Simple, Efficient, and Nondestructive DNA Extraction Protocol for Arthropods

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ABSTRACT We present a DNA extraction technique that does not require the destruction of the insect exoskeleton. This technique is, therefore, of great utility for extracting from museum specimens DNA suitable for the polymerase chain reaction and subsequent DNA sequencing.

KEY WORDS Gryllidae, polymerase chain reaction, DNA extraction

DNA IS A relatively stable molecule that may survive intact for an extremely long time under the right conditions (Higuchi et al. 1984, Pääbo 1989, Lindahl 1993). The maximal age of a DNA-yielding specimen is a function of the storage conditions of the specimen, particularly the initial storage conditions. If there is a small amount of nondegraded DNA, the utility of an extraction technique is limited by its efficiency. Until now, extraction of DNA from museum specimens required the grinding of source specimens, resulting in the destruction of all or much of the specimen.

A method that removes DNA from a specimen for analysis while at the same time preserving the morphology of the specimen intact is, therefore, of great utility for molecular systematics and population biology. We describe a fast and simple protocol for the extraction of DNA from arthropods that does not require the destruction of the exoskeleton. Because the specimens from which DNA is extracted remain intact, they retain their value as museum specimens (Whitfield & Cameron 1994).

Materials and Methods

The exoskeleton is perforated several times with small insect pins, placed in an eppendorf tube containing 600 μl extraction solution of 8% dodecyltrimethylammonium bromide (DTAB), 1.5 M NaCl, 100 mM Tris-Cl (pH 8.8), 50 mM EDTA, and kept in a 68°C water bath overnight. The tube is agitated several times to mix. We have also recovered DNA by merely submersing the abdomen of a dried pinned specimen of *Nemobius fasciatus* in the DTAB solution without removing it from the pin. The specimen is removed and washed with chloroform to remove the DTAB. The specimen may then be placed back in ethanol or air dried and placed directly back into the collection box.

The DTAB solution is added to 600 μl of chloroform, the tube is inverted several times, and centrifuged at 10,000 *g* for 2 min. The upper aqueous layer is transferred to another tube, chloroform-extracted again, and the aqueous layer removed. Water (900 μl) is mixed with 100 μl cetyltrimethylammonium bromide (CTAB) solution (5% CTAB, 0.4 M NaCl), and this is added to the aqueous layer from the chloroform extraction; the tube is then inverted gently several times and allowed to sit at room temperature (20–25°C) for 2 min. The tube is then spun at 10,000 × *g* for 10 min. The supernatant is discarded and the pellet resuspended in 300 μl 1.2 M NaCl to exchange the CTAB. The DNA is precipitated using 750 μl of 100% ethanol and spun at 10,000 × *g* for 10 min. The ethanol is discarded and the DNA pellet is washed with 300 μl of 70% ethanol and centrifuged again, dried in a speed-vac for 10–15 min and resuspended in 50 μl of 0.5× Tris EDTA buffer pH 8.0. This DNA solution is then used for polymerase chain reaction (PCR) amplification.

Polymerase chain reaction amplification of double-stranded DNA is performed in 100 μl of a solution containing 67 mM Tris-Cl (pH 8.8), 2 mM $MgCl_2$, 2 μg/ml bovine serum albumen, 0.125 μl of Amplitaq polymerase 5 U/μl (Perkin-Elmer/Cetus, Norwalk, CT), 0.25 mM each of dATP, dGTP, dCTP, and dTTP, 0.5 μm of each primer, and 5 μl of template DNA. Each cycle of the PCR consists of denaturation at 94°C for 1 min, primer annealage for 1 min at 55°C, and extension for 1 min 40 s at 72°C. This cycle is repeated 25–40 times, depending on the initial concentration of DNA template.

The PCR primers are based on conserved regions in the genes coding for the transfer RNA of leucine and lysine which flank the COII gene in *Drosophila yakuba* (Clary & Wolstenholme 1985). PCR products are prepared for sequencing

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using the USBioclean DNA isolation kit (United States Biochemical, Cleveland, OH). Sequencing reactions are performed using the chain termination sequencing method with the Sequenase sequencing kit (United States Biochemical) with slight modifications. Double stranded DNA (2–4 μg) are mixed well with 2.5 pmol of primer to a total volume of 8 μl, 1 μl of DMSO is then added, and the tube is placed in a boiling water bath for 5 min. The tube is flash cooled in 100% ethanol stored at −70°C; we have found it convenient to fill the wells of interchangeable dry bath heating blocks with the ethanol and keep them at −70°C until needed. The tubes are spun at high speed for several seconds in a microfuge and 2 μl of sequencing buffer (2×) is immediately added. The annealing reaction is pipetted repeatedly until completely thawed. The sample is allowed to sit at room temperature for 5 min. To the annealed template is added the following: 1 μl dithiothreitol, 2 μl of label mix diluted 1/50, 0.8 μl of ^{35}S-dATP, 0.5 μl DMSO, and 2 μl Sequenase diluted 1/8. This extension reaction is incubated at room temperature for 3 min. A portion (3.5 μl) of the reaction is aliquoted into each of the four termination tubes. The tubes are incubated at 35°C for 5 min after which 4 μl of stop solution is added. The reaction tubes are stored at −20°C or for immediate use incubated at 95°C for five min, and placed on ice. A volume of 2.5 μl of the reaction is then electrophoresed on a 6% polyacrylamide gel at 55 W, 1200 V, and 45 mA for 1 h. The gel is fixed in 10% methanol/10% acetic acid for 15 min and dried in a vacuum gel drier at 80°C for 45 min, then exposed using standard autoradiographic procedures.

Results and Discussion

This technique is adopted from a DNA extraction protocol for blood samples (Gustinich et al. 1991), which contain very small amounts of DNA; the method therefore must be efficient. It uses DTAB, a denaturing cationic detergent, in conjunction with CTAB, to precipitate DNA selectively. Using this technique, we have successfully extracted DNA from a wide variety of arthropod taxa including members of Acarina, Collembola, Heteroptera, Neuroptera, Lepidoptera, Odonata, Plecoptera, and Orthoptera. We have also extracted DNA from mammalian and cestode tissue. Typically, specimens used were stored in 100% ethanol and kept at −70°C and ranged in age from 1 to 5 yr; but we have had success with specimens much older. In this brief article we report successful DNA extraction and sequencing from a dried pinned specimen of *N. fasciatus,* (DeGeer) (Orthoptera: Gryllidae), collected in 1952 and from an ethanol-preserved specimen of *Apteronemobius darwini* (Otte & Alexander) (Gryllidae) (paratype) (Fig. 1), collected in 1962. These DNA samples were subsequently used to PCR-amplify and sequence DNA fragments 700 bp in length representing the cytochrome oxidase 2 gene. After 1 yr, no effect of the extraction on the morphology of these specimens has been detected.

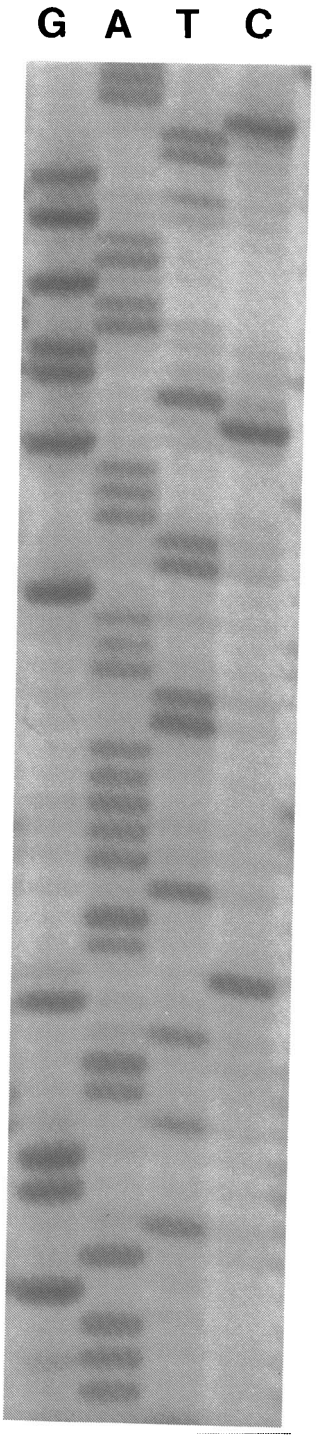

Fig. 1. DNA sequencing autoradiograph of a portion of the cytochrome oxidase 2 gene of *A. darwini.* PCR amplification used the TL2-J-3037 and the TK-N-3785 primers from Simon et al. (1994).

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References Cited

Clary, D. O. & D. R. Wolstenholme. 1985. The mitochondrial DNA molecule of *Drosophila yakuba;* nucleotide sequence, gene organization, and genetic code. J. Mol. Evol. 22: 252–271.

Gustinich, G., G. Manfioletti, G. Del Sal & C. Schnieder. 1991. A fast method for high-quality genomic DNA extraction from whole human blood. BioTechniques 11: 298–301.

Higuchi, R., B. Bowman, M. Freiberger, O. A. Ryder & A. C. Wilson. 1984. DNA sequences from the quagga, an extinct member of the horse family. Nature (Lond.) 312: 282–284.

Lindahl, T. 1993. Instability and decay of the primary structure of DNA. Nature (Lond.) 362: 709–715.

Pääbo, S. 1989. Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. Proc. Natl. Acad. Sci. U.S.A. 86: 1939–1943.

Simon, C., F. Frati, A. Beckenbach, B. Crespi, P. Flook & H. Liu. 1994. Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entomol. Soc. Am. 87: 651–701.

Whitfield, J. B. & S. A. Cameron. 1994. Museum policies concerning loans for molecular systematic research. Mol. Phylogenet. Evol. (in press).

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