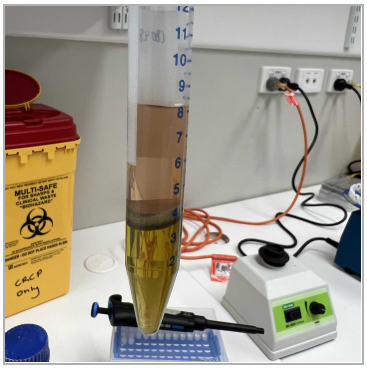

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We use this protocol and it's working

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# HV-CTAB-PCI 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

HV-CTAB-PCI DNA Extraction Protocol.docx

## GUIDELINES

Clean the working bench before starting DNA extraction.

Make sure to leave a small amount of aqueous layer when transferring the aqueous phase to prevent carry-over of the organic layer.

## MATERIALS

- TriGene Virucidal Disinfectant **In Vitro Technologies**
- Liquid nitrogen **Contributed by users**
- CTAB (Hexadecyltrimethylammonium bromide) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #52365-50G**
- 1M HCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1090571000**
- Tris **P212121**
- EDTA **Contributed by users**
- Sodium chloride **P212121**
- Chloroform **Merck MilliporeSigma (Sigma-Aldrich) Catalog #1024452500**
- Isoamyl Alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I9392-500ML**
- Liquified Phenol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P9346-500ML**
- Protease **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P5147-100MG**
- Isopropanol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #I9516-500ML**
- 70% Ethanol **Thermo Fisher Scientific Catalog #AJA726-5PL**

## SAFETY WARNINGS

Phenol and chloroform are hazardous chemicals. Please use appropriate personal protective equipment and lab safety protocol, e.g., always work in fume hoods when handling those chemicals.

## ETHICS STATEMENT

Dugong samples were obtained under The University of Queensland Animal Ethics Permit SBS/181/18, Scientific Purposes Permit WISP14654414, Moreton Bay Marine Parks Permit MPP18-001119, and Great Barrier Reef Marine Park Permit G14/36987.1.

## BEFORE START INSTRUCTIONS

Prepare the working reagents of Lysis Buffer 1, Lysis Buffer 2, Phenol-Chloroform-Isoamyl Alcohol (21:20:1), and TE Buffer. Autoclave the buffers.

# Disinfection of working bench

1 Clean the working bench with TriGene disinfectant.

## Faecal Sampling and Processing

2 Scrape 1 g of faecal material from the outer surface of a faeces and put it into a 15 mL centrifuge tube.

1 g of faeces

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

## Cell Lysis, Protein Digestion, and Purification

4 Add 1 mL of Lysis Buffer 1 (LB1: CTAB 2 %, Tris– HCL 100 mM, EDTA 20 mM, NaCl 1.4 M, pH 7.5) 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 15 mL tube.

1 mL of Lysis Buffer 1 (LB1)

5 Add another 1 mL of LB1 to the mortar to mix in any leftover faecal material on the mortar and transfer the liquid back into the 15 mL tube. Repeat this step once more, and then add 2 mL of LB1 to the 15 mL tube containing the faecal homogenate. Thus, a total of 5 mL of LB1 would be added to the ground faeces.

1 mL of LB1 + 1 mL LB1 + 2 mL LB1

6 Vortex the faecal homogenate and incubate in a thermomixer for 3 h, with occasional mixing, at 60°C for cell lysis. 3h

03:00:00 of incubation at 60 °C

7 Centrifuge the sample at 3,150 *g* (4,000 rpm) for 12 min and pipette 4 mL of the supernatant into a new 15 mL tube. 12m

00:12:00 of centrifugation
4 mL of supernatant

8 Add 4 mL of phenol: chloroform: isoamyl alcohol (21:20:1) to the supernatant, then gently mix the tube. Centrifuge the sample for 3 min at 3,150 *g* (4,000 rpm) and pipette 3 mL of the aqueous phase into a new 15 mL tube. 3m

4 mL of phenol: chloroform: isoamyl alcohol
00:03:00 of centrifugation
3 mL of aqueous phase

9 Add 330 µL of Lysis Buffer 2 (LB2: CTAB 10 %, NaCl 0.5 M, pH 5.5) to the aqueous phase, and leave it to lyse further at 60°C for 4 h. 4h

330 µL of Lysis Buffer 2
04:00:00 of incubation at 60 °C

10 Add 104 µL of protease to the sample and leave it to digest proteins at 60°C for another 1 h. 1h

104 µL of protease
01:00:00 of incubation at 60 °C

11 Add 3434 µL of phenol: chloroform: isoamyl alcohol (21:20:1) to the mixture, and then gently mix the tube well, and centrifuge the sample for 12 min at 3,150 *g* (4,000 rpm). Then, pipette 3 mL of the aqueous phase into a new 15 mL tube. 12m

3434 µL of phenol: chloroform: isoamyl alcohol
00:12:00 of centrifugation
3 mL of aqueous phase

## DNA Precipitation and Purification

12 Add one volume of isopropanol (i.e., 3 mL) to precipitate the DNA overnight at -20°C. 12m

3 mL of isopropanol
-20 °C Overnight

13 Centrifuge the sample for 20 min at 8000 *g* (5,200 rpm), and then get rid of all the supernatant. 20m

00:20:00 of centrifugation

14 Add 400 µL of 70% ethanol to the pellet to wash it. Vortex and then centrifuge the sample at 3,150 *g* for 12 min and get rid of the supernatant. 12m

400 µL of 70 % ethanol
00:12:00 of centrifugation

15 Dry the pellet in a fume hood at room temperature for 15 min. 15m

00:15:00 at Room temperature

## DNA Resuspension

16 Resuspend the pellet in 250 µL of TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8).

250 µL of TE buffer

## Storage of DNA Extracts

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

-20 °C
-80 °C