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

Benjamin Hume,Maren Ziegler,Christian Voolstra

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

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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 Phuision HF PCR Master Mix is used, PCR reaction ammounts 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. apdapter sequence ligation).

[‡]If an alternative polymerase system to the Phuision 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_5.8SII 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:

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(MiSeq adapter) + SYM_VAR_5.8SII: 5' (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG)GAATTGCAGAACTCCGTGAACC 3' (Hume et al 2018, in revision)

(MiSeq adapter) + SYM_VAR_REV: 5' (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG)CGGGTTCWCTTGTYTGACTTCATGC 3' (Hume et al 2018, in revision)

References

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