



## Invasive Animals CRC

### Field sampling protocol for trace DNA samples

This protocol outlines optimal storage and transport methods for hair and scat samples destined to be used for DNA analysis. It's based on our experience in dealing with these samples, but is a work in progress and is likely to change with time. We'd welcome your feedback if you've found some methods more easy/difficult, and will incorporate your comments in later versions.

Cheers,

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#### **Some general principles**

Trace samples contain small quantities of DNA that may also be of low quality. Correct collection and handling techniques can make a big difference to the success of a project. The usual sources of DNA are plucked hairs and scats (faeces), but other sources exist (e.g. saliva). Each sample type has its own requirements for optimal storage and these are detailed on pages 2 - 4. However, some general things to consider are:

1. Liaise with the laboratory team prior to sample collection. Each laboratory has its own way of doing things and individuals may prefer to process samples collected in a particular manner. They may also be happy to send out sampling tubes already prepared for collection.
2. Avoid cross-contamination of samples.
  - Wear a new pair of disposable gloves to collect each sample.
  - Use a fresh blade for each sample. If this is not possible, either flame sterilise instruments (tweezers/scissors) between samples (with ethanol), or if there is a fire risk wipe with ethanol or 20% bleach.
  - Store samples so that they will not come into contact with other samples or tissues from the target species (or related species).
3. Collect samples as soon as possible after deposition – ideally within 24 hours.
4. Minimise the time between sample collection and DNA analysis. This increases the chance of obtaining quality genotypes. It requires coordination between field and laboratory teams, so good communication is essential.
5. Store samples quickly and appropriately. DNA degrades in the presence of enzymes, microbes, and certain chemical conditions that are naturally present in hair and scat samples. Sample storage is all about minimising these impacts. This can be done by freezing, drying, or immersion in solutions that inhibit biochemical activity.

6. Record relevant field/site data at time of collection. Typically this will include
  - collectors name,
  - sex,
  - body condition,
  - any other ID you may have for that animal/sample,
  - reproductive state,
  - location information (as much detail as possible is important, ideally digital lats and longs or GPS co-ord's but failing that, at least a site description (eg 10.3km north of town X along road Y),
  - any other potentially relevant information.

## **Optimal methods to collect and store tissue, hair and scats**

### **Tissue (skin, flesh etc)**

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#### General

Tissue yields substantial quantities of DNA and should be collected wherever possible. However, because it requires animals to be captured there are many practical and/or ethical reasons why it may not be appropriate.

#### Collection

For mammals, ear tissue is generally the most convenient but any tissue type is suitable. If possible (and especially if an animal is dead), collect samples in duplicate and label sample tubes accordingly eg 01a, 01b. For each individual, place a small amount of tissue (less than half a gram, or approx. 0.5 - 1cm<sup>2</sup>) into each of the tubes. When you've finished collecting, retain one complete set of samples and send the other to the laboratory for genetic analysis.

#### Storage

*Liquid storage (see Appendix for recipes)*

Samples can be stored in either DMSO buffer or tissue lysis buffer. Our labs have had good success with both, but it would be wise to check with the laboratory team as to which they prefer. 95% Ethanol is an excellent tissue preservative but samples cannot be posted (although courier is OK). For this reason, we advise not to store samples in ethanol.

A 3:1 ratio of liquid to sample is a good rule of thumb so about 1.5ml buffer and <500mg tissue in a 2ml tube would work. The samples can be stored at room temperature if required but a fridge (4°C) would be better especially if for significant duration.

#### *Freezing*

Freezing at -20°C or in liquid nitrogen preserves the integrity of DNA very well, but requires quick access to a freezer (or equivalent) once a sample is taken. A major drawback of freezing samples in the field is that ideally they should remain frozen until

they reach the lab because repeated freeze-thaw cycles will degrade the DNA.. This may require special transport arrangements to be made.

## **Hair**

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### General

Hair is an excellent source of high quality DNA and can be easily stored and transported. We recommend that where practical, hair be used in preference to scats. It will result in more efficient laboratory analysis, is more likely to produce informative results, and will generally be less expensive.

DNA is contained in the follicle or root-bulb at the base of the hair. Cut or broken hair without the follicle will not contain DNA and cannot be used for individual identification. Hair follicles should appear to someone with good eyesight as obvious bulbs or enlarged areas at the end of the hair (try holding it up to the light or against a uniform background). They are often pale coloured. You may want to try plucking hairs from your study animal (or yourself) to get an idea of what they should look like.

### Hair-storage

Hair can be conveniently stored in plain paper envelopes or paper bags. These should be breathable (not lined or waxed). Labels can be written directly onto the bag/envelope. Samples should be kept in a dry cool place out of sunlight. If operating in a moist environment, consider placing the paper bag inside a zip-lock bag with a spoonful of silica crystals. These are inexpensive and available from scientific suppliers (e.g. Merck Chemicals 1kg, \$67, catalogue number 1.01969.1000), dried flower suppliers, and of course – ebay. Silica crystals are pink or orange when desiccated and turn progressively more blue as they absorb moisture. Blue silica can be easily re-desiccated in an oven. In the absence of silica, do not store the sample in a plastic bag.

### Removal of hairs from sticky traps.

It is essential that hair follicles are collected along with the hair shaft, so care needs to be taken when removing them from sticky traps. The device you use to collect hairs, and removal of the hair from snares/sticky traps in the field may make laboratory analysis more efficient. We suggest you speak to the laboratory conducting the analysis to arrange the most mutually convenient approach.

If you are removing hairs in the field, we have had good success with the product “Goo-gone” as an antidote to sticky glues and tapes. Quantities of Goo-gone can be purchased online at ebay for less than \$10. We have not examined the effect of Goo-gone on DNA quality, so if hairs are coated with Goo-gone it’s probably prudent to blot them on tissue before storing them. If you are working with sticky glue it may be wise to have some Goo-gone handy for clean ups.

### Numbers of hairs to collect.

In some cases it may be unclear whether more than one individual has deposited hair on a trap. If the hair of more than one is present it presents a problem for genetic analysis because composite genotypes or “identities” will be generated.

A useful way to get around this is to use a sand (or snow) pad beneath the hair trap. This has the added advantage of allowing an assessment of how well sand plots index abundance. Sand plots also enable hairs of non-target species to be excluded from the genetic analysis – though morphological analysis of hairs should be used if possible.

If hairs in trap could represent more than one individual, it is important to:

- a) separately store a number of hairs individually (6 or more depending of number of hairs present), and also
- b) store several hairs together which you have reason to think come from the same individual

This conservative approach requires a lot more genetic analysis than if single animals are assumed to have left hair – so adds expense. Whether it's necessary will depend on the behaviour of the animals, their density, and the frequency of sample collection. Discussion with lab staff and some pilot sampling should make it clear whether it's necessary.

## **Scats**

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### General

Although scats contains inferior DNA to hair, the ease of scat collection makes them useful in some situations (particularly for canids). The main difficulty with scats is that they contain substances that interfere with downstream genetic analysis. This makes results harder to obtain, more technically demanding and therefore more expensive. However, excellent genotype results can be obtained from scats.

### Scat storage.

Scats can be dried or immersed in a liquid that inhibits enzymatic activity.

### Drying

Drying should be done relatively rapidly (within a couple of days), and may require use of a drying room/oven and/or silica crystals. Excessive heat degrades DNA, so temperatures below 30°C are best. Place scats in labelled, breathable paper bags for the drying process. Once dry, the paper bag should be placed in a zip-lock bag along with a spoonful of silica crystals.

### Liquid storage

A useful storage liquid for scats is DET buffer. It may be easier for you to arrange the laboratory conducting the genetic analysis to provide you with sample tubes ready to receive samples. DET contains 20% DMSO (Dimethyl sulphoxide), which is toxic if ingested and may cause irritation if it contacts skin. Use latex gloves and work in a well ventilated area. Samples stored in DET should be kept in a cool place out of direct sunlight. If available, use a fridge.

For both drying and DET buffer it is not necessary to use the entire scat for DNA analysis, and you may want to conduct dietary analysis on the scat anyway. The most useful part of the scat is the surface, which is believed to be covered in epithelial cells from the digestive tract. We suggest you scrape off *ca.* 100mg from the outside of the scat with a sterilised blade and place it either in the paper bag or vial containing DET. Alternatively, if the laboratory staff are willing to remove the epithelial cells, break off a portion of the scat (say one third or less), and place it either in the paper bag or vial.

## Appendix - Storage solutions

If you have access to molecular biology consumables you may want to make up your own storage solutions. However, it may be easier for your collaborators in the DNA laboratory to supply them to you.

DMSO Storage buffer (Seutin *et al.*, 1991).

20% DMSO,  
0.25 M sodium-EDTA,  
NaCl to saturation,  
pH 7.5

Lysis buffer (Longmire *et al.* 1997):

0.1 M Tris-HCl (pH 8.0),  
0.1 M sodium-EDTA (pH 8.0),  
0.01 M NaCl,  
0.5% SDS