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## Polymerase Chain Reaction (PCR) - DNA barcoding

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**Protocol status:** Working

**We use this protocol and it's working**

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### 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 H<sub>2</sub>O; dNTP mix (1.25 mM); buffer 10X (200 mM Tris-HCl (pH = 8,4) + 500 mM KCl); MgCl<sub>2</sub> (50 mM); Primer Fish F1 and Fish R1 (5μM); Taq DNA polymerase.



## Before start

The reactions in 25  $\mu$ L final volume.

1. Add 15  $\mu$ L sterile H<sub>2</sub>O, 2.8  $\mu$ L dNTP mix (1.25 mM);
2. Add 2.5  $\mu$ L buffer 10X (200 mM Tris-HCl (pH = 8,4) + 500 mM KCl);
3. Add 2.5  $\mu$ L de MgCl<sub>2</sub> (50 mM);
4. Add 0.5  $\mu$ L of each primer (5 $\mu$ M);
5. Add 0.2  $\mu$ L Taq DNA polymerase (5U/ $\mu$ L) and 1  $\mu$ L of genomic DNA (100ng/ $\mu$ L)

