



Protocols for Collecting and Storing DNA Samples

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Introduction

The use of genetics in wildlife biology has rapidly spread. However, to ensure that studies can get the most out of their genetic data, samples must be handled and stored properly. Below is an attempt to synthesize the best ways to collect various samples for DNA analysis. There are common elements in each approach that reflect the importance of **sterile procedures, labeling, and preservation** of the sample.

When stored correctly, DNA can be extracted from various samples, including those considered sub-optimal (e.g. scat and hair). When improperly stored there are several dynamics that can harm DNA. First, naturally occurring enzymes found in animal cells, bacteria etc. will begin to degrade the DNA hindering future analysis. Most of these harmful enzymes require an aqueous environment to function optimally. Therefore the goal of our sample storage is to inhibit these enzymes often by drying or freezing the sample. Second, DNA in cells can degrade due to environmental factors (such as freeze/thaw cycles, UV light or excessive heat) that can physically degrade, damage, or sheer the DNA. Fortunately, there are ways to store all types of samples that can prevent chemical and physical degradation. Below we discuss the ways to store DNA allowing for optimal data to be generated from the sample.

****Contact Kristy Pilgrim, kpilgrim@fs.fed.us if you have any questions regarding sample collection and storage**

Collecting Data/Labeling Samples & Chain of Custody Form

Sample collection is useless (and might as well be skipped) unless the samples are well labeled and documented. When submitting samples to the lab, please include a hard copy of our chain of custody form. The following data should be recorded for all samples on a field form and/or on the vial itself:

- 1) Unique Sample ID
- 2) Collection Location.
- 3) Collection Date
- 4) Sample Number
- 5) Types of samples taken (e.g., ear plug, scat, muscle, hair, blood etc.) with their respective numbers
- 6) Collectors Initials
- 7) Any comments on condition of samples, etc.
- 8) Sex (if known)



****note, for HAIR and SCAT samples, remember that moisture is the enemy when it comes to DNA!!! Do not put these samples in a refrigerator or freezer.***

Hair

When collecting hair, the target tissue is often not the hair shaft, but the root cell attached to the base of the hair (the follicle). The root cell can often be seen by the naked eye, and appears as the white bulb at the end of many guard or thick hairs. However, when collecting samples from the field, collect all hairs available even if you can't see follicles. {If pulling hairs from an animal, it is important that **the hairs are pulled not cut**, to capture root cells; aim for a tuft of approximately 20-50 hairs}.

1) Prior to the field obtain:

- a) Sterile (new) 50 ml Polyethylene vials or paper envelopes (coin envelopes work well)
- b) Silica desiccant (indicating)
- c) Forceps or tweezers
- d) Ethanol (95%) for cleaning tweezers
- e) Kim-Wipes (or other tissue paper to wipe tweezers and prevent contamination)
- f) Permanent Ink Markers (Sharpie Brand)
- g) Clean (new) latex gloves
- h) *Fill vials halfway with desiccant

2) Sample Treatment (Field)

- a) Put on latex gloves.
- b) Clean the forceps with the Ethanol and wipe thoroughly with the Kim-Wipes
- c) Place the hair into the vial or envelope
- d) Label the vial/envelope with Sample ID, Date, Location, etc.

3) Sample Treatment (Field Camp several hours later)

- a) Hair samples in vials, check the silica desiccant. If the color of the desiccant has turned from the original color, change the silica desiccant under sterile conditions. Desiccant that has turned color is water saturated and is no longer working to preserve the sample.
- b) Hair samples in paper envelopes should be placed in a ziplock bag or plastic Tupperware with silica desiccant; check desiccant after a few hours and replace if it has turned color
- c) Store samples at room temperature, in a dry environment out of direct sunlight until shipped to the lab.



Scat—Carnivore

Scat can be a useful source of DNA, but it's critical to dry the scat out to prevent degradation (and growing mold etc.). When scat is found, collect the scat (fresher tends to work better) and place it in a new unwaxed brown paper bag, (or plastic vial/ specimen cup with desiccant). Which option you use may depend of the size of the scat. Only place one scat in each bag/container to ensure that samples are from only one individual. If two scat piles exist, use two different bags/containers. After scat is collected, take it to a warm, dry place and allow it to dry for 1-4 days (either in paper bags or vials with desiccant). We recommend keeping the scat out of direct sunlight as it dries. Dried scat in vials is preferable, but not always practical for large scats; it is fine to submit scats in the brown paper bags.

1) Prior to the field obtain:

- a) Clean (new) brown paper bags
- b) Extra latex gloves
- c) 50 ml polypropylene screw-cap vials or specimen cups filled 1/3 with silica desiccant

2) Sample Treatment (Field)

- a) Put on new gloves
- b) Place the scat in the paper bag or vial
- c) Label with Sample ID, Date, Location, etc.

3) Sample Treatment (Field Camp several hours later)

- a) If the scat is in a vial, check the silica desiccant!! If the color of the desiccant has turned from the original color, change the silica desiccant under sterile conditions. Desiccant that has turned color is water saturated and is no longer working to preserve the sample. This is very common for scat samples which often have a lot of moisture. Check the desiccant in the vials often, (every few hours initially) to replace exhausted desiccant until the color no longer changes. Make sure scat is surrounded by desiccant to effectively dry it. It is ok if the desiccant beads stick to the scat.
- b) If the scat is in a brown paper bag, ensure scat is in a warm, dry room to dry out for several days.
 - Optional--the bag with the scat can be placed in a Tupperware container with silica desiccant to help it dry (check the desiccant often, and replace until it stops turning color).
 - Optional--after the scat is dry (good indicator is that it's not very stinky), can be transferred to a 50mL vial/specimen cup filled ~1/3 with desiccant if desired
- c) Store samples at room temperature, in a dry environment out of direct sunlight until shipped to the lab.



Scat—*Herbivore (large animal)*

Scats from herbivores (frequently referred to as pellets) can also be a useful source of DNA. In addition to collecting pellets, if the pellets are fresh/moist, these pellets can also be swabbed to gather DNA from the mucous. If swabbing samples, use 3 separate Dacron (or similar) swabs to sample different pellets from the same pile, then store all swabs dry in a coin envelope or plastic packaging that came with the swab. Ideally, the swabs should be lightly stained, but not covered with fecal material.

Collect up to 6 individual pellets (if swabbing pellets, these should be ones that weren't swabbed) and place in paper envelopes. Pellets and swabs (if collected) should be sent to the lab.

- 1) Prior to the field obtain:
 - a) Pre-labeled envelopes
 - b) Dacron Swabs or similar
 - c) Extra latex gloves
- 2) Sample Treatment (Field)
 - a) Put on new gloves
 - b) Place up to 6 individual pellets not swabbed into paper envelopes.
 - c) Optional: Swab 3 different pellets with 3 swabs and place in coin envelope/swab packaging

Scat—*Herbivore (small animal)*

For smaller animals (such as rodents or bat), we recommend that the pellets are placed in 1.5ml or 2.0ml vials containing either silica desiccant or lysis buffer (contact us as we may be able to provide this depending on the size of the project). If the samples will be shipped, we recommend using desiccant or screw-cap vials if using lysis buffer to avoid leakage.

- 1) Prior to the field obtain:
 - a) Pre-labeled vials (pre-filled with desiccant beads or lysis buffer)
 - b) Extra latex gloves
- 2) Sample Treatment (Field)
 - a) Put on new gloves
 - b) Place up to 10 pellets in a pre-filled tube



Urine

Urine can be a source of DNA. This sample is usually collected in the snow during winter surveys. In the field, obtain yellow snow in a vial or plastic container. Try to maximize urine and minimize extra snow collected.

- 1) Prior to the field obtain:
 - a) 50 ml screw-cap vials/ specimen cups or similar.
 - b) Extra latex gloves
 - c) Urine preservation buffer (available from Norgen Biotek; catalog 18124/18126)
- 2) Sample Treatment (Field)
 - a) Put on new gloves
 - b) Scoop yellow snow into vial
 - c) Add dose of urine preservation buffer; can be done as soon as come back from the field
 - d) Store urine with preservation buffer in refrigerator

It's best to store the urine with preservation buffer in the refrigerator until shipping; ship samples in a cooler with blue ice.



Tissue/Meat/Ear Punches

Tissue can be stored several ways. Our two preferred methods are: 1) in a silica desiccant, and 2) frozen. We avoid storing tissues in ethanol as the concentration **MUST** be 95% ethanol for effective preservation (and a wet tissue will change this %), and ethanol can present challenges for the field and shipping. If you want to store tissues in ethanol, please contact us first.

****The most important thing for effective tissue preservation when using desiccant is to have the size of the vial allow for the tissue to float freely in the desiccant. Use a color-changing desiccant so that the desiccant can be swapped out or more added for wet tissues. Continue to monitor and change the desiccant until the color doesn't change.**

Obtain a piece of muscle tissue (avoid sampling organs) and place in vial with desiccant. Ear punches from study animals can be placed directly into vials with desiccant. When sampling from a carcass, target a pencil-eraser/dime-sized piece (~1 – 2cm³) to cut. If the carcass is old/decomposing, try and find a piece of tissue that doesn't look rotten. If dealing with a carcass such as from roadkill, a sample of tongue is often a convenient tissue to obtain. DNA from old carcasses can sometimes yield DNA, so always take a sample. If you have special circumstances or questions, please contact the lab.

Silica Desiccant—this is our preferred method as samples are easily stored at room temperature**

- 1) Prior to the field obtain:
 - a) Clean (new) 2ml-50ml polypropylene, screw-cap vials (the size depends on the size of the tissue)
 - b) Silica desiccant with color indicator
 - c) Forceps/tweezers; razor blade or other cutting tool
 - d) Ethanol (95%) for cleaning tweezers/forceps and blades
 - e) Kim-Wipes (or other tissue paper to wipe tweezers and prevent contamination)
 - f) Permanent Ink Markers (e.g., Sharpie Brand)
- 2) Prior to Capture
 - a) Add desiccant to vials; fill to about 1/3
 - b) Clean the tweezers/forceps and blade with the ethanol and wipe with Kim-Wipes
- 3) Sample Collection
 - a) Slice the tissue and place in sample vial with desiccant
 - b) Label sample vial
- 4) Sample Treatment (later)
 - a) **It's important that the tissue is fully immersed in the desiccant; ideally the tissue should be free to move as the tube is turned (avoid cramming tissue in the vial and topping off with desiccant as the tissue will almost certainly rot).**
 - b) Check the silica desiccant. If the color of the desiccant has turned from the original color, change the silica desiccant, or add more if the size of the vial allows under sterile conditions. Desiccant that has turned color is water saturated and is no longer working to preserve the sample. Check the desiccant in the vials often, (every few hours initially) to replace exhausted desiccant until the color no longer changes.
 - c) Store samples in the desiccant at room temperature. Keep the sample out of direct sunlight.

Tissue samples stored in desiccant can be shipped at room temperature to the lab



Frozen

- 1) Prior to the field obtain:
 - a) Clean (new) 2ml-50ml polypropylene, screw-cap vials or for larger tissues use a Whirlpack bag
 - b) Forceps/tweezers; razor blade or other cutting tool
 - d) Ethanol (95%) for cleaning tweezers/forceps and blades
 - e) Kim-Wipes
 - f) Permanent Ink Markers (e.g., Sharpie Brand)
- 2) Prior to Capture
 - a) Clean the tweezers/forceps and blade with the ethanol and wipe with Kim-Wipes
- 3) Sample Collection
 - a) Slice the tissue and place in sample vial/Whirlpack
 - b) Label sample vial/Whirlpack
 - c) Place sample in freezer

Ship samples in a cooler with ice packs /dry ice. DNA can degrade if it goes through freeze thaw cycles, therefore, avoid thawing of tissue once it has been frozen.

Blood

For mammal genetic studies, it is critical that whole blood be collected since mammalian red blood cells themselves do not contain DNA. Fish and birds have DNA in their erythrocytes, though collection of whole blood is preferred.

- For submitting blood samples to the lab, blood stored in blood storage vials (e.g. purple-top EDTA-tube) should be shipped on dry ice.
- **) A more convenient method is to transfer some whole blood (by dropper or syringe) to a Whatman FTA Micro Card. Ideally, enough blood should be applied to the filter paper such that a little bit of red color comes through the back. The cards should be completely air dried (at room temperature) and then placed in bags with desiccant and stored in a dark location at room temperature until they are sent to the lab.

The above 2 methods are preferred although there also protocols for storing blood with lysis buffer. Please consult the lab prior to using a lysis storage buffer.



Additional Information

Below is a list of some supplies that may be useful. Note that the part numbers and prices change often. Other vendors work as well.

Supply List

Item	Vendor	Part/Catalog Number	Est. Price
1.5ml SealRite Tube	USA Scientific	1615-5500	Bag of 500 for \$20
2.0ml SealRite Tube	USA Scientific	1620-2700	Bag of 500 for \$25
2ml Polypropylene tube with screw caps	USA Scientific	1420-9710	Case of 500 for \$115
4ml Cryovial Storage Tubes with screw caps	USA Scientific	1440-9100	500 for \$250
15ml Polypropylene tube with screw caps	USA Scientific	1475-2501	500 for \$90
50ml Polypropylene vials	Fisher Scientific	05-539-7	Case of 500 for \$260
Desiccant—Sorbead Orange**	eCompressedair; Delta Adsorbents		10 lb bag for \$150
100% Ethyl Alcohol (Denatured)	Fisher Scientific	A407P-4	4L for \$185
Latex Gloves (powder free)	Fisher Scientific; Various		Pack of 100 ~\$20-80
Nitrile Gloves (powder free)	Fisher Scientific; Various		Pack of 100 ~\$20-80
Urine Preservation Dose	Norgen Biotek Corp https://norgenbiotek.com/product/urine-preservation	18124 (single dose) 18126 (50 doses)	\$12/dose
Forceps/Tweezers	Fisher Scientific; Various		
Permanent Ink Markers (Sharpies)	Buy Locally		
Plastic Bags (WhirlPak)	Forestry Supply	79226	500 for \$55
Gun Brushes	Brownells (www.brownells.com)	084-401-030	12 for \$15.99
Whatman FTA Micro Card (Whatman – WB120210)	Fisher Scientific	09-923-344	100 for \$450
Swabs—Polyester (Dacron or similar)	Various		
ATL Lysis Buffer	Qiagen	19076	200ml for \$96

**we recommend using Sorbead Orange desiccant; it contains a color indicator without the use of cobalt (a heavy metal)