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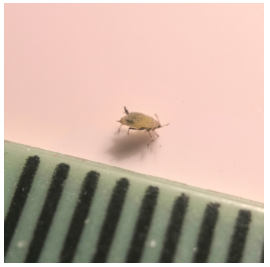
High quality DNA extraction from very small individual insects



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We use this protocol and it's working

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Abstract

Genomic studies of natural populations frequently benefit by having data from single individuals. This approach has a number of advantages: it avoids the risk of accidently pooling together individuals of closely related species, and it enables better resolution of haplotypes by separating non-identical individuals. However, especially in the case of very small invertebrates, recovering sufficient nucleic acid from individual samples can be challenging.

Here we present a method suitable for extraction of DNA from single individual aphids (weighing approximately 0.5mg). We are able to recover 100's of ng of high-quality DNA suitable for whole-genome sequencing using short-read technologies. DNA extraction using Cetrimonium bromide (CTAB) is a tried and tested method, and the protocol presented here is adapted from that of Marzachì et al. (1998). We present some modifications to optimize this popular method to maximize yield and quality from small single insect samples.

We have used this method across a range of aphid species, and also other sap-sucking hemipteran insects. It has enabled sequencing and de-novo assembly of a range of species from single individuals collected from the field, and also resequencing of individuals of the model aphid *Myzus persicae*. This is compatible with the method of ambient storage of dehydrated filed collected samples we describe in Wouters et al. (2020).

Marzachì, C, Veratti, F, & Bosco, D (1998). Direct PCR detection of phytoplasmas in experimentally infected insects. *Annals of Applied Biology*, *133*(1), 45-54.

Wouters, R; Mugford S, & Hogenhout, S (2020). Ambient sample storage system of field-collected insect samples for genomics.
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Guidelines

Note: For the preparation of high-molecular weight DNA it is necessary to take some precautions to avoid shearing the DNA. Cut the end from the pipette tips when pipetting DNA, the wider orifice will prevent shearing. Mix by gentle shaking and inversion, and not by vortexing. Avoid repeated freeze-thaw cycles. DNA can be stored at 4 degrees for several weeks.

Materials

CTAB buffer:
2% w/v CTAB (Cetrimonium bromide),
1.4 M NaCl,
20mM EDTA,
100 mM Tris-HCl pH 8.0,
and added fresh before use: 0.2 mg/ml RNAse (DNAse-free, 100mg/ml stock).

Isopropanol (2-Propanol) precooled to -20 degrees C.

70% Ethanol.

10mM Tris pH 7.5,
or TE buffer:
10mM Tris-HCl pH 7.5, 1mM EDTA

Plastic Pestles- for example Fisher Cat# 12-141-368

Dry ice and/or liquid nitrogen

Safety warnings

Liquid nitrogen: Warning Hazard Statement: When spilled the liquid will vaporize rapidly forming an oxygen-deficient vapor cloud. Contact with cold liquid or gas may cause frostbite.

Dry Ice: Warning Hazard Statement: May displace oxygen and cause rapid suffocation. Contact with product may cause severe cold burns or frostbite.

Chloroform: Warning Hazard Statement: Harmful if inhaled or swallowed. Causes respiratory tract, eye and skin irritation. Suspect cancer hazard - may cause cancer. May cause damage to the following organs: kidneys, liver, heart, skin, eyes, central nervous system

1 Grind a single frozen aphid in a 1.5ml Eppendorf tube, with a plastic pestle precooled in liquid nitrogen. Ensure that tissue remains frozen throughout by working with the Eppendorf tube sitting in a box of dry ice.

2 Remove the tube from the dry ice and immediately add 100 µl CTAB buffer. Leave the plastic pestle in the tube during addition of buffer to ensure tissue stuck to the pestle is not lost, mix thoroughly by agitating the pestle. Remove the pestle, taking care to leave as much material inside the tube as possible. Incubate at room temperature for 1 hour, with occasional mixing by inverting the tube.

3 Add 1 volume (100 µl) of chloroform, mix by gentle shaking and inversion for 10 minutes.

4 Centrifuge in a bench-top microfuge (5,000 G) for 10 minutes. Transfer the upper aqueous phase to a new tube. Take care not to disturb the interface. Be conservative, and leave some of the upper phase behind rather than carry over chloroform contamination.

5 Add 1 volume of ice-cold Isopropanol. Add one drop at a time. Mix the tube by inversion. Mix by inversion and incubate at -20°C for 60 minutes.

6 Centrifuge in a bench-top microfuge (15,000 G) for 20 minutes. Discard the supernatant without disturbing the pellet.

7 Wash the pellet in 0.5 ml of 75% ethanol. Centrifuge in a bench-top microfuge (5,000 G) for 20 minutes to collect the pellet and remove the ethanol. Air dry the pellet for 15 min.

8 Resuspend the pellet in 20 µl 10mM Tris pH 7.5, or TE.

9 Asses the quality of DNA by electrophoresis, and spectrophotometry. A single, high-molecular weight band should be visible, a smear at lower molecular weight indicates fragmentation and/or RNA contamination. Absorbance ratio of 260:280 and 260:230 should both be 1.8 or greater.