ANIMAL HANDLING METHODOLOGY

Cheetah is immobilised using e.g. blow-dart or hand syringe (squeeze box).
The following procedures are completed during anesthesia:

1. Temperature is taken. Heart and breath rates monitored regularly.
2. Within the first few minutes, blood samples are collected for various biological analysis, including genetic, over-all health and disease studies. A minimum of 25 ml of blood is collected from the saphonous vein, including, heparin, serum and EDTA.
3. An aseptically prepared skin biopsy of 1/4cm2 is collected and placed in biopsy transport medium.
4. An overall health examination is completed including, coat, eyes, ears, nails, teeth and presence of external parasites.
5. Measurements and weight recorded.
6. Census collar, transponder placed (juveniles eartagged).
7. Cheetah released once fully recovered from anesthesia.

PREPARATION OF SKIN BIOPSIES

Purpose: To obtain viably frozen samples of aseptically prepared skin biopsies. These will be frozen and transported in vapour-phase liquid nitrogen carriers to Dr. O'Brien's lab where they will be treated to establish permanent fibroblast cell culture lines. These lines will serve as permanent sources of DNA and protein products of the animals samples.

1. Piece of clean, shaved skin either using a scalpel or punch biopsy tool (a $1cm^2$ piece or 4 punches).
2. Open a tube of *biopsy transport medium* – wipe the outer surface of the lid with alcohol first before opening. Drop the piece(s) of skin into the tube, make sure they are immersed in the fluid and cap tightly. Biopsies can be kept several hours up to 1-2 days in this medium.
3. When ready to proceed, wipe neck of transport tube with alcohol swab before opening carefully, pour off most of the fluid, open a small sterile petri dish, then dump the skin pieces(s) with the remaining medium into the dish. Replace lid.
4. Pick up sterilized scissors and forceps.
5. Remove lid of dish and cut upskin with scissors while holding the forceps. Cut into the smallest pieces possible (a 6mm biopsy pucn should be cut into at least 4 pieces).
6. Have a thawed cryotube of *freeze medium* ready – label, wipe neck with alcohol swab, and loosen the cap.
7. With forceps, transfer pieces of skin from dish into cryotube – make two tubes if there is enough skin.
8. Tightly cap cryotube.
9. Label cryotube with: date, skin, sex of animal and cheetah accession number.
10. Freeze overnight in freezer, then place in Liquid nitrogen.

Biopsy transport medium supplied by CCF as well as *freeze medium.*

SHORT PROCEDURE FOR BLOOD PROCESSING FOR GENETIC ANALYSIS

Purpose: To obtain plasma, white blood cells ("buffy coat") and red blood cells, from heparinized blood, processed and frozen within 24 hours after collection.

1. Centrifuge at 2200-2500 rpm for 10 minutes. Plasma should be clear (pink to yellow). If not, spin longer.
2. With disposable transfer pipet remove most of the plasma (do not disturb the "buffy coat") and fill two 2cc cryotubes and put the rest in 4-5cc cryotubes.
3. With transfer pipet, reach down through remaining plasma and remove the "buffy coat" (interface between plasma and red blood cells containing white cells, you will be removing some RBCs as well). Pool the buffy coats from all the tubes into "blue top" tube.
4. Add equal amount of "easy blood" to buffy coats and mix gently.
5. Label tube with date, cheetah accession number and sex of animal.
6. From the bottom of the original tube(s) remove red blood cells with transfer pipet and dispense 1.5-2cc to each of 2 cryotubes. Label and freeze upright. (The highest concentration of red blood cells will be at the bottom of the tube where they have packed during centrifugation.) Remainder of RBCs may be discarded.

PROCESSING OF SERUM

1. Centrifuge red tops at 2200-2500rpm for 10 minutes.
2. Serum should be clear (pink to yellow). If not, spin longer.
3. With disposable transfer pipet remove serum and fill one 1cc (to be sent to GVLabs), and two 2cc cryotubes, transfer remaining serum into 4-5cc cryotubes.
4. Label with date, contents, cheetah accession number and sex of animal.
5. Place in freezer.

DIRECTIONS FOR MAKING A BLOOD SMEAR

1. Have 3 clean slides ready.
2. Collect blood from the EDTA (purple top) after it has been rocked gently to ensure blood is well mixed, using sterile needle and syringe.
3. Put a small drop of blood close to the end of a slide. A small drop is important to prevent the smear from being too thick.
4. Lay the slide down on a flat surface.
5. Take a second slide and dip the back of one end into the drop of blood, as shown in the diagram:
6. Let the drop spread across the back edge of the top slide.
7. Then slowly push the top slide the full length of the bottom slide to spread the drop of blood over the surface of the bottom slide.
8. Repeat this procedure to made a second blood smear.
9. Allow the slides to air dry.
10. Fix in Methonol.
11. Label with date, cheetah accession number, sex of animal.