A non-destructive enzymatic method to extract DNA from a single arthropod specimen

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Abstract

Preparation of insect specimens for morphological studies classically employs chaotropic salts which can cause disruption of important structures. Also, extraction of nucleic acids for genetic studies leads to destruction of the specimen. Thus, in this paper is proposed a new technique, based on the use of proteinase K, which allows DNA extraction while keeping intact the entire morphology of insect specimens. **The** presented technique can contribute to taxonomic and systematic studies on different groups of arthropods. Also, with the global emergency and reemergence of diseases transmitted by arthropod vectors, genetic and morphological investigations performed in an individual-scale would unveil important aspects of environment and host-pathogen interactions.

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Protocol

Step 1.

Place an insect in a 1,5 mL sterile tube containing the appropriate amount of digestion buffer (200 mM Tris HCl, 250 mM NaCl, 25 mM EDTA, 0,5% de SDS, 400ug/mL of Proteinase K - add proteinase K at the moment to initiate digestion)

necessary to submerge the specimen (for *Aedes* specimens, 200 uL);

Step 2.

Incubate the insect under 56^{0}C for 16 hours;

Step 3.

Collect the digestion buffer with a micropipette and transfer to another sterile tube. Observe the arthropod specimen in a stereomicroscope to verify the clarification process. If necessary, add fresh digestion buffer and incubate under 56^{0}C until obtaining appropriate clarification for morphological studies. Discard digestion buffer and cover the specimen with 80% of ethanol and kept at ambient temperature;

Step 4.

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Extract DNA from digestion buffer collected after 16 hours of specimen incubation with the employment of a PCR fragment purification kit (Qiagen, Thermo Scientific, Promega, BioRad), following manufacturer instructions, and dissolve DNA in 30uL

Step 5.

Amount and quality of DNA vary according to the size and source of specimen

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