



B155 The Characterization of New Chloroplast Markers to Determine the Biogeographical Origin and Crop Type of *Cannabis Sativa* Samples

Madeline G. Roman, BS*, Sam Houston State University, Huntsville, TX 77340; Rachel M. Houston, PhD, Sam Houston State University, Huntsville, TX 77340; Bobby Larue, Jr., PhD, Sam Houston State University, Huntsville, TX 77340; David A. Gangitano, PhD, Sam Houston State University, Huntsville, TX 77340

Learning Overview: After attending this presentation, attendees will understand a novel research project which seeks to: (1) provide a comprehensive genetic tool to identify *cannabis sativa*, (2) distinguish between hemp and marijuana samples, and (3) determine the biogeographical origin of samples.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing investigative leads for law enforcement and providing evidence for linking cases, distributors, growers, or individual samples.

Marijuana (*Cannabis sativa*) is the most commonly used illicit drug in the United States, and despite its schedule I classification by the federal government, 29 states and the District of Columbia have legalized its use for medicinal or recreational purposes. This state-specific legalization has created a new problem for law enforcement, which must now investigate the diversion of legally-obtained *Cannabis* to states where it remains illegal. In addition, illegal trafficking of the drug at the border with Mexico remains a substantial issue for law enforcement agencies.

C. sativa crops can be broadly classified as marijuana (a drug containing the psychoactive chemical delta-9-tetrahydrocannabinol, or THC) or hemp (the non-drug form of the plant, cultivated for oil and fiber), and differentiation between crop types is important for determining whether the crop is a controlled substance. In addition, investigation of trafficking routes into and within the United States requires genetic association of samples from different cases/seizures, and chloroplast DNA (cpDNA) markers may help to provide investigational leads or link cases with common growers or distributors.

This project seeks to exploit polymorphisms in *C. sativa* cpDNA to provide the forensic community with a comprehensive analytical tool that will allow genetic determination of biogeographic origin, discrimination between drug-type *Cannabis* and fiber-type *Cannabis* samples (marijuana vs. hemp), authentication of medical marijuana samples, and association between cases for *C. sativa* samples. By comparing published cpDNA sequences, 58 polymorphisms were identified, including several “hotspot” regions. This presentation will discuss the process of finding these polymorphisms and designing and optimizing custom Sanger sequencing, fragment, and SNaPshot™ analyses to genotype the chosen “hotspot” regions. Haplotype results from hemp and marijuana samples from four countries (United States, Canada, Mexico, and Chile) will be discussed, as well as phylogenetic analyses evaluating the utility of the chosen polymorphisms in grouping samples from different geographical origins and crop types. This is an ongoing project, and future research will focus on expanding the number of polymorphic markers, designing and validating a method to sequence the informative markers on a massively parallel sequencing platform, and building a database of cpDNA haplotypes for *C. sativa* samples.

Forensic Plant Science, Chloroplast DNA Barcoding, *Cannabis sativa*