qPCR assay for Aquarickettsia spp. V.1

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OPEN ACCESS

External link:
http://quantitative PCR

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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

DISCLAIMER

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* 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*.

Protocol status: In development
We are still developing and optimizing this protocol

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PROTOCOL integer ID: 76799

MATERIALS

1. Primers *of tlc1* gene of*A. rohwerii*
   - 0.3 µM Forward : 5'-GGCACCTATTGTAGTTGCGG-3',
   - 0.3 µM Reverse: 5'-CATCAGCTGCTGCCTTACCT-3'

2. Primers of Calmodulin (CaM) in the Caribbean *Acropora spp.*
   - 100 µM forward: 5'- GCC CTAATTTCTGATCGATTCAA-3',
   - 100 µM Reverse: 5'-GCAGACAGAAGGGCCACT-3'

3. PowerUp™ SYBR™ Green Master Mix (ThermoFisher Scientific **A25742**)
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.
- Wipe down PCR hood with bleach and ethanol.
- Place consumables such as tubes, plates, plate sealers, and water in PCR hood and turn on UV light for 20 min
- 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.

qPCR thermocyler program settings

2

	A	B	C	D
	Procedure	Temperature	Time	Cycle
	Initial denaturation	95 C	3 min	1
	Denaturation	95 C	10 sec	40
	Annealing	60	30 sec	40

	A	B	C	D
	Extension	72	30 sec	40

Prepare PCR master mix

3

	A	B	C
	Component	Volume per Rxn	x rxn + 10%
	PCR water	2.94 uL	
	Sybr master mix	5 uL	
	Forward primer (100 um)	0.03 uL	
	Reverse primer (100um)	0.03 uL	
	Template DNA	2 uL	NA
	Total reaction volume	10 uL	NA

- Add DNA last to each well
- Once the PCR master-mix reagents are combined, mix gently and spin down to collect mixture and remove bubbles that may interfere with downstream amplifications.
- Place samples in thermocycler.