Sep 05, 2024

# Probe qPCR assay for Charybdis japonicum

This protocol is a draft, published without a DOI.

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**External link: https://www.biosecurity-toolbox.org.nz/**

**Protocol Citation:** Jackson Treece, Gert-Jan Jeunen, Jo-Ann Stanton, Neil Gemmell 2024. Probe qPCR assay for Charybdis japonicum . **protocols.io https://protocols.io/view/probe-qpcr-assay-for-charybdis-japonicum-dki64uhe**



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

**Created:** September 02, 2024

**Last Modified:** September 05, 2024

**Protocol Integer ID:** 106814

**Keywords:** charybdis, charybdis japonicum, invasive crustacean, invasive crab, charybdis qpcr, charybdis japonicum qpcr, biosecurity toolbox, marine biosecurity, marine biosecurity toolbox

**Funders Acknowledgement:**
**New Zealand Ministry for Business, Innovation and Employment**
**Grant ID: CAWX1904**

## Abstract

*Charybdis japonica* or the Asian Paddle Crab is a non-native crab species to Aotearoa, New Zealand. It was first detected in 2000 and has since spread along the eastern Northland coast where it is a threat to local native species. This test was developed to aid in detecting the invasive crustacean's presence using environmental DNA sampling.
The probe based qPCR assay targets a 208bp portion of the COX-1 gene with an indicative limit of detection of 1.0E-4 ng/µl genomic DNA input or 92 molecules/µl with a synthetic standard (IDT gBlock). Species specificity was successfully challenged against a panel of 14 New Zealand native crab species. Further challenge with local species is recommended.

## Materials

1 Meridian Bioscience SensiFAST Probe Lo-ROX Mix (2x)
PCR quality water
Template DNA
Oligonucleotides (10uM concentration):
Forward primer: Cj_1295F ATGGCATGCCTCGTCGTTAT
Reverse primer: Cj_1498R CTGCAGGTGGGTAAGAGTGG
FAM Probe: Cj_1323P CCCCGATGCATACACTACGT

## Master Mix

2

10.0 µl SensiFAST Probe 2x Mix
0.8 µl 10µM Forward primer
0.8 µl 10µM Reverse primer
0.2 µl 10µM FAM Probe
6.2 µl PCR Water
2.0 µl Template
**20µl total reaction volume**

## Thermal Conditions

3 95°C - 3 minutes
**95°C - 15 seconds**
**60°C - 30 seconds x45 cycles**

QuantStudio 3 (ThermoFisher Scientific) used during assay development.

## Control Sequence

4 Sythesized positive control sequence (IDT gBlocks)

tattactgccattcttctactcctctcgcttcctgtgttagctggagctattactatattattaacagaccgaaacttaaatacttcattct
tcgaccctgctgggggtggggatcctgttctttatcaacacttgttttgattctttggacatcctgaagtttatattttaatcttacctgcc
ttcggaataatttcacatattgtcagacaagaatctggtaaaaaggaatcatttggtactttgggtataatttatgctataatggctatt
ggcattttaggctttattgtatgagctcatcacatgtttacagttggaatagatgtagatactcgagcatattttacatcagctactata
attattgctgtgcctactggaattaaaatttttagatgacttagaactcttcacggcactcaaattaactatagaccttctatattatgg
gccctaggatttatttttcttattcacagtaggaggtcttacaggagtagttttagcaaactcttccattgatattattcttcacgacacat
actatgtggtagcccactttcattacgttctttctataggagctgtatttggaattttcgccggcattgcccactgattccccttatttac
cggactttcattaaatcctaaatgaataaaaattcattttactattatatttattggtgtaaatattactttctttcctcaacacttttttagga
ctaaatggcatgcctcgtcgttattctgactaccccgatgcatacactacgtgaaatattgtctcttctataggatcaatagtatcact

tattgctatactaatttttataattgtaatttgagaagcacttatctcaaatcgacctgttctattttctccttccttccttcttctattgaat
gaaaccactcttacccacctgcagatcactcatacatagaaatccccctaatctctaacttctaaaatggcagatagatgtatagg

## Species for Assay Development and Specificity Testing

5 Many thanks to the National Institute of Water and Atmospheric Research (NIWA), the Marine Invasives Taxonomic Service (MITS) and Deakin University for contributions from their invertebrate tissue collection. Donation ID: 2023-032 D

| | Species | Accession |
|---|---|---|
| | Austrohelices crassa | MITS_74324 |
| | Carcinus maenas | N/A (Deakin) |
| | Charybdis japonica | MITS_70207 |
| | Elamena producta | NIWA_89937 |
| | Halicarcinus cookii | NIWA_81032 |
| | Halicarcinus whitei | NIWA_147705 |
| | Heterozius rotundifrons | NIWA_163347 |
| | Liocarcinus corrugatus | NIWA_73086 |
| | Nectocarcinus antarcticus | NIWA_74132 |
| | Notomithrax minor | NIWA_92580 |
| | Notomithrax peronii | NIWA_72993 |
| | Ovalipes catharus | NIWA_60579 |
| | Pilumnus lumpinus | NIWA_75667 |
| | Pilumnus novaezealandiae | NIWA_92423 |
| | Pinnotheres atrinicola | NIWA_74906 |