Nov 19, 2020

TaqMan qPCR assay for detecting Batrachochytrium dendrobatidis (Bd)

DOI
https://dx.doi.org/10.17504/protocols.io.bn2zmgf6

Omneya Ahmed Osman[1], Mats Töpel[1], Tomas Larsson[1], Alexander Eiler[1]

[1]eDNA solutions

Omneya Osman
IVL Swedish research Institute

DOI: https://dx.doi.org/10.17504/protocols.io.bn2zmgf6

Protocol Citation: Omneya Ahmed Osman, Mats Töpel, Tomas Larsson, Alexander Eiler 2020. TaqMan qPCR assay for detecting Batrachochytrium dendrobatidis (Bd). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bn2zmgf6

Manuscript citation:
Boyle DG et al. (2004). Rapid quantitative detection of chytridiomycosis (Batrachochytrium dendrobatidis) in amphibian samples using real-time Taqman PCR assay. Dis Aquat Org 60133–139.

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

Created: October 27, 2020

Last Modified: November 19, 2020

Protocol Integer ID: 43833

Keywords: BD, qPCR, TaqMan probe, detecting batrachochytrium dendrobatidi, fungus batrachochytrium dendrobatidi, batrachochytrium dendrobatidi, details of real time pcr assay, real time pcr assay, species in the country, listed species, water sample

Abstract

The fungus Batrachochytrium dendrobatidis (Bd) was first detected in Norway in 2017, and thus indicate the arrival of an invasive black-listed species in the country. Here we report the details of real time PCR assay which was used to screen for B. dendrobatidis from water samples collected from different locations in Norway.

Guidelines

Laboratory work space and equipment were sterilised 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 Water CATALOG NUMBER**10813012**

SsoAdvanced Universal Probes SupermixBio-rad LaboratoriesCatalog #172-5280

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

qPCR strip or qPCR plate

BioRad qPCR machine CFX96

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

3h 30m

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

3h 30m

Real time PCR

2 A primer set of ITS1-3 Chytr (5′- CCTTGATATAATACAGTGTGCCATATGTC-3),
5.8S Chytr (5′- AGCCAAGAGATCCGTTGTCAAA-3′) and
probe Chytr MGB2 (FAM-5′ TTCGGGACGACCC-3′-NFQ- MGB) (Boyel *et al.* 2004).

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

SsoAdvanced Universal Probes Supermix: https://www.bio-rad.com/webroot/web/pdf/lsr/literature/bulletin-10023650.pdf

2.1 A serial dilution of 100, 50, 25, 10, 1, 0.1 and 0.01 genomic equivalents (GE) of the DNA extract of Bd standard was tested and 5 dilutions (100, 25, 10, 1, 0.1 GE) were used for the qPCR assay.
Pipette 5 µl of DNA template to reach 20 µl final volume in each well.

For negative controls use 5 µl of RNase/DNase free water.

30m

Real time PCR

2h

2.2 PCR master mix without internal control (IC)

1h 30m

	Reagent	Working sol	Final conc	Volume (µL)

	SsoAdvanced Universal Probes Supermix	2x	1x	10
	ITS1-3 Chytr	10 uM	0,9 µM	1,8
	5.8S Chytr	10 uM	0,9 µM	1,8
	TaqMan probe	10 uM	0,25 µM	0,5
	DEPC-water			0,9
	template			5
	Σ			20

* Fluorogenic data should be collected during this step through the FAM channel.

2.3 PCR master mix with IC

1h 30m

		Working solution	Final concentration	Volume (µL)
	SsoAdvanced Universal Probes Supermix	2x	1x	12,5
	ITS1-3 Chytr	10 uM	0,9 µM	2,250
	5.8S Chytr	10 uM	0,9 µM	2,250
	TaqMan probe	10 uM	0,25 µM	0,625
	IC primer/probe			1
	IC-DNA**			0,5
	DEPC-water			0,875
	template			5
	Σ			25

* Fluorogenic data should be collected during this step through the VIC channel to detect IC and FAM to detect TaqMan probe.
** IC- DNA was diluted (1:20 dilution) before usage in the master mix.

PCR program

2h

3

2h

		Step	Time	Temp (C)
		Enzyme activation	2 min	95
	Cycling x50 (step3 & 4)	Denaturation	10 sec	95
		Extension and Data collection *	1min	60

qPCR instrument

15m

4 QPCR was performed on BioRad qPCR machine CFX96.

15m

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

5 **Quantification cycle (Cq)**

Positive control of 100 genome equivalent is expected to give a signal at Cq value 27.

In case of internal control, Cq values of 27±3 are within the normal range. A control sample with IC and water should be evaluated at each qPCR run.