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Metabarcoding PCR Protocol

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Protocol status: Working

We use this protocol and it's working

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Abstract

18S Metabarcoding PCR Protocol using Invitrogen Platinum Hot Start PCR 2X Master Mix (REF 13000014) buffer.



Preparing the PCR tubes

- 1 Label PCR tubes and place them under a UV light for  00:10:00 .

10m

Preparing the master mix

- 2 Add  10 μL of **Invitrogen Platinum Hot Start PCR 2X Master Mix (REF 13000014)** **buffer** and  8 μL of **molecular water** to a 1.5 mL tube PER NUMBER OF SAMPLES *i.e.*, *for 4 samples, add 40 μL of buffer and 32 μL of molecular water to a 1.5 mL tube.*
- 3 Mix well using a vortex, then centrifuge.

Metabarcoding PCR

- 4 Transfer  18 μL of the **master mix** to each PCR tube.
 - 5 Transfer  5 μL of the **DNA template** to each PCR tube.
 - 6 Transfer  2 μL of the **Metabarcoding Primer** to the assigned PCR tube. Immediately place primers on a PCR Cooler Rack after use.
 - 7 Mix the samples well using a vortex, then briefly centrifuge them using a microcentrifuge or platefuge to ensure all contents are at the bottom.
 - 8 Place samples in a thermo cycler and set to a PCR protocol. Set the thermo cycler to use a heated lid and wait until the lid and block are close to the temperature. See 8.1 for thermo cycler specifications.
Tip: In the Bik Lab, select protocol "METAB-HS" on thermo cycler.
- 8.1 METAB-HS Protocol:
Lid: 105°C
Volume: 25 μL
1. 94°C, 3:00
2. 94°C, 0:45
3. 50°C, 1:00



- 4. 72°C, 1:30
- 5. GOTO step 2, 34X
- 6. 72°C, 10:00
- 7. 4°C, ∞

9 Close the thermo cycler lid well.