

## Sample protocol for material to be used in DNA barcoding

Effective DNA barcoding depends on the quality of the biological material. Following this simple sampling protocol will ensure proper preservation of biological samples for DNA studies.

### For mammals, fish, birds and large invertebrates

1. Freeze whole individual specimens in plastic bags; use a write-on label to record vessel/expedition name/code, locality or station number, latitude and longitude, date, species name and collectors name. Store labelled specimens in freezer.
2. For large specimens that are impractical to freeze and return to the lab, take a small piece of muscle tissue from any location on the body (a half thumb size piece of muscle tissue) and freeze in a labelled clip-top mini-grip bag or a cryo-vial. Label the bag/vial with vessel name/code, locality or station number, date, species name, and name of scientist making the identification. Photograph the whole specimen before discarding, and cross reference the digital photo to the tissue sample code. It is essential that species-diagnostic characters can be seen on the photograph.
3. Avoid formalin work areas for handling specimens.

### For small fish and invertebrates

1. Use 96% pure ethanol (~80-85% for fragile arthropods) for fixation and preservation. Do not use denaturated alcohol.
2. Label all samples with locality, coordinates, date and collector.
3. If sample contain considerable amount of water (e.g. kick samples etc.), exchange the sample liquid with fresh ethanol after a day or two if possible.
4. Always fill sample containers full with ethanol to avoid damage to material during transport. Record specimen collection data on a waxy paper label, use pencil. Add label to ethanol filled jar. Record the vessel/expedition name/code, locality or station number, date, species name, and name of scientist making the identification.
5. Keep samples cool and dark (to avoid DNA degradation).

If ethanol is impractical or unavailable in large amounts, the samples (or specimens) can be subsampled in ethanol (as above). Cross reference labelling with unique identifiers is important to link subsamples or tissue samples with primary samples.

Formaldehyde solutions degrade DNA. If samples must be fixed in formaldehyde, make sure that they are kept cool and transferred to ethanol as soon as possible (at the latest within 14 days). Also make sure that the ethanol is exchanged with fresh ethanol after a few days. Note the formalin fixation on the sample record

Specimens that must be kept dry for morphological studies (such as butterflies) should be kept frozen (at -20°C or lower temperatures) or quickly dried in an oven or incubator.

### **For vascular plants**

High-quality DNA is most easily obtained from plants when the tissue is dried rapidly. Botanists now routinely use silica gel for field preservation of leaf material for DNA analysis. Silica gel can be purchased from most biological supply companies.

1. The ratio of silica gel to leaf tissue should be approximately 5-10:1. For best results, tissue should be completely dry within 24 hours.
2. Choose green, healthy leaves from a single individual plant. In general, a minimum of 2-3 square cm is necessary for 1-2 DNA extractions, but some small individuals will have less leaf material. A good rule of thumb to follow when deciding how much leaf material to sample is: more is always better.
3. Keep track of the individual from which you sampled leaf material. Small tags work well and they do not interfere with regular pressing activities. It is critical that voucher specimens be preserved from individuals that have associated material collected for DNA studies. A DNA sample that does not have an associated voucher specimen has extremely limited value in biological research.
4. Place the leaf tissue (or other green tissue if there is not a lot of leaf tissue available) into a small sealable bag with the silica gel. Tear or cut the leaf material into smaller pieces before inserting into the bag – this increases the surface area of the leaf that is exposed to the silica, and the drying process occurs more rapidly.
5. Write the collection number of the individual on a small piece of paper, and insert this into the Ziploc bag. Also write this information on the outside of the bag with a permanent marker.
6. Store the silica gel packets in a sealed bag or container to keep moisture out, and ensure that they do not get re-hydrated over time. For long term storage, silica gel packets can be stored in a freezer.

Animal protocol prepared by Torbjørn Ekrem, Museum of Natural History and Archaeology, Trondheim, Norway ([Torbjorn.Ekrem@vm.ntnu.no](mailto:Torbjorn.Ekrem@vm.ntnu.no)) & Peter Smith, National Institute of Water & Atmospheric Research, Hamilton, New Zealand ([p.smith@niwa.co.nz](mailto:p.smith@niwa.co.nz)).

Plant protocol prepared by Jeff Saarela, Canadian Museum of Nature, Ottawa, Ontario, Canada ([jsaarela@mus-nature.ca](mailto:jsaarela@mus-nature.ca)).