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Efficiency of Different DNA Extraction Methods for Fish Tissues: A Comparative Analysis

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Abstract: *Extraction of high quality DNA is the first step for many downstream procedures such as pcr, sequencing etc. An experiment was carried out to compare the efficacy among five different DNA extraction methods (i.e. Urea-SDS, Rapid MT, SNET, TNES and Salt out method) applied to extract DNA from Labeo rohita and Tenualosa ilisha. The quantity and quality of the extracted DNA was compared using UV-spectrophotometer and gel electrophoresis. Urea-SDS method produced good quality of DNA from both fish species (value of A_{260}/A_{280} for L. rohita was 1.77 ± 0.06 and for T. ilisha was 1.74± 0.04). Among all methods, Rapid MT method produced highest quality of DNA from L. rohita (value of A_{260}/A_{280} was 1.82 ± 0.04) while DNA produced from T. ilisha was not of good quality (value of A_{260}/A_{280} was 1.69 ± 0.05). The lowest quality of DNA (1.63 ± 0.00) was extracted from L. rohita by TNES method while highest quality of DNA (2.00 ± 0.06) was extracted from T. ilisha by Salt out method. Salt out method proved to be the most efficient. SNET method was the superior method in terms of quantity of isolated DNA from both fish tissue samples (value of DNA concentration of L. rohita was 177.85 ± 49.85 ng/µl and T. ilisha was 200.72 ± 28.97 ng/µl) whereas Rapid MT method performance was the poorest for the same case (value of DNA concentration of L. rohita was 39.35 ± 8.33 ng/µl and T. ilisha was 37.77 ± 5.63 ng/µl). The methods can be ranked on the basis of quantity of DNA from both fish tissues as following order: SNET>Urea-SDS>Salt out>TNES>Rapid MT. So, SNET method can be employed in fish DNA extraction where yield is more important and on the other hand, Urea-SDS method can be employed where quality of extracted DNA is most important.*

Keywords: *DNA extraction, Labeo rohita, Tenualosa ilisha.*

I. Introduction

Extraction of high quality DNA from various sources such as tissue samples is the primary crucial step in molecular biology and the quality and quantity of template DNA influence the attainment of several molecular experiments including PCR, enzyme digestion and recombinant selection [1]. Most of the methods for DNA extraction from animal tissues need sufficient amount of materials and time consuming and produce variable yields. Preference should be given for extracting DNA from any source should be high yielding, safe and rapid [2]. Yield and quality of DNA obtained from fish by methods that are successful in other animals can be very different in fish. Also, the same method can have different output for different fish species [3].

Although several methods of extraction DNA from different fish tissues, scales and fins using conventional and commercial kits have been compared [3-16] but still comparative efficiency of different methods of DNA extraction for tropical fish species has not been assessed.

In the current study, we compared modified version of five different techniques for DNA isolation i.e. Urea-SDS method [12], Rapid MT method [17], SNET method [18], TNES method [4], and Salt out method [18]. All five methods involved proteinase K digestion during the lysis of cell but the lysis buffer varies in other contents. Also, some of the methods used phenol-chloroform extraction while the other used salt for the purpose of organic phase separation. Sample preparations, homogenization procedures, lysis and incubation periods, precipitation and resuspension procedures were same in all methods. Objective of the current study included efficiency of five different methods of DNA extraction from the muscle tissues of two different fishes i.e. *Labeo rohita* and *Tenualosa ilisha*.

II. Materials and methods

2.1 Collection of Samples

L. rohita and *T. ilisha* were collected from Jatrabari fish market and immediately carried in frozen condition to the DNA barcoding laboratory of Department of Zoology, University of Dhaka. Fish muscle tissue samples of 20 mg were taken from *L. rohita* and *T. ilisha* separately and subjected to DNA extraction using five

different methods in duplicate. For DNA extraction from single tissue sample each of the methods is described here.

2.2 Urea-SDS Method

This was a modified method that was used to extract DNA from Mackerel fish [12]. Fish tissue sample was suspended in 100 µl of TESU6 buffer (10mM Tris-HCL pH 8.0 + 20 mM EDTA pH 8.0 + 2% SDS + 6M Urea +25 µg/ml proteinase K) and homogenized, mixed by vortex and then incubated at 55^0C in a shaking incubator with oscillation of 200 rpm for 15 min. After that 10 µl of 5 M NaCl was added and mixed gently by inversion followed by addition of equal volume of Phenol:Chlorophorm:Isoamyl Alcohol (25:24:1) and mixed by inversion. The mixture was centrifuged at 10,000 g for 5 minutes and the aqueous phase was collected. Equal volume of chilled isopropyl alcohol was added to it and inverted for several times and kept at -20^0C. After overnight precipitation and centrifugation at 10,000 g for 5 minutes, the supernatant was decanted. Then the DNA precipitate was washed with chilled 70% alcohol. Finally the DNA was re-suspended in 60 µl of nuclease free water.

2.3 Rapid MT Method

Rapid procedure Tail-tip DNA extraction was adapted and named as Rapid MT method [17]. With 20 mg of fish tissue taken in microcentrifuge tube, 660 µl of buffer (100 mM Tris-HCl, 5 mM EDTA, 0.2% SDS, 200 mM NaCl) and 10 µl of Proteinase K (20 mg/ml) was added and then the tissue was homogenized, mixed by vortex and then incubated overnight at 55^0C in a shaking incubator with oscillation of 200 rpm. After centrifugation of the mixture at 12,000 g for 15 minutes, the upper aqueous layer was transferred to another tube. After that 400 µl isopropanol was added and mixed by inverting the tube for 25 times. The DNA was pelleted (20 s at 12,000 g), rinsed with 70% alcohol and the alcohol was decanted and dried. Finally DNA was resuspended in 60 µl nuclease free water.

2.4 SNET Method

Customized version of the method for preparation of genomic DNA from mouse tails and other small samples [18]. Fish muscle tissue was suspended in 500 µl of buffer (20 mM Tris-Cl, 5 mM EDTA, 400 mM NaCl, 1% (w/v) SDS, 400 µg/ml Proteinase K), homogenized overnight at 55^0C in a shaking incubator with oscillation of 200 rpm. Equal volume of Phenol: Chlorophorm:Isoamyl Alcohol (25:24:1) was added to the dissolved liquid, placed in shaking incubator at room temperature for 30 min and centrifuged at 14000 rpm for 5 min. Upper aqueous layer was transferred to a new microcentrifuge and equal volume of isopropanol (chilled) was added and centrifuged again at 8000 rpm for 15 minutes. The isopropanol was removed and the remaining was washed with 70% ethanol. Air dried DNA was resuspended in nuclease free water.

2.5 TNES Method

Volume of the reagents originally mentioned were customized and followed in this method with other modifications [4]. Tissue sample of 20 mg was placed in 800 µl of buffer (10 mM Tris-HCl, 125 mM NaCl, 10 mM EDTA, 0.5% SDS, 4 M Urea) and 10 µl of RNase (10mg/ml) was added and homogenized and then incubated at 42^0C for 1 h. After that10 µl of Proteinase K (10 mg/ml) was added and maintained at 42^0C overnight. 800 µl of Phenol: Chlorophorm: Isoamyl Alcohol (25:24:1) was added to the microcentrifuge tube containing the mixture which was then inverted for 15 minutes. The microcentrifuge tube was then centrifuged for 15 min at 10,000 rpm and top aqueous layer was recovered. The DNA was precipitated in 1 M NaCl and two volumes absolute ethanol by centrifugation in 10,000 rpm. The DNA was washed with 70% ethanol, air dried and was resuspended in 60µl of nuclease free water.

2.6 Salt out Method

Followed by Sambrook [18] and adapted in this study. Here, 550 µl of buffer (50 mMTris-HCl, 50 mM EDTA, 100 mMNaCl and 1% SDS), 20 mg of fish tissue and 7 µl of proteinase K (20 mg/ml) was taken in a single microcentrifuge tube, homogenized and incubated overnight at 50^0C in a shaking incubator with oscillation of 200 rpm. 600 µl of 5M NaCl was added to the solution and centrifuged for 10 min at 12,000 rpm and remove the aqueous layer carefully to a new microcentrifuge. After that 700 µl of freezing absolute ethanol was added and incubated at -20^0C for 2 hours. The DNA pellet was obtained by centrifugation at 12,000 rpm for 10 min. The DNA was washed with 70% ethanol and the pellet was retained. Air dried DNA was resuspended in nuclease free water.

2.7 Quantification and Visualization of Extracted DNA

The quality and quantity of DNA isolated from each of the methods was evaluated by gel electrophoresis and spectrophotometer. The DNA solution was visualized in 1% agarose gel using fluorescence

of ethidium bromide in UV light, by direct comparison with a standard marker (50bp DNA ladder, Promega). The fluorescence was documented using Gel documentation system [19]. For the quantification of DNA, spectrophotometry (Nanodrop 2000) was used. The concentration of DNA was inferred from the absorbance (OD). Additionally, the quality of isolated DNA were assessed using OD_{260}/OD_{280} [20].

III. Results and Discussion

Fish DNA isolation has been very elusive compared to isolation of DNA from other animal tissues. However, the application of fish DNA to various downstream analysis has accelerated the development of various methods of DNA isolation. In this study, we have subjected five such methods to comparative analysis. The methods were analyzed for their time, cost and labor requirement and the quality and quantity of the outcome.

Each methods uses proteinase K digestion but other contents in lysis buffer vary among different methods [21]. Proteinase K digestion has been widely used for the preparation of unpurified PCR quality DNA and moreover the protease is active in a pH range of 4.3–12.0, with optimal activity at pH 8.0. Proteinase K has a broad temperature profile, retaining >80% of its activity at temperatures of 20–60°C [22]. Mentionable that Proteinase K denatures above 65^0C [23]. Proteinase K is also fully active in 0.5% (w/v) SDS and is frequently used in the presence of detergents like SDS [24]. By lysing cell nuclei with detergents and digesting the lysates with proteinase K, protein could be dislodged from DNA, and then easily removed with phenol extraction [23]. And in the present study for digestion of tissues in different methods, temperature of 42^0C to 55^0C was employed. Although SDS is used in all of the methods to disrupt cell membrane, urea was used in two of the methods as chaotropic agents to enhance the process of cell lysis. The methods also vary in the time required for cell lysis and precipitation of DNA. Either absolute ethanol or isopropanol is used for the purpose of DNA precipitation. For removal of proteins and other macromolecules either Phenol: Chlorophorm: Isoamyl Alcohol (25:24:1) or high concentration of chaotropic salt [25] (Table 1).

Table-1: Separation Principle Used by Different Methods of DNA Isolation

Methods	Separation principle employed
Urea-SDS	Lysis with proteinase K, urea, SDS; proteins removed by NaCl and PCI; DNA precipitated using absolute ethanol
Rapid MT	Lysis with proteinase K, SDS and NaCl; DNA precipitated directly from supernatant using isopropanol after centrifugation of cell lysate
SNET	Lysis with proteinase K, SDS and NaCl; proteins removed by PCI extraction; DNA precipitated using isopropanol
TNES	Lysis with proteinase K, SDS, urea and NaCl; proteins removed by PCI extraction; DNA precipitated using NaCl and absolute ethanol
Salt out	Lysis with proteinase K, SDS and NaCl; proteins removed through salting out using NaCl; DNA precipitated using absolute ethanol

The time and labor required to complete the whole process of DNA isolation also varies among the methods. Urea-SDS method required only 15 minutes for tissue lysis as urea is quite denaturing for protein and at least it disrupted most likely any protein multicomplexes [4], however precipitation of DNA in absolute ethanol took overnight [26]. So, the total time required by this method was more than 15 hours. The method was also labor intensive requiring multiple centrifugation and phase separation steps. Rapid MT method also required overnight for tissue digestion but took a lower amount of labor in the later processes. This method had only one centrifugation and Phenol: Chlorophorm: Isoamyl Alcohol (25:24:1) was not used but for the extraction of protein, isopropanol was used and DNA washing was done with 70% alcohol which required much less time than other methods. Similar to Rapid MT method, tissue lysis in SNET method required overnight incubation for separation of DNA multiple centrifugation used with Phenol: Chlorophorm: Isoamyl Alcohol (25:24:1) and chilled isopropanol [18, 27]. TNES method also required overnight incubation for tissue lysis but did not take any additional time for precipitation because NaCl salt was used along with absolute ethanol during precipitation. Salt out method is unique from other methods as Phenol: Chlorophorm: Isoamyl Alcohol (25:24:1) was not used in any step and highly concentrated NaCl, which is a chaotropic salt was used to extract the proteins from the cell lysates [28]. However, the method required overnight incubation, multiple centrifugation and in addition, 2 hours for precipitation of DNA in absolute ethanol. Also, because no measure was taken in earlier steps to remove RNA molecules, this method required the application of RNase at the end. In our study, we did not use RNase and obtained RNA impurities indicated by absorbance (A_{260}/A_{280}> 2) of the isolated DNA. In agarose gel, all of the five methods showed distinct bands of DNA along with smear of DNA of shorter length (Fig. 1).

As summarized in Table 2, the DNA extracted by these methods were relatively pure. Good-quality DNA will have a ratio of 1.7–2.0 [29]. Urea-SDS method produced good quality of DNA from both fish species (value of A_{260}/A_{280} for *L. rohita* was 1.77 ± 0.06 and for *T. ilisha* was 1.74± 0.04). Among all methods, Rapid MT method produced highest quality of DNA from *L. rohita* (value of A_{260}/A_{280} was 1.82 ± 0.04) while DNA produced from *T. ilisha* was not of good quality (value of A_{260}/A_{280} was 1.69 ± 0.05). So, the lowest quality of

DNA (1.63 ± 0.00) was extracted from *L. rohita* by TNES method while highest quality of DNA (2.00 ± 0.06) was extracted from *T. ilisha* by Salt out method. Salt Out method proved to be the most efficient. It has been reported that high molecular weight DNA can be prepared with no organic solvents in salt out protocol that is useful for restriction enzyme digestion and Southern blotting and moreover the DNA is also stable enough to be stored in a refrigerator for long periods without subsequent degradation [23]. High concentration of NaCl disrupts protein structures by interfering with hydrogen bonding, Van der Waals interactions and hydrophilic/hydrophobic interactions, because the high concentration of salt competes with the proteins and other macromolecules for the aqueous solvent, effectively dehydrating the protein/macromolecules and purified sample can be obtained after removing insoluble cellular proteins by centrifugation or filtration [30]. Moreover, NaCl might preserved the structure of DNA molecules [31]. Surprisingly, Rapid MT method was solely successful and better performed in isolating DNA from *L. rohita* fish tissue sample than Urea-SDS method when quality was considered. TNES method yielded lowest quality of DNA from both tissue samples which might be due to the poor solubility of SDS in solutions with high salt concentration and precipitation at low temperature.

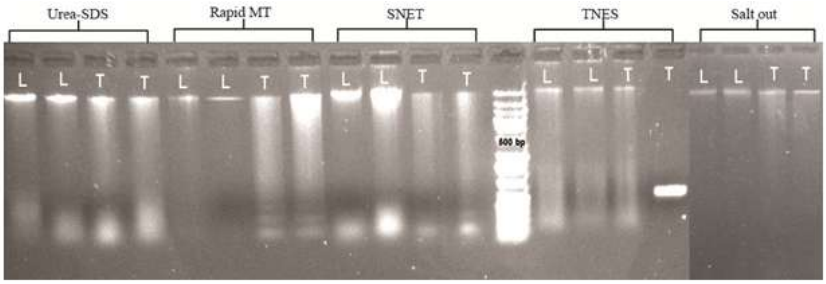

Fig. 1 Agarose gel photo showing DNA bands from five different methods. L=*Labeo rohita* and T = *Tenualosa ilisha*

One-way analysis of variance (ANOVA) was performed to pin-point the significance of differences in outcome of different methods and for different samples. The P value suggests that no significant difference exists for DNA isolation of different tissue type using same method however, difference exist for DNA isolation of same tissue using different methods.

Table-2: Comparative DNA yields by different methods of DNA isolation

Methods	Measure of DNA	Tissue type		P value
		Labeo rohita	*Tenualosa ilisha*	
Urea-SDS	Conc. ± SD (ng/µl)	89.30 ± 22.58	111.5 ± 25.40	0.453
	A_{260}/A_{280} ± SD	1.77 ± 0.06	1.74 ± 0.04	0.674
Rapid MT	Conc. ± SD (ng/µl)	39.35 ± 8.33	37.77 ± 5.63	0.845
	A_{260}/A_{280} ± SD	1.82 ± 0.04	1.69 ± 0.05	0.086
SNET	Conc. ± SD (ng/µl)	177.85 ± 49.85	200.72 ± 28.97	0.631
	A_{260}/A_{280} ± SD	1.68 ± 0.00	1.72 ± 0.03	0.188
TNES	Conc. ± SD (ng/µl)	59.77 ± 13.95	67.10 ± 30.00	0.784
	A_{260}/A_{280} ± SD	1.63 ± 0.03	1.67 ± 0.02	0.253
Salt out	Conc. ± SD (ng/µl)	86.90 ± 10.76	75.82 ± 3.72	0.303
	A_{260}/A_{280} ± SD	2.00 ± 0.06	1.90 ± 0.01	0.140
P value	Conc.	0.020*	0.005*	
	A260/A280	0.002*	0.005*	

Because the P value is outside the allowed region of significance for different tissue type the average of DNA concentration and the ratio of absorbance was calculated for each of the methods. Value of the concentration of DNA in table 2 clearly shows that SNET method was the superior method in terms of quantity of isolated DNA from both fish tissue samples (value of DNA concentration of *L. rohita* was 177.85 ± 49.85 ng/µl and *T. ilisha* was 200.72 ± 28.97 ng/µl) whereas Rapid MT method performance was the poorest for the same case (value of DNA concentration of *L. rohita* was 39.35 ± 8.33 ng/µl and *T. ilisha* was 37.77 ± 5.63 ng/µl). The methods can be ranked on the basis of quantity of DNA from both fish tissues as following order: SNET>Urea-SDS>Salt out>TNES>Rapid MT. The SNET method yielded the highest concentration of DNA which might be due to high concentration of NaCl (400 mM) and Proteinase K (400 µg/ml).

IV. Conclusions

Among the five DNA extraction methods, the SNET method is the superior in terms of yield. On the other hand, Urea-SDS method is the superior in terms of quality of extracted DNA and can be used in high

fidelity experiments. So, for most of the downstream applications for fish genetics studies, both the SNET method and Urea-SDS method could be employed.

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