

Protocols for Sample Collection, Shipment and Record Keeping for the Southeastern Cooperative Project on Fish Genetics

I. Isozyme analysis:

1. Depending on the size of the samples and/or prior agreement, whole fish samples or dissected tissues or fin clips can be shipped to the laboratory. Whole fish samples, if they are smaller than 5 inches, can be wrapped in food grade aluminum foil along with a label (use pencil), and collectively placed in a labeled Ziploc bag.
2. Tissue samples should be collected in a 2.0 ml Nalgene cryo-vial (Fisher Scientific Catalogue # 03-337-7W) or 2.0 ml Corning cryo-vial (Fisher Scientific Catalogue # 09-761-71). The vial only needs to be filled 1/3 to 1/2 of the way (with the muscle, liver or eye tissue only). **Please do not fill to the top.**
3. Tubes should be labeled individually with the fish number, the type of fish, water body of origin and type of tissue. Please use a **permanent marker or sharpie** when writing on the tubes. To make the writing even more secure, the writing area can be wrapped with scotch tape. We suggest labeling the tubes before going out into the field.
4. All tubes from each sampling site should be grouped together and the bag should be labeled with the pertinent information such as type of fish, number of fish sampled, name of population and date sampled.
5. During collection, appropriate data should be recorded, for example length, weight, date collected, person collected etc.
6. Dissecting tools must be wiped clean between each tissue and each fish. Instruments should be rinsed and wiped between each tissue type and each fish. The dissection area should be wiped between samples. This prevents cross-contamination of samples.
7. Tissues needed for isozyme analysis are muscle, liver and eye unless otherwise specified. (e.g. LMB only requires muscle and liver)
8. Tissues should be obtained from a live specimen. If this is not possible, fish in the field should be buried in crushed ice until the return to the facility. Do not keep specimens in ice more than 6 hours. Please do not freeze the fish and take the samples later.
 - The fish samples must be at least two inches long (total length), if the samples are less than two inches in total length, please contact **Dr. Huseyin Kucuktas**.
 - Samples collected out in the field should be quickly frozen with liquid nitrogen or in an ethanol/dry ice bath.
 - When taking the liver sample, it is important that the gall bladder is not included. The bile will break down the tissue and degrade the enzymes. Gall bladder has a yellow or green color to it.
 - When taking muscle samples, take only the muscle; not the skin. Clean scales from the muscle samples.
 - When taking the eye samples, cut around the eyeball, try to remove all muscle around it. If the eye is large to fit in the tube, cut it in half, remove the lens and then put it in the tube.
9. When sample collection is complete, the samples should be either stored in liquid nitrogen or in a -70°C freezer. If this is not available, they could be stored at -20°C in a frost freezer for a short time. If the tissue samples are stored in a frost-free freezer, the freeze-thaw cycle of the freezer will destroy the enzyme activity. If you are unsure about your freezer or do not have adequate storage, the samples can be shipped to us immediately.

II. Mitochondrial DNA analysis, DNA Fingerprinting, and PCR analysis

1. Tissue samples for DNA analysis can be obtained from live or recently dead fish. For DNA analysis, including mitochondrial DNA, RAPD, RFLP and microsatellite analyses, the best tissue to use is blood, and muscle. However, fin clip is most widely used because sampling is relatively fast, simple, and is non-lethal. Fin clips from a population may be placed in separate vials (one sample per vial) containing 95% ethanol.
2. All tubes and bottles used for this purpose must be leak resistant. All caps and lids must be tightly secured to prevent leakage. Caution must be exercised with labels on the specimen collection bottles as ethanol dissolves the mark of most type of permanent markers.
3. Prevention of cross-contamination between the fish and between the tissues is essential- especially for the tissues being sent for PCR analysis. In this analysis, it is possible that even a single molecule of DNA from another individual could be detected, making the data questionable for that particular sample. When taking liver or fin, make sure the dissection area is clean between each fish. Again, instruments and hands should be wiped and rinsed between each fish.

III. Shipping Samples

1. Samples that are stored in liquid nitrogen should be delivered to the laboratory personally. Frozen samples **must be** shipped on dry ice, with next day delivery option. Samples stored in ethanol can be shipped at room temperature (again- all tubes and bottles used for this purpose must be leak resistant. All caps and lids must be tightly secured to prevent leakage). The Auburn Fish Genetics Laboratory should be notified prior to delivery or shipping.
2. Please contact **Dr. Huseyin Kucuktas** for shipping samples and any other issues regarding to Southeastern Fish Genetics Projects. Other personnel at the laboratory may also be contacted for shipping samples.
3. Always include a [project initiation](#) form.
4. Please indicate any deadlines or special circumstances requiring rapid analysis of samples. The laboratory will make the greatest possible effort to accommodate any of these special needs. This information is critical for prioritizing sample analysis.
5. Individual states and shipping biologists will be informed that the samples arrived, Auburn will inform the individual states and shipping biologist of what information was received concerning the samples and indicate receipt of any information concerning the schedule of analysis to ensure that such information was transferred properly and clearly.

Our address is as follows:

Department of Fisheries and Allied Aquacultures

Attn: Dr. Huseyin Kucuktas

203 Swingle Hall

Auburn University, AL 36849

Tel: (334) 844-4085

Fax: (334) 844-9208

E-mail: kucukhu@auburn.edu