G37 Using Multiplexed Microsatellite Markers of Cannabis sativa to Determine Genetic Diversity

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After attending this presentation, attendees will understand previously described microsatellite markers known to discriminate between individual plants were multiplexed into a single reaction and validated in 2 separate laboratories with over 30 plant individuals.

This presentation will impact the forensic community and/or humanity by providing the forensic community with a genetic test, which they can use to track origin in order to connect samples to each other to associate distributors.

Cannabis sativa L. (marijuana) plants can be easily identified through morphological examination and chemical analysis; however there is a need for a DNA test for use as a means of association between individual plants and even as a method to track distribution networks.

Cannabis sativa L. is the most frequently used illegal drug in the United States. *Cannabis* has been used throughout history for its stems in the production of hempen fiber for rope and fabric, for its seed for oil and food and for its flowers and leaves as a psychoactive drug. Microsatellite markers have been chosen for a DNA test because these markers have distinct advantages over other genetic methods. STRs have multiple alleles at a single locus, can be standardized such that reproducibility between laboratories can be easily achieved, have a high discrimination power and can be multiplexed.

In this project, seven *Cannabis* primers selected from a set of primers previously described by the group [1] and four *Cannabis* primers from a set previously described by Gilmore's group [2] were multiplexed into a single reaction. The multiplex reactions were independently analyzed in two separate labs for 30 different cannabis plants. Both an ABI 3100 and an ABI 310 were used for the analysis. Trinucleotide repeats were chosen to reduce the incidence of artifacts that may affect interpretation. The forward primers in some of the primer sets were fluorescently tagged with 6-FAM dye and some of forward primer sets were tagged with HEX dye. Hemp DNA extracts were provided by Tariq Mahmood of the Alberta Research Council in Alberta, Canada. The hemp samples were amplified in a single optimized reaction to determine base pair size for each allele. The primers were then combined into a single multiplexed reaction. The samples were amplified on a 9700 Thermal cycler with the following parameters: a 5minute incubation at 94°C then twenty-five cycles of 94°C for 30 seconds, 54°C for 30 seconds and 72°C for 30 seconds, eight cycles of 94°C for 30 seconds, 52°C for 30 seconds and 72°C for 30 seconds, a 60 minute extension time at 60°C and a final 4°C chill. The samples were prepared and electrokinetically injected for capillary electrophoresis on the ABI Prism 3100. The data generated was imported into GeneScan 3.7 and the base pair size analysis performed using Genotyper 3.7.

Previous studies using these microsatellite markers were able to distinguish clones from non-clones. Efforts to construct a comprehensive genomic map of *Cannabis sativa*, where the positions of these microsatellite loci on various chromosomes/linkage groups could be defined are presented. Efforts to determine the level of polymorphism and to measure the genetic relationships between different *Cannabis* plants are also presented.

There were a total of 30 individual *Cannabis sativa* plants analyzed, 15 with a low Δ^9 tetrahydrocannabinol (THC) content and 15 with a high THC content.

This study determined the practicality of multiplexing primers sets to differentiate individual plants within the Cannabis sativa species. Using previously described primer sets the authors were able to produce a working multiplex, which could differentiate fourteen individual Cannabis samples of unique origin. During testing, the authors determined that there was no significant difference in base pair size between alleles typed using the single locus amplification and the multiplexed amplification. Each cannabis sample gave a unique profile showing clear differences between the generated genotypes.

References:

1. H. AlGhanim and J.R. Almirall, Development of Microsatellite Markers in *Cannabis sativa* for DNA Typing and Genetic Relatedness Analyses, *J. of Analytical and Bioanalytical Chem.,* **2003**, 376: 1225-1233.
2. S. Gilmore and R. Peakall, Isolation of microsatellite markers in *Cannabis sativa* L. (marijuana). *Molecular Ecology Notes* **2003,** 3: 105-107.

Cannabis, STRs, Multiplex

 ** Presenting Author*