B134 An Evaluation of Hotspot Chloroplast Regions to Differentiate Crop Type and Biogeographical Origin of *Cannabis Sativa*

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Learning Overview: After attending this presentation, attendees will understand the basic principles behind utilizing DNA barcoding and Massively Parallel Sequencing (MPS) techniques to develop genotyping methods to aid in the differentiation of crop type and biogeographical origin of *Cannabis sativa.*

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the applicability of chloroplast markers and an MPS panel that could potentially assist law enforcement agencies in distinguishing legal hemp and illicit marijuana and also aid in the linkage of illegal cases and drug trafficking.

Cannabis sativa (marijuana and hemp) is one of the most controversial crops worldwide. In the United States, the state-specific legalization of marijuana and recently legalized hemp poses a problem for law enforcement. The study also contributed to the development of a *C. sativa* chloroplast DNA (cpDNA) database, which will need to be expanded to include more chloroplast markers as well as genotypes from samples obtained around the world.

DNA barcoding is a technique that examines specific regions of DNA to identify inter- and intra-species genetic differences. Both cpDNA and mitochondrial DNA have been studied for species identification, but cpDNA is particularly beneficial for plant barcoding markers. However, due to the limited discriminatory ability of common barcoding markers in plant identification, this study sought to discover more informative polymorphic regions that could be used for cannabis differentiation. By comparing published whole genome cpDNA sequences, seven polymorphic hotspot regions were identified. Capillary Electrophoresis (CE) -based methods were developed to quickly genotype the polymorphic loci from the hotspot regions in hemp samples from the United States and Canada, marijuana samples from Mexico and Chile, and medical marijuana samples from Chile. The effectiveness of the assays to distinguish sample groups was assessed using haplotype analysis, pairwise comparisons, and principal component analysis. While CE offers a reliable and robust technique, it has disadvantages such as limited multiplexing capability and size-based genotyping. MPS is an emerging technology in the field of forensic genetics that processes distinct advantages such as high-throughput multiplex capacity and the potential to provide deeper interrogation of sequence-based polymorphisms. Therefore, this study also reports on the development of a MiSeq® FGx® assay targeting seven hotspot regions in the *C. sativa* chloroplast genome.

This study developed six fragment analysis assays and four SNaPshot™ assays for 25 polymorphisms in the seven hotspot regions. The genotype results of 166 samples from five different regions indicated a clear separation of Canadian hemp from other groups. Results also demonstrated that increasing the number of markers was able to improve the discriminatory power of the assays. Moreover, this study reports preliminary results from sequencing 14 hemp and marijuana samples from different countries using a novel MPS assay. A total of 49 polymorphisms were observed in the seven hotspot regions, 16 of which have not been previously reported using CE. Additionally, isoalleles, which were able to differentiate two samples that had the same haplotype using CE-based methods, were discovered. However, no combination of markers was capable of definitively distinguishing the crop type and biogeographical origin of *C. sativa*, and a more extensive database is needed to evaluate the true discriminatory power of these markers.

Overall, this research investigates the seven hotspot regions in the *C. sativa* chloroplast genome from five different origins and developed DNA barcoding and MPS assays that can potentially be used for exclusionary purposes in marijuana identification and geosourcing.

***Cannabis Sativa*, DNA Barcoding, Massively Parallel Sequencing**

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