



B112 Expansion of an AFLP DNA Marijuana (*Cannabis sativa*) State, National, and International Database

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The goal of this presentation is to demonstrate how AFLP can be used to create databases of non-human origin and how they can be applied to the forensic and law enforcement community.

This presentation will impact the forensic community by demonstrating how AFLP can be used to create databases of non-human origin and how they can be applied to the forensic and law enforcement community.

Amplified Fragment Length Polymorphism (AFLP) analysis is a polymerase chain reaction (PCR) based DNA typing method in which amplification of restriction fragments are used to individualize single source biological samples. The authors are currently in the latter stages of completing state, national, and international AFLP marijuana (*Cannabis sativa*) databases composed of law enforcement seizure samples. The database has forensic significance in that it has the potential to identify and link clonally propagated marijuana plants with cultivators, distributors, and users as well as tracking certain "strains" that illegally enter into and are circulated throughout the United States. In order to determine the genetic variability of marijuana and its statistical correlation, a large marijuana population database is being created from seizure samples provided by state and local authorities in Connecticut, Vermont, Florida, Iowa, Kentucky, Wyoming, Tennessee, West Virginia, Canada, and Taiwan. These seizure samples were used to create state (100 samples), national (150 samples), and international (200 sample) databases.

The database was created by using four selective primer sets (EcoRT-ACT FAM/MseI-CAA, EcoRI-ACT FAM/MseI-CAT, EcoRI-AAG JOE/MseI-CAT, and EcoRI-AAG JOE/MseI-CTA: A1, A4, F4, and F5 respectively) from the Applied Biosystem's AFLP™ Plant Mapping Kit and separating PCR products by gel electrophoresis on an ABI 377 DNA Sequencer. 100 predetermined fragments are then scored in Genotyper® (ABI) and converted to a binary code sequence that represents the samples genetic "profile." This combination of "1s" and "0s" are then imported into the database, which is used as a valuable search tool for identifying samples that are consistent with clonality. However, due to the fact that there is the possibility of two or more unrelated or half-sibling samples being represented by the same binary code, samples whose profiles match are then superimposed upon each other in Genescan® (ABI) to determine whether any minor peaks outside the defined categories are detected.

As with many forensic applications, statistics play an important role when a database of this type is used in court. Two types of statistical analyses are being conducted a) the counting method and b) the confidence interval. To determine the random match probability (RMP), the conservative counting method of $1/N$ (N =the total number of unique profiles in the database) is used. Since this statistical method may be used, the greater the size of the database the more meaningful the statistical values will be. With this in mind, the authors are hoping to eventually create a 500 unique profile database. For an even rare conservative statistical approach two methods may be used. $1-a^{-1-N}$ ($\alpha=0.05$ for the 95% confidence interval) can be applied to profiles not previously observed within the database, while for those profiles that have been observed, $P \pm 1.96(\sqrt{P(1-P)/N})$ may be employed. Again, since a 95% confidence interval is being used, the latter two statistical methods are much more conservative than the more commonly used counting method.

In demonstrating the usefulness of this technique and database, two case study examples will be presented in detail.

AFLP, Marijuana, Population Database