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DNA SAMPLING PROTOCOLS

Sample Collection Supplies

1. Select the appropriate sample collection box based on the type of sample to be collected.

Sample Type	Collection Box	Short-term Storage
Blood	Blood Buffer	Room Temperature
Blood	Filter Paper in Envelopes	Dry, Room Temperature
Blood quills	Tissue Preservation Buffer	Room Temperature
Bone	Envelopes or Dry Microtubes	Dry, Room Temperature
Egg shell membranes	Envelopes or Dry Microtubes	Dry, Room Temperature
Feathers from museum skins	Envelopes	Dry, Room Temperature
Feathers from nests	Tissue Preservation Buffer	Room Temperature
Feathers from nests	Envelopes	Dry, Room Temperature
Fin Clips	Ethanol	Room Temperature
Fin Clips	Filter Paper, Envelopes or Dry Microtubes	Dry, Room Temperature
Hair	Envelopes or Dry Microtubes	Dry, Room Temperature
Muscle	Tissue Preservation Buffer	Room Temperature
Scat	Ethanol	Room Temperature
Scat	Silica Beads/Gel	Room Temperature
Teeth	Envelopes or Dry Microtubes	Dry, Room Temperature

2. Check to ensure that your sample collection kit contains numbered microtubes (i.e., 1 to 100), a Sample Collection Form, and a Sharpie marker. Additionally, some kits may contain sampling scissors and forceps.

3. On the Sample Collection Form, record data associated with the respective sample (i.e., species, sample ID or band number, collection location, collection date, sex and age if known, and collector). Additional data may be required, depending upon study.

4. Label the outside of the box (i.e., species, collection location, collection dates, collector name, and contact telephone number).

Dropping Off Samples

1. Return samples to the Sample Drop Box located in rm. 102 (Molecular Ecology Lab) of the Alaska Science Center, 1011 E. Tudor Rd., Anchorage, AK 99503. Samples may also be returned by regular mail.

2. Record samples in the *Samples Received Log*.

3. Complete *Sample Data Entry Form* for entry into the BIOTA Inventory system.

Collection of Materials for DNA Analysis

Blood *(Blood/1 Vial/Blood Buffer/Room Temperature)*

For birds, blood is the preferred tissue type for nuclear DNA work, but less valuable than muscle for work that includes mtDNA analyses. We have provided 1.5 mL eppendorf tubes with a blood buffer storage solution (Longmire Buffer). The chemicals are not toxic, but this solution should not be ingested. The buffer and any blood/buffer combination can be stored at room temperature until you return from the field, at which point they should be frozen. Only a few drops of blood are needed from each bird, enough to turn the buffer red (usually about 5 drops). Blood can be obtained by pricking the tarsus vein and transferring the drops to the tubes by way of capillary tubes **(DO NOT USE heparinized tubes)**. Using a capillary bulb, blood can be blown from the capillary tube into the buffer after collection. If the blood clots inside the capillary tube, break off the tube inside the buffer vial and just leave it there. Blood can also be collected from the brachial or jugular vein with a sterile syringe. Use a new, sterile syringe for each individual.

Blood on filter paper *(Blood/Whatman Filter Paper/Dry/Room Temperature)*

5-6 drops of blood can be placed on filter paper (Whatman). The paper should be kept separate from other samples to avoid contamination. Allow the damp filter paper to dry and store separately in either: (1) a ziploc bag with silica gel, or (2) a separate envelope.

Blood Quills. *(Blood Quills/1 Vial/Tissue Preservation Buffer/Room Temperature)*

Blood quills are put into tubes containing tissue preservation buffer provided by our lab. Pull two or more blood quills from the bird. We recommend sampling wing coverts rather than emerging primaries and secondaries, which are more critical to flight. We extract DNA from the bloody "skin end" of the quill, so if the quills will not fit into the tube, trim off the feather tips, leaving the bloody ends (calamus) to put into the tube.

We strongly suggest that latex gloves be used when sampling, and that instruments be cleaned with 10% bleach between sampling. This prevents between-sample contamination and protects the collector from infectious diseases and any preservatives that may have been used in the skin's preparation.

Tubes can be stored at ambient temperature for shipping.

Egg Shell Membranes *(Egg shell membranes/1 Envelope/Dry/Room Temperature)*

DNA yields from eggshell membranes are very good, provided there is vascularization on the membrane. The easiest field technique is to collect each membrane and place it in a separate plastic bag: placing all membranes in the same bag causes cross contamination of samples. We do not use the hard shell at all, so that portion can be left in the field. Do not store feathers and eggshells from the same nest in the same bag. Give the nest a number, and then label each feather or egg sample with that number (e.g., nest number 100 has feather sample number 100 and membrane sample numbers 100(1), 100(2), etc.).

Feathers from nests. *(Feathers/1 Envelope/Dry/Room temperature)*

We get the best results with contour or tail wing feathers (those with a substantial sheath or rachis) deposited in nests or shed by birds during molt. The DNA is actually in the calamus, so feathers without the calamus cannot be used to extract DNA. We are unable to obtain DNA from down feathers. Please collect as many

contour feathers from each nest as possible (we use 5 feathers per DNA extraction from geese and at least that many for passerines, but like to have extra in case it does not work the first time around). Feathers can be removed after the nest has failed or hatched, or when first discovered if you don't plan to revisit the nest. Keep feathers dry after collection, since moisture can cause decay of feathers and subsequently the DNA. Place feathers in paper envelopes or, if bone dry, in plastic bags. **Store feathers from different nests in separate bags/envelopes**. Feathers do not need to be frozen. Envelopes can be placed in a plastic bag with 2 Tbsp. silica gel to aid in maintaining a dry environment. {See Pearce et al. (1997) for additional considerations when sampling feathers or egg membranes. However, note that chelex extractions are not recommended unless it is anticipated there will be no long-term use of the extracted DNA.}

Feathers from museum skins. *(Feathers/1 Vial/Tissue Preservation Buffer/Room Temperature)*

Success in extracting DNA from museum skins is variable, depending upon the way the skin was prepared. We have developed protocols for extracting from samples prepared using a number of preservatives (and combinations), including gasoline, arsenic and borax. We generally get better extractions from feathers plucked from museum skins (along with skin at the base of the feather), rather than from snips of skin alone. This may be due to inhibitors from preservatives used on the skins.

To minimize damage to museum skins, try to collect from areas that are less noticeable. Sampling can often be more easily done along suture lines, such as in the area of the cloaca. Feathers from the wing area (such as the marginal coverts) usually yield good DNA (perhaps because preservatives were used less often on the wings), but sampling from this area is difficult to do without affecting the integrity of the skin. Please abide by the instructions of the curators. We like to have at least 5 feathers and associated skin if possible.

Pluck feathers and associated skin from a small area. It helps sometimes to use forceps or tweezers. Take care to support the skin with one hand while gently pulling the sample; this will help to keep the skin from ripping. Place each sample in a sampling envelope and record museum numbering system, and species, sex, age, date and collection location. Often the museums will have much of this information on databanks. Keep the envelope dry.

We strongly suggest that latex gloves be used when sampling, and that instruments be cleaned with 10% bleach between sampling. This prevents between-sample contamination and protects the collector from infectious diseases and any preservatives that may have been used in the skin's preparation.

If we are preparing skins, we always collect tissue samples from the carcass. We collect heart, breast muscle, and blood as it pools around the heart, and store each in separate tissue preservation buffer vials (see protocols below, and separate multiple tissue sampling protocol).

See Mundy et al. (1997) for additional information about collecting from museum skins.

Fin Tissues. *(Tissue/1 Vial/Ethanol/Room Temperature) or (Tissue/1 Container/Dry/Room Temperature)*

Fifty fin samples for population genetic analysis and three or four reference samples (whole fish) for phylogenetic analysis need to be collected from each location. There are two methods for sample collection: dry or in vials with 100% ethanol (EtOH). The *preferred method of collection* is to store the tissue in a vial with *100% ethanol (EtOH).* (If this is not possible, the following dry method can be used.)

Use clean scissors or a clean scalpel blade to cut a small piece of tissue from one of the fins of the live fish. Tissue size should be approximately 5 mm^2 (about the size of this block ■). A wedge from the upper or lower lobe of the tail fin works fine. Because adipose fins contain a lot of complex lipids, they are not an easy target for DNA extraction, although some DNA can be extracted from this tissue. Eroded fins from dead salmon carcasses are highly degraded, and DNA is usually not readily extracted from such tissue. A well-dried 5 cm^2 piece of skin tissue works best under these conditions.

The date of collection, fish species and stock, type of collection method, and fish length, sex, and age (YOY, juvenile, adult will suffice) should be collected with each fin where possible.

If samples are to be sent through the mail, ethanol should be drained from the samples immediately prior to mailing; the samples will be rehydrated upon receipt at the Molecular Ecology Laboratory.

Once the samples have been collected, please contact Talia Wiacek, Biological Science Technician, (907) 786-3494, Talia_Wiacek@usgs.gov.

Dry Sample Collection (Alternative). For the dry method, whirl-pack bags, cryo-tubes, or scale envelopes lined with high quality filter paper work well.

Either in the field after collection, or in the office immediately upon return from the field, samples should by air-dried on filter paper or paper towels until all mucus and moisture in the fin has evaporated and the fin feels dry to the touch. Sun drying in the field works best and can be done quickly. Drying fins inside usually takes 18-24 hours at room temperature. Fungus and bacteria immediately invade the fins upon collection and these factors break down the cell walls of the tissue and the DNA exudes into the surrounding medium, making DNA extraction in the lab difficult, if not impossible. DNA from moist-stored fins are often OK for up to 6-8 hours (it depends on the original condition and size of fin clip), but samples are best when packed on ice if drying is to be delayed for over 4 hours.

Dried fin clips should be repackaged separately (make sure the baggy or envelope is dry as well) and attached to field notes for shipment. Dry samples can be sent surface mail without special packaging.

Hair and Hard Tissue *(Hair, Bone, or Teeth/1 Envelope/Dry/Room Temperature)*

Hairs and hard tissue samples, such as bone or teeth, should be kept as dry as possible. DNA is present only in hair follicles, so hairs without follicles are not useful for genetic analyses. DNA is extracted from tooth pulp, so the whole tooth is preferred. These can be stored in containers or envelopes.

Muscle (*Tissue/1 Vial/Tissue Preservation Buffer/Room Temperature)*

Muscle tissue samples are the preferred samples for work that includes mtDNA analyses along with nuclear DNA analyses, particularly for birds. Among muscle tissue samples, heart is the most preferred for birds, since the mtDNA yield is very high relative to nuclear yield. DNA can also be extracted from tongue, skin, hair, teeth and bone. Soft tissue samples can be stored at room temperature in the field in the tissue preservation buffer. Any muscle or skin tissue will work and can be stored in this buffer solution. **Please make sure that the storage buffer completely covers the tissue sample**. Also, make sure to clean instruments between sampling different birds to prevent cross-contamination, using a 10% bleach solution **followed by a water rinse**. A sample about the size of a pencil eraser is all that is needed, but make sure the sample is entirely submersed in the buffer.

Scat *(Scat/1 Vial/EtOH/Room Temperature) or (Scat/1 Vial/Silica Beads/Room Temperature)*

Host DNA is very difficult to obtain from scat samples. Because of the low amounts of host DNA compared to bacterial and diet sources of DNA, extreme caution must be used to prevent contamination of one scat sample with scat from another individual. Therefore, we provide gloves, and tongue depressors with each sampling vial to be used only for one vial then discarded. We provide two different types of preservative for scat samples: (1) Liquid ethanol in a 50 ml tube, or (2) Silica beads/gel in a 50 ml tube. Unpublished research from our laboratory and others suggest that ethanol is superior to silica beads/gel for preserving scat samples for DNA analysis.

Regardless of which preservative you have, use a new pair of gloves and a new tongue depressor for each sample handled. If you are directed to aliquot one scat sample between the two preservative types, you do not have to change gloves and tongue depressors. Try to place an amount of scat approximately the size of a golf ball into the collection tube. Do not fill the tube; it is important to leave enough space for the sample and preservative to mix (easier done with the liquid ethanol than the silica beads). Samples are okay left at ambient temperature, but should be kept away from heat and out of sunlight for a few days. It is best to return them to the lab and freeze them as soon as possible. Unpublished data from several laboratories, including ours, indicate DNA yields decline dramatically in samples over about a week old, regardless of collection method. Scat samples preserved within 24 hours of defecation yield the highest amount of host DNA.

Chemical Descriptions and Hazards

(For more specific details on each chemical ingredient, see the attached Material Safety Data Sheets.)

Blood Buffer

Tris/HCL. May cause irritation to skin and mucous membranes on contact. Wash contacted area with plenty of water and contact physician if irritation persists. Ingestion of large doses may cause interior irritation, nausea, weakness and collapse. If ingested, drink copious amounts of water and call a physician.

EDTA. May cause irritation to skin and mucous membranes on contact. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, drink copious amounts of water and call a physician

NaCl (Sodium chloride). May cause irritation to skin and mucous membranes on contact. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, drink copious amounts of water and call a physician

SDS (Sodium dodecyl sulfate). May cause irritation to skin and mucous membranes on contact. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, do not induce vomiting. Drink copious amounts of water and call a physician.

Tissue Buffer

Tris/HCL. See above.

EDTA. See above.

NaCl (Sodium chloride). See above.

N Lauroyl sarcosine. May cause irritation to skin and mucous membranes on contact. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, do not induce vomiting. Drink copious amounts of water and call a physician.

Urea. May cause irritation to skin or eyes. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, do not induce vomiting. Drink copious amounts of water and call a physician.

Silica Beads. Silica is an inhalation hazard. Do not breath silica dust or leave in open areas.

Ethanol. Ethanol is highly flammable; do not place near an open flame. High vapor concentrations or consumption can cause narcotic effects. Do not breath vapors or consume this ethanol.

Additional Sampling Information

Mundy, N. I., P. Unitt, and D. S. Woodruff. 1997. Skin from feet of museum specimens as a non-destructive source of DNA for avian genotyping. *The Auk* 114(1):126-129.

Pearce, J. M., R. L. Fields, and K. T. Scribner. 1997. Nest materials as a source of genetic data for avian ecological studies. *J. Field Ornithol.*, 68(3):471-481.