B95 The Development and Validation of a New 13-Loci Short Tandem Repeat (STR) Multiplex 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).

Forensic DNA typing is typically performed on human DNA samples; however, the molecular analysis of plant DNA is increasingly being studied and considered for use in criminal justice systems around the world. Plant DNA can be used to link a suspect/victim to an area or, in the case of marijuana, can be used to aid in the investigation of drug cases. 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. In 2003, the first polymorphic STR markers were published for *Cannabis sativa*. Previous research has shown the utility of these markers in individualizing marijuana samples; however, the technique has been scarcely used in crime laboratories due to lack of standardization and validation.

For this purpose, a new 13-loci STR multiplex method was developed, optimized, and validated according to the International Society of Forensic Genetics (ISFG) and Scientific Working Group on DNA Analysis Methods (SWGDAM) guidelines. The 13-loci multiplex mainly consisted of previously described tri- and tetra-nucleotides *Cannabis* STRs: ANUCS501, 9269, 4910, 5159, ANUCS305, 9043, B05, 1528, 3735, CS1, D02, C11, and H06. Validation studies were comprised of: (1) species specificity; (2) sensitivity; (3) Hardy-Weinberg and linkage equilibrium in a reference population; (4) heterozygous Peak Height Ratios (PHR); (5) inter-loci balance; (6) stutter ratios; and, (7) precision and accuracy. In addition, a sequenced allelic ladder consisting of 55 alleles was designed to accurately genotype 101 *C. sativa* samples from three seizures provided by a federal agency.

Using an optimal range of input DNA (0.125ng-0.5ng), 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 55 million with a sensitivity of 125pg of template DNA. The 13-STR panel was found to be specific for *C. sativa*; however, non-specific peaks were produced with *Humulus lupulus*.

In conclusion, the results of this research demonstrate the robustness and applicability of this 13-locus STR system in identification of *Cannabis* samples for intelligence purposes.

Forensic Botany, Cannabis Sativa, Short Tandem Repeats