## A16 A High Throughput Protocol for Using Soil Molecular Biology as Trace Evidence

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After attending this presentation, attendees will understand how soil sample handling and storage can alter soil microbial community fingerprints using capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) and fatty acid methyl ester (FAME) analysis.

This presentation will impact the forensic science community by presenting a method of using soil molecular biology for comparative analysis, which will ultimately lead to more robust crime scene reconstruction.

Trace evidence, although often found in small quantities, can be vital in a forensic investigation. The primary contribution of this form of physical evidence is typically to trace the movement of an object or a person. Soil as trace evidence is the main focus of this experiment. Soil has complex mineralogical, physical, chemical, hydrological, and biological properties that can be specific to its location. These properties can be an accurate way to determine whether a person or an object has been at a certain location.

In order to test whether the storage and/or treatment before storage has an effect on microbial communities within the soil, samples were taken from a depth of zero to five cm at four different locations in Nebraska representing a variety of soil types of varying texture and organic matter content. The samples from each location were divided into seven subsamples. Microbial DNA and fatty acids from the first subsample were extracted immediately, while the other subsamples went through a selected storage treatment under varying conditions (-80 °C, - 20 °C, 4 °C, freeze dried, air dried, oven dried). Soil fatty acids and microbial DNA from the stored samples were analyzed at a later date. This process was done for each of the four selected soil types. Fresh samples were collected and processed in the same manner within two weeks of initial collection at each location to determine whether soil microbial communities fluctuate significantly over short periods of time (similar to the time between the commission of a crime and the

collection/analysis of evidence). Soil samples will also be collected seasonally to identify the effects of seasonal impacts on microbial communities in the soil.

The polymerase chain reaction was used to amplify and fluorescently tag amplicons of the V3 region of 16S rDNA. This region is highly conserved throughout prokaryotes, yet it has enough variability to allow for different conformations during capillary electrophoresis single-strand conformation polymorphism (CE-SSCP). The CE-SSCP peaks and their relative heights represent the microbial community and its diversity for an individual soil sample. These sets of peaks were compared to determine if soil samples can be matched, thereby locating a crime scene. Fatty acid methyl ester (FAME) analysis was used as a second method to fingerprint soil samples. Results will be presented to show the effectiveness of these two methods to detect small variations in the soil microbial community.

**Capillary Electrophoresis, Fatty Acid Methyl Ester, 16S rDNA**

 ** Presenting Author*