B142 The Development of the Field Isolation and Amplification of DNA Assay (FIA-DNA) Kit: A Revolutionary Method for Species Identification of Unknown Samples

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After attending this presentation, attendees will better understand the impact of wildlife forensics and genetic analysis on conservation and wildlife management plans for threatened and endangered species.

This presentation will impact the forensic science community by presenting a novel technique for genetic species identification of biological samples in the field for both international conservation and law enforcement efforts.

A major challenge in biodiversity conservation is the identification of unknown samples collected in the field. Most endangered species are elusive; therefore, many monitoring programs rely on non-invasive sampling using scat or hair. One difficult hurdle is identifying unknown biological samples in the field due to the reliance on genetic analysis in a laboratory. The use of specialized software and equipment is generally tied to labs located in developed countries such as North America, Europe, India, and Japan; however, sample collection and management are often conducted in rural and developing countries in Africa, South America, Asia, and remote areas of North America where most large animal populations remain.

This can cause delays in obtaining important information and is often infeasible for many on-the-ground conservation efforts with limited resources. Because there is no method of on-site species identification for these samples, money and time are wasted sending non-target samples to labs for expensive and time-consuming analyses. The goal was therefore to develop an FIA-DNA Kit for genetic field identification of species for both international conservation efforts and management of Pennsylvania wildlife without the need for Polymerase Chain Reaction (PCR) and agarose gel electrophoreses.

Once successfully implemented, the FIA-DNA Kit will allow field researchers and wildlife managers to perform a simple genetic analysis for species identification without requiring samples to be sent to a laboratory. The FIA-DNA Kit makes use of Loop-Mediated Isothermal Amplification (LAMP) and visual product detection using calcein, a novel technique which eliminates the need for a thermal cycler for DNA amplification and detection. This technique was coupled with a modified extraction protocol using Whatman® Non-Indicating Fast Technology for Analysis (FTA) Elute Micro Cards, allowing for portability and ease of use outside of the laboratory setting.

Preliminary results tested efficacy on snow leopard *(Panthera uncia*), bobcat (*Lynx rufus*), and coyote (*Canis latrans*) samples for species-specific identification. This method using LAMP amplification of DNA has exhibited 100% specificity for more than 30 previously extracted snow leopard scat samples. Coupling LAMP amplification with the modified FTA extraction protocol has yielded successful, repeatable detection in more than 50% of tested scat samples to date. This method can be completed in less than one hour with minimal equipment, using Ultraviolet (UV) fluorescence within the reaction tube to confirm species identification.

Results from initial field deployment tests of the FIA-DNA Kit at the Powdermill Nature Reserve in Rector, PA, will be discussed. The FIA-DNA Kit was tested for precision in identifying a wide range of scat samples, eliminating the time and cost of lengthy lab-based analyses. Due to LAMP amplification's ease of use, rapid reaction time, and isothermal conditions, which do not require a thermal cycler, this kit will be able to be used in the field to effectively identify samples to a species level.

The ability to pre-screen samples will also increase the efficiency and turnaround time for the genetics laboratory. Because the FIA-DNA Kit is easily modified to test for other species by developing primers, it will prove invaluable for other time-sensitive applications, including population surveys of game species, poaching cases, and outdoor crime scenes involving unidentified biological samples. Due to the expanding field of wildlife forensics, the FIA-DNA Kit holds endless potential for wide applicability across genetic and forensic disciplines.

LAMP, Species Identifiction, Wildlife Forensics