



# eDNA Marker Development Protocol

Version 1.2 – June 2021

The following is a brief guide to the biological materials needed for development of an eDNA species-specific marker. Marker development cannot be completed until all of the following have been provided:

1. **Range map of the target species and any relevant citations with distribution and/or genetic information**
2. **List of non-target species**
  - a. This should include closely related, sympatric species and other species of the same taxon (i.e. fish, amphibians, mollusks) overlapping in distribution
3. **Tissue samples from the target species**
  - a. 3-5 tissue samples from each population across its range
4. **Tissue samples from non-target species**
  - a. Closely related species: ~5 tissue samples of each species from across the range of overlap with target species (or at a minimum across the area of interest)
  - b. Distantly related species: 1 tissue sample of each species
5. **eDNA samples from known positive sites of target species**
  - a. Collect at least 5 from different sampling locations
  - b. For added marker robustness, collect eDNA samples from across the range of target species at known positive sites
6. **eDNA samples from known negative sites**
  - a. Collect 5 total (ideally these would be taken at locations with similar community composition as known positive sites)

Please keep in mind that this is to act as a standard guideline to the biological materials needed. Depending on the species of interest, the exact tissues needed may vary.

For questions about marker development, please contact: Tommy Franklin, eDNA Program Leader at NGC ([thomas.franklin@usda.gov](mailto:thomas.franklin@usda.gov)) or Daniel Mason ([daniel.mason1@usda.gov](mailto:daniel.mason1@usda.gov)).