Y28 Using Loop-Mediated Isothermal Amplification (LAMP) to Identify At-Risk Species in the Field

Brooke Driscoll, Orchard Park, NY 14127; Nickolas P. Walker, BS, East Amherst, NY 14051; Jan E. Janecka, PhD, Duquesne University, Pittsburgh, PA 15282*

Learning Overview: After attending this presentation, attendees will better understand LAMP and its use as a field method for identifying species of interest for wildlife forensics.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by offering a species identification technique for trafficked animal samples that can be performed directly in the field.

Species identification through DNA analysis is relevant to population genetics, species conservation, and wildlife forensics. Unfortunately, many species of interest are endemic to areas with little access to advanced scientific technology. This means that DNA testing must be outsourced to other areas or testing must wait until the researchers are in a different location. Additionally, the biological substrate for the species of interest may contain limited amounts of DNA. This pertains mainly to highly elusive species that can only be readily studied using scat and to trafficked samples such as teeth, claws, and scales. All of these substrates pose greater DNA processing difficulties when compared to the more traditional blood samples. To better aid in the research of endangered and at-risk species, a method is needed that can accurately identify species through unconventional biological substrates with minimal laboratory instrumentation.

A promising solution to this issue is LAMP. This amplification method has similar sensitivities to traditional Polymerase Chain Reaction (PCR) but can be done at isothermal temperatures. This eliminates the need for a thermocycler during the reaction. LAMP has also been studied with fluorescent, in-tube visualization methods that reduce the need for agarose gel. The entire process is relatively fast, requiring only a 30-minute incubation. Using these methods, it is possible that species could be identified in a field environment.

The main question to be answered by this research is: can a field-accessible kit be developed for non-invasive species identification using the LAMP method? Previous research has shown that isothermal amplification can be performed with minimal laboratory equipment, but a functioning kit has not been assembled that can accurately identify species in the field. The species of interest in this study is the snow leopard, which is highly elusive and difficult to study. A non-invasive sampling source for DNA is often scat, which poses difficulties for DNA analysis due to inhibitors. Creating a method based off of this substrate will allow the method to be more adaptable to other low-level DNA substrates.

The method is conducted primarily using a heat block, a fluorescent dye, components of the Lucigen® LavaLAMP™ kit, and an animal sample. The suspected snow leopard scat is collected in Mongolia and China, dried with a desiccant material, and shipped to the United States for testing. Approximately 100 of these scat samples will be rehydrated for testing. The DNA is tested by dipping Whatman® No. 1 filter paper into the scat, then into a lysis buffer, and then placed directly into the LavaLAMP™ amplification mix. Prior to incubation, calcein and manganese (II) chloride are added as fluorescent indicators. The samples are heated on a heat block to 70° Celsius for 30 minutes. Following amplification, the samples are immediately visualized under Ultraviolet (UV) light, with fluorescence being indicative of amplification. The expected results of this study are that the scat samples can be identified with high accuracy and reliability without the use of a thermocycler or agarose gel. Initial results have shown that the method is reliable with scat samples and can be visualized using the calcein fluorescence. Once further optimized, the method will be applied in China so that samples will not need to be sent to the United States for analysis. This kit will allow wildlife forensics and species conservation efforts to be conducted in resource-poor areas to accurately identify endangered and trafficked species.

LAMP, Wildlife Forensics, Field

*Presenting Author