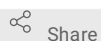


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# qPCR assay for detecting round goby invasive fish species *Neogobius melanostomus*

Omneya Ahmed<sup>1</sup>, Alexander Eiler<sup>1</sup><sup>1</sup>eDNA solutions

1 Works for me



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dx.doi.org/10.17504/protocols.io.bq3umynw

eDNA solutions

Tech. support phone: +0700264843 email: omneya@ednasolutions.se



Omneya Ahmed Osman

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## ABSTRACT

Members of Ponto-Caspian gobies are predatory fish which have colonized freshwaters and brackish waters in Europe and North America causing a lot changes in the native ecosystems.

Several eDNA assays were developed to identify round goby fish. We are going to describe two successful assays.

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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 TEXT

UltraPure<sup>®</sup>; DEPC-treated Water Thermo Fisher Catalog #10813012

TaqMan<sup>™</sup> Environmental Master Mix 2.0 <https://www.thermofisher.com/order/catalog/product/4396838#/4396838>

Interbal control [http://www.primersdesign.co.uk/assets/files/internal\\_control\\_handbook\\_dna.pdf?timestamp=1504081027](http://www.primersdesign.co.uk/assets/files/internal_control_handbook_dna.pdf?timestamp=1504081027)

SAFETY WARNINGS

Negative controls of DNase/RNase free water in each qPCR assay.

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BEFORE STARTING

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.

DNA extraction

2h 30m

1

A tissue of round goby was extracted with Qiagen DNeasy blood and tissue extraction kit

<https://www.qiagen.com/us/shop/pcr/dneasy-blood-and-tissue-kit/>

The quality of DNA and 260/280 ratio were checked by NanoDrop instrument for nucleic acid measurements.

2

To check for inhibitors in DNA samples, internal control was used,

[http://www.primersdesign.co.uk/assets/files/internal\\_control\\_handbook\\_dna.pdf?timestamp=1469446474](http://www.primersdesign.co.uk/assets/files/internal_control_handbook_dna.pdf?timestamp=1469446474)

OR: <https://www.thermofisher.com/order/catalog/product/4308321>.

3

Primers

| A                             | B                                    | C  | D           | E      | F          |
|-------------------------------|--------------------------------------|--|-------------|--------|------------|
| <i>Neogobius melanostomus</i> | Primer                               | Reference                                  | Temperature | Length | GC content |
| Neo_Mel_COI_F01               | 5'-CTTCTRGCTCCTCTGGWGTG-3'           | Andersen et al., 2016                      | 59.6 -62.8  | 22     | 54-59      |
| Neo_Mel_COI_R01               | 5'-CCCWAGAATTGASGARATKCCGG-3'        |  | 58.9 -63.9  | 23     | 47-56      |
| Neo_Mel_COI_P01               | 5'-FAM-CAGGCAACTTRGCACATGCAG-BHQ-3'  |  | 60.1 - 62.9 | 21     | 52-57      |
| NeoMel_IK_F1                  | 5'- TATGTGATGATCGGACAGC-3'           | Adrian-Kalchhauser & Burkhardt-Holm, 2016. |             | 19     | 53-56      |
| NeoMel_IK_R1                  | 5'- GTTCTCTAGTCAGCTCGCT-3'           |  |             | 19     | 45-51      |
| NeoMel_IK_Probe               | 5'-FAM-CATCTTTCTCGGCTTATCCCCA-BHQ-3' |  |             | 23     |            |

#### 4 2 µl Standard DNA dilution

30m

To determine both limit of quantification and limit of detection, genomic DNA of round goby was serially diluted from  $1e^2$ - $1e^{-4}$ .

#### 5 The composition of PCR mixture

1h 30m

| A                                      | B              | C                | D                        |
|--|----------------|------------------|--------------------------|
| PCR reagent                            | Stock solution | Working solution | Final concentration (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                                 |                |                  | 0.5                      |
| DNase/RNase free water                 |                |                  | 8.5                      |
| Template                               |                |                  | 2                        |
| Total volume                           |                |                  | 25                       |

RNase/DNase free water was used as a negative control. Lower volume of PCR mixture was tested and efficiency was evaluated.

#### 6 °C Amplification conditions

| A         | B                 | C           | D           |
|-----------|-------------------|-------------|-------------|
|           | <b>Step</b>       | <b>Time</b> | <b>Temp</b> |
| 50 cycles | Enzyme activation | 10 min      | 95          |
|           | Denaturation      | 15 s        | 95          |
|           | Data collection   | 1 min       | 60          |

Enzyme activation temperature depends on the type of qPCR master mixture.

7 QPCR was performed in BioRad qPCR machine CFX96.



Analysis of qPCR results was performed through CFX maestro software

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

8



The analysis of PCR internal 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 26 or 27±3 are within the normal range.