The Effectiveness of Various Strategies to Improve DNA Analysis of Formaldehyde-Damaged Tissues from Embalmed Cadavers for Human Identification Purposes

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ABSTRACT

Short tandem repeats (STRs) have been the gold standard markers for DNA HID for over 15 years. However, many samples treated with formalin fixatives are not always successfully genotyped using STR analysis. Other methods that pre-amplify the low amount of good quality DNA, repair the damaged DNA template, or use alternate genetic markers to amplify smaller target regions may generate more probative genetic information from these samples than standard STR typing.

This study examined whether pre-treatment with Whole Genome Amplification (WGA) or DNA repair prior to STR typing, or bi-allelic markers such as single nucleotide polymorphisms (SNPs) and Insertion/Deletions (INDELs) may provide the most probative information from formalin damaged (FD) samples for human identification (HID) purposes. Results demonstrate that the more productive approach for FD samples may be to utilize INDEL panels or SNP markers using Massive Parallel Sequencing (MPS) technologies.

INTRODUCTION

Formaldehyde causes significant damage to DNA in tissues by forming cross-links between DNA and proteins causing obstacles to the polymerase chain reaction (PCR)(1,2). Formaldehyde also causes degradation of DNA strands into progressively smaller fragments, often less than 200 base pairs (bp) in length, causing amplification of larger STR markers to fail (3, 4).

WGA has been proposed as a possible solution for the treatment of low amounts of template DNA in forensic samples (3). However, to date, no particular WGA method has been shown to consistently outperform the others when amplifying low amounts and/or degraded starting template, leading to more complete and balanced downstream STR profiles (3,5). Therefore, DNA repair may be the only viable option to overcome the significant DNA damage and degradation found in FD samples.

When WGA pre-amplification or DNA repair fails to yield robust STR results, the use of bi-allelic markers such as SNPs and INDELs may yield more genomic information (6). Greater success using these marker systems is primarily due to the smaller amplicons (<200bp), which are less susceptible to the effects of DNA degradation (6-8). However, these methods may not overcome the substantial chemical damage also found in FD samples.

RESULTS

The genotyping success of five embalmed tissue samples using the GlobalFiler® Amplification Kit, the INDEL multiplex, and the HID-Ion AmpliSeq™ Identity Panel was evaluated using the number of correct alleles detected (concordant to reference samples) and the resulting Random Match Probability (RMP) values calculated for each STR profile.

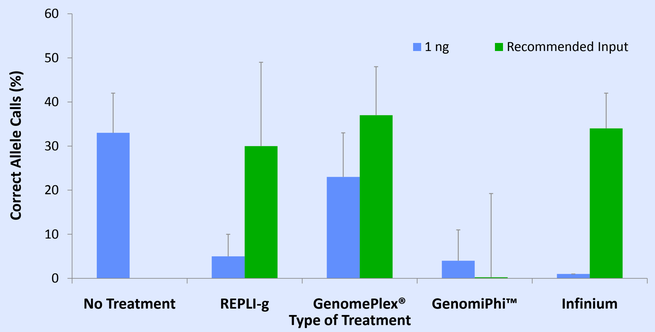

Figure 1. Average Genotyping Success of the five samples before (no treatment) and after treatment (WGA or repair) with inputs of 1ng and recommended DNA amounts (10ng for GenomiPhi™, 100ng for REPLI-g, GenomePlex®, and Infinium). Data presented as average ± SD.

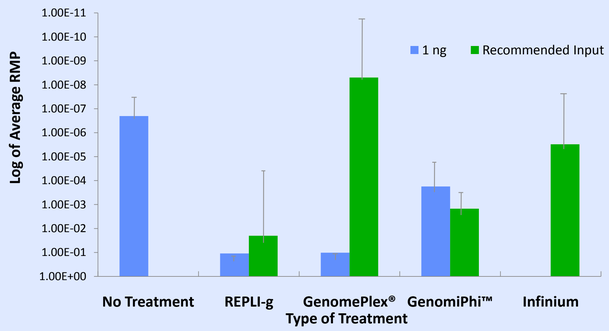

Figure 2. Average RMP values generated from the STR profiles of the five FD samples before (no treatment) and after treatment (WGA or repair) with inputs of 1ng and recommended DNA amounts (10ng for GenomiPhi™, 100ng for REPLI-g , GenomePlex®, and Infinium). Data presented as average ± SD.

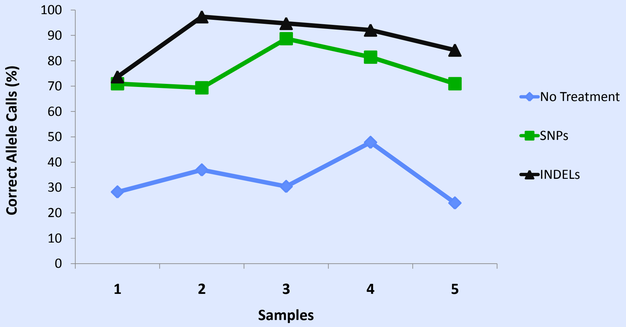

Figure 3. Genotyping Success for the five FD samples used in this study when non-treated samples were amplified using the GlobalFiler® STR Kit, the INDEL multiplex, and the HID-Ion AmpliSeq™ Identity Panel.

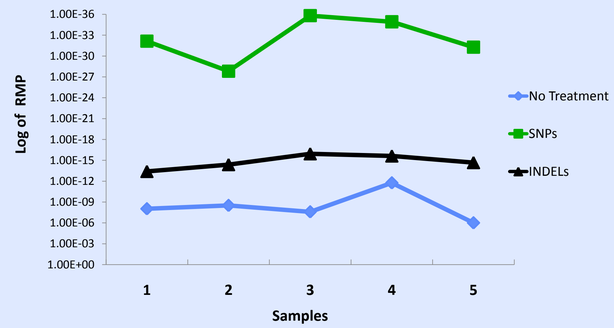

Figure 4. RMP values generated for the five FD samples used in this study when non-treated samples were amplified using the GlobalFiler® STR Kit (no treatment), the INDEL multiplex and the HID-Ion AmpliSeq™ Identity Panel.

DISCUSSION & CONCLUSIONS

- The results of this study suggest that rather than attempting to improve the quantity and quality of severely damaged and degraded DNA template in FD samples prior to STR typing, a more probative approach for HID purposes may be to utilize INDEL panels or SNP markers using MPS technologies to provide more robust and discriminatory DNA identifications.
- When comparing the average STR success and RMP values of the five samples after each treatment using 1ng of input DNA , none of the WGA methods or DNA repair produced more complete or more discriminatory STR profiles than untreated samples (Fig. 1 and 2).
- Of the four treatments tested, GenomePlex® was the only method that generated more reportable alleles on average (>23 % alleles) and lower RMP values compared to the untreated samples. However, this result was only true with 100 ng DNA.
- Although more bi-allelic markers are needed to match the RMP values of STR kits, both INDEL and SNP typing methods resulted in higher genotyping success rates than STRs when 1 ng of untreated DNA was amplified (Fig. 3 and 4). However, a higher percentage of INDEL markers than SNPs were successfully amplified.
- The HID-Ion AmpliSeq™ Identity Panel generated the most discriminatory profiles for identification purposes (Fig. 4).
- The SNP panel contains 85 more bi-allelic markers than the INDEL multiplex (124 versus 39 respectively). Therefore, a greater discriminatory power is expected with the SNP panel.

MATERIALS AND METHODS

- Five tissue samples (two jejunum, kidney, stomach, and spleen) were dissected from three male embalmed cadavers.
- DNA extraction was performed using the QIAamp DNA FFPE Tissue Kit (Qiagen).
- Four different treatments were performed on each of the five samples using the kits' recommended total DNA input amount (10 ng or 100 ng as applicable) and a forensically relevant template amount of 1ng.
- WGA was performed using the GenomePlex® Complete WGA Kit (Sigma-Aldrich), Illustra™ Ready-To-Go™ GenomiPhi™ V3 (GE Healthcare Life Sciences), REPLI-g FFPE Kit (Qiagen), as per manufacturers' protocols.
- DNA Repair was performed using the Infinium HD FFPE Restore Kit (Illumina) as per manufacturer's protocol.
- DNA extracts and treated DNA products (2 µL) were quantified using the Quantifiler® Trio DNA Quantification Kit (ThermoFisher Scientific).
- DNA extracts and treated products (0.8 ng) were amplified using the GlobalFiler® PCR Amplification Kit (ThermoFisher Scientific). Separation and detection of amplified products was performed on a 3500 Series Genetic Analyzer (ThermoFisher Scientific).
- INDELs (0.75 ng) were amplified using a prototypical 39-loci INDEL multiplex.
- SNPs (1 ng) were amplified on the Ion Torrent Personal Genome Machine (PGM) (ThermoFisher Scientific) using the HID-Ion AmpliSeq™ Identity Panel (v2.3) (ThermoFisher Scientific).

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