



OPTIMIZATION OF DNA EXTRACTION PROTOCOLS FROM SHRIMP (*Penaeus monodon*) TISSUE

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Abstract: DNA analyses and DNA-based diagnoses are going forwardly in the commercial deals with shrimp (*Penaeus monodon*) as well as in the basic research. In this regard extraction of DNA is obvious. However, numerous protocols are available DNA extraction from animal tissue. Therefore, which technique is suitable one for shrimp tissue is indispensable to justify. In the current study, a protocol was optimized for extraction of DNA from shrimp collected from Batiaghata, Khulna. Muscle Tissue (MT) and Hepatopancreas Tissue (HT) were distinctly sampled on 15th March, 2007 from only one fresh individual for each of six protocols applied. Guanidium isothiocyanate treatment involving two groups of commercial preparation (DNAzol from invitrogen® and DNA Extraction Buffer (DEB) from Genei®) with or without RNase, as well as Proteinase K isolation technique and sodium dodecyl sulfate (SDS) isolation technique were applied to extract DNA. The quantity (μgml^{-1}) and quality were determined by UV spectrophotometry at $A_{260\text{nm}}$ and $A_{260/280}$ respectively. The current study provided that the quantity of DNA obtained with almost every protocol was higher when the hepatopancreas tissue was used. Among six protocols, DEB with RNase ($102.5 \mu\text{gml}^{-1}$ for MT, $302.5 \mu\text{gml}^{-1}$ for HT) resulted the highest concentration of DNA with acceptable level of purity ($A_{260/280}$ 1.86 for MT and 1.70 for HT).

Key words: DNA extraction, DNA concentration, extraction protocols, optimization.

Isolation of Total DNA is prerequisite for any DNA based analysis. *Penaeus monodon* (Giant tiger prawn), the valuable aquatic species, is such an organism that the DNA based analysis is effective to detect the disease as well as to analyze population genetics (Milligan, 1998). There are numerous procedures for extraction of DNA. However, depending on the ultimate intended use of the DNA sample, total DNA isolations may be readily scaled up or down to accommodate the available tissue sample. In case of shrimp mainly the application of a DNA extraction protocol will be viable when the quantity and quality of extracted DNA is suitable for PCR (Polymer Chain Reaction) or other diagnostic purposes. In this study, total six treatments (guanidium isothiocyanate treatment involving two commercial preparations from Invitrogen (DNAzol) and Genei (DNA extraction solution) with or without RNase, as well as Proteinase K and SDS isolation techniques) were applied to extract DNA from shrimp cells and optimized a standard one.

Giant tiger prawn (*Penaeus monodon*) of 15.05 ± 1 g body weight and 10 cm total length was collected on 5th March, 2007 from Batiaghata of Khulna district. Muscle and Hepatopancreas tissues were separated and used for DNA extraction. The quantity (μgml^{-1}) of DNA determined by

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UV spectrophotometry at 260 nm and the quality of DNA was justified by the absorbance ratio 260/280 nm (Sambrook *et al.*, 1989; Aquadro *et al.*, 1998). The concentration (C) was therefore determined (Aquadro *et al.*, 1998; Anon, 2005) by:

$C = A_{260} \text{ (observed)} \times 50 \mu\text{gml}^{-1} \times \text{dilution factor}$ for the measured sample, given that a $50 \mu\text{gml}^{-1}$ solution of double stranded DNA has an absorbance of 1.0 at 260 nm.

Where, $\text{Dilution factor} = \frac{\text{amount of solvent for dilution}}{\text{amount of original sample solution}}$. In this study, dilution factor was 50

(2500/50 μl).

The standardization and optimization of the DNA extraction protocols relied on the concentration and purity i.e. whether the protein, ethanol or RNA contamination (from the ratio of $A_{260\text{nm}}$ and $A_{280\text{nm}}$) existed (Table 1).

Table 1: Concentration ($\mu\text{g/ml}$) and purity of DNA obtained from different protocols.

Protocol no.	Protocol name	Source of tissue	$A_{260\text{nm}}$	Concentration (μgml^{-1})	$A_{260/280}$	Time required (h)
01	DNAzol isolation of total DNA	M	0.01	32.5	2.6	3
		H	0.15	385	1.53	
02	DNAzol with RNase treatment (modified from 1)	M	0.05	117.5	2.24	3.5
		H	0.02	45	1.64	
03	DEB treatment	M	0.02	60	2.09	3
		H	0.07	162.5	1.14	
04	DEB with RNase treatment (modified from 3)	M	0.04	102.5	1.86	3.5
		H	0.12	302.5	1.70	
05	Proteinase K isolation of total DNA	M	0.09	220	1.28	10-16
		H	0.17	415	1.64	
06	SDS isolation of total DNA	M	0.04	90	1.34	2
		H	0.08	200	1.33	

M=Muscle; H=Hepatopancreas

The DNA concentration in hepatopancreas tissue samples was higher perceptibly than that of muscle tissue samples. Because the chemical composition of tissue samples derived from the diversity of sources may alter the array of available isolation techniques (Milligan, 1998). Likewise, the hepatopancreas of shrimp is bloody in colour. So, it may contain many chromatin that are supposed to contribute in the spectrum absorbance other than nucleic materials. Among the three uppermost amount of DNA extracted from both muscle and hepatopancreas, in wet-weight per unit, were yielded from Protocol 5, Protocol 4 and Protocol 1 respectively. However, regardless the rest others, among them what's the standard and optimal to further extraction relies on the purity assessment. The DNA quality can be said the best if $A_{260/280}$ is around 1.8 (Hillis *et al.*, 1990; Chomczynski *et al.*, 1997; Aquadro *et al.*, 1998; Ligozzi and Fontana, 2003; Anon, 2005). Lower ratios indicate protein or phenol contamination (Hillis *et al.*, 1990; Aquadro *et al.*, 1998) and in contrast, higher ratios indicate RNA contamination (Anon, 2005). Protocol 1 derived total nucleic acids (of DNA including RNA) $32.5 \mu\text{gml}^{-1}$ from muscle tissue and $385 \mu\text{gml}^{-1}$ from hepatopancreas tissue. The treatment was by only guanidium isothiocyanate (DNAzol) excluding RNase reagents. Functionally, nucleic acids absorb ultraviolet radiation in the wavelength range, 200-320 nm (i.e. in the UV-B and UV-C region of the electromagnetic spectrum). The absorbance maximum in this region is around 260 nm and is due to absorption by the purine and pyrimidine bases. The shape of the spectrum is therefore the same for both RNA and DNA. The obtained quantity of DNA, therefore, being comparatively standard was spotted with the question of quality. $A_{260/280}$ for MT (2.60) sample indicated slightly RNA contamination whereas for HT sample it was almost satisfactory one resulting 1.53 of $A_{260/280}$ indicating free of RNA contaminant

but possibility of protein contamination. It is evident from previous studies (Chomczynski and Sacchi, 1987; Puissant and Houdebine, 1990; Chomczynski and Mackey, 1995; Ligozzi and Fontana, 2003) that the DNAzol procedure is based on the use of a novel guanidine-detergent (guanidine isothiocyanate) lysing solution that hydrolyzes RNA and allows the selective precipitation of DNA from a cell lysate. However, According to Schneider (2005) “guanidine-hydrochloride separates proteins from ribonucleic acids very well, but carbohydrates and RNA may be contained in the DNA solution. However, the presence of RNA in the DNA sample can be eliminated by treating the sample using RNase.” Though the Protocol 2 using the RNase yielded less quantity of DNA from HT sample ($45 \mu\text{gml}^{-1}$) with compared to its original protocol (DNAzol isolation) (Protocol 1), MT sample resulted somehow an large amount ($117.5 \mu\text{gml}^{-1}$) of DNA with $A_{260/280}$ 2.24 that seems abnormally. It might be from any secondary organic compounds. The purity test by $A_{260/280}$ of Protocol 4 (1.86 for MT and 1.70 for HT sample) with the DNA quantity ($102.5 \mu\text{gml}^{-1}$ from MT and $302.5 \mu\text{gml}^{-1}$ from HT) was good to show better and reliable one. Moreover, the $A_{260\text{nm}}$ was reliable (Anon, 2005). It is notable that the responsible reagent of DEB to extract the DNA from tissue is also guanidine isothiocyanate.

Protocol 5 in which proteinase K was used with phenol-chloroform treatment yielded the highest quantity of DNA ($220 \mu\text{gml}^{-1}$ from MT and $415 \mu\text{gml}^{-1}$ from HT) with reliable and recommended $A_{260\text{nm}}$ (Anon, 2005). Perhaps the most widely used method of isolating DNA, particularly from animal sources, is based on lysis of the tissue in a detergent buffer usually containing SDS, followed by proteinase digestion of cellular proteins often with proteinase K, less often with pronase E (Hillis *et al.*, 1990). This method was developed with 1:1 phenol: chloroform and several times ethanol treatment (Minton and Mayer, 1994). However, 1.28 $A_{260/280}$ for MT could be possible if the protein and/or phenol contamination pertained whereas 1.64 $A_{260/280}$ for HT sample was good for purity. The theory says that there is no chance of protein contamination due to the protein digestion by proteinase K (Milligan, 1998). Moreover, the incubation at 55°C for at least one hour denatures the DNase and facilitates the protein dissociation, and further treatment with phenol-chloroform (Sevag procedure) is used to deproteinize (Anon, 2005). In Protocol 5, the incubation period at the elevated temperature was over-night and Sevag procedure was also done. Therefore, the phenol contamination was very likely to occur in that case. This problem is commonly encountered in this protocol (Wilson, 1999) if it is done with hurry alcohol removing. Furthermore, it may not remove compounds such as some organic acids that are inhibitors to protein digestion (Milligan, 1998).

SDS isolation technique (Protocol 6) that produced $90 \mu\text{gml}^{-1}$ for MT and $200 \mu\text{gml}^{-1}$ for HT derived from worthy $A_{260\text{nm}}$. However, the purity assessment (1.34 $A_{260/280}$ for MT and 1.33 $A_{260/280}$ for HT) lightened protein or ethanol contamination. There are evidences (Goodwin and Lee, 1993; Edwards *et al.*, 1991) that this protocol is used commonly for all, however, mainly for animal DNA extraction resulting high quality DNA though some complex compounds are associated. From the current study with shrimp tissue, the quality was not satisfactory.

The time estimation, from the work proceeding and described protocol, directed that the SDS isolation technique (Protocol 6) required least duration (approx. 2 h) whereas Proteinase K isolation technique (Protocol 5) most (approx. 10-16 h). Rest all were in the range of 3-3.5 h.

Being comparatively easy procedures and from the data obtained DNAzol treatment of total DNA (Protocol 1) appears as the standard one. This protocol was effective as well as straight forward comparing with other commercial kits. However, it should not be pertinent to take DNAzol treatment as an optimized single rather proteinase K isolation (Protocol 5) of total DNA was also effective one for shrimp tissue. Though highest amount of DNA was obtained from the proteinase K isolation technique it should not be used in PCR operation due to probable phenol contamination in most evidence. Last of all, from my experiment the use of DNA extraction buffer

(DEB) with RNase (Protocol 4) was the most suitable with reasonable yield and purities. However, quantity estimation of DNA would have been more reliable if gel electrophoresis and UV spectrophotometry were done simultaneously. So, further study is required for the final conclusion regarding the suitability of the DNA extraction protocols under investigation.

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