Molecular Ecology 1996, 5, 456–457

TECHNICAL NOTE

Marine mammal faeces as a source of DNA

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Remote collection of skin biopsies from free-ranging marine mammals has been to date, the most effective and noninvasive means of tissue collection for DNA studies (Amos & Hoelzel 1990; Brown *et al.* 1991; Weinrich *et al.* 1991). Although it has been shown that DNA extraction from faeces is possible (Höss *et al.* 1992), there are no records of DNA extraction from the faeces of marine mammals, perhaps because it is expected their faeces have a shorter life span compared with the faeces of (dry) land mammals. We have been able to extract DNA from the faeces of a free-ranging dugong *(Dugong dugon)*. We present a sequence from this DNA, a region of the mitochondrial DNA which we have used for a genetic population structure study of the dugong. Extraction of DNA from faeces of marine mammals provides an attractive alternative to biopsy sampling. It is less invasive and more economical.

The dugong faecal sample was collected from Borroloola (15°8′S 136°7′E) as part of a feeding study (A.R. Preen, pers. comm., 1995). Dugong feeding trails are easily recognizable and their faeces float to the surface of the water. Several intact faeces were collected after floating for an undetermined time (a few hours?) and individually contained in plastic bags. It was about 3 h before they were frozen and, although they stayed cold, they were defrosted during a 2-day transit, refrozen, then thawed for DNA extraction.

Up to 2 g of faeces was suspended in 2 mL of lysis buffer (40 mM Tris; 2 mM EDTA; 0.2 M NaCl; 10% SDS). Proteinase K (20 mg/mL) was added in 50-μL aliquots until the lysate was clear, or almost so (Lovell-Badge 1987). The lysate was phenol/chloroform extracted (Sambrook *et al.* 1989), followed by ethanol precipitation (Crouse & Amorese 1989). Pellets were resuspended in TE [10 mM Tris-Cl (pH 8.0); 1 mM EDTA (pH 8.0)] with DNase-free RNase (Sigma) added to a final concentration of 20 μg/mL.

The quality and quantity of DNA was checked on an 0.6% agarose gel. In total, there was ≈ 2 μg of high molecular weight as well as partially degraded, genomic DNA (note that this is likely to contain DNA from the dugongs diet as well as epithelial cells from the dugongs intestine). Up to 20 ng of the genomic DNA was used as template for the polymerase chain reaction (PCR). Optimal PCR conditions were established using the PCR Optimization kit (Boehringer Mannheim) which provided a series of buffers of various pH and $MgCl_2$ concentrations. The greatest yield of PCR products were given when the buffer pH of 9.2 was used, regardless of the $MgCl_2$ range (between 1.0 and 2.0 mM). PCR reagents include: 0.8 mM final concentration of dNTPs, 1 x buffer (from the optimization kit, which included various $MgCl_2$ concentrations), 10 pmol of each primer, 0.2% BSA. After an initial denaturation/annealing of template to primers 0.025 units of *Taq* polymerase (Promega) was added.

The sequence for the conserved control region (forward) primer (L15926) was obtained from Kocher *et al.* (1989). The dugong-specific reverse primer, 5′-CCT GAA GTA GG/AA ACC AGA TGT C-3′ was designed considering guidelines as suggested by Hoelzel (1992). These primers amplify 194 bases of the hypervariable portion of the control or D-loop region of mitochondrial DNA.

Amplification was performed in a Corbett Research FTS-1 Thermal Cycler. We initially denatured the template/primer mix at 95 °C for 2 min followed by chilling on ice for 2 min to anneal. Contents were settled by a brief centrifugation before the polymerase was added. The PCR cycles were as follows: one cycle of 35 s at 94 °C, 45 s at 45 °C, 1 min 30 s at 72 °C; followed by 29 cycles of 20 s at 95 °C, 5 s at 45 °C, 10 s at 55 °C, 1 min 30 s at 72 °C; with a final hold temperature of 72 °C for 5 min.

The PCR product was cleaned using Wizard PCR preps (Promega) and cloned using the TA Cloning kit (Invitrogen) before being sequenced using Sequenase (USB).

We aligned the sequence from the Borroloola dugong (Fig. 1) to sequences of a dugong from the Torres Strait

Keywords: DNA extraction, marine mammal faeces, dugong, control region sequences, PCR

Received 25 September 1995; revision accepted 15 January 1996

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1    CCAGTACGGTAGGATTCATGCTCTAAAGCCTAAGTAATTAATCTCCATTATACAACCTCT 60
2    ............................................................
3    .A.....A...T..G.A......CT.GA.AGT.TAT.CCTTC.A.TGCAGATTCC.AA.C

1    ACACCATGGATATTGTCCAGTCCATGTACCTCTTGATTTTGCATAGTACATTCAAC-CCT 119
2    .............................T.......C..................-...
3    ...---........C.T........TC.TTC......A........C....CATG.T...

1    TTATCGTACATAGCACATCTC-TGAGATAGTTCTCGTCAACACGCTTATCACCTCCAATG 178
2    .....................-......................................
3    .A................TA.T...A..CA.C..........T..............T.A

1    AACAGTCCTTGACTAC                                             194
2    ................
3    GG..........TC..
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Fig. 1 Aligned control region or D-loop sequences of a dugong from (1) Borroloola, (2) Torres Strait and the Florida Manatee, (3). GenBank accession numbers U48623, U48624 and U48622, respectively.

(muscle sample) and the Florida manatee (*Trichechus manatus;* also a muscle sample). The Borroloola dugong had a unique haplotype (specific gene sequence), not found in any of the other 102 dugongs we have sequenced in this region for our study of dugong population structure. Because it is a unique sequence, we consider it unlikely that our product arose from contamination.

Dugongs are elusive in the field. To collect skin biopsies they must be chased in shallow water with small boats until they surface. Although the biopsy method was successful when we applied it to the dugong, it is also extremely time consuming and not as economical, easy and noninvasive as collecting faeces. Moreover, the international shipment of faeces is not governed by the same restricting laws that apply to shipment of tissues from protected species (faeces transport not prohibited by the Wildlife Protective Act in Australia). Collecting faeces as a source of DNA has proved to be an attractive, noninvasive method for sample collection from free-ranging marine mammals such as the dugong.

Acknowledgements

We thank A.R. Preen (James Cook University) for collection of the faecal sample. A. Harris (CSIRO Marine Labs) for collection of the Torres Strait sample and to S. Wright (Florida Department of Environmental Protection) for provision of the manatee sample. We thank the Australian Fisheries Management Authority and the Australian Postgraduate Research Award, for their financial support.

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