

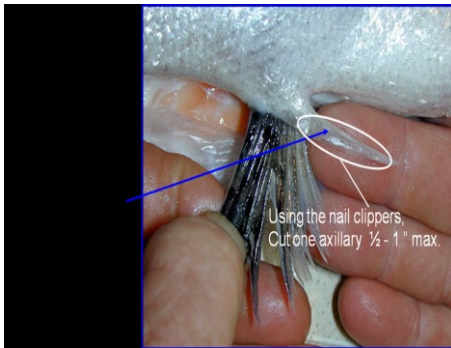
Non-lethal Sampling Finfish Tissue for DNA Analysis

ADF&G Gene Conservation Lab, Anchorage

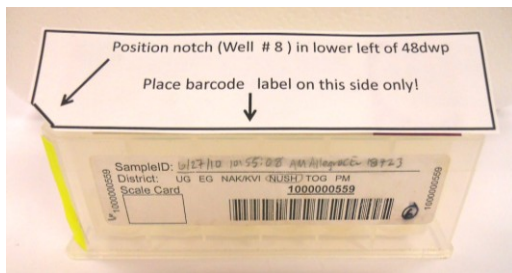
I. General Information

We use axillary tissue samples from individual fish to determine the genetic characteristics and profile of a particular run or stock of fish. The most important thing to remember in collecting samples is that **only quality tissue samples give quality results**. Tissues need to be as “fresh” and as cold as possible and recently moribund. This will be a dry sampling method and samples must be covered with EtOH either at ½ session (2 hrs. lapsed time) or directly after ending a sampling session (approx. 4 hrs. lapsed time maximum) **NO LATER - NO EXCEPTIONS!**

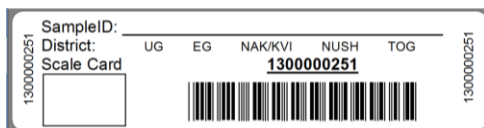
Sample preservative: Ethanol (EtOH) preserves tissues for later DNA extraction without having to store frozen tissues. Avoid extended contact with skin.



Axillary process, clip one per fish per well.



Apply barcode label on notched side of each plate. Label should be facing sampler.



Barcode will be color coded for each year. The first 2 digits indicate year; **2013**.

II. Sample procedure:

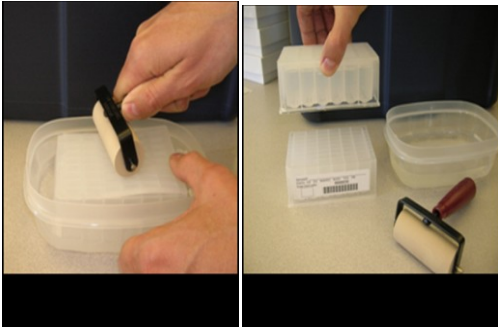
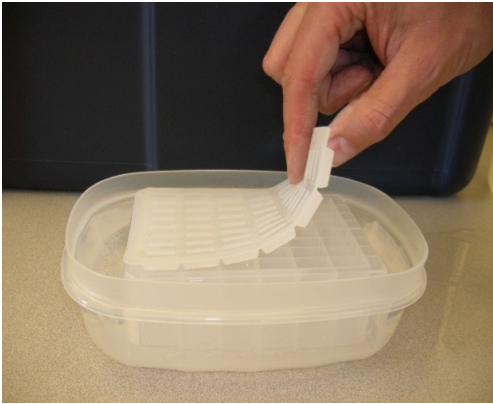
1. Tissue type: Axillary process (see photo).
2. Dry sampling method; tissue samples will be collected directly into **DRY** 48 deep well plate (48dwp) and stowed in a **cool place** out of the sun until each sampling session is complete **or** up to 4 hours maximum without EtOH. If you find it works best to take a break and fill 48dwp sooner with EtOH, that's GREAT!
3. Prior to sampling session, label 48dwp with pre-printed barcode labels provided by GCL. Labeling 48dwp should be done in a dry area to insure the labels stick well to the 48dwps. Sheet labels will be packed in ZipLoc bag to keep labels dry at all times. Apply barcode label on notched side (see picture left) of each plate.
4. For each 48dwp pre-printed barcode label, record the following information:

Date, Time Stamp, Allegro number: fill information on SampleID: _____ line provided (**see label left**).

District: Scratch out pre-printed district indicating where you are sampling (ex: **EG**) Egegik.

Scale Card (Letter): enter letter from corresponding scale card in [box] provided on each label.
5. Prior to sampling; wipe excess slime with fingers from the axillary process to avoid any excess water or fish slime in the 48dwp.
6. Using nail clipper, clip off one axillary process from each fish (see photo above) and place one axillary clip per well within the 48dwp. **NEVER** put two clips in one well within the 48dwp.
7. Periodically, wipe or rinse the clippers so not to cross contaminate samples.
8. Steps for filling 48dwp with EtOH (**see picture diagram**):





III. Supplies included with sampling kit:

1. Dog clipper - used for cutting axillary process.
2. Sample template – guide for holding 48dwp while sampling.
3. 48 deep well plate (48dwp) – holds 48 fish per plate. One fish per well only!
4. Rubber mat – mat caps each 48dwp for shipment.
5. Roller or 2x4 – use to seal the plastic mat on 48dwp for field storage and transport.
6. Re-useable “filler” plate with holes – for filling 48dwp with EtOH. Will need rubber mat to make this work.
7. Plastic container – use when filling each 48dwp with EtOH (see diagram).
8. Ethanol (EtOH) – in 5 gallon can and/or Nalgene bottle(s).
9. Squirt bottle – to top off any wells with EtOH (snip nozzle for increase flow rate) “**OPTIONAL**”.
10. Sampling instructions and laminated “return address” labels.

- a. After samples are taken; either at $\frac{1}{2}$ session (2 hrs. lapsed time) or directly after ending a sampling session (approx. 4 hrs. lapsed time maximum); fill the 48 dwps $\frac{3}{4}$ way with EtOH covering the samples.
 - b. To fill 48dwp with EtOH, use the 48dwp filler plate with pre-drilled holes in bottom for metered EtOH amount into each plate. The plastic container will hold the EtOH for each plate. Pour the EtOH into the container up to the pre-marked line that determines the proper level of EtOH. Only fill the 48dwps with samples from your recent sampling session. One plate will hold 48 fish total.
 - c. With EtOH in container; fill EtOH to “**lip**” level on container and submerge the pre-drilled 48dwp filler plate into container. Using a wood 2X4 or roller provided, secure the plastic mat on 48dwp. This will create a vacuum and hold the proper level of EtOH needed per 48dwp sampling.
 - d. Lift the “filler” plate and stack directly on top of the 48dwp with tissue samples. The top plate will stack into place over the bottom plate filled with samples. With the filler plate secure, gently peel off the mat and wait 15-30 seconds for the EtOH to transfer into the lower 48dwp. EtOH will be evenly distributed into the 48dwp wells below covering the samples taken.
 - e. Repeat the filling step until you have all plates from your sampling session filled with EtOH. Quick glance; make sure **all tissue is covered with EtOH** before rubber mat seals each plate. Top off if needed. Leave room at top of each well for mat indentation (depth of rubber mat/well) for proper seal of mat.
 - f. Using squirt bottle, top off any wells to cover tissues in EtOH and secure rubber mat securely onto 48dwp using wood 2x4 or roller. The squirt bottle is for day use only since it will leak if unattended.
9. Ethanol/tissue ratio should be **slightly less than 3:1** to thoroughly soak the tissue. EtOH levels have been pre-calculated with the 48dwp “filler”plate.
 10. Seal each 48dwp with rubber mat using the wood 2x4 or mat roller for a proper seal.
 11. Store all mat sealed 48dwp’s containing tissues upright at room temperature away from heat. In the field: keep samples out of direct sun, rain and store mat sealed 48dwp in a dry, cool location. Freezing not required.
 12. **Tissue samples must remain in 48dwp with ethanol** until shipped to Anchorage. Please follow packing instructions provided for shipment.

IV. Shipping: Hazmat paperwork is required for return “wet” shipment of these samples.

Ship samples to:

ADF&G – Genetics
333 Raspberry Road
Anchorage, Alaska 99518

Lab staff: 1-907-267-2247
Judy Berger: 1-907-267-2175
Freight code: 11100066-11160950