B55 From Fragment Isolation to DNA Amplification: A Detailed Protocol for Using Plant and Insect Material in Forensics

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After attending this presentation, attendees will be aware of a tested protocol for the isolation, extraction, and amplification of DNA from soil biological fragments.

This presentation will impact the forensic science community by providing an alternative to bulk meta-genomics for the analysis of biological fragments recovered in soil samples.

In the current era of massively parallel sequencing, there has been an influx of studies documenting the biological diversity in soil samples using a bulk metagenomic approach. Insect and plant fragments have been collected for hundreds of years and more recently documented based on their geographic region by the National Bureau of Agriculturally Important Insects and the United States Department of Agriculture. Within a collected sample, there can be substantial diversity in the biological fragments. In most cases, it is not possible to classify them using morphology for taxonomic identification; however, by using conserved primers targeted to specific groups of organisms such as insects, plants, fungi, and bacteria, individual taxa can be amplified and sequenced to provide a detailed picture of the biological community. Although a metagenomic approach facilitates the collection of large amounts of data from small and even very degraded samples, the current usefulness of this technique mainly lies with cross-sample comparisons; the Operational Taxonomic Units (OTUs) identified are compared among samples to determine whether it is likely they came from the same source/location. Species-level taxonomic identification is not possible using this approach, but such data could prove useful for soil geoattribution considering biological organisms only inhabit specific ecosystems.

A molecular-based approach may provide a more accurate and reliable tool for species identification. One commonly used technique for molecular identification is DNA barcoding. DNA barcoding is based on the notion that there is substantial variation at the DNA level among organisms to permit discrimination (i.e., each species on Earth will have its own unique barcode sequence). As DNA is embedded in every cell, a DNA barcode approach can be used to identify insect and plant fragments found within a soil sample. The current project was focused on developing a protocol to successfully isolate DNA from *individual* biological fragments commonly recovered from soil samples. To facilitate taxonomic identification of the samples, the appropriate DNA barcoding region was amplified according to established protocols of expert researchers in the field.[1]

This presentation will outline the details of the protocol including: (1) fragment isolation, washing, and documentation (photography and weighing); (2) extraction of the total genomic DNA; (3) determination of the DNA quality in each sample; (4) Polymerase Chain Reaction (PCR) optimization for the amplification of the insect (*CO1*) and plant (*matK* and *rbcL*) barcoding regions from a broad range of samples; and, (5) techniques used to screen PCR amplicons prior to sequencing.

Reference(s):

1. Kress W.J., Erickson D.L., editors. *DNA Barcodes: Methods and Protocols*. New York: Humana Press, 2012.

Individual Biological Fragment, DNA Extraction, PCR

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