## B19 Development of a Nationwide AFLP DNA Database for Marijuana *(Cannabis Sativa)*

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The goal of this presentation is to identify DNA polymorphisms that will enable the individualization of marijuana samples and build a database of the DNA profiles.

The Connecticut State Forensic Science Laboratory is validating Amplified Fragment Length Polymorphism (AFLP) analysis as a means of DNA typing/individualizing marijuana (*Cannabis sativa*) samples. An important application of this research is to identify and link clonally propagated marijuana plants from different cases, suspects, locations or growing operations to one another. The authors have demonstrated that cloned plants generated by Dr. Gary Shutler (formerly of the Royal Canadian Mounted Police) exhibit identical AFLP profiles and that different marijuana "varieties" have dissimilar profiles. However, the estimation of a random match probability requires knowledge about the amount of genetic variation present among local and national marijuana "populations." Therefore, an AFLP database from statewide and nationwide marijuana samples is being generated. This database will enable the authors to survey genetic diversity present within and between grower-identified varieties of marijuana, as well as to look for differences between marijuana grown locally and marijuana that is smuggled into the U.S. from various countries. Furthermore, profiles will be made available to the forensic community for comparative purposes.

Thus far, 68 unique AFLP profiles have been obtained from samples seized in Canada, Connecticut, and Vermont, and many more samples from additional states nationwide are in the process of being acquired. Four selective primer pair combinations of the AFLP Plant Mapping Kit (Applied Biosystems Inc.) were chosen to generate the data for the database. These include EcoRI-ACT FAM/MseI-CAA, EcoRIACT FAM/MseI-CAT, EcoRI-AAG JOE/MseI-CAT, and EcoRI-AAG JOE/MseI-CTA. In order to facilitate data management, specific fluorescent peaks of each profile were selected to be automatically scored by Genotyper software (ABI) and converted to binary code. Although all of the DNA fragments (~75-100) in an AFLP profile are informative and would be used to demonstrate a match in court, binary coding is a useful search tool for screening the database for candidate matches, and assessing the degree of similarity between particular profiles. For the four selective primer pairs, a total of 100 DNA fragment peaks were selected using more than 100 marijuana plant profiles, of which 60% were unique. Peaks were chosen based on their variability, peak height, interference from neighboring peaks, and amplification consistency. The average size of each selected peak, plus or minus 0.25-0.50 base pairs (to account for variation in size measurement precision), and a minimum peak height value (50 relative fluorescence units) were used to define bins or "categories." Several non-variable "control" peaks per primer pair profile were also selected to help the analyst gauge amplification yield/efficiency. Genotyper scores for the presence or absence of peaks within the defined categories and converts the data into binary code. The authors plan to build a database of at least 500 unique AFLP profiles; therefore additional seizure samples are being sought. Since the use of the counting method (1/N where N equals the number of individuals within the database) to estimate the frequency of observed profiles, more unique profiles are identified and more value can be assigned to a match between two evidentiary marijuana samples.

**Marijuana, DNA Database, AFLP**

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