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qPCR assay for the Detection of Coralliophila galea

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We use this protocol and it's working

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Abstract

This real-time PCR assay is designed to detect *Coralliphila galea* (formerly *Coralliphila abbreviata*) in DNA-extracted samples. Primers were developed to target the COX1 and 12S genes of *C. galea*, using sequences obtained from the National Center for Biotechnology Information (NCBI). The assay was validated using tissue samples from *C. galea* and gut content samples from *Panulirus argus* and *Panulirus guttatus* that consumed *C. galea*. Additionally, validation included a primer efficiency test via serial dilutions, gene alignment via Sanger sequencing, and melt curves confirmed no primer dimers. This assay offers a potential tool for detecting *C. galea* in environmental DNA (eDNA) samples, enabling non-invasive monitoring in coral restoration sites.

Attachments

C. galea - C01 - Pri... 153KB

C. galea - 12S - Pri... 102KB

Guidelines

Primer storage: Keep at -20 °C for long-term storage
SYBR Green PowerUp™ SYBR™ : Keep at -20 °C for long-term storage

See attachments "C. galea - 12S primer Design.pdf" and "C. galea - CO1 primer Design.pdf" for primer gene annotations and *Insilico* primer testing results.

Materials

Primers :
*Only one primer set is required for the assay. *Assay is the same for each primer set, you can choose either of them or run both for confirmation of results**

1. Cgalea_12S primers
Forward primer: 5'-AAACCCTTGAGGGAAACTGG-3'
Reverse primer: 5'-CCAGGTTCAACGCGGATCAT-3'

2. Cgalea_CO1 primers
Forward primer: 5'-GTGCTCCCGACATAGCTTTT-3'
Reverse primer: 5'-AGTCCCAACACCCCTTTCTA-3'

Reagents:
3. Thermo Fisher SYBR Green PowerUp™ SYBR™ Green Master Mix for qPCR (Cat# A25742)
4. DNase/RNase-free water/PCR grade water

Dry Goods:
5. Optical 8-cap strips for 0.2 ml tubes (Biorad TCS0803)
6. PCR Plate (Biorad MLL9651)
7. Sterile 1.5 mL screw-top microcentrifuge tubes
8. Sterile filter pipette tips

Equipment:
9. Quantitative PCR instrument
10. Micro centrifuge and/or reagent reservoir
11. Vortex
12. Laminar flow hood for PCR setup

Troubleshooting

Before start

To ensure accurate and reliable results, it is critical to verify the quality and purity of DNA extractions prior to performing this assay. High-quality DNA that is free of inhibitors, contaminants, and degradation is essential for optimal amplification efficiency and sensitivity in real-time PCR. It is recommended to assess DNA concentration and purity using spectrophotometric methods (e.g., A260/A280 ratios).

Prepare for qPCR

1

- Remove PCR reagents from the freezer and allow reagents to thaw on ice or at room temperature.
- Wipe down the PCR hood with 10% bleach and 70% ethanol.
- Place consumables such as tubes, plates, plate sealers, and water in the PCR hood and turn on the UV light for 20 minutes.
- Once everything is thawed, vortex PCR reagents, spin them down, and place them on ice.

1. SYBR Green Master Mix - Gently Mix, then spin down
2. Primers - Vortex for 15 sec, then spin down

- Keep reagents cool or on ice during the duration of the protocol.

Prepare qPCR Master Mix

2 Prepare enough master mix for the number of reactions needed. Each combination of sample and target (gene) should be run at least in duplicates. Add a few reactions to your calculations to account for pipetting errors.

Assay is the same for each primer set, you can choose either of them or run both for confirmation of results

	A	B	C
	Reagent	**Volume (µL)**	**Final concentration**
	PCR grade water	2	NA
	SYBR MM 2x	5	1x
	F primer (10 µm)	.5	0.5 µm
	R primer (10 µm)	.5	0.5 µm
	Total =	**8**	
	Sample (DNA Extraction)	2	NA
	Total with DNA =	**10**	

- Combine all the PCR master-mix reagents in a microcentrifuge tube
- Mix **GENTLY** and spin down to collect mixture and remove bubbles

Adjustments to the ratio of PCR-grade water to sample elution during qPCR setup may be made in cases of low DNA concentration. For example, eDNA samples may be prepared using 4 µL of sample elution without the addition of PCR-grade water, and 6 µL can be added to the wells instead of the standard 8 µL

Setup the qPCR plate

3

- Add 8 µL of master mix to each well. Aiming for the bottom of the well will help to visualize what wells had the master mix and DNA added.
- Add DNA to each well (2 µL). Aiming for the top of the well will help to visualize which wells had the master mix and DNA added.
- Close the plate with optical clear caps or seals.
- Spin down the plate to mix the DNA and mastermix.
- Place the plate in the qPCR machine and start the machine using the specified settings.

Setup the qPCR machine settings

4 Select SYBR green and long run

	A	B	C	D
	Procedure	Temperature	Time	Cycle
	Initial Denaturation	95 ºC	10 mins	1X
	Denaturation	95 ºC	15 sec	40X
	Annealing & Extension	60 ºC	1 min	40X

Insure that the qPCR machine is recording fluorescence at the end of each extension cycle

Protocol references

Coralliophila galea voucher MNHN-IM-2013-9565 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial: ACCESSION PP094199
https://www.ncbi.nlm.nih.gov/nuccore/PP094199.1/

Coralliophila galea voucher RMNH.5004317 12S ribosomal RNA gene, partial sequence; mitochondrial ACCESSION KY829343
https://www.ncbi.nlm.nih.gov/nuccore/KY829343