COMPARATIVE TOLERANCE OF SHORT TANDEM REPEAT AND MASSIVELY PARALLEL SEQUENCING CHEMISTRIES TO INHIBITED SAMPLES

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Victim identification is one of the most important goals after a mass disaster event or in a missing persons case. Often times these human remains are very challenging samples to identify as they may be highly degraded and fragmented, burnt, decomposed, or containing inhibitory substances. Capillary electrophoresis-based short tandem repeat (STR) markers are traditionally used for DNA identification, but massively parallel sequencing (MPS) has recently emerged as an alternative approach for identifying human remains. The purpose of this study was to compare the tolerance of a commercial STR kit and a MPS-based system to common inhibitors that are frequently encountered in skeletal and decomposed tissue samples requiring identification in forensic and missing persons casework.

DNA (1ng and 0.1 ng) was spiked with various concentrations of five inhibitors (humic acid, melanin, hematin, collagen, calcium). Samples (N = 150) were amplified with GlobalFiler® PCR Amplification Kit (ThermoFisher Scientific) and genotyped on the ABI 3500 Genetic Analyzer. A subset of samples (N = 25) were also sequenced using single nucleotide polymorphism (SNP)-based HID-Ion AmpliSeq™ Identity Panel (ThermoFisher Scientific) performed using the Ion Personal Genome Machine (PGM) (ThermoFisher Scientific).

In general, STR results show a decrease in the number of alleles being amplified and detected as inhibitor concentrations increase. As expected, the average peak height and average heterozygote peak height ratios showed a decreasing trend as inhibitor concentration increases. Samples with 0.1 ng DNA input resulted in considerably less complete STR profiles than 1 ng DNA samples at all inhibitor concentrations, suggesting that samples amplified with less DNA template are more susceptible to the effect of PCR inhibition.

MPS sequencing results suggest that the HID-Ion AmpliSeq™ Identity chemistry may not be as tolerant to PCR inhibitors than STR amplification kits. Samples with the same inhibitor concentrations generated considerably worse results via MPS. The lowest inhibitor concentrations for humic acid, melanin, and hematin resulted in complete STR profiles but performed poorly when the samples were sequenced via MPS. However, the highest inhibitor concentrations for collagen and calcium resulted in poor STR profiles but performed well with MPS resulting in complete SNP profiles. Overall, the chemistry in commercial STR kits is more tolerant to common inhibitors found in biological samples than the MPS sequencing chemistry tested in this study.