

EVALUATION OF A 13-LOCI STR MULTIPLEX SYSTEM FOR *CANNABIS SATIVA* GENETIC IDENTIFICATION

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Marijuana (*Cannabis sativa*) is the most commonly used illicit substance in the United States. The development of a validated method using molecular techniques 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 US-Mexico border).

For this purpose, a previously reported STR multiplex method was optimized and evaluated according to ISFG and SWGDAM guidelines. A new real-time PCR method was developed to accurately quantitate the amount of *Cannabis* DNA extracted and 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. The combined power of discrimination of this multi-locus system was 1 in 70 million with a sensitivity of 0.25 ng 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 *Fst* 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. 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.