Video Article

Extracting DNA from the Gut Microbes of the Termite (Zootermopsis nevadensis)

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Abstract

Termites are among the few animals known to have the capacity to subsist solely by consuming wood. The termite gut tract contains a dense and species-rich microbial population that assists in the degradation of lignocellulose predominantly into acetate, the key nutrient fueling termite metabolism (Odelson & Breznak, 1983). Within these microbial populations are bacteria, methanogenic archaea and, in some ("lower") termites, eukaryotic protozoa. Thus, termites are excellent research subjects for studying the interactions among microbial species and the numerous biochemical functions they perform to the benefit of their host. The species composition of microbial populations in termite guts as well as key genes involved in various biochemical processes has been explored using molecular techniques (Kudo et al., 1998; Schmit-Wagner et al., 2003; Salmassi & Leadbetter, 2003). These techniques depend on the extraction and purification of high-quality nucleic acids from the termite gut environment. The extraction technique described in this video is a modified compilation of protocols developed for extraction and purification of nucleic acids from environmental samples (Mor et al., 1994; Berthelet et al., 1996; Purdy et al., 1996; Salmassi & Leadbetter, 2003; Ottesen et al. 2006) and it produces DNA from termite hindgut material suitable for use as template for polymerase chain reaction (PCR).

Protocol

Procedural summary for termite whole-gut DNA extraction:

1. Chill termites on ice, remove gut using sterile tweezers and stabilize gut samples in buffer.
2. Homogenize samples in PVPP/SDS/phenol buffer.
3. Extract and purify DNA from crude lysate using Qiagen DNeasy columns.

Protocol:

1. On ice, remove the guts from worker caste termites using sterile forceps.
2. Immediately transfer the guts and contents to a sterile, nuclease-free tube containing 50 mL ice-cold 1x molecular biology grade TE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH 8.0). Freeze samples at -20°C, or proceed directly with homogenization.
3. Transfer the gut samples and buffer to a sterile, nuclease-free 2 ml screw capped tube pre-loaded with 500 mg of sterile zirconia/silica beads (0.1 mm) and 700 ml of 1x TE buffer containing 1% w/v polyvinylpolypyrrolidone (PVPP).
4. Add 50 ml of 20% Sodium dodecyl sulfate (SDS) and 500 ml of phenol to the samples.
5. Homogenize (bead beat) on the highest setting using three cycles of 30 sec homogenization and 30 sec of chilling on ice.
6. Sediment insoluble material for 1 min at 8,000x *g*.
7. Purify 300-ml aliquots of the aqueous (uppermost) layer with Qiagen DNeasy columns using the method described for crude lysate purification.
8. Quantify nucleic acid content and freeze samples at -20°C for later use.

Discussion

In our experience, DNA extracted from the microbial communities of wood-feeding termite species like Zootermopis nevadensis is sufficiently pure for PCR template after one round of extraction and purification. However, some termites such as litter-feeding and soil-feeding species may have a higher concentration of humic acids in their gut contents and may require additional purification of gut microbial DNA. The total DNA yield from the guts of 5 Z. nevadensis workers is in the range of 10-30 ug. For termite species significantly smaller or larger than this species, more or fewer specimens may be needed to obtain a similar amount of DNA.

This method can easily be adapted to allow RNA extraction. For RNA extraction, substitute 1x RNAprotect Bacteria reagent from Qiagen (catlog no. 76506) for the ice-cold TE buffer described in step 2 of the protocol. Qiagen RNeasy reagents and columns (catlog no. 74104) should be used in place of the DNeasy purification procedure described above. As DNA can be purified from RNAprotect-stabilized samples and only 300 uL of the approx. 700 uL aqueous layer retrieved in step 7 is required for nucleic acid purification, this method can be used to retrieve both DNA and RNA in parallel from a single sample.

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