CITES Cheetah Trade Resource Kit

DNA Sampling Guidelines

This section includes information about how to sample a variety of different sources of DNA and how to store the samples to ensure the highest likelihood of extracting high quality DNA.

Contents

1. Why Collect DNA Samples?

Collecting DNA samples from seized wildlife specimens allows definitive identification of the species and whether animal parts originally came from the same animal.

DNA samples can also be used to investigate relationships between animals – for example to determine whether a group of cubs are siblings or whether they are unrelated.

Samples also have the potential to help identify links between criminal cases, for example samples taken from a crime scene where a cheetah has been poached, may be linked to a seizure of illegally traded cheetah specimens and to implicated suspects, thereby promoting successful prosecutions.

The collection of DNA samples is an important tool for understanding and combatting the illegal wildlife trade.

This kind of information can help with targeting interventions to prevent other animals being taken from the same source populations. Over time these data can also help us to identify trends in where animals are being taken from and to, which may then be used to predict areas that may be targeted in future. These data can also help us to identify patterns within the illegal wildlife trade which can then be used to identify major international criminal networks.

2. Analysis of DNA Samples

DNA samples should be analysed by laboratories that meet, or exceed, minimum quality assurance standards (see A review of wildlife forensic science and laboratory capacity to support the implementation and enforcement of CITES https://cites.org/sites/default/files/eng/cop/17/WorkingDocs/E-CoP17-25-A4.pdf) This will ensure that high quality data is obtained from the samples, which will help in the pursuit of convictions as well as in determining the origin of the cheetah(s).

Where samples may be used in criminal prosecutions ensure that chain of custody records are carefully and thoroughly maintained throughout the collection and analysis of samples.

If it is necessary to export the DNA samples for analysis in a different country, this must be done in a way that is compatible with national legislation regarding the admissibility of the DNA evidence into prosecutions and court cases.

The CITES Secretariat has developed an electronic directory of laboratories conducting wildlife forensic testing. The directory is available on the Wildlife forensics page of the CITES website , see Wildlife forensics | CITES

Any samples sent for analysis in a different country must have the appropriate CITES export and import permits, see CITES Permit system | CITES and Exemptions and special procedures | CITES.

3. Health and Safety

Many wildlife diseases can be transmitted to humans and so due care and attention should be paid to health and safety when handling biological materials. Some basic, but vital precautions are:

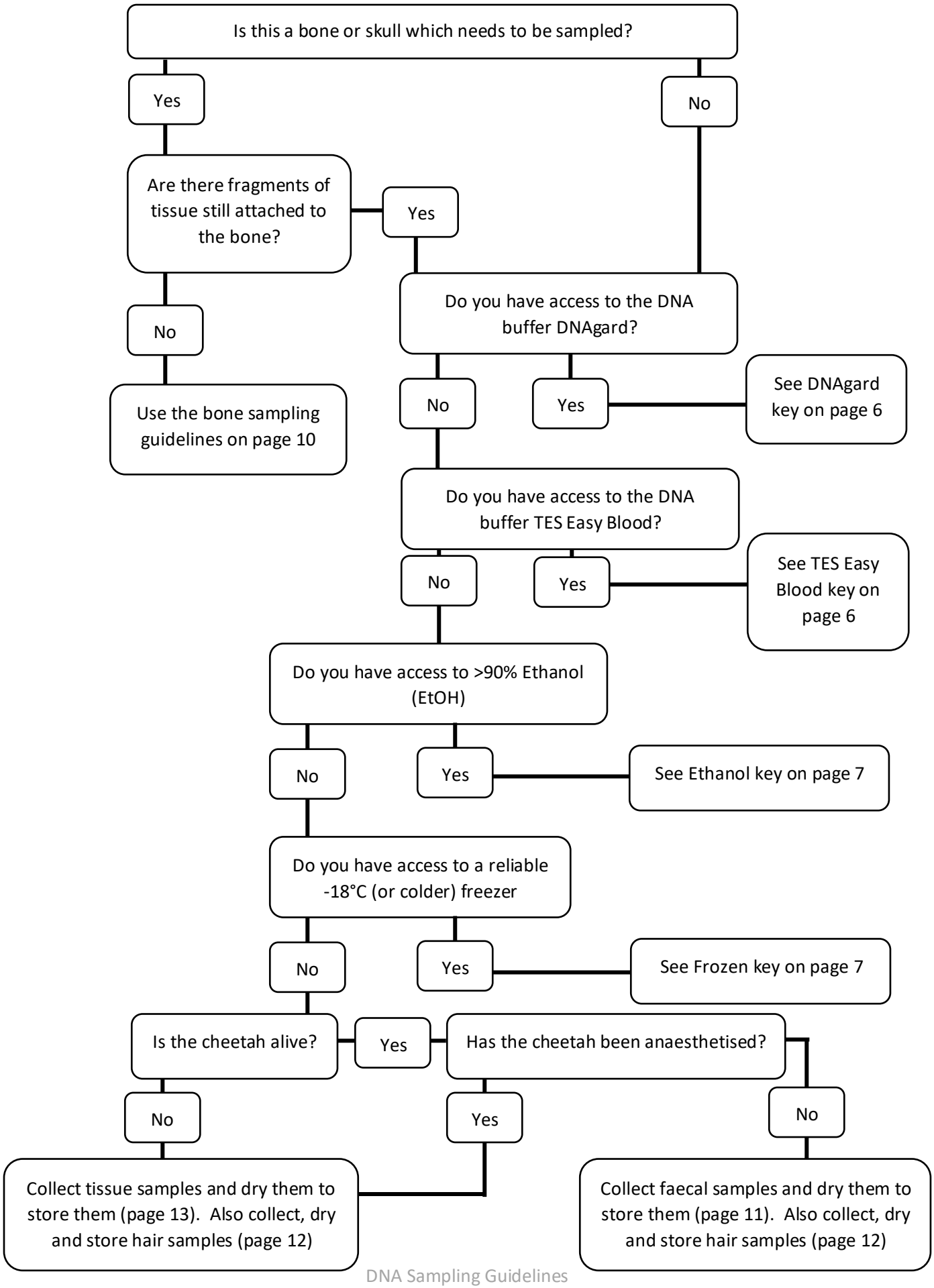
- Wear disposable gloves when collecting and handling samples, and discard them once sampling is complete
- Wash hands thoroughly after collecting or handling samples (including when gloves have been worn).
- Do not eat, drink, smoke or touch your face whilst collecting and handling samples – only do so after removing gloves and washing hands.

4. Preventing Contamination

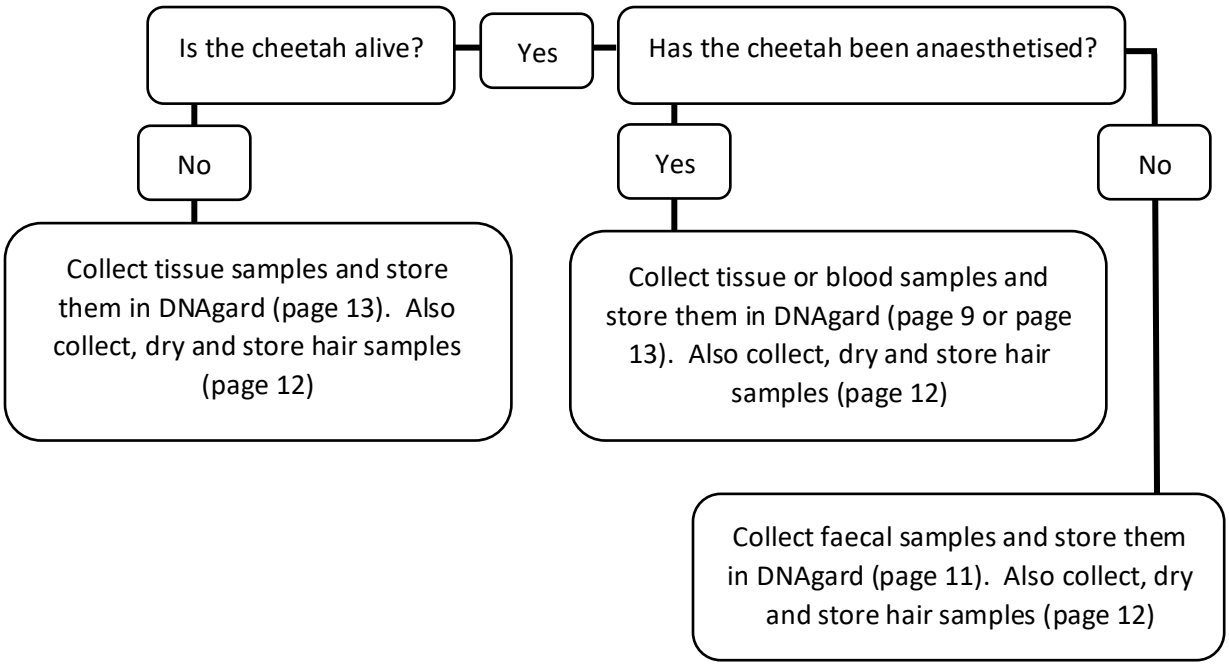
It is very important to prevent samples from being contaminated, as contamination may reduce accuracy of results. There are several basic steps which can be taken to reduce the chances of a sample being contaminated:

- Handle samples as little as possible
- Wear gloves when collecting and handling samples
- Use a fresh pair of gloves for each sample – never use one pair of gloves for multiple samples as this risks contaminating samples with the DNA of other individuals
- Ensure all sample collection vessels are fully and legibly labelled
- Ensure all sample collection vessels are properly sealed after collection
- Ensure samples are kept somewhere safe, clean and dry.

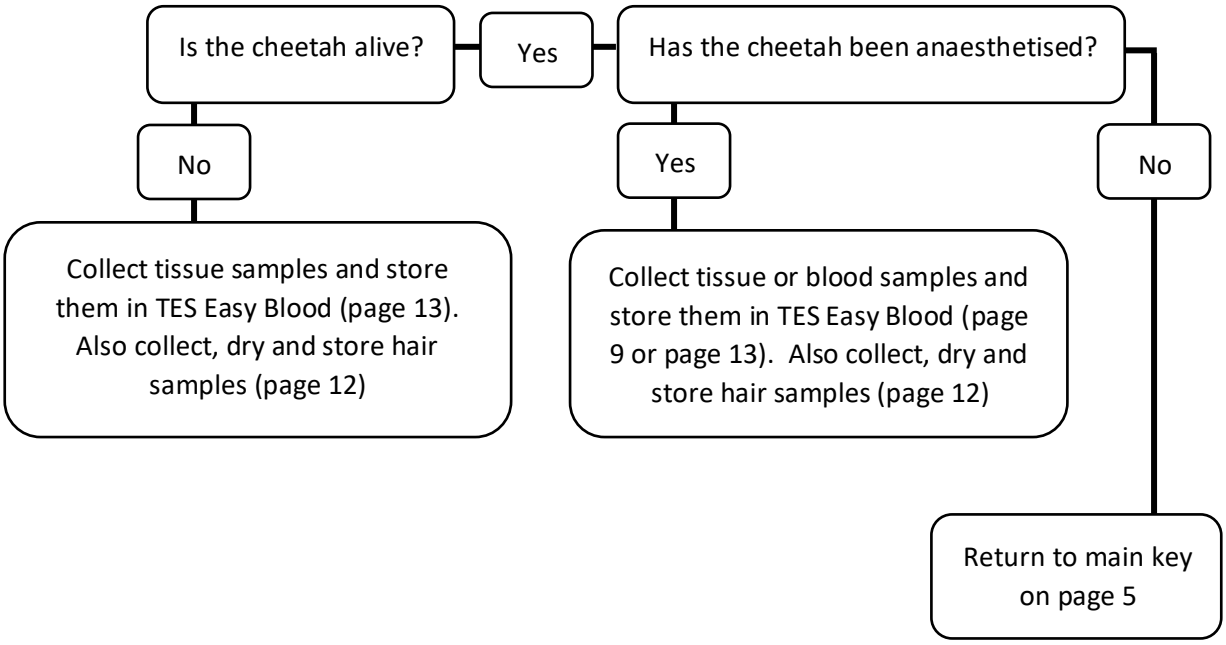
5. Which DNA Sampling Technique to Use?



DNAGard

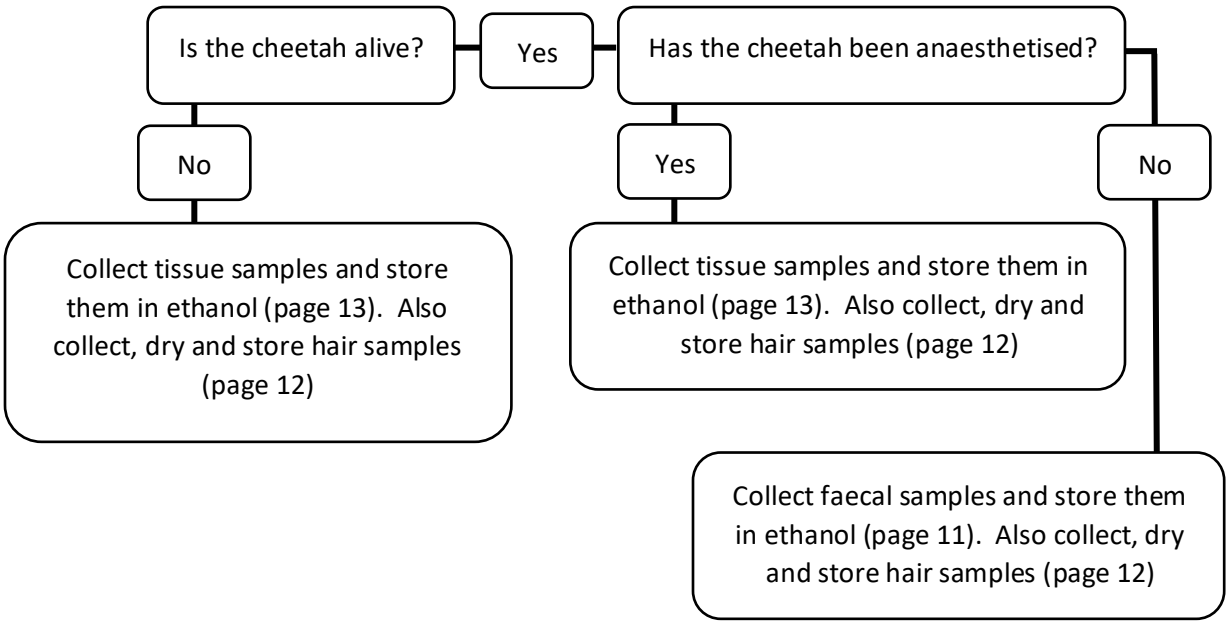


TES Easy Blood

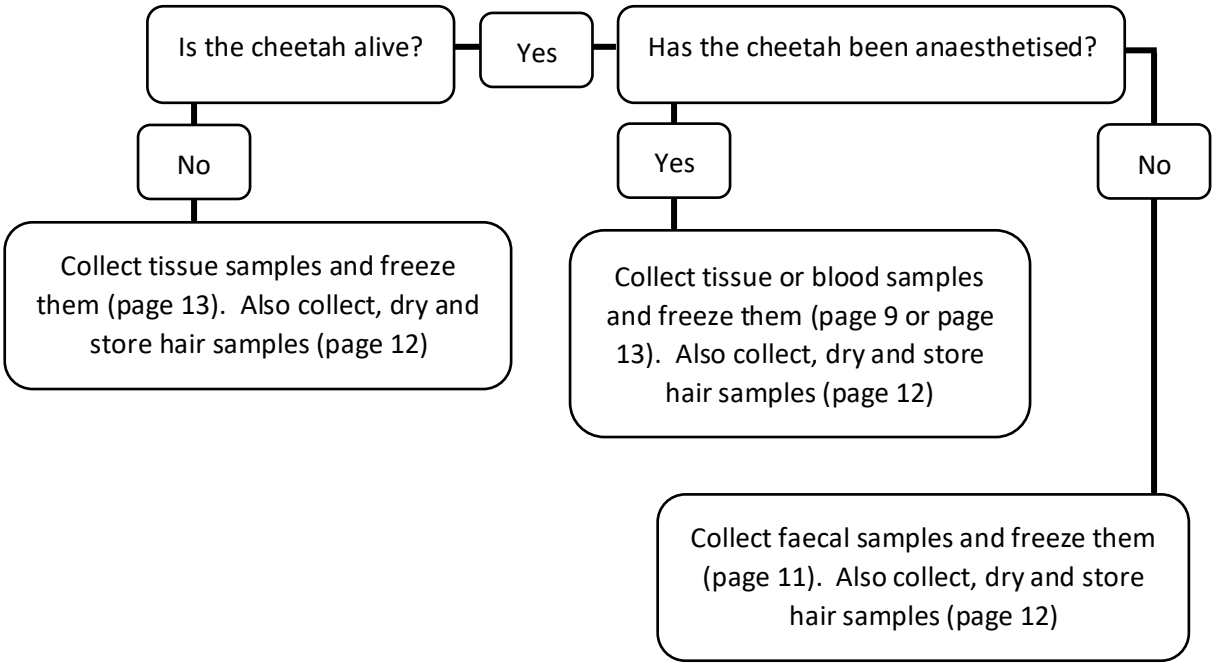


***Note**:* Animals should not be anaesthetised only to collect DNA samples, however if an animal needs to be anaesthetised for other reasons (eg to treat an injury) then blood and/or tissue samples should be taken at that time.

Ethanol



Frozen



***Note**:* Animals should not be anaesthetised only to collect DNA samples, however if an animal needs to be anaesthetised for other reasons (eg to treat an injury) then blood and/or tissue samples should be taken at that time.

6. Labelling Samples

Ensure that all samples are labelled with:

- Date
- Geographic location (as accurate as possible)
- Species
- Sex and approximate age of animal (if known, see Section: *Identification Guides* for guidance)
- Sample type (e.g. hair/tissue/blood etc)
- Storage medium (e.g. dried/95% ethanol etc)
- Person taking sample
- Animal reference number
- Seizure reference number

Where possible, the sample collection container should be labelled using a permanent marker with all of the above information. However, if the container is too small, ensure at least the date, animal reference number and seizure reference number are written on the container.

Unless the samples are being left to dry, it is then a good idea to put the sample tube inside a sealable polythene bag which is labelled on the outside in permanent marker with all of the above information. For extra security also write the details in pencil on a piece of paper and put inside the sealed polythene bag.

A record of the samples collected and all their associated information should be kept in a database which can be easily and reliably accessed.

7. Blood

7.1.a. How to obtain a blood sample

If the animal is anaesthetised	The easiest vein for obtaining a blood sample is the cephalic vein which runs down the front of the forelegs. Using a new, clean, sterile hypodermic needle and syringe, carefully puncture the vein and extract 10 ml of blood
If the animal is dead	If the cheetah is freshly dead then it may be possible to extract some blood from one of the veins in its extremities, for example from the ear. In animals that have been dead for longer periods of time, the blood will have clotted and so it will not be possible to extract samples from the veins, instead it can be taken from the chambers of the heart during the post-mortem (necropsy). However if the animal is dead it is usually easier to take tissue samples rather than blood samples.

7.1.b. Storage

DNAgard	Follow a ratio of buffer to blood of 1:4 (ie add 1ml of DNAGard to 4ml of whole blood). Ensure the sample and buffer are well mixed, and that the total volume of the sample + buffer does not exceed 2/3 of the total capacity of the container. Store at room temperature in a cool dark place for up to 6 months, or longer if refrigerated at 4°C.
TES Easy blood	TES is a buffer which can be used to store tissue and blood samples. Its formulation is 1.2g Tris HCl, 3.7g Na2 EDTA and 2g sodium dodecyl sulfate (SDS) with distilled water added to 100ml. Store the blood sample in a sterile tube with TES at 1:1 ratio. The sample can then be stored at room temperature for a short period of time (less than 7 days before DNA extraction) or for longer if the sample is refrigerated at 4°C or frozen.
Frozen	If the blood sample is to be frozen it should be transferred to a reliable -18°C freezer as soon as possible after collection. If a -80°C freezer or liquid nitrogen storage is available these can also be used. Ensure sample tubes are safe to use at the freezer temperature so that they do not shatter. Once frozen, samples should not be allowed to thaw until the DNA is to be extracted – repeated thawing and refreezing can damage the samples and make them unusable.

8. Bone

If a bone has been seized, then it should be sampled – this will enable the species of animal that the bone came from to be definitively identified. If there is some tissue remaining on the bone, then this can be sampled by removing it from the bone (using a clean, sterile implement) and storing the samples as described below under *Tissue* on page 1113.

8.1.a. How to obtain a bone sample

Skull

If the sample is to come from a skull, then often the easiest area to obtain a sample from are the very fine bones inside the skull nasal cavity. Holding the skull above a new, clean sheet of paper, use a clean, sterile implement to knock and break the fine bones inside the nasal cavity so that pieces fall onto the paper. Using the paper as a funnel, put these shards into a new, clean paper envelope for storage. Do not use the same piece of paper for sampling multiple skulls as this would contaminate the samples.

Other

Other bones can be sampled by chipping a small piece of bone off from the main section. Using a clean, sterile implement remove a piece of bone, preferably from the head of the bone (including some cartilage where some is still attached) but from any part of the remaining bone if the head of the bone is no longer attached. Put the bone chips into a new, clean paper envelope for storage.

8.1.b. Storage

Bone samples should be allowed to dry out and so store them in a new, clean, dry paper (<u>not</u> plastic) envelope. Ensure the envelope is sealed and labelled. For long term storage, keep the samples in a clean, dry place, preferably in a container with silica gel to remove any remaining moisture. Silica gel beads change colour when they became saturated with moisture therefore they need to be checked regularly and fresh silica added if the colour has changed.

9. Faeces

Collection of faeces is a non-invasive method of collecting DNA samples for analysis. As the faeces travels down the alimentary canal, gut cells are shed into the faecal matter and these cells can be used for DNA sampling.

9.1.a. How to obtain a faecal sample

If the cheetah is in a good state of health it should regularly produce faeces. Samples should be collected as soon as possible after the faeces have been produced.

DNA is found at random within the faecal material therefore as much faecal matter as possible should be collected and placed in a new, clean, sterile collection tube, large enough to accommodate the sample and storage buffer. It is important that during the collection the sample is not disturbed and includes the outer layers, mucus and blood (if present). Collect at least two samples, stored in separate containers. Ensure containers are fully labelled as described under *Labelling Samples* on page 8.

9.1.b. Storage

DNAgard — Ensure the faecal sample is completely covered with DNAgard solution at avoid any moisture seeping into the sample (moisture within the sample is the main cause of DNA degradation and should be avoided until DNA extraction in the lab). Store at room temperature in a cool, dry, dark place for up to 6 month or longer if refrigerated at 4°C

Ethanol — Fill the sample tubes with ethanol. The ethanol used should be minimum 90% concentration. Ensure the sample is completely covered and well immersed in the liquid, ideally in a 1 part faeces to 10 parts ethanol ratio. The samples should then be stored at room temperature, in a cool, dry, dark place. The ethanol in the tube will evaporate over time, thus samples should be regularly checked and the ethanol level topped up when needed.

Frozen — Put the samples into a reliable -18°C freezer (or -80°C if available) as soon as possible after collection. Ensure the collection tubes are freezer safe, to the temperature of the freezer used, to ensure the tubes do not shatter. Samples can be kept in liquid nitrogen, but again the collection tubes must be cryo safe or they will shatter and the samples will be lost.

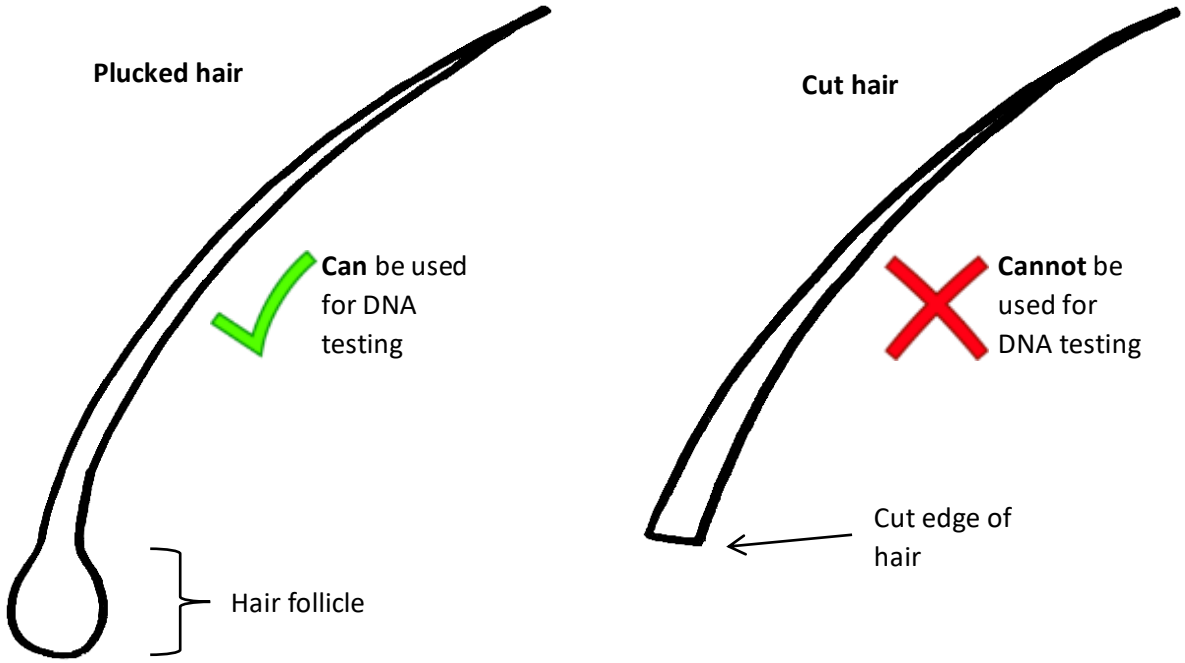
Samples must be kept completely frozen until the DNA is to be extracted. Thawing and refreezing will damage the samples and so should be avoided. If samples are to be transported, keep in a well-insulated container with freezer blocks etc and return to a freezer as soon as possible.

Dried — Store the samples in a new, clean paper envelope and put them somewhere clean, warm (but not hot) and dry to allow the samples to dry out. Do not expose the samples to direct heat as this will damage the DNA. The sample should <u>not</u> be kept in a plastic container as this will prevent the sample from drying properly. Store in a container containing silica gel if possible, as this will extract moisture from the air and help the drying process. Silica gel beads change colour when they became saturated with moisture, therefore they need to be checked regularly and fresh silica added if the colour has changed.

10. Hair

Hair can be a convenient way of obtaining DNA samples as it can be taken from a live animal while it is awake; however the hair must be plucked from the animal, not cut, in order to yield a sample.

If the hair does not have its follicle attached, it does not have any value for DNA testing.



10.1.a. How to obtain a hair sample

Take a small bunch of hair (often tail hair is easiest as it is longer and easier to grasp) and pull quickly away from the animal in order to pluck it out. If the animal is awake be sure to complete the protocol quickly so minimise distress to the animal. Also ensure the cheetah is not able to reach the person taking the sample – if the cheetah is able to reach the person (e.g. if they have their hands or fingers inside the cheetah's cage) then the cheetah may strike out and cause an injury to the person. An alternative method is to create a "hair trap" by putting packing tape or Sellotape, with the sticky side up, on the bars of the cheetah's crate or the fence of its enclosure, in a location where the cheetah will brush against it allowing some hairs to stick to the tape and be collected.

At least 20 plucked hairs are required for a sample to yield sufficient quantities of DNA. Where possible, collect two samples and store separately.

10.1.b. Storage

Hair samples should be allowed to dry out and should be stored in a new, clean, dry paper (not plastic) envelope. Ensure the envelope is sealed and labelled. Store the samples in a clean, dry environment. For long term storage keep the samples somewhere clean and dry, with silica gel beads where possible to extract moisture from the air. Silica gel beads change colour when they became saturated with moisture therefore they need to be checked regularly and fresh silica added if the colour has changed.

11. Tissue

Tissue samples can be taken from sections of skin or muscle. Tissue samples are reliable means of obtaining DNA samples and so should be used wherever possible. However, if samples are being taken from, for example, a seized skin then the preservation techniques used on the skin may prevent successful extraction of DNA.

Do not take tissue samples from a live cheetah whilst it is awake as this will cause the animal to become extremely stressed. Animals in captivity can die from stress and a newly seized cheetah will already be in a particularly heightened state of stress, causing further distress could result in serious consequences including the death of the animal.

11.1.a. How to obtain a tissue sample

If the animal is anaesthetised	Cut a small section, approx. 5mm x 5mm from the edge of the animal's ear. Put the sample in a new, clean, sterile sample tube.
If the animal is dead	Cut two sections of skin or muscle, approx. 1cm x 1cm from the animal. Put the samples in new, clean, sterile sample tubes.

If the animal is alive at the time of sampling, the wound will bleed. Using a clean swab apply pressure to the wound until it stops bleeding. Apply a topical anti-septic or anti-biotic to the wound to prevent infection. The wound should be monitored over the ensuing days to ensure it does not become infected.

11.1.b. Storage

DNAgard	Ensure tissue sample is completely covered with DNAgard solution. Store at room temperature in a cool, dry, dark place
TES Easy Blood	TES is a buffer which can be used to store tissue and blood samples. Its formulation is 1.2g Tris HCl, 3.7g Na2 EDTA and 2g sodium dodecyl sulfate (SDS) with distilled water added to 100ml. If TES is used, add to the sample tube to completely cover the sample. The sample can then be stored at room temperature for a short period of time (less than 7 days before DNA extraction) or for longer if the sample is refrigerated or frozen.
Ethanol	Tissue samples can be stored in ethanol (min. 90% concentration). Fill the sample tube with ethanol, ensuring the sample is fully immersed and covered by the ethanol, ideally one part sample to nine parts ethanol. Store at room temperature in a cool, dry place.
Frozen	Transfer the tissue sample to a reliable -18°C freezer as soon as possible after collection. If a -80°C freezer or liquid nitrogen storage is available these can also be used. Ensure sample tubes are safe to use at the freezer temperature so that they do not shatter. Frozen samples should not be allowed to thaw until the DNA is to be extracted – thawing and refreezing can damage the samples and may make them unusable.
Dried	For tissues to be dried and stored successfully, small thin samples need to be taken so that they will dry fully before decomposition starts. Cut samples into approx. 2mm wide strips. Store in paper envelopes in a clean, warm (but not hot), dry place until the sample is completely dry. For long term storage, keep the samples in a clean, dry place, preferably in a container with silica gel to extract moisture from the air. Silica gel beads change colour when they became saturated with moisture therefore they need to be checked regularly and fresh silica added if the colour has changed.