Evaluation of five common DNA 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 remains may also contain inhibitory agents such as 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. MPS expands our current technologies as more genetic information can be retrieved from each sample and more markers (e.g. iiSNPs, STRs, aiSNPs) can be analyzed simultaneously.

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 and decomposed 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 each substrate.

Sample	Substrate Amount	Inhibitor	Inhibitor Amount[1]
Blood	15 µL	Hematin	27.5 mM
Hair	1 hair (with root)	Melanin	750 ng
Bone	50 mg	Calcium	22.5 mM
Bone	50 mg	Humic Acid	3750 ng

[1]Amount of inhibitor in the sample prior to DNA extraction.

DNA Extraction All samples (N=72) were extracted using a previously reported organic protocol [1], PrepFiler™ BTA (Applied Biosystems™) [2], DNA IQ™ (Promega) [3], and DNA Investigator (QIAGEN) [4]. Bone samples were also extracted using two different total demineralization protocols [5&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 consisting of 35 STRs, 41 iiSNPs, and 34 Y-SNPs. 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.

RESULTS

CE STRs

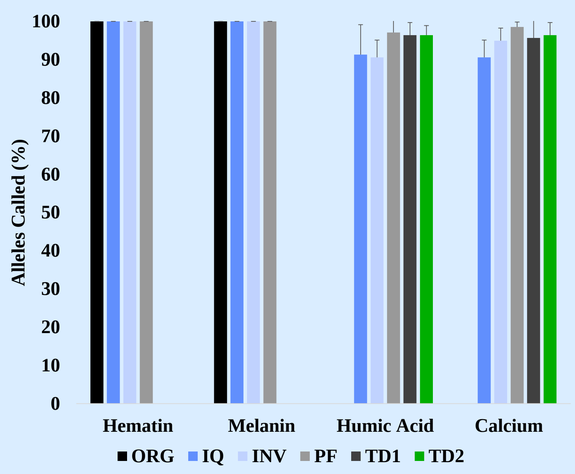

Figure 1. Percentage of alleles reported for all substrates spiked with their respective inhibitors and extracted using commercial extraction kits (DNA IQ, DNA Investigator, and PrepFiler), and either a general organic method or two total demineralization techniques. Data presented as average ± SD (N=3).

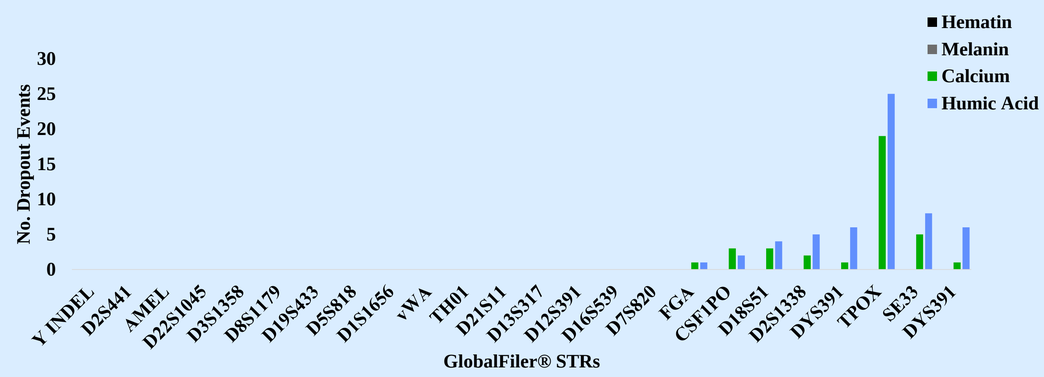

Figure 2. Percentage of alleles that dropped out at each locus using the GlobalFiler® PCR Amplification kit for all bone samples (N = 40). Loci are in order of increasing length.

MPS STRs/SNPs

STRs	Hematin	Melanin	Humic Acid	Calcium
IQ				
INV				
PF				
ORG				
TD1				
TD2				

SNPs	Hematin	Melanin	Humic Acid	Calcium
IQ				
INV				
PF				
ORG				
TD1				
TD2				

	100%
	98-99%
	96-97%
	Not Tested

Figure 3. Percentage of reportable alleles for each extraction method and each substrate.

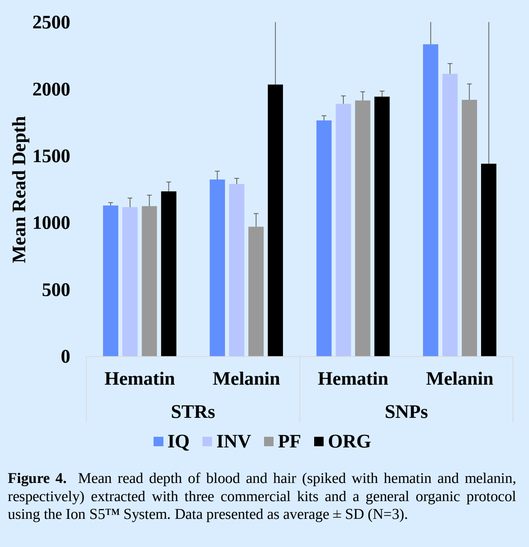

Figure 4. Mean read depth of blood and hair (spiked with hematin and melanin, respectively) extracted with three commercial kits and a general organic protocol using the Ion S5™ System. Data presented as average ± SD (N=3).

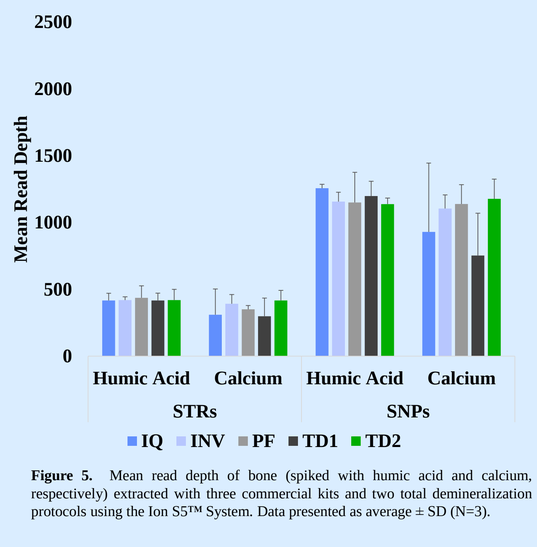

Figure 5. Mean read depth of bone (spiked with humic acid and calcium, respectively) extracted with three commercial kits and two total demineralization protocols using the Ion S5™ System. Data presented as average ± SD (N=3).

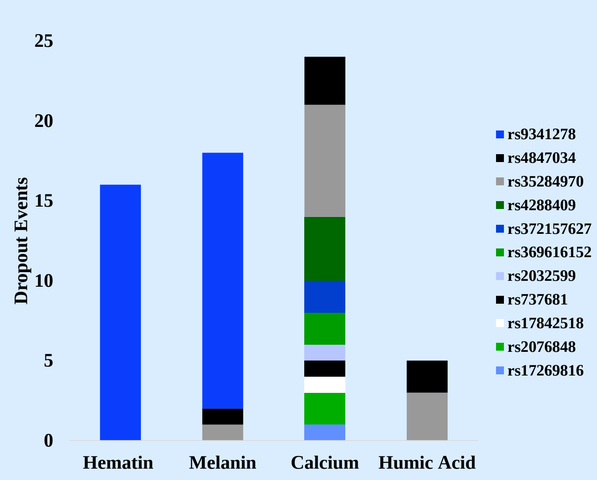

Figure 6. SNP dropout events in samples with each of the four inhibitors using the Ion S5™ System.

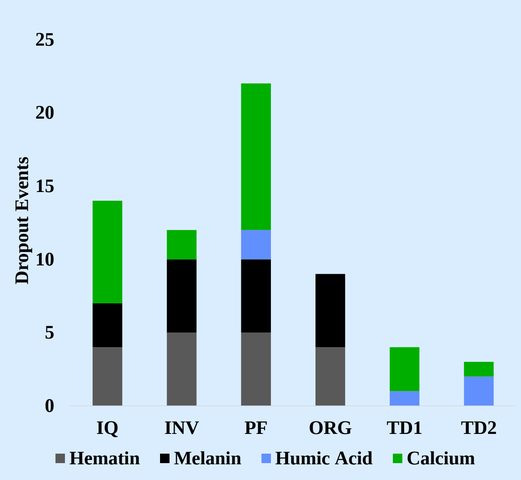

Figure 7. SNP dropout events for each of the six extraction methods using the Ion S5™ System.

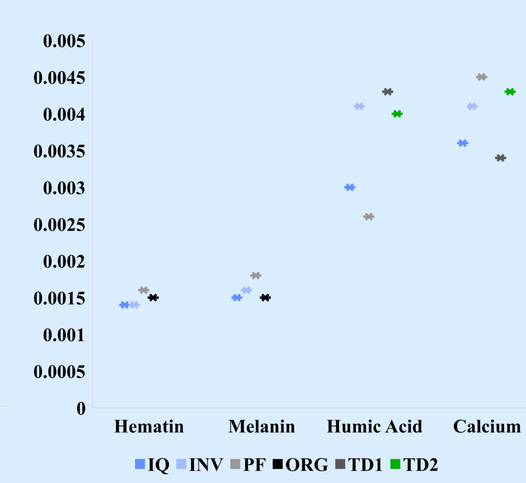

Figure 8. SNP noise within each of the four inhibitors using the Ion S5™ System. Noise was defined as PCR/sequence error and calculated by dividing the number miscalls by the number of total calls.

CONCLUSIONS

- **CE-based STR Analysis**
  - All extraction kits/protocols performed well with the sample types used.
  - Blood and hair samples spiked with hematin and melanin resulted in complete profiles for the four extraction methods used (**Fig. 1**).
  - Bone samples spiked with humic acid and calcium resulted in 90-99% of alleles called for the five extraction methods used (**Fig. 1**). There was no statistical difference between the extraction methods for the number of reportable alleles.
  - Average peak height ratios ranged from 62-91% for all sample types and methods (data not shown).
  - Average peak heights (RFUs) ranged from ~1270-2330 RFUs for bone samples. However, samples extracted with the DNA IQ kit displayed significantly lower APHs than the DNA Investigator and PrepFiler kits ($p < 0.05$) (data not shown).
  - TPOX was the locus most prone to dropout regardless of the extraction method used. TPOX failed to amplify in 55% of the bone samples; additional loci affected by dropout included other longer amplicons such as D21S1338, SE33, and DYS391 (**Fig. 2**).
- **MPS-based STR/SNP Analysis**
  - No statistical significance was observed between extraction methods for mean read depth, heterozygote balance, or the number of reportable alleles.
  - All sequence-based STRs and SNPs resulted in near complete profiles **(Fig. 3).**
  - Heterozygote allele balance averaged above 67% for all samples (data not shown).
  - Allele balance increased by ~10% for blood (hematin) and hair (melanin) compared to bone (data not shown).
  - For all samples, SNPs averaged higher mean read depth than STRs (**Figs. 4&5**).
  - Blood (hematin) and hair (melanin) samples produced higher mean read depth for STRs and SNPs than bone samples (**Figs. 4&5**).
  - Calcium (bone) demonstrated the highest occurrence of SNP dropout events of all inhibitors, both in the total number of events and the number of SNPs affected (**Fig. 6**). The majority of SNP dropout occurred with the PrepFiler extraction method, but dropout was observed across all commercial extraction kits (**Fig. 7**).
  - SNP noise was slightly higher in bone samples than blood and hair samples. In general, all noise was extremely low (**Fig. 8**).
- **General Conclusions**
  - Samples extracted with the DNA Investigator and PrepFiler kits demonstrated significantly higher peak heights than DNA IQ ($p < 0.05$) for CE-based STRs.
  - Blood and hair samples produced full CE-based STR profiles with higher APHs and APHRs than bone samples.
  - All samples generated more complete STR profiles with MPS than CE-based STR analysis.
  - No significant difference was found between any of the extraction methods used for sequence-based STRs and SNPs. All extraction methods produced clean DNA extracts that were fully amenable with the Precision ID chemistry and Ion S5™ System.
  - SNP dropout occurred within each inhibitor, but calcium (in bone) produced the majority of SNP dropout events.

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