Assessment of five common DNA extraction methods for the analysis of human remains using the Ion S5™ and MiSeq FGx™ Systems

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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.

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

CE STRs

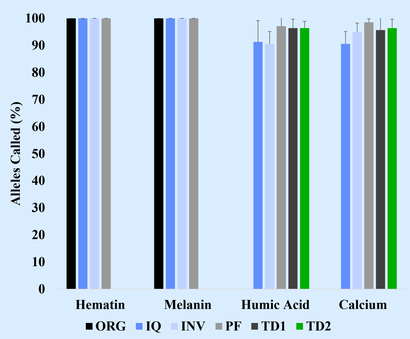

Figure 1. Percentage of alleles reported for all substrates spiked with their respective inhibitors and extracted using commercial extraction kits (DNA IQ, DNA Investigator, and PrepFiler), and either a general organic method or two total demineralization techniques. Data presented as average ± SD (N=3).

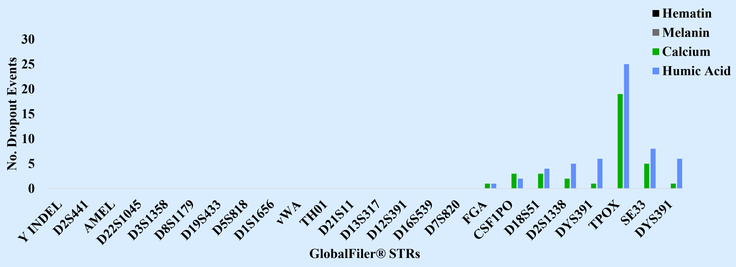

Figure 2. Number of alleles that dropped out at each locus using the GlobalFiler® PCR Amplification kit for all sample types (N = 72). Loci are in order of increasing length.

MPS STRs/SNPs

Table 2. Reportable alleles (%) for STRs averaging three replicates per extraction method

	EXTRACTION KIT	S5	MiSeq
Hematin	DNA IQ	100	100
	DNA INV	100	98.28
	PREPFILER	100	100
	ORGANIC	100	100
Melanin	DNA IQ	100	100
	DNA INV	100	100
	PREPFILER	100	100
	ORGANIC	100	100
Caclium	DNA IQ	98.51	100
	INVESTIGATOR	100	100
	DNA INV	99	100
	TD1	100	100
	TD2	100	100
Humic Acid	DNA IQ	100	100
	DNA INV	100	100
	PREPFILER	100	100
	TD1	100	87.27
	TD2	100	100

Table 3. Reportable alleles (%) for SNPs averaging three replicates per extraction method

	EXTRACTION KIT	S5	MiSeq
Hematin	DNA IQ	100	100
	DNA INV	100	99.65
	PREPFILER	100	100
	ORGANIC	100	100
Melanin	DNA IQ	100	100
	DNA INV	100	100
	PREPFILER	100	100
	ORGANIC	100	100
Caclium	DNA IQ	99.67	100
	DNA INV	100	100
	PREPFILER	100	100
	TD1	100	100
	TD2	100	100
Humic Acid	DNA IQ	100	100
	DNA INV	100	100
	PREPFILER	100	100
	TD1	100	100
	TD2	100	100

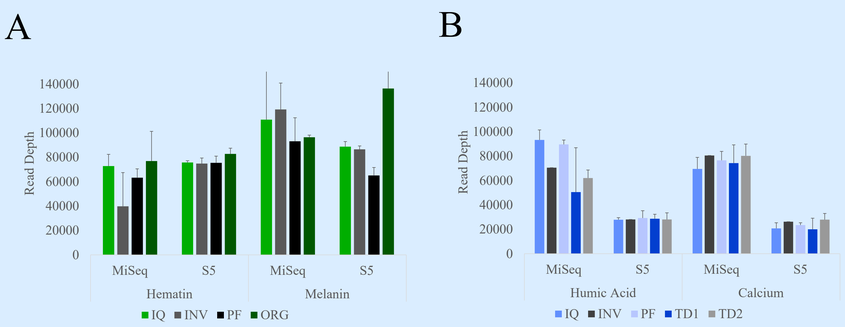

Figure 3. STR read depth of **A.)** blood (spiked with hematin) and hair (spiked with melanin) extracted with three commercial kits and an organic method and **B.)** bone (spiked with humic acid and calcium) extracted with three commercial kits and two total demineralization methods, while comparing two sequencing platforms (MiSeq vs. S5). Data presented as average ± SD (N = 3)

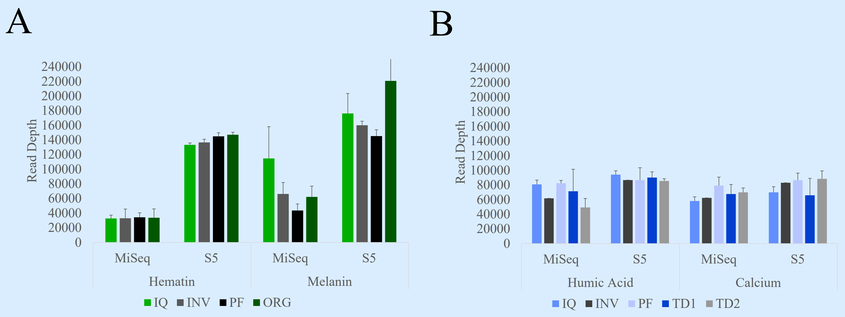

Figure 4. SNP read depth of **A.)** blood (spiked with hematin) and hair (spiked with melanin) extracted with three commercial kits and an organic method and B.) bone (spiked with humic acid and calcium) extracted with three commercial kits and two total demineralization methods, while comparing two sequencing platforms (MiSeq vs. S5). Data presented as average ± SD (N = 3)

CONCLUSIONS

CE-based STR Analysis

- All extraction kits/protocols performed well with the sample types tested.
- 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 Analysis

- There was no notable difference between extraction methods for sequence-based STRs and SNPs.
- All STRs and SNPs for both S5 and MiSeq platforms resulted in near complete profiles (**Tables 2&3**).
- The average heterozygote balance for both platforms averaged above 67% (data not shown).
- Heterozygote balance increased by ~10% for blood (hematin) and hair (melanin) compared to bone (data not shown).
- In general, SNPs averaged higher read depth than STRs (**Figs. 3&4**).
- Blood (hematin) and hair (melanin) samples produced higher read depth for STRs and SNPs than bone samples (**Figs. 3&4**).

General Conclusions

- 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 notable 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.
- Very little STR and SNP dropout occurred with either sequencing platform.

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