USGS/BRD
MOLECULAR ECOLOGY LABORATORY
Lab 102 and 103, 1011 E. Tudor Road
ALASKA SCIENCE CENTER
Anchorage, AK 99503
(907) 786-3582; Fax (907) 786-3636

Sandra Talbot
Research Geneticist (Wildlife)
office: (907) 786-3683
sandy_talbot@usgs.gov

Judy Gust
Geneticist
office: (907) 786-3949
judy_gust@usgs.gov

Kevin Sage
Geneticist
office: (907) 786-3975
kevin_sage@usgs.gov

John Pearce
Research Wildlife Biologist
office: (907) 786-3893
john_pearce@usgs.gov

DNA SAMPLING PROTOCOLS

Updated 23 May 2006

Sample Collection Supplies

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

Sample Type	Collection Method	Short-term Storage	Page
Blood	Blood Buffer	Room Temperature	2
Blood	Filter Paper in Envelopes	Dry, Room Temperature	2
Blood quills	Tissue or Blood Preservation Buffer*	Room Temperature	2
Bone	Envelopes or Dry Microtubes	Dry, Room Temperature	3
Buccal swab	Longmire Buffer	Room Temperature	3
Egg shell membranes	Envelopes or Dry Microtubes	Dry, Room Temperature	3
Feathers from nests	Tissue Preservation Buffer	Room Temperature	3
Feathers from nests	Envelopes	Dry, Room Temperature	3
Feathers from museum skins	Envelopes	Dry, Room Temperature	3
Fin Clips	Ethanol	Room Temperature	4
Fin Clips	Filter Paper, Envelopes or Dry Microtubes	Dry, Room Temperature	4
Hair	Envelopes or Dry Microtubes	Dry, Room Temperature	4
Muscle	Tissue Preservation Buffer	Room Temperature	5
Scat	Ethanol (95% - 100%)	Room Temperature	5
Scat	Silica Beads/Gel	Room Temperature	5
Teeth	Envelopes or Dry Microtubes	Dry, Room Temperature	5

***We recommend the use of Longmire Buffer (Longmire et al. 1988) as a blood preservation buffer for storage of samples in the field. We and other researchers have determined experimentally that "Queen's Buffer" (Seutin et al. 1991) and "SET" lysis buffer (0.15M NaCl, 0.05M Tris, 0.001M EDTA, pH 8.0) can cause difficulties in preservation and extraction of DNA from some species and sample types, if samples are not extracted within a few days (Talbot et al., unpublished data, Conrad et al. 2000), and particularly when working with microsatellite loci. We have not observed this difficulty with blood samples stored in Longmire Buffer at ambient temperatures for up to several years (Talbot et al. unpublished data).**

2. Check to ensure that your sample collection kit contains 1.7 mL microcentrifuge tubes containing the appropriate preservation buffer, 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. 105 (Molecular Ecology Lab-105 the Alaska Science Center, 1011 E. Tudor Rd., Anchorage, AK 99503. [Samples may also be returned by regular mail (Alaska Science Center, U.S. Geological Survey, 1011 E. Tudor Road, MS701, Anchorage, AK 99503). Collaborators contact Kevin Sage or Sandra Talbot for FedEx account number.]

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

3. Include a hard copy of the sample collection information with the samples, or preferably, send an electronic copy of the information to judy_gust@usgs.gov.

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, Longmire et al. 1988). 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 - 10 drops, about 30 to 60µL). 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)**. Use a capillary tube bulb to expel blood from capillary tubes (do not use your mouth) 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. In a pinch (field situations), paper towel can be used, following the same protocol. Whatman FTA cards are an alternative; they are impregnated with a solution that lyses cell membranes and denatures proteins on contact. However, we have not experienced any difficulties with plain filter paper. A drop or two of 95-100% ETOH as a "chaser" on a blood stain on filter paper will suffice to inactivate DNAses (that degrade DNA) if the samples cannot be kept dry.

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

Blood quills are put into tubes containing either tissue preservation buffer, or blood 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) in 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.

Bone. *(Bone/1 Envelope/Dry/Room Temperature)*

Hard tissue samples, such as bone, should be kept as dry as possible. These can be stored in containers or envelopes.

Buccal Swab. (*epithelial cells/1 swab/original packaging, Longmire Buffer, or silica gel/Room Temperature)* We have determined that buccal swabs are useful for collecting samples non-invasively from birds (Handel et al. 2006), mammals and amphibians (Simac et al. in prep). For wood frogs, the "buccal swab" technique is actually an "epithelial swab" technique, because we sample epithelial cells from the backs of frogs or tails of tadpoles, rather than the buccal area. To collect buccal cells from birds or mammals, roll the collection swab firmly on the inside of the "cheek", approximately 20 times on each. Make certain to move the brush over the entire "cheek" side. For frogs/tadpoles, gently rub the back or the tail approximately 10 times on each side of the body (dorsal), rolling the swab. To avoid injuring amphibian skin, try not to rub over the same area multiple times.

The samples can be stored dry if you are returning to the lab right away. If storing dry, air dry the swab at for 10-15 minutes at room (field) temperature. Store the dry swab in the original packaging at ambient temperature for up to one week. If you are going to be in the field longer than a week (and can't send the samples back to the lab), place the swab in a 1.7 mL vial containing 350 mL of blood preservation buffer (Longmire Buffer). You will need to cut the swab to size, cap and label the vial. An alternative method of storing for longer periods is to place the swab (after collection of cells and drying) into a small coin envelope, cutting the end of the stick so it will fit, close the envelope, and place the envelope into a Ziploc bag containing silica gel (provided). We prefer the Longmire Buffer storage procedure. Samples stored in silica gel or blood preservation buffer can be stored at ambient temperature.

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 envelope, then into a plastic ziploc bag containing silica gel: placing all membranes in the same envelope 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 envelope. 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 or live birds. *(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 (or plucked from live birds). The DNA is actually in the calamus, so feathers without the calamus cannot be used to extract DNA. Although in the past, we were unable to obtain DNA from down feathers, we have recently been able to extract DNA from down feathers of raptors and are doing experiments to see if our techniques are more broadly applicable (Latta et al., in prep). 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 coin envelopes or, if bone dry, in plastic bags. **Store feathers from different nests or birds 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. Note, however, that chelex extractions are not recommended unless no further use of the extracted DNA is anticipated.}

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, dependin upon species, 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 Sara Graziano, Geneticist (Fisheries), (907) 786-3466, Sara_Graziano@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 *(Hair/1 Envelope/Dry/Room Temperature)*

Hairs should be kept as dry as possible. DNA is present only in hair follicles, so hairs without follicles are not useful for genetic analyses. 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 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. Data from several laboratories, including ours, indicate DNA yields decline dramatically in samples over about a week old at the time of collection, regardless of collection method. Scat samples preserved within 24 hours of defecation yield the highest amount of host DNA.

Teeth *(Teeth/1 Envelope/Dry/Room Temperature)*

Hard tissue samples, such as teeth, should be kept as dry as possible. DNA is extracted from tooth pulp, so the whole tooth is preferred. These can be stored in containers or envelopes.

Storage Chemical Descriptions and Hazards

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

Blood Buffer

Longmire Blood Preservation Buffer (Longmire et al. 1988): recommended for field storage of blood

100mM Tris HCl pH 8.0
100mM EDTA
10 mM NaCl
0.5% SDS (Sodium Dodecyl Sulfate)

Recipe: 100 mL 1M Tris- HCl, pH 8.0.
200 mL 0.5M EDTA
2.5 mL 4M NaCl
5.0 grams SDS
Bring to 1 Liter with dH_20

Storage: Room temperature

Disposal: Sink

Hazards:

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 (recommended for field storage of tissue samples)

4.0 M Urea
0.2 M NaCl
10mM EDTA
0.5% N-Lauroyl-Sarcosine
100mM Tris HCl pH 8.0

Recipe: 240.24 grams Urea
11.69 grams NaCl
5.0 grams N-Lauroyl-Sarcosine
3.72 grams EDTA
100 mL 1M Tris- HCl, pH 8.0.
Bring to 1 Liter with dH_20

Storage: Room temperature
Disposal: Sink

Hazards:

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 can be an inhalation hazard, especially for those with asthma. Do not breathe 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 breathe vapors or consume this ethanol.

Additional Sampling Information

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