B47 Differentiating Marijuana From Hemp Using an NGS Panel and Rapid Genotyping Assays Targeting Cannabinoid Synthase Genes

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Learning Overview: The goal of this presentation is to delve into the advantages of employing previously discovered potential markers in cannabinoid synthase genes identified by Next Generation Sequencing (NGS). These markers hold promise in facilitating the effective differentiation of various crop types of *Cannabis sativa* through the development of rapid genotyping methods.

Impact Statement: *Cannabis sativa*, known for its cultivation as hemp or marijuana, remains a topic of controversy in the United States. The 2018 Agriculture Improvement Act, legalizing hemp, presents a challenge for law enforcement to distinguish between the two crop types. This presentation demonstrates the use of novel polymorphisms discovered via NGS to develop two rapid genotyping assays, which could help law enforcement differentiate hemp, hemp seed, and illicit marijuana samples.

Genetic markers play a crucial role in identifying crop types, especially in sample types unsuitable for chemical analysis (e.g., trace residues, small leaf fragments, immature crops). Cannabinoid synthase genes are key enzymes in determining a crop's chemical composition or chemotype. However, existing markers often overlook the analysis of pseudogenes in the cannabis genome. Cannabinoid synthase genes are found to be highly repetitive in the cannabis genome and contain many incomplete coding sequences (pseudogenes) that can have 91–95% nucleotide identity to functional synthase genes. The risk of amplification of pseudogenes needs to be carefully evaluated in all synthase-gene targeting methods for genetic testing of cannabis. To address this, we previously developed a custom NGS panel targeting all synthase genes (THCAS, CBDAS, and CBCAS) as well as their pseudogenes. To ensure a comprehensive analysis, a diverse array of sample types was incorporated. These samples included reference *C. sativa* samples sourced from the National Institute on Drug Abuse (NIDA) and the National Institute of Standards and Technology (NIST), along with CBD, CBG, delta-8-THC, delta-10-THC, THCO, and CBDV hemp samples containing. Additionally, the study included hemp seed samples and extracts from seized marijuana provided by the Drug Enforcement Agency (DEA). The identified novel polymorphisms were then incorporated into two fast-genotyping assays.

The study developed a SNaPshot™ assay and a TaqMan™ quantitative Polymerase Chain Reaction (qPCR) **Single Nucleotide Polymorphism (**SNP) genotyping assay for hemp-marijuana differentiation. SNaPshot™, with four markers on the THCAS gene, THCAS pseudogene, and CBDAS gene successfully differentiated five hemp types and hemp seeds from seized marijuana across various locations in the United States, Chile, and the Mexico-United States border. Furthermore, this assay yielded valuable CBD information in the marijuana samples. On the other hand, the TaqMan™ qPCR SNP genotyping assays, using two markers on the THCAS gene and THCAS pseudogene, showed efficient differentiation of the majority of hemp samples from marijuana, offering the benefits of reduced analysis time and lower costs. However, these assays were unable to distinguish hemp seeds from marijuana or provide CBD information of the sample.

In summary, this presentation showcases the extensive findings from the custom-target NGS panel, leading to the development of two robust, rapid genotyping assays. The comprehensive evaluation of synthase genes enhances researchers' understanding of the diversity within and between *Cannabis sativa* crop types. This research offers valuable insights for forensic science and law enforcement in their efforts to accurately differentiate hemp and marijuana varieties.

Cannabis; Cannabinoid Synthase Gene; Pseudogene

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