SIMPLE BIOPSY TECHNIQUE FOR SAMPLING SKIN FOR DNA ANALYSIS OF SEA TURTLES

Recent advances in molecular biology have allowed genetic approaches to reveal information on population structure, breeding systems and individual identity in studies on sea turtles. A variety of methods have been used to collect and preserve samples for genetic studies, including collection of liver, heart or muscle taken shortly after death and either frozen or stored in saturated salt preservative or alcohol. Since sea turtles possess nucleated red blood cells, it is possible to obtain DNA non-lethally from blood samples which can be frozen or stored at ambient temperature in a lysis buffer. However, considerable training is required to sample blood effectively from live sea turtles under field conditions, and hatchlings or embryos are sometimes sacrificed for tissue as an alternative source of DNA.

Clearly, a sampling technique that is simple and non-lethal is preferable for use with endangered and threatened species, such as sea turtles. Small biopsy darts are routinely used to collect tissue non-lethally for marine mammal DNA studies (Amos and Hoelzel 1991). We tested a similar method to obtain small skin tissue plugs from sea turtles, which involved the use of an inexpensive and disposable tool commonly used in human medicine.

Disks of skin 6 mm in diameter were sampled from live juvenile green turtles (Chelonia mydas) 40-60 cm in carapace length. In addition, skin plugs were taken from frozen green, leatherback (Dermochelys coriacea), and loggerhead (Caretta caretta) turtles 30-70 cm in carapace length that were being held for necropsy. All samples were rapidly and easily taken using an Acu-Punch 6 mm biopsy punch available from Acuderm, Inc. (Fort Lauderdale, Florida 33309 USA).

The plastic handle of the punch was held by the thumb and index finger, with the circular surgical blade resting against the smooth skin located in the dorsal axial region of the hind flipper (the skin that adjoins the ventral surface of the marginal scutes and runs contiguous to the flippers). Small turtles were placed on their back to facilitate access to the biopsy site. However, adult turtles, such as on a nesting beach, can be easily sampled in a prone position at this recommended biopsy site.

A circular cut 2-4 mm deep was rapidly made by rotating the tool once or twice while gently pressing down. After withdrawing the blade, the resulting disk of tissue was removed with forceps and stored in 20% DMSO saturated with salt. In the case of live turtles, the skin was cleaned with 90% alcohol prior to sampling. Virtually no bleeding occurred following the biopsy. On the advice of a consulting veterinarian, a suture was not deemed necessary. Increased bleeding may occur if the biopsy is taken at other locations, such as the pectoral region.

Extraction of DNA from each of the 0.02-0.04 g tissue "plugs" was carried out using the proteinase K digestion protocol of Maniatis et al. (1982) as modified by Hillis and Davis (1986). All samples yielded visible DNA pellets which were washed in ice cold 70% ethanol and resuspended in sterile water. The d-loop region of mtDNA was amplified by the polymerase chain reaction (PCR; Innis et al. 1990) and resulted in a single-band product of the correct size (cf. Allard et al. 1994).

This work demonstrates that DNA can be obtained of sufficient quality and quantity for PCR analysis from small skin tissue biopsies of sea turtles, using a quick, simple, and relatively non-invasive sampling procedure. Smaller biopsy punches (as small as 1.5 mm) are also available and might be used for hatchlings without creating undo tissue trauma.

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