

Molecular species of *Diaphanes* sp from Nusa Kambangan Island as a source of luciferase molecular marker on cancer diagnostic

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Abstract. The luciferase, an important element, is currently being developed as an early detection for cancer biomarker. This study aims to obtain the gene sequence for luciferase and discover the percentage of similarity between the luciferase gene from *Diaphanes javanus* Gorham and gene bank reference fireflies. The study employed a descriptive analysis method. The results of the isolation, restriction and amplification of the luciferase gene produced a single band and nucleotide readings. The luciferase contig sequence for *Diaphanes* sp. was compared to the with the gene bank reference sequence. The highest similarity of the luciferase gene in *Diaphanes* sp was showing high similarity. The similarity rate can help to determine the direction of the development of the *Diaphanes* sp luciferase gene in native species of Indonesia.

1. Introduction

The firefly species *Diaphanes* is part of the bioluminescent animals which can be utilized in many aspects by humankind. The existence of *Diaphanes* firefly as directional help tool for ships at sea has been widely known. The strength of this insect's bioluminescence is actually a form of physiological and metabolism protection of the firefly against their environment. The existence of the firefly's bioluminescence was used by ship captains to be tools for direction and location in several specific areas [1] Nowadays the luminescence of fireflies is also utilized as the molecular marker for the diagnosis of cancer malignancy [2]. The explanation of the oxygen metabolism of this insect can explain the degree of luminescence in the physiological sense from the metabolism reaction of tissues, particularly metabolism of cancer tissue [3]. The enzymatic reaction of this Luciferase bioluminescence becomes a huge opportunity as cancer diagnosis [4]. It might even describe cancer prognosis non-invasively for cancer patients [5]. The use of fireflies as a source of biomarker enzyme in the Nusa Kambangan Island in the southern area of Java, Indonesia. [6] Tried to track the best firefly luminescence of Indonesia. The luciferase enzyme as the source of optimum luminescence from native fireflies of Indonesia.



2. Research methodology

2.1. Molecular determination

In this research, the identification of firefly species from the Nusa Kambangan Island [7] employed the PCR technique using general Firefly PCR Primer, namely: LCO-HCO and Lep. The technical stages of the firefly species identification are as follows [8]:

- DNA isolation technique for the firefly DNA was conducted by employing Genomic DNA extraction using ZR Tissue and Insect DNA MiniPrep Kit (Zymo Research) 2. [9]
- PCR amplification using MyTaq HS Red Mix (Bioline)
- PCR products purification with Zymoclean Gel DNA Recovery Kit (Zymo Research)
- Bi-directional Sequencing

2.2. Amplification and cloning luciferase gene

Amplification luciferase gene employed using primer Dia_Jav F-ACGCGCTAATATCATTGCA dan Dia_Jav R-TCCGTTAGAATATAGTAAACCGAAG. PCR products purification with Zymoclean™ Gel DNA Recovery Kit (Zymo Research). Clone to pTA2 Vector with Toyobo Target Clone -Plus- (Toyobo). Transform to E. coli Zymo 5α with Mix and Go Competent Cells™ (Zymo Research). PCR Colony with primer T3 and T7 promotor using KOD FX Neo (Toyobo). Plasmid isolation with ZR Plasmid MiniPrep (Zymo Research). Bi-directional Plasmid Sequencing.

3. Research results

The location of the existence of Diaphanes fireflies from Nusa Kambangan Island is in the southern area of Java, Indonesia.

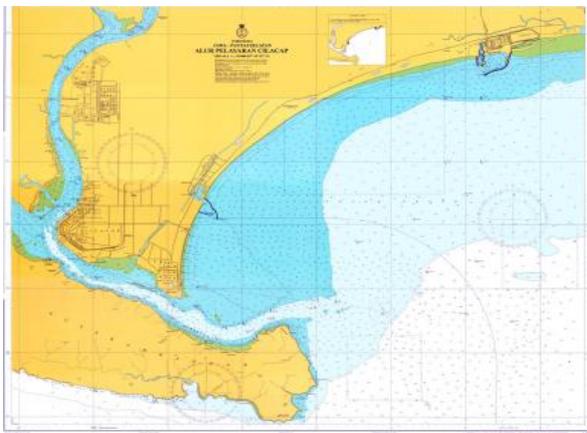


Figure 1. Nusakambangan.



Figure 2. Diaphanes sp from Indonesia [10].



Figure 3. The process of flickering fireflies.



Figure 4. Diaphanes sp From Nusakambangan.

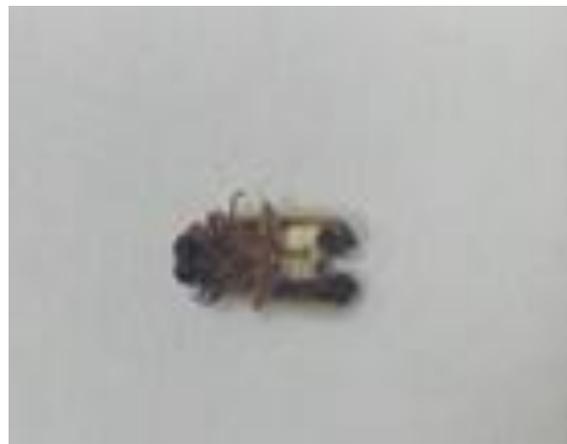


Figure 5. Diaphanes sp From Nusakambangan.

3.1. Molecular determination of firefly species from Nusa Kambangan Island

The results obtained are as follows: Sample Name: The molecular determination of this species was tested by employing the PCR technique using general Firefly PCR Primer, namely: LCO-HCO and Lep. [11]. The DNA count result employing NanoDrop Genomic DNA are as follows: Conc.(ng/μl) A260/280 A260/230 Volume (μl) 1 Firefly 563.7 1.79 0.98 3

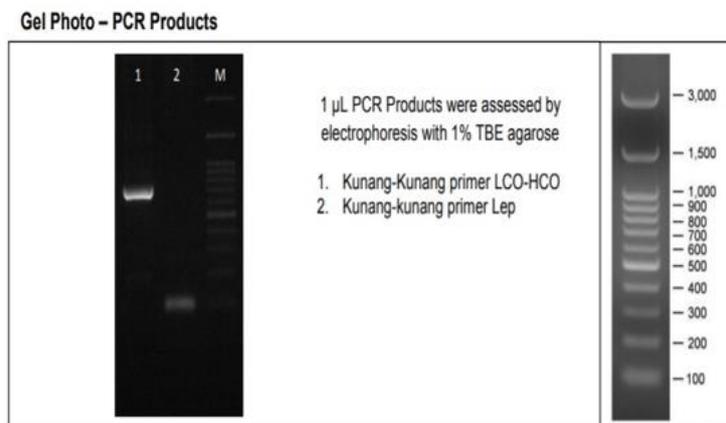


Figure 6. Gel photo PCR product.

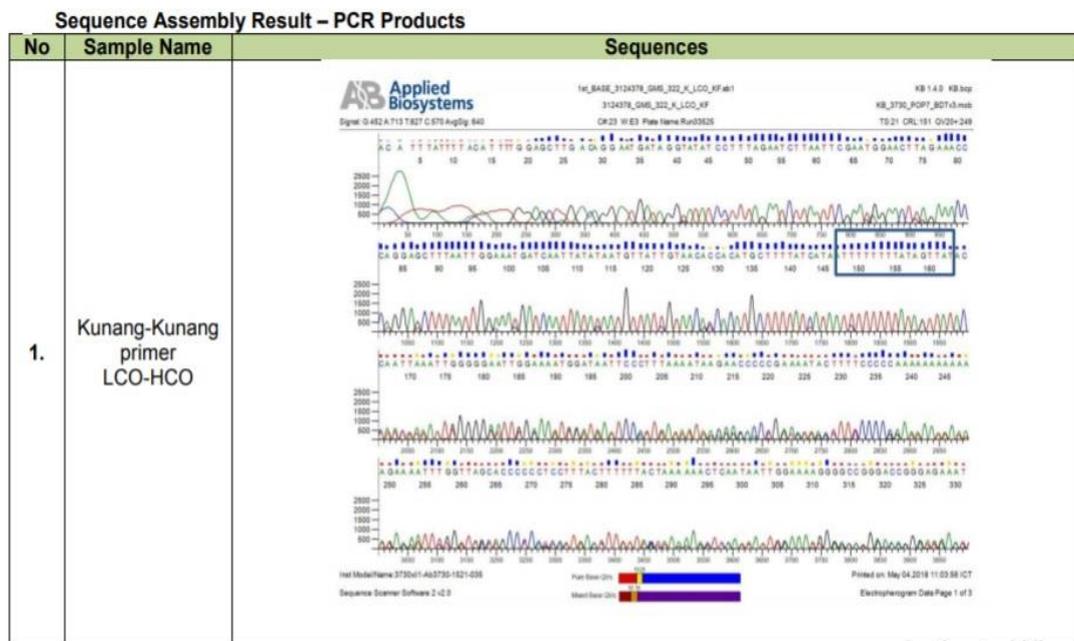


Figure 7. Sequence assembly result PCR product no sample name sequences.

3.2. Amplification and cloning of the luciferase gene

The amplification of the luciferase gene was successfully conducted using the primers Dia_Jav F and Dia_Jav R. Optimal luciferase gene amplification was conducted with the annealing temperature of 51°C and amplicon length of 1300 bp. The successfully amplified Luciferase gene was then cloned into pTA2 Vector and this stage is currently a work in progress.

4. Conclusion

The fireflies from Nusa Kambangan were successfully identified using LCO-HCO primer as the species *Diaphanes sp.* Luciferase gene isolation was successful in the length of 1300 bp and the cloning stage into pTA2 Vector is currently in progress.

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