H21 Wildlife Forensic Investigation: Identifying an Unidentified Specimen Using NADH Subunit 2 (*ND2*) and Cytochrome B (*cytb*) Genes

Hailey Mcclenon, 2906 Palm Street, Apt 1, Houston, TX 77004; Ashraf Mozayani, PharmD, PhD, Texas Southern University, 3100 Cleburne Avenue, Houston, TX 77004; and Hector Miranda, PhD, Texas Southern University, 3100 Cleburne Street, Houston, TX 77004*

After attending this presentation, attendees will understand the principles in determining the true identity of an unidentified carcass donated by the Houston Museum of Natural History; the use of DNA markers as an indispensable tool in the practice of wildlife forensics and monitoring of endangered species; and the elements for the application of mitochondrial gene markers *cytb* and *ND2* in wildlife crimes.

This presentation will impact the forensic science community by explaining the application of two commonly used mitochondrial DNA markers to establish a true DNA profile and create a personalized barcode based upon the species' closest relatives as a reference to the animal's geographic origin which, when applied, could potentially decrease the current amount of slaughter and harvest involved in illegal trade routes within the black market.

The unidentified species sample was received by the Department of Biology at Texas Southern University. To determine the fidelity of DNA markers to identify the exact wildlife species beyond doubt, two mitochondrial gene markers, cytb and ND2, were used. DNeasy Tissue Kit was used to isolate genomic DNA. *Cytb* and *ND2* were sequenced using primer pairs L14990/H15646, L15517/H16404 for *cytb* and L5216/H5766, L5758/H6313 for *ND2*. Polymerase Chain Reaction (PCR) was carried out in a total volume of 25µL containing 10ng-50ng of template DNA, 4µL of deionized water, 4µL 10mM of each dNTP, and 8µL of AmpliTaq® 360 Gold Polymerase Master Mix. The PCR cycles were as follows: one cycle of 10m at 95°C, 35 cycles of 20s at 95°C, 20s at 60°C, and 50s at 72°C. The process was completed with a final elongation at 72°C for 10m. These parameters were adjusted to optimize PCR product quality. Amplifications were performed on Veriti® Thermal Cycler. The PCR products were about 1,000bp for *ND2* and 1,100bp for *cytb* gene. The PCR products were sequenced using ABI® 3730, then viewed and examined in Geneious Pro. Basic Local Alignment Search Tool (BLAST) was used to compare DNA sequences in Genbank (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The results suggested the specimen was a Malayan Peacock-Pheasant (Polyplectron malacense) with 100% identities for ND2 and cytb genes. This species is classified as vulnerable on the International Union for the conservation of Nature Red List. This approach of using two, instead of just one, gene marker for wildlife forensics can greatly contribute to the confidence in wildlife monitoring and trafficking.

Wildlife Forensics, NADH Subunit 2 (*ND2*), Cytochrome B (*cytb*)

*Presenting Author