Protocol: Non-destructive DNA extraction from Psyllids

This protocol is used to extract DNA from psyllids by piercing them with a pin, thereby retaining a voucher specimen (exoskeleton) after extraction. Psyllids were stored in 95% Ethanol and 5% glycerol beforehand. This protocol uses the QIAGEN DNeasy Blood & Tissue kit and was modified from Percy (2003) Evolution: 57, 2540-56. This protocol was produced as part of the Scottish Government CRF Psyllid Project and the POnTE project. Contact Jennifer Sjölund (jennifer.sjolund@sasa.gsi.gov.uk) for more information.

Before starting

- Prepare kit buffers according to manufacturer's instructions.

Psyllid sample preparation

1. Remove psyllid from tube, blot on paper.
2. Place on top of a piece of mounting material, and under a dissecting microscope.
3. Pierce the psyllid using a 0.1 mm pin in two places; through the abdomen, and partly through the top of the thorax.
4. Place in a 1.5 ml/2 ml safe-lock microcentrifuge tube

DNA extraction: Day 1

5. Add 180 µl buffer ATL and 20 µl Proteinase K to each sample.
6. Vortex and spin down.
7. Incubate overnight at 56 °C in a slow shaking heat block.

DNA extraction: Day 2

8. Vortex for 15 s and spin down.
9. Add 200 µl buffer AL. Vortex and spin down.
10. Incubate at 56 °C for 10 min.
11. Add 200 µl Ethanol (96-100 %). Vortex and spin down.
12. Transfer liquid to spin column placed in a 2 ml collection tube, leaving the psyllid specimen at the bottom of tube. Centrifuge at 8000 rpm for 1 min.
    - *Store remaining psyllid voucher specimen in 95% ethanol and 5% glycerol.*
13. Discard flow through and collection tube. Place in a new collection tube.
14. Add 500 µl buffer AW1. Centrifuge for 1 min at 8000 rpm.
15. Discard flow through and collection tube. Place in a new collection tube.
16. Add 500 µl buffer AW2. Centrifuge for 3 min at 14000 rpm.
17. Discard flow through and collection tube. Place in a 1.5 ml microcentrifuge tube.
18. Add 100 µl of buffer AE. Incubate for 1 min at room temperature. Centrifuge for 1 min at 8000 rpm.

Project: Pest Organisms Threatening Europe (POnTE)
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