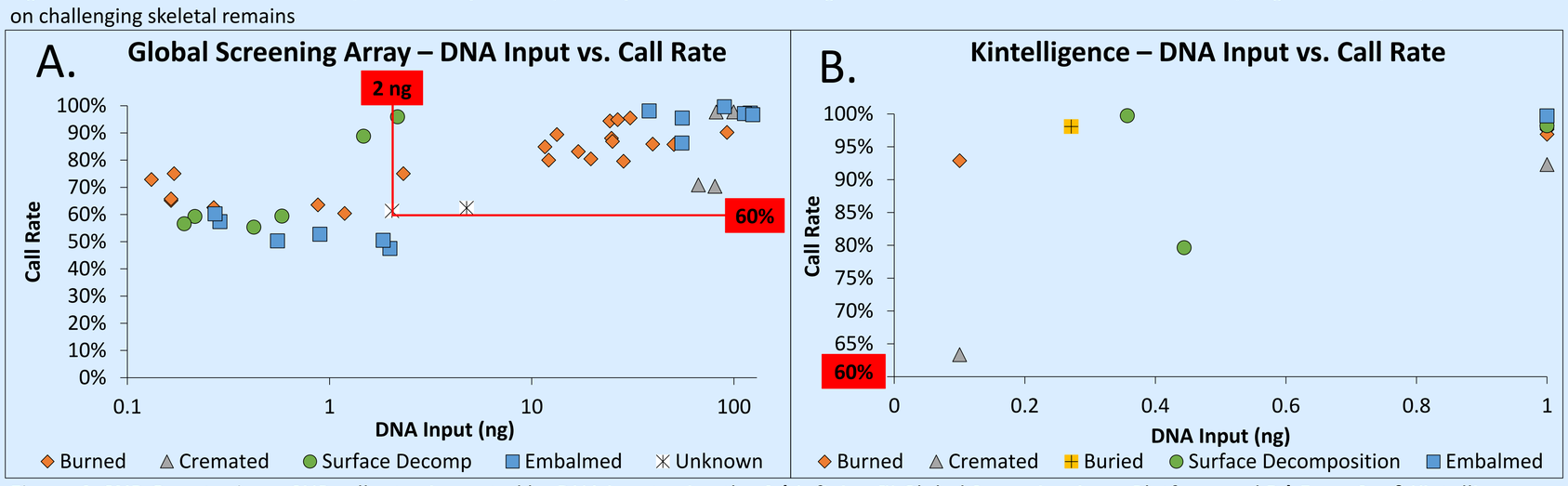

Figure 4: SNP Genotyping – SNP call rates impacted by DNA input using the **A.)** Infinium™ Global Screening Array Platform and **B.)** ForenSeq® Kintelligence Kit

DISCUSSION

Traditional STR Genotyping vs. NGS Methods:

- The NGS method (ForenSeq® Signature Prep Kit, Primer Mix B) provided a 95% allele call rate for the 20 CODIS core loci with at least 100 pg of sample input **(Figure 1A)**; meanwhile the traditional STR typing (Investigator® 24plex QS) did not follow this trend**.** This is likely due to the impact the degradation of these samples have on traditional STR genotyping versus NGS methods.
  - This can be confirmed by examining **Figure 1B**, where all NGS samples have at least an 80% allele call rate despite the degradation index unlike the STR genotyping samples that have lower call rates for more degraded samples.
- The ForenSeq® Signature Prep Kit, Primer Mix B, provides an additional tool for aiding investigations by predicting ancestry and phenotypic (hair and eye color) traits **(Figure 2A & 2B).** However, there is room for error in these predictions; therefore, traditional HID methods should still be implemented.
- Coupling the Enhanced PCR buffer with the ForenSeq® Signature Prep Kit provided a statistically significant increase (p – value < 0.05) in intensity (number of reads) and SNP call rates for the 10 samples processed **(Figure 3A & 3B).**

SNP Genotyping Methods:

- **Infinium™ Global Screening Array Platform (Figure 4A) –**
  - Provided adequate SNP call rates for potential FGG leads with as little as 200 pg of DNA input
  - 2 ng of DNA input provided at least a 60% SNP call rate
  - Degradation did not appear to effect SNP call rates (data not shown)
- **ForenSeq® Kintelligence Kit (Figure 4B) –**
  - Provided adequate SNP call rates for potential FGG leads with as little as 100 pg of DNA input
  - The recommended 1 ng of DNA input provided an average SNP call rate of ~97%

CONCLUSIONS

1. In addition to improved success in eligibility for searching and submitting profiles to NDIS, Next Generation Sequencing methods also provided ancestry and phenotypic predictions aiding in HID investigations.
   - Utilizing the Enhanced PCR Buffer can improve recovery of highly challenging skeletal samples with increased total reads and SNP call rates
2. SNP genotyping methods on challenging skeletal remains resulted in call rates that are potentially beneficial in developing forensic genetic genealogy leads.
3. Although traditional STR genotyping should be attempted on samples suspected to perform well; **highly degraded or low-template skeletal samples should be processed using a more novel investigative approach such as Next Generation Sequencing or SNP genotyping methods. These methods appeared unaffected by degradation and demonstrated success with as little as 100 pg of DNA input.**

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