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We use this protocol and it's working

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DNA extraction from insect gut-dwelling fungi

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ABSTRACT

This protocol is good for DNA extraction from microbial fungi isolated from aquatic insect guts. It works for small input tissues (starting from one fungal thallus). The product can be used for PCR and Sanger sequencing directly, which is ideal for fungal barcode analyses. This protocol is modified from a method described in Gottlieb and Lichtwardt 2001 (Mycologia Vol. 93, No. 1, pp. 66-81).

1 Suspend fungal thalli and spores in a 1.5 mL centrifuge tube with 2x CTAB buffer (200 µL).

2 Freeze and thaw the sample three times by submerging the tube into liquid nitrogen and incubating it at 65 °C using a heat block.

3 After the final thaw, crush the fungal tissues using a disposable pellet pestle (for 1.5 mL microtube).

4 Cool the sample for 00:01:00 On ice . 1m

5 Add 4 µL Proteinase K [M] 20 mg/mL to the tube and mix them by inverting the tube three times.

6 Warm the tube at 65 °C for 00:30:00 using a heat block. 30m

7 Centrifuge at 12000 x g, Room temperature, 00:02:00 and transfer the supernatant to a new tube. 2m

8 Add Chloroform: Isoamyl alcohol (24:1) with an equal volume of the supernatant.

9 Shake the mixture slowly for 00:10:00 on a rotator and leave the tube on the rack for 00:02:00 at room temperature. 12m

10 Centrifuge at 13000 x g, Room temperature, 00:04:00 and transfer the top layer to another tube with wide-bore tips, avoiding breaking DNAs. 4m

11 Transfer the supernatant to a new tube and repeat steps 8-10.

12 Add 4 µL RNase A ([M] 10 mg/mL) and incubate at 37 °C for 00:40:00 . 40m

13 Add **Isopropanol (**stored at -20 °C) with a 66% volume of the product from the last step and mix by inversion.

14 Leave the tube at 4 °C for at least 05:00:00 (or overnight) until fully precipitated. 5h

15 Centrifuge at 13000 x g, Room temperature, 00:10:00 with the cap opening side pointing to the centrifuge center. 10m

16 Immediately dump the supernatant and transfer the tube (with the cap open & upside down) on the paper towel to absorb the remaining supernatant.

17 Leave the cap open on rack.

18 Add 150 µL 70% ethanol (stored at -20 °C) to the tube.

19 Cap it and use the finger to tap the tube gently.

20 Centrifuge at 13000 x g, Room temperature, 00:01:00. 1m

21 Repeat step 16.

22 Leave the tube at 37 °C with the cap open and a piece of Kimwipe paper on top for 00:30:00 or until the DNA becomes dry. 30m

23 Add 50 µL H_2O to the dried DNA in the tube and wait for 00:10:00. 10m

24 Pipette and store the DNA in the tube at -20 °C.