

## Protocols for Collecting and Storing DNA Samples

*Michael K. Schwartz  
Research Ecologist  
Rocky Mountain Research Station  
Missoula Montana*

*mkschwartz@fs.fed.us  
(406) 542-4161*

*Kristy Pilgrim  
Laboratory Supervisor  
Rocky Mountain Research Station  
Missoula Montana*

*kpilgrim@fs.fed.us  
(406) 542-3255*

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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 tissue for field operations. There are common elements in each approach that reflect the importance of **sterility** (to prevent contamination of samples), **labeling**, and **preservation** of the sample

When stored correctly DNA can be extracted from tissues that are relatively old. When improperly stored there are several dynamics that can harm DNA. First, naturally occurring enzymes found in animal cells 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 tissue storage is to inhibit these enzymes often by drying or freezing the sample. Second, once the cell containing DNA is broken the DNA becomes subject to a harsh environment. Abusing the sample by exposing it to freeze / thaw (even partial thaw) cycles or excessive heat will physically degrade, damage, or shear the DNA. Fortunately, there are ways to store all types of tissues that prevent chemical and physical degradation. Below we discuss the ways to store DNA allowing for optimal data to be generated from the sample. The most preferred method is listed first, followed by other options for storing tissues.

### Collecting Data and Labeling Samples

Sample collection is useless (and might as well be skipped) unless the samples are well labeled. The following data should be recorded for all samples on a field form and/or on the vial itself:

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

## Non-Invasive Samples (Hair, Scat, Urine)

*\*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. Therefore, it is important that **the hairs are pulled not cut**, to capture root cells. When pulling hairs from an animal aim for a tuft of approximately 20-50 hairs. Below are the steps recommended for best treatment of hair follicles:

- 1) Prior to the field obtain:
  - a) Clean (new) 50 ml Polyethylene vials
  - b) Silica desiccant (indicating) of size 10-18 mesh
  - 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) Rite-in the Rain Paper and a Pencil
- 2) Prior to Capture
  - a) Clean the forceps with the Ethanol and wipe thoroughly with the Kim-Wipes
  - b) Label the vial.
  - c) Label a piece of Rite in the Rain Paper with Pencil.
- 3) Sample Treatment (Field)
  - a) Put on latex gloves. Pull a small tuft of hair preferably with a forceps (20-50 hairs).
  - b) Place the sample gently into the vial.
  - c) Fill the vial half way (to all the way) with silica desiccant (we often pre-fill the vials)
- 4) Sample Treatment (Field Camp several hours later)
  - a) Check the silica desiccant. If the color indicator is used and the silica is the original blue color the samples are in good shape. If the silica is white, pink or gray change the silica desiccant under sterile conditions. White, pink or gray silica desiccant is water saturated and is no longer working to preserve the sample. If the silica does not have a color indicator (i.e., it is originally white) then examine the sample to see if any excessive moisture is seen in the vial. If any moisture is apparent, replace the silica with fresh silica desiccant (but be gentle as the root cells are fragile).
  - b) Store samples in the desiccant at room temperature out of direct sunlight.
- 5) OPTIONAL Sample Treatment
  - a) If the samples are dry, place the hair in a dry envelope and label. Keep the samples in a dry place (and out of direct sunlight). Using a dry envelope should

not become the standard protocol, however, if the field crew should run out of silica desiccant filled vials, hair can be stored in dry envelopes.

### Scat

Scat can be a useful source of DNA. If scat is found while at a den site, collect the relatively fresh scat (i.e., leave the white, "bleached" scat) and place it in a new unwaxed paper bag. Only place one scat in each bag to ensure that samples are from only one individual. If two scat piles exist, use two different bags. After scat is collected, take it to a warm, dry place and allow it to dry for 1-4 days. We recommend keeping the scat out of direct sunlight as it dries. After drying, place the scat in a vial filled with silica desiccant (preferable), or 95-100% Ethanol.

- 1) Prior to the field obtain:
  - a) Clean (new) paper bags
  - b) Rite-in the Rain Paper and a Pencil
  - c) Extra latex gloves
  - d) 50 ml polypropylene screw-cap vials
  - e) Silica desiccant 10-18 mesh
- 2) Sample Treatment (Field)
  - a) Put on new gloves
  - b) Place the scat in the bag
  - c) Label the bag and a small piece of Rite-In the Rain paper
  - d) After the scat is dry, place in a desiccant, or Ethanol filed 50 mL vial.

### Urine

Urine can also be a source of DNA. This sample is usually collected in the snow during winter surveys. In the field, obtain yellow snow (the darker the color the better since the snow will dilute the sample). Place in 50ml polypropylene screw-cap vial (straight sample, **no** ethanol or desiccant) and **keep refrigerated**.

### **Organs/Meat/Solid Tissue/Ear Punches**

Solid tissue can be stored several ways. The three preferred ways are: 1) in a silica desiccant, 2) in 95-100% Ethanol, or 3) frozen. With live-trapped animals place the tissue in a well labeled vial. If a carcass is found collect a small piece (size of a pencil eraser – 0.75 cm<sup>3</sup>) of muscle (meat) or spleen tissue when the carcass is fresh (often, a piece of tongue works well). DNA from old carcasses can sometimes also be used, so take a sample. Place this sample in a well-labeled vial. Below are the details for collecting tissue.

#### *Silica Desiccant*

- 1) Prior to the field obtain:
  - a) Clean (new) 2ml polypropylene, screw-cap vials.
  - b) Silica desiccant 10-18 or 6-16 mesh (preferentially with color indicator).
  - 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 (e.g., Sharpie Brand)
- 2) Prior to Capture
  - a) Clean the forceps with the Ethanol (Do not dunk the forceps in the ethanol, pour the ethanol onto the forceps - this minimizes contamination)
  - b) Wipe the forceps thoroughly with the Kim-Wipes (or other clean tissues)
  - c) Label the vial
- 3) Sample Treatment (Field)
  - a) Place the sample in the vial
  - b) Fill the tube half way with silica desiccant (we often pre-fill tubes)
- 4) Sample Treatment (Field Camp several hours later)
  - a) Check the silica desiccant. If using the color-indicator type of silica desiccant (preferred) check its color. If it has turned color (often from blue to gray, white, or pink) replace the silica with clean silica desiccant. Gray, white or pink silica desiccant is water saturated and is no longer working to preserve the sample. Some silica desiccants are sold white. In this case the field biologist must decide if the sample has been exposed to excessive moisture and in need of new silica desiccant. We rarely have to replace the desiccant and try to minimize handling of the sample
  - b) Store samples in the desiccant at room temperature. Keep the sample out of direct sunlight.

#### *Ethanol*

- 1) Prior to the field obtain:
  - a) Clean (new) 2ml polypropylene, screw-cap tubes.
  - b) Forceps or tweezers
  - c) Ethanol (95%) for cleaning tweezers
  - d) Rite-in the Rain Paper and a Pencil
  - e) Kim-Wipes (or other tissue paper to wipe tweezers and prevent contamination)
  - f) Permanent Ink Markers (Sharpie Brand)
- 2) Prior to Capture

- a) Clean the forceps with the Ethanol and wipe thoroughly with the Kim-Wipes
  - b) Label the vial with the Permanent Ink Marker (Pigma Micron 05 permanent ink pens work well for labels.
  - c) Label the Rite-In-the Rain in pencil with the sample's number and date collected (Ethanol has a bad habit of removing even permanent ink, thus added labeling is needed; also ensure that the label is dry before immersing in alcohol.)
- 3) Sample Treatment (Field)
- a) Place the sample in the vial.
  - b) Place the Rite-In-the Rain label in the vial.
  - c) Fill the vial most of the way with ethanol. (We pre-fill our vials in the lab)
  - d) Store samples at room temperature or in cooler places if possible (i.e. freezer or fridge)

### *Frozen*

- 1) Prior to the field obtain:
- a) Clean (new) 2ml polypropylene, screw-cap tubes or for larger tissues use a whirlpack (or other) plastic bag.
  - b) Forceps or tweezers
  - c) Ethanol (95%) for cleaning tweezers
  - d) Rite-in the Rain Paper and a Pencil
  - e) Kim-Wipes (or other tissue paper to wipe tweezers and prevent contamination)
  - f) Permanent Ink Markers (Sharpie Brand)
- 2) Prior to Capture
- a) Clean the forceps with the Ethanol and wipe thoroughly with the Kim-Wipes
  - b) Label the vial with the Permanent Ink Marker
- 3) Sample Treatment (Field)
- a) Place the sample in the vial.
  - b) Label the vial.
  - c) Store samples in a cooler with ice packs / dry ice. When return store in freezer. It is important to note that DNA degrades rapidly when 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. Using sterile procedures, blood can be drawn and stored in well-labeled purple-top (EDTA-tube) or red-top blood collection vials, and frozen.

Blood can also be collected on Whatman FTA Micro Cards (catalog no. WB12 0210). Ideally, a fair amount of blood should be collected (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 DNA lab prior to using a lysis storage buffer.

### Added Information

Below is a list of supplies that may be carried into the field. Next to each item is the estimated cost and a vendor for which to purchase the item. Note that the part numbers and prices change often. Other vendors work as well.

### Field Equipment List

Item	Vendor	Part Number	Price
2ml Polypropylene tube with screw caps	Fisher	05-669-8	Case of 500 for \$120
50ml Polypropylene vials	Fisher	05-539-7	Case of 500 for \$200
Desiccant—Sorbead Orange**	eCompressedair, #704-947-1967		10 lb bag for \$54
Silica Desiccant (6-16 Mesh) Silica Desiccant (10-18 Mesh)	Fisher,	S161-500, S161-212	500 g for \$32. 2.5 kg for \$135
100% Ethyl Alcohol (Denatured)	Fisher	A407P-4	4L for \$68
Latex Gloves (powder free)	Fisher	19-120-2945 (B,C, or D)	Pack of 100 for \$19
Nitrile Gloves (powder free)	Fisher	19-050-221 (B,C,orD)	Pack of 100 for \$22
Forceps	Forestry Supply	53782	\$13
Permanent Ink Markers (Sharpies)	Buy Locally		
Plastic Bags (WhirlPak)	Forestry Supply	79226	500 for \$55
Whatman FTA Micro Card	Whatman	WB12 0210	

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

### Vendor Phone Numbers

*Fisher Scientific:* 1-800-766-7000

*Forestry Suppliers Inc.:* 1-800-647-5368

*Cole Palmer:* 1-800-323-4340

*USA/Scientific:*1-800-522-8477