MBA DTOL - DNA extraction and barcoding of Marine Metazoans

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Darwin Tree of Life | DTOL - Marine Biological Association

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ABSTRACT

This document provides a guide to the protocols used by the Marine Biological Association for DNA extraction and barcoding of marine metazoans sampled for the Darwin Tree of Life project. The selection of organisms described are due to these species requiring more optimized techniques than the high-throughput method otherwise used by the Natural History Museum (dx.doi.org/10.17504/protocols.io.8epv5x7qjg1b/v1). Please note that this is a working document, with more organism-specific barcoding methods being added as they become optimized. The methods described are generally successful for the selected groups, but may still require further optimization for some samples.

NOV 16, 2023

OPEN ACCESS

Protocol Citation: Rebekka Uhl 2023. MBA DTOL - DNA extraction and barcoding of Marine Metazoans . **protocols.io** https://protocols.io/view/mba-dtol-dna-extraction-and-barcoding-of-marine-me-c45syy6e

Protocol status: Working
We use this protocol and it's working

Created: Nov 16, 2023

Last Modified: Nov 16, 2023

PROTOCOL integer ID: 91026

Funders Acknowledgement:

Wellcome Sanger Trust

DNA extraction: General

1 **DNA extraction:**

Unless otherwise stated, DNA extraction for marine metazoans is done with the Qiagen DNAeasy Mouse Blood and Tissue kit.
https://www.qiagen.com/us/Resources/ResourceDetail?id=aa250d94-fc4b-4e27-bb74-d32391ff8a48&lang=en

The provided protocol is followed, apart from the final elution step:
Pre-heated elution buffer (56C) is used to increase yield.
An elution volume of 60ul is used to increase the final DNA concentration.
The buffer is left to incubate on the membrane for 5 minutes.

If sample yields are relatively high (>25ng/ul), gDNA samples are usually diluted by 1:10 to reduce contamination during PCR amplification.

DNA Barcoding: Ascidians

2 **Reference:** Adapted from Salonna et al., 2021
Salonna, M., Gasparini, F., Huchon, D., Montesanto, F., Haddas-Sasson, M., Ekins, M., McNamara, M., Mastrototaro, F. and Gissi, C., 2021. An elongated COI fragment to discriminate botryllid species and as an improved ascidian DNA barcode. *Scientific reports*, *11*(1), p.4078.

Primers: COI barcode

	A	B	C
	F	dinF	CGTTGRTTTATRTCTACWAATCATAARGA
	R	Nux1R	GCAGTAAAATAWGCTCGRGARTC

PCR reaction mix:

	Mastermix	**1x**
	GoTaq 5x buffer	10ul
	MgCl2 (25mM)	4ul
	dNTPs (10uM each)	1ul
	Primer (10uM)	2ul (each)
	Mol. H2O	29.75ul
	Taq	0.25ul
	DNA template	1ul (usually 1:10 dil. of extracts)
	Total	50ul

Thermocycling conditions:
95C, 3mins, 30x[95C, 30s; 50C, 30s; 72C, 1.5, mins], 72C, 5mins

If unsuccessful, try repeating PCR with x35 cycles instead (generally required)

DNA barcoding: Fish

3 **Reference:** Adapted from Moran et al., 2016
Moran, Z., Orth, D.J., Schmitt, J.D., Hallerman, E.M. and Aguilar, R., 2016. Effectiveness of DNA barcoding for identifying piscine prey items in stomach contents of piscivorous catfishes. *Environmental Biology of Fishes*, *99*, pp.161-167.

Primer: COI barcode

	A	B	C
	F	FishF2_t1	TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC
	R	FishR2_t1	CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA

PCR reaction mix

	Mastermix	**1x**
	GoTaq 5x buffer	5ul
	MgCl2 (25mM)	3ul
	dNTPs (10uM each)	0.625ul
	Primer (10uM)	0.25ul (each)
	Mol. H2O	14ul
	Taq	0.125ul
	DNA template	2ul
	Total	25ul

Thermocycling conditions:
94C, 2mins, 35x[94C, 30s; 52C, 40s; 72C, 1min], 72C, 10min

DNA barcoding: Hydroids

4 **References:** Adapted from Beckman et al., 2023 and Cunningham & Buss, 1993

Beckmann, L.M., Soto-Angel, J.J., Hosia, A. and Martell, L., 2023. Odd family reunion: DNA barcoding reveals unexpected relationship between three hydrozoan species. *PeerJ*, *11*, p.e15118.

Cunningham, C.W. and Buss, L.W., 1993. Molecular evidence for multiple episodes of paedomorphosis in the family Hydractiniidae. *Biochemical Systematics and Ecology*, *21*(1), pp.57-69.

DNA extraction:
Left in heat block for 1.5hrs (exoskeleton remains in tube – does not mean DNA is not extracted!)

Relatively varied DNA concentrations - usually required 1:10 dilution (if >10ng/ul) to reduce contamination.

Primers: 16s barcode

	A	B	C
	F	SHA	ACGGAATGAACTCAAATCATGT
	R	SHB	TCGACTGTTTACCAAAAACATA

PCR reaction mix:

	Mastermix	**1x**
	RedTaq with MgCl2	25ul
	Primer (10uM)	1ul
	Mol. H2O	22ul
	DNA template	1ul (usually 1:10 dil. of extracts)
	Total	50ul

Thermocycling conditions:
94C,5mins, 35x[94C, 30s; 50C, 30s; 72C, 1mins], 72C, 7 mins

If failed, increase reaction above to x39 cycles (some may better with x35 some better with x39 due to extent of contaminants)

DNA barcoding: Barnacles*

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***Note:** This method has been optimized for barnacle barcoding, but may also be useful for other crustaceans as universal COI primers are used (this has not been trialled).

Reference: Adapted from Benny et al., 2007

Chan, B.K., Tsang, L.M. and Chu, K.H., 2007. Morphological and genetic differentiation of the acorn barnacle Tetraclita squamosa (Crustacea, Cirripedia) in East Asia and description of a new species of Tetraclita. *Zoologica Scripta*, *36*(1), pp.79-91.

Primer: COI barcodes

	A	B	C
	F	LCO1409	GGTCAACAAATCATAAAGATATTGG
	R	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA

PCR reaction mix:

	Mastermix	**1x**

GoTaq 5x buffer	10ul
MgCl2 (25mM)	4ul
dNTPs (10uM each)	1ul
Primer (10uM)	1ul (each)
Mol. H2O	31.7ul
Taq	0.3ul
DNA template	1ul
Total	50ul

Thermocycling conditions:
94C,3mins, 33x[94C, 30s; 47C, 30s; 72C, 40s], 72C, 3 mins

DNA barcoding: Copepods

6 **References:** Adapted from Figueroa et al, 2020 and Rossel et al., 2023

Figueroa, N.J., Figueroa, D.F. and Hicks, D., 2020. Phylogeography of Acartia tonsa Dana, 1849 (Calanoida: Copepoda) and phylogenetic reconstruction of the genus Acartia Dana, 1846. *Marine Biodiversity*, *50*, pp.1-20.

Rossel, S., Kaiser, P., Bode-Dalby, M., Renz, J., Laakmann, S., Auel, H., Hagen, W., Arbizu, P.M. and Peters, J., 2023. Proteomic fingerprinting enables quantitative biodiversity assessments of species and ontogenetic stages in Calanus congeners (Copepoda, Crustacea) from the Arctic Ocean. *Molecular Ecology Resources*, *23*(2), pp.382-395.

DNA extraction:
Samples were incubated in 30 µl chelex (InstaGene Matrix, Bio-Rad) for 50 min at 56°C, followed by a denaturation of the enzymes for 10 min at 96°C. Centrifuge samples @10,000rpm for 1min and carefully pipette out supernatant (avoid Chelex beads!). The resulting sample was used directly as DNA template for PCR amplification.
Sample concentrations varied between 1-50ng/ul.

Primers: COI barcode

A	B	C
F	LCO1409	GGTCAACAAATCATAAAGATATTGG
R	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA

PCR reaction mix:

Mastermix	**1x**
GoTaq 5x buffer	10ul
MgCl2 (25mM)	4ul
dNTPs (10uM each)	1ul
BSA (20mg/ml)	1ul

	Primer (10uM)	2ul (each)
	Mol. H2O	24.75ul
	Taq	0.25ul
	DNA template	5ul
	Total	50ul

Thermocycling conditions:
94C, 5mins, 38x[94C, 45s; 42C, 45s; 72C, 1,20s], 72C, 7 min