

## PROTOCOL FOR COLLECTION OF TISSUES FOR GENETIC ANALYSIS

There are two different methods for the collection and storage of fish tissues depending on the kind of biological analysis: FREEZING and ETHANOL. Freezing is the best method for the collection and storage of specimens for electrophoresis and other biological analyses, like biochemistry and physiology, because of the liability of enzymes in vitro.

Proper cryogenic storage will preserve enzyme activity and minimize breakdown. Genetic studies with samples preserved on ethanol, can only be used for molecular analysis such as DNA amplification and sequencing.

The general procedures for processing the samples are as follows:

### A. CLEANLINESS OF SAMPLES:

Contamination must be avoided. Clean the knife or razor blades for cutting each fish. (Contamination might occur if a cutting tool is used on different fish without cleaning. If contamination occurs, PLEASE annotate this in the sample's information).

### B. ETHANOL COLLECTION

#### SAMPLING

1. From each individual, tissues required are heart or skeletal muscle.
2. Cut some pieces of tissue and place it in vial with 96% ethanol (70% ethanol is also enough). Smaller pieces are better than larger ones, to ensure penetration of ethanol.
3. Place vial in plastic bag and label.

#### SHIPPING

1. Shipment can be carried out by any mail regular system or other deliveries, to the address below

### C. FREEZING COLLECTION

#### SAMPLING

1. From each individual, tissues required are liver, heart, and skeletal muscle.
2. All samples must be frozen, as soon as possible, after collecting. If this is not possible, you may keep the sample on ice until arriving in the laboratory. Then, keep the samples immediately in the freezer.
3. A small portion of approximately 3 x 3 x 3 cm each side is sufficient. (The whole fish is not required since it takes too much space and weight).
4. Tissue extracts must be placed into labeled plastic bags. Complementary data about age and sampling location are needed.

## SHIPPING

1. Samples must be shipped on dry ice. (A sty-foam cooler is sufficient). If you don't have dry ice, alternatively, you can cover up the cooler with ice and bring into the freezer for some days before shipping.
2. Shipment must be carried out by Air Cargo System in regular flight as personal cargo (this way is faster and cheaper than the other deliveries).
3. Send us, by fax, the "Air Waybill" document. This document is necessary for us to pick up the parcel at the Barcelona airport. As personal cargo, I can remove the parcel directly from the Spanish customs, on the same day, 2 hours after the arrival of the flight .

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