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Metabarcoding PCR Protocol

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Mirayana Marcelino Barros[1], Tiago Pereira[2], Alejandro De Santiago Perez[1], Hunter Powell[3], Jenna Brown[4]

[1]University of Georgia; [2]Department of Marine Sciences, University of Georgia; [3]UGA; [4]University of Georgia- Holly Bik

Jenna Brown
University of Georgia- Holly Bik

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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 uL of buffer and 32 uL 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.