



VERSION 1

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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>

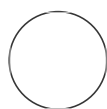
qPCR assay for *Aquarickettsia* spp. V.1

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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* 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*.

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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 μ M Forward : 5'-GGCACCTATTGTAGTTGCGG-3',
 - 0.3 μ M Reverse: 5'-CATCAGCTGCTGCCTTACCT-3'
2. Primers of Calmodulin (CaM) in the Caribbean *Acropora spp.*
 - 100 μ M forward: 5'- GCC CTAATTTCTGATCGATTCAA-3',
 - 100 μ M 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 thermocycler program settings

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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

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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.