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We are still developing and optimizing this protocol

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Organ Biopsy Protocol (Mammals): Post-mortem Sampling V.2

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Revive and Restore

ABSTRACT

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The following protocol illustrates how to collect and ship living tissue from a deceased wild or captive mammal for long-term cryopreservation. Somatic tissues, including organs and skin, are optimal for harvesting live fibroblast cells. Collected tissues can either be immediately cryobanked at -196°C or processed for cell culture and later cryobanked at -196°C.

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GUIDELINES

The collection of samples post-mortem should be opportunistic and follow all applicable regulations. Tissues must be collected as close to the time of death as possible to avoid tissue decomposition and proliferation of bacteria. If the animal dies suddenly or if the animal must be euthanized, refrigerate but **do not freeze** the animal carcass until you perform the biopsy procedure, if possible. For any animal carcass found in the wild, time, ambient temperature, and storage methods are critical factors that can impact how quickly a sample must be collected.

If the animal has been found in a cooler climate, the maximum length of time to collect a biopsy sample is **24 hours.** If the carcass has been stored at approximately 4°C in a cooler or fridge, biopsy samples can be collected up to **72 hours** postmortem. If you do not know the time of death of the animal, try to collect multiple biopsies from different areas of the animal that do not show visible decay.

Take as many samples as permissable. Samples must be **larger than 3mm^3** to yield enough living fibroblasts cells for the eventual creation of cell lines. Collecting more that one sample type per individual increases the chances of successful biobanking in case one sample is not successful.

Use a sterile container large enough to hold the entire specimen. Use of a commercial kit is recommended. Other options include a large 50ml centrifuge tube, a clean Tupperware with a tight-sealing lid, or a durable ziplock bag. Include only **one** biopsy sample **per vial**. If you are running low on materials, up to two biopsy can be including in one vial. **DO NOT** mix biopsies from different individuals in the same vial.

For urgent questions regarding protocol steps or collection guidelines, please contact the Revive & Restore Biobanking Team at informedbiobanking@reviverestore.org

MATERIALS

Included in Commercial Sampling Kit:

Biopsy vials with media

Biopsy tool or ear punch

- 3, 4, 5, or 6 mm Integra Biopsy Punch Dermal #33-32, 33-34, 33-35, or 33-36

Parafilm

Insulated Tupperware

Vinyl lunch bag

Ice packs

Styrofoam container

Biopsy form

Pre-paid FedEx shipping label

User-supplied Materials:

Sterile saline (optional)

Sterile scalpel

Disposable gloves

Battery powered or electric clippers (optional)

Gauze

Rubbing alcohol

Disposable tweezers

Sterile scissors (optional)

Field notebook

Pencil/Pen

Permanent/alcohol-resistant marker

Newspaper or bubble wrap

SAFETY WARNINGS

Steps for personal safety must be considered before going into the field. Some wildlife diseases are transmissible to humans. Refer to your agency's health and safety guidelines for personal protective equipment (PPE). At a minimum, field personnel should wear disposable gloves and a fresh pair should be used between handling different specimens to avoid cross contamination. Sampling instruments and equipment should be thoroughly cleaned and disinfected or disposed of after use.

Tissue Handling

If using a commercial kit, biopsied tissues are stored in vials containing a nutrient-rich media to keep the cells alive and a mixture of antibiotics to prevent bacterial growth. If vials with media are not readily available, samples can be placed in a tube containing sterile saline. All samples must be collected under aseptic conditions to avoid contamination and stored at 4°C. Using sterile tweezers, scalpels, and scissors, as well as cleaning the sampling site with alcohol or surgical scrub (e.g. chlorhexidine) will decrease the chances of contamination. Take as many samples as permissable.

Collect in order of priority:
1) Ear or skin (*See Skin Biopsy Protocol*)
2) Trachea
3) Tongue
4) Lung
5) Kidney
7) Placenta

Cold Storage

Avoiding temperature fluctuations is very important for preserving the tissue samples. Use ice packs for transport of specimens from the field and ensure refrigeration is available immediately after returning from the field site. Do not use wet ice or other commercial therapeutic packs. Chill ice packs in the freezer the night before collection. Frozen ice packs will remain cold in an insulated container for up to 24 hours. If the field site is more than 24 hours from refrigeration, plan to bring a portable electric refrigerator. Vials containing tissue should not have direct contact with the ice packs. **Never freeze tissue biopsies** before shipping to a biobanking facility.

Shipping

Samples must be shipped to a biobanking laboratory via overnight express. You must use an insulated container and ice packs to maintain a temperature between 4-8°C. Considering that samples may be in queue at the receiving facility for a period of time before they are processed, **ship your samples as soon as possible.** Shipment Monday through Wednesday will guarantee arrival at the receiving facility before the weekend. **Do not ship samples on Friday**. Most facilities will not be available to receive shipments on weekends. If samples are collected Thursday through Sunday, samples must be stored in the fridge until Monday. Consult with the receiving facility for any questions regarding shipping timelines before sending samples.

Preparation

1 Pre-chill icepacks in the freezer the day before planning to collect and ship samples.

2 Record all information indicated in the biopsy form, including a picture of the animal for identification and GPS location where the animal was found.

3 Proper protective equipment must be worn (gloves, etc). Sterility must be maintained as much as possible.

Tissue Collection

4 Use a sterile container large enough to hold the entire specimen. Use of a commercial kit is recommended. Other options include a large 50ml centrifuge tube, a clean Tupperware with a tight-sealing lid, or a durable ziplock bag. Gather containers and have them readily available.
Step 4 includes a Step case.
Internal Organ
Tongue (for sport hunted species)

step case

Internal Organ

5 Wet the entire sampling area of the carcass in rubbing alcohol and blot with sterile absorbent gauze.

6 With sterile scissors or scalpel, cut through the body wall and aseptically retrieve the desired tissues using sterile tweezers and scissors.

Tissue Preservation

7 Using the disposable tweezers, immediately transfer sample to biopsy container. Ensure that tissue is fully submerged in liquid. Close the cap tightly. ***Each biopsy should be placed in a separate vial.***

Photo credit: Endangered Wolf Center

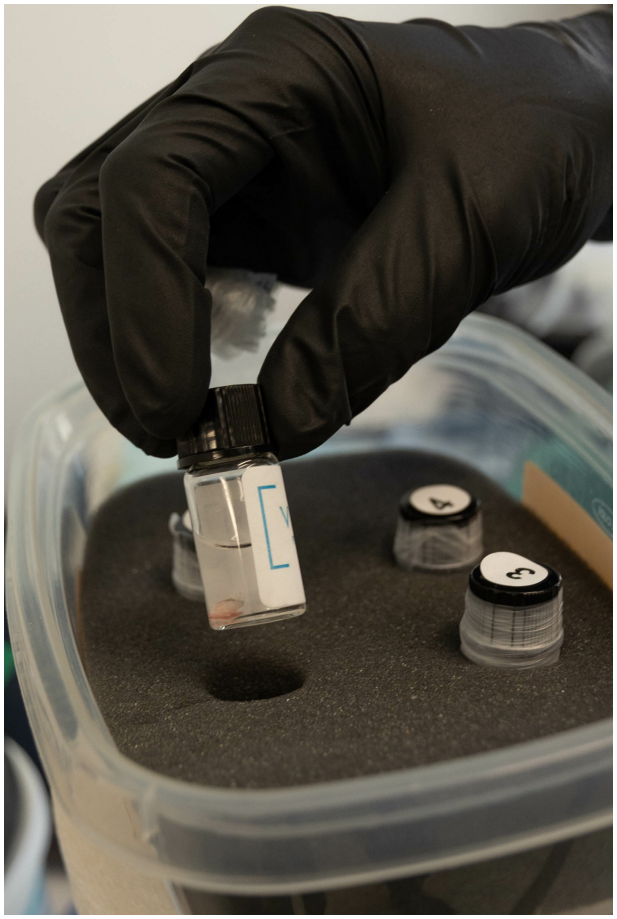

Photo credit: Endangered Wolf Center

8 Using an alcohol-resistant marker, label the biopsy vial with an identifier that matches ***exactly*** what is indicated on the biopsy form. Check to make sure that each vial is easy to identify with the information provided on the form.

Required information to include on the form:

- Scientific name of animal
- Sex of individual denoted as ♂ (male) or ♀(female)
- Date of tissue collection
- Tissue type
- Any other identification number of individual

9 Add a thin layer of Parafilm around the vial covering the seal.

How to apply Parafilm:

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10 Place samples on cold ice packs in an insulated cooler for transport back to your facility. Use the insulated Tupperware to transport your vials. If you do not have the Tupperware on hand, use about 3 inches of newspaper or bubble wrap to ensure that the tubes are not in direct contact with the ice packs. **Do not freeze samples.**

Do not ship samples via overnight express on Thursday-Sunday. For samples collected on these days, store samples upright in a refrigerator until you are ready to ship them*

Shipping

11 When you are ready to ship the samples, make sure the ice packs are frozen ahead of time. Below are video instructions on how to pack your box:

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12 Open the shipping box and place one frozen ice pack at the bottom of the styrofoam container inside.
Photo credit for the following photos: ViaGen Pets & Equine, 2021

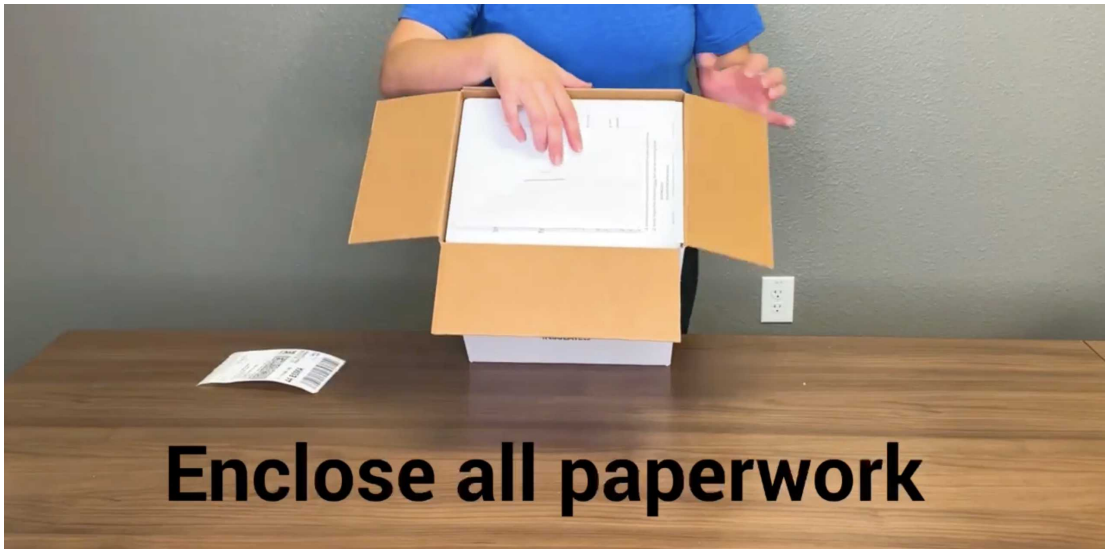
13 Place the biopsy vials inside the insulated Tupperware container included in your kit. If you do not have the insulated Tupperware container, wrap each vial in bubble wrap or newspaper and secure with tape to avoid direct contact with the icepacks.

14 Close the lid and place the Tupperware container into the vinyl lunch bag provided in your kit.

15 Place the vinyl lunch bag into the styrofoam container, on top of the first ice pack.

16 Place the second frozen icepack on top of the vinyl lunch bag.

17 Close the lid to the styrofoam container. Enclose all paperwork to the top, including the Biopsy Form. Close the shipping box and place the prepaid shipping label to the top.



18 Contact the receiving facility when you have shipped your sample and provide the tracking number so that personnel know when to expect your shipment.