A89 PCR Optimization of a Highly Polymorphic Marijuana STR Locus on Collection Cards for High-Throughput Screening

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After attending this presentation, attendees will have a better understanding of evidence archival for plant DNA and how plant DNA can be useful as forensic evidence.

This presentation will impact the forensic science community being that this is the first time collection cards and automation for plant data basing has been presented in a useful forensic context that could be implemented in all forensic laboratories and for crime scene personnel for marijuana typing.

The genetics of marijuana has long been undefined and a better understanding of the different relationships of *Cannabis* cultivars would be useful in trying to understand different grow operations and for tracing drug distribution patterns. As such, a DNA-based bioinformatics classification program using a variety of genetic markers and methodologies is being initiated. A series of different markers have been identified and published in the scientific literature in recent years; however, evaluating which markers would be ultimately the best to use (based on power of sample discrimination) is challenging at the population level. A polymorphic hexanucleotide repeat STR locus (NMI01) was selected as a genetic marker to screen our samples for initial classification by DNA. As our samples are sorted into groups, we will add more markers to determine if further individualization of the samples can be accomplished as deemed necessary from the data.

As an initial step, one goal was to define a convenient, long-term archival system for plant DNA typing of marijuana (*Cannabis sativa* L.). Forensic evidence collection and archival of plant evidence is typically performed by collecting leaf samples in coin envelopes and air drying the sample for later trace evidence analysis. Collection cards, however, are utilized for human DNA database archival of blood and saliva fluids and are considered valuable for long-term preservation of DNA evidence and for high through-put processing by PCR. Samples on these cards are stable for several years at room temperature storage. These cards are also pre-treated with chemicals that lyse cells, denature proteins and protect nucleic acids from nucleases, oxidation and ultra violet irradiation damage as well as preventing mold and bacterial growth.

Collection cards were selected and utilized the manufacturer protocol for preparation of a 3 mm diameter punch removed from the card after the plant leaf had been applied to the card. This preparation procedure took approximately 30 - 60 minutes to perform for sample batches of ten samples at a time, processed by hand. As long as green material from the fresh leaf was visibly transferred, a DNA profile was obtained from the card punch. Further parameters such as size of card punch, number of reagent washes and time between drying the punch and performing PCR will be assessed to determine if processing time can be shortened.

For the PCR reaction, a PCR kit was utilized and supplemented with custom-labeled PCR primers and PCR conditions as specified in Hsieh et al., 2003.[1] In order to conserve reagents, the PCR reaction volume for the kit was reduced in-scale from 50 to 25 microliter reaction volumes without any effect on profile quality. As a positive control, fresh bud marijuana material was used that genotyped consistently as a 22/26. Ivy (*Hedera helix L.*) and catnip (*Nepeta cataria*) were used as negative card controls. Results show that collection cards are a simple and effective means for capturing plant nucleic acids and for simplifying marijuana genotyping for high throughput processing by PCR. Further steps to stream-line processing with collection cards will be reviewed. In addition, the level of genetic diversity that we identify within our sample database with the NMI01 locus will be discussed and compared against other published data sets.

Reference:

[1] Hsieh et al. 2003. A highly polymorphic STR Locus in *Cannabis sativa*. Forensic Science International. 131: 53-58.

Cannabis, Plant, DNA

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