Addendum to Class Protocols #001, 002, 003, 004, 005, 007, 008, 009, 010, 012

Wildlife Research Permit or Collection Licences

Adopted 22 July, 2013

Invasive DNA Collection from Wildlife Species

Specific Activities

Procedures for Invasive DNA collection including buccal swabs, whole blood, tissue biopsy darting and hair/feather pluck.

Objectives

To collect DNA invasively for research and management purposes.

Primary Contact / Authority

Director of Fish and Wildlife Policy

Applicable Personnel

- Project leads must have appropriate experience in mammal species and/or bird species capture and handling methods.
- All team members must be educated about possible zoonotic diseases that small mammal species can carry and how to protect themselves against transmission.
- Individuals collecting DNA through the use of tissue biopsy darts must have wildlife chemical immobilization training to mitigate shot placement hazards.

Species

All mammals, birds, amphibians, reptiles

Applicable Geographic Range

Provincial

Methods

- Buccal swabbing is the preferred method to collect DNA from captured wildlife and should be considered prior to utilizing more invasive procedures to minimize stress and injury to the individual.
- Bird, reptile and amphibian species have nucleated erythrocytes (red-blood cells) in addition to nucleated leukocytes (white blood cells). As a result, small blood volumes from such species will provide high quantities of DNA template.
- Researchers must refer to and follow class protocols for the capture, and handling of their target species, as listed on the Alberta research permit and collection license web site:

http://srd.alberta.ca/FishWildlife/WildlifeResearchCollection/Default.aspx

DNA from buccal (epithelial) swabbing

Buccal swabs are commonly used in epidemiological and forensic investigations to acquire human DNA (Handel et al. 2006). They were applied recently in field and laboratory studies of nonhuman mammals (e.g., Brooks et al. 2003; Mitrec˘ic´ et al. 2008), reptiles and amphibians (e.g., Poschadel and Mo¨ller 2004), birds/nestlings (Brubaker et al. 2011; Yannic et al. 2011) and even fish (Smalley and Campanella 2005).

As a reliable method to collect high quantities of quality DNA, this method is preferred over more invasive and stressful procedures such as bleeding, tissue biopsy and feather plucks.

- DNA buccal swabs come in various sizes. A swab size that reflects the mouth opening of your target species must be considered prior to sampling to prevent injury.
- The buccal swab is inserted into the mouth of an individual and gently rotated against the cheeks and across the tongue of the individual three to five times. The swab is then placed in a collection tube.
- DNA can also be extracted from amphibians by gently swabbing their skin.

DNA from Whole Blood

Small Birds: Collection from Toe Nail Clip

1. Support leg of bird at knee joint and clip an accessible toe nail with nail clippers, scissors, or other appropriate instrument. Cut should extend to beginning of vascular area in the nail bed, but no deeper than necessary. If blood is not present, gently squeeze the toe until blood appears. Ensure that the toenail clipper is thoroughly cleaned with alcohol between birds.

2. Press drop of blood forming at the site of the nail clip directly to the centre of a Whatman FTA-card collection site. Allow blood on card to dry for approximately 20 minutes. Sample only 1 bird per card and do not saturate the card, one drop is sufficient. FTA cards can be stored at room temperature in a dry location.

3. If Whatman FTA cards are not used, blood drops can be collected in a tube containing 95% alcohol in a sealed eppindorf collection tube, shaken and stored at room temperature. Approximately 50 microliters of blood (1-2 drops) is sufficient per 1 ml of ethanol. Collecting more blood may result in sample degradation.

4. Soft direct pressure should be applied to the site of the clip with a sterile, non-adhesive bandage. Cotton should not be used as it may stick to the blood clot and pull the clot away when the cotton is removed. Cornstarch, flour or baking soda may also be placed on the bleeding site to act as a coagulant.

Collection from Veins

1. Only persons trained in the removal of blood from veins are authorized to employ this procedure.

2. For DNA collection, purple topped blood collection vaccutainers must be used. These tubes contain EDTA, Clotting tubes and those with Heparin, are unsuitable for DNA extraction.

3. Do not remove blood volumes exceeding 1% of the individual's body weight.

DNA from Tissue Biopsy Darting of Large Mammals

1. Personnel using tissue biopsy darting must be certified in Wildlife Chemical Immobilization techniques to receive the necessary training, to properly, and safely, dart wildlife without causing additional injury.

2. Tissue biopsy needles must be used that are 2.2mm - 5.0mm outside diameter (roughly a 18-20 gauge needle) and 15-25 mm in length. This covers both the Palmer and Dan Inject style biopsy darts.

3. The dart is to be fired from a CO_2 rifle, with adjustable firing power. A range finder must be used to estimate distance and allow power adjustment prior to darting.

4. Once darted, and the needle repels from the animal, the needle must be collected, and the animal no longer pursued.

DNA from Feather Plucks or Hair pulls

1. Before taking any samples, ensure that you are wearing clean gloves. If you are collecting from multiple individuals, change gloves between each collection.

2. **Feathers:** Collect 4-5 chest feathers. Large feathers are NOT required. DNA is contained in epithelial cells at the base of the feather under the surface of the skin. Avoid plucking feathers from the opposite end or plucking in clumps, as this may cause the shafts to break off above the skin, rendering them unusable. Pluck a feather as close to the skin as possible, use sanitized tweezers if necessary.

3. **Hair:** Collect 10-20 hairs from each animal. DO NOT cut the hair, DNA is found in the hair root and follicle, which can only be obtained by pulling the hair out of the skin. The hair shaft does not contain genomic DNA.

References

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Mitrec˘ic´ D, Mavric´ S, Vrabec Branica B, Gajovic´ S (2008) Mice genotyping using buccal swabs: an improved method. Biochem Genet 46:105–112.

Poschadel JR, Mo¨ller D (2004) A versatile field method for tissue sampling on small reptiles and amphibians, applied to pond turtles, newts, frogs and toads. Conserv Genet 5:865–867.
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