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The detection of freshwater pearl mussel

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We use this protocol and it's working

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Abstract

qPCR assay to detect freshwater pearl mussels *Margaritifera margaritifera* in order to monitor populations in their natural environment.

Attachments

XLSX

qPCR_Freshwater Pear...

44KB

Guidelines

Laboratory work space and equipment were sterilized by UV-light and DNase solution and 70% ethanol. Filter pipet tips were used in all steps of the laboratory work.
Negative controls of DNase/RNase free water were used in each qPCR assay.

Materials

MATERIALS

UltraPure™ DEPC-treated Water **Thermo Fisher** **Catalog #**10813999

Qiagen DNeasy power water sterivex kit **Catalog #**14600-50-NF

STEP MATERIALS

SsoAdvanced Universal SYBR® Green Supermix **Bio-Rad Laboratories** **Catalog #**172-5270

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Troubleshooting

Safety warnings

Handling high concentration of positive controls was performed in a post-PCR room which is physically separated from the pre-PCR room to avoid contamination.
Always add your samples first and seal them before adding the serial dilutions of positive control (standard) at the end.

1 DNA extraction was performed using Qiagen DNeasy power water sterivex kit. The quality of the extracted DNA was estimated using Nanodrop.

Qiagen DNeasy power water sterivex protocol can be found in this link: https://www.qiagen.com/se/resources/resourcedetail?id=c5fe7d5f-070a-4ebe-ac04-4bbf05a13e91&lang=en

Primers

2 Stoeckle et al., 2016 designed primers targeting 16S rRNA gene of *Margartifera margaritifera.*
The sequence of the primer set is:
MarMa_16S2.1: 5′- GCAACACGGAAAACCCC TG -3′
MarMa_16S1.2: 5′- GGCT GCGCTCATGTGAATTA -3′.

3 The concentration of freshwater pearl mussel standard was measured by PicoGreen assay (http://tools.thermofisher.com/content/sfs/manuals/PicoGreen-dsDNA-protocol.pdf) before preparing standard serial dilutions.
Serial dilutions, 10, 1, 0.1, 0.01 and 0.001 ng, were prepared to generate standard curve in each qPCR run.
Unio pictorum or Äkta målarmussla was used to test the specificity of qPCR detection.

Plate setup

4 Setup and design your qPCR plate in the template of qPCR machine before preparing your qPCR run.
At least 3 or 4 replica of standard dilutions should be included in each run.
Three replica of each sample was performed.

PCR reaction

5 A master mix was prepared and calculated according to the number of standard dilutions, samples and negative controls to run.
The PCR mixture per sample was as follows:

	Reagent	working sol	final conc	µl
	SsoAdvanced Universal SYBR® Green Supermix	2x	1x	10

	MarMa_16SF primer	10 uM	0,25 uM	0,5
	MarMa_16SR primer	10 uM	0,25 uM	0,5
	DEPC-water			6
	DNA template			3
	∑			20

Sample or qPCR plate should be carefully sealed with no marking on the tube to facilitate plate reader step.

PCR program

6

	Amplification step	Time	Temp	
	Enzyme activation	15 sec	98 °C	
	Denaturation	10 sec	98 °C	40 cycles
	Annealing	10 sec	60 °C	
	Extension	20 sec	72 °C	
	Melt curve analysis		63 to 99°C	

7 QPCR was performed in BioRad qPCR machine CFX96.
Analysis of the results was done by CFX maestro software
https://www.bio-rad.com/en-se/product/cfx-maestro-software-for-cfx-real-time-pcr-instruments?ID=OKZP7E15

QPCR efficiency, limit of detection and quantification range of standard were checked for each run.
Results can be evaluated through data analysis windows where there is a quantification, standard melt curve and RFU spreadsheet.