Nov 26, 2018

Polymerase Chain Reaction (PCR) - DNA barcoding

DOI
dx.doi.org/10.17504/protocols.io.vwae7ae

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Protocol Citation: Karen Guimaraes 2018. Polymerase Chain Reaction (PCR) - DNA barcoding. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.vwae7ae**

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

Created: November 26, 2018

Last Modified: November 26, 2018

Protocol Integer ID: 18082

Guidelines

PCR conditions: 95°C (2min), 35 cycles of 94°C (30sec), 54°C (30sec) and 72°C (1min), followed by 72°C (10min).

Materials

Sterile H2O; dNTP mix (1.25 mM); buffer 10X (200 mM Tris-HCl (pH = 8,4) + 500 mM KCl); MgCl2 (50 mM); Primer Fish F1 and Fish R1 (5µM); Taq DNA polymerase.

Before start

The reactions in 25 µL final volume.

1. Add 15 µL sterile H2O, 2.8 µL dNTP mix (1.25 mM);
2. Add 2.5 µL buffer 10X (200 mM Tris-HCl (pH = 8,4) + 500 mM KCl);
3. Add 2.5 µL de MgCl2 (50 mM);
4. Add 0.5 µL of each primer (5µM);
5. Add 0.2 µL Taq DNA polymerase (5U/µL) and 1 µL of genomic DNA (100ng/µL)