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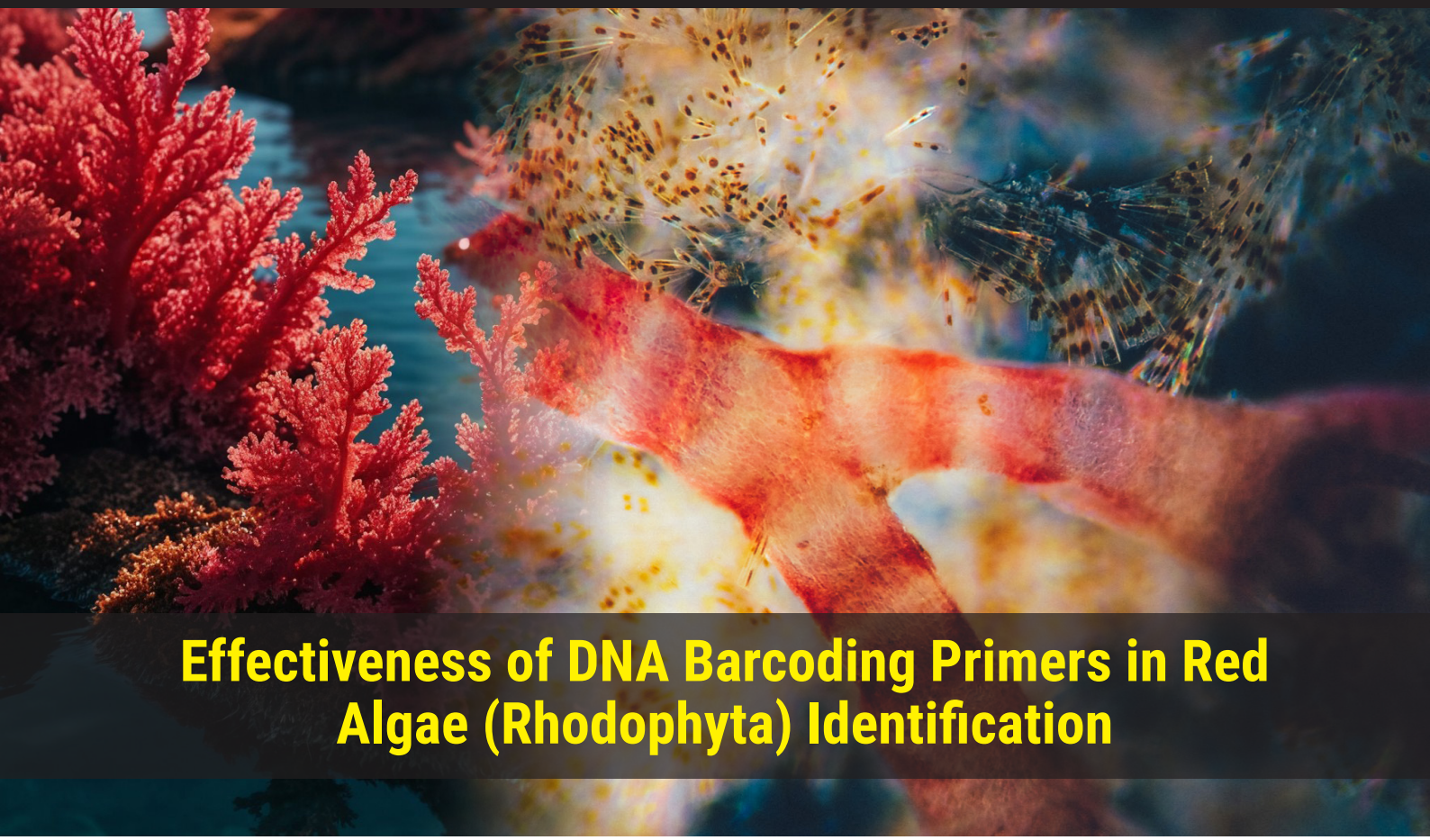

# Effectiveness of DNA Barcoding Primers in Red Algae (Rhodophyta) Identification

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## ABSTRACT

Red algae (Rhodophyta) are vital primary producers in marine ecosystems and are economically significant due to their wide use in food, pharmaceutical, and cosmetic industries. The significant utilization of red algae indicates that these organisms require conservation and protection from extinction, therefore, accurate identification is a must. Traditional morphological approaches face challenges due to their simplicity and plasticity; however, molecular techniques, such as DNA barcoding can overcome these limitations. This study evaluated the effectiveness of using ITS1, Cox2-3, rbcL 1, and rbcL 2 primers for barcoding seven red algae species, focusing on amplification success and sequencing quality. All of the above-mentioned primers have demonstrated a noteworthy amplification rate of success, with 100% efficacy observed for ITS1 and rbcL 2. However, only Cox2-3, rbcL 1, and rbcL 2 primers exhibited a high-quality read based on the sequencing quality score, indicating their reliability in capturing the target sequence for identification. The results strongly suggested that rbcL 2 is the optimal choice for identifying Rhodophyta due to its high amplification rate and high-quality sequencing results.

**Keywords:** DNA barcoding, genetic identification, primer, seaweed

## INTRODUCTION

Rhodophyta, commonly known as red algae, is a division within the subkingdom Biliphyta, classified under the plant kingdom, Plantae (Ruggiero *et al.,* 2015). Red algae play a pivotal role in marine ecosystems as primary producers, significantly contributing to the maintenance of coral reefs, and providing a structural habitat for a diverse range of microorganisms through the secretion of calcium carbonate in their cells (Rajasulochana & Preethy, 2015). Certain species in this group are economically valuable because they produce carrageenan, a polysaccharide derived from algae. Carrageenan is extensively utilized across several industries, including food, pharmaceuticals, and cosmetics. The considerable utilization of red algae indicates that these organisms require conservation and protection from extinction (Samman & Achmad, 2023).

Traditional morphological identification techniques encounter challenges in diversity study. This is especially the case for red algae species that display simple, environmentally plastic or convergent characteristics, manifesting as morphological features that are difficult to define precisely (Zuccarello & Paul, 2019). In contrast to environmental and developmental factors, DNA-based molecular tools are not susceptible to such influences (Dev *et al.,* 2020). The application of a DNA-based information repository for conservation initiatives allows for the formulation and substantiation of policies through informed decision-making (Hogg *et al.,* 2022).

Over the past two decades, DNA barcoding, a novel approach utilizing DNA markers, has emerged as a reliable and rapid method for organism identification in the scientific community (Letsiou *et al.,* 2024). The DNA regions used as universal markers originate from sequences located in the nucleus, such as the Internal Transcribed Spacer 1 (ITS1), or from those within specialized organelles, such as the mitochondrial DNA (mtDNA) Cox2-3 spacer markers gene. The ITS-1 region, located between the 18S and 5.8S rDNA coding regions, is a commonly utilized marker in phylogenetic studies due to its high variability (Lee *et al.,* 2024). Similarly, the Cox2-3 spacer has been validated as a valuable marker for studying intraspecific relationships, given that this noncoding region exhibits a higher mutation rate than the surrounding genes (Zuccarello *et al.,* 2006). Moreover, the RuBisCo ribulose-1,5-bisphosphate carboxylase oxygenase-large subunit (rbcL) gene was utilized for phylogenetic studies due to its high amplification efficiency and restriction to photosynthetic organisms (Wongsawad & Peerapornpisal, 2014).

Prior research has shown that ITS1, Cox2-3, and rbcL primers are effective for delineating species boundaries within the red algae group (Achmad *et*

*al.,* 2024; Mshiywa *et al.,* 2024; Osathanunkul *et al.,* 2018; Satriani *et al.,* 2023). Universal barcode markers must be evaluated across a more expansive spectrum, given the morphological and geographical variations and the reticulate evolution observed in plant species (Bafeel *et al.,* 2011). The success of polymerase chain reaction (PCR) is a prerequisite for barcoding, as this technique is the exclusive means of amplifying the target sequence. Consequently, maintaining high PCR success rates and its product read quality remains an important scientific objective. This study examined the effectiveness of ITS1, Cox2-3, and rbcL primers for barcoding seven species of red algae, particularly emphasizing the success rate of amplification and sequencing quality scores.

## MATERIALS AND METHOD

The study was conducted at the Plant Biotechnology Laboratory, using seven red algae samples from Ambon and Lampung cultivation area and collected by a team from the Southeast Asian Regional Centre for Tropical Biology (SEAMEO BIOTROP) Bogor, Indonesia. Sample descriptions used in the study is listed in Table 1.

Total genomic DNA was extracted according to the cetyltrimethylammoniumbromide (CTAB) method described by Doyle *et al.,* (1990), modified by adding 3% PVPP. DNA concentrations were quantified using NanoPhotometer N50-Touch (Implen, Germany), and the quality of extracted DNA was confirmed by electrophoresis on 1% agarose gels prepared with 1× TAE Buffer and stained with DNA loading dye. DNA was stored at -20 $^{o}$C for later analysis.

Table 1 The codes of the seven samples utilized in the study

| No. | Code | Common Name |
|---|---|---|
| 1 | LK15 | Lampung 2015 |
| 2 | LK18 | Lampung 2018 |
| 3 | BPBL | BPBL Ambon Culture |
| 4 | SCL | Sacol Lampung |
| 5 | KMT | Kotoni Maluku Tenggara |
| 6 | KW | Kotoni Wanci |
| 7 | SW | Spinosum Wanci |

The specific DNA regions, ITS1, Cox2-3, rbcL 1, and rbcL 2 primers were then amplified by PCR using each primer pair. The rbcL 2 primer was designed on the basis of the complete *Kappaphycus alvarezii* and *Kappaphycus striatus* chloroplast genome available in the National Center for Biotechnology Information (NCBI) database to ensure specificity and accuracy in targeting the desired regions using the PrimeQuest website (https://www.idtdna.com/PrimerQuest). Each PCR mixture (50 µL) consisted of a 5 µL DNA template, 2 µL of each primer (10 mM), 25 µL MyTaq HS Red Mix (Bioline Reagent, Ltd., United Kingdom), and 16 µL Nuclease Free Water (NFW). The primers and PCR conditions are detailed in Table 2.

The PCR product was visualized by 1% agarose gel electrophoresis with the addition of 3 µL FloroSafe DNA Stain. Gel images were acquired with Vilber Lourmat ETX-20.M UV transilluminator (Vilber, France). The amplified PCR products were examined for the presence or absence of the band on an agarose gel to determine the percentage of primers amplification rate. The DNA amplification results were then sent to APICAL Scientific Malaysia for sequencing purposes. The Chromatogram of each sequencing product was displayed using GeneStudio software and quality scores for all sequences were determined using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/).

Table 2 PCR primers and programs used for DNA amplification

| Primer Set | Primer sequence (5'-3') | PCR condition | Reference |
|---|---|---|---|
| ITS1 | F : GGTGAACCTGCGGAAGGATCATTG | 95 °C, 1 min; 30 × (95 °C, 15 s; 59 °C, 15 s; 72 °C 30 s); 72 °C, 3 min; ∞ 4 °C | (Osathanunkul *et al.*, 2018) |
| Cox2-3 | R : CCGAGATATCCATTGCCGAGAGTC | 95 °C, 1 min; 30 × (95 °C, 15 s; 56 °C, 15 s; 72 °C 30 s); 72 °C, 3 min; ∞ 4 °C | (Zuccarello *et al.*, 1999) |
| | F : GTACCWTCTTTDRGRRKDAAATGTGATGC | | |
| | R : GGATCTACWAGATGRAAWGGATGTC | | |
| rbcL 1 | F : AACTCTGTAGTAGAACGNACAAG | 94 °C, 4 min; 35 × (94 °C, 1 min; 52 °C, 1 min; 72 °C 1 min); 72 °C, 10 min; ∞ 4 °C | (Satriani *et al.*, 2024) |
| | R : GCTCTTTCATACATATCTTCC | | |
| rbcL 2 | F : CATATAAAGTCGATGCTGTG | 95 °C, 1 min; 30 × (95 °C, 15 s; 50 °C, 15 s; 72 °C 30 s); 72 °C, 3 min; ∞ 4 °C | (Designed in this study) |
| | R : CACCTGTAGCAGCAATA | | |

Table 3 Primer screening using ITS, Cox2-3, rbcL 1, and rbcL 2 primers in seven red algae samples

| Primer set | Amplification product | | | | | | | Amplification rate (%) |
|---|---|---|---|---|---|---|---|---|
| | LK15 | LK18 | BPBL | SCL | KMT | KW | SW | |
| ITS1 | + | + | + | + | + | + | + | 100.0 |
| Cox2-3 | + | + | + | + | + | - | + | 85.7 |
| rbcL 1 | + | + | + | + | + | - | + | 85.7 |
| rbcL 2 | + | + | + | + | + | + | + | 100.0 |

Notes: (+) = Amplified successfully; (-) = Cannot be amplified.

## RESULTS

### Amplification Rate Analysis

The rbcL 2 primer designed in this study was able to amplify all DNA samples and had higher amplification or success rate than rbcL 1 primer from the literature reference (Table 3). Table 3 also shows that ITS primer was able to amplify all DNA samples (Table 3; Fig. 1). On the other hand, sample "KW" could not be amplified by using Cox2-3 and rbcL 1 primers (Table3; Fig.2), which may have been caused by the incompatibility of the primers with the sample (Roux, 2009). It is worth noted from this study that PCR setup and temperature optimization enhance the amplification success during the PCR process.

### Sequencing Quality Score Analysis

FastQC is a very popular tool used to provide an overview of basic quality control for next-generation sequencing data (Wingett & Andrews, 2018). The Sequence Quality Score in FastQC is a critical analysis module that assesses the quality of sequences in a FASTQ file. FastQC can visually view the quality of the segment. A warning is raised if the most frequently observed mean quality is below 27, and an error is raised if the most frequently observed mean quality is below 20 (Shi & Xu, 2016).

The quality score of sequence reads was analyzed using fastQC software (Table 4). As we can see, sequences analyzed by using rbcL 1 & 2 primers had an average score over 36, which means the sequences had a good quality. A low-quality score (0-20) is indicative of sequences with chromatograms devoid of read peaks (Fig. 3, left section for ITS sequences). The discernible peaks between each sequence read are regarded as sequences exhibiting superior sequencing quality, with a score above 20, and may serve as an identification reference (Fig. 4).Table 4 The quality score of sequencing results in ITS, Cox2-3, rbcL 1, and rbcL 2 primers in seven red algae samples

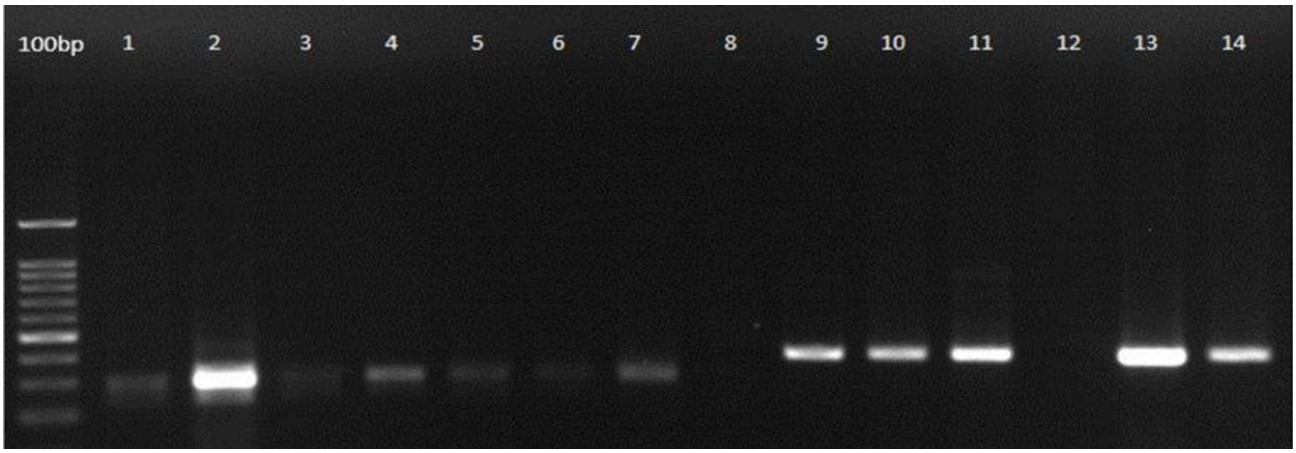


Figure 1 Amplified ITS1 (1-7) and Cox2-3 spacer (8-14) in red algae samples

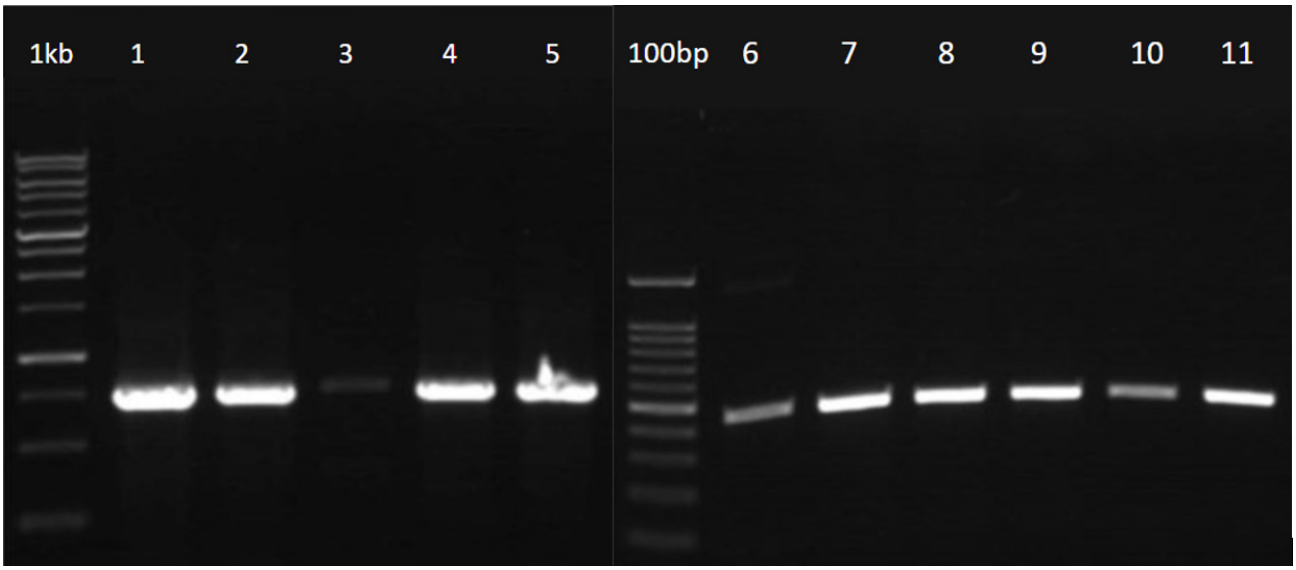


Figure 2 Amplified rbcL 1 (1-5) and rbcL 2 (6-11) in red algae samples

Table 4 The quality score of sequencing results in ITS, Cox2-3, rbcL 1, and rbcL 2 primers in seven red algae samples

| Primer set | Quality score | | | | | | | | | | | | | | Ave. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | LK15 | | LK18 | | BPBL | | SCL | | KMT | | KW | | SW | | |
| | F | R | F | R | F | R | F | R | F | R | F | R | F | R | |
| ITS1 | 13.4 | 10.3 | 13.3 | 17.8 | 0.0 | 0.0 | 25.1 | 12.4 | 0.0 | 14.9 | 12.5 | 12.8 | 0.0 | 0.0 | 9.46 |
| Cox2-3 | 40.8 | 39.9 | 21.2 | 13.0 | 44.6 | 44.1 | 43.9 | 41.7 | 44.9 | 42.0 | - | - | 42.9 | 45.1 | 33.15 |
| rbcL 1 | 46.1 | 46.4 | 45.3 | 46.5 | 46.2 | 47.3 | 45.6 | 46.0 | 46.2 | 45.6 | - | - | 40.2 | 26.7 | 37.72 |
| rbcL 2 | 41.8 | 39.4 | 41.9 | 45.7 | 42.8 | 42.3 | 44.7 | 44.7 | 47.0 | 47.6 | 45.0 | 45.3 | 43.1 | 42.4 | 43.86 |
| Average | 35.5 | 34.0 | 30.4 | 30.8 | 33.4 | 33.4 | 39.8 | 36.2 | 34.5 | 37.5 | 14.4 | 14.5 | 31.6 | 28.6 | |

Notes: <20 = low-quality QC score; 20-30 = normal quality QC score; >30 = high-quality QC score.

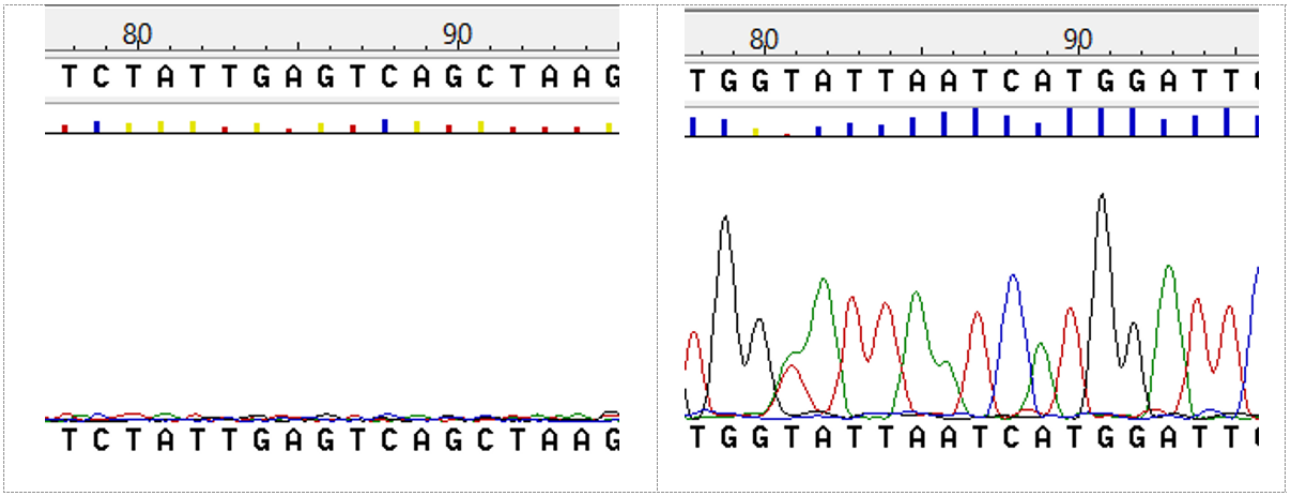


Figure 3 Sequencing chromatogram of LK18 DNA amplified by ITS1 (left) and Cox2-3 (right) primers

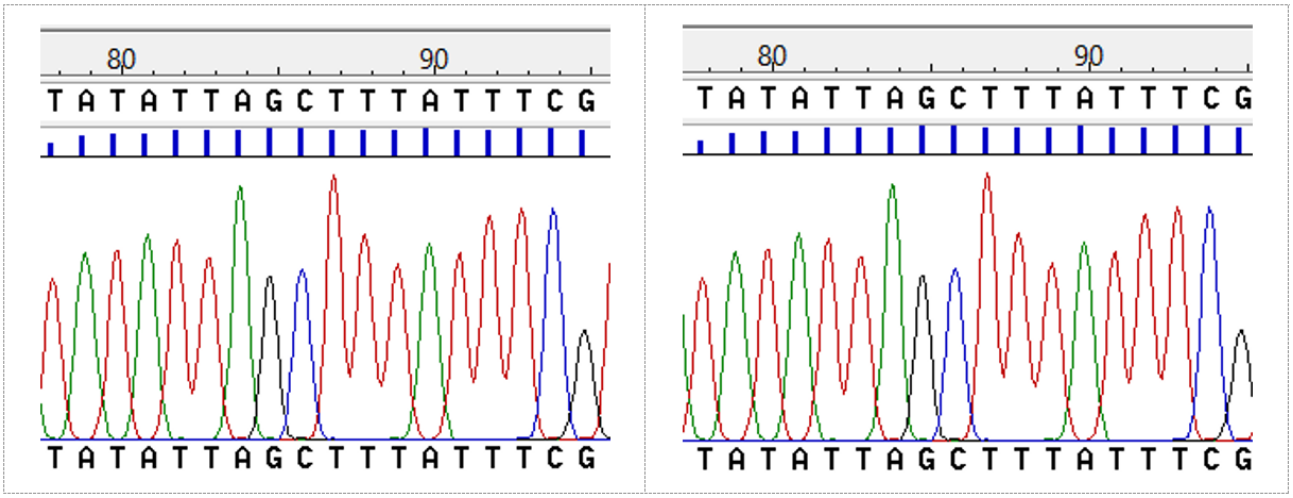


Figure 4 Sequencing chromatogram of LK18 DNA amplified by rbcL 1 (left) and rbcL 2 (right) primers

## DISCUSSION

Accurate identification of many red algae to the species level using only morphological characters can be difficult. DNA barcoding developed approximately twenty years ago, is an approach that has significantly contributed to the development of the molecular biology field study (Hebert *et al.,* 2003). In its development, DNA barcoding has helped in the process of identifying plant species, both aquatic and terrestrial plants.

In this study, we successfully identified red algae using several DNA barcoding primers. As a result of this study, we found that rbcL 2 primers showed advantages over other primers. PCR results using rbcL 2 primer was able to amplify all DNA samples with an optimal sequence QC value (Table 3). This result is in accordance with the results of other studies which stated that rbcL has been effectively used in various ecological studies, including marine environments, demonstrating its adaptability and effectiveness in different biological contexts (Turk Dermastia *et al.,* 2023).

One of the important factors in the success of DNA barcoding is determined by the success of the PCR process. In PCR experiments, the primers are the key to the success of the experiment. Primers have a very important role in the process of PCR amplification (Bustin *et al.,* 2020). If the primers are too short they might hybridize with non-target sites and give undesired amplification products. In addition, the suitability of temperature is also a determining factor in the success of PCR (Roux, 2009).

Phillips *et al.,* (2019) proposed that several factors must be considered and weighed when selecting a DNA barcode, such as universal PCR amplification, range of taxonomic diversity, power of species differentiation, and bioinformatics analysis and application. Generally, for one gene marker, at least two or more pairs of primers are used, especially for ITS, Cox, and rbcL, because all of those primers are among the most commonly used universal primers (Kowalska *et al.,* 2019). As a widely used and effective tool, DNA barcoding will become more useful over time in the field of any plants.

The present barcode reference libraries are insufficient in marine macro-algal identification for Indonesian species, therefore, more efforts for DNA barcoding program of the local species is necessary to facilitate the environmental monitoring efforts, especially red algae species. Building a comprehensive local barcode reference library could contribute to resolving macro-algal taxonomy and systematics and address biogeography pertaining to the invasion of non-indigenous species. This could also result in the development and application of cost-effective and better biodiversity monitoring projects.

Some species of red algae have health, industrial, and environmental benefits (Tanaka *et al.,* 2020; Subramanian *et al.,* 2020). In species like *Kappaphycus alvarezii* barcoded in the present study and the ones occurring on the Brazilian coast (Nogueira *et al.,* 2019), various haplotypes were known to contain variable composition of antioxidants (Araujo *et al.,* 2020). Hence, the generated barcodes will be useful for taxonomic non-experts of food, pharmaceutical, and cosmetics. Further studies could be carried out to explore the possibility of linking DNA barcodes to inter and intra-species biochemical constituents of seaweeds.

## CONCLUSION

Based on the result of this study, we recommend the use of rbcL 2 primer to identify red algae species because its average QC score is over 30, which is the high-quality category. Strengthening the local barcode libraries by barcoding all species could facilitate cost-effective biodiversity surveys and effective environmental barcoding programs in the near future. The generated barcode and the use of certain primers can facilitate further research (climate change, species distribution) and also for various industrial (pharmaceutical, biofuel, cosmetic, seafood, etc.) applications.

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## REFERENCES

Achmad, M. J., Akbar, N., Ismail, F., Samman, A., Subhan, B., Paembonan, R. E., & Arafat, D. (2024). DNA barcoding of red algae (Rhodophyta) in Ternate Island Sea, North Maluku, Indonesia. Jurnal Ilmiah Perikanan dan Kelautan, 16(1). http://doi.org/10.20473/jipk.v16i1.44436

Araújo, P. G., Nardelli, A. E., Fujii, M. T., & Chow, F. (2020). Antioxidant properties of different strains of Kappaphycus alvarezii (Rhodophyta) farmed on the Brazilian coast. Phycologia, 59(3), 272–279. https://doi.org/10.1080/00318884.2020.1736878

Bafeel, S. O., Arif, I. A., Bakir, M. A., Khan, H. A., Al Farhan, A. H., Al Homaidan, A. A., ... & Thomas, J. (2011). Comparative evaluation of PCR success with universal primers of maturase K (matK) and ribulose-1, 5-bisphosphate carboxylase oxygenase large subunit (rbcL) for barcoding of some arid plants. Plant Omics, 4(4), 195-198.

Bustin, S. A., Mueller, R., & Nolan, T. (2020). Parameters for successful PCR primer design. Quantitative Real-Time PCR: Methods and Protocols, 5-22.

Dev, S. A., Sijimol, K., Prathibha, P. S., Sreekumar, V. B., & Muralidharan, E. M. (2020). DNA barcoding as a valuable molecular tool for the certification of planting materials in bamboo. 3 Biotech, 10, 1-12. https://doi.org/10.1007/s13205-019-2018-8

Doyle, J. J., & Doyle J. L. (1990). Isolation of plant DNA from fresh tissue. Focus, 12, 13-15.

Guo, L., Sui, Z., Zhang, S., Ren, Y., & Liu, Y. (2015). Comparison of potential diatom 'barcode'genes (the 18S rRNA gene and ITS, COI, rbcL) and their effectiveness in discriminating and determining species taxonomy in the Bacillariophyta. International Journal of Systematic and Evolutionary Microbiology, 65(4), 1369-1380.

Hebert, P. D., Cywinska, A., Ball, S. L. & deWaard, J. R. (2003). Biological identifications through DNA barcodes. Proc Biol Sci, 270, 313–321.

Hogg, C. J., Ottewell, K., Latch, P., Rossetto, M., Biggs, J., Gilbert, A., ... & Belov, K. (2022). Threatened species initiative: Empowering conservation action using genomic resources. Proceedings of the National Academy of Sciences, 119(4), e2115643118. https://10.1073/pnas.2115643118

Kowalska, Z., Pniewski, F., & Latała, A. (2019). DNA barcoding–A new device in phycologist's toolbox. Ecohydrology & Hydrobiology, 19(3), 417-427.

Kress, W. J. & Erickson, D. L. (2008). DNA barcodes: genes, genomics, and bioinformatics. Proc Natl Acad Sci U S A, 105, 2761– 2762.

Lee, J. H., Jeon, H. J., Seo, S., Lee, C., Kim, B., Kwak, D. M., ... & Han, J. E. (2024). The use of the internal transcribed spacer region for phylogenetic analysis of the microsporidian parasite Enterocytozoon hepatopenaei infecting whiteleg shrimp (Penaeus vannamei) and for the development of a nested PCR as its diagnostic tool. Journal of Microbiology and Biotechnology, 34(5), 1146. https://doi.org/10.4014/jmb.2401.01010

Letsiou, S., Madesis, P., Vasdekis, E., Montemurro, C., Grigoriou, M. E., Skavdis, G., ... & Tzakos, A. G. (2024). DNA Barcoding as a Plant Identification Method. Applied Sciences, 14(4), 1415. https://doi.org/10.3390/app14041415

Mshiywa, F. M., Edwards, S., & Bradley, G. (2024). Rhodophyta DNA barcoding: Ribulose-1, 5-bisphosphate carboxylase gene and novel universal primers. International Journal of Molecular Sciences, 25(1), 58. https://doi.org/10.3390/ijms25010058

Nogueira, M.C.F., Henriques, M.B.(2020). Large-scale versus family-sized system production: economic feasibility of cultivating Kappaphycus alvarezii along the southeastern coast of Brazil. J Appl Phycol 32, 1893-1905. https://doi.org/10.1007/s10811-020-02107-2.

Osathanunkul, M., Osathanunkul, R., & Madesis, P. (2018). Species identification approach for both raw materials and end products of herbal supplements from Tinospora species. BMC Complementary and Alternative Medicine, 18, 1-6. https://doi.org/10.1186/s12906-018-2174-0

Phillips, J. D., Gillis, D. J., & Hanner, R. H. (2019). Incomplete estimates of genetic diversity within species: Implications for DNA barcoding. Ecology and Evolution, 9(5), 2996-3010.

Rajasulochana, P., & Preethy, V. (2015). Biotechnological applications of marine red algae. Journal of Chemical and Pharmaceutical Research, 7(12), 477-481.

Roux, K. H. (2009). Optimization and troubleshooting in PCR. Cold Spring Harbor Protocols, 2009(4), pdb-ip66.

Ruggiero, M. A., Gordon, D. P., Orrell, T. M., Bailly, N., Bourgoin, T., Brusca, R. C., Cavalier-Smith, T., Guiry, M. D., & Kirk, P. M. (2015). PloS one, 10(4), e0119248. https://doi.org/10.1371/journal.pone.0119248

Samman, A., & Achmad, M. J. (2023). Diversitas dan distribusi alga merah (Rhodophyta) di Perairan Pulau Ternate. Jurnal Kelautan Tropis, 26(1), 148-154. https://doi.org/10.14710/jekk.v%vi%i.13342

Satriani, G. I., Soelistyowati, D. T., Alimuddin, A., Arfah, H., & Effendi, I. (2023). Molecular assessment of Kappaphycus alvarezii cultivated in Tarakan based on Cox2-3 spacer. Squalen Bulletin of Marine and Fisheries Postharvest and Biotechnology, 18(1), 52-64. https://doi.org/%2010.15578/squalen.736

Satriani, G. I., Soelistyowati, D. T., Arfah, H., & Effendi, I. (2024). Identification of Kappaphycus alvarezii Seaweed based on phylogenetic and carrageenan content. Jurnal Akuakultur Indonesia, 23(1), 1-11. https://doi.org/10.19027/jai.23.1.1-11

Shi, H., & Xu, X. (2016). Learning the Sequences Quality Control of Bioinformatics Analysis Method. In 2016 International Conference on Education, E-learning and Management Technology (pp. 464-468). Atlantis Press.

Subramaniam, D., Hanna, L. E., Maheshkumar, K., Ponmurugan, K., Al-Dhabi, N. A., & Murugan, P. (2020). Immune stimulatory and anti-HIV-1 potential of extracts derived from marine brown algae Padina tetrastromatica. Journal of Complementary and Integrative Medicine, 17(2). https://doi.org/10.1515/jcim-2019-0071

Tanaka, Y., Ashaari, A., Mohamad, F. S., & Lamit, N. (2020). Bioremediation potential of tropical seaweeds in aquaculture: low-salinity tolerance, phosphorus content, and production of UV-absorbing compounds. Aquaculture, 518, 734853. https://doi.org/10.1016/j.aquaculture.2019.734853

Theissinger, K., Fernandes, C., Formenti, G., Bista, I., Berg, P. R., Bleidorn, C., ... & Zammit, G. (2023). How genomics can help biodiversity conservation. Trends in Genetics, 39(7), 545-559. https://doi.org/10.1016/j.tig.2023.01.005

Turk Dermastia, T., Vascotto, I., Francé, J., Stanković, D., & Mozetič, P. (2023). Evaluation of the rbcL marker for metabarcoding of marine diatoms and inference of population structure of selected genera. Frontiers in Microbiology, 14, 1071379.

Wingett, S. W., & Andrews, S. (2018). FastQ Screen: A tool for multi-genome mapping and quality control. F1000Research, 7.

Wongsawad, P., & Peerapornpisal, Y. (2014). Molecular identification and phylogenetic relationship of green algae, Spirogyra ellipsospora (Chlorophyta) using ISSR and rbcL markers. Saudi Journal of Biological Sciences, 21(5), 505-510. https://doi.org/10.1016/j.sjbs.2014.01.003

Zuccarello, G. C., Burger, G., West, J. A., & King, R. J. (1999). A mitochondrial marker for red algal intraspecific relationships. Molecular Ecology, 8(9), 1443-1447.

Zuccarello, G.C., Buchanan, J., & West, J. A. (2006). Increased sampling for inferring phylogeographic patterns in Bostrychia radicans/B.moritziana (Rhodomelaceae, Rhodophyta) in the eastern USA. Journal of Phycology, 42, 1349–1352. http://dx.doi.org/10.1111/j.1529-8817.2006.00292.x

Zuccarello, G. C., & Paul, N. A. (2019). A beginner's guide to molecular identification of seaweed. Squalen Bull. of Mar. and Fish. Postharvest and Biotech, 14(1), 43-53. https://doi.org/10.15578/squalen.v14i1.384