B140 Autosomal, Chloroplast, and Mitochondrial Data of a United States Cannabis DNA Database

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After attending this presentation, attendees will understand the basic principles behind employing both autosomal and lineage (mitochondrial and chloroplast) markers for individualizing and sourcing marijuana samples.

This presentation will impact the forensic science community by demonstrating the applicability of an autosomal and lineage panel that could not only assist law enforcement agencies in verifying legal marijuana products but also aid in the linkage of illegal cases. These methods could also serve as additional tools 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).

Since *Cannabis sativa* (marijuana) is a controlled substance in many parts of the world, the ability to track biogeographical origin of cannabis could provide law enforcement with investigative leads regarding its trade and distribution. Population substructure and inbreeding cause individual marijuana plants to become more genetically related. This genetic relatedness can be helpful for intelligence purposes. Using autosomal, chloroplast, and mitochondrial DNA allows not only for prediction of biogeographical origin of a plant, but also allows its genetic identification.

A previously validated 13-autosomal Short Tandem Repeat (STR) multiplex was used to genotype 496 samples. Samples were analyzed from four different sites: 21 seizures at the United States-Mexico border, Northeastern Brazil, hemp seeds purchased in the United States, and the Araucarian area of Chile. In addition, a previously reported multi-loci system was modified and optimized to genotype five chloroplast and two mitochondrial markers. For this purpose, two methods were designed: a homopolymer STR pentaplex and a Single Nucleotide Polymorphism (SNP) triplex with one chloroplast (cscp001) marker shared by both methods for quality control. For successful mitochondrial and chloroplast typing, a novel real-time Polymerase Chain Reaction (PCR) quantitation method was developed and validated to accurately estimate the quantity of the chloroplast DNA (cpDNA) using a synthetic DNA standard. In addition, a sequenced allelic ladder was designed for the homopolymer STR pentaplex.

For autosomal typing, distinguishable profiles generated from 381 samples that yielded full STR profiles and 44 duplicate genotypes within seizures were observed. Phylogenetic analysis and case-to-case pairwise comparisons of 21 seizures at the United States-Mexico border, using F_{ST} as genetic distance, revealed the genetic association of nine seizures that formed a reference population.

For mitochondrial and chloroplast typing, subsampling was performed and 141 samples were genotyped. Complete haplotypes (STRs and SNPs) were observed for 134 samples. As expected, extensive haplotype sharing was observed; five distinguishable haplotypes were detected. In the reference population, one haplotype was observed 39 times in addition to two other unique haplotypes. Haplotype sharing was observed between the United States border seizures, Brazil, and Chile, while the hemp samples generated a distinct haplotype.

Results revealed that both autosomal and lineage markers could discern population sub-structure. Phylogenetic analysis of the four populations using the neighbor joining method and F_{ST} as genetic distance were estimated with the GDA software. Parsimony analysis was then performed with the PAUP* software. The STRUCTURE software was employed to investigate the population structure among groups. And finally, the R package, adegenet, was used to visualize the genetic distance of the populations using Principal Component Analysis (PCA).

In conclusion, the results of this research demonstrate the utility of both autosomal and lineage genotyping methods for characterizing marijuana samples.

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