



# "Custom Development of Molecular and Genetic Barcoding Tools for Predator Identification in Texas Horned Lizard (*Phrynosoma cornutum*) Populations"



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## Abstract & Background

The Texas Horned Lizard (*Phrynosoma cornutum*) has undergone dramatic population declines across its native range due to habitat loss, invasive species, and predation.



Figure 1: Hatchling THL with tracker tags



To support reintroduction, graduate researchers from TCU's biology program glue harmonic tags onto the backs of hatchlings, (Figure 1) making them easier to locate in the wild and to identify the most viable reintroduction areas.

Hatchlings experience high predation after reintroduction, and we often find their tags in the scat of various predators. Identifying the primary predators of reintroduced populations is critical for improving hatchling survival and informing conservation strategies.

Previous studies suggest coachwhip snakes (*Masticophis flagellum*) are the major predators of these Texas Horned Lizards.

We extracted DNA from scat that contained tracker tags and used coachwhip specific primers to determine how common coachwhip predation is at the reintroduction sites.

## Data & Discussion

Additional predators besides the coachwhip are found at reintroduction sites. Tissue samples were collected from various mammals and other native Texas snake species, and processed via DNA sequencing. (Figure 2)

The MfCOI primer was tested against all of them to ensure that only the coachwhip samples would amplify during PCR reactions. (Figure 4)

Figure 5: *Masticophis flagellum* eating a THL *Phrynosoma cornutum* adult

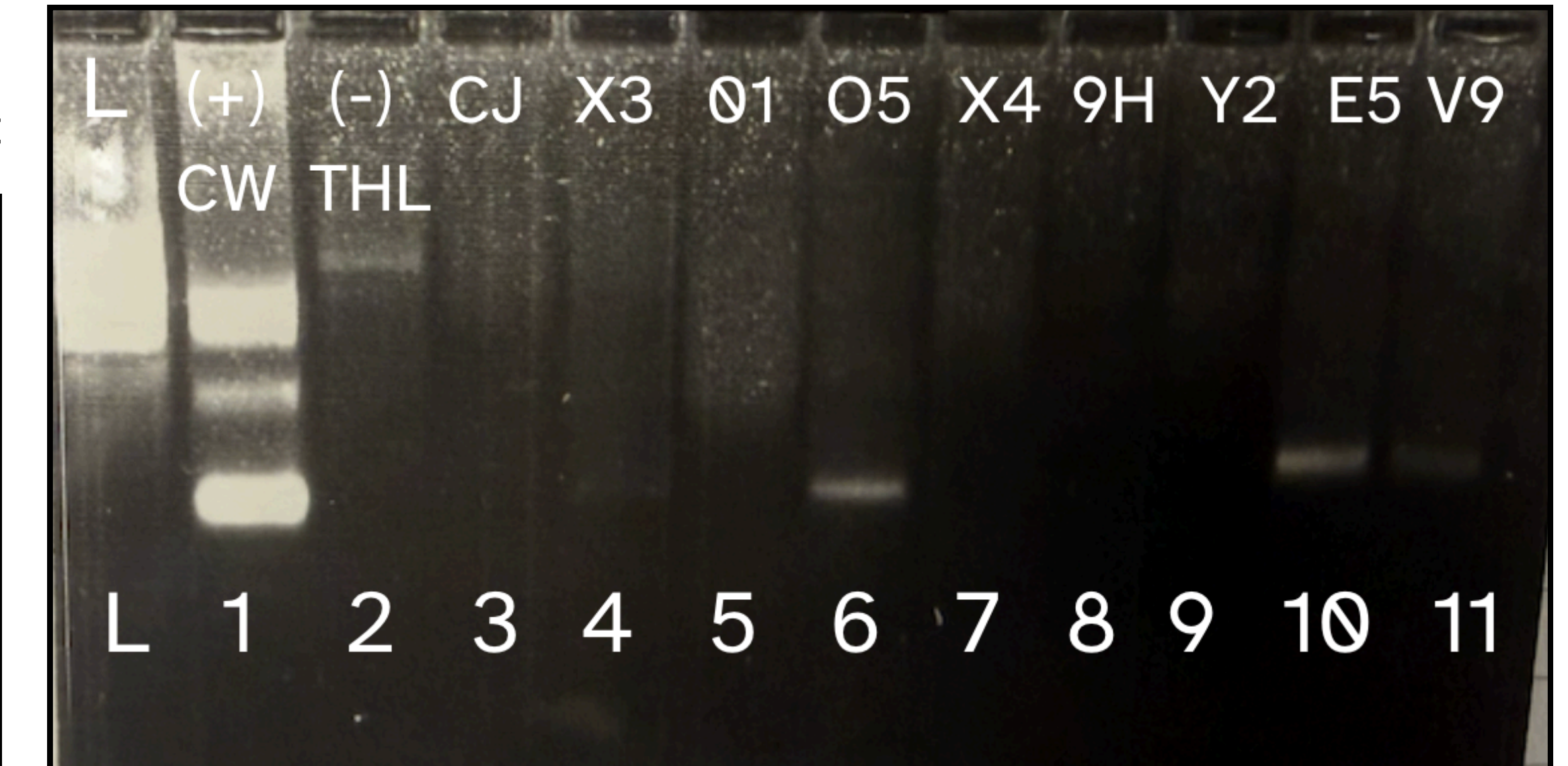


Figure 6: Example of Results from 8 samples tested with the custom MfCOI Primer. Samples with positive bands show the presence of coachwhip DNA, located in wells 4, 6, 10, and 11

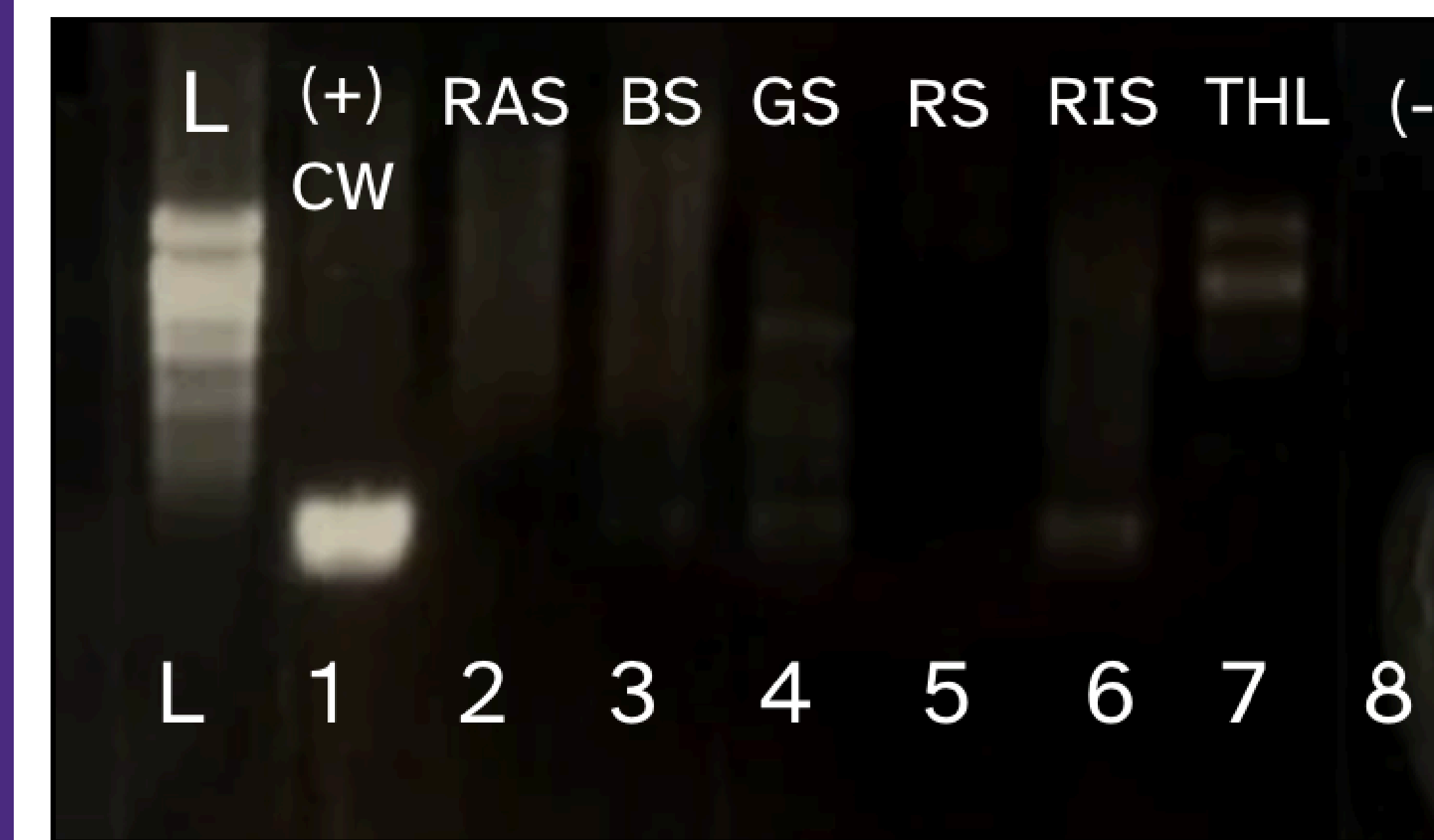
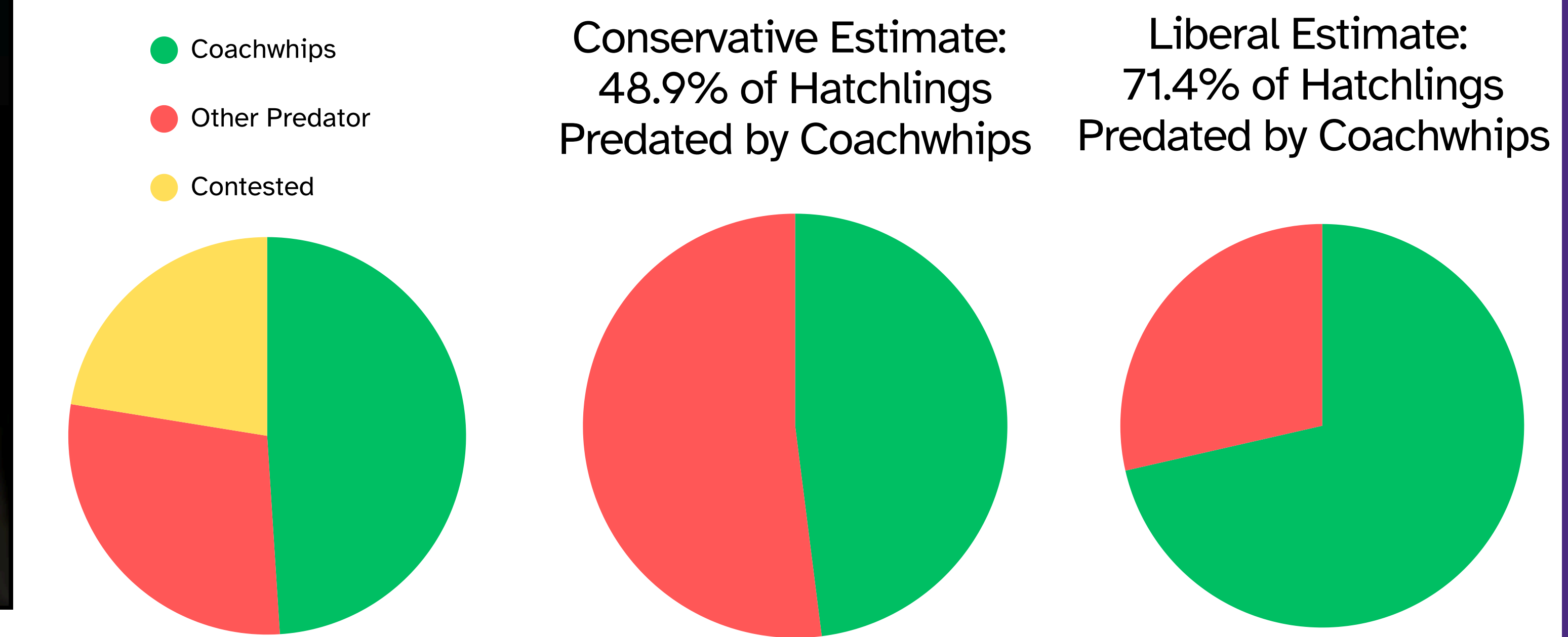


Figure 4: PCR Amplification Confirmation for MfCOI Primer

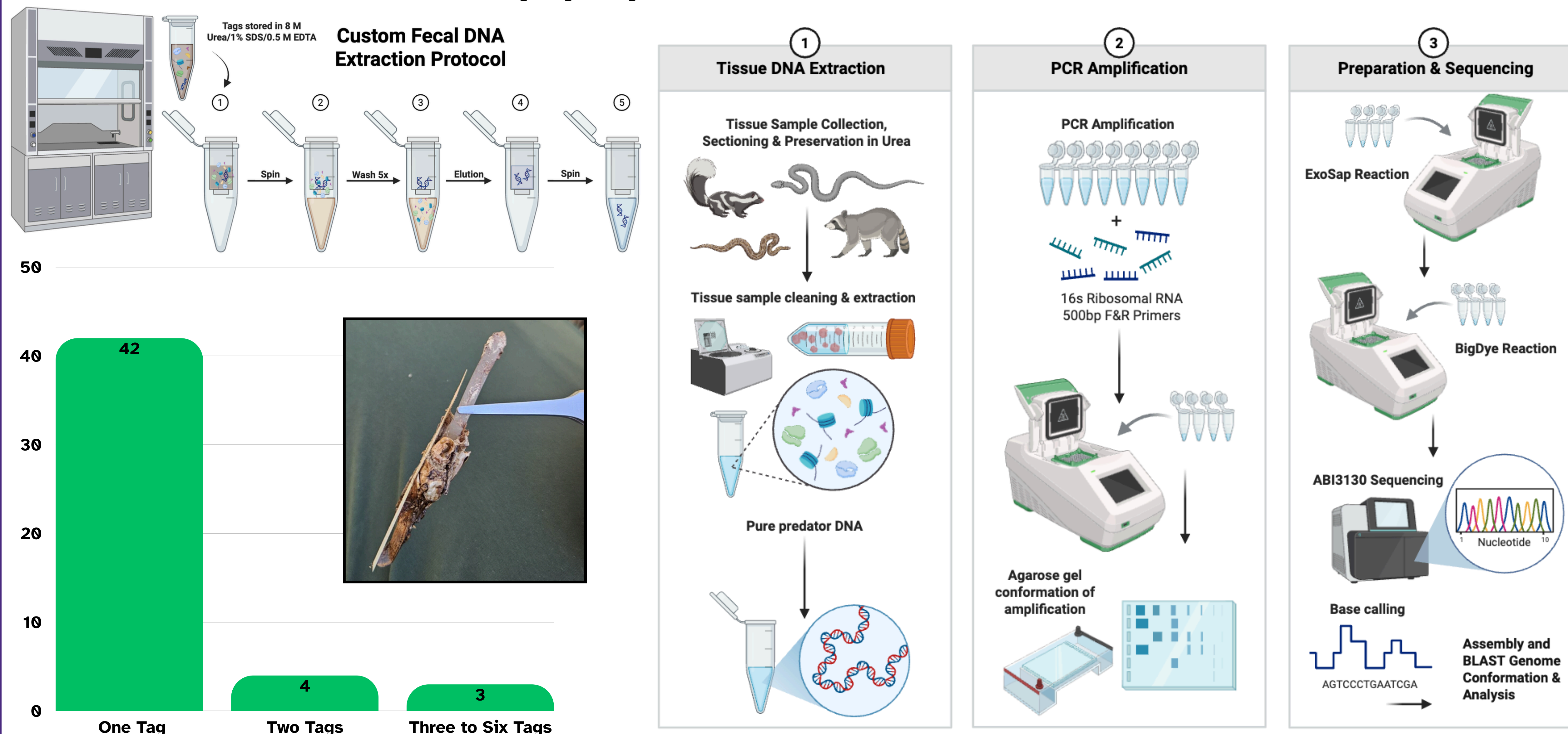
Rat Snake: RAS (*Pantherophis obsoletus*)  
Bullsnake: BS (*Pituophis catenifer*)  
Western Diamondback Rattlesnake: RS (*Crotalus atrox*)  
Green Snake: GS (*Ophedrys aestivus*)  
Ribbon Snake: RIS (*Thamnophis proximus*)



Out of 49 fecal samples with tracker tags that were tested, the Coachwhip (*Masticophis flagellum*) accounted for approximately 50-71% of all predation events.

## Methods

Figure 2: Methods and biological processes for predator tissue extraction, identification, and extraction of predator DNA from fecal samples found with hatchling tracker tags. We extracted DNA from the predator scat and developed a coachwhip-specific genetic marker and a horned lizard-specific primer to screen 75 fecal samples with tracking tags (Figure 3) from the 2024 fall season.



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