Galliform DNA Storage and Extraction

1.0 Storage Conditions

1.1 Avian Erythrocyte Storage Buffer Link

http://ravel.zoology.wisc.edu/sgaap/Protocols_html/Protocols.htm

1.2 Avian Tissue Storage Buffer

From Travis Glenn's lab. Good for storing avian tissue samples at room temperature (keep out of light). Samples have been successfully extracted from this buffer at ages ≥ 1 year.

100mMol Tris, pH 8.0; 100mMol EDTA, pH 8.0; 10 mMol NaCl; 1% SDS

Tris = $H_2NC(CH_2OH)_3$ FW 121.14
EDTA = $(HO_2CCH_2)_2NCH_2CH_2N(CH_2CO_2H)_2$ FW 292.25
NaCl = salt. FW 58.44
SDS = sodium dodecyl sulfate = $CH_3(CH_2)_{10}OSO_3Na$ FW 288.38

100 ml 1.0M Tris; 200 ml 0.5M EDTA; 2 ml 5.0M NaCl; 100 ml 10% SDS; 598 ml H20

Mix thoroughly in 1 L water and store in dry, cool place (cap the bottle). You may want to put it into a squirt bottle or use some sort of pipette to dispense the buffer. I would recommend using 1.5 ml centrifuge tubes with a screw cap for storage. Keep these in a cool dry place, as well. They should hold all your tissue materials. I would go ahead and put the eggshells in this potion, too.

1.3 Avian Feather Storage

Store feathers in 70% EtOH.

1.4 Effects of Storage Conditions of Galliform Egg Membranes (Reference: Baker, GC (2005, in press) Non-invasive genetic methods for the study of megapodes and other galliformes. *Proceedings of the Third International Symposium on Galliformes*.)

DNA can be extracted from the egg membranes of Galliformes, once a vascular network has formed in the chorioallantois membrane. This is after 7 days in *Gallus* and may be of a similar time period in other Galliformes. Eggshells stored immediately after hatching can be equally well preserved in silica gel, ethanol or storage buffer (20% DMSO; 0.25M EDTA (pH 8.0) in saturated NaCl). Eggshells left in sand for 1 month may also yield recoverable DNA provided that the egg membrane has not been destroyed by arthropods. The DNA of egg membranes left in megapode incubation mounds will become degraded, over time, but storage of 1 week old membranes in silica gel or ethanol may preserve enough DNA for successful PCR amplification. Once DNA has begun to degrade, DMS0- based storage buffer is detrimental to the preservation of DNA and in cases were samples have been left in sand or compost it is recommended that they be stored in 100% ethanol or in silica gel.

2.0 DNA Extraction Methods

2.1 Chelex 100 extraction. (Reference: Baker, GC (2005, in press) Non-invasive genetic methods for the study of megapodes and other galliformes. *Proceedings of the Third International Symposium on Galliformes*.)

The method given is for extraction of DNA from eggshell membranes, but also works effectively for feather tips (use 3-4 feathers) and alcohol-preserved tissue (use 1mm^2 muscle or other tissue).

Duplicate 2 cm^2 pieces of chorioallantois membrane were excised and incubated in 0.5ml STE (100mM NaCl; 10mM Tris; 1mM EDTA, pH 8.0) for 10 minutes. The membranes were cut into approx 1mm^2 fragments, placed into microfuge tubes, and washed twice with fresh STE. The STE was discarded and replaced with 800µl Chelex 100® (5% in water). Proteinase K was added to a final concentration of 125µg/ml and incubated @ 55°C for 100 minutes. The Protease was denatured by incubation at 95°C for 10 minutes and samples were vortexed for 1 minute and centrifuged for 1 minute at full speed. A 400µl aliquot of the supernatant was transferred to a fresh tube containing 400µl Chelex 100® (5%) and re-spun for 1 minute. A 2µl sample of the supernatant was used for amplification. A further 400µl sample was precipitated with in 0.3M sodium acetate (pH 5.3) and 1.8 volumes ethanol and resuspended in 50µl TE and stored at -20°C. A 1µl aliquot was tested for amplification by PCR.

2.2 **Avian DNA Extraction Link**
http://ravel.zoology.wisc.edu/sgaap/Protocols_html/Protocols.htm