

An improved primer set and PCR amplification protocol with increased specificity and sensitivity targeting the *Symbiodinium* ITS2 region using the SymVar primer pair

Version 2

Benjamin Hume, Maren Ziegler, Christian Voolstra, Julie Poulain, Xavier Pochon, Sarah Romac, Emilie Boissin, Colomban de Vargas, Serge Planes, Patrick Wincker

Abstract

The Internal Transcribed Spacer 2 (ITS2) rRNA gene is a commonly targeted genetic marker to assess diversity of *Symbiodinium*, a dinoflagellate genus of algal endosymbionts that is pervasively associated with marine invertebrates, and notably reef-building corals. Here we tested three commonly used ITS2 primer pairs (SYM_VAR_5.8S2/SYM_VAR_REV, ITSintfor2/ITSReverse, and ITS-DINO/ITS2Rev2) with regard to amplification specificity and sensitivity towards *Symbiodinium*, as well as sub-genera taxonomic bias. We tested these primers over a range of sample types including three coral species, coral surrounding water, reef surface water, and open ocean water to assess their suitability for use in large-scale next generation sequencing projects and to develop a standardized PCR protocol. We found the SYM_VAR_5.8S2/SYM_VAR_REV primers to perform superior to the other tested ITS2 primers. We therefore used this primer pair to develop a standardised PCR protocol. To do this, we tested the effect of PCR-to-PCR variation, annealing temperature, cycle number, and different polymerase systems on the PCR efficacy. The *Symbiodinium* ITS2 PCR protocol developed here delivers improved specificity and sensitivity towards *Symbiodinium* with apparent minimal sub-genera taxonomic bias across all sample types. In particular, the protocol's ability to amplify *Symbiodinium* from a range of environmental sources will facilitate the study of *Symbiodinium* populations across biomes.

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Protocol

Step 1.

Title

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PCR reaction[†]

Reagent	μl
Phusion High-Fidelity PCR Master Mix 2X	12.5
SYM_VAR_5.8SII @ 10μM	1
SYM_VAR_REV @ 10μM	1
DMSO	0.75
Water	8.75
genomic DNA 5-10 ng	1
Final Volume	25

† If an alternative polymerase system to the Phusion HF PCR Master Mix is used, PCR reaction amounts may need to be adjusted according to the polymerase manufacturer's guidelines

PCR cycling conditions[†]

98 °C for 2 mins

98 °C for 10 sec

56 °C for 30 sec X 35 cycles*

72 °C for 30 sec

72 °C for 5 min

* for next generation sequencing applications, consideration for the number of PCR cycles (35) should include any additional cycles required as part of the library preparation (e.g. adapter sequence ligation).

†If an alternative polymerase system to the Phusion HF PCR Master Mix is used, temperatures and times of cycling steps may need to be adjusted according to the polymerase manufacturer's guidelines.

Primer sequences

Forward primer: SYM_VAR_5.8S2 5' GAATTGCAGAACTCCGTGAACC 3' (Hume et al 2013)

Reverse primer: SYM_VAR_REV 5' CGGGTTCWCTTGTYTGACTTCATGC 3' (Hume et al 2015)

This primer pair can be adapted for Illumina MiSeq sequencing through the addition of MiSeq adapter sequences to the 5' end of the sequences. For example:

(MiSeq adapter) + SYM_VAR_5.8S2: 5'
(TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG)GAATTGCAGAACTCCGTGAACC 3' (Hume et al 2018)

(MiSeq adapter) + SYM_VAR_REV: 5'
(GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG)CGGGTTCWCTTGTGTGACTTCATGC 3' (Hume et al 2018)

References

Hume B, D'Angelo C, Burt J, Baker AC, Riegl B, Wiedenmann J (2013). Corals from the Persian/Arabian Gulf as models for thermotolerant reef-builders: Prevalence of clade C3 *Symbiodinium*, host fluorescence and *ex situ* temperature tolerance. *Mar Pollut Bull* **72**: 313-322.

Hume BCC, D'Angelo C, Smith EG, Stevens JR, Burt J, Wiedenmann J (2015). *Symbiodinium thermophilum* sp nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. *Sci Rep-Uk* **5**.

Hume BCC, Ziegler M, Poulain J, Pochon X, Romac S, Boisson E, de Vargas C, Planes S, Wincker P, Voolstra CR (2018). An improved primer set and amplification protocol with increased specificity and sensitivity targeting the *Symbiodinium* ITS2 region. *PeerJ* 6, e4816. doi:10.7717/peerj.4816
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