Version 2

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DNA extraction protocol (Salting out) Modified V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bpgkmjuw

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes how to extract DNA with modified salting out method.

This protocol is part of the Bark Beetle Mycobiome (BBM) Research Coordination Network. For more information on the BBM international network: Hulcr J, Barnes I, De Beer ZW, Duong TA, Gazis R, Johnson AJ, Jusino MA, Kasson MT, Li Y, Lynch S, Mayers C, Musvuugwa T, Roets F, Seltmann KC, Six D, Vanderpool D, & Villari C. 2020. Bark beetle mycobiome: collaboratively defined research priorities on a widespread insect-fungus symbiosis. Symbiosis 81: 101–113 https://doi.org/10.1007/s13199-020-00686-9.

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Modified Salting Out - mycelium only

Modified from MU Patwary, EL Kenchington, CJ Bird, E Zouros. 1994. The use of random amplified polymorphic DNA markers in genetic studies of the sea scallop Placopecten magellanicus (Gmelin, 1791). Journal of Shellfish Research 13(2): 547-553.

1st Day

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1. Preheat the water bath at **55°C**
2. Prepare **Lysis Buffer** as following (For 1 Reaction)
    a. Add 140 µl of the **extraction buffer** (Standard extraction buffer)
    b. Add 17.5 µl of **SDS 10%**
    c. Add 2 µl of **proteinase K** (20 mg/ml)
3. Add **~50-100 mg** of mycelium in a 1.5 mL centrifuge tube (Use less amount if it has pigments)
4. Freeze it using liquid N_2 and crush it with a sterilized micro pestle
5. Add **159.5 µl** of Lysis Buffer as soon as possible and mix it by pipetting
6. Repeat step 2 to all tubes (If you extract more than one tube)
7. Incubate at **55°C** overnight

2nd Day

8. Turn on and cool centrifuge to **4°C**
9. Preheat incubator or hot plate to **37°C**
10. Add 2 µl of **RNase A** (10 mg/ml) and leave it act for 10 minutes at 37°C (15 minutes at RT)
11. Add 40 µl of a **saturated solution of NaCl** in water (>6M, autoclaved)
12. **Vortex** for 20 min
13. **Centrifuge** at 14000 rpm for 30 min
14. Transfer the **supernatant** to a new tube
15. Add one volume (200 µl or more if needed) of **chilled isopropanol**
16. **Mix** it by flipping the tube or vortex 5 seconds
17. Keep in the **freezer or ice** for 10 minutes
18. Precipitate the DNA by **centrifugation** at 14000 rpm at 4°C for 20 min
    a. Be sure to orient tubes so that you know where the pellet will be
    b. Never vortex from this point on
19. **Discard** the supernatant by pouring it out with one single movement, without disturbing the pellet. Always pour from the side of the vial opposite to the pellet. Do not turn over the vial again until dry.
    a. Leave it to dry 10 min
    b. Dry by leaving upside down on paper towel with cap held down, tap out excess liquid
20. **Wash** the pellet with 500 µl of 70% EtOH
21. **Centrifuge** at 14000 rpm at 4°C for 10 min
22. **Discard** the supernatant
    a. Be quick in discarding and ensure that the tube is oriented such that the pellet will not dislodge
23. **Dry** the pellet in the vacuum
    a. Start with small increments of time (3 min) and keep going until dry. Over-drying will make re-suspension difficult.

Alternatively, dry the pellet in a 37°C drying oven.

24. Add 20 to 40 µl of **sterile H_2O (PCR water)** to the DNA
25. **Let it stay** at 4^{O}C (Refrigerator) for 20-30 minutes and mix well by tapping the tubes. Centrifuge briefly before storing in the freezer.

Buffers and solution ingredients/concentrations

- Extraction buffer for Standard method: 0.1 M EDTA, 0.05 M, Tris pH 8. Autoclave before use.
- Saturated NaCl: greater than 6M solution with visible salt still not in solution. Autoclave before use.
- Store 70% EtOH and isopropanol in freezer

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