B46 Comparative Tolerance of Short Tandem Repeat (STR) and Massively Parallel Sequencing (MPS) Chemistries to Inhibited Samples

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After attending this presentation, attendees will better understand the difference in tolerance to common Polymerase Chain Reaction (PCR) inhibitors between the chemistries of a commercial DNA amplification kit for Capillary Electrophoresis (CE) -based STR typing and a Single Nucleotide Polymorphism (SNP) -based identity kit sequenced via MPS with the Ion Personal Genome Machine® (PGM™) platform.

This presentation will impact the forensic science community by demonstrating how common inhibitors found in challenging bone and tissue samples, such as those that may be recovered during a mass disaster or missing person's case, affect different genotyping successes employing conventional STR typing used in forensic laboratories and newer MPS methods.

Victim identification is one of the most important goals after a mass disaster event or in a missing person's case. Oftentimes, these human remains are very challenging samples to identify as they may be highly degraded and fragmented, burnt, decomposed, or containing inhibitory substances. CE-based STR markers and mitochondrial analyses are traditionally used for DNA identification, but MPS has more recently emerged as an alternative approach for identifying human remains. The purpose of this study is 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 0.1ng) was spiked with various concentrations of five inhibitors (humic acid, melanin, hematin, collagen, and calcium). Samples (N=150) were amplified with the GlobalFiler® PCR Amplification Kit and genotyped on the ABI 3500 Genetic Analyzer. A subset of samples (N=25) was also sequenced using the Human Identification (HID) -Ion AmpliSeq™ Identity Panel with the Ion PGM™.

In general, STR results showed a decrease in the number of alleles being amplified and detected as inhibitor concentrations increased. As expected, the average peak height and average heterozygote peak height ratios showed a decreasing trend as inhibitor concentration increased. Samples with 0.1ng DNA input resulted in considerably poorer STR profiles than 1ng 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 as 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.

Data also showed that when highly inhibited samples were pooled for library amplification and loading onto the same chip for sequencing, carry-over inhibition from highly inhibited samples affected the performance of samples with little or no inhibition. In fact, when samples with the highest amounts of inhibitor were processed, library amplification failed completely. Overall, the chemistry in commercial STR kits is more tolerant to most of the common inhibitors found in biological samples than the MPS sequencing chemistry tested in this study.

Massively Parallel Sequencing, STR Typing, PCR Inhibitor