

## IDENTIFICATION OF PLANT TRACE EVIDENCE USING MOLECULAR TECHNIQUES

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The identification of plant materials associated with trace evidence can provide useful leads during criminal investigations. Historically, botanical trace evidence has been analyzed by the morphological and histological characteristics of the plant material. However, many plant materials found in trace evidence cannot be identified to the species level based on their morphological features. We have utilized molecular techniques to identify trace amounts of plant materials to the family, genus and species level. The *rbcL* gene of chloroplast DNA and the Internal Transcribed Spacer (ITS) region of ribosomal DNA are moderately conserved sequences within the plant kingdom and are used to identify plants at the family, genus and species level. We have used the DNA sequence variation of these two loci to identify the individual plant components found in trace evidence.

The methods developed to analyze the trace evidence involve the following steps:

- Extract the DNA from the trace evidence sample with a variety of methods. Removal of the secondary plant metabolites that can inhibit PCR is particularly important.
- Amplify the plant DNA at several loci (*rbcL* and ITS) developed by molecular systematists for plant identification purposes.
- Clone the amplified DNA into appropriate hosts for storage and screening purposes.
- Screen the clones using restriction enzyme patterns to identify different plant clones.
- Sequence the amplified products associated with the plant clones.
- Identify the plant using the NCBI BLAST search algorithm and the PAUP alignment software.

Using molecular techniques, plants have been identified from as little as a 1 mm punch of dried plant tissue or from 5 grains of fresh pine pollen. A minimum of 10 picograms of DNA has been used to identify plant material. Additionally, methods have been developed to identify plant materials in a heterogeneous mixture from soil and dust collected from a variety of environments. Using these methods we have identified plants from trace materials collected from air filters, clothing, roadside debris, and packaging materials. It is envisioned that the molecular analysis of botanical trace evidence will be useful in providing investigative leads for solving crimes such as kidnapping, distribution of drugs, murder, and acts of terrorism.