Comparative Tolerance of Short Tandem Repeat and Massively Parallel Sequencing Chemistries to Inhibited Samples

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INTRODUCTION

Mass disasters can be categorized as environmental, natural disasters, medical, vehicle, industrial, and terrorist attacks (1-3). Missing persons cases can also be referred to as mass disasters taking place over a longer period of time (4).

Mass disasters, missing persons, and forensic cases may present with fragmented and highly decomposed or skeletonized human remains, comingled, contaminated with environmental elements, and/or intense heat damage (1). DNA in these samples may be highly degraded, damaged, and/or inhibited because remains may be exposed to adverse conditions, which can also increase the rate of decomposition (3).

Highly degraded, damaged or environmentally effected samples can be problematic and may reduce the success of downstream DNA typing for identification purposes. Inhibitors are chemical or biological matrix interferences that can affect DNA extraction and/or PCR amplification processes during DNA analysis (5). Common co-extracted inhibitors include humic acid, hematin, collagen, calcium, melanin, indigo, bile salt, and urea and have different mechanisms by which they inhibit DNA amplification (5-9). This study will focus on PCR inhibitors most commonly encountered in skeletal and decomposed remains.

Human Identification (HID) is traditionally performed using capillary electrophoresis-based STR typing and mitochondrial DNA analyses. Massively parallel sequencing (MPS) has offered an alternative method to Sanger sequencing for mitochondrial genome sequencing, STR and SNP typing for HID purposes (10). With MPS, DNA molecules are sequenced in parallel, which increases throughput and can also provide more genetic information for each sample than conventional STR typing including, but not limited to, sequence variations (or SNPs) within STRs, ancestral, and phenotypic information (11-15).

The Ion Personal Genome Machine (PGM) is a MPS platform that can be used in combination with commercial library preparation kits for SNP-based HID panels, ancestry panels and whole genome mitochondrial analysis for forensic and investigative purposes. The aim of this study was to determine the comparative tolerance to inhibitors for the chemistries used for the SNP-based HID panel via MPS and conventional STR analysis.

MATERIALS AND METHODS

Sample Preparation DNA samples (N=3) included control DNA 2372 Component A Male (NIST, Gaithersburg, MD) and DNA sourced from buccal swabs provided by two anonymous donors. Samples were collected in accordance with the Sam Houston State University Institutional Review Board (#2015-12-26123).

Inhibitor Preparation A range of inhibitor concentrations was used to test the tolerance of STR typing and MPS-based methods for HID purposes. Final concentrations for each inhibitor were prepared based on previously published studies in literature (Table 1). All subsequent working solutions were made with deionized water. Inhibitors were added to the PCR amplification to achieve the desired final inhibitor concentration in each 25 µL PCR reaction. The concentrations chosen for PCR amplification are shown in Table 1.

STR Amplification Amplification was performed using the GlobalFiler® PCR Amplification Kit (ThermoFisher Scientific) in a 25 µL reaction volume as per manufacturer instructions.

Capillary Electrophoresis PCR products were separated and detected via capillary electrophoresis using the 3500™ Genetic Analyzer (ThermoFisher Scientific). STRs were analyzed using GeneMapper ID-X v. 4.1 (ThermoFisher Scientific).

RESULTS AND DISCUSSION

Table 1. The various concentrations of the five PCR inhibitors tested in this study.

Inhibitor	Units	Inhibitor Concentrations					
		0	1	2	3	4	5
Humic Acid	ng/µL	0	50	100	200	225	250
Melanin	ng/µL	0	25	35	40	45	50
Hematin	µM	0	300	500	1000	1050	1100
Collagen	ng/µL	0	50	100	112.5	130	160
Calcium	µM	0	250	350	500	650	850

STR Inhibitor Tolerance

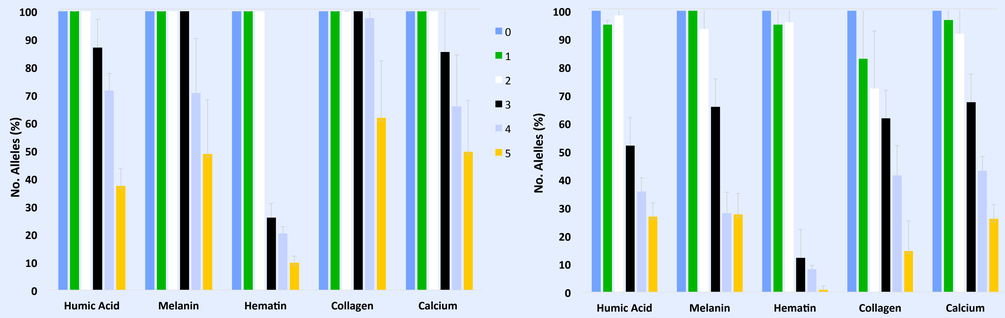

Figure 1: Percentage of STR alleles reported with 1 ng (left) and 0.1 ng (right) of DNA input, at 5 different concentrations with 5 inhibitors (Table 1). Concentration 0 = no inhibitor. Data presented as average ± SD. (N = 3)

- STR success decreased as the concentration of each of the 5 inhibitors increased at both 1 and 0.1 ng of DNA input (Fig. 1).
- Peak heights decreased as the concentration of inhibitor increased for both 1 and 0.1 ng of DNA input. Heterozygote peak height ratios did not appear to notably decrease as locus size increased (data not shown).
- STR analysis (GlobalFiler® kit) was most vulnerable to samples inhibited with high levels of humic acid and hematin.

MPS Inhibitor Tolerance

Table 2. Percentage of STRs vs SNP alleles reported for five PCR inhibitors at five concentrations.

Humic Acid	ng/µL	5	7	10	25	50
	STRs	100.0%	100.0%	100.0%	100.0%	100.0%
	SNPs	100.0%	71.8%	44.4%	3.2%	0.8%
Melanin	ng/µL	3	4	5	10	12
	STRs	100.0%	100.0%	100.0%	100.0%	100.0%
	SNPs	100.0%	100.0%	88.7%	33.9%	4.0%
Hematin	µM	1	3	5	7	10
	STRs	100.0%	100.0%	100.0%	100.0%	100.0%
	SNPs	100.0%	54.8%	3.2%	0.0%	0.0%
Collagen	ng/µL	50	100	113	130	160
	STRs	100.0%	100.0%	100.0%	97.8%	48.8%
	SNPs	100.0%	100.0%	100.0%	100.0%	100.0%
Calcium	µM	250	350	500	650	850
	STRs	100.0%	100.0%	87.2%	47.4%	36.6%
	SNPs	100.0%	100.0%	100.0%	87.9%	73.4%

100%
70 - 99 %
40 - 69 %
< 39 %

- In general, the HID-Ion AmpliSeq™ Library Kit and Identity Panel chemistry was less tolerant to the PCR inhibitors most relevant to skeletal samples than STR analysis using the GlobalFiler® STR Amplification kit.
- HID-Ion AmpliSeq™ Library and Identity Panel chemistries were the most susceptible to humic acid, melanin, and hematin (Table 2 and Fig. 2 B).
- SNP typing with the HID-Ion AmpliSeq™ Library Kit and Identity Panel chemistry via MPS was more tolerant than STR analysis with collagen and calcium (Table 2).
- Overall STR analysis produced more balanced amplification than the SNP markers as measured by heterozygote peak height ratios (Fig. 2 A &B).
- Samples inhibited with hematin, humic acid, and melanin resulted in the least balanced SNP typing results. However the HID-Ion AmpliSeq™ Identity Panel continued to produce balanced amplification for collagen and calcium samples as the level of inhibition increased (Fig. 2 B).

Heterozygote Allele Balance

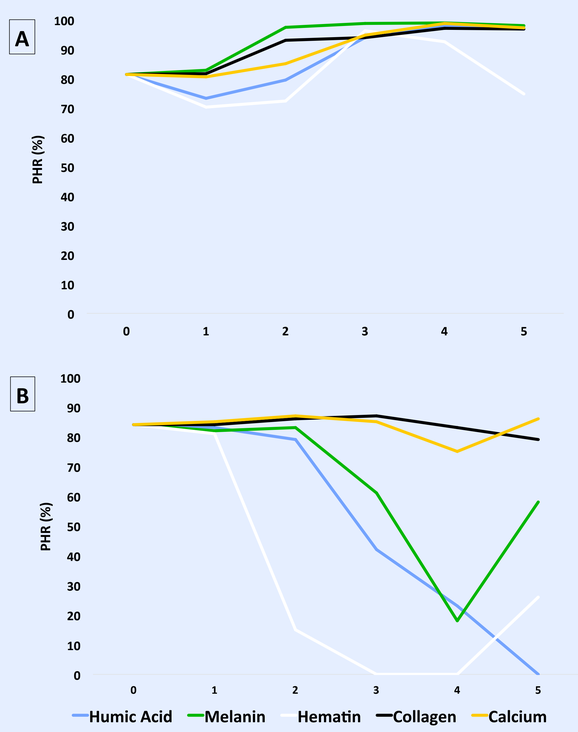

Figure 2: Heterozygote allele balance, with 1 ng DNA input for five inhibitors at five different concentrations using the A) STRs (GlobalFiler®), and B) SNPs (AmpliSeq™ Identity Panel) . Concentration 0 = no inhibitor.

MATERIALS AND METHODS

Massively Parallel Sequencing For this preliminary study, MPS was performed with a DNA template amount of 1 ng. Samples were prepared for amplification using the HID-Ion AmpliSeq™ Library Kit and Identity Panel (ThermoFisher Scientific). After amplification, primer sequences were partially digested. Following the partial digestion of the primer sequences barcode adapters were ligated to the amplicons (a different barcode for every sample).

After the ligation of the barcode adapters, Agencourt® AMPure® XP Reagent was added to each library and placed on a magnetic rack. Ethanol (70% solution) was added to the libraries to purify the DNA adhered to the beads. After the ethanol was removed from the libraries, Low TE was added to elute the DNA from the beads.

Following library purification, the samples were quantified using the Ion Library TaqMan® Quantitation Assay (ThermoFisher Scientific) using the 7500 Real-Time PCR System (ThermoFisher Scientific).

After quantification, libraries were diluted to 20 pM and pooled together. The pooled library was added to the Ion Chef™ System (ThermoFisher Scientific) and loaded onto 316 barcoded semiconductor chips. Once the Ion Chef™ run was complete, the chips were placed on the Ion PGM™ to be sequenced.

Data analysis including ISP loading, percent enrichment, percent library, and total usable reads was completed using Torrent Suite Software v4.6. SNP calls, coverage, and number of reads were analyzed using HID_SNP_Genotyper v4.3 plugin.

CONCLUSIONS

- STR chemistry (GlobalFiler®) was more tolerant to inhibitors with 1 ng DNA than 0.1 ng.
- GlobalFiler® was more tolerant to the PCR inhibitors tested in this study compared to the Ion PGM sequencing chemistry for the HID-Ion AmpliSeq™ Library Kit and Identity Panel.
- With MPS the inhibitors most vulnerable were hematin, humic acid, and melanin, but was more tolerant to samples spiked with collagen and calcium than STR analysis.
- Samples inhibited with collagen and calcium behaved similarly, while humic acid, melanin, and hematin showed similar patterns of inhibition and amplification success..

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