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Silica Gel Drying of Sea Cucumber Tissue as an Alternative to Extraction Buffer or Ethanol for Preservation of DNA

Arom Mucharin[1,2,*], Mats Gustafsson[2], Tomas Cedhagen[2] and Peter Funch[2]

[1] *National Science Museum, Technopolis, Khlong 5, Khlong Luang, PathuThani, 12120, Thailand*
[2] A*arhus University, Department of Bioscience, Section of Aquatic Biology, Building 1135, Ole Worms allé 1, DK-8000 Aarhus C, Denmark*
*Corresponding author: arom@nsm.or.th

ABSTRACT

The material for DNA extraction of sea cucumber which involve refrigeration may not always be convenient because of certain conditions that cannot be achieved. So, we tried out drying the sea cucumber body wall. A comparison is made with traditional ethanol preservation and a modern molecular method in 3 different sampling methods: storage in 96 % Ethanol under refrigeration; storage in RNAlater (Quiagen) under refrigeration and drying in excess silica gel at ambient temperature. The result clearly shows that there is at least some DNA in every samples. Even though it is not an optimial method, silica gel drying can be used for sea cucumber DNA sampling.

Keywords: Silica gel drying, sea cucumber, DNA, sequencing.

INTRODUCTION

Protocol for collection of the material for DNA extraction often involve refrigeration, which may be difficult to achieve under field conditions, particularly in tropical climate as such conditions soon will degrade the DNA. Plant scientists often succesfully dry their samples, including the ones intended for DNA extraction. Even zoologists can dry organisms such as small calcareous foraminiferans and later successfully extract DNA from them (T. Cedhagen, pers. comm.). Here we tried out drying also for animal tissues, specifically sea cucumber body wall. A comparison is made with traditional ethanol preservation and a modern molecular method.

METHODS AND MATERIALS

Sea cucumbers of the order Apodida were collected subtidally in the Andaman Sea and the Gulf of Thailand, cleaned in sea water and kept in a zip-lock plastic bag with some sea water. All specimens were then transferred to isotonic (7 %) magnesium chloride for 2–5 hours (depending on the size of the specimen) as part of the standard procedure for Sea cucumber collection.

The body wall was cut with a scalpel into pieces of ca. 10x10 mm size. Each sample was divided into three subsamples that were subsequently exposed to three different methods:
1. storage in 96 % Ethanol under refrigeration
2. storage in RNA*later* (Quiagen) under refrigeration
3. drying in excess silica gel at ambient temperature

DNA was extracted using the DNeasy Tissue Kit (Quiagen). The purified DNA was visualized with 1% agarose gel electrophoresis (Fig. 1).

RESULTS AND DISCUSSION

The gel electrophoresis picture clearly shows that there is at least some DNA in every sample. The DNA from tissues preserved in RNA*later* gave a slightly larger amount of DNA if compared with the other methods. Tissue preserved in 96 % Ethanol and Silica gel yielded a similar amount. A greater degree of degradation is observed in the silica gel dried subsample of sample 005/1, but not in the other samples even if the samples of the same genus, Synaptula, and were collected from the same area.

Even though it is not an optimial method, silica gel drying can be used for sea cucumber DNA sampling.

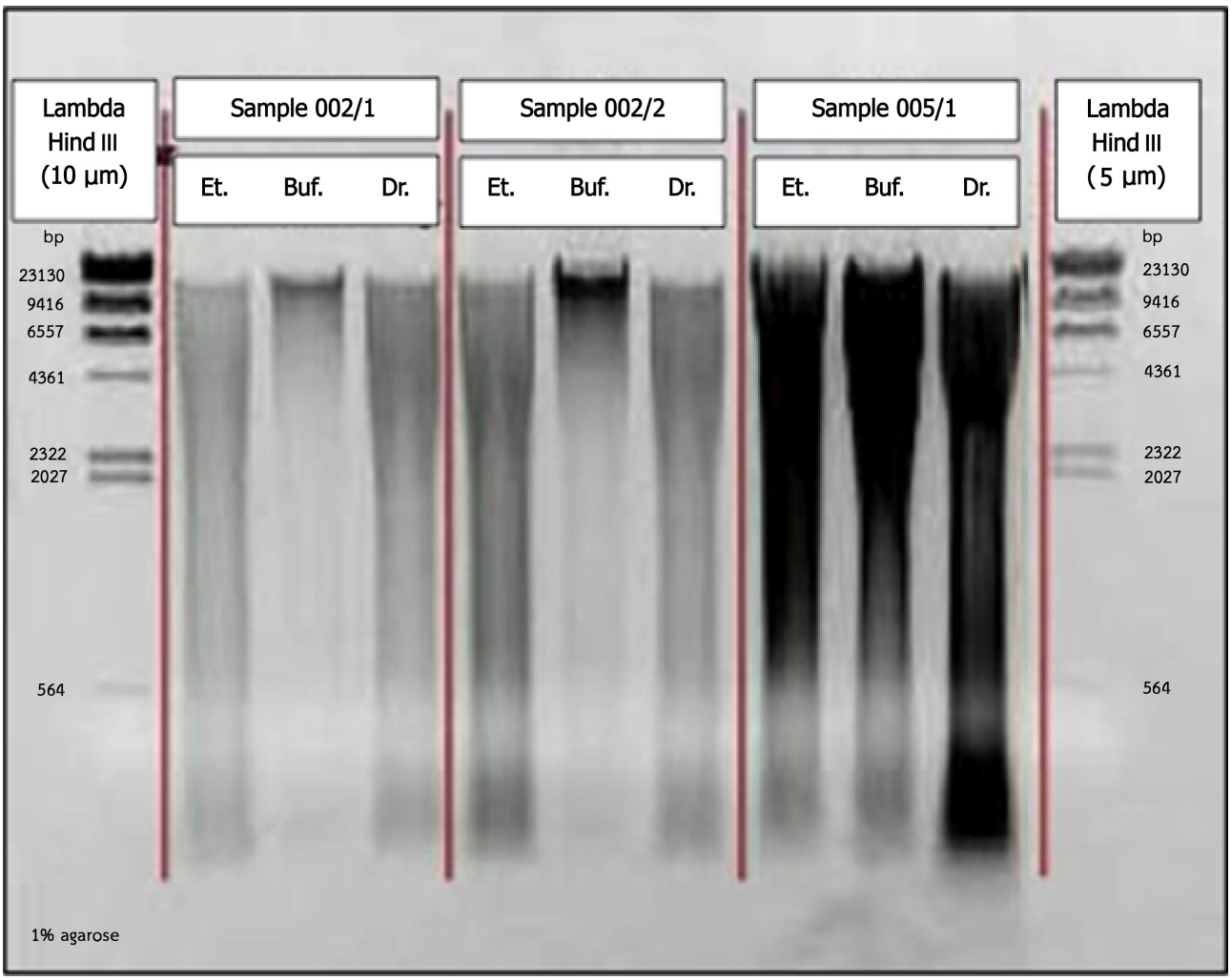

Figure 1. Purified DNA from fieldwork specimens visualized with agarose gel electrophoresis. Et: 96 % Ethanol, Buf: RNA*later*, Dr: Driying in silica gel.

REFERENCES

Houreau, T.B. E. and Boissin 2010. Design of phylum-specific hybrid primers for DNA barcoding: addressing the need for efficient COI amplification in the Echinodermata. *Molecular Ecology Resources* 10: 960–967.

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