Salt Extraction

This protocol is designed for DNA (nuclear and mitochondrial) extraction from small amounts of tissue or non-mammalian vertebrate blood.

*****For skins*****
Two days prior to the extraction steps below, do several 70% EtOH soaks and washes. Add 1 mL of 70% EtOH to tube with skin clip. Rapidly wash by pipetting up and down several times. Pipette off the ethanol. Add 1mL of 70% EtOH and let sit at room temperature for 3-6 hours. Repeat the wash. Do this several times over a 24 hour period.

The next day, do one last 70% EtOH wash, pipette off all of the ethanol. Add 1 mL of either STE or 1XPBS buffer. Rapidly wash, remove buffer, and add another 1 mL of buffer. Let sit for 4-8 hours in the refrigerator, then repeat. Leave overnight in the fridge with buffer. The next morning do one last buffer wash and pipette it all of. Remove the skin clip and scrape off the hair. Cut into several small pieces on a sterile glass slide or a piece of parafilm. Begin the extraction steps below at step 3.

1. Place <20 mg of tissue in a 1.5ml microcentrifuge tube.

2. Rapidly wash tissue with 1ml of cold STE buffer two times. Remove the wash liquid with a P1000 pipetman.

3. Add 550ul tissue lysis buffer and 11ul proteinase K. Incubate at 60Â°C for 5 hours to overnight. (Additional proteinase K can be added if the tissue is still solid following incubation, or a gentle vortex may break up remaining tissue).

4. Remove from incubator and add 5.5ul RNase A (10mg/ml). *OPTIONAL*

5. To precipitate proteins from tissue solution, add 350ul 5M sodium chloride (make sure NaCl is relatively new and that the salt is not precipitated out). Mix well by inverting tube. Centrifuge in the microcentrifuge for 30 minutes @ 14000 RPM (total volume is about 900ul).

7. Transfer approximately 1/2 the supernatant (410-420ul) to each of two (2), 1.5ml tubes. The pellet contains proteins and other cellular debris which can be disposed of.

8. Add 900ul of 100% cold, absolute ethanol to each tube. Invert tube gently several times. At this point you may be able to see the DNA as a clearish, white precipitate.

9. Incubate samples at -20°C for 2 hours to overnight (recommended).

10. To pellet the DNA, centrifuge samples for 30 minutes @ 14000 RPM in the microfuge.

11. Pour off the supernatant carefully. Be careful not to disturb the DNA pellet. Remove excess ethanol by touching the edge of the microcentrifuge tube to a clean Kimwipe.

13. Add 1ml cold 70% ethanol remove excess salts from the DNA pellet. Once again, be careful not to disturb the DNA pellet.

14. Microcentrifuge for 5 minutes at 6000 RPM.

15. Gently pour off the 70% rinse ethanol.

16. Kimwipe the edge of the tube to remove excess ethanol.

17. Repeat steps 13 - 16 again (optional, recommended).

18. Cover the Eppi tubes with a Kimwipe and place in 37°C incubator until dry. -OR- place Eppi tubes in Speed-Vac to dry -OR- leave on bench top overnight.

19. Suspend DNA pellet in 50-100 ul of T(1/10)E (or other EDTA-based buffer) and incubate at 55°C for 1 hour (People usually combine the two tubes from Step 7 at this point for a total volume of 100-200 ul)

20. Label the sample and store at -20°C.

21. Proceed to PCR

Optional Step
A 1/100 dilution (or 1/10, 1/50, etc.) of the above DNA solution will sometimes result in more successful PCRs (see PCR protocols). Suspend 5ul DNA in 495ul distilled water and label appropriately.