May 20, 2024

Cobia PCR of sex-specific markers

GigaScience

DOI
dx.doi.org/10.17504/protocols.io.4r3l2q7r3l1y/v1

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GigaScience Press

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.4r3l2q7r3l1y/v1

External link: https://doi.org/10.1093/gigascience/giae034

Protocol Citation: Zhi Weng Josiah Poon 2024. Cobia PCR of sex-specific markers. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.4r3l2q7r3l1y/v1**

Manuscript citation:
Shen X, Hu J, Yáñez JM, Bastos Gomes G, Poon ZWJ, Foster D, Alarcon JF, Shao L, Guo X, Shao Y, Huerlimann R, Li C, Goulden E, Anderson K, Fan G, Domingos JA. Exploring the cobia (Rachycentron canadum) genome: unveiling putative male heterogametic regions and identification of sex-specific markers. Gigascience. 2024 Jan 2;13:giae034. doi: 10.1093/gigascience/giae034.

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

Created: May 18, 2024

Last Modified: May 20, 2024

Protocol Integer ID: 100091

Keywords: cobia pcr of sex, specific markers pcr for sex, specific markers in cobia, cobia pcr, specific markers pcr, cobia, rachycentron canadum, specific marker, pcr

Abstract

PCR for sex-specific markers in Cobia (*Rachycentron canadum*)

Troubleshooting

Panama population (Taq PCR Core Kit (QIAGEN))

1 (c*ephx1_1*) and (c*ephx1_2*)
20 µL reaction containing:
- 2.08 µL of 10X Taq Buffer
- 0.42 µl of dNTPs (10 µM)
- 0.67 µL of each primer (10 µM)
- 0.17 µL of Taq DNA polymerase (5 units/µL)
- 30 ng of extracted DNA
- made up to final volume with nuclease-free water

Thermal cycling:
- 3 mins at 94°C
- 30 cycles of 1 min at 94°C, 1 min at 60°C (c*ephx1_1*) and 61°C (c*ephx1_2*), and 1 min at 72°C
- 10 mins at 72°C

Brazil population (Taq PCR Core Kit (QIAGEN))

2 (c*ephx1_1*)
20 µL reaction containing:
- 2.08 µL of 10X Taq Buffer
- 0.42 µl of dNTPs (10 µM)
- 0.67 µL of each primer (10 µM)
- 0.17 µL of Taq DNA polymerase (5 units/µL)
- 35 ng of extracted DNA
- made up to final volume with nuclease-free water

Thermal cycling:
- 3 mins at 94°C
- 30 cycles of 1 min at 94°C, 1 min at 66°C (c*ephx1_1*), and 1 min at 72°C
- 10 min at 72°C

(c*ephx1_2*)
20 µL reaction containing:
- 2.08 µL of 10X Taq Buffer
- 0.42 µl of dNTPs (10 µM)
- 0.67 µL of each primer (10 µM)
- 0.17 µL of Taq DNA polymerase (5 units/µL)

- 20 ng of extracted DNA
- made up to final volume with nuclease-free water

Thermal cycling:
- 3 mins at 94°C
- 20 cycles of 1 min at 94°C, 1 min at 65°C (c*ephx1_2*), and 1 min at 72°C
- 10 mins at 72°C

Australia population (PlatinumTM Taq DNA Polymerase High Fidelity (Invitrogen))

3 20µL reaction containing:
- 2 µL of 10X Buffer
- 0.4 µL of dNTPs (10 µM)
- 0.8 µl of MgSO4
- 0.4 µL of each primer (10 µM)
- 0.08 µL of Taq DNA polymerase
(5 units/µL)
- 32 ng of extracted DNA
- made up to final volume with nuclease-free water

Thermal cycling:
- 2 mins at 94°C
- 30 cycles of 15 secs at 94°C, 30 secs at 62°C (c*ephx1_1*) and 62°C (c*ephx1_2*), and 1 min at 72°C
- 10 mins at 72°C

Japan population (Q5 High-Fidelity 2X master Mix)

4 25µL reaction containing:
- 12.5 µL of Q5
- 1 µL of each primer (10 µM)
- 15 ng of extracted DNA
- made up to final volume with nuclease-free water

Thermal cycling:
- 30 secs at 98°C
- 30 cycles of 10 secs at 98°C, 30 secs at 61°C (c*ephx1_1* and c*ephx1_2*), and 30 secs at 72°C
- 2 mins at 72°C