



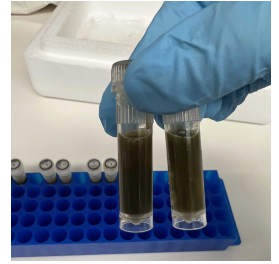
Mar 20, 2023

QIAamp DNA Extraction Protocol

 [PLOS One](#)

DOI

<https://dx.doi.org/10.17504/protocols.io.yxmvm212ng3p/v1>



Vicky Ooi¹, Lee McMichael², Margaret E. Hunter³, Aristide Takoukam Kamla^{4,5}, Janet M. Lanyon¹

¹School of Biological Sciences, The University of Queensland, St Lucia, Queensland, Australia;

²School of Veterinary Science, The University of Queensland, Gatton, Queensland, Australia;

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

⁴Aquatic Animal Health Program, College of Veterinary Medicine, University of Florida, Gainesville, Florida, USA;

⁵African Marine Mammal Conservation Organization, Dizangue, Littoral, Cameroon



Vicky Ooi

Moss Landing Marine Laboratories

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.yxmvm212ng3p/v1>

External link: <https://doi.org/10.1371/journal.pone.0278792>

Protocol Citation: Vicky Ooi, Lee McMichael, Margaret E. Hunter, Aristide Takoukam Kamla, Janet M. Lanyon 2023. QIAamp DNA Extraction Protocol. [protocols.io https://dx.doi.org/10.17504/protocols.io.yxmvm212ng3p/v1](https://dx.doi.org/10.17504/protocols.io.yxmvm212ng3p/v1)

Manuscript citation:

Ooi V, McMichael L, Hunter ME, Kamla AT, Lanyon JM (2023) A new DNA extraction method (HV-CTAB-PCI) for amplification of nuclear markers from open ocean-retrieved faeces of an herbivorous marine mammal, the dugong. PLoS ONE 18(6): e0278792. doi: [10.1371/journal.pone.0278792](https://doi.org/10.1371/journal.pone.0278792)

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: March 18, 2023

Last Modified: March 20, 2023

Protocol Integer ID: 79036

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, Non-invasive DNA Extraction, dna from dugong faece, several faecal dna extraction method, dna from dugong skin, dna extraction method, effective dna extraction method, dna extraction, genetic studies of dugong, novel dna extraction protocol, faecal dna, collected faecal sample, genetic studies of wildlife, faecal sample, dugong faece, markers from faece, amplification success of mtdna, first time from dugong faece, mitochondrial dna, direct sampling of animal, studies of other large herbivore, ndna, alternative source of dna, mtdna, cryptic marine herbivores in remote location, extraction, other large herbivore, nuclear dna, cryptic marine herbivore, dna, dugong skin, nuclear markers from large quantity, fresher faeces with shorter duration, species, wild dugong, capture of sloughed intestinal cell

Funders Acknowledgements:

Sea World Research and Rescue Foundation Inc.

Grant ID: SWR/1/2015; SWR/6/2016

Disclaimer

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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 Extractio...

24KB

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

⊗ Liquid nitrogen



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 μ 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.

 500 μ L of InhibitEX buffer

- 4 Add another 500 μ 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.

 500 μ L 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 μ L of Proteinase K into a new 2 mL tube.

 25 μ L of Proteinase K



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

 800 μ L of supernatant

- 9 Add 800 μ L of Buffer AL to the mixture and vortex for 15 s.

15s

 800 μ L 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 μ L each tube).

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

 400 μ L of 99.9 % ethanol in each tube

- 13 Carefully apply 600 μ L 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 μ L 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 μ L of Buffer AW1. 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 collection tube containing the filtrate.

1m



🧪 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

Protocol references

Protocol: Isolation of DNA from Stool for Human DNA Analysis, QIAamp Fast DNA Stool Mini Kit Handbook.