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Cobia PCR of sex-specific markers

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

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Abstract

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

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