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Working

Species-specific quantitative PCR (qPCR) protocol developed to identify Haplosporidium pinnae

Version 1

PLOS One

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ABSTRACT

The aim of this study has been to develop species-specific quantitative PCR (qPCR) protocol carrying out a fast, specific and effective molecular diagnose of *Haplosporidium pinnae*. In this sense, the detection limit for qPCR was equal to 30 copies of SSU rDNA / ng of DNA using plasmid alone and when 100ng DNA of non-infected oyster were added.

EXTERNAL LINK

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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PROTOCOL STATUS

Working

MATERIALS

NAME	CATALOG #	VENDOR
Water		
primers		
PowerUp SYBR Green Master Mix	A25741	

MATERIALS TEXT

Primers used in qPCR:
Forward HpF3: 5´GCGGGCTTAGTTCAGGGG 3´
Reverse FpR3: 5´ACTTGTCCTTCCTCTAATAATAAGG3´

1 The qPCR assay for H. pinnae detection and quantification was carried out in duplicate using the species-specific primer pair (HpF3/HpR3) in a Mx3000P Thermocycler (Agilent).

2 Amplification reactions were performed in a total volume of 10 µl comprising:

3 2 µl of genomic DNA (100 ng),

4 5 µl of PowerUpTM SYBRTM Green Master Mix (Applied Biosystems),

5 0.2 µM each specific primer and

6 adjusted to 10µl with distilled water.

7 Negative control (without DNA or with H. pinnae non-infected fan mussel DNA, previously confirmed by qPCR),

8 positive controls (samples with positive cPCR amplification for H. pinnae) and

9 standard curve (plasmid HpF3/HpR3) were included in each qPCR assay.

10 After testing various annealing temperature, the final qPCR program was: 1 cycle for 2 min at 50ºC; 1 cycle for 2 min at 95ºC, 40 cycles of amplification at 95ºC for 15 s, 60ºC for 18 s and 72ºC for 1 min and followed by a dissociation stage for determining the melt curve (Quantitative SYBR Green with dissociation curve).

11 Two standard curves were calculated using serial dilutions of the plasmid and plasmid diluted with DNA of fan mussel;

12 the efficiency (E) was from the slope of the standard curve following formula [11] E=10-1/slope-1.

13 Melting curve was generated with temperatures increments of 0.5ºC s-1 starting at 60ºC and ending at 95ºC in order to ensure that a single PCR product was amplified for the primers.

14 Amplification was also confirmed by electrophoresis on 2% agarose gels, in 1% TAE (Tris acetate EDTA buffer), stained with Red Safe (Nitron Biotechnology) and scanned in a GelDoc-It Imagen System Ultraviolet Transilluminator (BioImaging Systems).