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qPCR assay for Aquarickettsia spp. V.2

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ABSTRACT

qPCR for the quantification of *Aquarickettsia spp. (*Klinges et al., 2022) a putative parasite found in the coral *A. cervicornis.* This protocol has been altered by incorporating a recently published *A. cervicornis* CAM control gene (Palacio-Castro et al., 2021) targeted to detect differences across *A. cervicornis* genotypes because it is a single-copy gene in *A. cervicornis*.

MANUSCRIPT CITATION:
J Grace Klinges, Shalvi H Patel, William C Duke, Erinn M Muller, Rebecca L Vega Thurber, Phosphate enrichment induces increased dominance of the parasite*Aquarickettsia*in the coral*Acropora cervicornis*,*FEMS Microbiology Ecology*, Volume 98, Issue 2, February 2022, fiac013,https://doi.org/10.1093/femsec/fiac013

Palacio-Castro, A.M., Dennison, C.E., Rosales, S.M.*et al.*Variation in susceptibility among three Caribbean coral species and their algal symbionts indicates the threatened staghorn coral,*Acropora cervicornis,*is particularly susceptible to elevated nutrients and heat stress.*Coral Reefs***40**, 1601–1613 (2021). https://doi.org/10.1007/s00338-021-02159-x

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

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GUIDELINES

- PrimeTime MM - (keep in -20 for long storage)
- Forward coral host primer (Acropora) at 10 uM (keep in -20 for long storage) - Primer sequences from https://doi.org/10.1007/s00338-021-02159-x
- Reverse coral host primer (Acropora) at 10 uM (keep in -20 for long storage) - Primer sequences from https://doi.org/10.1007/s00338-021-02159-x
- Forward Aquarickettsia primer at 10 uM (keep in -20 for long storage) - Primer sequences from
- Reverse Aquarickettsia primer at 10 uM (keep in -20 for long storage) - Primer sequences from

MATERIALS

Reagents

1. Primers of tlc1 gene of A. rohwerii
- 10 µM Forward : 5' - AGGAGTTTGGAAAGCACAAG - 3',
- 10 µM Reverse : 5' - GCTACCAAATAACATAGCAGAC - 3'
- 10 µM Probe : TGCAAACTTATACTGGCCTTGCAAGT

2. Primers of Calmodulin (CaM) in the Caribbean Acropora spp. (adpated from https://doi.org/10.1007/s00338-021-02159-x)
- 10 µM forward: 5’ - GGTTATTTACAAGCCCAACCAAG - 3’,
- 10 µM Reverse: 5’ - ACAGAAGGGCCACTGAAATAG - 3’
- 10 µM Probe : ACTCCAGATTTCAAGTCTGATGCCCT

3. PrimeTime™ Gene Expression Master MIx (IDT 1055770)
4. DNase/RNase free water/PCR grade water
5. Optical 8-cap strips for 0.2 ml tubes (Biorad TCS0803)
6. white PCR Plate (Biorad MLL9651)
7. Sterile 1.5 mL screw-top microcentrifuge tubes
8. Sterile filter pipette tips

Equipment

- Quantitative PCR instrument
- Microcentrifuge and/or reagent reservoir
- Vortex
- Laminar flow hood for PCR setup

Prepare for qPCR

1 20m

- Remove PCR reagents from freezer and allow reagents to thaw on ice or at room temperature.
- Wipe down PCR hood with 10% bleach and ethanol.
- Place consumables such as tubes, plates, plate sealers, and water in PCR hood and turn on UV light for 00:20:00
- Once everything is thawed vortex PCR reagents, spin them down, and place them on ice.
- Keep reagents cool or on ice during the duration of the protocol.

Prepare PCR 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.

A. cervicornis (CAM) master mix

	A	B	C
	Component	Volume per Rxn	x rxn + 10%
	PCR water	2.4 uL	
	PrimeTime MM	5 uL	
	Forward primer (10 uM)	0.2 uL	
	Reverse primer (10 uM)	0.2 uL	
	Probe (10 uM)	0.2 uL	
	Total MM volume per reaction	8 uL	

A. rohwerii (tlc1) master mix

	A	B	C
	Component	Volume per Rxn	x rxn + 10%
	PCR water	2.4 uL	
	PrimeTime MM	5 uL	
	Forward primer (10 uM)	0.2 uL	
	Reverse primer (10 uM)	0.2 uL	
	Probe (10 uM)	0.2 uL	

	A	B	C
	Total MM volume per reaction	8 uL	

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

Setup the qPCR plate

3

- Add 8 uL of master mix to each well. Aiming for the bottom of the well will help to visualize what wells had master mix and DNA added.
- Add DNA to each well (2 uL). Aiming for the top of the well will help to visualize what wells had 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 in the qPCR machine and start the machine using the specified settings.

qPCR thermocyler program settings

4 Select SYBER green and long run

	A	B	C	D
	Procedure	Temperature	Time	Cycle
	Initial denaturation	95 C	3 min	1
	Denaturation	95 C	15 sec	40
	Annealing	60 C	1 min	40
	Extension	72 C	30 sec	40