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The 1313bp luciferase gene sequence in *Diaphanes javanus*

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ABSTRACT

Fireflies use a characteristic communication using light in their mating process. The light is produced by peroxisomes in photocytes which contain the luciferase enzyme. The luciferase enzyme is regulated by the luciferase gene (*LUC*). The luciferase gene is an important element that is currently being developed as a bioindicator and biosensor. This study aimed 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 data collected were in the form of single electrogram bands, chromatogram sequences and percentages of similarity. The results of the isolation, restriction and amplification of the luciferase gene produced a single band and nucleotide readings which were at a length of 1313pb. The luciferase contig sequence for *Diaphanes javanus* was compared to the with the gene bank reference sequence. The highest similarity of the luciferase gene in *Diaphanes javanus* was shown by*Diaphanes pectinealis* at 98% with the number of genes successfully isolated 67% of the total luciferase gene. The lowest similarity rate was 64% when compared to *Photuris pennsylvanica*. The similarity rate can help determine the direction of the development of the *Diaphanes javanus* Gorham luciferase gene in Indonesia.

Key words : *Diaphanes javanus, Gorham, Firefly, Luciferase gene, Percentage of similarity*

Introduction

Communication is the means for an organism to relay information (Carazo and Font, 2010). Communication is also needed to adapt to environmental conditions. All forms of communication are mediated by signals. Signals based on the sensory nervous system are classified into four types: chemical, acoustic (sound), tactile (touch) and visual signals (Syefarth and Dorothy, 2016; Higham and Eileen, 2013).

One of the most influential signals is the visual signal. A visual signal is an information mediator received by the sense of sight. The advantages of visual signals are that they have relatively far ranges because of the fast information transmission process. Visual signals can also be produced with relatively little energy and are the easiest to observe and tend to be easy to understand (Higham and Eileen, 2013). One of the examples of a visual signal that has been continuously studied in the last decade is luminescence (Docter *et al*., 2016)

Luminescence is a highquantum light that does not emit heat (endothermic) and originates from certain parts of certain organisms (Cevenini *et al.*, 2016). The luminescence produced by living organisms as a form of communication in mating, a method of predation, and a form of defense in avoiding preda-

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tors (Haddock *et al.*, 2010) Luminescence and various other phenotypic characteristics are related to the genetic materials contained in an organism (Burga and Ben, 2012). The gene that codes and regulates luminescence is the luciferase gene (England *et al.*, 2016)

The luciferase gene is found in nearly all bioluminescent organisms. The luciferase gene is scattered throughout 700 genera from 16 phyla, inhabiting the soil, air, or, most commonly, bodies of water (Muthukumaran *et al.*, 2013). One of the organisms that have the luciferase gene and is easy to find in Indonesia is the firefly (Cevenini *et al.*, 2016). There are 15 genera of fireflies in Indonesia, namely *Australoluciola, Drilaster, Emasia, Falsophaeopterus, Flabellotroteta, Luciola, Medeopteryx, Mimophaeopterus, Ototretinae, Picodrilus, Trisinuata* (Janisova and Milada, 2012; Ballantyne and Christine, 2013) *Pteroptyx* (Sari *et al*., 2014) *Diaphanes, Lamprigera, Pyrocoelia* (Mairawita *et al.*, 2016; Jeng, 2008). Most of them are endemic fireflies in Sumatra, Kalimantan, and Papua, but some can be found on Java Island such as *Diaphanes javanus.*

The luciferase gene is presently being studied in earnest in the fields of biochemistry and molecular biology. This gene regulates the secretion of the luciferase enzyme. The luciferase enzyme is non-toxic and can be expressed dynamically (Johnson *et al.*, 2014). The luciferase gene could be inserted as a bioindicator (bioimaging)for cancer and abnormal cell growth (Dong *et al.*, 2013). The marking of diseases using luciferase could be an indicator of the prognosis of a certain disease (Dusthacker *et al.*, 2012). Another example of the application of the luciferase gene is as a biosensor for detecting a number of microbial contaminations or environmental contamination (Cevenini *et al.*, 2016). The luciferase gene can also detect the primary parainfluenza virus in a non-invasive way (Burke *et al.*, 2015) and help understand the trigger for plant DNA rearrangement signals when induced by pathogens (Seternes *et al.*, 2016).

Based on the sequence data and further studies, the luciferase gene's regulation in prokaryotic organisms such as in bacteria (*Vibrio harveyi* and *V. fisheri*) has been discovered. The luciferase gene in bacteria is regulated by the Lux operon which consists of the lux genes R andI as the promoter genes and lux C, D, A, B, E, and G as structural genes (Tehrani *et al.*, 2011). Different from the luciferase found in prokaryotic organisms, luciferase in eukaryotic organisms originate from a heterocyclic substrate, not and an aliphatic aldehyde (Docter *et al.*, 2016). The luciferase enzyme in eukaryotic organisms also has another superiority: it is synthesized by a single gene (Li *et al.*, 2003). Gene regulation in eukaryotic organisms has not yet been completely discovered, especially in Indonesia, and the isolation and determination of the luciferase gene in eukaryotic organisms have never been conducted, including in fireflies. The luciferase gene from fireflies' eukaryotic cells has potential to become the main reference in various studies because it is more compatible when inserted into eukaryotic cells (Cadena and Paul, 2016). Its low mutagenesis rate gives way to a larger possibility of the luciferase gene in fireflies being consistent. This advantage enables it to be developed into a marker gene that could avoid bias and is more sensitive because the light wavelength it produces tends to be more stable.

The importance of using luminescence light is the foundation of this study to determine the luciferase gene in fireflies living in Indonesia. The determination could become an alternative reference for applications in various fields. The data about *Diaphanes javanus's* luciferase gene sequence is expected to become material for buildingan *in vitro* gene expression cloning construction. The knowledge pertaining to the expression pattern of the luciferase gene in *Diaphanes javanus* is hoped to substitute commercial luciferase promoters so that the application of markers with luciferase could be more affordable. The gene sequence of *Diaphanes javanus's* luciferase could also become the pioneer for data about luciferase originating from Indonesia. The benefits of this study from the scientific development point of view are that it could increase the knowledge and information of the data sequence fragment the luciferase gene fragment from indigenous Indonesian fireflies.

Methodology

The Location and Time

This study was conducted between January and September 2016 in the Laboratory of Biochemistry and Microbiology, Jakarta State University, the Laboratory of Molecular Biology, Syarif Hidayatullah State Islamic University, and Sentra Biosains Dinamika Laboratory, Jakarta.

Equipment and Materials

The equipment used in this study were a digital scale, Erlenmeyer flasks, micropipettes, micro tips and tubes, measuring cylinders, a micropestle, mortar, aluminum foil, a microwave oven, a nanodrop spectrophotometer, a PCR Advanced, electrophoresis equipment, a transilluminator, a vortex, a centrifuge, a Biometra thermoshaker, a freezer (-20°C), Geneious software, Oligo Calc., Finch Tv 1.4.1.

The materials used were an Innuprep DNA micro Analytic Jena AG isolation kit, TAE 1x, nuclease free water, 20 µM Afluc F Primer, 20 µM Afluc R Primer, Solis BioDyne 5x Hot FIREpolmaster m*ix*, loading dye, PCRBIO Ladder I 1 kb, agarose powder and ice gel.

Research Method

The method used in this study was a descriptive analysis method. The study was conducted in three phases, 1) designing a specific primer and isolating the luciferase gene from the firefly, 2) sequencing the luciferase gene so that the nitrogen base sequence could be documented, 3) determining the percentage of similarity between the luciferase gene from the sequencing and other gene bank reference species of fireflies.

Results

The Afluc Primer Pair

The (Total) DNA Genome of *Diaphanesjavanus*

The average size of genomes in Coleopterais greater than 12 Mb (NCBI, 2016). The results of the electrophoresis test of the DNA genome of *Diaphanes javanus*can be seen in Figure 2. Isolates with an average weight of 8 mg yield an average of 106.32 ng DNAwith an average DNA purity of 1.45 at an absorbance of 260/230 and 1.47 at an absorbance of 260/280.

Restriction and Amplification of the Luciferase Gene

The luciferase gene restriction results using the Afluc primer have not yet been able to isolate a sequence of target DNA which was approximately 2 kb, but the results of the restriction could be said to be quite successful. This was signified by the DNA genome being cut from the isolate.

The Luciferase Structure and Gene Sequence

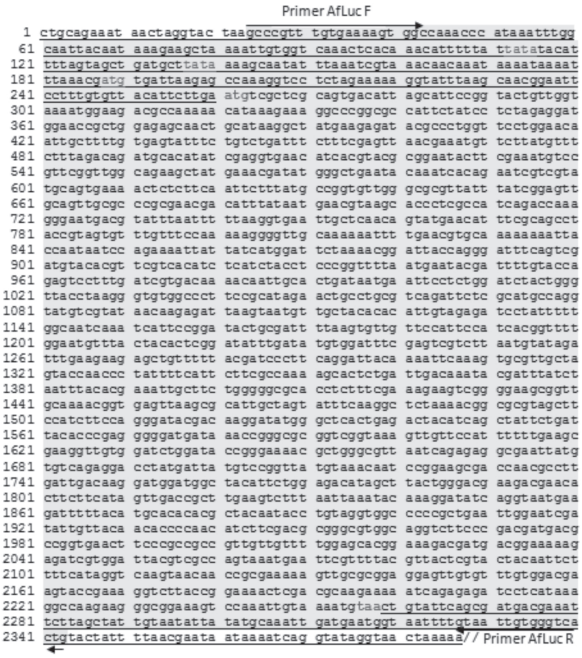

Fig. 1. The Diagram of the Afluc Primer Cutting from *Photinuspyralis.*

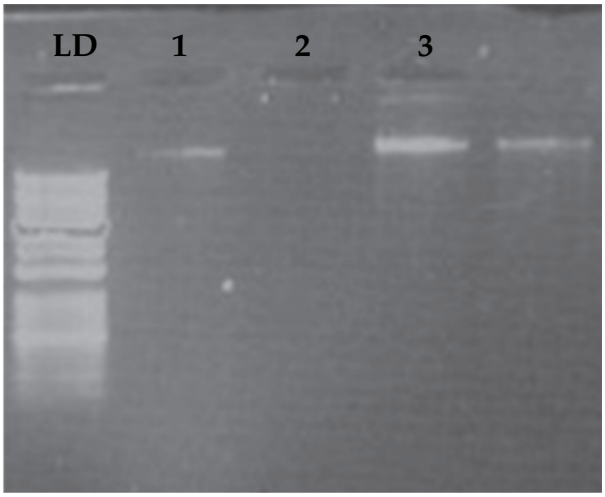

Fig. 2. The DNA electropherogram for the *Diaphanes javanus* genome, LD; 0.5 ug Ladder I Biosystem, 1; 100 ngDNA genome isolate, 2; Second repeat, 3; Third repeat.

Similarity Percentage

The the *Diaphanesjavanus* luciferase gene sequence when compared to the sequence of other fireflies in the gene bank. The difference in luciferase sequence similarity between *Diaphanesjavanus* and other species of firefliesis presented in Table 1.

Discussion

The luciferase gene sequence from *Photinus pyralis* was selected as the reference in creating an Afluc primer design because it has a coding sequence(cds) region located between the 253rd and 2251st nucleotide. The cds region from the *Photinus pyralis* sequence data was bracketed by the sequence of another (non cds) nucleotide which is the foundation for manufacturing the primer so that the entire cds from the luciferase gene could be obtained. Meanwhile, the sequence data for *Diaphane spectinealis* was available in the gene bank from the genus *Diaphanes;* therefore, this study employed the sequence from *Photinus pyralis.*

The luciferase protein synthesis initiation point (codon start) was between the 253rd and 255th nucleotide, while the codon stopwas between the 2249th and 2251st (TAA) nucleotide. In the luciferase gene sequence in *Photinus pyralis*, two TATA boxes were discovered in the upstream region, between the 102nd and 137th sequence. Another initiation point was also found in the region before the cds region, in the 132nd sequence. Based on this indicators, the Afluc primer pair was designed for before the sequence where the TATA boxes and initiation points were found. This was done in consideration of the glycosylation site of the luciferase protein which was found in the N-terminal region of the luciferase amino acid residue. If this amino acid residue is translated into the codon form, it would be located at the beginning of the cds sequence of the luciferase gene. The Afluc primer pair was selected because it fulfilled all the requirements as a good primer pair, as listed (Ye *et al*., 2012).

The DNA genome fragment was described through the DNA band that had its length reduced, the one approximately 1300pb. This differed from the statement by Green and Joseph (2012) who said that the temperature for adhering the PCR primer is usually the median of the meltingtemperature (Tm). The Afluc primer pair that had a Tm of 48 and 58, in other words, had a median of 53°C did not yield the restriction resulting DNA band; therefore, the PCR optimation was conducted at a number of temperatures. The Afluc primer pair was at last obtained at a specific adhesion temperature of 51°C. The results of the electropherogramfrom the PCR process is presented in Figure 3.

The DNA isolation and amplification formulation succeeded in isolating and determining the luciferase gene from *Diaphanes javanus* with an average base length of 1309pb. The sequence from one individual, individual number 2 was selected as the

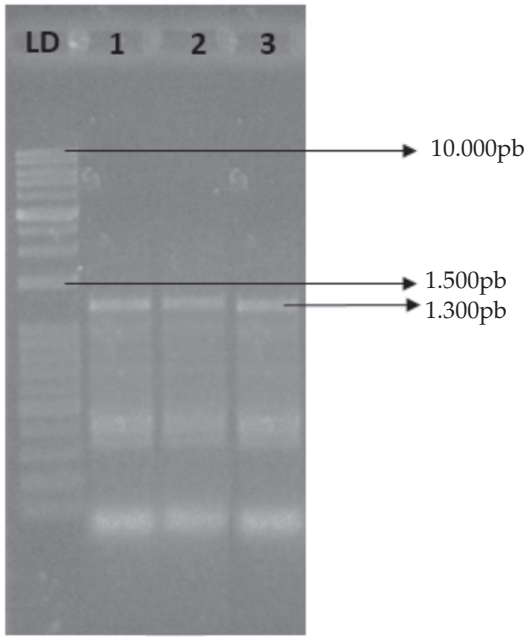

Fig. 3. The electropherogram results for the restriction using the Afluc primer, LD; 0.5 ug Ladder I Biosystem, 1; 50 ng DNA genome *Diaphanes javanus,* 2; second repeat, 3; third repeat.

Table 1. The similarity rate of the luciferase gene sequence between *Diaphanesjavanus*and other species of fireflies

No.	Species	1	2	3	4	5	6	7	8
1	*Diaphanesjavanus*	-	98	83	80	82	81	78	76
2	*Diaphanespectinealis*	95	-	94	90	82	81	76	76
3	*Lampyrisnoctiluca*	89	84	-	84	81	81	76	74
4	*Nyctophylacaucasica*	87	86	85	-	80	81	74	72
5	*Photinuspyralis*	83	83	84	78	-	80	74	72
6	*Luciolanoctiluca*	65	68	68	68	78	-	73	72
7	*Hotariaparvula*	66	67	66	67	69	66	-	71
8	*Photurispennsylvanica*	64	62	61	59	55	54	54	-

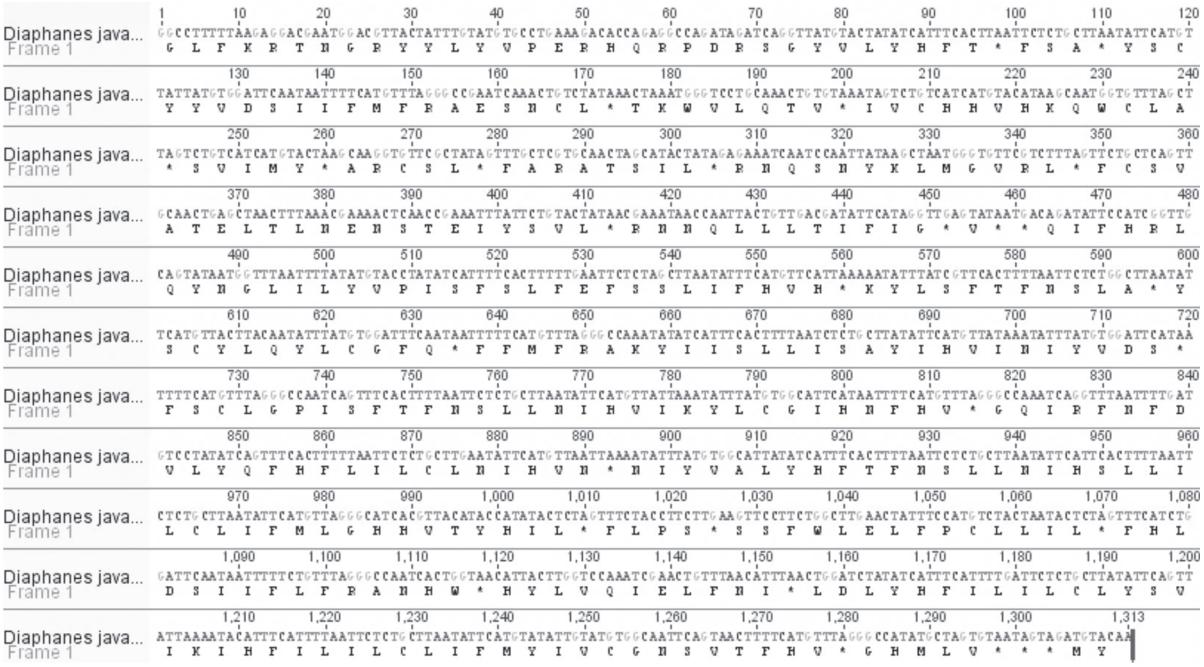

Fig. 4. The nucleotide sequenceand amino acid residue sequence from the *Diaphanesjavanus* luciferase gene.

subject for the similarity comparison with other sequences because it had the best DNA quality and the largest total coveringsequence at 1313pb. Unfortunately, *Diaphanes javanus*'s luciferase gene sequence that was obtained did not contain all the cds from the start codon (ATG) to the stopcodon (TAA). The luciferase gene sequence from *Diaphanes javanus* coded 437 residual amino acid (Figure 4). The luciferase residual amino acids that were isolated from *Diaphanes javanus* were less numerous than those of *Diaphanes pectinealis.* The residual aminoacids from the *Diaphanes pectinealis* luciferase gene numbered 652 amino acids (Li *et al., 2006*). Based on the difference in the number of amino acids, it was assumed that the luciferase gene isolated using the Afluc primer was 67% of the total luciferase gene. The *Diaphanes javanus* luciferase sequence that was recovered was not yet enough to identify the coding region (exon) and noncoding region (intron). However, the Afluc primer used in this study succeeded to obtain a larger isolate than the LnocF1 and LnocR2 primer pairs that had a PCR product of 1008pb (Day, 2006). From Table 1, it can be seen that the similarity rate between *Diaphanes javanus* and other species of firefly which are the gene bank's reference resulted in a relatively different value, ranging between 54% and 98%. The greatest difference between the *Diaphanes javanus* luciferase gene sequence similarity rate was demonstrated by a firefly in the same genus, namely *Diaphanes pectinealis*, at 98%, while the lowest similarity rate was demonstrated by *Photuris pennsylvanica* at 54%.

Conclusion

The luciferase gene from the firefly *Diaphanes javanus* could be isolated using the Afluc F (5'GCCGTTTGTGAAAAGTGGCC3') and Afluc R (5'GTAATTGTGGGTCACTG3') primer at a length of 1313pb. The luciferase gene sequence of *Diaphanes javanus* has a 98% similarity in sequence with *Diaphanes pectinealis* and a54%similarity with *Photuris pennsylvanica*.

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