

## WARM SPRINGS CONSERVATION GENETICS LAB

### Standard Operating Procedure

#### Collecting tissues samples for genetic analyses

##### **Field notes**

Collections that lack precise documentation are almost worthless, and the Conservation Genetics Lab (CGL) reserves the right to refuse specimens that are not properly documented or preserved. Therefore, *proper field documentation is required for each collection submitted to CGL*. Such documentation is best achieved when the researcher responsible for each collection maintains a field note book. Field notes are a part of the specimen collection and are kept for reference by CGL; therefore while field notes are tedious and time consuming to compile, they are an invaluable reference source about the collection. Important information that should be recorded in field notes are *field number, state and locality data, sampling site, drainage, latitude and longitude (UTM data preferred), date, names of people and agencies who collected samples, genus and species, length (metric preferred), weight (metric preferred), tag number (when appropriate) and preservation type*.

##### **Species identity and vouchers**

Taxonomic certainty is required when identifying specimens from which tissues are taken. Voucher specimens should be associated with each tissue sample if doubts exist about species identification.

##### **Detailed sampling protocol**

Tissue types and methods in which they are processed vary among researchers. DNA has been obtained from formalin-fixed tissues, but the process is difficult; therefore, tissue for genetic analysis should be made prior to formalin fixation.

The amount of tissue required to conduct genetic studies can be relative small, and such tissue can be obtained from areas (e.g., fins) where the removal does not affect the fish. For example, a 5mm<sup>3</sup>-piece of tissue (about the size of a pencil's eraser) is more than sufficient for several separate DNA extractions that can be amplified by polymerase chain reaction (PCR) and used for most phylogenetic and population genetic studies.

The right pelvic fin of fish greater than 2 cm typically yields enough DNA for most genetic studies and is fairly non-destructive to the rest of the fish. If the pelvic fin is large, a hole punch can be used to take a smaller sample

A 5mm<sup>3</sup>-piece of tissue should be preserved in approximately 1 mL of non-denatured\* EtOH ( $\geq 95\%$ ); thus, 1.5 mL polypropylene tubes are ideal for storage of tissue samples. Each tube should be marked to identify tissue sample. Do not write on outside of vial or vial cap with a permanent marker such as a Sharpie because any EtOH that might leak during transportation will dissolve the identifier. CGL recommends placing a tissue tag printed on a laser printer in each

tube. *CGL will provide tissue vials containing EtOH and an individual tissue tag to any agency upon request.*

The initial tissue dehydration process dilutes the EtOH resulting in a final EtOH concentration often <70%. For this reason, changing the EtOH on samples within a few days after collection, then again after a month (or until leaching is completed) often is necessary. If researcher does not have EtOH, samples should be sent to CGL in one month of collection for proper fixation. Failure to properly fix and maintain fin clips over time will result in degradation of the sample.

Tissue samples preserved in EtOH can be stored at room temperature, refrigerated, or frozen at -20 °C or -80 °C (a cool constant temperature will minimize EtOH evaporation); Care should be taken to keep the samples out of direct exposure to sunlight or high intensity, fluorescent lighting.

### **Tissue sample contamination**

Care should be taken to prevent cross-contamination among tissue samples. Ideally, sterile surgical gloves should be worn to prevent contamination with human tissue, and instruments should be cleaned and sterilized after each use. However, such precautions are often inconvenient for field researchers. Contamination of fish tissue with human tissue is of little concern because the PCR primers, which have a sequence that is different from that of humans, will only work on closely related DNA sequences. Wiping the cutting instrument (e.g., scissors or scalpel) after every use and ensuring that remnants of tissue or blood are not present before dissecting the next specimen will eliminate most sample contamination issues.

\*Do not use denatured alcohol (e.g., ethyl rubbing alcohol) because the denaturing agents (xylene, toluene, denatonium benzoate, methanol, naphtha, pyridine or acetone) have unpredictable effects on the long-term stability of the tissue, rendering DNA analysis difficult.

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##### **Truncated sampling protocol**

- Using forceps, cut a small (about the size of a pencil's eraser or ●) piece of tissue from right pelvic of fish. Wipe cutting instrument before dissecting the next specimen.
- Place tissue in 1.5ml tissue tube containing > 1 mL of non-denatured\* EtOH ( $\geq 95\%$ ) and a tissue tag. Avoid use of Sharpies because any EtOH that might leak during transportation will dissolve the identifier.
- In field notebook, record tissue tag # and other identifiers such as field number, state and locality data, sampling site, drainage, latitude and longitude (UTM data preferred), date, names of people and agencies who collected samples, genus and species, length (metric preferred), weight (metric preferred), tag number (when appropriate) and preservation type.
- Either recharge vials with fresh EtOH in a few days (but no latter than a month after tissue collection) or return to CGL in a month after collection.
- Tissue samples preserved in EtOH can be stored at room temperature, refrigerated, or frozen at  $-20\text{ }^{\circ}\text{C}$  or  $-80\text{ }^{\circ}\text{C}$  (a cool constant temperature will minimize EtOH evaporation); Care should be taken to keep the samples out of direct exposure to sunlight or high intensity fluorescent lighting.

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