B93 The Development and Validation of a Dual-Genus Quantitative Polymerase Chain Reaction (qPCR) Assay for African and Asian Elephants for Forensic Purposes

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After attending this presentation, attendees will understand how a qPCR assay has been developed that will detect and quantitate African and Asian elephant DNA simultaneously. Attendees will also learn how to efficiently create qPCR assays for wildlife identification and quantitation.

This presentation will impact the forensic science community by providing a standardized qPCR assay that detects and quantitates elephant DNA for use in wildlife investigations. The development of this assay fills a research gap in wildlife forensic science.

Real-time PCR is most commonly used in the forensic community to quantify small amounts of human DNA in evidentiary samples. In the growing field of wildlife forensic genetics, real-time PCR is utilized primarily for identifying the species of origin from illegally traded animal byproducts. Although DNA sequencing of mitochondrial DNA is the most common approach for species identification, it can be costly. Utilization of a multiplex real-time PCR assay can be a fast, inexpensive, and robust approach to species identification that can aid law enforcement in prosecuting crimes against animals.

African (*Loxodonta africana)* and Asian (*Elephas maximus*) elephant populations are categorized under Appendix I and II of the Convention on the International Trade of Endangered Species (CITES), respectively. CITES is an agreement that regulates plant and animal species throughout the world to ensure that international trade of their products does not impact their survival. An Appendix I listing includes species that are threatened with extinction; thus, trade of these plants and animals is highly restricted. Species not facing extinction that require extra attention and regulations so they don't become exploited and over-utilized are listed in Appendix II. The primary reason for the decline of these two animals is the illegal trade of their ivory. Other reasons for the decline in the elephant population are deforestation and human conflict.

In wildlife crime laboratories, species of origin can often be determined by morphology. This method is limited by the expertise of the taxonomist and the condition of the animal product. Ivory is commonly carved into small figurines and trinkets. Elephant meat, hair, and hide are traded, which can make it difficult to identify the species. These limitations have led to the development of genetic tests to identify species of origin in wildlife investigations. The *cytochrome b* (*cyt b*) region of the mitochondrial genome is well established as a genetic marker for species identification. African and Asian elephants do have highly similar genomes; however, variation exists in portions of their *cyt b* gene.

In this study a dual-genus, real-time PCR assay to identify elephant DNA for forensic purposes was developed. By eliciting information from the variable areas of the *cyt b* gene in elephants, both genera of animals can be differentially identified and quantified in a robust and cost effective assay. Costs were decreased by scaling down reaction volumes and using one set of primers. Following the assay development, a rigorous developmental validation was conducted according to current community recommendations set forth by the Scientific Working

Group for DNA Analysis and Methods (SWGDAM). The completion of this work provides an assay that can generate data of evidentiary quality for wildlife crime laboratories.

Elephant, qPCR, DNA