DEVELOPMENT OF A NATIONWIDE AFLP DNA DATABASE FOR MARIJUANA (CANNABIS SATIVA)

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The Connecticut State Forensic Science Laboratory is validating Amplified Fragment Length Polymorphism (AFLP) analysis as a means of DNA typing individual marijuana samples. An important application of this research is the use of AFLPs to identify clonally propagated marijuana plants and link cloned specimens from different cases, suspects or locations to one another. We have demonstrated that cloned plants, generated by Dr. Gary Shulter of the Royal Canadian Mounted Police, do indeed exhibit identical AFLP profiles. However, estimation of the random match probability requires the creation of a large population database, therefore we are generating an AFLP database from statewide and nationwide seized marijuana samples. This database will enable us to survey the amount of genetic variation present within and between grower-identified "varieties" or "strains" of marijuana, as well as to look for differences between marijuana grown locally and marijuana that is smuggled into the United States from various countries. Furthermore, profiles will be made available to the forensic community for comparative purposes.

Thus far, approximately 50 unique AFLP profiles have been identified from samples seized in Canada, Connecticut and Vermont. We are in the process of acquiring many more samples from additional states nationwide. We are defining an AFLP "profile" as a series of specifically selected fluorescent peaks generated using four primer pair combinations of the AFLP Plant Mapping Kit (Applied Biosystems Inc.). Although all DNA fragments (~75-100) in each AFLP profile are potentially informative, certain ones were chosen that could be automatically scored by Genotyper® software (ABI) and converted into binary code. For the primers EcoRI-ACT FAM and MseI-CAA, 31 DNA fragment peaks were selected based on ~100 marijuana plant profiles using criteria such as variability, peak height, interference from neighbouring peaks, and amplification consistency. The peaks are indicated as present or absent by Genotyper® depending on whether or not they fall within defined "categories". Category definitions include the average size of the peak, plus or minus a fraction of a base pair to account for variation in size measurement precision, and a minimum peak height value (relative fluorescence units). Converting AFLP profiles into binary code allows the data to be easily compared (identify match or mismatch) and analysed to look for geographic correlations or relationships between samples. We aim to build our database of at least 500 unique AFLP profiles; therefore we are seeking additional seizure samples. Since we anticipate using the counting method (1/N where N equals the number of individuals with the database) to estimate the frequency of observed profiles, the more unique profiles that are identified, the more value that can be assigned to a match between two evidentiary marijuana samples.