

STEP 6

Obtaining tissue samples for DNA analysis

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In current research, DNA analysis is performed intensively by many scientists. Many museums collect and store tissue samples for DNA analysis, besides whole specimens.

In this section, methods of collection and storage of tissue samples in our museum are discussed.

Tissue samples should be collected after assigning the number tags (STEP 5) and before applying formalin (STEP 7) to the specimens, so that each tissue sample is assigned the same individual number as the specimen. Moreover, formalin consti-

tutes a limiting factor for DNA analysis.

In taxonomic study of fishes, scientists examine the left side of the fish body. Therefore, muscle tissue samples should be collected from the right side of the body, so that the important characters for taxonomy such as the lateral line and pectoral fins are preserved. Enough genomic DNA can be extracted from at least a 5-mm² muscle tissue section. However, if possible, it is better to obtain a 1-cm² muscle tissue section, as reserve. In addition, the muscle tissues samples should not contain other material such as scurf, fat, and blood. This is because the scurf will produce a smear on the tissue sam-



Fish (right side of body) with tissues excised for samples. White part indicates the point of excision.



Tissue sample and data label (specimen number and species name) in a screw-cap sampling glass bottle.



Screw-cap sampling glass bottles used for tissue samples.



Data (specimen number, species name, and sampling site) written on a screw-cap.

ples when they are immersed in ethanol, and the fat and blood cells will hinder DNA purification.

It is difficult to collect muscle tissues from some small fish species. In this case, the right pelvic (abdominal) fin is collected as an alternative for muscle tissue. Fishes have a pair each of pectoral fins and pelvic fins. Pelvic fins have relatively low mutations as compared with pectoral fins and are not used as an alpha-level taxonomy. Thus, they can be excised.

The obtained muscle or fin tissue samples are put into screw-cap sampling glass bottles filled with 99.5% ethanol.

A 20-cc screw-cap sampling glass bottle is well suited for a 1-cm² muscle tissue section. A large tissue sample should not be put into a small bottle to prevent penetration of ethanol into the whole tissue. A small note indicating the individual number and species name should be included with each tissue sample. The note should be written with a regular or a mechanical pencil on waterproof paper.

For better storage of the tissue samples, ethanol should be changed regularly, as it becomes hazy because of water oozing out from the tissues.

In our museum, the screw-cap sampling glass bottles containing the tissue samples are stored in boxes. This arrangement makes it easy to locate a

particular sample in order to write the same individual number as the specimen and the species name on the cap. For this purpose, an ethanol-proof pen should be used, since the ink of a normal felt pen will get washed away by ethanol, erasing all information. The tissue sample boxes are stored in the deep freezer for subsequent analysis.

Previously, the liver was used to extract enough DNA from fresh fish samples, since it contains the largest amount of DNA. However, it contains a large amount of fat, as well as sugar and protein. Therefore, nowadays, a liver tissue sample is not suitable for conducting DNA analysis.



Tissue samples in screw-cap sampling glass bottles in a box. The box is placed in deep freezer after closing the lid.

Pre-fixation

→ Step 7