

## B54 Using Hybridization Capture to Obtain Mitochondrial Genomes From Forensically Relevant Canids: Assessing Sequence Variation for Species Identification

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**Learning Overview:** After attending this presentation, attendees will have learned the common methods to identify vertebrate species in non-human forensic casework and the limitations of those methods when trying to distinguish closely related species. Based on these results, attendees will be introduced to other regions of the mitochondrial genome that can offer species resolution for closely related canids.

**Impact on the Forensic Science Community:** This presentation will impact the forensic science community by introducing attendees to a method that can be straightforwardly used to generate full mitochondrial genomes: hybridization capture baits designed for a closely related species that isolate target DNA fragments for next generation sequencing. This method works well with low template and highly degraded samples and could be applied to other species groups.

The majority of DNA casework processed by forensic laboratories focuses on human samples, but material from canids (i.e., dogs, wolves, coyotes) can also be encountered. For example, dogs can be the victim of a crime or link a potential suspect to a victim or crime scene via their hair and biological fluids. Undomesticated canids, such as wolves and coyotes, can also be the center of forensic investigations in the United States, given that some species are endangered, meaning that hunting them in certain jurisdictions is illegal. Given many wolf species are highly similar morphologically, identification in the field by wildlife officers is often not straightforward and can be further complicated when incomplete specimens are available. Thus, molecular-based approaches are often used for canid species identification.

While some established methods using mitochondrial DNA targets can discriminate between *Canis* species, they are either not compatible with highly degraded samples (e.g., Cytochrome C Oxidase I [COI] barcode region is ~650bp) or they cannot differentiate closely related subspecies (most recent common ancestor is ~20,000 years ago). Although some United States laboratories regularly perform veterinary/wildlife casework, including canid identifications, their validated methods and the reference genetic databases they use are not publicly available. Thus, this study aimed to assess the utility of alternative regions in the mitochondrial genome for discriminating among forensically relevant canid species. To achieve this, a commercially available hybridization capture panel designed for the domestic dog (*Canis lupus familiaris*) to enrich entire canid mitochondrial genomes from Abor Biosciences™ was utilized. Briefly, this panel consists of biotinylated RNA “baits” that are complementary to the dog’s mitochondrial genome, permitting isolation of the whole mitochondrial genome from even highly degraded samples for downstream next generation sequencing. Given the baits will anneal when the template sequence is ~80% identical, it was hypothesized that the assay for the domestic dog would permit the recovery of full mitochondrial genomes from closely related wolves and coyotes. This study used this panel to successfully sequence full mitochondrial genomes for 53 samples (total input quantities as low as ~10ng), representing four United States forensically relevant canid species (coyote, wolf, Mexican wolf, and dog). While the full mitochondrial genome permitted discrimination between species and subspecies, this study also identified four ~200bp fragments from ND1, ND5, COI, and CYTB genes that could resolve the canids sampled in this study. The utility of these regions should be more fully assessed in future studies prior to implementation into casework, using forensic-type samples representing canids from diverse geographic areas.

### Hybridization Capture, Mitochondrial Genomes, Wildlife Forensics