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### **B59 Botanical DNA Evidence in a Case of Robbery and Property Crime: Application of High Resolution Melting Analysis of *Triticuma Aestivum* L. Grains**

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After attending this presentation, attendees will understand the importance of the use of genetic botanical evidence in casework.

This presentation will impact the forensic science community by increasing fundamental understanding of the application of real-time Polymerase Chain Reaction (PCR) technology and DNA barcoding to the discriminatory analysis of plants.

Forensic botany is an emerging discipline that has evolved rapidly during the past few years. Botanical evidence is usually found at crime scenes and sometimes is the only available element for criminal investigations in cases where other evidence is absent. Different molecular techniques have been applied to analyze botanical evidence including Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat Amplification (ISSRs), chloroplast and mitochondrial DNA, and Short Tandem Repeats (STRs). In recent years, research has been focused on DNA barcoding to determine the species of different organisms through sequencing of conserved DNA regions such as Cytochrome C Oxidase I (COI) or Internal Transcribed Spacer (ITS). Another application of DNA barcoding is the use of real-time PCR combined with High Resolution Melting (HRM) analysis to discriminate specific conserved DNA regions of closely related botanical species. For melting temperature ( $T_m$ ) determination, an intercalating fluorescent dye is added to the real-time PCR reaction and a derivative melting curve is generated. Distinct nucleotide sequences of a conserved DNA region will provide different  $T_m$ .

A robbery and crime property case at a farm was investigated using real-time PCR combined with HRM analysis. After burning two yards of a wheat field, the suspects stole several tons of a brand new variety of wheat, transported it in a truck, then distributed it among people living in a nearby community. The truck was found by the police and wheat grains were collected from the vehicle.

Genetic analysis of wheat grains collected from the truck (evidence) was performed, and the results were compared to those obtained from wheat plants collected at the farm (crime scene). Control wheat grains were also included in the analysis. Briefly, wheat DNA was extracted from the grains using the Plant DNAzol<sup>®</sup> kit by Invitrogen<sup>™</sup> and quantified with a fluorometer Qubit<sup>®</sup> 2.0 by Invitrogen<sup>™</sup>. HRM analysis using ITS 2-3 primers was performed in an Illumina's<sup>®</sup> Eco real-time PCR system. HRM analysis showed indistinguishable melting curves for wheat grains collected in the truck (evidence) and grains collected at the farm (crime scene).

The HRM strategy enabled the "molecular traceability" of the wheat grains to the crime scene, demonstrating the usefulness of this approach for the identification of closely related species and its application as a potential forensic molecular tool. Two suspects were arrested and prosecuted.

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#### **Forensic Botany, DNA Typing *Triticum Aestivum* L, DNA Barcoding**