B36 The Use of AFLP Technology to Prove the Genetics of Patented Varieties of Sutera

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After attending this presentation, attendees will have an understanding of how forensic botany and plant DNA testing can be applied to civil casework.

This presentation will impact the forensic community and/or humanity by illustrating how forensic botany and, in particular, plant DNA testing can be used to individualize samples and answer forensically relevant issues.

AFLP, or amplified fragment length polymorphism, testing has been used for the genetic analysis of many organisms including plants, animals, insects and bacteria. This form of DNA testing requires a minimum of twenty nanograms of high quality plant DNA and can be used on any plant species. Since it is a multilocus test system, band patterns can become quite complex, therefore, it is simplest to make AFLP data interpretation from nonmixed samples (*i.e.*, single plant sources). Using only a single PCR primer set and a limited number of markers, one can easily determine if samples do not match. Additional PCR primer sets are used to confirm matches by increasing the number of markers observed and compared to approximately one hundred in total. The basic AFLP process involves restriction enzyme digestion of the plant DNA, followed by addition of adaptors (*i.e.*, short DNA sequences used for PCR primer recognition), two rounds of PCR amplification to reduce the complexity of DNA fragments to a manageable number of bands to interpret, separation of fluorescently labeled fragments on a capillary electrophoresis platform and a final inter- pretation of the data using Genescan® and Genotyper® software programs. Previous work has described validation studies on a model plant, mari- juana, to determine if AFLP may be useful for tracing drug distribution pat- terns based on the detection of clonally propagated plants (cloned plants, like identical twins, have the same genetic profile).

The authors were approached by an attorney representing a plant breeder that believed his patented variety of *Sutera*, an ornamental land- scaping plant, had been illegally propagated and was being distributed by a major seed company. The seed company, Ball Seed, was positive that no illegal propagation could have occurred. The plant breeder claimed that the only way Ball seed could have achieved plants with the particular flower color they exhibited was by crossing their variety with his patented variety based on the genetic information listed on the patent applications.

To resolve this issue, AFLP technology, was used to screen the dif- ferent plant samples and determine if they shared the same or different AFLP-DNA profiles. Seven different plant samples (EU10396 Giant Cloud, Giant Snowstorm, Lavender Storm, Blizzard, Cabana, Giant Snowflake and SC-1) plus controls (positive, negative) were tested with two different selective PCR primer sets. These data were sufficient to determine which samples were consistent with a matching versus a mis- matching AFLP-DNA profile. Ultimately, it was determined that the Giant Cloud variety of *Sutera* was not genetically the same as SC-1 and that no intentional patent violation had occurred. In fact, additional AFLP testing resolved the matter by sorting out a seed sample that had been misidentified on the original patent that explained the ultimate discrepancy between the genetics and the flower color.

The application of AFLP technology to civil cases to resolve questions of plant patent infringement is illustrated well by this case. While both parties were initially adamant about what they believed had occurred, the use of plant DNA testing by AFLP was able to quickly and efficiently resolve the situation and provide a rationale explanation for the plant flowering trait that was observed. This type of case was particularly amenable to AFLP typing since it used fresh plant material to generate the DNA for further testing. Previous work with marijuana has shown that the starting plant DNA needs to be of sufficient quantity and quality for AFLP to be useful.

Forensic Botany, AFLP Testing, Plant DNA

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