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Laurel Wilt / *Raffaelea lauricola* screening from wood pieces with QIAGEN – QIAamp Fast DNA Stool Mini Kit

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

Protocols Bark Beetle Mycobiome

Bark Beetle Mycobiome Research Coordination Network

ABSTRACT

This protocol describes DNA extraction of wood samples for the detection of R. lauricola.

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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1.Have ready autoclaved 2 ml screw-cap tubes (purple lids) filled with 4-5 zirconia beads.

2.Put the wood pieces(sawdust, slivers, chunks) inside the tubes. Fill 60-70mg of Sawdust tissue. *Overfilling on this step will cause

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issues in your lysate transfer*

3.Label tubes on the cap and on the side with sharpie.

4.Set Heat Block to 95°C.

5.Add 1 ml of InhibitEx buffer. Change tips for each sample because of bubbles.

6.**Homogenize the samples**. 2 cycles, 30 secs (be sure tubes are close tightly).

7.Incubate sample for 10 min at 95°C. Once completed, turn off heat block to prepare for the second incubation period.

8.Centrifuge the tubes for 1 minute @ 8,000 rpm.

9.Add 25 ul of proteinase K (green cap) vortex lightly or mix well and incubate overnight at 56°C.

10.**Homogenize the samples**. 2 cycles, 30 secs (be sure tubes are close tightly).

11.Centrifuge the tubes for 2 minutes @ 8,000 rpm.

12.If there is still wood debris floating, centrifuge again for 2 min @ 8,000 rpm.

13.Pipet ~300 - 600 ul of the supernatant and add it to a **new tube** (1.5 ml Eppendorf). Do not transfer any particles but do transfer all liquid solution.

14.Add 200ul of AL buffer (lysis) to the new Eppendorf tube and vortex or mix samples well by hand.

15.Incubate at 70°C for 10 min.

16.Add 600 ul of EtOH (96-100%) to the same Eppendorf tube and vortex.

17.Add this liquid solution from the Eppendorf tube to the column (QIAamp Mini Spin Column), centrifuge for 1 minute @ 14,000 rpm and discard flow-through (use a beaker to dispose the liquid).

18.Repeat step 17 until all of the lysate has been transferred to the column. Remember to dispose of the flow-through after each centrifuge run.

19.Set the heat block to 70°C. Place the ATE buffer in the heat block, so it is ready for step 23.

20.Add 500 ul of the buffer AW1 to the column, centrifuge for 1 minute @ 14,000 rpm, discard flow-through.

21.Add 500 ul of the buffer AW2 to the column, centrifuge for 3 minute @ 14,000 rpm, discard flow-through.

22.Place column in a new, labeled, 1.5 ml Eppendorf tube (DNA will be stored in this tube). Label the top and sides of the Eppendorf tube with a sharpie.

23.Pipet directly 50 ul of the **HOT** buffer ATE onto the column membrane, let it stand for 5 min, centrifuge for 1 minute @ 14,000 rpm.

24.Repeat step 23, pipet directly 50 ul of the **HOT** buffer ATE onto the column membrane, let it stand for 5 min, centrifuge for 1 minute @ 14,000 rpm.

25.You should have 100ul of DNA in a labeled Eppendorf tube.

26.Store DNA at -20 °C in a box with the type of DNA stored, date of storage and any important information for the project supervisor and other lab members working on the project.

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