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Isolation of sperm vesicles from adult male mayflies and other insects to prepare high molecular weight genomic DNA samples

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Abstract

We describe here a simple and efficient protocol for genomic DNA isolation from adult males of insects: e.g., Ephemeroptera, Odonata, Orthoptera and Dictyoptera. To minimize contamination of external DNA source, the sperm vesicles were isolated from male individuals from which high molecular weight genomic DNA was extracted. According to this protocol, the genomic DNA samples obtained were high quality (intact), and abundant enough for genotyping analyses and molecular cloning. The protocol reported here enables us to process a huge number of individuals at a time with escaping from cross-contamination, and thus it is quite useful for conducting genetic studies at least in some species of insects. The large yield of high molecular weight DNA from single individual may be advantageous for non PCR-based experiments. As a case study of the protocol, partial coding sequences of histone H3 and EF-1α genes are determined for some insects with PCR-amplified DNA fragments.

Introduction

Isolation of genomic DNA from wild animals is a key step for genetic analyses. Quality of genomic DNA is especially critical for constructing DNA library and also for genotyping using DNA markers such as RFLP, AFLP and RAPD to keep reproducibility and reliability of the results. Contamination of exogenous DNA, e.g., parasitic organisms and the stomach contents, should be avoided. Generally, muscle tissues were widely used as a source of genomic DNA for winged insects [1], and it is a simple and reliable way to prepare DNA samples from muscle tissues of insects for PCR-based analyses. It is, however, time-consuming to dissect individual body to harvest large amount of the muscle tissue suitable for non PCR-based studies such as genomic DNA library construction. In this report, we describe a simple protocol to prepare high molecular weight genomic DNA for genetic analyses from the sperm vesicles of adult males of some species of winged insects. The protocol consists of two steps: firstly, the sperm vesicles are isolated from posterior abdomen of adult male individuals, and secondly, the total DNA is extracted by conventional method as used before [2–5]. Here, a practical method for isolating sperm vesicles from adult male mayfly is demonstrated, and some results drawn from these DNA samples are shown.