

FISH DNA SAMPLE COLLECTION PROTOCOL

Genomic Variation Laboratory

Updated 5/2007

1) **Collecting tissue from the fish:** Cut a small piece of fin tissue from the caudal (preferred) or adipose fin of a live fish using clean scissors or a scalpel blade. Hands of the collector should be cleaned of mucus and scales between handling fish, and scissors/knife should be rinsed between samples. 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.

2) **Transferring tissue to storage container:** Each tissue sample is stored separately in individual containers: coin envelopes for dry fin clips, or cryotubes for wet tissues or fin clips.

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 UTM's 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. **Wet tissue:** Alternatively, collected tissues may be deposited into a preservative-filled (typically DMSO or 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). 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) Place the fin clip into a small glass or plastic vial containing high strength (80% to 95%) ethanol. The ethanol will preserve the tissue and the DNA at room temperature, so does not need to be refrigerated.

(2) Label each vial with a permanent (Sharpie) marker. Ensure each sample can be identified later by including the following information on each label: locality, sample number, collection date, and species. (see below example §).

3) **Recording data:** The date of collection, detailed locality information (accurate description of locality is critical – include GPS info if possible), collector(s) name, species, subspecies, type of collection (e.g. fin clip), fork length, and sex, should be written on data sheets. Use the following abbreviations for species identity: CAGT = California golden trout, LKGT = Little Kern golden trout, KRRT = Kern River rainbow trout, and RBT = rainbow trout. Use “CAGT/RBT” format to indicate fish that clearly appear to be hybridized with rainbow trout.

4) **Storing samples:** Samples must be kept out of extreme sun/heat (e.g. dashboards, hot warehouses), especially those in ethanol, as this may damage the DNA.

5) **Shipping samples:** Repackage dried fin clips separately and attach field notes for shipping. Dry samples can be sent surface mail with no special packaging.

† approximate size of fin clip:



§ sample cryovial label:

S. Fork Kern R. @Kennedy Mdw. #12 7/26/2004 CAGT
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