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qPCR assay for detecting *Triturus cristatus*

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We use this protocol and it's working

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Disclaimer

Use at own risk!

Abstract

eDNA assay to detect great crested newt *Triturus cristatus*

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

UltraPure™ DEPC-treated WaterThermo FisherCatalog #10813012

SsoAdvanced Universal Probes SupermixBio-rad LaboratoriesCatalog #172-5280

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.

DNA extraction

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 kit: https://www.qiagen.com/se/resources/resourcedetail?id=c5fe7d5f-070a-4ebe-ac04-4bbf05a13e91&lang=en

2 **Internal control**

When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process.

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

3 Primers

	A	B	C	D
	Primer/probe	Sequence	Fragment	Gene
	TCCBL (fwd)	CGTAAACTACGGCTGACTAGTACGAA	81	cyt-b
	TCCBR (rev)	CCGATGTGTATGTAGATGCAAACA		
	TCCB.probe	CATCCACGCTAACGGAGCCTCGC		

4 **Standard dilution**

DNA of *Triturus cristatus* was serially diluted from $1e^{2}$-$1e^{-4}$ for qPCR experiment. *Triturus vulagaris* was also tested as related species.

5 PCR mixture

	A	B	C	D
		Stock solution	**Working solution**	**Final concetration (µl)**

	A	B	C	D
	TaqMan Environmental Mastermix 2	2X	1X	10
	Forward primer	10 µM	0.4 µM	0.8
	Reverse primer	10 µM	0.4 µM	0.8
	TaqMan probe	2.5 µM	0.1 µM	0.2
	Internal control (IC) primer/probe mix			1
	IC-DNA			0.5
	Water			3.7
	Template			3
	Total			20

3 µl of RNase/DNase free water was used for negative controls

6 Amplification conditions

	A	B	C	D
		Step	**Time**	**Temp (℃)**
		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

https://www.bio-rad.com/en-se/product/cfx-maestro-software-for-cfx-real-time-pcr-instruments?ID=OKZP7E15

8 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.