## B173 Individualization of *Acer rubrum* Using Amplified Fragment Length Polymorphism

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Attendees will learn how the AFLP technique is a rapid, inexpensive, and efficient method that can be used to genotype DNA from botanical material. Within the closed set of samples used in this study, the AFLP profiles were species specific and unique.

AFLP is a rapid, cost-effective, simple, and robust method that can be used to genotype DNA of any origin and complexity. The technique can be used by forensic scientists to type DNA from nonhuman biological evidentiary material (plants, insects, and animals). This presentation will impact the forensic community and/or humanity by adding to the repertoire of techniques available to forensic scientists and increase the probative value of nonhuman DNA evidence.

Amplified fragment length polymorphism (AFLP) is a powerful method that combines techniques from classical hybridization-based and PCR-based genotyping strategies. AFLP was used to genotype leaf material from red maple trees, *Acer rubrum*, which are widely distributed throughout much of the United States and Canada. Duplicate samples from 40 *Acer rubrum* trees were collected from four different sites in central New Jersey. Samples from five additional species were collected for comparison. One set *Acer rubrum* samples was used to establish a DNA database. The second set of *Acer rubrum* samples and the comparison samples were analyzed and compared to the AFLP profiles in the database to determine the discriminative capacity of the technique.

The AFLP protocol was performed using components from the AFLP Core Reagent and Preamp Primer Mix I kits (Invitrogen, Rockville, MD). Genomic DNA was double-digested by two restriction endonucleases: *EcoRI* and *MseI*. The DNA fragments were ligated to *EcoRI* and *MseI* oligonucleotide adapters to generate primer binding sites. In this manner a select set of DNA fragments can be amplified without knowledge of the sequence. Two consecutive PCR reactions (preamplification and selective amplification) were performed. In the preamplification reaction DNA fragments were amplified using primers complementary to the adapters and adjacent restriction sites with one selective nucleotide at the 3' ends: *EcoRI* (5'-GACTGCGTACCAATTC**A**-3'; *MseI* (5'GATGAGTCCTGAGTAA**C**-3'). During selective amplification the products of the preamplification reaction were amplified using a primer pair with three selective nucleotides at the 3' ends: *EcoRI* [5'(dyeD4) GACTGCGTACCAATTC**ACT**-3', Proligo, Boulder, CO] labeled with the fluorophore (D4WellRed, Beckman Coulter); *MseI* (5'GATGAGTCCTGAGTAA**CAT**-3', Invitrogen). The primer design and amplification strategy ensured that only a subset of the EcoRIMseI fragments was preferentially amplified.

The DNA fragments were separated by capillary electrophoresis using the CEQ 8000 DNA Fragment Analyzer. Data were analyzed using the CEQ 2000XL (Beckman Coulter) and Twin Peaks (authors, proprietary) software.

Within this closed set, AFLP profiles were species specific and unique. The individualization of plant matter will enable forensic scientists to derive more information from evidentiary material and to help link a suspect or a victim to a particular crime scene (source tree).

**DNA, AFLP, *Acer rubrum***

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