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Detection of marbled crayfish *Procambarus fallax*

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Protocol status: Working
We use this protocol and it's working

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Disclaimer

Use at own risk!

Abstract

Taqman QPCR assay for marbled crayfish *Procambarus fallax*

Guidelines

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.

Materials

UltraPure™ DEPC-treated WaterThermo FisherCatalog #10813012
TaqMan™ Environmental Master Mix 2.0 https://www.thermofisher.com/order/catalog/product/4396838#/4396838
Interbal control http://www.primerdesign.co.uk/assets/files/internal_control_handbook_dna.pdf?timestamp=1504081027

Safety warnings

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.

DNA extraction

2h 30m

1 A tissue of marbled crayfish was extracted with DNeasy blood and tissue extraction kit **https://www.qiagen.com/us/shop/pcr/dneasy-blood-and-tissue-kit/**
The quality of DNA was checked by nanodrop.

2 Internal control:
http://www.primerdesign.co.uk/assets/files/internal_control_handbook_dna.pdf?timestamp=1469446474

3 Primers

	A	B	C	D	E	F
	Procambarus fallax	mtDNA-CO1	181 bp	Temp (C)	Length	GC(%)
	Profal_COI_F01	5'-AGTTGAGAGGGGAGTAGGAAC-3		56.5	21	52.4
	Profal_COI_R01	5'-AGTTATACCAGCTGCCCGTA-3'		57.4	20	50
	Profal_COI_P01	5'-FAM-AACTGTTTATCCTCCTTTAGCTTCTGC-BHQ1-3'		62.6	27	40.7

4 2 µL Standard dilution 30m

DNA of marbled crayfish was serially diluted from $1e^{2}$-$1e^{-4}$ for qPCR experiment.

5 PCR mixture 2h 30m

	A	B	C	D
		Stock solution	Working solution	Final concetration (µl)
	TaqMan Environmental Mastermix 2	2X	1X	10
	Forward primer	10 µM	0.4 µM	1
	Reverse primer	10 µM	0.4 µM	1
	TaqMan probe	2.5 µM	0.1 µM	1
	Internal control (IC) primer/probe mix			1
	IC-DNA			1

	A	B	C	D
	Water			8
	Template			2
	Total			25

2 µl of RNase/DNase free water was used for negative controls
PCR mixture can be lowered to 12 ul instead of 25 ul showed the same efficiency.

6 - °C Amplification conditions

	A	B	C	D
		Step	**Time**	**Temp (°C)**
		Preheat	5 min	50
		Enzyme activation	10 min	95
	50 cycles	Denaturation	30 s	95
		Extention and Data collection	1 min	60

7 qPCR was performed in BioRad qPCR machine CFX96.

Expected result

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=OKZP7E1502:30:00

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Expected result

Internal PCR control The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of DNA added to the PCR reaction and the individual machine settings. Cq values of 27±3 are within the normal range.
When amplifying sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

9 **Primer validation**

DNA of related crayfish species were tested to ensure primers specificity.

	A	B	C
	Related species	**Tested**	**Amplification**
	Faxonius rusticus	Yes	No
	Faxonius virilis	Yes	No
	Faxonius immunis	Yes	No
	Faxonius juvenilis	Yes	No
	Pontastacus leptodactylus	Yes	No
	Astacus astacus	Yes	No