Evaluation of five common extraction methods for analysis of human remain samples on massively parallel sequencing success

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INTRODUCTION

Often in missing persons cases bone, teeth, hair, and decomposed tissue are the only samples remaining for identification. Exposure to harsh environmental conditions may also cause DNA degradation, damage, and/or inhibition, making these samples challenging to process. Human skeletal remains are often inhibited by humic acid, melanin, hematin, collagen, and calcium. Inhibitors may be co-extracted with the DNA, can interfere with PCR, and may reduce downstream DNA typing success. Current DNA identification methods include capillary electrophoresis based short tandem repeats (STRs), which are currently the gold standard. Single nucleotide polymorphisms (SNPs) are single base changes in the genome that can also be used for human identification, bio-ancestry and phenotypic information.

Massively parallel sequencing (MPS) is a newer technology used in the forensic science field. It has the ability to expand our current technologies as more genetic information can be retrieved from each samples and simultaneous analyze different (and more) markers (Eg. iiSNPs, STRs, aiSNPs).

An effective DNA extraction method is critical to obtain clean DNA from difficult samples. However, little is known regarding the compatibility of common DNA extraction methods with MPS chemistries. The goal of this study was to evaluate the efficiency of various DNA extraction methods to remove PCR inhibitors from skeletal remains prior to MPS. Samples were extracted using various extraction methods commonly used in forensic laboratories.

MATERIALS AND METHODS

Sample Preparation Blood, hair, and bone were spiked with high amounts of inhibitor (Table 1).

Table 1. The final inhibitor concentration spiked on a substrate (25 µL volume for blood).

Sample	Inhibitor	Inhibitor Concentration
Blood	Hematin	27500 µM
Hair	Melanin	750 ng
Bone	Calcium	22500 µM
Bone	Humic Acid	3750 ng

DNA Extraction All samples (N=72) were extracted using previously a reported organic technique [1], two different total demineralization techniques [2&3], PrepFiler™ BTA (Applied Biosystems™) [4], DNA IQ™ (Promega) [5], and DNA Investigator (QIAGEN) [6].

STR Genotyping Samples were genotyped using the GlobalFiler® PCR Amplification Kit (Applied Biosystems™) on the 3500 Genetic Analyzer.

Ion S5™ Sequencing All sequencing reactions were performed with 1 ng DNA input using the Precision ID DL8 Kit and an early access degradation panel. Templating and chip loading were conducted using the Ion Chef™ System with Ion 530™ semiconductor chips. Sequencing was performed using the Ion S5™ System. Data analysis was conducted using Converge™ Software v2.0 and an in-house workbook.

MiSeq FGx™ Sequencing Each sample was amplified using the ForenSeq™ DNA Signature Prep kit (using Primer Mix A) according to manufacturers specifications [7]. Sequencing was performed using the Illumina system. Data analysis was conducted using STRaitRazor v2s [8].

RESULTS & DISCUSSION

CE STRs

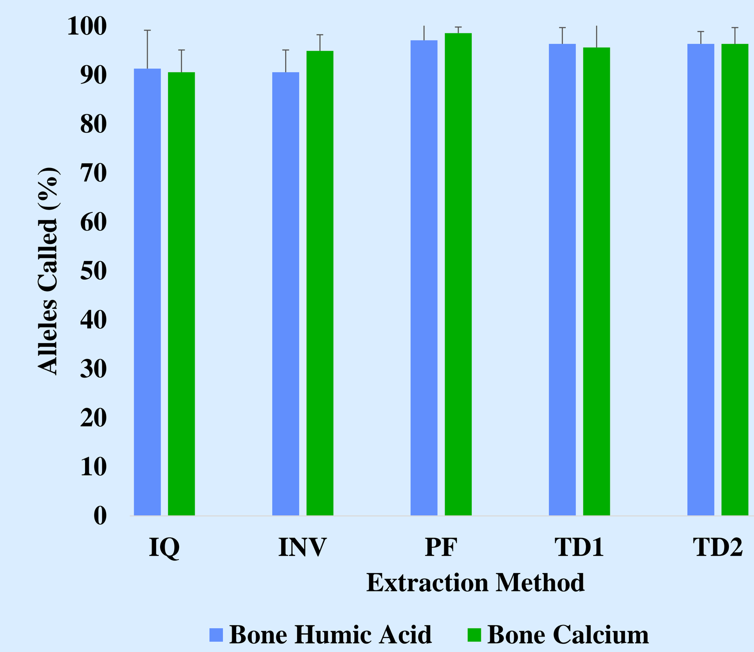

Figure 1. Percentage of alleles reported for bone samples spiked with humic acid (HA) and calcium (Cal) using commercial extraction kits (DNA IQ, DNA Investigator, and PrepFiler) and two total demineralization techniques. Data presented as average ± SD (N=3).

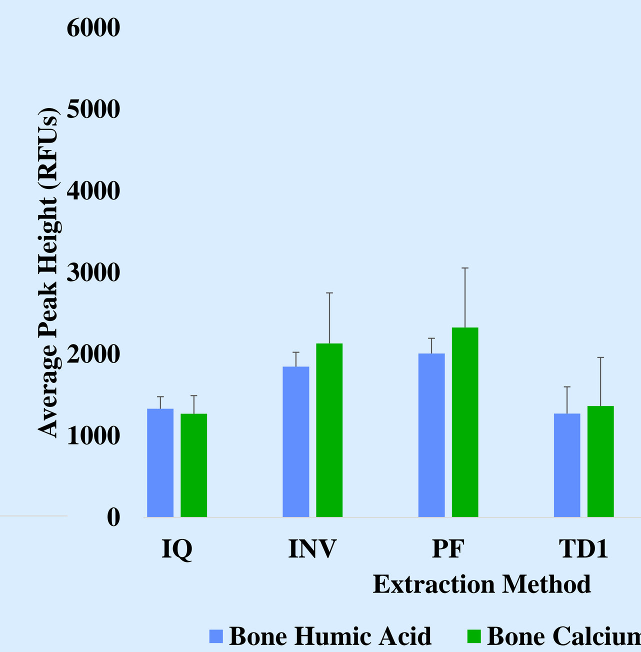

Figure 2. Average peak height (RFUs) for bone samples (spiked with humic acid and calcium) using commercial extraction kits (DNA IQ, DNA Investigator, and PrepFiler) and two total demineralization techniques. Data presented as average ± SD (N=3).

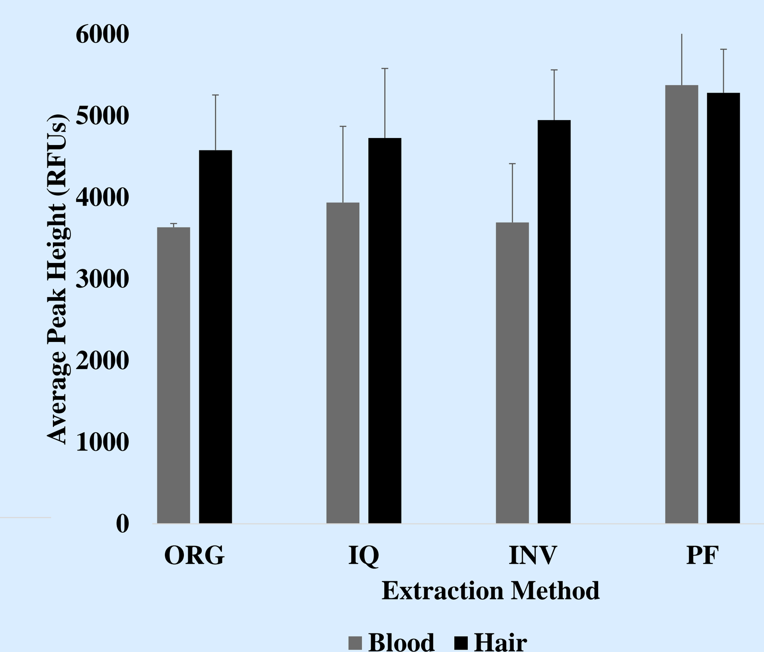

Figure 3. Average peak height (RFUs) for blood and hair samples (spiked with hematin and melanin, respectively) using commercial extraction kits (DNA IQ, DNA Investigator, and PrepFiler) and a general organic method. Data presented as average ± SD (N=3).

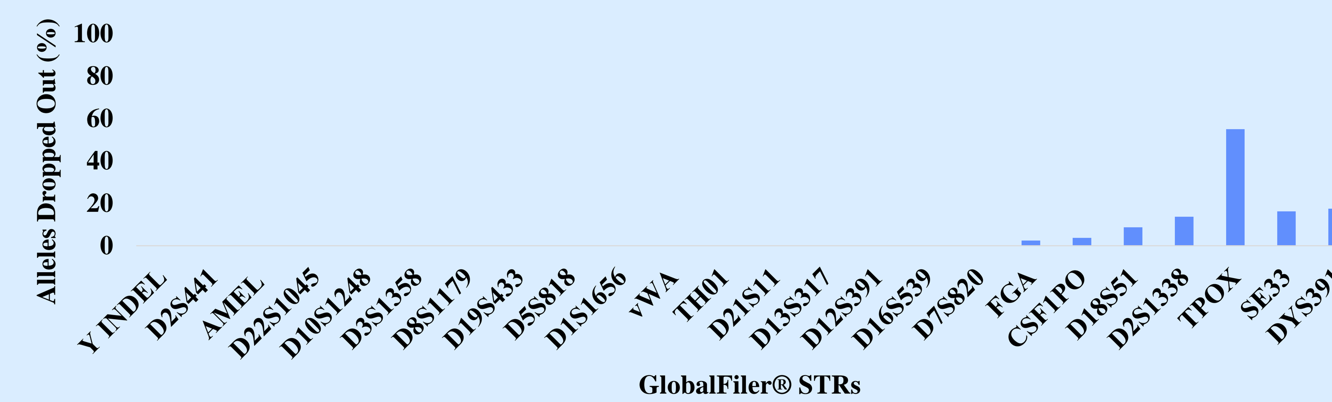

Figure 4. Percentage of alleles that dropped out at each locus using the GlobalFiler® PCR Amplification kit for all bone samples. Loci are in order of increasing length.

MPS STRs

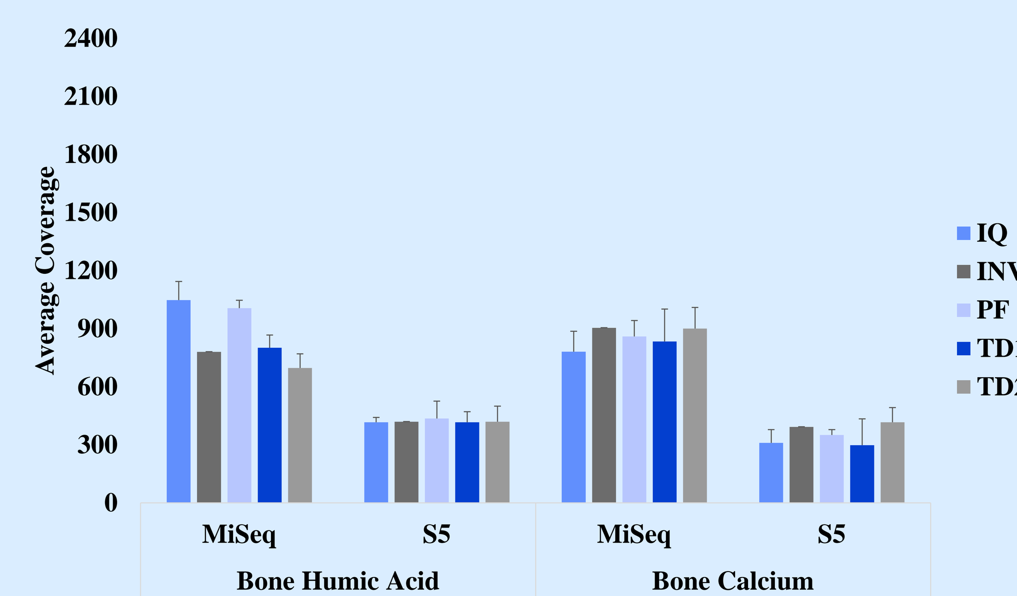

Figure 5. Average coverage of bone (spiked with humic acid and calcium) extracted with three commercial kits and two total demineralization techniques, while comparing two sequencing platforms (MiSeq vs. S5). Data presented as average ± SD (N=3).

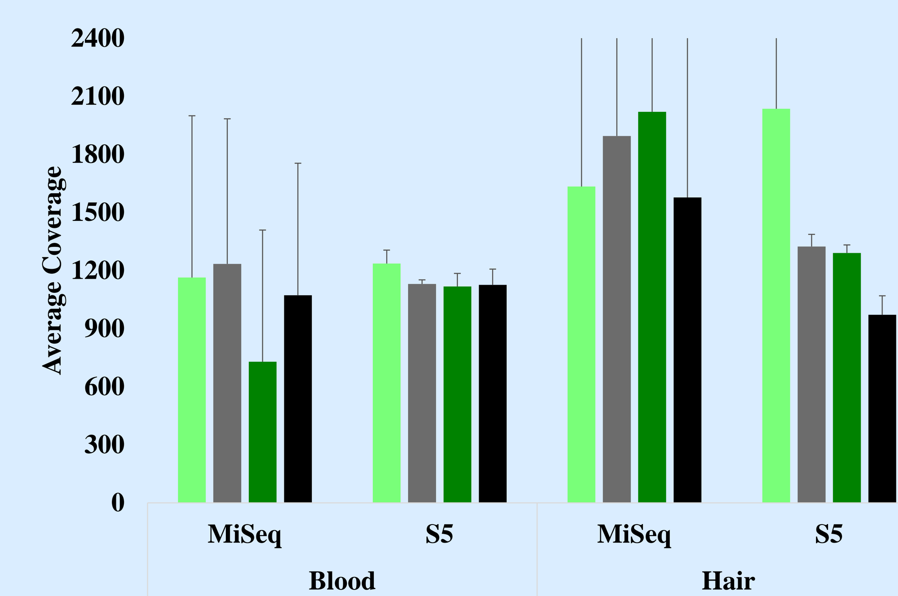

Figure 6. Average coverage of blood and hair (spiked with hematin and melanin, respectively) extracted with three commercial kits and a general organic method, while comparing two sequencing platforms (MiSeq vs. S5). Data presented as average ± SD (N=3).

CONCLUSIONS

- **CE-based STR Analysis**
  - Blood and hair samples resulted in complete profiles for the four extraction methods used (data not shown).
  - Bone samples spiked with humic acid and calcium resulted in 90-99% of alleles called for the five extraction methods used (**Fig. 1**).
  - Average peak height ratios ranged from 62-91% for all sample types and methods (data not shown)
  - Hair and blood demonstrated the highest APHRs (data not shown).
  - APHRs of bone samples were ~20% lower than blood and hair samples (data not shown).
  - Average peak heights (RFUs) ranged from ~1270-2330 RFUs for bone samples (**Fig. 2**).
  - APHs for blood and hair were ~1.5-4-fold higher than bone samples (**Fig. 3**).
  - All extraction kits/techniques performed well with the sample types used.
  - There was no statistical significance between the extraction methods for the APHR or APH (data not shown).
  - TPOX dropped out in 55% of bone samples, other loci affected by dropout included D21S1338, SE33, and DYS391 (**Fig. 4**).
- **MPS-based STR Analysis**
  - All STRs for both S5 and MiSeq platforms resulted in 100% of alleles called (data not shown).
  - The average heterozygote allele balance (AHAB) for both platforms averaged above 67%, with the S5 platform performing slightly more optimally (data not shown).
  - Allele balance increased by ~10% for blood and hair compared to bone (data not shown).
  - The MiSeq platform demonstrated higher average coverage for most samples except blood, in which the S5 platform performed similarly (**Figs. 5&6**).
- **General Conclusions**
  - No statistical significance was found between any of the extraction kits used. All extraction methods produced clean DNA extracts that were fully amenable to both MPS systems.
  - MPS generated more complete profiles for all samples than CE-based STR analysis.
  - Blood and hair samples produced full profiles, higher APHRs, and higher APHs than bone samples for CE-based STRs.
  - In general, the S5 produced slightly higher AHAB than the MiSeq platform.
  - In general, the MiSeq produced higher average coverage than the S5 platform.

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