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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 Reefs40, 1601–1613 (2021). https://doi.org/10.1007/s00338-

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021-02159-x

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

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PROTOCOL integer ID: 77144

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 Remove PCR reagents from freezer and allow reagents to thaw on ice or at room temperature.
- 20m

- 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

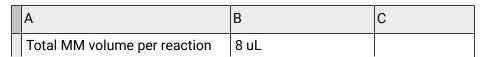
Prepare enough master mix for the number of reactions needed. Each combination of sample and target (gene) should be run at least in duplicates. Add a few reactions to your calculations to account for pipetting errors.

A. cervicornis (CAM) master mix

A	В	С
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	В	С
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	



- 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

- 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	В	С	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