A Simple Method to Extract DNA from Bryophytes for RAPD Analysis

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Traditionally, morphological characters have been used for the identification of bryophytes. Although this method is still employed by most bryologists, recent developments in polymerase chain reaction (PCR) for methods such as Randomly Amplified Polymorphic DNA analysis (RAPD) have opened up new methods for distinguishing closely related taxa. RAPD analysis serves as an additional method among several methods in taxonomic diagnosis, especially for taxonomically questionable taxa. Hadrys, et al (1992) have provided an overview of the potential of RAPD analysis for establishing systematic relationships at various taxonomic levels. Drawbacks to the RAPD technique has typically been the large amount of plant tissue needed for extraction of DNA. Also, the reliance of most DNA extraction procedures on hazardous organic chemicals such as phenol and chloroform are dangerous to use and can sometimes contaminate samples.

During the course of conducting DNA comparison studies on several species of *Frullania* and *Leucobryum*, the authors modified a method of DNA extraction first described by K. Edwards, et al. (1991). The following extraction procedure requires little material and is not subject to hazardous organic chemicals.

Obtain approximately 250 µg of tissue from the sample. This amount may vary depending upon the amount of material available. The tissue is then placed into a 1.5 ml Eppendorf microtube and enough liquid nitrogen is added to cover the sample. Once the liquid nitrogen has evaporated, a disposable grinder is used to macerate the tissue for approximately 15 seconds. Add 400 µl of extraction buffer (200 mM Tris HCL with a pH of 7.5, 250 mM NaCl, 25 MMEDTA, 0.5% SDS) to each tube and vortex for 5 seconds. All samples are then centrifuged at 10,000 rpm for 5-10 minutes. After centrifugation, the

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supernatant is carefully poured out of the tube, and the tube is dried overnight, upside down, with the lid open in a freezer. After all the supernatant has evaporated, 100 µl RNAse (10mg\ml) is added to each tube.

Once this procedure is completed, the extracted DNA can be qualitated by running 10 µl of sample mixed with 2 µl of 6X Buffer (0.25% bromophenol blue, 40% [w/v] sucrose in water) on a 1.0% agarose gel and visualizing by ethidium bromide staining (Maniatis, T., et al. 1982). DNA can also be quantitated using a spectrophotometer at 260 nm or using DNA dipsticks (Invitrogen Corp.).

Literature Cited

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