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We use this protocol and it's working

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## QIAamp DNA Extraction Protocol V.1

Vicky Ooi<sup>1</sup>, Lee McMichael<sup>2</sup>, Margaret E. Hunter<sup>3</sup>,  
Aristide Takoukam Kamla<sup>4,5</sup>, Janet M. Lanyon<sup>1</sup>

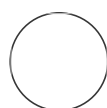
<sup>1</sup>School of Biological Sciences, The University of Queensland, St Lucia, Queensland, Australia;

<sup>2</sup>School of Veterinary Science, The University of Queensland, Gatton, Queensland, Australia;

<sup>3</sup>U.S. Geological Survey, Wetland and Aquatic Research Center, Sirenia Project, Gainesville, Florida, USA;

<sup>4</sup>Aquatic Animal Health Program, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA;

<sup>5</sup>African Marine Mammal Conservation Organization, Dizangue, Littoral, Cameroon



Vicky Ooi

### ABSTRACT

Dugong faecal DNA was extracted using the QIAamp Fast DNA Stool Mini Kit (#51604, Qiagen, Germany). The "Protocol: Isolation of DNA from Stool for Human DNA Analysis" was optimised for DNA extraction from dugong faeces.

**Keywords:** QIAamp, DNA extraction, DNA extraction method, DNA extraction methods, QIAGEN, QIAamp Fast DNA Stool Mini Kit, Faecal DNA extraction, DNA extraction of faeces, DNA, Faeces, scat, stool, non-invasive DNA extraction, QIAGEN

- 1 Use a sterile blade to scrape off 220 mg of faecal material from the outside surface of a stool and then transfer it into a 2 mL microcentrifuge tube.
- 2 Transfer the faecal material into a mortar and grind the faeces into powder with liquid nitrogen.
- 3 Add 500  $\mu$ L of InhibitEX buffer to the mortar containing the faecal material to further grind and mix in the buffer with the ground faeces. Transfer the liquid back into the 2 mL tube.
- 4 Add another 500  $\mu$ L of InhibitEX buffer to the mortar to mix in any leftover faecal material on the mortar. Transfer the liquid back into the 2 mL tube.
- 5 Vortex continuously for 1 min or until the solid material is thoroughly homogenised.
- 6 Centrifuge the sample at 20,000  $g$  (~14,000 rpm) for 2 min to pellet stool particle.
- 7 Pipette 25  $\mu$ L of Proteinase K into a new 2 mL tube.
- 8 Pipette 800  $\mu$ L of supernatant from the centrifuged homogenate into the 2 mL microcentrifuge tube containing Proteinase K.

- 9 Add 800  $\mu\text{L}$  of Buffer AL and vortex for 15 s.
  - 10 Incubate at 70°C for 10 min. Then, centrifuge briefly to remove drops from the inside of the tube lid.
  - 11 Split the lysate into two 2 mL tubes ( $\sim 813 \mu\text{L}$  each tube).
  - 12 Add 400  $\mu\text{L}$  of 95% ethanol to both tubes containing the lysate (thus, 800  $\mu\text{L}$  of ethanol overall) and mix by vortexing.
  - 13 Carefully apply 600  $\mu\text{L}$  of lysate to the QIAamp spin column. Close the cap and centrifuge at 20,000  $g$  ( $\sim 14,000$  rpm) for 1 min. Place the QIAamp spin column in a new 2 mL collection tube and discard the tube containing the filtrate.
  - 14 Repeat step 13 until all the lysate has been loaded on the column.
  - 15 Carefully open the QIAamp spin column and add 500  $\mu\text{L}$  of Buffer AW1. Centrifuge at 20,000  $g$  ( $\sim 14,000$  rpm) for 1 min. Place the QIAamp spin column in a new 2 mL collection tube and discard the collection tube containing the filtrate.
  - 16 Carefully open the QIAamp spin column and add 500  $\mu\text{L}$  of Buffer AW2. Centrifuge at 20,000  $g$  ( $\sim 14,000$  rpm) for 3 min. Place the QIAamp spin column in a new 2 mL collection tube and discard the collection tube containing the filtrate.
  - 17 Centrifuge at 20,000  $g$  ( $\sim 14,000$  rpm) for 3 min to eliminate the chance of possible Buffer AW2
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carryover.

- 18** Transfer the QIAamp spin column into a new, labelled 1.5 mL microcentrifuge tube and pipette 100  $\mu$ L of Buffer ATE directly onto the QIAamp membrane to elute the DNA from the spin column into the 1.5 mL Eppendorf LoBind microcentrifuge tube. Incubate for 1 min at room temperature, then centrifuge at 20,000  $g$  (~14,000 rpm) for 1 min to elute DNA.
- 19** Store the DNA isolate at -20°C for use within a week or at -80°C for longer-time storage.