Charles Darwin University Animal Ethics Committee

Standard Operating Procedure:

TISSUE SAMPLE COLLECTION AND STORAGE FOR GENETIC PURPOSES (WA DBCA)

Version No:	Version 1.3
Date of Approval by the CDU AEC:	04/02/2026
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Please note: this SOP has been developed for animal use in WA. Consideration should be taken to the specific conditions of the region in which your work is being conducted, and modifications to procedures made accordingly to ensure the best welfare of the animal and safety of the project participants. Any modifications required should be outlined in the project application.

CHARLES DARWIN UNIVERSITY AUSTRALIA

Standard Operating Procedure

SC25-09 TISSUE SAMPLE COLLECTION AND STORAGE FOR GENETIC PURPOSES (AUGUST 2025)

Animal welfare is the responsibility of all personnel involved in the care and use of animals for scientific purposes.

Personnel involved in an Animal Ethics Committee approved project should read and understand their obligations under the *Australian code for the care and use of animals for scientific purposes*.

Version 1.3

August 2025

GOVERNMENT OF WESTERN AUSTRALIA

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***Previously titled Tissue Sample Collection and Storage for Mammals**

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Approved by the DBCA Animal Ethics Committee: 

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Chairperson, Animal Ethics Committee

Department of Biodiversity, Conservation and Attractions

Contents

1 Acknowledgments

This Standard Operating Procedure was originally developed by Rebecca Bloomfield, Myrto Roberts, Holly Raudino and Mia Podesta with contributions from Manda Page, Kym Ottewell, Doug Coughran, Peter Spencer, Linette Umbrello, Aline Gibson Vega, Diana Prada, Robyn Shaw, Rujiporn Sun, Rowan Lymbery and Martin Dziminski.

2 Purpose

This Standard Operating Procedure (SOP) provides advice on the collection and storage of tissue samples for genetic studies. It includes information on animal welfare considerations, disease considerations, mitigations and procedures for obtaining tissue samples, types of tissues preferred for different genetic methods and correct sample storage procedures.

3 Scope

This SOP has been written specifically for scientific and education purposes, and endorsed by the Department of Biodiversity, Conservation and Attractions' (DBCA) Animal Ethics Committee (AEC). However, this SOP may also be appropriate for other situations.

This SOP applies to survey and monitoring activities involving invasive and non-invasive tissue sampling for genetic studies undertaken across Western Australia by DBCA (hereafter department) personnel. It may also be used to guide fauna related activities undertaken by Natural Resource Management groups, consultants, researchers and any other individuals or organisations. All department personnel involved in fauna research and management should be familiar with the content of this document. Genetic sampling of crocodiles and cetaceans are described in department SOPs *Biopsy Tissue Sample Collection for Crocodiles* and *Sampling Cetaceans Using a Remote Biopsy System.*

This SOP complements the *Australian code of practice for the care and use of animals for scientific purposes* (The Code). The Code provides the ethical framework and governing principles to guide decisions and actions of all of those involved in the care and use of animals for scientific purposes and should be referred to for all AEC approved projects. A copy of the Code may be viewed by visiting the National Health and Medical Research Council website https://www.nhmrc.gov.au/about-us/publications/australian-code-care-and-use-animals-scientific-purposes).

4 Rationale

A DNA profile provides a unique and highly discriminative method for animal identification. Genetic information gained from multiple animals can provide valuable insights into population dynamics and assist in the development of species conservation management strategies. Genetic information is used in multiple ways in species conservation, for example:

- Species identification – confirm species or sub-species designation, clarify taxonomic boundaries when morphological characters are unclear.
- Population structure – delimit appropriate management units, understand connectivity amongst these.
- Genetic diversity – genetically-diverse populations have higher adaptive capacity and resilience to change, while low genetic diversity may indicate that management is required.
- Tracking animal movement – identify how populations are connected, landscape characteristics that facilitate or impede movement.

- Inbreeding – high inbreeding may indicate populations are declining and require management.
- Population modelling – genetic data incorporated with trapping data to predict population trends.
- Disease – understanding diversity at immune genes can inform management strategies to improve resilience to novel pathogens.

Animal tissue yields substantial amounts of DNA and is the best biological sampling method for DNA analysis. It is suitable for use in a broad range of genetic applications and can often be available for re-use as molecular techniques advance over time, increasing the breadth of information that can be obtained from a single sample. Invasive tissue samples, when stored correctly, also have extended longevity compared to other DNA sources (e.g. non-invasive samples), enabling research questions or molecular analyses to be applied retrospectively.

Invasive tissue samples involve the removal of live tissue directly from the animal and usually require the animals to be captured and restrained therefore carries greater risks to animal welfare (Berry and Aitken, 2007). Recently, molecular technologies have been applied to non-invasively collected samples such as hair, scats, eggshells, sloughed skin and moulted feathers. Non-invasive samples can provide a source of DNA that can be used to identify species and have potential as an alternative animal marking method (individuals can be identified by their unique genetic 'fingerprint') in capture-recapture monitoring activities (e.g. Dziminski et al., 2020; Treloar et al., 2024). However, the low amount and/or quality of DNA obtained from non-invasive samples limits the reliability of genetic results and restricts the genetic questions or techniques that can be applied (Taberlet et al., 1999; Table 1). Non-invasive samples are not typically suitable for long-term storage (compared with invasive tissue samples), so may have limited value for retrospective analyses. While non-invasive approaches may be a suitable replacement depending on the genetic application, the threshold at which the advantage of non-invasive sampling surpasses that of live-trapping must be assessed on a case-by-case basis (Ferreira et al., 2018).

Table 1 Suitability of invasive vs. non-invasively collected samples (e.g. hair, scat, moulted feathers) for different genetic research applications

Sample Type	**Reference genome**	**Species Identification**	**Population structure**	**Genetic diversity / inbreeding**	**Immunogenetics**	**Mark-recapture**	**Virology / toxicology**	**Long-term storage**
Invasive	✓	✓	✓	✓	✓	✓	✓	✓
Non-invasive	×	✓	~	~	×	✓*	~	~

* The use of non-invasive samples as a replacement for live capture for mark-recapture analysis needs to be evaluated on a case-by-case basis as its effective use has only been demonstrated for a small number a species to date.

4.1 Collection guidelines

DBCA maintains a threatened species tissue repository associated with the Sid James Conservation Genetics Laboratory in Kensington. Whilst a valuable collection, there is limited

storage capacity and guidance is required on the number and frequency of tissue sample collection. The following guidelines pertain to threatened, priority or otherwise conservation-dependent species and are indicative only. In addition, the Western Australian Museum houses a long-term tissue storage facility and can be contacted for tissue samples from non-threatened species or small numbers of samples from threatened species. They do not have capacity to take large collections of tissue samples from single species, such as usually obtained during population monitoring or translocations.

Following consideration of animal welfare guidelines and any disease concerns, the following indicative sampling guidelines are recommended:

4.1.1 Translocations

- Tissue samples should be collected from all founder individuals in a translocation at a minimum.
- Following translocation, tissue samples should be collected at suitable intervals, depending on factors such as recruitment rate and species generation times, during population monitoring to address short, medium and long-term success criteria.
- More intensive sampling is preferred in the first year (or years, depending on species life history traits) post-translocation to assess founder contributions and maintenance of genetic diversity. Consider tissue sample collection from all captured individuals at this time step.
- Less intensive sampling is typically needed for medium – long-term success criteria. Aim to obtain ~20 samples per sub/population for genetic assessment at medium and long-term time steps.

4.1.2 General Population Monitoring

- Tissue samples should be collected at regular intervals for genetic assessment e.g., every five or 10 years.
- ~20 samples are generally required for genetic assessment per sub/population. These may be obtained in a single survey event or could be obtained cumulatively for less abundant species.

4.1.3 Biodiversity Survey

- Curators at the Western Australian Museum should be contacted in the first instance to discuss species expected to be encountered and determine whether tissue collection is required.
- Similarly, the DBCA fauna genetics group should also be consulted to determine the value of tissue sample collection for ongoing projects.

4.1.4 ‘Opportunistic’ sampling

- If a biological survey or monitoring is to be undertaken, and there is potential by-catch of threatened species, the DBCA fauna genetics group should be consulted as there may be opportunity to gain genetic samples from under sampled geographic regions. The procedures must be included in the AEC application and approved by the Committee.

5 Animal Welfare Considerations

To reduce the level of impact of tissue sampling on the welfare of animals, personnel must consider, address and plan for the range of welfare impacts that may be encountered. Strategies to reduce impacts should be identified during the planning stage to ensure that they can be readily implemented during tissue sampling, and to ensure that contingencies for managing welfare issues have been identified. Ensure that all personnel involved in the project are aware of the range of issues that they may encounter, the options that are available for reducing impact and improving animal welfare, and the process for managing adverse events.

Invasive tissue sampling inherently carries a greater risk to animal welfare than non-invasive tissue sampling. Department projects involving invasive tissue sampling for genetic purposes will require approval from the department's AEC however approval may also be required for non-invasive sampling if there is a risk of causing disturbance to the habitat that may alter the animal's behaviour or affect its welfare. More information on activities requiring approval can be found in the Scientific Use Licences for environmental scientists in Western Australia – interim policy and frequently asked questions, or by contacting the AEC executive officer. The key animal welfare considerations whilst taking tissue samples for genetic studies are listed below and highlighted throughout the document.

5.1 Injury and unexpected deaths

If adverse events including injury, unexpected deaths or unplanned requirement for euthanasia occur then it is essential to consider the possible causes and take action to prevent further issues. Adhering to the guidance in this SOP will assist in minimising the likelihood of adverse events. For projects approved by the department's AEC, adverse events must be reported in writing to the AEC Executive Officer as soon as possible after the event by completing an *Adverse Event Form* (refer to Guidance for Adverse Events). Guidance on first aid and field euthanasia procedures are described in the department SOPs for *First Aid for Animals* and *Euthanasia of Animals Under Field Conditions*. Where infectious disease is suspected, refer to the department SOP for *Managing Disease Risk and Biosecurity in Wildlife Management* for further guidance.

5.2 Level of impact

Potential animal welfare impacts when taking a tissue sample include:

- Distress caused by handling, discomfort, social isolation, separation of mother and young.
- Trauma from injury to the animal during restraint.
- Trauma, discomfort and pain associated with the sampling activity.
- Infection at site of tissue sample.
- Altered behaviour due to disturbance to the environment (non-invasive collection).

It should be noted that whilst these impacts are specifically associated with the procedure of tissue sampling for genetic analysis, an animal may also experience other impacts from associated procedures such as capture and handling. Investigators must be aware that the effects of a series of stressors, such as capture, handling, transportation, sedation, anaesthesia or marking can be cumulative.

5.2.1 Disease concerns

When handling animals, zoonotic disease and biosecurity must always be considered. If any animal is sampled that appears unwell or has suddenly died, samples can usually still be collected and appropriately stored. If an infectious disease is suspected, samples should not be shipped externally to other institutions until a diagnosis confirms the animal is clear of the suspected infectious disease. For further information refer to department SOP for *Managing Disease Risk and Biosecurity in Wildlife Management.*

6 Procedure Outline

6.1 Liaise with the laboratory

Prior to collection, it is important to liaise with the laboratory processing the samples as there may be specific requirements for sample collection and storage (Berry and Aitken, 2007). More detail on storage requirements is provided in Section 6.8 below. Confirming collection protocols prior to being in the field will ensure that the maximum value can be obtained from any given tissue sample.

6.2 Equipment

The specific equipment required for tissue sampling in the field may vary depending on target species. Single use items may be preferred to prevent contamination or infection between samples. If equipment is to be reused, items with a smooth surface rather than serrations are preferred to ensure that they can be sufficiently cleaned and sterilised. Some examples include:

- An ear notching/biopsy tool
  - reusable e.g. 2 mm Onyx scissor action ear punch
  - disposable e.g. Kai biopsy punches
- Fine-pointed forceps e.g. stainless steel fine-pointed forceps G-E963, Australian Entomological supplies
- Straight sharp pointed dissection scissors e.g. G-E140, Australian Entomological Supplies
- Disposable needles e.g. 23 – 26G needle
- Haematocrit tubes e.g. Kimble 75 ul heparinised tubes.

6.3 Storage tubes and other consumables

For ethanol storage of tissue samples, 2 ml sample tubes with screw caps (with O-ring) are recommended (e.g. interpath screw tubes and caps). For cryo-storage of tissue samples (-80°C), specialist crack-resistant cryotubes are required (e.g. Greiner Bio-One Cat No. 122279). Note, these tubes are not suitable for use with ethanol as they do not seal adequately to prevent leakage.

Other consumables include ethanol wipes, cotton wool, paper towel, and antibacterial or disinfectant wipes.

6.4 Cleaning and disinfection

Although sterile techniques are difficult in the field, cleanliness of all tissue sampling equipment is paramount to providing reliable genetic samples and to minimise the potential for disease transmission between animals. For further guidance regarding hygiene procedures refer to the department SOP for *Managing Disease Risk and Biosecurity in Wildlife Management*.

Cross-contamination of DNA between samples can render tissue samples non-viable. The most likely ways for contamination to occur is by re-using sampling instruments on multiple animals without sterilisation or by the animal handler touching the tissue sample, collecting instruments or storage vials, which will result in their own human DNA contaminating the sample.

To avoid cross-contamination:

- Where possible use sterile, disposable equipment, e.g. biopsy punches, scalpel blades or needles (one unit per animal).
- If instruments need to be reused, they must be cleaned and disinfected prior to reuse (see below). Multiple instruments are useful if several animals are processed.
- To avoid sample contamination with human DNA, avoid direct contact with the area to be sampled and the sample itself. Where practical, wear latex or nitrile gloves when collecting samples and replace gloves between animals.
- All equipment used to obtain the tissue sample should be cleaned and disinfected prior to returning the equipment for storage (see below).

6.4.1 Flaming

Flaming is the most common method for cleaning and disinfecting equipment but in fire risk areas it may not be possible or appropriate. In such cases, dissection scissors can be easier to clean than reusable biopsy punches and are less likely to retain tissue remnants (or consider using single-use biopsy punches). A 70% isopropyl alcohol medical swab is a suitable alternative to flaming or use one of the solutions in Section 6.4.2 below.

Method:

a) Wipe the equipment to be used for obtaining the sample with a tissue or ethanol swab to remove any dirt or tissue.
b) Dip the equipment into 70% ethanol. *Note: ethanol is a highly flammable substance, and care should be taken to not get ethanol on anything other than the equipment to be flamed.*
c) Clean up any spillages immediately, including any ethanol on hands and clothing, and if required wait until the spilled ethanol has evaporated before continuing with the procedure.
d) Remove equipment from the ethanol and flame the cutting part with a lighter or portable flame torch. *Note: the flame from ethanol is not visible in sunlight. Allow the equipment to cool before using it on an animal.*
e) DO NOT allow contact with any other biological material (including human fingers) before the above procedure is repeated and the next animal is sampled.

6.4.2 Cleaning and disinfecting solutions

For DNA tissue collection, it is necessary to disinfect equipment between individuals to prevent cross contamination. Where flaming is not used, the following steps are required to avoid cross contamination, while preserving DNA quality:

For single step disinfection, equipment can be soaked in 10% bleach for 10 minutes followed by a thorough rinse with two changes of deionised water.

a) Submerge tool in 10% bleach solution and shake vigorously for several seconds
b) Remove and gently shake equipment above the tube containing the liquid to remove excess bleach and use a Kimwipe to dry the equipment as much as possible (any remaining bleach will damage the DNA).
c) Submerge in distilled water and shake vigorously for 10 seconds. Drain off water and repeat, finally drying the equipment with a Kimwipe.
d) DO NOT allow contact with any other biological material (including human fingers) before the next animal is sampled.
e) Alternatively, use a new piece of equipment for each individual and disinfect all equipment at the end of the sampling session.

6.5 Animal handling

a) Techniques for handling animals vary depending on the species involved and the experience and skills of the personnel. General advice on animal handling is contained in the department SOP for *Hand Restraint of Wildlife*.
b) Use handling bags appropriate for the species and duration of containment as advised in the department SOP for *Animal Handling and Restraint using Soft Containment*.
c) If an animal is injured during handling/sampling refer to the department SOP for *First Aid for Animals*.
d) If an animal is seriously injured, refer to the flowchart in the department SOP for *Euthanasia of Animals Under Field Conditions* to make the decision on whether to euthanise or seek veterinary care (refer to Guidance for Adverse Events).

6.6 Sample Collection

Only trained and experienced personnel (or those under direct supervision) should take tissue samples from live animals. Common approaches for tissue sampling from different taxa are listed in Table 2.

Table 2 Invasive tissue sampling approaches commonly used for different groups of live vertebrates.

Taxonomic group	Tissue Type	Section
Non-volant mammals	Ear notch/punch, 1-2 mm, maximum 2	6.6.1
Bats	Wing punch from a non-vascularised area, 1-2 mm, maximum 2 punches	6.6.2
Birds	Blood (brachial or medial metatarsal vein) Feathers	6.6.10 6.7.2
Lizards	Tail tip (excluding some geckos) Toe clip	6.6.3 6.6.8

Snakes	Tail tip (excluding blind snakes) Scale clip	6.6.3 6.6.4
Testudines (turtles, tortoises)	Blood Skin sample from trailing web of hindfoot	6.6.6 6.6.5
Amphibians	Swabbing Toe clip Fin clip	6.6.7 6.6.8 6.6.9

Mammals

6.6.1 Ear notch/punch (non-volant mammals) tissue sampling

Ear punching or notching provides both tissue and hair samples by cutting a small piece of an individual's ear using an ear punch, notching tool or dissection scissors.

Ear punching (using a punch or notching tool) may be appropriate for medium to large-sized mammals, however, care should be taken to ensure that the ear notching tool remains sharp across repeated use so that a clean, sharp cut/punch can be made. For smaller mammals (e.g. rodents) obtaining a triangular notch or a sliver of the external margin of the ear using dissection scissors may be safer and quicker to reduce handling stress.

a) Restrain the animal, exposing the ears and leaving the rest of the body in the handling bag, taking particular care to ensure eyes are covered.
b) Clean the ear with antiseptic or an alcohol swab, to prevent dirt and bacteria from the ear's surface being pushed into the open wound by the ear punch.
c) It is important that the handler does not touch the area where the sample will be taken from to avoid cross contamination of DNA.
d) A small piece of clean card placed flush against the ear can assist in achieving a clean cut.
e) Take a small tissue sample from the margin of the ear, where it is thinnest and where there are few blood vessels. Specific location will vary between species, dependent on their anatomy. Shining a torch through the ear can enable blood vessels to be seen, so that they can be avoided.

ANIMAL WELFARE: Care should be taken for species with marginal veins, such as woylies, it is advisable to take a tissue sample from further in on the ear to avoid a significant risk of bleeding.

f) Tissue should be taken from the margin of the ear to avoid punching a complete hole through the ear, which may be snagged on vegetation and cause injury to the animal. Exceptions include the fitting of identification tags, or when ear punches are taken from species with marginal veins (see above).
g) Use sterile tweezers to remove the tissue from the ear, ear punch tool or clean card.
h) Place the tissue sample in a screw-cap tube, labelled (Section 6.9) and half filled with ethanol (Section 6.8).
i) Re-secure the animal in the handling bag and allow it to recover before releasing.

6.6.2 Wing Punch (microbats) tissue sampling

a) Animals need to be restrained and placed on their backs (Figure 1).
b) When working during cold nights, heat loss during tissue sampling may be an issue, especially for small mammals that can suffer quick heat loss. It is recommended to preheat the sampling surface by placing a heating pad (e.g. glove containing hot water), which is removed before laying down the animal.
c) Extend the wing to be sampled and using a 3 mm biopsy punch perforate the wing on a non or minimally vascularised area. Avoid any major veins. Two biopsy punches are required, one from each wing.
d) Add the punches to a labelled tube with 100% ethanol.

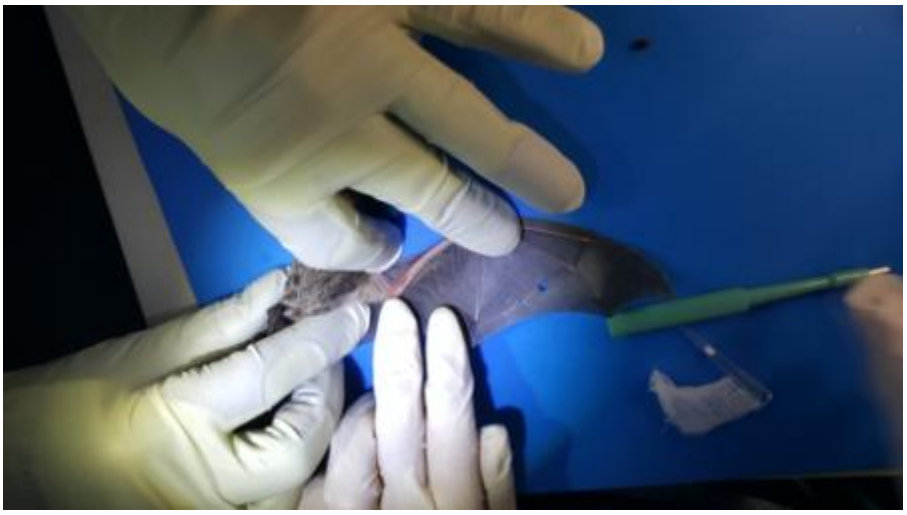

Figure 1 Performing a wing biopsy for DNA extraction. Photo: Katrina Anderson

Reptiles and Amphibians

6.6.3 Tail tipping (lizards and snakes)

For most species with elongate tails, tissue samples can be obtained via tail tip. Tail tips should NOT be taken from species with functional tail tips (e.g. knob-tailed and giant cave geckos, and blind snakes). The amount of tail tip taken will depend on the species, as some will yield a larger amount of tissue. Samplers should use discretion on the amount of tail sampled and liaise with the genetic laboratory on the size of the sample required for DNA extraction (Table 3).

a) Restrain the animal, making sure that the head is immobile (refer to the department SOP for *Hand Restraint of Wildlife* for different restraining methods).
b) The scales need to be cleaned prior to cutting with an alcohol swab, as dirt/bacteria can be pushed into open wounds as the scissors moves through the tissue.
c) Using a sharp pair of surgical scissors, cut the required length of tail tip.
d) If the animal is bleeding apply pressure with a dry gauze swab until the bleeding stops. Apply a topical antiseptic spray to the area that has been clipped to prevent infection.

e) Re-secure the animal in the handling bag/bucket and allow it to recover before releasing.
f) Long sections of tail should be cut into smaller pieces to allow the preservative to effectively penetrate the tissues, which increases utility of the tissue samples.

Many reptiles (including geckos, pygopods and skinks) voluntarily drop their tails, and pinching the tail triggers the natural autotomy response. No long-term effects are anticipated with this method as species with caudal autonomy have a vasoconstriction reflex, which stops blood loss (Gamble, 2014). Should any lizard voluntarily drop its tail during capture or removal from a trap, this can be collected as the tissue sample instead.

Table 3 General sample requirements for tail tipping snakes and lizards.

Species	Sample requirement
Lizards and large snakes (>100 mm snout-vent length)	~10 mm tail tip OR dropped tail if applicable
Small snakes (<100 mm snout-vent length)	5-10 mm tail tip
Species with functional tails	Tail tip not appropriate, alternate method to be used

6.6.4 Scale clipping (large snakes)

a) Restrain the animal, with the help of a second person, making sure that the head is immobile (refer to the department SOP for *Hand Restraint of Wildlife* for different restraining methods).
b) The scales need to be cleaned prior to cutting with an alcohol swab, as dirt/bacteria can be pushed into open wounds as the knife moves through the tissue.
c) At half the width of the ventral scale (i.e. the middle), insert the tip of the scissors under the posterior edge of the scale to be clipped, push it forward beneath the width of the entire scale, and cut. Make another such incision, either on the left or the right of the first excision. Insert the scissors under the entire section and cut. If using a scalpel, insert the blade under the scale and, holding the blade parallel to the body, undertake a single clean cut.
d) If the animal is bleeding apply pressure with a dry gauze swab until the bleeding stops or cauterize the wound to seal. Apply a topical antiseptic spray to the area that has been clipped to prevent infection.
e) Re-secure the animal in the handling bag/bucket and allow it to recover before releasing.

6.6.5 Skin (freshwater turtles)

Skin samples are taken from the trailing web of the hindfoot. After cleaning the skin with an alcohol wipe, a small sliver of skin (approximately 2 mm^2) is cut from the trailing web with a sterile scalpel or pair of dissecting scissors and stored in 100% ethanol.

6.6.6 Blood (freshwater turtles and tortoises)

a) Blood samples (~100 µl) are collected from the neck (jugular) or tail vein or the brachial sinus with a 23-25G needle and a 1-3 ml syringe.
b) The area is cleaned with an alcohol wipe, and the blood is transferred to a vial containing 100% ethanol and the sealed vial shaken.

c) The needle is subsequently disposed of in appropriate sharps containers.
d) The site of blood collection should also have firm pressure applied for a minute to aid clot formation.

6.6.7 Skin (frogs) and buccal swabbing (frogs, lizards)

Skin and buccal swabs may provide a less invasive way to sample DNA from amphibians, however reliability of gathering enough DNA during swabbing can be an issue. Furthermore, there are welfare considerations around buccal swabbing, which requires the mouth to be forced and held open for a prolonged period. This can cause visible distress to the frog and may be difficult for small species. These techniques may have a low reliability of obtaining enough DNA for SNP genotyping (RADseq) techniques used in conservation genomic analyses, however, if trialled and if successful, may have a better welfare outcome than toe-clipping.

Poschadel and Möller (2004) describe a minimally invasive technique of buccal swabbing, using cotton buds, which can be applied to a range of reptiles and amphibians.

6.6.8 Toe clipping (frogs, lizards)

Toe-clipping of frogs involves the amputation of one or more digits (Heyer et al., 2014). Advantages are low cost and collection of a large amount of high-quality DNA. Disadvantages are pain, skill required by operator, infection risk to the individual and risk of disease transmission to other individuals, reduced survival, hinders amplexus in frogs (mating) as the first three digits of the forelimb of the male are important for gripping the female, and hinders ecdysis in frogs (hindlimb – fourth digit of both sexes is used for removing shed skin). More information can be found in Australian Society of Herpetologists Inc. Position Statement No. 1 Toe clipping of lizards (2007) and Philpott et al. (2007). Less invasive methods, such as skin or buccal swabbing, should be considered before toe-clipping or toe-tipping (removing the disc from hylid species or just the tip of the toe), which may have better welfare outcomes than removing the entire toe.

a) Restrain the animal, ensuring the leg is sufficiently restrained before processing with clipping (refer to the department SOP for *Hand Restraint of Wildlife* for different restraining methods).
b) The toe needs to be cleaned prior to clipping with an alcohol swab (lizards) or sterile saline (frogs).
c) Using a sterilised scalpel or surgical scissors, cut no more than 5 mm from the end of the toe. Note: for smaller species a smaller sample may be necessary.
d) If the animal is bleeding apply pressure with a dry gauze swab until the bleeding stops. Apply a topical antiseptic, appropriate to the target species, to the area that has been clipped to prevent infection.
e) Re-secure the animal in the handling bag/bucket and allow it to recover before releasing.

6.6.9 Fin clipping (tadpoles)

Fin clipping or tail tipping small tissue samples from tadpole tails provides a minimally invasive method to collect genetic material (Othman, 2020).

Birds

6.6.10 Blood (birds)

Blood can be collected from a number of sites in birds, including the brachial or ulnar wing vein, or medial metatarsal (foot) vein or jugular vein. The preferred method will depend on the species, the size of the bird and the experience and competence of the personnel collecting the sample.

6.6.10.1 Brachial (ulnar) vein blood extraction

a) Hold the bird in a secure manner so that the inner underside of the wing is accessible.
b) Clean the area and move the feathers out of the way with an alcohol wipe to expose the vein.
c) The person collecting the blood must take great care to firmly stabilise the extended wing (at the elbow) and minimise any attempts at wing movement, while an assistant restrains the remainder of the animal.
d) Using the free hand, the person collecting the blood introduces the needle parallel to and into the vein where it crosses the elbow (Figure 2). Gentle pressure proximal to the elbow can be applied during this process so the vein stands up and is more visible.
e) Once the site is fully prepared, use an appropriately-sized needle (usually 23 – 26G) with the bevelled edge up to puncture the skin. Do this by inserting the needle directly into the vein through the overlying skin. Take care to only insert the needle as far as it needs to puncture the vein.
f) As drops of blood start to form on the wound, collect it with a heparinised capillary tube.
g) Do not collect more than what is necessary for the purpose of the study as this increases handling time. For example, the collection of 15ul of blood is more than sufficient for subsequent genetic sequencing.

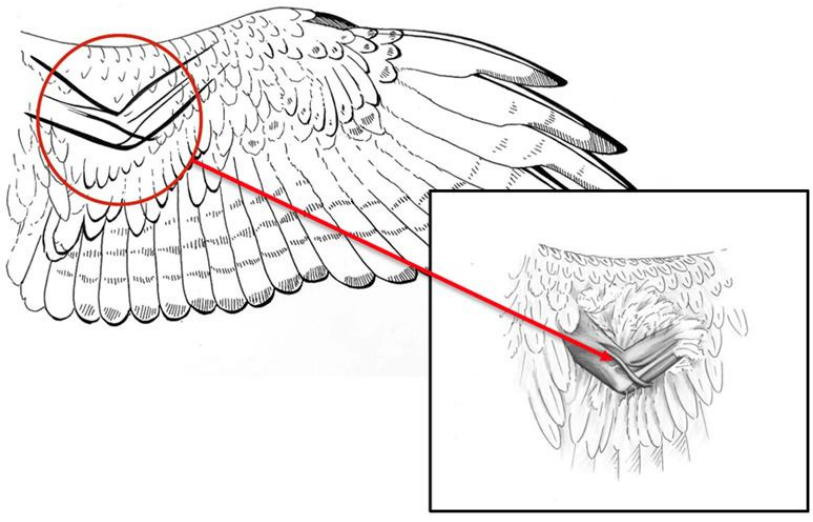

Figure 2 Location of brachial vein. Source: Evers et al. (2021)

h) After blood collection, remove the needle and apply pressure to the vein with a gauze swab for at least 1 minute then recheck to ensure no ongoing blood loss. Kaltostat is an alginate based wound dressing product that can also be applied if ongoing blood loss is occurring.

i) The blood in the capillary tube should be transferred to a vial containing 100% ethanol. Blood will clot in ethanol, so it is paramount that the sealed vial with the sample be vigorously shaken immediately to keep the blood as smaller particles. This allows better long-term storage and easier pipetting.
j) If only a small quantity of blood was collected that cannot be transferred out of the capillary tube, break the part of the capillary tube containing the sample into the 100% ethanol tube. The needle and capillary tube should subsequently be disposed of in appropriate sharps containers.

ANIMAL WELFARE: The brachial vein is particularly prone to hematoma formation, even when correct technique is used. Incorrect technique, inadequate restraint and insufficient post-sampling pressure to the sampling site will increase the likelihood of a hematoma, which can result in significant blood loss as well as pain and loss of function. Additionally, if no blood is exposed after puncturing the skin and vein, **do not** attempt the same wing as it will increase the likelihood of a hematoma. Instead, attempt the brachial vein on the other wing or consider taking a sample from a different vein or different individual.

6.6.10.2 Medial metatarsal vein blood extraction

The medial metatarsal (caudal tibial) vein is located along the medial aspect of the tibiotarsus (leg), crosses over the medial aspect of the tibiotarsus-tarsometatarsal joint (hock), and progresses down to the dorsomedial aspect of the foot. The medial metatarsal vein is a commonly used venipuncture site for waterfowl, although it also has application in larger parrots, pigeons, and other avian families.

a) Cleaning and disinfection techniques as used for the brachial vein should also be undertaken at this site.
b) Locate the junction at the hock between the thin feathered skin and thicker “reptilian” skin. This site provides good access to the vein in many different species.
c) It may be necessary to remove feathers near this junction, wet the feathers with alcohol, or occlude the vein to enhance visualisation of the vessel and facilitate blood collection.
d) A needle should be introduced into the vein at a shallow angle with or without a syringe attached. In contrast to the ulnar vein, hematoma formation at this site is less common because of the surrounding musculature and relatively non-expansible skin in the area.
e) Apply haemostasis as described for the brachial vein.

Figure 3 Blood collection into a capillary tube from the medial metatarsal vein of a larger psittacine patient. Photo: Angela Lennox, DVM (Kramer and Harris, 2010).

6.7 Non-invasive methods

As discussed in Section 1, non-invasive methods do not always provide sufficient quality and quantity of DNA for all genetic applications. However, non-invasive methods may be appropriate for some species and genetic applications, e.g. for obtaining genetic information on rare, cryptic, non-trappable species, or when obtained opportunistically. DNA degrades rapidly from non-invasively collected samples, so the collection of multiple samples is encouraged to provide the greatest opportunity for a successful outcome. Care should be taken to handle samples with gloves (replacing between each individual sample collection) or by other means to avoid contamination of samples with human DNA.

6.7.1 Collection of sloughed or shed skin

When whales are surface-active (tail slap, pectoral fin slap, or breach), they dislodge small pieces of skin, which can be used for genetic analysis. Such skin fragments can be collected from the water column using a net. However, this technique is not suitable for small cetacean species, such as dolphins (Wursig *et. al*., 1999).

Shed skins from lizards and snakes can be a suitable source of DNA for genetic analyses. Shed skins should be allowed to dry in a cool dark place with ample desiccant such as silica gel beads prior to storage to prevent mould growth. Dry shed skins should be stored in sealed plastic bags or plastic containers at -20°C or -80°C.

6.7.2 Scats, feathers, eggshells

Scats, moulted feathers and eggshells are also common non-invasively collected samples.

a) To avoid cross-contamination, samples should be collected into individual sample containers/envelopes (one scat per container) unless it is obvious that samples belong to the same individual (e.g. are from a pile of scats or fragments from the same eggshell).
b) Samples may be preserved by freezing immediately (-20°C or -80°C) or otherwise samples should be dried in a cool dark place with ample desiccant (silica gel beads). Large scats

may not dry effectively so can be stored in 100% ethanol as a preservative instead.

6.7.3 Carcasses

For freshly deceased animals and if time permits, collection of samples from the liver that would normally only be obtained through euthanasia can be helpful to maximise material available for different genetic research activities, such as long-read genome sequencing or RNA sequencing. DNA degrades rapidly once tissue starts decomposition or if exposed to acids such as those from the bile duct or the stomach (O'Meally and Livingston, 2011), in some cases, may be completely digested within a few hours (Mulcahy et al., 2016). Therefore, collection of tissue samples from the internal organs should only be made if the time of death of the animal is known and if dissection can occur immediately. Such soft-tissue samples are best frozen or for RNA sequencing studies, can be preserved in RNALater. Slightly larger tissue samples can be taken from deceased animals than would normally be taken from live animals (~5-10 mm^3). If large samples are taken, make sure to score the surface of the sample to allow penetration of the liquid preservative. If samples are taken from individuals that are to be retained as voucher specimens it is important to obtain the sample prior to fixing the body, as formalin denatures DNA.

If time is not available or the specimen is older, an ear notch or small piece of muscle tissue taken from the thigh will suffice. If possible, avoid sampling from areas of the carcass that have been exposed to the sun for long periods and/or to scavengers like flies, birds and burrowing invertebrates. Direct sunlight damages proteins and DNA, and scavengers can contribute their own proteins and DNA to the tissue, making interpretation of biochemical studies difficult (O'Meally and Livingston, 2011).

When collecting samples from carcasses, it is best to collect duplicate samples, one to retain and one to send for analysis (Berry and Aitken, 2007).

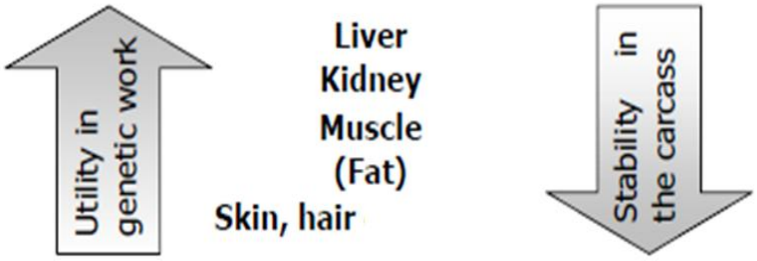

Figure 4 Ordering of tissues for genetic work preference. Image: O'Meally and Livingston, 2011

6.8 Storage

The method of storage depends on the specimen type and aim of the analysis. For routine population genetic analysis methods (e.g. mtDNA, nuclear DNA), ethanol-preserved tissues are typically suitable, but for advanced genome and RNA sequencing analysis methods, high quality, cryopreserved tissues are required (Blom, 2020). Prior to field collection of samples, it is advisable to consult with the laboratory undertaking the analyses to confirm the appropriate sample collection and storage methods.

Samples should be preserved in a clean screw-cap tube (one tissue sample per tube) and labelled (Section 6.9) to ensure they are not at risk of coming into contact with other samples or tissues (Berry and Aitken 2007).

Cryopreservation (flash-freezing in liquid nitrogen) is the gold standard method of preservation for DNA and RNA samples, but not likely to be readily available in the field and there is a risk of thawing during transport, which can contribute to DNA degradation. Therefore, ethanol is the preferred fixing solution used in the widest range of field scenarios (Table 4). For circumstances where ethanol cannot be used in the field, alternative liquid preservatives such as dimethyl sulfoxide (DMSO) or nucleic acid preservation (NAP) buffer can be used (Appendix 1). As DMSO acts as universal solvent, it must not be used in conjunction with other hazardous substances. Larger soft tissue types may be better preserved by creating multiple incisions that allow faster penetration of a liquid buffer, with the volume of preservative to sample at least 10:1. For short term storage in the field, stable, cool conditions are preferable to unstable conditions where samples may experience large temperature fluctuations.

Table 4 DNA preservation methods dependent on specimen type and genetic analysis method. Preservation in 95-100% ethanol is the most common approach that covers the widest range of samples. Consult with the genetics laboratory prior to tissue sampling to confirm the appropriate storage methodology

Tissue type	Genetic analysis type	Preservative	Storage
Heart, liver, kidney, muscle	Museum grade specimens - MtDNA, nuclear DNA, long-read genome sequencing, RNA sequencing	Liquid Nitrogen, -80°C **(Gold Standard)**	Long-term: -80°C
Muscle, ear notch, wing punch, tail tip, scale clip, fin clip etc	MtDNA, nuclear DNA	95-100% analytical grade ethanol (**Preferred**); 20% DMSO solution or NAP buffer	Short-term: stable room temp, 4°C Long-term: -20°C
Heart, liver, kidney, muscle	RNA sequencing	RNALater (ThermoFisher Scientific)	Short-term: stable room temp, 1 day at 37°C, 1 week at 25°C, 1 month or more at 4°C, and long-term at -20°C or -80°C.
Blood	MtDNA, nuclear DNA	Longmire's or Queen's buffer	Short-term: stable room temp, 4°C Long-term: -20°C

6.8.1 Ethanol

Ethanol (ethyl alcohol) is the most frequently used method for tissue preservation. A 95-100% ethanol solution is the best preservative for long-term storage for DNA analysis. 100% ethanol is also preferred where samples of an aquatic nature are concerned as water is likely to compose a significant portion of the sample. It is essential to confirm that the ethanol is non-methylated.

Containers can be filled with ethanol prior to going in the field, which minimises the amount of liquid transported. Samples can be stored at room temperature for short periods of time however refrigeration is preferred (Berry and Aitken, 2007). Ensure that container lids are completely sealed to avoid leakage or evaporation. Parafilm (https://www.interpath.com.au/products/amcor-parafilm-laboratory-film-no-2) stretched around the lid can also aid in sealing containers to avoid evaporation once tissue samples have been collected. It is advisable to regularly check collection tubes for evaporation and top up with ethanol if necessary. If samples have become completely dry before re-filling, make a note of this on any sample metadata sheets provided to the laboratory. During transport, avoid placing containers in direct sunlight or leaving in hot cars. For long-term storage, tissue should be stored in a -20°C freezer (or -80°C freezer if available) until DNA extraction (Gonser and Collura, 1996).

6.8.2 Freezing

Freezing at -20°C or in liquid nitrogen best preserves the integrity of DNA and is critical for RNA preservation. However, it may not be practical in field situations as it requires quick access to a freezer or liquid nitrogen once the sample has been taken. Samples must also remain frozen until they reach the laboratory as repeated thawing and freezing will degrade DNA (Berry and Aitken, 2007). However, once in the laboratory, freezing, particularly at -80°C, is recommended for long-term storage of cryopreserved tissue samples.

6.9 Labelling and recording data

Labelling is of the utmost importance when taking biological samples for genetic analysis. All individual samples MUST be labelled with the following as a bare minimum:

- Individual ID written on the cap and side of tube or microchip barcode sticker (these are to be linked to additional metadata); and
- Collection Date.

Other information (metadata) should be provided in a spreadsheet referencing the individual/barcode ID (Appendix 2):

- Species/sub-species;
- Collector's name;
- Location information – population/site;
- Additional location information (e.g. trap ID, transect number);
- GPS location including datum;
- Sex of animal;
- Other observations (age, weight, size, breeding status, etc);
- Translocation source/destination;
- Preservation material (e.g. 100% ethanol, DMSO); and

- Animal Ethics project approval number.

Permanent marker can rub off when it comes into contact with ethanol; therefore, as a precaution it is advised to insert a clean waterproof label written with pencil inside the tube with the individual ID number to ensure the sample can be cross-referenced to the sample metadata. If possible, the label should be prepared before collecting the sample.

6.10 Transport

Liquid nitrogen, and preservative solutions with greater than 70% ethanol content, are classified as dangerous goods.

Under International Air Travel Association (IATA) Special Provision A180 ethanol (UN1170) is not restricted for sending by air, where the total amount of ethanol is under 1 L per parcel and each container within the parcel contains no greater than 30 ml. Generally, most tissue samples are placed in vials/tubes with a maximum volume of 1.5-2 ml, which is well under the limit of 30 ml per container. Calculate the total volume of ethanol and ensure each package is under the 1 L limit (this would only be reached if hundreds of tissue samples are to be sent). The outside of the parcel must be labelled with “Scientific research specimens, not restricted, IATA Special Provision A180 applies”.

Under IATA Special Provision A180, small volumes of ethanol can be sent via Australia Post. Samples stored in DMSO buffer, tissue preservation buffer solution with less than 70% ethanol or less than 30 ml of total volume of alcohol can be transported via Parcel Post providing that they are leak proof (seal lids with parafilm wrapped around the cap) and with sufficient absorbent material (e.g., paper towel, absorbent pads/mats such as those found in spill kits or unused cleaning cloth like chux wipes) to contain the volume of liquid if spilt. Vermiculite should not be used as it is a health hazard. Packages should be triple sealed to prevent leakage and should be labelled appropriately. To protect sample tubes during transit they can be wrapped in bubble wrap or packed with foam peanuts.

For samples stored in liquid nitrogen, check with Australia Post whether your item can be exempt for dangerous goods. If not, seek alternative posting companies that are equipped with handling hazardous samples.

7 Competencies

A person who is competent has the knowledge, skills, and experiences that allow them to capture and handle animals successfully to obtain tissue samples, and appropriately manage adverse events as required. Department personnel, and other external parties covered by the department’s AEC, undertaking fauna-related activities require approval from the committee and will need to satisfy the competency requirements (Table 5). This is to ensure that personnel involved have the necessary knowledge and experience to minimise the potential impacts of tissue sample collection on the welfare of animals. Other groups, organisations or individuals using this SOP to guide their fauna monitoring activities are encouraged to also meet these competency requirements as well as their animal welfare legislative obligations.

It should be noted that sampling design details such as intensity and scope of the study being undertaken will determine the level of competency required and Table 5 provides advice for

standard monitoring only.

Table 5 Competency requirements for Animal Handlers of projects involving tissue sample collection

Competency category	Competency requirement	Competency assessment
Knowledge	Broad understanding of the framework governing the use of animals in research and environmental studies in Western Australia	Training (e.g., DBCA Fauna Management Course or equivalent training). In applications, provide details on the course provider, course name and year.
	Species identification, understanding species biology and ecology	Personnel should be able to correctly identify the likely species to be encountered at the site(s) being studied and understand the species' biology and ecology. This knowledge may be gained through sufficient field experience and consultation of field guides and other literature.
Animal handling and tagging skills/experience required	Experience handling target species	Personnel should be experienced at hand restraint of species sampled. This experience is best obtained under supervision of more experienced personnel. In applications, provide details on the longevity, frequency and recency of experience.
	Experience in collecting tissue for DNA analysis	Personnel should be familiar with the animal welfare principles of tissue sampling for DNA analysis. Personnel should be familiar with how to operate tissue sampling equipment. This experience is best obtained under supervision of more experienced personnel.
	Experience managing disease risk and biosecurity in wildlife management	Personnel should be familiar with hygiene procedures. This knowledge may be gained through sufficient field experience and consultation of literature.

In conjunction with possessing the required understanding and knowledge of the tissue sampling technique and animal welfare requirements, a guide to the experience and skill requirements for an animal handler to be considered competent to collect and store tissue samples are as follows (noting that some personnel with experience may still require initial supervision in unfamiliar locations or with species that they have not encountered previously):

- Currency of tissue collection and storage experience: within the past 15 years.

- Minimum three individuals of similar species sampled under supervision.
- Minimum two individuals of similar species sampled.

8 Approvals

In Western Australia any person using animals for scientific purposes must also be covered by a licence issued under the *Animal Welfare Act 2002*, which is administered by the Department of Primary Industries and Regional Development. Projects involving wildlife may require a licence/authorisation under the *Biodiversity Conservation Act 2016* (examples below).

- Fauna taking (scientific or other purposes) licence (Reg 25)
- Fauna taking (biological assessment) licence (Reg 27)
- Fauna taking (relocation) licence (Reg 28)
- Section 40 Ministerial Authorisation to take or disturb threatened species.

Personnel should consult the department's Wildlife Licensing Section for further guidance Contact the department's Wildlife Licensing Section for more information. It is your responsibility to ensure you comply with the requirements of all applicable legislation.

9 Occupational Health and Safety

The following departmental SOPs for wildlife survey and monitoring activities are relevant to occupational health and safety:

- Department SOP *Managing Disease and Biosecurity Risk in Wildlife Management*
- Department SOP *First Aid for Animals*
- Department SOP *Hand Restraint of Wildlife*

Departmental personnel, contractors and volunteers have duties and responsibilities under the *Occupational Safety and Health Act 1984* and Occupational Safety and Health Regulations 1996 to ensure the health and safety of all involved. Fieldwork is to be undertaken in line with the department's corporate guidelines, policies and standard operating procedures, including but not limited to, risk management and job safety analyses. Further information can be found at

https://dpaw.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/SOPs.aspx

If department personnel or volunteers are injured, please refer to the departmental Health, Safety and Wellbeing Section's 'Reporting Hazards, Near-misses and Incidents' intranet page, which can be found at https://dpaw.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/Reporting-Hazards,-Near-Misses-and-Incidents.aspx

Relevant departmental Job Safety Analysis (JSA) reports that evaluate risks and mitigation for field or laboratory activities associated with sample collection should be completed, signed, and referred to regularly. Pre-start checks should be completed before any hazardous work commences. Further information on JSAs and pre start checks are available at https://dpaw.sharepoint.com/Divisions/corporate/people-services/HS/SitePages/Job-Safety-Analyses.aspx. Information on safety requirements and risk mitigation for hazardous chemical use (e.g. ethanol, DMSO) is available in the Material Safety Datasheet (MSDS)

associated with the product. Hard copies of MSDSs for materials associated with laboratory work are held in the Sid James Conservation Genetics Laboratory in Kensington and MSDSs for hazardous chemicals are available online at https://www.safeworkaustralia.gov.au/safety-topic/hazards/chemicals/safety-data-sheets.

10 Further Reading

The following SOPs have been mentioned in this advice, and it is recommended that they are consulted when undertaking tissue collection and storage:

- Department SOP *Biopsy Tissue Sample Collection for Crocodiles*
- Department SOP *Sampling Cetaceans Using a Remote Biopsy System*
- Department SOP *Animal Handling and Restraint using Soft Containment*
- Department SOP *Hand Restraint of Wildlife*
- Department SOP *Permanent Marking of Mammals using Ear Notching*
- Department SOP *Permanent Marking of Reptiles by Scale Marking*
- Department *Managing Disease Risk and Biosecurity in Wildlife Management*
- Department SOP *First Aid for Animals*
- Department SOP *Euthanasia of Animals Under Field Conditions*

For further advice refer also to:

National Health and Medical Research Council (2013) *Australian code for the care and use of animals for scientific purposes*, 8th edition. Canberra: National Health and Medical Research Council.

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12 Glossary of Terms

Adipose: Adipose tissue is the main reservoir of fat in animals.

Animal handler: A person listed on an application to the department's Animal Ethics Committee that will be responsible for handling animals during the project.

Sample: A small part of tissue, intended as representative of the whole.

Cetacean: Group of marine mammals encompassing whales, dolphins and porpoises.

DNA (deoxyribonucleic acid): A macromolecule found in all living cells that contains genetic identifying information.

Invasive tissue sample: A sample of living tissue which generally involves the capture and restraint of a live animal, increasing the risk of compromising animal welfare.

Non-invasive: A sample collected passively which does not require the living animal to be handled, captured or restrained.

Tissue: An aggregate of similar cells and cell products forming a definite kind of structural material with a specific function, in a multicellular organism.

Appendix 1: Alternative preservation solutions

Dimethyl sulfoxide (DMSO) and other buffers are not frequently used, they are included in this document as an alternative preservative solution (i.e. when ethanol is not available). Most of these chemicals may cause irritation to skin and mucous membranes on contact. Check chemical material safety datasheets (MSDSs) or appropriate Job Safety Analysis (JSA) prior to use and ensure you wear appropriate PPE (personal protective equipment) such as air mask, gloves and eye protection. Wash contacted area with plenty of water and contact physician if irritation persists. If ingested, drink copious amounts of water and call a physician.

1. DMSO-salt solution

DMSO works by osmotic dehydration and are especially recommended for marine samples (e.g., Maiers et al. 1998, Häussermann 2004;). The mechanism is twofold: DMSO allows the salt to penetrate the tissue more readily while the salt draws water out of the cell by osmosis (O'Meally and Livingston, 2011).

At the concentrations used, the components of DMSO have low toxicity and present low risks of fire and explosion. DMSO acts as a universal solvent, enhancing the absorption of substances through the skin or respiratory passages. It is therefore advised to not be used in conjunction with other hazardous substances, such as formalin or ethanol. DMSO may limit the application of some DNA techniques, for instance, it cannot be used for protein work.

20% DMSO buffer can be made up according to the following instructions (Seutin *et. al*., 1991):

1. Add 20ml DMSO to 60 ml distilled water
2. 0.25 M sodium-EDTA
3. NaCl to saturation (about 25 g of salt at 20-25°C)
4. Leave a thin layer of undissolved salt in the stock solution to compensate for changes in solubility due to temperature (and it ensures the solution is saturated with NaCl).
5. The final volume should be approximately 100 ml.

2. Nucleic Acid Preservation (NAP) buffer

Materials	**Equipment**
EDTA disodium salt dihydrate	Scale
Sodium citrate trisodium salt dihydrate	Weigh boat or paper
Ammonium sulfate	Magnetic stirrer with heating plate
Ultra-purified, molecular grade water	Stirring rod
H_2SO_4 to adjust the pH	pH reader
	Bottle or flask

To make NAP buffer:

1. Combine 7.44 g of EDTA, 7.35 g of sodium citrate tri-sodium salt dihydrate, and 700 g of ammonium sulfate in 1 L of water in bottle or flask. Stir on low to moderate heat until the ammonium sulfate dissolves completely, which usually takes hours.
2. Cool to room temperature, then adjust pH to 5.2 with H_2SO_4.
3. Store at room temperature or keep refrigerated until aliquoted.
4. Aliquot 1.5 mL of buffer into 2 mL tubes for preservation of up to 150 mg of sliced tissue.

3. Longmire's Tissue lysis buffer

Lysis buffer is good tissue storage medium when the samples are collected for DNA analysis; lysis buffer is, however, not a suitable storage medium if the sample is collected for protein or RNA analysis (Longmire *et. al*., 1997). Lysis buffer allows greater yields of high molecular weight DNA to be obtained in comparison to other methods (Longmire *et. al*., 1997).

The solution can be made up according to the following instructions (Longmire *et. al*., 1997):

1. 50 ml of 2 M Tris-HCl, pH8
2. 200 ml of 0.5 M EDTA, pH8
3. 2 ml of 5 M NaCl
4. Bring to 1 L with distilled water
5. 25 ml of 20% SDS (w/v)

4. Tissue preservation buffer solution

Tissue preservation buffer solution is recommended for field storage of tissue. The solution can be made up according to the following instructions:

1. 240 g of 4 M urea
2. 11.5 g of 0.2 M NaCl
3. 5 g of 0.5% N-Lauroyl-Sarcosine
4. 3.72 g of 100 mM Tris-HCl pH8
5. Bring to 1 L with distilled water

5. Queen's Lysis Buffer (1X)

- 0.01M Tris
- 0.01M NaCl
- 0.01M di-sodium-EDTA
- 1.0% n-lauroylsarcosine
- pH .0

To make 100ml of 10X Queens Lysis Buffer:

1. Add 1.21g Tris, 0.4g NaCl, 3.73g K_2EDTA, 1g N-Lauryl sarcosyl to ~95ml distilled water.
2. Bring pH to 8.0 and make up to 100ml with distilled water.

6. RNALater

RNALater solution is used for preservation of RNA under field conditions when access to liquid nitrogen is not possible. RNALater can be purchased from commercial suppliers e.g. https://www.thermofisher.com/au/en/home/brands/product-brand/rnalater.html. Tissues should be submerged in 5 – 10x the volume of RNALater to ensure effective preservation.

Appendix 2: Recommended tissue sample metadata

As many of the following fields should be filled out as possible. Fields in red are ESSENTIAL.

TISSUE_ID	Allocated by DBCA Fauna genetics team
TAG_ID	Field ID [matching sample container label] - e.g. ear tag number, microchip number, sequential ID number
GENUS	Taxonomic name
SPECIES	Taxonomic name
SUBSPECIES	Taxonomic name
LOCATION	Population location name
LOCATION_2	Additional location information e.g. transect number, contextual site information
COMMON_NAME	Species common name
COLLECTOR	Name of collector and organisation
MICROCHIP	Microchip number
COLLECTION_DATE	Date of tissue collection
SEX	Sex of animal
AGE	Life stage e.g. juvenile, sub-adult, adult
EASTING	GPS location in UTM
NORTHING	GPS location in UTM
MAP_ZONE	UTM Map Zone
DATUM	GPS datum e.g. WGS84
DEC_LAT	GPS location in decimal lat/longs
DEC_LONG	GPS location in decimal lat/longs
LAT	GPS location in lat/long
LONG	GPS location in lat/long
LOCATION_ACCURACY	GPS location accuracy OR free text comment on relative accuracy of location e.g. if lat/long is estimated from google maps, give information on whether location is precise or approximate
TRANSLOCATION SOURCE	Translocations only: Indicate source population animal taken from
TRANSLOCATION DESTINATION	Translocations only: Indicate source population animal taken to
STATUS	Translocations only: Indicate whether individual is a founder
STORED_IN	Information on tissue preservative e.g. 100% ethanol, 20% DMSO
Collector's data sheet	Hyperlink to collector's datasheet on Sharepoint
FAMILY	Taxonomic name
OTHER	Additional free text
OTHER2	Additional free text