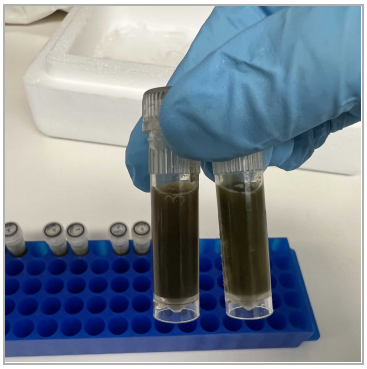

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QIAamp DNA Extraction Protocol

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ABSTRACT

Non-invasively collected faecal samples are an alternative source of DNA to tissue samples, that may be used in genetic studies of wildlife when direct sampling of animals is difficult. Although several faecal DNA extraction methods exist, their efficacy varies between species. Previous attempts to amplify mitochondrial DNA (mtDNA) markers from faeces of wild dugongs (*Dugong dugon*) have met with limited success and nuclear markers (microsatellites) have been unsuccessful. This study aimed to establish a tool for sampling both mtDNA and nuclear DNA (nDNA) from dugong faeces by modifying approaches used in studies of other large herbivores. First, a streamlined, cost-effective DNA extraction method that enabled the amplification of both mitochondrial and nuclear markers from large quantities of dugong faeces was developed. Faecal DNA extracted using a new 'High Volume-Cetyltrimethyl Ammonium Bromide- Phenol-Chloroform-Isoamyl Alcohol' (HV-CTAB-PCI) method was found to achieve comparable amplification results to extraction of DNA from dugong skin. As most prevailing practices advocate sampling from the outer surface of a stool to maximise capture of sloughed intestinal cells, this study compared amplification success of mtDNA between the outer and inner layers of faeces, but no difference in amplification was found. Assessment of the impacts of faecal age or degradation on extraction, however, demonstrated that fresher faeces with shorter duration of environmental (seawater) exposure amplified both markers better than eroded scats. Using the HV-CTAB-PCI method, nuclear markers were successfully amplified for the first time from dugong faeces. The successful amplification of SNP markers represents a proof-of-concept showing that DNA from dugong faeces can potentially be utilised in population genetic studies. This novel DNA extraction protocol offers a new tool that will facilitate genetic studies of dugongs and other large and cryptic marine herbivores in remote locations.

ATTACHMENTS

QIAamp DNA Extraction Protocol.docx

GUIDELINES

Follow the guidelines as per the "Protocol: Isolation of DNA from Stool for Human DNA Analysis" from the QIAamp Fast DNA Stool Mini Handbook (Version: February 2020) unless noted otherwise.

MATERIALS

- QIAamp® Fast DNA Stool Mini Kit **Qiagen Catalog #51604**
- 99.9 % Ethanol **Contributed by users**
- Liquid nitrogen **Contributed by users**

Faecal Sampling and Processing

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.

220 mg of faeces

2 Transfer the faecal material into a mortar and grind the faeces into powder with liquid nitrogen.

Cell Lysis, Protein Digestion, and Purification

21m 15s

3 Add 500 µL 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.

500 µL of InhibitEX buffer

4 Add another 500 µL 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.

500 µL of InhibitEX buffer

5 Vortex continuously for 1 min or until the solid material is thoroughly homogenised. 1m

00:01:00

6 Centrifuge the sample at 20,000 *g* (~14,000 rpm) for 2 min to pellet stool particle. 2m

00:02:00

7 Pipette 25 µL of Proteinase K into a new 2 mL tube.

25 µL of Proteinase K

8 Pipette 800 µL of supernatant from the centrifuged homogenate into the 2 mL microcentrifuge tube containing Proteinase K.

800 µL of supernatant

9 Add 800 µL of Buffer AL to the mixture and vortex for 15 s. 15s

800 µL of Buffer AL
00:00:15

10 Incubate at 70°C for 10 min. Then, centrifuge briefly to remove drops from the inside of the tube lid. 10m

70 °C
00:10:00

11 Split the lysate into two 2 mL tubes (~813 µL each tube).

12 Add 400 µL of 99.9 % ethanol to both tubes containing the lysate (thus, 800 µL of ethanol overall) and mix by vortexing.

400 µL of 99.9 % ethanol in each tube

13 Carefully apply 600 µL of lysate to the QIAamp spin column. Close the cap and centrifuge at 20,000 *g* (~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. 1m

600 µL of lysate
00:01:00

14 Repeat step 13 until all the lysate has been loaded on the column.

15 Carefully open the QIAamp spin column and add 500 µL of Buffer AW1. Centrifuge at 20,000 *g* 1m

(~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.

500 µL of Buffer AW1
00:01:00

16 Carefully open the QIAamp spin column and add 500 µL of Buffer AW2. Centrifuge at 20,000 *g* (~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. 3m

500 µL of Buffer AW2
00:03:00

17 Centrifuge at 20,000 *g* (~14,000 rpm) for 3 min to eliminate the chance of possible Buffer AW2 carryover. 3m

00:03:00

Elution

21m 15s

18 Transfer the QIAamp spin column into a new, labelled 1.5 mL microcentrifuge tube and pipette 100 µL 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. 2m

100 µL of Buffer ATE
00:01:00 of incubation at room temperature : 15 °C to 25 °C
00:01:00 of centrifugation

Storage of DNA extracts

19 Store the DNA isolate at -20°C for use within a week or at -80°C for longer-time storage.

-20 °C
-80 °C