Comparing Genomic DNA Extraction Methods/ Protocols to obtainHigh Quality DNA from *Ulva lactuca*

ABSTRACT

Selecting DNA extraction protocol is crucial for the quality of DNA to be isolated from a sample, it also entails the success of other downstream application like PCR amplification and sequencing. In this study, three (3) extraction protocols were used to extract DNA from a seaweed, the *Ulva lactuca.* The protocols that were used in this study were optimized to extract genomic DNA for other organisms, for instance, the sodium dodecyl sulfate (SDS) method was used for insects, the tri-phosphate extraction (TPE) method is used for rice, while cetyltrimethylammonium bromide (CTAB) method is commonly used for plants. Some of the components of the protocols were modified like for instance changing the pH of Tris-HCl. The experiment consists of two trials with two replicates in each trial. For the first trial, the TPE method got the highest DNA concentration of 304.1 ng/ul but it did not pass the purity ratio of $A_{260/280}$ (1.8-2.0) by only having 1.46, however, two replicates pass the $A_{260/280}$ ratio and both are the replicates 1 of SDS and CTAB protocol having 1.81 and 1.80 $A_{260/280}$ ratio and the concentrations of 127 ng/ul and 77.0 ng/ul, respectively. For the second trial, both the replicates using CTAB protocol pass the $A_{260/280}$ ratio with 1.82 and 1.81, and DNA concentration of 24.2 ng/ul and 1,993.6 ng/ul, respectively. This concludes that, still the conventional genomic DNA extraction protocol for plants, which is the CTAB method can also be used to isolate high quality DNA from a green seaweed, the *Ulva lactuca.* However, some of the components of CTAB method poses health risks and possible environment hazard, the TPE and SDS method can offer a less harmful way to extract DNA, though further optimization on the components of the two protocol is highly encouraged.

Comment [LEGA1]: Key words?

INTRODUCTION

In today's world of DNA analysis, the importance of high quality, purified DNA cannot be underestimated. Finding a suitable DNA isolation system to satisfy your downstream application needs is vital for the successful completion of experiments (Mandrekar, 2016). The analysis of genomic DNA is crucial for various applications, including the identification and study of marine plants such as *Ulva lactuca.* Different methods for extracting genomic DNA have been developed, each with its advantages and limitations (Jeukens*et al*., 2014). To choose most suitable extraction method, factors such as the required quantity and quality of DNA, the nature of the sample, and the presence of interfering substances must be considered (Semagn, 2013).

The DNA extraction protocol can be crucial when attempting to isolate the most representative DNA sample. It has been shown that due to differences in thecell wall and membrane structures, the effectiveness of DNA extraction can depend on procedure used (Felczykowska*et al*., 2015), while successful application of molecular techniques relies on an efficient recovery of nucleic acids (Hurt *et al*.,

2001). In recent years, the continuous developments in molecular detection technology, which has a higher sensitivity and specificity, shorter detection time and increased automation, and performs an important role in the early and rapid detection of infectious disease pathogens. (Liu*et al.,* 2023). Thus, it is important to choose methods that yield both, good quality and high quantity of the extracted DNA.

Ulva lactuca, commonly referred to as sea lettuce, is a green macroalgae species that is widely distributed in coastal ecosystems around the world. It plays a vital ecological role as a primary producer and provider of habitats, and it also holds potential for various biotechnological applications, including biofuel production, pharmaceuticals, and food additives. However, to fully explore the genetic potential of ~~*Ulva*~~ *U. lactuca*, it is essential to develop robust genomic DNA extraction methods that can yield high-quality DNA appropriate for subsequent molecular analyses. While numerous DNA extraction protocols have been developed for diverse organisms, there is no universally guaranteed method that ensures high yields and purity, particularly for challenging species like ~~*Ulva*~~ *U. lactuca*. This species possesses complex cell wall compositions and abundant secondary metabolites. Hence, it is crucial to carefully select an appropriate DNA extraction method tailored to the specific characteristics of ~~*Ulva*~~ *U. lactuca* to achieve successful genomic studies (Bischof *et al*., 2016).

One common approach for extracting genomic DNA is the TPE (tri-phosphate extraction) method. The TPE method utilizes tri-phosphate buffer to extract genomic DNA specifically from rice (Kim *et al.,* 2016). This method is known for its efficiency in extracting adenosine 5'-triphosphate (ATP) from tissues and cells, a crucial component in molecular biology studies. This method plays a vital role in quantifying ATP levels, which is essential for assessing cell viability, energy dynamics, and cellular processes(Bischof, 2002).Another widely used method is the CTAB (cetyltrimethylammonium bromide) extraction method (Semagn, 2013). The CTAB method involves the use of CTAB as a detergent to remove proteins and other contaminants from the DNA samples, resulting in high quality DNA extracts (Dong *et al.*, 2021).Additionally, the SDS (sodium dodecyl sulfate) extraction method can also be used for extracting genomic DNA (Semagn, 2013). The SDS method relies on the use of SDS detergent to solubilize cell membranes and release DNA from the cells. The CTAB and SDS methods are renowned for their effectiveness in isolating high-quality and quantity of genomic DNA from plant tissues. These methods are instrumental in obtaining DNA samples suitable for various molecular analyses, such as PCR amplification, sequencing, and genetic characterization (Dong et al., 2021).

This study will try tocompare the three (3) methods of genomic extraction to obtain genomic DNA from a seaweed, which is the ~~*Ulva*~~ *U. lactuca*. By employing and comparing the three (3) methods, this study will be of great help for the determination and preparation of seaweeds/ macroalgae for its precise species identification, evolutionary studies, and exploration of their ecological roles since DNA extraction is the primary step for the molecular identification or DNA barcoding of any organism.

MATERIALS AND METHODS

Collection and Preparation of Sample

The seaweed sample (~~*Ulva*~~ *U.lactuca*) was collected in the UEP Town, Catarman, Northern Samar. After collection, the sample were rinsed with seawater, then, it was placed in a tube with absolute ethanol. Prior to every extraction method, the seaweed sample were air-dried until the excess alcohol dries out from the blade, about 2 mg of ~~*Ulva*~~ *U.lactuca*were used for lysis.

Cetyltrimethylammonium bromide(CTAB) Genomic DNA Extraction Method

The protocol of Zucarello and Paul (2019) were followed for the CTAB genomic DNA extraction.

Table 1. Component of CTAB Buffer/ Reagent

Comment [LEGA2]: Call in the text

Component	Quantity
Tris-HCl (pH 8.0)	14 mL
NaCl	56 mL
EDTA (pH 8.0)	7 mL
CTAB	2.8g
B-mercaptoethanol	1.4 mL
PVP	2.8g

Sodium dodecyl sulfate(SDS)Genomic DNA Extraction Method

The procedure for SDS protocol was followed from Waldschimdt*et al* (1997) with modifications were made. The components of the SDS buffer are shown below.

Table 2. Component of SDS Buffer/ Reagent

Comment [LEGA3]: Call in the text

Component	Quantity
Tris-HCl (pH 8.0, 1st trial)(pH 8.0, 2nd trial)	120 ul
SDS	480 ul
NaCl	360 ul
EDTA (pH 8.0)	48 ul
Proteinase K	48 ul
Sterile Distilled Water	1,344 ul

Two (2) mL of blade sample were ground in a 2 mL microcentrifuge tube. For first trial, 800 buffer were added, while for the second trial 1 mL buffer was added to facilitate lysis and release of DNA from the cell. Then, the samples in a tube were incubated in a water bath at 65°C for 30 minutes. An equal volume of 24:1 chloroform: isoamyl was added and mixed by vortexing. In addition, samples undergone centrifugation (refrigerated) at 11,000 rpm for 20 minutes. The aqueous phase was then separated and transferred to a new 1.5 mL microcentrifuge tube. Equal volume of cold isopropanol were also added to the tube and was incubated overnight at -20°C. After that, the tubes/ samples had undergone centrifugation at 15,000xg for 30 minutes. Then, a pellet should appear after the centrifugation, wash the pellet once or twice with wash buffer. Air dry the pellet, make sure that it is completely dried. Lastly, resuspend the pellet using TE buffer for further downstream application of the isolated DNA.

Tri-phosphate (TPE) Genomic DNA Extraction Method

The procedure was followed from Kim *et al* (2016) with modifications were made. The components of the TPE buffer are shown below.

Table3. Component of TPE buffer for 100 mL reagent solution.

Component	Quantity
1M Tris-HCl	10 mL
Potassium chloride	7.45g
0.5M EDTA	2 mL
Sterile distilled water	70 mL and fill the remaining to reach 100 mL

Comment [LEGA4]: Call in the text.

Example: The components of the TPE buffer are shown in table 3

About 2 mg of seaweed sample was placed in a 2 mL microcentrifuge tube. The sample were lysed to facilitate the release of DNA from the cell. For the first trial the TPE buffer added were 200 ul and for the second trial 400 ul was used. After the addition of the TPE buffer, the sample in a tube were shaken through hand motion vortex mixer. Then, sample were incubated at 65°C for about 30 minutes. After incubation, 1 mL of sterile distilled water were added for the dilution of extract, then, it was shaken through hand motion vortex mixer. Samples undergone centrifugation at 13,000 xg for 10 minutes at room temperature. Lastly, take-out the sample tubes carefully from the centrifuge and transfer as much supernatant as you need without disturbing tissue debris to a new 1.5 mL microcentrifuge tube. Extracted DNA can be stored at 4°C for at least eight (8) months. The extraction was done in two trials with two (2) replicates in every trial.

Quantitation of Extracted/ Isolated DNA

One (1) ul of each isolated DNA from three (3) different method or protocol were poured in Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific) to determine the quantity and concentration of isolated DNA.

Agarose Gel Electrophoresis

To check the quality and size (bp) of the isolated DNA, 1% agarose were diluted in 0.5X TAE buffer/running buffer with 1.2 ul gel red. To complete the electrophoretic analysis, the agarose gel with the isolated genomic DNA (DNA + bromophenol blue/ loading buffer) was run at a constant voltage of 120V, 100 watts for 30 minutes, with Quick Load ® 1kb+ DNA ladder (New England BioLabs ®, Inc.).

RESULTS AND DISCUSSION

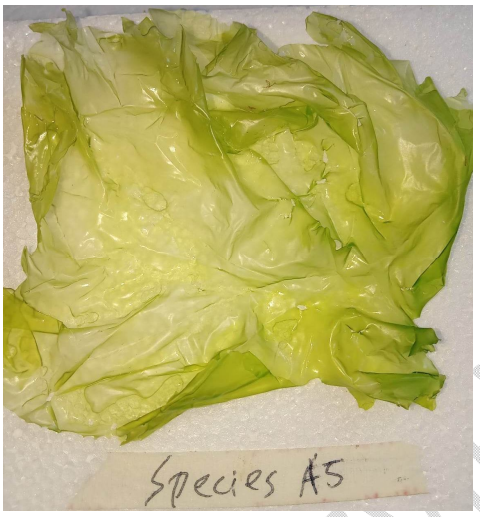

Figure 1. *Ulva lactuca*

Comment [LEGA5]: Call in the text.

It is not advisable to start with a topic

Three (3) extraction protocol were used to extract high quality DNA from *Ulva lactuca.* Table 4 shows the concentration of the isolated DNA, it was found out in the first trial that the Replicate 1 of the TPE method had obtained the highest concentration of 304.1 ng/ul, however its purity ratio at $A_{260/280}$ was below the 1.80-2.00 purity range, since a ratio of 1.8-2.0 is generally accepted as "pure" for DNA, however, if the ratio is appreciably lower (<1.6), it may indicate the presence of proteins, phenol, or other contaminants that absorb strongly at or near 280 nm (Aguilar, *et al.,* 2016). Among all the replicates for the first trial, two passed the $A_{260/280}$ purity range, it was the replicate 1 of the SDS method (Waldschmidt *et al.,* 1997) with 127 ng/ul which had 1.81 $A_{260/280}$ ratio, and the replicate 2 for the CTAB Method (Zucarelloand Paul, 2019) with 77 ng/ul which had 1.80 $A_{260/280}$ ratio.

Table 4. Concentration of isolated DNA from *Ulva lactuca*during 1st trial

Protocol	Replicate	Concentration (ng/ul)	A 260/280	A 260/230
TPE Method	1	304.1	1.46	0.75
	2	147.6	1.12	0.72
SDS Method	1	127.0	1.81	0.69
	2	33.1	1.64	1.37
CTAB Method	1	77.0	1.80	1.15
	2	66.1	1.66	0.82

For the second trial as shown in Table 5, both the replicates for the CTAB method had passed the $A_{260/280}$ ratio, with replicate 2 using CTAB method had extracted the highest concentration of 1,993.6 ng/ul and the replicate 1 had only 24.2 ng/ul. Also, all the replicate 1 of all the three-extraction protocol were treated with RNAse, which is clear evidence as shown in Figure 2 that there is no interference of RNA contamination in the first three wells in the gel where all the replicate 1 for all the genomic extraction protocol were placed.

Table 5. Concentration of isolated DNA from *Ulva lactuca*during 2nd trial

Protocol	Replicate	Concentration (ng/ul)	A 260/280	A 260/230
TPE Method	1	279.2	1.46	0.73
	2	112.9	1.32	0.81
SDS Method	1	916.2	1.42	0.52
	2	534. 9	1.49	0.55
CTAB Method	1	24.2	1.82	4.78
	2	1993.6	1.81	0.54

Another determiner for the purity of the isolated DNA is the $A_{260/230}$ ratio, it is widely used as a secondary measure for DNA purity. Expected $A_{260/230}$ ratio values should be between 2.0-2.2 (Gallagher, 1998; Aguilar *et al.,* 2016), as shown in the 1st and 2nd trial in Tables 4 and 5, all of the isolated genomic DNA did not passed the $A_{260/230}$ ratio. However, the $A_{260/230}$ ratio is considered a questionable DNA quality indicator because of the instability of this value when a saline elution buffer is used to dissolve the DNA. This is due to the higher increase of salt concentration than the DNA concentration in the sample (Stulnig and Amberger, 1994; Aguilar *et al.,* 2016). In this experiment, Tris-EDTA buffer was used as elution buffer.

Also, electrophoresis on agarose gel accompanied by densitometry analysis of band intensity evaluates the DNA integrity as well as the contamination by RNA (Aguilar *et al.,* 2016). Isolated genomic DNA appears as a unique well-defined high molecular weight (HMW) band which was shown in Figure 2, wherein sizes are more than 1 kb/ 1000 bp.

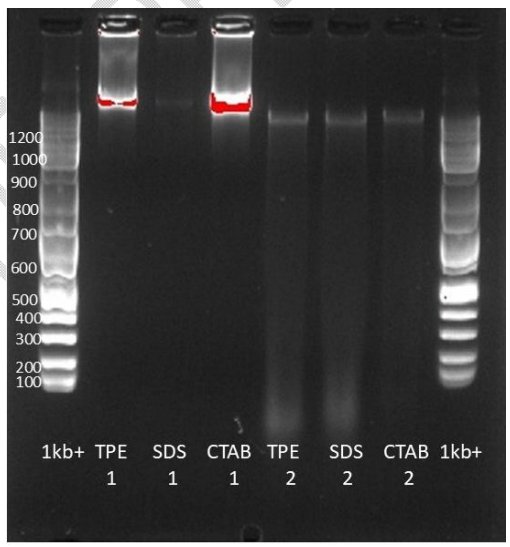

Figure 2. Gel visualization

Shown in Figure 2 is the gel visualization of second trial on the evaluation of band intensity through agarose gel electrophoresis, all of the replicate 1 for the three extraction protocols were treated with 1 ul RNAse, to prevent the contamination of

the RNA. The replicate 1 of TPE and CTAB have shown an intense band, while the replicate 1 for SDS had shown a faint band. On the other hand, all the replicate 2 for all the extraction protocols were not treated with RNAse, slightly faint bands and DNA fragmentation were also observed in all replicate 2.

Conclusion

Both the DNA purity and integrity are the determiners of the quality and usability of the isolated DNA, this can be done using spectrophotometer and gel electrophoresis. As shown in the results of this study the CTAB method yielded a good quality of isolated DNA that pass the $A_{260/280}$ ratio of 1.8-2.0. CTAB protocol was an established genomic DNA extraction method for plants, since the sample used in this experiment is a marine plant, it is expected to extract a genomic DNA. In addition, two other genomic DNA extraction protocol was also utilized to compare which among the three will yield DNA which will pass the purity ratio. Fortunately, all three methods yield an amount of concentration as shown using Nanodrop Spectrophotometer. Further optimization of some components of the TPE and SDS genomic DNA extraction methods to yield high quality DNA that will pass the purity and integrity analysis.

References:

Aguilar, G.L., Sanchez-Lopez, A.M., Barberan-Aceituno, C., Carrillo-Avila, J.A., Lopez-Guerrero, J.A., & Aguilar-Quesada, R. (2016). DNA Source Selection for Downstream Application Based on DNA Quality Indicators Analysis. Biopreservation and Biobanking, 14 (4). https://doi.org/10.1089/bio.2015.0064.

Bischof R (2002). Comparison of Eight Methods of Genomic DNA Extraction from Babassu. DOI:http://dx.doi.org/10.4238/2015.

Bischof R, Ramoni J, &Seiboth B (2016). Cellulase and Beyond: The First 70 Years of the Enzyme Producer Trichoderma reesi. 15(1). Doi: 10.1186/s 12934-016-0507-6.

Dominguez S. (2019). Two Efficient Methods for Isolation of High-Quality Genomic DNA from Entomopathogenic Fungi. Journal of Microbiological Methods. Vol 148 (55-63).

Dong T, Ma X, Sheng XQ, Chu Y, Song Q, Zou B, & Zhou G (2021). Point-of-Care DNA Testing by Automatically and Sequentially Performing Extraction, Amplification and Identification in a Closed-Type Cassette. Sens Actuators B Chem. 327:128919. DOI:10.1016/j.snb.2020.128919.

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin, 19(1), 11-15. Retrieved from www.ncbi.nlm.nih.gov.

Felczykowska, A., Krajewska, A., Zileinska, S., & Los, J. M. (2015). Sampling, Metadata and DNA Extraction – Important Steps in Metagenomic Studies. Acta Biochimica Polonica, 62, 51-160.

Gallagher, S. (1998). Quantitation of nucleic acids with absorption spectroscopy. Current Protocols in Protein Science, 1: A-4K.

Hurt R, Qiu X, Wu L, Roh Y, Palumbo A, Tiedje JM, & Zhou J (2001). Simultaneous Recovery of RNA and DNA from Soils and Sediments. Appl Environ Microbiol. 67(10):4495-4503.

Jeukens J., Boyle B., Kukavica-Ibrulj I., Ouellet M. M., Aaron S. D., Charette S. J., et al., (2014). Comparative Genomics of Isolates of a Pseudomonas Aeruginosa Epidemic Strain Associated with Chronic Lung Infections of Cystic Fibrosis Patients. PLoSONE9:e87611.10.1371/journal.pone.0087611.

Kim, S.R., Yang, J., An, G., & Jena, K.K. (2016). A simple DNA preparation method for high quality polymerase chain reaction in rice. Plant Breeding and Biotechnology, 4:99-106. https://doi.org/10.9787/PBB.2016.4.1.99.

Liu, Q., Jin, X., Cheng, J., Zhou, H., Zhang, Y., & Dai, Y. (2023). Advances in the application of molecular diagnostic techniques for the detection of infectious disease pathogens (Review). Molecular Medicine Reports, 27, 104. https://doi.org/10.3892/mmr.2023.12991

Mandrekar, P. (2016). Introduction to Nucleic Acid Purification Basics and Their Application to Different Sample Types [Webinar].

Murray, M. G., & Thompson, W. F. (1980). Rapid isolation of high molecular weight plant DNA. Nucleic Acids Research, 8(19), 4321-4325. Retrieved from www.academic.oup.com.

Semagn, K. (2014). Leaf Tissue Sampling and DNA Extraction Protocols. In: Molecular Plant Taxonomy: Methods and Protocols. Methods in Molecular Biology; Besse P (ed). Vol. 1115, Humana Press, Springer Science + Business Media, New York.

Stulnig, T.M. and Amberger, A. (1994). Exposing contaminating phenol in nucleic acid preparations. “Biotechniques, 16 (3): 402-404.

Waldschmidt, A.M., Salomao, T.A., Goncalves de, E., Campos, L. (1997). Extraction of genomic DNA from *Melipona quadrifasciata*(Hymenoptera: Apidae, Meliponinae).

Winnepenninckx, B., Backeljau, T., & De Wachter, R. (1993). Extraction of high molecular weight DNA from molluscs. Trends in Genetics, 9(12), 407. Retrieved from www.sciencedirect.com.

Zucarello, G.C., and Paul, N.A. (2019). A Beginner's Guide to Molecular Identification of Seaweed. Squallen Bulletin and Fisheries Postharvest and Biotechnology, 14 (1): 43-53. DOI://http://doi.org/10.1557/squalene.v14i1.384.