COMPARISON OF THREE DNA EXTRACTION PROTOCOLS BY ASSESSING DNA YIELD AND PURITY ON CATFISH, *CLARIAS* BATRACHUS (LINNAEUS, 1758)

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Abstract

There are various DNA extraction kits available on the market for animal tissue. In the present study, three DNA extraction kits, utilizing the silica solution-based extraction method, the GS solution-based method, and the Proteinase K solution-based method, were employed to extract total genomic DNA from *Clarias batrachus*, and their extraction efficiencies were compared. The quantity and quality of the extracted DNA were evaluated using a Nanodrop spectrometer. The Silica solution-based method yielded DNA concentrations of 19.1 ng/µl, 20.8 ng/µl, and 25.9 ng/µl, with A260/280 purity ratios of 2.05, 2.06, and 1.99, and A260/A230 contamination ratios of 0.06, 0.05, and 0.06, respectively, for three specimens of catfish. The GS solution-based method produced DNA concentrations of 167.2 ng/µl, 75.6 ng/µl, and 372.0 ng/µl, with purity ratios of 1.92, 2.09, and 1.99, and contamination ratios of 0.80, 0.85, and 1.30. The proteinase K solution-based method resulted in DNA concentrations of 124.2 ng/µl, 164.0 ng/µl, and 85.6 ng/µl, with purity ratios of 2.14, 2.09, and 2.14, and contamination ratios of 1.67, 1.73, and 1.35. Time analysis showed that the Proteinase K solution-based method was the fastest, requiring 22 minutes, while the Silica solution-based method and the GS solution-based method took 93 minutes and 82 minutes, respectively. This study highlights the differences in extraction efficiency, quality, and time consumption among the three methods.

Keywords: Three DNA extraction methods, catfish, DNA yield and purity, time consumption

Introduction

DNA extraction is a cornerstone technique in molecular biology, essential for diverse applications such as genetic research, species identification, and biodiversity studies (Alberb *et al.*, 2002). The efficiency and accuracy of DNA extraction directly affect the quality of genetic material, which is critical for downstream applications like PCR, sequencing, and genotyping, which in turn impact the reliability of subsequent analyses (Reddy & Nakano, 2012).

The method chosen for DNA extraction plays a significant role in determining the quality and quantity of DNA obtained, thereby influencing the reliability of subsequent analyses (Miller *et al.,* 1988). Effective DNA extraction is crucial not only for understanding genetic diseases and developing diagnostics and therapeutics but also for forensic science, genome sequencing, pathogen detection, and paternity testing (Wang and Wang, 2018)

Typically, DNA extraction involves several fundamental steps: disruption of cellular structure to create a lysate, separation of DNA from cellular debris, purification of DNA using a matrix, removal of contaminants through washing, and elution of the DNA (Promega product guides, 2024). Various techniques, including physical, enzymatic, and chemical methods, are

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employed for lysing cells and releasing nucleic acids (Wilson, 2001). The effectiveness of DNA extraction methods can vary depending on the species and the specific kit used (Kreader, 1996).

Nowadays, DNA sequencing for species identification of fish is widely used. The fundamental requirement for DNA sequencing is the extraction of DNA from the tissue of the investigated species. Different extraction kits may provide different concentrations and purities of nucleic acid.

The present study aimed to evaluate and compare modified versions of three different DNA extraction methods, focusing on their yield (DNA concentration) and quality (purity and contamination levels), with the following objectives to assess how well each method performs concerning the unique requirements of catfish *Clarias* species tissue samples, considering factors such as time efficiency and ease of use, and to identify the most suitable technique for optimizing DNA extraction in this context.

Materials and Methods

Sample collection

Three individuals of catfish, *Clarias batrachus,* were purchased available at Hledan market, Kamaryut Township in Yangon in May 2024, and immediately carried in frozen condition to the molecular laboratory of the Department of Zoology, University of Yangon. In the laboratory, the fish was confirmed as *Clarias batrachus* using FishBase software.

Preparation of the DNA extraction

For DNA extraction, the muscle tissue was excised from each collected fish specimen and preserved in 70% ethanol to maintain DNA integrity. The samples were labeled as MAF 1, MAF 2, and MAF 3 (Fig.1). Each tissue sample was processed using three distinct DNA extraction methods for comparative analysis of their efficiency. A 25 mg portion of tissue was carefully weighed from each sample and assigned to one of three extraction methods such as (i) Silica-based extraction method product of HiMedia Laboratories Pvt.Ltd, (India): Samples were labeled MAF 1 Sili, MAF 2 Sili , and MAF 3Sili , (ii) GS solution-based extraction method product of Selangor Darul Ehsan, (Malaysia, MD-TNA-100): Samples were labeled MAF 1 GS,MAF 2 GS, and MAF 3 GS, and (iii) Proteinase K-based extraction method product of Qiagen, (Hilden, Germany): Samples were labeled MAF 1 QG,MAF 2 QG, andMAF 3 QG.

The DNA extraction procedures for catfish tissue were carried out according to the protocols specific to each method.

(A) (B)

Figure. 1 (A) Collected catfish species. (B) Fixation and labeling of specimens

Silica solution-based extraction method

Firstly, a new 1.5 ml microcentrifuge tube was prepared and labeled. In that tube, 25 mg of fish tissue sample was suspended in 100 µl of GT buffer, ground by a grinder to be homogenized, and then 800 µl of GT Buffer was added and centrifuged at 12000 rpm for three minutes. Secondly, a new 1.5 ml microcentrifuge tube was prepared with 40 µl of Silica solution. After adding Silica, 600 µl of upper clear solution was transferred from the mixture tube, vortexed for 20 seconds, and then centrifuged at 12000 rpm for three minutes. The supernatant was decanted, and 500 µL of GT Buffer was added to the tube. The mixture was then vortexed for 20 seconds and centrifuged for 3 minutes at 12000 rpm.

The supernatant was decanted, and 1000 µl of 70% Ethanol was added and mixed by vortex for 20 seconds, and centrifuged for three minutes. Furthermore, decanted the supernatant and then pipetted the remaining ethanol along with dried the pellet to ensure no ethanol remains in the tube. In addition to 1000 µl of DEPC ddH_2O was added, mixed by vortex, incubated at 55 ºC for ten min in the incubator, and 12000 rpm for three min. Finally, 500 µl of supernatant was transferred into a new 1.5 ml microcentrifuge tube.

GS solution-based extraction method

A total of 300 µl of GS solution was added into a 1.5 ml microcentrifuge tube with 25 mg of fish tissue sample, that was grinded by disposable grinder, mixed by vortex briefly, incubated the sample tube at 65^0 C for ten minutes, added 150 µl of GS solution 2 into the tube, mixed by inverting five to ten times, incubated the prepared sample on ice for ten minutes and centrifuged at 10,000 rpm for ten minutes.

A new 1.5 ml microcentrifuge tube containing 500 µl of isopropanol was prepared, transferred 300 µl of supernatant was transferred into the isopropanol-prefilled 1.5 ml microcentrifuge tube and mixed by inverting five to ten times. After that, the tube was centrifuged at 10,000 rpm for ten minutes, the supernatant was slowly decanted, and washed the pellet by adding 800 µl of 75% ethanol into the tube, again centrifuged the tube at 10,000 rpm for five minutes, removed supernatant and let the pallet to be dried at room temperature. Finally, the dried pellet was resuspended with 100 µl of GS solution 3.

Proteinase K solution-based extraction method

A total of 180 µl buffer ATL was added into a 1.5 ml microcentrifuge tube with 25 mg of fish tissue sample and 20 µl proteinase K solution, and then mixed by vortex and incubated at 56^0 C until completely lysed. It was Vortexed occasionally during incubation and vortexed for 15 seconds directly before proceeding to step 2. Added 200 µl buffer AL and mixed thoroughly by vortex. 200 µl of ethanol was added to the tube and vortexed thoroughly.

The mixture was transferred the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube and centrifuged at ≥6000 x g (8000 rpm) for 1 min, and the mixture was discarded from the collection tube. The spin column was placed in a new 2 ml collection tube, and 500 µl added Buffer AW1 was added. It was centrifuged for 1 min at ≥6000 x g, and then the discarded collection tube with the mixture. The spin column was placed in a new 2 ml collection tube, and 500 µl Buffer AW2 was added and centrifuged again for 3 min at 20,000 x g (14,000 rpm). Discarded the flow-through and collection tube. The spin column was transferred to a new 1.5 ml

microcentrifuge tube. Finally, eluted the DNA by adding 200 µl Buffer AE to the center of the spin column membrane. It was incubated for 1 min at room temperature (15-25°C) and centrifuged for 1 min at ≥6000 x g.

Recording on time-consuming for each method per sample

In our analysis, we recorded the time taken for steps that were the lysis step, washing step, DNA rehydration, and elution step for each extraction method applied to individual samples according to the time-consuming steps during this conducting experiments. The average time taken (mean) and standard deviation were calculated for each method to assess the variation in processing times. These calculations allowed us to compare the efficiency and consistency of each method based on the time required for processing.

Evaluation of DNA Quality

In this study, based on spectrophotometric evaluation, DNA can be considered pure when the absorbance ratio of A260/280 is 1.8 to 2 and the secondary absorbance ratio of A260/230 is 1.8 to 2.2 for all methods according to (Green and Sambrook, 2012). The quality of DNA was measured using the Nanodrop spectrometer (One C spectrophotometer, USA). The average values were calculated for DNA concentration, purity ratio, and contamination ratio.

Results

In the present study, the total genomic DNA was extracted from three individuals of catfish *Clarias batrachus* species by using three different methods. The quantity and quality of total genomic DNA were measured using 4 µl of each sample on the Nanodrop spectrometer. The DNA concentrations and purity ratios were calculated to assess the quality and quantity of the extracted DNA.

In using Silica solution-based DNA extraction method, the DNA concentration of MAF 1Sili, MAF 2 MAF 3 Sili were 19.1 ng/µl, 20.8 ng/µl, and 25.9 ng/µl, purity ratio of DNA at A260/280 reading were 2.05, 2.06 and 1.99, and the contamination ratio at A260/A230 reading were 0.06, 0.05 and 0.06 respectively from 4 µl of sample (Table 1).

In using GS solution-based DNA extraction method, the DNA concentration of MAF 1GS, MAF 2 GS and MAF 3 GS were 167.2 ng/µl, 75.6 ng/µl, and 372.0 ng/µl, purity ratio of DNA at A260/280 reading were 1.92, 2.09 and 1.99, and the contamination ratio of DNA at A260/A230 reading were 0.80, 0.85 and 1.30 (Table .2).

In using proteinase K solution-based DNA extraction method, the DNA concentration of MAF 1 QG, MAF 2 QG and MAF 3 QG were 124.2 ng/µl, 164.0 ng/µl, and 85.6 ng/µl, purity ratio of DNA at A260/280 reading were 2.14, 2.09 and 2.14, and the contamination ratio of DNA at A260/A230 reading were 1.67, 1.73 and 1.35 (Table. 3).

Table 1. The result of the Silica solution-based extraction method for three samples on Nanodrop spectrometer

Sample	Silica-based extraction method		
	DNA concentration	Purity ratio (A260/280)	Contamination Ratio (A260/230)
MAF 1 Sili	19.1	2.05	0.06
MAF 2 Sili	20.8	2.06	0.05
MAF 3 Sili	25.9	1.99	0.06

Table 2. The result of the GS solution-based extraction method for three samples on Nanodrop spectrometer

Sample	GS solution-based extraction method		
	DNA concentration	Purity ratio (A260/280)	Contamination Ratio (A260/230)
MAF 1GS	167.2	1.92	0.8
MAF 2GS	75. 6	2.09	0.85
MAF 3GS	372	1.99	1.3

Table 3. The result of Proteinase K solution-based extraction method for three samples on Nanodrop spectrometer

Sample	Proteinase K-based extraction method		
	DNA concentration	Purity ratio (A260/280)	Contamination Ratio (A260/230)
MAF1QG	124.2	2.05	1.67
MAF 2 QG	164	2.06	1.73
MAF 3 QG	85.6	2.14	1.35

Table 4. The average time taken for each method per sample

Time taken steps	Silica solution-based	GS solution-based	Proteinase K solution-based
Lysis(min)	25	15	10
Washing(min)	30	45	10
DNA rehydration(min)	18	20	0
Elution(min)	20	2	2
Average	23.25	20.5	5.5
SD	±5.38	±18	±5.25
Total (min)	93	82	22

The average time taken for each method per sample

The average time taken per sample were record for each method, including time spent on lysis, washing, DNA rehydration and elution. The average and standard deviation were as follow 23.23±5.38 minutes in Silica-based extraction method, 20.5±18 minutes in GS solution-based extraction method, 5.5±5.25 minutes in Proteinase K solution-based conduction (Table 4). The total duration required per sample was 93 minutes, 82 minutes and 22 minutes. The longest method was Silica-based extraction method.

Comparison of average DNA Concentration, purity ratio and contamination ratio across of three methods

When comparing the average DNA concentration across three methods, GS solution-based method was the highest DNA concentration with average value (204.93 ng/µL), significantly more than the other two methods. Proteinase K-based method with a moderate concentration (124.6 ng/µL) and Silica solution-based method was the lowest concentration (21.93 ng/µL). (Fig. 2)

When comparing the average DNA purity ratio across three methods, GS solution-based method was the highest purity ratio with average value (2.00), Silica solution-based method was slightly lower purity (2.03) and Proteinase K-based method was the lowest purity (2.08). (fig 3)

When comparing the average DNA purity ratio across three methods, Proteinase K-based method was lower contamination with average value (1.58) and GS solution-based method had a moderate contamination level (0.98). Silica-based method showed the highest contamination (0.06) respectively. (fig 4)

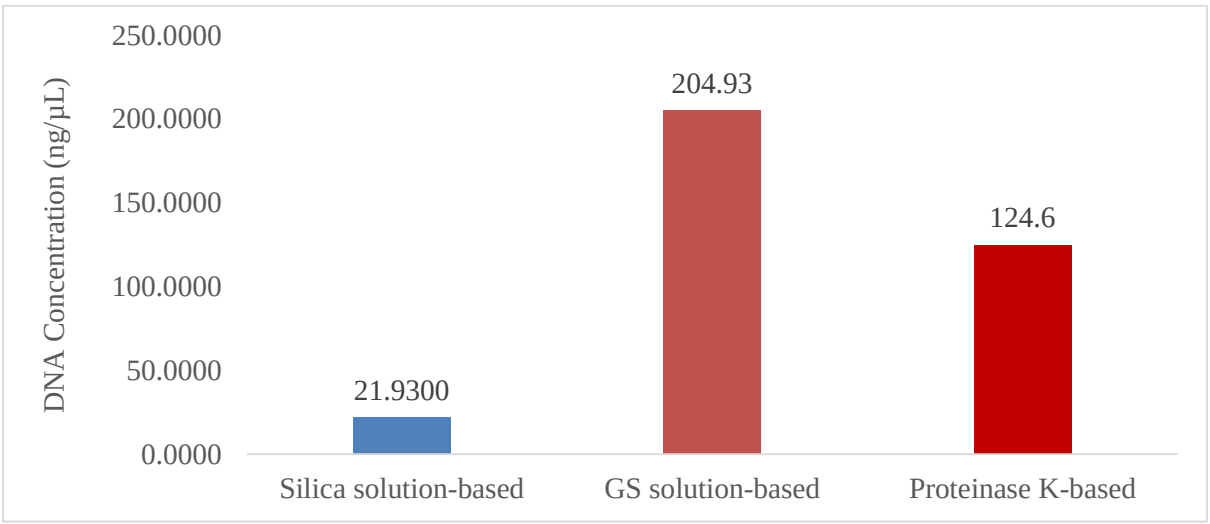

Figure. 2. Comparison on average three methods of DNA concentration

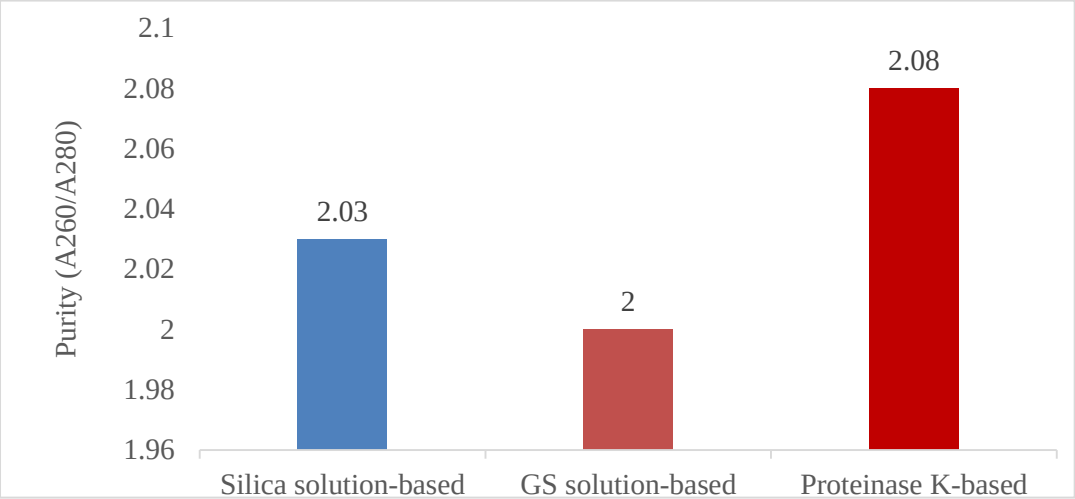

Figure. 3 Comparison on average three methods of DNA purity ratio

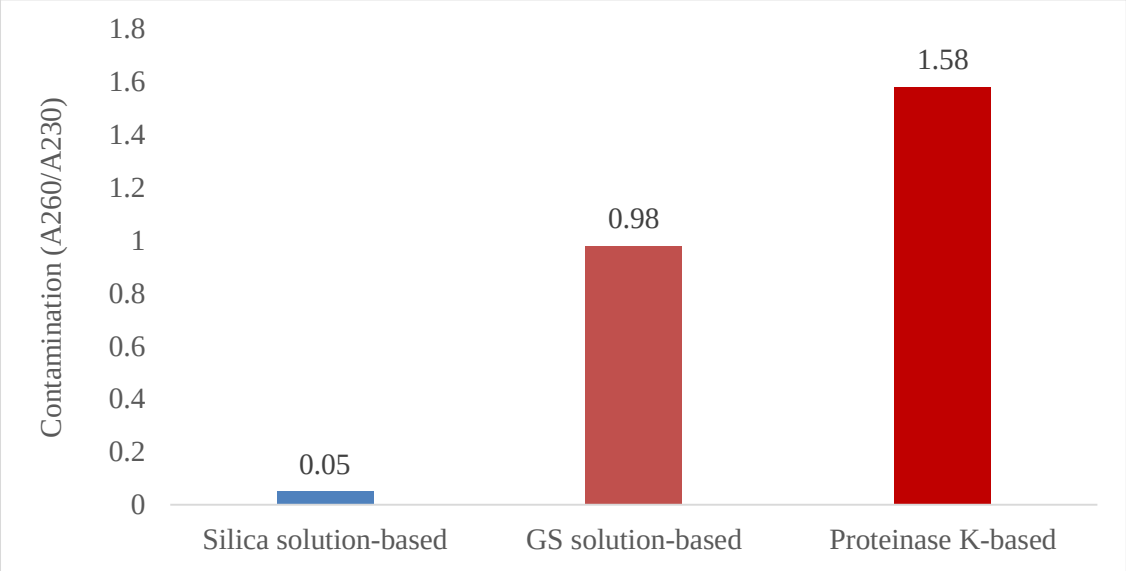

Figure. 4. Comparison on average three methods of contamination ratio

Discussion

The present study evaluated the efficiency of three different DNA extraction methods on catfish specimens: Silica solution-based, GS solution-based, and Proteinase K solution-based methods. DNA concentrations and purity ratios were assessed using a Nanodrop spectrometer, with results obtained from 4 µL of each sample.

The goal was to determine the most suitable method for downstream applications such as PCR and sequencing, where both the quantity and purity of DNA are crucial factors for successful PCR and sequencing. Contaminants such as proteins, salts, and organic compounds can inhibit enzymatic reactions. (Wilson. 1997)

Therefore, when we choose the ideal extraction method, we should consider not only sufficient DNA yield but also ensure high purity. In present study, the Silica solution-based method was the highest contamination level and also the lowest DNA yield.

The GS solution-based method was the highest DNA concentration but higher contamination level than Proteinase k-based, may be less desirable for some applications, but the best purity ratio (2.00). The Proteinase K-based method was the lowest contamination levels among three methods. This indicates that this method was the least number of contaminants like salts and organic compounds.

The time required for DNA extraction can vary based on the ingredients and components of the extraction kits used. Different kits have different protocols, reagents, and technologies that can impact the efficiency and duration of the extraction process. (Wilfinger *et al.,* 1997).

In present study, when comparing time taken for each method per sample for all specimens, including lysis, washing, DNA dehydration and elution.

Silica solution was the most time-consuming, with a total duration of 93 minutes because it might be depended on ingredients of method but offers a balance between speed and consistency.

GS solution took 82 minutes in total during per sample. The lysis time was relatively short, but the washing phase was longer. The brief elution time was a quick final step and had significant variability in time taken steps, making it less reliable. Proteinase K-based was the fastest method, took only 22 minutes.

The choice of extraction method often depends on the specific application and priorities. However, we should consider the overall balance of concentration, purity, and time consistency. Generally, methods with lower SDs are more reliable for time estimation and we can have more confidence in predicting the time for each step.

The Proteinase K and Silica-based methods compared to the GS solution-based method. and the fastest overall, both in terms of average time taken per step and total time. Therefore, the Proteinase K-based method is the best option if saving time is important and a slightly lower DNA yield is acceptable and it was the most efficient and consistent.

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Conclusion

This study successfully compared three DNA extraction protocols on *Clarias batrachus*. The GS solution-based method produced the highest yield but moderate contamination. The proteinase K-based method offered high purity and fast processing. The silica solution-based method had lower yield and higher contamination. All methods showed variations in DNA quality, despite using the same species. Further research is needed to understand these variations and optimize extraction methods.

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