DEVELOPMENT AND VALIDATION OF A NEW 13-LOCI STR MULTIPLEX SYSTEM FOR *CANNABIS SATIVA* GENETIC IDENTIFICATION

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Marijuana (*Cannabis sativa* L.) is a plant cultivated and trafficked worldwide as a source of fiber (hemp), medicine, and intoxicant. The development and validation of a method using molecular techniques such as short tandem repeats (STRs) could serve as an intelligence tool to link multiple cases by means of genetic individualization/association of *Cannabis* samples. For this purpose, a new 13-loci STR multiplex method was developed, optimized, and validated according to ISFG and SWGDAM guidelines. The 13-loci multiplex mainly consisted of previously described tri- and tetra-nucleotides *Cannabis sativa* STRs: ANUCS501, 9269, 4910, 5159, ANUCS305, 9043, B05, 1528, 3735, CS1, D02, C11, and H06. Validation studies included a) species specificity, b) sensitivity, c) Hardy-Weinberg and linkage equilibrium in a reference population, d) heterozygous peak height ratios (PHR), e) inter-loci balance, f) stutter ratios, and g) precision and accuracy. In addition, a sequenced allelic ladder consisting of 55 alleles was designed to accurately genotype 101 *C. sativa* samples from 3 seizures provided by a federal agency.

Using an optimal range of input DNA (0.125 – 0.5 ng), validation studies revealed minimal artifacts and stutter (average stutter ratio of 0.021 across all loci), relatively balanced heterozygous peaks (average PHR of 0.83 across all loci), and a well-balanced electropherogram (inter-loci balance range: 0.500 – 1.296). The combined power of discrimination of this multi-locus system was 1 in 77 million with a sensitivity of 125 pg of template DNA. The 13 STR panel was found to be species specific for *C. sativa*; however, non-specific peaks were produced with *Humulus lupulus*.

The results of this research demonstrate the robustness and applicability of this 13-loci STR system in identification of *Cannabis* samples for intelligence purposes.