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**A SIMPLE, RAPID AND INEXPENSIVE DNA EXTRACTION METHOD FROM
GREEN TIGER SHRIMP (*PENAEUS SEMISULCATUS*)**

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ABSTRACT

DNA extraction from cells is one of the most widely used molecular biology techniques. The current investigation presents a simple method for extracting DNA from the *Penaeus semisulcatus*. The traditional methods for the isolation of DNA are more time consuming and the reagents used are costly. Due to some restrictions on the use of commercial kits for DNA extraction, including its high price and its rare accessibility, the study tried to establish a cheap, quick and easy protocol for DNA extraction. In this method, samples were briefly incubated in hot NaOH and then neutralized by Tris buffer. The method was found to be rapid, reliable, and inexpensive for the isolation of Polymerase Chain Reaction (PCR) quality DNA from shrimp tissues. These advantages make it useful for high-throughput applications. Although it is a very rapid and simple method, the quality of DNA is adequate for PCR applications. This procedure required as little as 30 mg of tissue and produced highly purified DNA OD₂₆₀/OD₂₈₀ ratios between 1.70 and 1.76. Therefore, this method was used to extract both genomic and mitochondrial DNAs. It is also a reproducible, simple, and rapid technique without enzyme for routine DNA extractions. This procedure is also simple, rapid, cheap and may be used with minor modifications for large-scale DNA extractions for a variety of experimental goals.

Keywords: DNA extraction; DNA purification; 16S rRNA gene; *Penaeus semisulcatus*

INTRODUCTION

The extraction of biomolecules, DNA, RNA, and protein is the most crucial method used in molecular biology. It is the starting point for downstream processes. The DNA-based methods are highly dependent on the DNA extraction and purification techniques (Abbaszadegan, et al., 2007; Pinto, et al., 2007). DNA isolation is a process of purification of DNA from a sample using a combination of physical and chemical methods. The first isolation of DNA was done in 1869 by Friedrich Miescher. Currently, it is a routine procedure in molecular biology or forensic analyses (Vasuki, *et al.*, 2003; Dahm, 2008). Isolation of a sufficient amount of high quality DNA is a prerequisite to such applications and the selection of an appropriate DNA extraction method plays a pivotal role in this regard. Many different methods can be applied for DNA extraction from diverse sources of samples, with modifications depending on the selected tissue (Psifidi, *et al.*, 2010; Bryant, *et al.*, 1997; Jackson, *et al.*, 1990).

The DNA extraction method must be simple, quick and efficient. Safety, cost and DNA quality must also be considered (Marechal-Drouard and Guillemaut, 1995, 1992). DNA quality is critical because the efficiency of PCR amplification can be reduced by

inhibitors from the matrix. DNA extraction has therefore been highlighted as a limitation of culture-independent methods. Reliable and highly efficient DNA extraction methods have been a core element of molecular research fields. Tremendous efforts to develop an efficient method to extract, and thereby isolate, DNA with high sensitivity and selectivity from complex samples in a time-saving manner have met with success.

The primary candidate was a DNA extraction technique for isolation of RAPD quality DNA from potatoes; termed by the author “One-Minute DNA Extraction” technique (Hosaka, 2004). The extracted DNA is purified using a phenol/chloroform mixture, and precipitated in isopropanol. The extracted DNA is of high quality and suitable for molecular analyses, such as PCR, restriction enzyme digestion, genomic DNA blot hybridization, and genomic DNA library construction (Duval, et al., 2010; Keb Llanes, *et al.*, 2002). The successful application of molecular techniques relies on the effective recovery of DNA from samples (Lee, et al., 2003). Therefore, a reproducible, simple and rapid protocol for DNA extraction is essential for molecular studies on these marine shrimp. To minimize the time and cost, and to maximize the sensitivity and reproducibility of the

procedure, a rapid, simple and safe method of DNA extraction is developed and tested by the authors.

MATERIALS AND METHODS

Sampling and DNA extraction:

Samples for DNA extraction, purchased from local fishermen and they were kept in 96% alcohol. About 300 mgr of shrimp body muscle tissue was separated and were powdered using liquid nitrogen. About 30mg of powdered tissue, were transferred to several vials individually. 400µl of 50 mM NaOH was added to each vial and then shut the door vials with Parafilm, which are for 10, 15, 20, 25, 30 minutes were treated in boiling water. After cooling, 80µl Tris 0.5M pH 7.5 was added to the sample solution and mixed well. Then, Samples centrifuged for 3, 5, 15 minutes at 14000 rpm. 400µl the supernatant transferred to a new vial and stored until analysis in -20°C. 2µl of above sample directly were used as a template in PCR. The remaining 350µl, 200µl mixture of Chloroform: Isoamylalcohol (24:1) (Takada Hoshino, et al., 2005) was added and then, the mixture was centrifuged for 10 min at 12000 rpm. Supernatant transferred to a new vial and the precipitated DNA rinsed by isopropanol or 70% ethanol. The precipitate was dissolved in 30 -50µl double-distilled water. The quantity and quality of DNA was evaluated

with a spectrophotometer at a wavelength of 260nm and 260nm to 280nm ratio wavelength.

PCR amplification of mitochondrial and nuclear regions:

Amplification of the mitochondrial 16S rRNA/tRNA^{val} and the nuclear Internal Transcribed Spacer I (ITS-I) using specific primers to assess extracted DNA by boiling method, was used. For amplification of the 16S rRNA/tRNA^{val}, 16 Scru F (5' TATGGCTGTTTTTAAGCCTAATTC 3') and 16 Scru R (5' CGTTGAGAAGTTCGTTGTGCA 3') primers were used (Calo-Mata, *et al.*, 2009). ITS-I using the primers ITS-I F (5' GTTCCGTAGGTGAACCTGC 3') and ITS-I R (5' TGATCCCCCGTTAAGAGTC 3') were amplified (designed in the laboratory). Primer pairs 16 Scru F/R and ITS-1 F/R respectively was amplified of approximately 300 and 530 bp.

PCR for the Amplification each three regions in are action mixture containing 2µl DNA template, PCR buffer 2.5µl, 1.5µl MgCl₂ (50mM), 10 pm of each primer, 1µl dNTP mix (10mM) and 1.5 units of enzyme Taq pol. (Fermentas) was performed in a volume 25µl. PCR Amplification according to the Heat's 16 srRNA/tRNA^{val} was performed: 94°C (3 seconds), during 30 cycles 94°C (50seconds), 55°C (45 seconds) and 72°C (55seconds). The

final extension was performed for 5 minutes at 72°C. The thermal profile was used for amplification of ITS-I, except that the temperature was 58°C primer binding. The thermal profile for amplification of mitochondrial DNA Amplification and 60°C primer annealing was also 90 seconds. The product of PCR, was electrophoresed on 1% agarose gel.

RESULTS

Using spectrophotometric measurement of DNA quantity and quality, for example, that for 20, 25 and 30 minutes were boiled, have been reported (**Table 1**). Electrophoretic pattern of DNA on 1% agarose gel as a smear was observed (not shown). In general, DNA was extracted by boiling method, five different times (10, 15, 20, 25 and 30 min). 2 microliter of all treatments after 10 minutes and centrifuged at 14000 rpm, directly as a template for amplification of 16S rRNA/Trna and ITS-I were used. In all the above cases, PCR was successful. The resulting DNA different treatments boil, chloroform: isopropanol by Isoamylalcohol was added and the precipitate was washed with 70% ethanol and distilled water were dissolved in an appropriate volume. 2 microliter of DNA, the boiling of five treatments, the area was used as a template for Amplification. Agarose gel electrophoresis of PCR products showed

that the samples for 20, 25 and 30 minutes have been boiled, can be successful as a template for amplification of the target are as to be used. Amplification mitochondrial and genomic regions used.

In order to quantify the extracted DNA sample by spectrophotometry the measurement for the intensity of absorbance of the DNA solution at wavelengths 260 nm and 280 nm is used as a measure of DNA purity. DNA absorbs UV light at 260 and 280 nanometers; a pure sample of DNA has a ratio of 1.8 at 260/280 and is relatively free from protein contamination. A DNA preparation that is contaminated with protein will have a 260/280 ratio lower than 1.8 (**Table 1**).

According to the results, about 50 mgr of shrimp tissue using liquid nitrogen, and then add the powder to distilled water 400 µl were treated for 30 minutes in the water is boiling. Then it was centrifuged for 15 minutes at a speed of 14000 rpm. Then 2 µl for Amplification of the mitochondrial length 523 bp using primers 16S rRNA F/R and 2 µl for Amplification of the genomics length 333 bp using primers ITS-1 F/R was used as a template. Results of electrophoresis of PCR products showed that the region has been successfully amplified (**Figure 1**).

RESULTS AND DISCUSSION

According to the method used for extraction of genomic and mitochondrial DNA, the electrophoresis was determined to be successful and applicable. The procedure can be done with a quick, inexpensive and easy way of extraction. The experiment was repeated three more times, and then the

results were compared with TESⁱ extraction method (Chen, *et al.*, 2009; Sambrook, et al., 1989) interestingly, similar results were observed in the current study; The results are reported in **Figures 1 and 2** (genomics Primer products is marked with subscript 1 and mitochondrial primer products is marked with subscript 2).

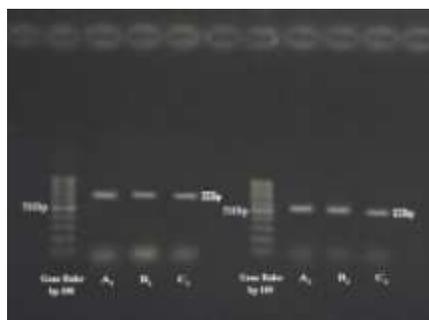


Figure 1: Gel electrophoresis of boiling extraction method

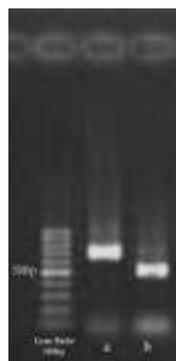


Figure 2: Gel electrophoresis of TES extraction method
a: PCR product from ITS-1 primer , b: PCR product from 16 Scru primer

Table 1: Boiling time and DNA optical absorption

Vial	Boiling time	OD ₂₆₀	A ₂₆₀ /A ₂₈₀
A	20 min	0.462	1.70
B	25 min	1.053	1.76
C	30 min	0.910	1.75

Generally, DNA extraction from prokaryotes and eukaryotes follows two steps: rupturing the cell to release DNA contents, followed by extraction of DNA from a lysate using a phenol/chloroform extraction and ethanol DNA precipitation protocol (Sambrook, *et al.*, 1989). This method was used to extract both extract genomic and mitochondrial DNA. The proposed method is reproducible, simple and rapid for routine DNA extractions. Furthermore, the low cost of this method makes it attractive for large-scale studies. This procedure is simple, rapid and cheap; in addition, it may be used with minor modifications for large-scale DNA extractions for a variety of experimental goals.

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