H105 DNA Extraction and Sample Storage Considerations for Using Metagenomic Sequencing Approaches to Evaluate Soil Microbial Communities Associated With Human Death Scenes

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After attending this presentation, attendees will understand how to collect bacterial evidence from human cadaver-associated soils using optimized DNA extraction methods, specifically testing the use of commercially available DNA extraction kits and the efficacy of short-term sample storage on the quality of genomic DNA to be used in downstream molecular applications (e.g., metagenomics).

This presentation will impact the forensic science community and practitioners interested in using bacterial communities found in soils associated with human decomposition by comparing five methods of DNA extraction. Soil, particularly cadaver-soil, provides crucial physical and forensic evidence that may be used to link a criminal to a crime scene, identify an unknown corpse, or determine if a body has been moved from its original burial location. The sensitivity and precision in Polymerase Chain Reaction (PCR) amplification of the extracted DNA is paramount for forensic lab protocols; however, complex organic and inorganic substances in soil samples make standardizing methodologies for DNA extraction difficult. Recent studies suggest there are important variations in methods that provide optimal extraction of high-quality genomic DNA for downstream molecular applications, such as PCR and next generation sequencing.

The goals of this study were to evaluate soil extraction and storage methods to determine an effective, relatively rapid, and standardized method for obtaining ample concentrations of high-quality DNA. Five DNA extraction methods were compared to assess the yield and purity of DNA from soil collected beneath five body zones of replicate human cadavers. Furthermore, the efficacy of short-term DNA storage at 6°C and in an RNA stabilization solution on DNA yields was compared between two commercial extraction kits using non-cadaver-associated soils.

The cadaver-soil samples were obtained from cadavers placed at the outdoor research facility Freeman Ranch located at the Forensic Anthropology Research Facility (FARF) at the Forensic Anthropology Center at Texas State (FACTS) University in San Marcos, TX. Samples were collected from the soil located under and juxtaposed to five body zones: the cranium, the right and left foot, and the right and the left olecranon. Four commercial soil DNA extraction kits and one lab-optimized DNA extraction method were used to extract genomic DNA (in duplicate) from each of the cadaver-soil samples. A spectrophotometer for optical density measurements was used to assess DNA yields (ng/µl) and quality; specifically, the purity of DNA extractions were evaluated by measuring the ratio of Ultraviolet (UV) absorbance at 260/280nm and 260/230nm for protein and humic acid contamination, respectively. Following quantification of DNA, each sample was tested for the presence of the desired 16S rRNA gene regions using targeted amplification by PCR. The results of the DNA extraction method comparison study revealed DNA yields of 50-107ng/µl with the purity of samples ranging from 1.59-1.93 (260/280nm) and 0.75-1.82 (260/230nm). As an additional comparison, DNA was extracted from non-cadaver soil that was preserved using either cold storage (6oC) or an RNA stabilization solution for 20 days. Non-cadaver-soil samples stored in an RNA stabilization solution had a greater mean DNA yield compared to soil samples stored at 6oC for both commercial extraction kits.

This research provides new information and specific parameters by which cadaver-soil microbial DNA can be evaluated and is important for downstream molecular applications, which could be used to improve the estimates of postmortem timelines. These results further aid in formulating standardization protocols for forensic laboratories to achieve reproducible DNA results.

Necrobiome, Soil Bacteria, Standardized Protocols