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ANALYSIS OF EFFICIENCY OF SEVEN GENOMIC DNA ISOLATION TECHNIQUES THROUGH APPLICATIONS OF DNA BARCODE, PCR AND NANODROP FROM FINS OF *Notopterus notopterus* (PALLAS, 1769)

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Abstract

Notopterus notopterus is a single species of Genus *Notopterus*. In Pakistan, *N. notopterus* is an important fish for food and ornamental trade. The unlimited catch and water pollution are major causes of rapid decline in *N. notopterus* population. However, genetic study is crucial for its conservation. DNA isolation is the first key step of genetic studies. With this objective, we compared the efficiency of seven genomic DNA isolation techniques. DNA was isolated from fins of *N. notopterus* because fins were used in small quantities and there was no detrimental effect on fish. Isolated DNA concentration and purity were measured with NanoDrop. PCR amplification and barcoding of mitochondrial COI gene were also used to analyse the purity of isolated DNA. Mitochondrial COI was selected because it is universally used genetic marker for genetic studies of species. Among all methods, GeneJET Genomic DNA Purification Kit was found significantly higher in terms of isolated DNA concentration (894 ng.µl^{-1}), yield (178.3 µg.µl^{-1}), purity (1.7 ng.µl^{-1}), successful PCR amplification and barcode sequencing with 612 base pairs of *N. notopterus* as compared to investigated six traditional DNA isolation methods. GeneJET Genomic DNA Purification Kit was proved the best in terms of cost and labour and the least hazardous for a handler to perform. Present study has also revealed that the traditional DNA isolated methods are the secondary choice for isolation of DNA from fish fins. Moreover, information about the best genomic DNA isolation technique, from this study can be significant for many molecular techniques such as PCR amplification and gene barcode sequencing among others.

INTRODUCTION

DNA isolation is extremely essential for molecular identification, genetic diversity and genetic evaluation of fish species. Recently, DNA isolation techniques consider fascinating, if it is non-destructive for the organism. DNA isolation from fins is beneficial in case of fish species because fins are non-destructive tissues and required in small quantity [1]. So, fish fins can be used without any possible harm to the animals.

Traditional DNA isolation techniques like phenol chloroform method, TNES method and Urea SDS (Sodium Dodecyl Sulphate) methods are commonly used to extract DNA [2]. Though, this type of traditional methods produces suitable results but these are laborious and overwhelming. The reagents which are used in traditional methods can contaminate the isolated DNA chemically [3]. Nowadays, many commercial kits are introduced for DNA isolation. However, it is necessary to compare the efficiency of commercially available DNA isolation kits with traditional DNA isolation techniques [2]. According to the best of our

knowledge, comparative study of seven different DNA isolation techniques from different fins of *Notopterus notopterus* has not been published yet. Therefore, this is the first attempt to compare the efficiency of commercially available DNA isolation kits with six traditional DNA isolation techniques and isolated the DNA from fins of *N. notopterus*. Considering the variety of fish species and fish products from the food industry, the identification of universal DNA isolation method from different fin tissues of fish would be valuable and useful for molecular applications.

The basic objective of the present study was to isolate the high concentration, maximum yield and purity of DNA from the fins (dorsal fins, pectoral fins, anal fins and caudal fins) of *N. notopterus* that could be subjected to effective PCR amplification and gene barcoding. With this objective, one commercially available GeneJET Genomic DNA Purification Kit was compared with six traditional DNA isolation methods (i.e. Phenol chloroform method, TNES method, Rapid MT method, Urea SDS method, Salt out method and SNET method) and analysed the efficiency of isolated DNA in terms of high concentration, yield, purity, amplification of PCR and mitochondrial COI gene sequencing of *N. notopterus* species, available in Pakistan.

MATERIALS AND METHODS

The study was approved in meeting of Board of Studies (BOS) Zoology dated 06-03-2020 and then case was approved by Advance Study and Research Board (ASRB) dated 26-11-2020. The Advance Study and Research Board (ASRB) issued letter in approval of study with No. Acad/Scholar's File/582.dated 23-01-2021. The experiments were carried out according to guidelines of the Advance Study and Research Board (ASRB) of Bahauddin Zakariya University Multan, Pakistan.

List of Materials

Chemicals and Instruments Used

Liquid nitrogen, digestion solution, proteinase K, Tris-HCL, EDTA, SDS, urea, absolute ethanol, NaCl, isopropanol, RNase, phenol, chloroform, isoamyl alcohol, primes Fish F1 and Fish R1, Master Mix, nuclease free water, NanoDrop, PCR tubes, dissection box, eppendorf tube, shaking incubator, vortex mixer, centrifugation machine, PCR machine, agarose gel, gel electrophoresis, illuminator.

Study Area and Sample Collection

A total of seventy *Notopterus notopterus* specimens were collected from Marala Headworks, River Chenab, Pakistan (74°46'E, 32°67'N) (Fig. 1).

The collected specimens were transported to the Fisheries Research Laboratory, Institute of Pure and Applied Biology, Bahauddin Zakariya University Multan, Pakistan. Fish fin tissues (dorsal fins, pectoral fins, anal fins, and caudal fins) selected for DNA isolation were removed and put in a sterile 2 mL Eppendorf tube for ensuing analysis. The seven different DNA extraction techniques; GeneJET Genomic DNA Purification Kit, TNES method, Phenol chloroform method, SNET method, Urea SDS method, Salt out method and Rapid MT method were used for DNA extraction.

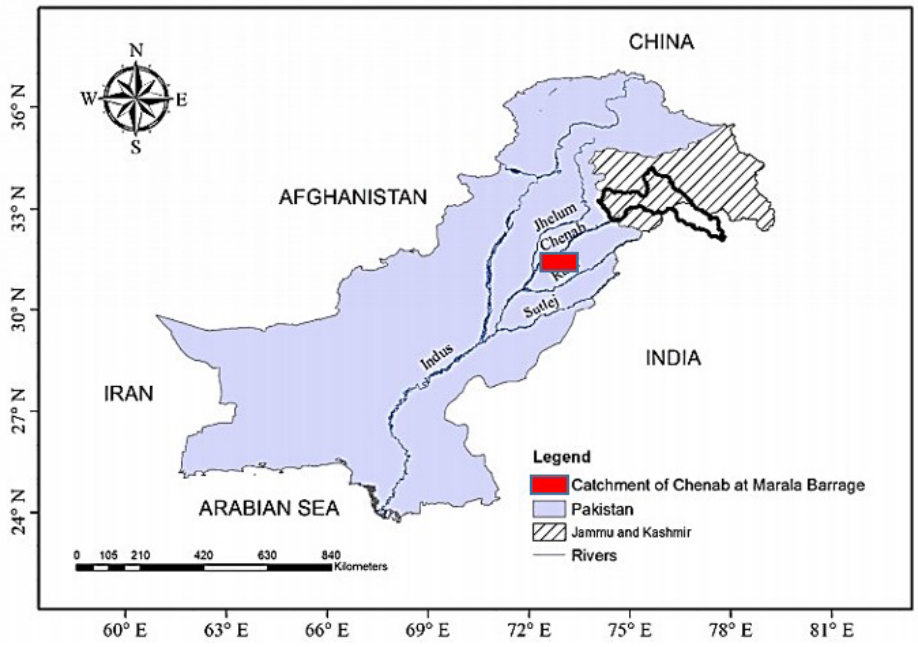

Figure 1. Sampling site and study area, Marala Headworks, River Chenab, Pakistan

DNA Isolation

DNA was isolated and quantified at the fisheries and Molecular Biology Laboratory of Institute of Pure and Applied Biology. From the literature, six traditional techniques Phenol chloroform method [4]; TNES method [5]; SNET method [6]; Urea SDS method [7]; Salt out method [6]; and Rapid MT method [8] of DNA isolation were selected. Seventh, a commercially available GeneJET Genomic DNA Purification Kit was proposed, evaluated and compared. The descriptions of procedures of seven DNA isolation techniques are described as under.

GeneJET Genomic DNA Purification Kit

GeneJET Genomic DNA Purification Kit (Thermo Scientific #K0721. Pub. No. MAN0012663) was used to isolate DNA. Fifty milligram fish fin tissues were clipped in liquid nitrogen and homogenized. The mixture was put into micro-centrifuge tube and added 180 µl digestion solution. Then 20 µl Proteinase K was added, mixed with vortex mixer and incubated at 56°C. After that 20 µl RNase solution was added and mixed with vortex mixer and incubated at room temperature for 10 minutes. Then 200 µl lysis solution was added and mixed with vortex mixer for 15 seconds. After that 400 µl ethanol (50%) was added and mixed. Then lysate was transferred into GeneJET Genomic DNA Purification column. The column was centrifuged at 6000 rpm for 1 minute. GeneJET Genomic DNA Purification column was put into a new 2 ml micro-centrifuge tube. Then 500 µl Wash Buffer I was added containing ethanol. It was centrifuged at 8000 rpm for 1 minute. Wash Buffer II 500 µl with ethanol was added into GeneJET Genomic DNA Purification Column. It was centrifuged at 12000 rpm for 3 minutes. The GeneJET Genomic DNA Purification Column was transferred into micro-centrifuge tube and added 200 µl elution buffer to elute genomic DNA. After that it was incubated at room temperature for 2 minutes and centrifuged at 8000 rpm for 1 minute. Then purified DNA was collected and stored at -20°C.

The principle of separation of different DNA isolation methods are provided in Table 1.

Table 1. The principle of DNA isolation methods

Method	Separation principle employed
GeneJET Genomic DNA Purification Kit	Liquid nitrogen, digestion solution and Proteinase K used for lysis, absolute ethanol used for precipitation of DNA
Phenol Chloroform method	SDS and proteinase K used for lysis; absolute ethanol used for precipitation of DNA
TNES method	SDS, urea, proteinase K and NaCl used for lysis; PCl used to removed proteins; absolute ethanol and NaCl used for precipitation of DNA
Urea SDS method	SDS, urea and proteinase K used for lysis; PCI and NaCl used to remove proteins; absolute ethanol used for DNA precipitation
SNET method	proteinase K, NaCl and SDS used for Lysis with; PCI extraction used to remove proteins; isopropanol used for DNA precipitation
Rapid MT method	SDS, proteinase K and NaCl used for Lysis; after cell lysate centrifugation using isopropanol precipitate of DNA was directly formed from supernatant
Salt out method	SDS, NaCl and proteinase K used for Lysis; NaCl used to remove proteins; absolute ethanol used for precipitation of DNA

TNES Method

Fish fin tissues 50 mg was put in 800 µl of buffer (Tris-HCl 10 mM, NaCl 125 mM, EDTA 10 mM, SDS 0.5%, urea 4 M), homogenized it by adding 10 µl of RNase and incubated at 42^0C for 1 hour. 10 µl Proteinase K was added after incubation of 1 hour. Then incubate it overnight at 42^0C. Then 800 µl of phenol, chloroform and isoamyl alcohol were added with the ratio of 25:24:1 respectively. After that it was centrifuged at 10,000 rpm for 15 minutes. DNA pellets were formed in 1 M NaCl. Then 70% ethanol was added to wash DNA and left it for air dried. Then 60 µl of nuclease free water was added to re-suspend DNA pellets [5].

Phenol Chloroform Method

The fish fin tissues 50 mg were taken and homogenized in DNA extraction buffer. Proteinase K 12 µl was added in paste and mixed it with vortex mixer and incubated it at 37^0C for 1 hour. Then again it was incubated at 55^0C for 1 hour. After that it was centrifuged at 5000 rpm for 10 minutes. Then collected the supernatant and added phenol, chloroform and isoamyl alcohol with ratio 25:24:1 respectively. Then again it was centrifuged at 12000 rpm for 10 minutes and collected the supernatant. Add chloroform and isoamyl alcohol with ratio 24:1. Then again it was centrifuged at 12000 rpm for 10 minutes and collected the

supernatant. 0.1 volume 3 M sodium acetate and equal volume of 100% ice cold ethanol were added. Then put it into micro-centrifuged tube at -20^{0}C for 1 hour. After that it was centrifuged at 1000 rpm for 10 minutes. The pellets of DNA were formed at base of the tube. DNA pellets were collected and added 100 µl of 70% ethanol and centrifuged at 1000 rpm for 10 minutes. Then 60 µl nuclease free water was added to dissolve the pellets of DNA [4].

Urea SDS Method

Fish fin tissue sample of 50 mg was homogenized in 100 µl of TESU6 buffer (Tris-HCL 10 mM with 8.0 pH, EDTA 20 mM with 8.0 pH, SDS 2%, Urea 6 M), 12 µl proteinase K and mixed with vortex mixer. In a shaking incubator incubated at 55^{0}C for overnight with oscillation of 200 rpm. Then gently it was mixed with adding 10 µl NaCl (5 M). After that phenol, chloroform, isoamyl alcohol with ratio 25:24:1 was added and centrifuged it at 10,000 rpm for 5 minutes. Then collect the supernatant and added equal volume of isopropyl alcohol (chilled). It was gently mixed and kept it at -20^{0}C. Then centrifuge it at 10,000 rpm for 5 minutes. DNA pellets were formed at the base of the tube. Then DNA pellets were washed with 70% alcohol (chilled). After that 60 µl of nuclease free water was added to re-suspend DNA [7].

SNET Method

In 500 µl buffer (Tris-Cl 20 mM, NaCl 400 mM, SDS 1%, Proteinase K 400 µg/ml EDTA5 mM) 50 mg fish fin tissues were homogenized and left it for overnight in a shaking incubator at 55^{0}C with oscillation of 200 rpm. The phenol, chloroform, isoamyl alcohol was added with ratio 25:24:1 respectively. After that it was placed at room temperature in shaking incubator for 30 minutes. After that it was centrifuged at 14000 rpm for 5 minutes. Then collect the supernatant and added isopropanol (chilled) with equal volume. Then it was centrifuged at 8000 rpm for 15 minutes. DNA pellets were formed at the base of the tube. Then DNA pellets were washed with 70% ethanol. After that 60 µl of nuclease free water was added to re-suspend DNA pellets [6].

Rapid MT Method

Fish fin tissue 50 mg were taken and homogenized it in extraction buffer (NaCl 200 mM, SDS 0.2%, EDTA 5 mM, Tris-HCl 100 mM). Then 10 µl of Proteinase K was added and mixed with vortex mixer. Then it was incubated at 55^{0}C overnight. After incubation the mixture was centrifuged at 12,000 rpm for 15 minutes. After that 400 µl isopropanol was added in collected supernatant and mixed gently. Then centrifuged at 12,000 rpm for 20 seconds and pellets of DNA were formed at the base of micro-centrifuge tube. DNA pellets were washed with 70% alcohol and re-suspended the DNA pellets in 60 µl nuclease free water [8].

Salt out Method

Fish fin tissues 50 mg were homogenized in 550 µl buffer (EDTA 50 mM, Tris-HCl 50 mM, SDS 1%, NaCl 100 mM) and 7 µl proteinase K was added. Then incubated overnight in a shaking incubator at 50^{0}C with oscillation of 200 rpm and added 600 µl NaCl (5 M). After that it was centrifuged at 12,000 rpm for 10 minutes. Aqueous layer was collected and transfer into new micro-centrifuge tube. After that chilled 700 µl ethanol was added and put micro-centrifuge tube at -20^{0}C for 2 hours. It was then centrifuged at 12,000 rpm for 10 minutes. DNA pellets were formed and washed with 70% ethanol. Then nuclease free water was added to re-suspend the DNA pellets [6].

Quantification and Visualization of Extracted DNA In Terms of Concentration and Purity

NanoDrop was used to evaluate the concentration and purity of isolated DNA. NanoDrop provides accurate value of DNA concentration and purity at absorbance ratio of A_{260}/A_{280} [9]. Isolated DNA value 1.7–2.0 at absorbance ratio of A_{260}/A_{280} is considered pure, high quality free from protein and contamination [15]. Every sample was analysed three times.

Evaluation of PCR Amplification

The PCR amplification was also used to evaluate the purity and concentration of isolated DNA. PCR purified products were barcoded to observed the purity and concentration by calculating number of base pairs of each sequence and identify the *N. notopterus* fish species using mitochondrial COI genetic marker.

The PCR amplification was successfully completed using the primes Fish F1 and Fish R1 are provided in Table 2.

The total PCR reaction volume was 25 µl with a DNA template of 1.5 µl, 12.5 µl PCR Taq Nova-Red, PCR Master Mix (BLIRT S.A.), 0.1 µl forward primer, 0.1 µl reverse primer and 10.8 µl sterile (nuclease free) water. The condition for PCR thermal cycler, initial denaturation was set at 95°C for 2 minutes, further 30 complete cycles with denaturation was set at 95°C for 30 seconds, annealing at 54°C for 40 seconds and extension at 72°C for 1 minute. The final extension at 72°C was set for 7 minutes. The success of PCR amplification was checked on 2% (w/v) agarose gel by running the PCR products.

Table 2. The detail of primers used for PCR amplification

Target gene	Primer ID	Prime sequence 5′-3′	Temperature °C	GC %	Primer size (nt) bp
Mitochondrial COI	Fish F1 COI	TCAACCAACCACAAAGACATTGGCAC	61	46.15	26
Mitochondrial COI	Fish R1 COI	TAGACTTCTGGGTGGCCAAAGAATCA	61	46.15	26

DNA Barcoding Sequence Comparison of Seven Genomic DNA Isolation Methods

PCR purified products were sent to First BASE Laboratories Sdn Bhd, Malaysia. The *N. notopterus* barcoded COI sequences were BLAST with nucleotide database of NCBI (National Center for Biotechnology Information) and examined the accurate identity match. DNA barcoded sequences which provide 100% similarity of *N. notopterus* were submitted in the GenBank database as reference. The number of base pairs of each barcoded sequence was counted.

Statistical Analysis

Isolated DNA significant level of concentration and purity were analysed using one way analysis of variance (ANOVA) with LSD post hoc test. SPSS software was used for statistical analysis of seven different DNA isolation techniques. The different DNA isolation methods relative to the isolated DNA concentration and DNA purity were compared by using one-way ANOVA with LSD post hoc test. The statistically significant differences of the isolated DNA concentration and DNA purity of seven different DNA isolation techniques were evaluated at a level of 5% ($P<0.05$).

RESULTS AND DISCUSSIONS

Isolated DNA Concentrations and Purity Analysis

NanoDrop quantification of DNA concentration and purity was evaluated at optimal absorbance values at wavelength A_{260}/A_{280}. DNA absorbance range of 1.7–2.0 at A_{260}/A_{280} is considered pure [15]. High concentration of isolated DNA with GeneJET Genomic DNA Purification Kit was found (894 ng μl^{-1}) higher as compared to six investigated traditional DNA isolation methods. The purity of isolated DNA with GeneJET Genomic DNA Purification Kit was found (1.7-2 ng.μl^{-1}) higher as compared to six investigated traditional DNA isolation methods. The comparison of isolated DNA in terms of concentration and purity is provided in Table 3.

Comparison of mean isolated DNA concentration shown in Figure 2 and mean isolated DNA purity is shown in Figure 3.

The isolated DNA concentration and purity with GeneJET Genomic DNA Purification Kit were found significantly ($P<0.05$) higher as compared to investigated traditional DNA isolation methods. However, Urea SDS method, Rapid MT method, Phenol chloroform method, Salt out method and SNET method concentration and purity were not significantly ($P>0.05$) higher in all samples of fins as compared to GeneJET Genomic DNA Purification Kit.

Table 3. The isolated DNA comparison in terms of concentration and purity with seven different DNA isolation methods from fins (dorsal fin, pectoral fin, anal fin and caudal fins) of *Notopterus notopterus*

Method	DNA concentration and Purity range (ng.μl^{-1}) ± SD							
	Dorsal fin		Pectoral Fin		Anal Fin		Caudal Fin	
	Conc. ± SD	Purity± SD	Conc. ± SD	Purity± SD	Conc. ± SD	Purity± SD	Conc. ± SD	Purity± SD
GeneJET Genomic DNA Purification Kit	781-888 ±29.50	1.90-1.98 ±0.02	838-893 ±22.44	1.91-2.00 ±0.03	805-891 ±29.81	1.91-2.00 ±0.03	816-894 ±29.43	1.90-2.00 ±0.04
Phenol Chloroform method	521-650 ±49.48	1.70-1.88 ±0.06	576-693 ±35.98	1.71-1.98 ±0.09	705-809 ±35.15	1.19-1.89 ±0.27	590-694 ±32.68	1.70-1.92 ±0.06
TNES method	669-769 ±35.10	1.76-1.91 ±0.06	710-790 ±28.99	1.75-1.98 ±0.07	716-793 ±32.27	1.75-1.98 ±0.07	702-882 ±24.58	1.76-1.92 ±0.06
Urea SDS method	438-593 ±57.05	1.53-1.63 ±0.04	502-602 ±34.95	1.41-1.60 ±0.07	593-691 ±29.95	1.44-1.65 ±0.06	508-608 ±31.50	1.40-1.60 ±0.07
SNET method	305-495 ±58.84	1.13-1.43 ±0.11	205-499 ±88.09	1.31-1.47 ±0.06	405-499 ±37.67	1.25-1.49 ±0.07	446-594 ±52.23	1.41-1.54 ±0.05
Rapid MT method	201-309 ±39.31	1.21-1.34 ±0.04	311-49 ±67.16	1.41-1.54 ±0.06	81-191 ±34.92	1.11-1.46 ±0.11	302-454 ±53.33	1.12-1.39 ±0.08
Salt out method	113-220 ±37.02	1.11-1.38 ±0.09	221-355 ±39.69	1.12-1.35 ±0.06	145-325 ±60.05	1.11-1.38 ±0.08	205-321 ±41.61	1.11-1.39 ±0.10

SD = Standard Deviation; Conc. = Concentration

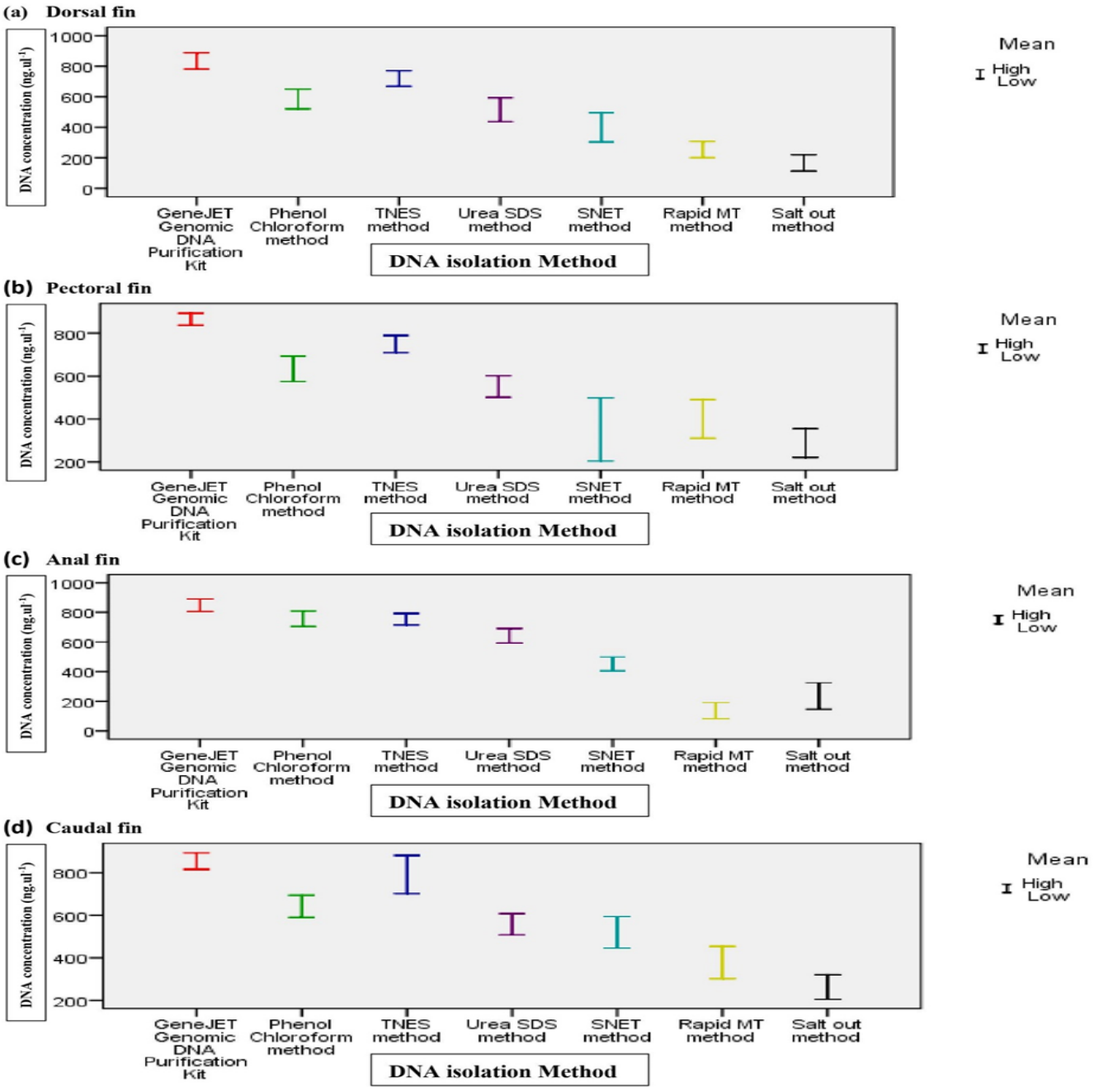

Figure 2. A comparison of mean isolated DNA concentration obtained with seven different methods (GeneJET Genomic DNA Purification Kit, Phenol chloroform method, TNES method, Urea SDS method, SNET method, Rapid MT method and Salt out method) from (a) dorsal fin; (b) pectoral fin; (c) anal fin;(d) caudal fin of *Notopterus notopterus*. High and low bars indicate the mean concentration values

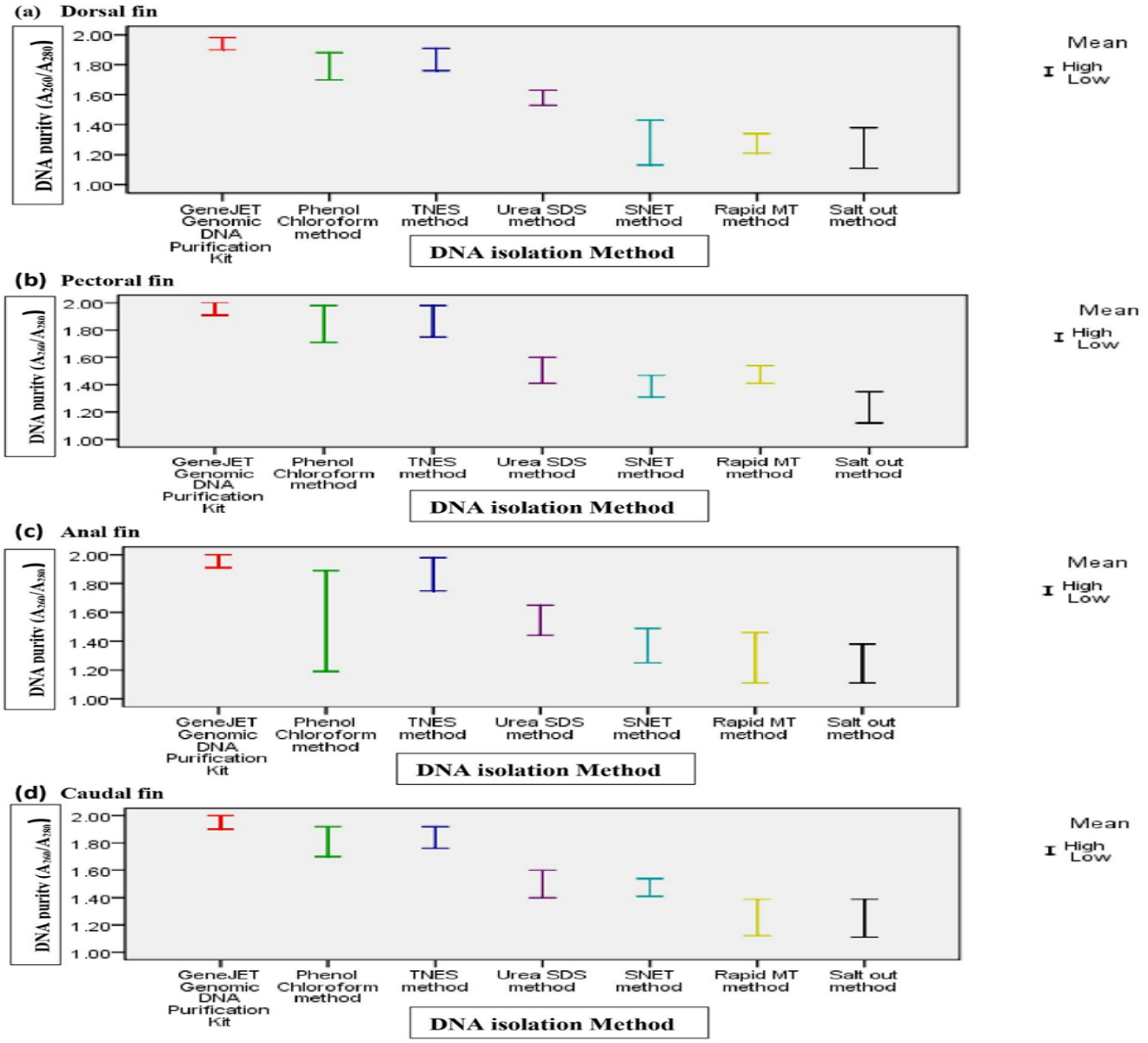

Figure 3. A comparison of mean isolated DNA purity obtained with seven different methods (GeneJET Genomic DNA Purification Kit, Phenol chloroform method, TNES method, Urea SDS method, SNET method, Rapid MT method and Salt out method) from (a) dorsal fin; (b) pectoral fin; (c) anal fin;(d) caudal fin of *Notopterus notopterus*. High and low bars indicate the mean purity values

Isolated DNA Purity Range Within, Below and Above Satisfactory Limits of Total Samples

The isolated DNA proportion from fins (dorsal fins, pectoral fins, anal fins, caudal fins) of *N. notopterus* species indicated that GeneJET Genomic DNA Purification Kit 100% samples were found within purity range (1.7-2.0) and considered pure and of good quality. Among traditional methods, 100% samples of TNES methods were observed within purity range (1.7-2.0). Comparison of Isolated DNA purity range within, below and above satisfactory limits of seven DNA isolation techniques are provided in Table 4.

Table 4. The isolated DNA comparison in terms of purity of isolated DNA within, below and above satisfactory limits

DNA isolation Method	Total samples	Number of samples within purity range (1.7-2.0)	Samples below purity (1.7)	Samples above purity (2.0)	%age of samples in purity 1.7-2.0 range
GeneJET Genomic DNA Purification Kit	40	40	0	0	100%
Phenol Chloroform method	40	39	1	0	97.5%
TNES method	40	40	0	0	100%
Urea SDS method	40	0	40	0	0%
SNET method	40	0	40	0	0%
Rapid MT method	40	0	40	0	0%
Salt out method	40	0	40	0	0%

PCR Amplification Success

PCR was successfully amplified with DNA which was isolated by all methods. As compared to all investigated methods the highest nucleotide base pair band range in all fins was observed in DNA which was isolated by GeneJET Genomic DNA Purification Kit. However, lowest base pair band range was observed in PCR product of DNA isolated with TNES method. The success of PCR amplification was checked on 2% (w/v) agarose gel by running the PCR products. The comparison of PCR amplification of DNA isolation methods in dorsal fins, pectoral fins, anal fins and caudal fins is shown in Figure 4.

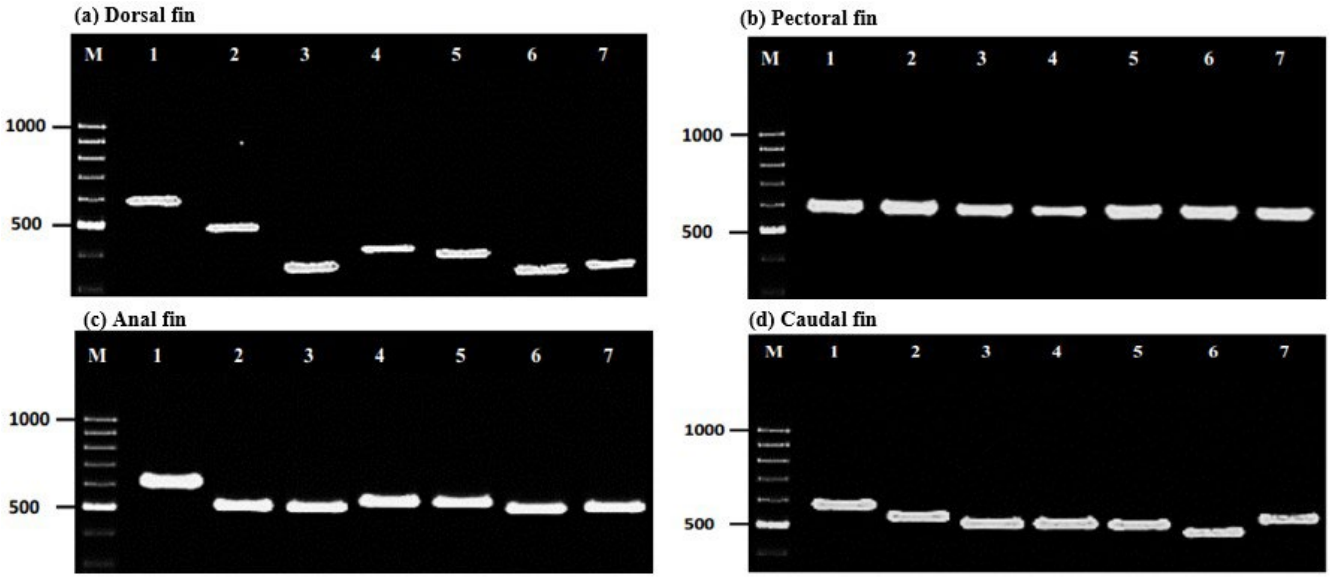

Figure 4. the comparison PCR amplification between seven different DNA isolation techniques. Lane 1; GeneJET Genomic DNA Purification Kit, Lane 2; Phenol chloroform method, Lane 3; TNES method, Lane 4; Urea SDS method, Lane 5; SNET method, Lane 6; Rapid MT method, Lane 7; Salt out method and M; Standard marker in (a) dorsal fin; (b) pectoral fin; (c) anal fin;(d) caudal fin of *Notopterus notopterus*

Mitochondrial COI Gene Barcoding and Data Analysis of BLAST

In present study, PCR amplified fish samples of seven investigated methods were barcoded. All barcoded sequences were BLAST (NCBI database) to compare the identity match. BLAST analysis revealed that only four barcoded sequences showed 100% similarity and accuracy with relevant sequences of GenBank databases of mitochondrial region and confirmed *N. notopterus* identity while none of the traditional DNA methods provided 100% barcode similarity and accuracy. The four DNA barcoded sequences which showed 100% similarity and accuracy were the product of DNA which was isolated with GeneJET Genomic DNA Purification Kit. These four barcoded sequences were submitted to the GenBank databases as reference with Accession MZ798274.1, MZ798275.1, MZ798272.1, MZ798273.1. Among all DNA barcoded

sequences, PCR products of DNA isolated with GeneJET Genomic DNA Purification Kit provided highest number of nucleotide 612 base pairs.

Economic Feasibility

The GeneJET Genomic DNA Purification Kit provided high yield (178.3 µg.µl^{-1}) of extracted DNA from a single sample. The cost per microgram calculation of isolated DNA showed that GeneJET Genomic DNA Purification Kit was estimated to be a cost effective and economically feasible approach in terms of concentration and yield of isolated DNA per microgram, per individual sample. Comparison relative to cost of isolated DNA is provided in Table 5.

Table 5. The comparison relative to the costs per microgram of isolated DNA per sample with seven different DNA isolation methods

DNA isolation Method	Per DNA extraction cost	Mean isolated DNA yield (µg.µl^{-1})	Cost per µg of extracted DNA
GeneJET Genomic DNA Purification Kit	2.00 USD	178.3	0.20 USD
Phenol Chloroform method	2.01 USD	142.3	0.20 USD
TNES method	2.00 USD	161.7	0.20 USD
Urea SDS method	1.66 USD	124.7	0.16 USD
SNET method	1.86 USD	104.3	0.18 USD
Rapid MT method	1.68 USD	72.25	0.16 USD
Salt out method	1.66 USD	61.05	0.16 USD

Safety Considerations

In seven different DNA isolation techniques, the used reagents were evaluated in terms of safety for handler. Out of seven assessed DNA isolation techniques, the GeneJET Genomic DNA Purification Kit was proved highly safe and had no health concerns for user while phenol and chloroform possibly had highest safety and health concerns for user. The phenol and chloroform are considered as extremely dangerous. The phenol burns the skin and poisons the eyes due to its high corrosive nature [16]. The chloroform is sensibly expected to be a carcinogen [17] and damage reproductive system [18]. The aforesaid safety hazards are perhaps the main reasons that's why numerous laboratories worldwide no longer use phenol chloroform DNA isolation technique while the use of commercially available kits does not cause any serious safety and health dangers, conferring to information provided by the manufacturers [19].

High quality DNA isolation provides the basis of genetic studies. Quality of isolated DNA mainly depends upon its isolation method and the organ used for its isolation [20]. Mostly soft tissues (i.e. muscles, blood and liver) are used for DNA extraction in case of fishes, but DNA isolation from muscles, blood and liver tissues is achieved with the sacrifice of animals [1]. Therefore, the isolation of DNA from muscles, blood and liver tissues for genetic studies are not desirable. For DNA isolation fin tissues are seemed to be attractive and desirable because fins are required in small quantity and are not detrimental to fish [14]. In present study, we used fins for DNA isolation and compared the efficiency of seven genomic DNA isolation methods. The main objective of the present study was to measure the efficiency differences of isolated DNA in terms of concentration, purity, and amplificability of isolated DNA from seven genomic DNA isolation methods. Finally, we compared the efficiency of the one commercially available DNA isolation kits with six traditional DNA isolation methods.

DNA concentration was determined by using the NanoDrop, we calculated the differences in the efficiency of each particular method (Table 3). The highest isolated DNA concentration was found with GeneJET Genomic DNA Purification Kit in caudal fin (894 ng.µl^{-1}) of *N. notopterus*. The lower amounts of isolated DNA were detected with Phenol chloroform method in anal fin (809 ng.µl^{-1}) while the lowest concentrations of isolated DNA were observed with Rapid MT method in anal fin (81 ng.µl^{-1}) of *N. notopterus* (Table 3).

The purity and quality of isolated DNA was determined by calculating the absorbance value at A_{260}/A_{280}, mostly samples were in the purity range of 1.7–2.0 (Table 4). The samples outside the purity range may have contamination of proteins and other constituents. The values of Table 4 indicates that GeneJET Genomic DNA Purification Kit and TNES method were found the best in terms of purity (1.7-2.0) and their all samples were 100% within purity range as compared to Phenol chloroform method, Urea SDS method, SNET method, Rapid MT method and Salt out method [4]. In Phenol Chloroform method 97.5% samples were found within purity range while Urea SDS method, SNET method, Rapid MT method and Salt out method were found sub-optimal but not found within purity range (Table 4).

The DNA suitability for PCR amplification was analysed using mitochondrial COI genetic marker to amplify the barcode fragment ranging from 400 to 700 bp of the mitochondrial COI gene. PCR amplification products provided different length of fragment (400 to 700 bp) [21]. The PCR analysis results for individual methods are shown in Fig. 4a-d.

DNA barcode sequence were analysed with relevant sequence of GenBank database [22]. The mitochondrial COI gene barcode sequence of 612 bp sequence in length was obtained in all samples of fin using GeneJET Genomic DNA Purification Kit. The BLAST analysis 100% similarity and accuracy with relevant sequences of GenBank databases of mitochondrial region [23] provides the efficiency evaluation of the isolated DNA method.

The statistical significance of each particular DNA isolation method was compared using the Post hoc test (non-parametric ANOVA) [24]. DNA concentration and purity was found significantly ($P<0.05$) higher with GeneJET Genomic DNA Purification Kit as compared to investigated traditional six DNA isolation methods. DNA concentration of Urea SDS method, Rapid MT method, Phenol chloroform method, Salt out method and SNET method was not significantly ($P>0.05$) higher in all samples of fins as compared to GeneJET Genomic DNA Purification Kit.

In terms of cost of the reagents and DNA yield, different DNA isolation methods varied considerably. The cheapest and economic method is GeneJET Genomic DNA Purification Kit [25], which has cost of 2.00 USD per sample (Table 5) while the most expensive DNA isolation method having a cost of 31.2 USD per sample is reported by Bowers et al. [26]. However, this cost of 2.00 USD per sample is economic and reasonable [27].

In consideration of cost of time and labour usually commercial kits are faster than that of traditional DNA isolation techniques. The estimated extraction time of Commercial GeneJET Genomic DNA Purification Kit is around 2 hours. This DNA isolation time is shorter as compared to other six investigated traditional methods with the exception of modified DNA isolation method as reported by Marsal et al. [25]. The data of this study reveals that the GeneJET Genomic DNA Purification Kit is proved the best in terms of processing time, cost and labour.

The different DNA extraction methods have variation in processing time. As compared to all seven evaluated DNA isolation methods, the incubation period for tissue digestion in GeneJET Genomic DNA Purification Kit is of 1 hour and in Phenol Chloroform method incubation period is of two hours which is very short while all other investigated methods need overnight incubation for tissue digestion. The GeneJET Genomic DNA Purification Kit is less time consuming and less laborious in terms of phase separation [5]. Urea SDS method needs 15 hours to complete the process of fin tissues digestion. In terms of the phase separation and centrifugation steps, it is arduous and time consuming. In Rapid MT method, SNET method, TNES method and Salt out method overnight incubation is essential for tissue needs [10]. These methods also require multiple centrifugation and absolute ethanol for DNA pellets [11].

Proteinase K is used for tissue digestion in each DNA isolation technique while DNA extraction buffer is needed differently according to DNA isolation method [12]. Proteinase K requires 20-60°C temperature for its activity. This 20-60°C wide temperature range is useful to obtain high concentration and purity of DNA [13]. Fin tissues digestion incubation at 42°C for 10 hours would be very important while in present study for the GeneJET Genomic DNA Purification Kit incubation time was only of one hour and for Phenol chloroform method incubation time was only two hours [1]. The use of Proteinase K less than 30µl did not fully break the tissue and contribute DNA of low quantity while in present study; we used just 7-20 µl of Proteinase K resultantly obtained purified and high quantity of DNA was obtained [14]. The purity of isolated DNA with optimal absorbance values at A_{260}/A_{280}. DNA with range of 1.7–2.0 is consider pure and of good quality [15]. Less volume of Proteinase K and less duration of incubation save time and money.

The conclusion of the present study revealed that the GeneJET Genomic DNA Purification Kit was most suitable and the best for DNA isolation from fins (dorsal fins, pectoral fins, anal fins and caudal fins) of *N. notopterus* as compared to six investigated traditional DNA isolation methods (Phenol chloroform method, TNES method, Rapid MT method, Urea SDS method, Salt out method and SNET method) in terms of maximum yield, high concentration and purity of DNA. The DNA isolated with GeneJET Genomic DNA Purification Kit was proved the best in PCR amplification and genome sequencing of mitochondrial COI as compared to six evaluated traditional methods. According to the best of our knowledge, this study is the first attempt to compare the purity of seven genomic DNA isolation techniques on the basis of gene barcode sequencing and the number of nucleotide base pairs are compared in each method. The GeneJET Genomic DNA Purification Kit is relatively safe, easy to use and applicable to obtain DNA. The traditional DNA isolation methods need additional labour and safety issues as compared to commercially used GeneJET Genomic DNA Purification Kit. However, the traditional DNA isolation methods are the secondary choice for the isolation of DNA from fish fins. In six investigated traditional DNA isolation techniques both TNES method and Phenol chloroform method were found good in terms of DNA yield, concentration, purity, PCR amplification and genome sequencing as compared to Rapid MT method, Urea SDS method, Salt out method and SNET method. The TNES method and Phenol chloroform method were found to be optimal while Rapid MT method, Urea SDS method, Salt out method and SNET method were found sub-optimal in terms of purity of isolated DNA among traditional DNA isolation methods. Moreover, information about the best genomic DNA isolation method, from this study can to be suitable for many molecular techniques as PCR amplification and gene sequencing among others.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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