SAC RESEARCH NEEDS ASSESSMENT FORM

Title of research need:	Develop species-specific mitochondrial primers for comingled samples that require taxonomic identification of one or more contributors
Describe the need:	Forensic DNA typing for taxonomic identification (aka barcoding) is used to authenticate seized samples by comparison to appropriate databases (1, 2, 3). Taxonomic identification of multiple contributors in a DNA mixture allows the makeup of complex samples to be elucidated. Identifying different species in a complex mixture can result in identification of illegal or protected species when mixed with legal or non-protected species. In addition, the ability to use eDNA samples to detect illegal and injurious species in imports of aquatic organisms is in demand at US ports of entry to facilitate legal trade. When unknown biological material is subjected to DNA sequencing using non-specific, or universal, mitochondrial primers, an uninterpretable mixed sequence may be obtained. Developing species- or taxon-specific mitochondrial primers would assist in restricting sequencing results to a single entity, resulting in the identification of the species/taxon of origin. Further, wildlife forensic source materials may be contaminated by human DNA prior to submission to the forensic laboratory and developing species/taxon-specific mitochondrial primers that do not amplify human DNA would assist in identifying the wildlife species present in the sample. Reliable and detailed data on taxon of origin in mixed samples can also enhance identification of illegal species in food supplements and traditional medicines (4) and that of cryptic species, or species that can be distinguished genetically but not necessarily morphologically (5, 6). By targeting species/taxon-specific DNA sequences, the sensitivity and accuracy of forensic testing is increased, enabling more specific identification of questioned samples that could not be achieved using standard sequencing methods (7).
Keyword(s):	Species identification, taxonomic identification, mitochondrial DNA, sequencing, complex mixtures, primers, DNA typing, barcoding, eDNA

Submitting subcommittee(s):	Wildlife Forensic Biology	**Date Approved:**	10/7/2022

Background Information:

1. Does this research need address a gap(s) in a current or planned standard? (ex.: Field identification system for on scene opioid detection and confirmation)

ANSI/ASB Standard 048, Wildlife Forensic DNA Standard Procedures states there must be a protocol to interpret “sequence mixtures” (5.1.c) and that for “laboratories that report on the composition of mixture samples, a method must be established that specifically addresses analysis and interpretation of such mixtures” (6.1.2).
ANSI/ASB Standard 047, Wildlife Forensics Validation Standard - Validating New Primers for Sequencing sets requirements for species specificity of primers (4.3.2), including “species likely to be present in combination with the species of interest on evidentiary items (4.3.2.b).

2. Are you aware of any ongoing research that may address this research need that has not yet been published (e.g., research presented in conference proceedings, studies that you or a colleague have participated in but have yet to be published)?

The U.S. Fish and Wildlife Service and US Geological Survey invasive species programs have funded research into targeted PCR testing for invasive and injurious aquatic species (e.g., Dreissenid mussels, Burmese python, among others). The intent of this research is to deploy rapid testing units at ports of entry to test water samples for the target species.

3. Key bibliographic references relating to this research need: (ex.: Toll, L., Standifer, K. M., Massotte, D., eds. (2019). Current Topics in Opioid Research. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88963-180-3)

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2. Bezeng, B. S., Davies, T. J., Daru, B. H., Kabongo, R. M., Maurin, O., Yessoufou, K., van der Bank, H., & van der Bank, M. (2017). Ten years of barcoding at the African Centre for DNA Barcoding. Genome, 60(7), 629–638.
3. Sheth, B. P., & Thaker, V. S. (2017). DNA barcoding and traditional taxonomy: an integrated approach for biodiversity conservation. Genome, 60(7), 618–628.
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5. Wilson-Wilde, L., Norman, J., Robertson, J., Sarre, S., & Georges, A. (2010). Current issues in species identification for forensic science and the validity of using the cytochrome oxidase I (COI) gene. Forensic Science, Medicine, and Pathology, 6(3), 233–241.
6. Vasconcelos, R., Montero-Mendieta, S., Simó-Riudalbas, M., Sindaco, R., Santos, X., Fasola, M., Llorente, G., Razzetti, E., & Carranza, S. (2016). Unexpectedly High Levels of Cryptic Diversity Uncovered by a Complete DNA Barcoding of Reptiles of the Socotra Archipelago. PloS One, 11(3), e0149985.
7. Tobe, S. S., & Linacre, A. (2010). DNA typing in wildlife crime: recent developments in species identification. Forensic Science, Medicine, and Pathology, 6(3), 195–206.

4. Review the annual operational/research needs published by the National Institute of Justice (NIJ) at https://nij.ojp.gov/topics/articles/forensic-science-research-and-development-technology-working-group-operational#latest? Is your research need identified by NIJ?

Forensic Biology/DNA, Scientific Research, Increased information about the discriminatory power and sensitivity of alternate biological analyses (e.g., proteomics, microbiome, plants, animals) to associate individuals with crime scene evidence.

5. In what ways would the research results improve current laboratory capabilities?

Identification of species-specific primer sets would allow labs to increase the number of samples that could be tested by not eliminating mixture samples. The success rate of identifying evidentiary samples would be increased, and rapid testing would enhance the interception of illegal species.

6. In what ways would the research results improve understanding of the scientific basis for the subcommittee(s)?

Availability of species-specific mitochondrial primers is insufficient to meet the needs of wildlife forensic laboratories and law enforcement agencies that submit samples for testing and identification. Many species of interest to wildlife law enforcement are difficult to obtain but important to detect in forensic samples, however, they are not of general interest to the wider conservation genetics community. The development of additional species-specific mitochondrial primers for taxonomic identification would greatly increase the ability of species of forensic value to be identified for casework purposes.

7. In what ways would the research results improve services to the criminal justice system?

Wildlife forensic laboratories are relied upon to identify the taxonomic origin of evidentiary materials. When the desired laboratory capabilities do not exist because further targeted research is required, investigations are delayed or abandoned that could otherwise be easily completed.

8. Status assessment (I, II, III, or IV): II

	Major gap in current knowledge	Minor gap in current knowledge
No or limited current research is being conducted	I	III
Existing current research is being conducted	II	IV

This research need has been identified by one or more subcommittees of OSAC and is being provided as an informational resource to the community.