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IN DEVELOPMENT

Skin Biopsy Protocol (Mammals): Non-lethal Sampling

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Revive and Restore

COMMENTS 7

ABSTRACT

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The following protocol illustrates how to collect and ship living tissue from a mammalian species under field conditions for long-term cryopreservation. Skin tissues are optimal for harvesting live fibroblast cells that can either be immediately cryobanked at -196°C, or processed for cell culture and later cryobanked at -196°C.

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PROTOCOL CITATION

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GUIDELINES

Skin biopsies can be taken when animals are immobilized for veterinary exams, radio-collaring, relocation, capture and release for captive breeding, and ear tagging. The collection of samples should be opportunistic and follow all applicable regulations.

Biopsies must be **larger than 3mm^3** to yield enough living fibroblasts cells for the eventual creation of cell lines. For large mammals, 5 or 6 mm biopsies are recommended. For small mammals, 3 to 4 mm biopsies are recommended. Biopsied tissues are stored in vial containing a nutrient-rich media to keep the cells alive and a mixture of antibiotics to prevent bacterial growth.

MATERIALS TEXT

Included in Commercial Sampling Kit:
Sterile scalpel
Biopsy vials with media
Insulated Tupperware
Vinyl lunch bag
Ice packs
Styrofoam container
Biopsy form
Pre-paid FedEx shipping label

User-supplied Materials:
Disposable gloves
Battery powered or electric clippers
Gauze
Rubbing alcohol
Disposable tweezers
Sterile scissors (optional)
Topical anesthetic (lidocaine, bupivacaine, or ropivacaine)
Biopsy tool or ear punch

- Large Mammal: 5 or 6 mm Integra Biopsy Punch Dermal #33-35, or 33-36
- Small Mammal: 3, 3.5, or 4 mm Integra Biopsy Punch Dermal #33-32, 33-33, or 33-34

CELOX hemostatic agent
Neosporin
Parafilm™ M Laboratory Wrapping Film, 2 in. W x 250 ft. L (5cm x 76m) **Thermo Fisher Catalog #1337416**
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. Field biologists should refer to their 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.

BEFORE STARTING

Tissue Handling

Biopsied tissues are stored at 4°C 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, biopsies can be placed in a sterile saline and kept at 4°C. All samples must be collected under aseptic conditions to avoid contamination. Using sterile tweezers, scalpels, and scissors, as well as cleaning the sampling site will decrease the chances of contamination. Collect as many samples as possible.

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.

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 GIS location where the animal was found.

3 For general sedation, anesthetize according to standard practices. Instructions for local anesthesia are in the proceeding steps (Refer to Handbook of Wildlife Chemical Immobilization by Terry Kreeger).

4 Shave sampling area with hair trimmer, removing as much hair as possible. Ear punches are most preferred for collection, but biopsies can also be collected from the neck, flank, or lower abdomen. Skip this step if the area (e.g. ear) is too small to trim.

5 Soak gauze with rubbing alcohol and rub shaved sampling area to thoroughly disinfect. Discard used gauze. Reserve some clean gauze for use later.

6 If using local anesthesia, apply a topical anesthetic such as lidocaine, bupivacaine, or ropivacaine to temporarily relieve pain. Consult a veterinarian for recommended products.

7 Use a sterile scalpel and manually shave off all remaining hair in the area. Skip this step if the area (e.g. ear) is too small.

8 Use a new piece of alcohol-drenched gauze to clean the shaved area again very well. Discard used gauze.

Tissue Collection

9 Remove the biopsy vials with media from the kit and have them readily available. There are different methods for obtaining skin biopsies. Select the method below that is most appropriate for the species being handled:
Step 9 includes a Step case.
Sterile scalpel
Tissue biopsy tool
Ear punch

step case

Sterile scalpel

Tissues can be taken from the neck, flank, or lower abdomen. For large mammals, 5 or 6 mm^3 biopsies are recommended. For small mammals, 3 or 4 mm^3 biopsies are recommended.

Using sterile tweezers and scalpel, grasp a piece of skin from the center of the shaved area and cut off a full thickness of dermis about the size of a pea. Discard/replace instruments between individual animals.

10 Apply CELOX hemostatic agent to stop the bleeding and antibiotic ointment to sampling area.

Tissue Preservation

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

12 Using an alcohol-resistant marker, label the biopsy vial with:

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

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

How to apply Parafilm:

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14 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

15 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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16 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

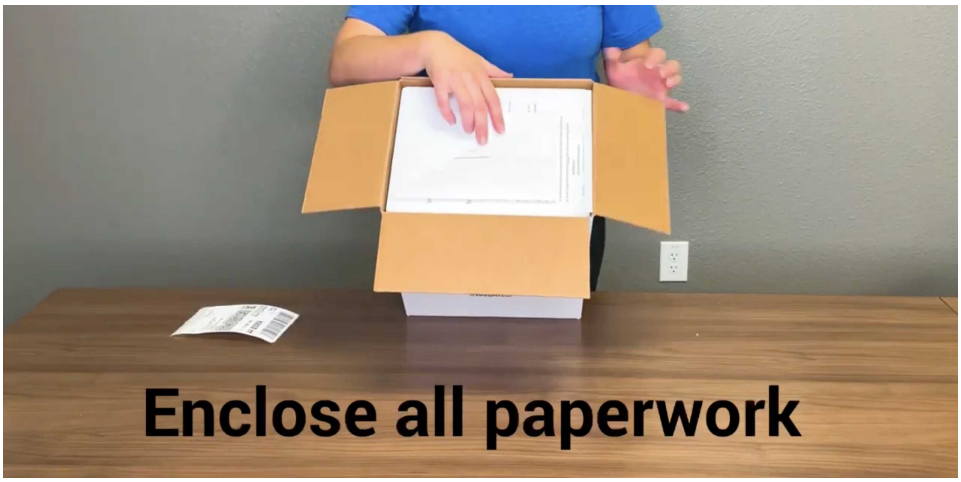
17 Place the biopsy vials inside the insulated Tupperware container included in your kit. If you do not have the insulated Tupperware container, wrap each vial in bubble wrap or newspaper and secure with tape to avoid direct contact with the icepacks.

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

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

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

21 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.



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