B58 Validation and Application of an STR Multiplex for Discrimination of *Cannabis Sativa* Individuals

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After attending this presentation, attendees will understand the efforts to multiplex ten STR markers previously described and known to discriminate between individual cannabis plants into a single reaction.

This presentation will impact the forensic community and/or humanity by demonstrating the practicality of multiplexing primers sets to differentiate individual plants within the cannabis sativa species.

Cannabis sativa L. plants can be easily identified through morphological examination and chemical analysis; however there is an interest within the law enforcement investigation community for DNA analysis of these plants with the potential to differentiate between individual plants. This method can be used as a means of associating criminal cases and tracking cannabis distribution networks. *Cannabis sativa* L. is the most frequently used of all illicit drugs in the United States. Cannabis has been used throughout history for its stems in the production of fiber, for its seed for oil and food and for its flowers and leaves as a psychoactive drug. Short tandem repeat, STR, markers are advantageous over other markers due to their reproducibility, high discrimination power and their multiplexing capacity.

In this research project, a total of ten previously described STR markers were multiplexed into a single reaction. Five primers were selected from a set of primers previously described by FIU group[1] four from a set previously described by Gilmore's group[2] and the highly polymorphic primer described by Hsieh et al[3]. Where appropriate, trinucleotide repeats were chosen to reduce the incidence of artifacts that may affect interpretation. The forward primers of the primer sets were fluorescently tagged with either 6-FAM dye, HEX or VIC dye. Hemp DNA extracts were provided by the Alberta Research Council in Alberta, Canada. Marijuana DNA extracts were provided by the Nederlands Forensisch Instituut in Den Haag, Holland. Marijuana leaves were provided by the law enforcement agencies in Florida. The DNA was extracted from the leaf samples using a plant DNA extraction kit. The multiplex reaction was used to analyze 25 different cannabis plants. The 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 and amplified on a thermal cycler followed by analysis on a capillary electrophoresis, CE. The results where then analyzed using appropriate software. Studies using these STR markers were able to distinguish clones from non-clones. 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 25 individual *Cannabis sativa* plants analyzed, 14 with a low Δ^9 tetrahydrocannabinol, THC, content and 11 with a high THC content, in addition to a set of different plants retrieved in the State of Florida at different geographic locations.

This project determined the practicality of multiplexing primers sets to differentiate individual plants within the *Cannabis sativa* species. Using previously described primer sets, a working multiplex which could differentiate 25 individual cannabis samples was obtained. During testing it was 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.

3 H.M. Hsieh, R.J. Hou, L.C. Tsai, C.S. Wei, S.W. Liu, L.H. Huang, Y.C. Kuo, A. Linacre, J.C. Lee, A highly polymorphic STR locus in *Cannabis sativa*, *Forensic Sci. Int*. 2003, 131: 53-58.

Cannabis DNA, STRs, Multiplex

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