Human remains can be evidence in forensic cases and identification of missing migrants, refugees, and victims of mass disaster. Analysis of human remains can be challenging when the samples have been exposed to harsh environmental conditions causing DNA degradation and/or impregnation of inhibitors. Common PCR inhibitors that are associated with human remains include humic acid in soil, melanin in hair and skin, hematin in red blood cells, collagen in soft tissue and bone, and calcium in bone. STRs are the most common genetic marker used for the identification of human remains due to their relatively small amplicon size (75 – 450 bp) and high power of discrimination. Single nucleotide polymorphisms (SNPs) are alternative markers for human identification, bio-ancestry determination, and phenotype prediction that may be more suitable for analysis of degraded DNA. With the development of massively parallel sequencing (MPS) technology, it is possible to multiplex large numbers of STRs and SNPs. This multiplex capability increases the power of discrimination, but more importantly enhances the ability to type a wider array of quality and quantity sample types.

As with any forensic genetics methodology, the front end of the process is critical for obtaining a result. Therefore, in order to realize the full potential of MPS for identifying human remains, sample preparation methods must be assessed and if necessary optimized with the MPS chemistries used for forensic analysis. The goal of this study was to evaluate the efficiency of various DNA extraction methods to remove PCR inhibitors associated with human remains. Samples were extracted using either an organic extraction method or bind, wash, and elute methods commonly used in crime laboratories, such as DNA IQ (Promega), PrepFiler (Life Technologies), and DNA Investigator (QIAGEN). DNA was extracted from samples spiked with various inhibitors and samples from decomposed cadavers maintained at the Southeast Texas Applied Forensic Science facility and then sequenced using the Precision ID GlobalFiler® Mixture ID panel on the Ion S5™ system and the ForenSeq™ DNA Signature Prep Kit on the MiSeq FGx™. The overall results show that all extraction methods tested are compatible with MPS systems. The success of typing of each system and a comparison of performance among STRs (based on marker type and amplicon size) and between STRs and SNPs will be presented so the community can gain a better understanding of the performance of MPS systems and their capabilities to analyze extremely challenging samples.