


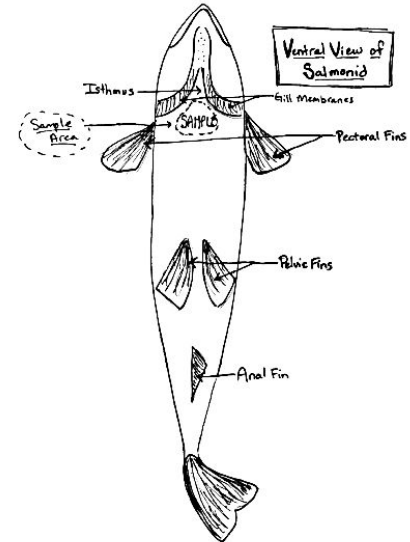
# Fish DNA Sampling Collection Protocol (adapted from UCD GVL)

- 1) **Tissue collection from fish:** There are three methods for collecting tissue samples for DNA analysis from fish; fin clips, muscle and heart tissue biopsies. Fin clips are best for alive and/ or recently dead fish. A fresh fish is defined as having clear eyes, not cloudy, and red to pink gills. Muscle tissue biopsies are best for dead fish that are defined as fresh yet have fins that are not intact, in a state of decay, and/ or have fungus on fins. If the fish is not fresh then a heart tissue biopsy must be taken. **When sampling a severely decayed fish is the only option, the heart tissue biopsy method must be used because the heart tissue is the last tissue type to decay.** For each method of genetic sampling, it is mandatory for each person handling a fish and taking a tissue sample to wear a new pair of sterile gloves for each sample taken to prevent cross contamination.

- A. **Fin Clip:** Cut a small piece of fin tissue from the caudal (preferred), dorsal or adipose fin of a fresh fish using clean scissors or a scalpel blade; use freshness of fin to decide where to clip. Tissue size should be at a minimum 5 sq. mm. (see below †), which is about the size of a hole punch. If the fin is too small to collect this size sample, take a portion of a pelvic fin. All forceps and scissors must be sterilized in a 20% bleach solution and rinsed in RO-H<sub>2</sub>O before being used again.

† Approximate size of fin clip: 

- B. **Muscle Tissue Biopsy:** Scrape any fungus, dirt, or parasites off of the skin in tissue biopsy sampling area (the blunt side of a knife works well for this). The biopsy must be taken from a muscle in the least state of decay. Take a new or laboratory cleaned (see Instrument cleaning protocol below) biopsy needle and insert the sharp metal punch portion of needle into the muscle. Once the biopsy needle is inserted, rotate the needle around to grab and disconnect the sample tissue. Slowly pull out the biopsy needle and check if there is muscle tissue inside the metal punch. If not, retry until muscle tissue is collected. When the biopsy is successful, eject the tissue into a cryotube filled with 95% ethanol and securely close.



- C. **Heart Tissue Biopsy:** Scrape any fungus, dirt, or parasites off of the skin in tissue biopsy sampling area (the blunt side of a knife works well for this). Alternatively, use sterilized scalpel or fillet knife (see Field Instrument Cleaning Protocol) to cut open fish just below isthmus where bone ends and organ cavity begins, the heart is located just below the surface. Take a new or laboratory cleaned (see Instrument cleaning protocol below) biopsy needle and insert the sharp metal punch portion of needle into the ventral side of fish and into the heart. Once the biopsy needle is inserted, rotate the needle around to grab and disconnect the sample tissue. Slowly pull out the biopsy needle and check if there is heart tissue inside the metal punch. If not, retry until heart tissue is collected. When the biopsy is successful, eject the tissue into a cryotube filled with 95% ethanol and securely close.

- 2) **Storage of tissue samples:** Each tissue sample is stored separately in individual containers: coin envelopes for dry fin clips, and cryotubes for wet fin clips or biopsy tissue samples.

- A. **Dry Fin Clips:** It is critical that samples be completely dry in order for DNA extraction in the lab to be successful.

- (1) Label a standard scale envelope (unbleached kraft paper) with all relevant details (date, water body, location (latitude and longitude or UTMs if available), species, individual fish identification number, length, weight, etc.)
- (2) Air dry the samples on filter paper until all mucus and moisture in the fin has evaporated and the tissue is dry to the touch. Place the fin clip in the envelope and loosely close the envelope. Do not seal the envelope, as air and moisture should be allowed to escape to help the fin sample dry out. Do not rubber-band envelopes together until samples inside are **completely dry**.

B. **Biopsy and wet tissue samples:** Alternatively, collected wet tissues may be deposited into a preservative-filled (at least 95% ethanol) cryotube. It is crucial that wet tissue samples be completely immersed and not exposed to air (vial should be filled to the top with EtOH after sample is present). Exposure of alcohol-stored tissue to air can cause cell wall fracturing and loss of DNA into the liquid buffer. A minimum 10:1 ratio of preservative to tissue is desired.

- (1) After placing tissue sample in cryotube filled with EtOH, it is best to keep cool and out of sun, preferably in a cooler until samples can be placed in laboratory refrigerator.
- (2) Label each vial with either pre-printed labels (preferred) or a permanent (Sharpie) marker on blank cryotube labels (less preferred since EtOH can wash off marker). Ensure each sample can be identified later by including the following information on each label: locality/ reach, sample number, collection date, and species.

### 3) Field Instrument Cleaning Protocol:

When instruments used for genetic tissue collection need to be reused, remove any tissue on the instrument, and clean by immersing in: 10% bleach solution vial, then DI water vial, and then 70% EtOH vial. Visually inspect to ensure no tissue is left on/ in instrument. If residual tissue is unable to be removed, DO NOT RE-USE.

For fillet knife used in Heart Tissue Biopsy, dip blade into 50% bleach solution vial and dry with clean paper towel.

### 4) Instrument Cleaning Protocol:

Materials needed:

- 10% household bleach solution (mixed with tap water)
  - DI water in wash bottle
  - 70% ETOH in wash bottle
  - Kimwipes or paper towels
- 1) Rinse all tissue and body fluids off of instruments, vials, petri dishes, and any other item being exposed to sample tissue using tap water.
  - 2) Submerge items in 10% bleach solution for at least 5 minutes. Be sure to completely expose all surfaces to bleach, including opening and closing scissors while submerged.
  - 3) Remove items from bleach and rinse with DI water. Make sure to remove all bleach.
  - 4) Rinse all items with 70-100% ETOH.
  - 5) Thoroughly dry items with a fresh Kimwipe, paper towel or air dry on clean paper towels.