B156 Massively Parallel Sequencing (MPS) of 12 Autosomal Short Tandem Repeats (STRs) in *Cannabis Sativa*

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Learning Overview: After attending this presentation, attendees will understand the basic principles behind applying Massively Parallel Sequencing (MPS) techniques to sequencing autosomal STR markers in *Cannabis sativa*.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating the applicability of an autosomal MPS panel that that could not only assist law enforcement agencies in verifying legal marijuana products, but also aid in the linkage of illegal cases. This method could also serve as an additional tool to previously established marijuana profiling programs used in federal agencies such as U.S. Customs and Border Protection (CBP) and the Drug Enforcement Administration (DEA). Importantly, the methods presented could also be applied to integrate any custom PCR multiplex into a MPS pipeline.

Massively parallel sequencing (MPS) is an emerging technology in the field of forensic genetics that provides distinct advantages compared to capillary electrophoresis CE. While CE offers a reliable and robust technique, it has disadvantages such as limited multiplexing capability with a maximum of 25 to 30 loci configurable across five dye channels. In addition, MPS has the potential to provide deeper interrogation of sequence-based polymorphisms, which in turn allows for a greater power of discrimination compared to size-based STR genotyping by CE. Currently, no targeted MPS workflows have been used for *C. sativa.* Targeted sequencing is necessary for forensic comparisons and custom MPS panels can be designed by manufacturers. However, *C. sativa* is not currently a supported species for commercial MPS panels and targeted sequencing panels need to be designed in-house.

This study offers a proof of concept that MPS technologies can be applied to genotype autosomal short tandem repeats (STRs) in *Cannabis sativa*. A custom panel for MPS was designed to interrogate 12 cannabis-specific STR loci by sequence rather than size. A simple workflow was implemented to integrate the custom PCR multiplex into a workflow compatible with the Ion Plus Fragment Library Kit, Ion™ Chef, and Ion™ S5 System. For data sorting and sequence analysis, a custom configuration file was designed for STRait Razor v3 to parse and extract STR sequence data. This study represents a preliminary investigation of sequence variation for 12 autosomal STR loci in 16 cannabis samples from three different countries.

Results demonstrated that MPS can be used to genotype autosomal STRs in *C. sativa* and revealed intra-repeat variation in eight loci where the nominal or size-based allele was identical, but variances were also discovered in the sequence of the flanking region. MPS performance including read depth, heterozygote balance, noise, and CE concordance was accessed. Complete concordance was observed between the two methods when comparing the length-based alleles extracted by STRait Razor and the allele number observed by CE. Although only a small number of cannabis samples were evaluated, this study demonstrates that more informative STR data can be obtained via MPS. In addition, this study reveals a workflow that can be used to integrate any custom PCR multiplex into a MPS workflow.

Overall, this research investigates the sequence variation of 12 autosomal STR loci in 16 *C. sativa* samples and provides a proof of concept that MPS can be applied to genotype *C. sativa* samples.

Forensic Botany, *Cannabis sativa*, Massively Parallel Sequencing