B190 Evaluation of a 13-Loci Short Tandem Repeat (STR) Multiplex System for *Cannabis Sativa* Genetic Identification

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After attending this presentation, attendees will understand the basic principles behind using an STR multiplex method for individualizing marijuana samples.

This presentation will impact the forensic science community by providing an STR panel that could not only assist law enforcement agencies in verifying legal marijuana products but also aid in the linkage of illegal cases. This method could also serve as an additional tool to previously established marijuana profiling programs used in federal agencies such as the United States Customs and Border Protection (CBP) and the Drug Enforcement Administration (DEA).

Marijuana (*Cannabis sativa*) is the most commonly used illicit substance in the United States. Although the federal government considers marijuana a Schedule I substance, it has become legalized for recreational use in four states (Colorado, Washington, Oregon, and Alaska). As a result of legalization, law enforcement faces a unique challenge in tracking and preventing the flow of legal marijuana to states where it is illegal. Moreover, there is significant traffic of illegal marijuana from Mexico. When identifying marijuana for legal purposes, the Scientific Working Group for the Analysis of Seized Drugs (SWGDRUG) recommendations require the confirmation of THC via Gas Chromatography/Mass Spectroscopy (GC/MS), the microscopic confirmation of the presence of cystolithic hairs, and a positive Duquenois-Levine color test.[1] These tests are sufficient for prosecuting an individual for possession of marijuana but do not provide any meaningful intelligence as to the origin or individualization of the plant; however, there are many methods that can be used to individualize and determine the origin of a marijuana sample. These methods include, but are not limited to, palynology, chemical profiling, Isotope Ratio Mass Spectrometry (IRMS), and DNA analysis.[2-6] DNA has been shown to provide higher resolution for the individualization of marijuana plants as compared to the other techniques.[7]

The development of a validated molecular method such as STRs could aid in the individualization of cannabis samples as well as serve as an intelligence tool to link multiple cases (e.g., illegal traffic at the United States-Mexico border). For this purpose, a modified STR multiplex method was optimized and evaluated according to the International Society for Forensic Genetics (ISFG) and the Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines.[8] A new quantitative Polymerase Chain Reaction (qPCR) method was developed to accurately quantitate the amount of cannabis DNA extracted. A sequenced allelic ladder was also designed to accurately genotype 199 *C. sativa* samples from 11 seizures provided by a federal agency.

Distinguishable DNA profiles were generated from 127 samples that yielded full STR profiles, and four duplicate genotypes within seizures were found. From the analysis of STR profiles and the lack of clonal material, it can be concluded that the analyzed cannabis samples, most likely from Mexico, were propagated from seeds. The combined power of discrimination of this multi-locus system is 1 in 70 million with a sensitivity of 0.25ng of template DNA. The 13-STR panel was found to be species-specific for *C. sativa*; however, non-specific peaks were generated for *Humulus lupulus* (Hops). Phylogenetic analysis and case-to-case pairwise comparison of 11 cases using F_{ST} as genetic distance revealed the genetic association of four groups of cases. Moreover, due to their genetic similarity (common origin), a subset of samples (N=97) was found to form a homogeneous population in Hardy-Weinberg and linkage equilibrium. Using this homogenous population, a reference cannabis population database with associated allele frequencies for forensic purposes was developed.

In conclusion, the results of this research demonstrate the applicability of this 13-loci STR system not only in associating cannabis cases for intelligence purposes, but also in potentially detecting the presence of plant material generated via clonal propagation.

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