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Extraction and Purity DNA of *Culex* spp Mosquito in Kemelak Village, Bindung Langit, Ogan Komering Ulu

Farah Nuriessa Aputri[1], Laila Hanum[2*], Ayu Safitri[3] and Yuanita Windusari[4*]

[1]*Department of Biosciences Program, Faculty of Science, Sriwijaya University, Jalan Padang Selasa 524, Palembang, South Sumatra 30139, Indonesia.*
[2] *Department of Biology Faculty of Mathematics & Natural Sciences, Sriwijaya University. Jalan Raya Palembang Prabumulih km 32, Indralaya, Indonesia.*
[3] *Department of Environmental Science, Graduate Program, Sriwijaya University. Jalan Padang Selasa 524, Palembang, South Sumatra 30139, Indonesia.*
[4] *Departement of Public Health Mathematics & Natural Sciences, Sriwijaya University. Jalan Raya Palembang Prabumulih km 32, Indralaya, Indonesia.*

*Corresponding author

E-mail address: ywindusari@yahoo.com (Yuanita Windusari).
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Abstract

Culex spp are mosquito vectors that have a very wide distribution capability and are carriers of pathogens that can interfere with human and animal health. The wide distribution makes Culex spp a dangerous threat. DNA extraction is one of the important steps in obtaining genetic information and genetic analysis. Good quality DNA is used for activities such as the use of molecular markers, genome library creation, and sequencing. This study aims to determine the quality, concentration and purity of Culex spp mosquito DNA in Kemelak Bindung Langit Village, OKU Regency. It is hoped that the sample can be used for further research analysis on Mitochondria D-Loop Sequences in Culex spp mosquitoes. Quantitative measurement of DNA in the form of concentration and purity of DNA using Nanodrop Thermo cycle while qualitative DNA using electrophoresis technique. The results of the isolation of the mosquito genome DNA, obtained clear DNA bands without any degradation (smear) and the concentration results for the four samples ranged from 10-100 ng/µL and the DNA purity was good, ranging from 1.8 to 2.00.

Keywords: Culex; extraction DNA; moleculer; concentration and purity of DNA.

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1. Introduction

The *Culex* spp is one of the mosquito vectors that has a wide distribution capability. *Culex* carries pathogens that can harm human and animal health. Some of these *Culex* borne diseases, in particular West Nile, Japanese encephalitis and lymphatic filariasis, pose significant risks to human health [1].

Lymphatic filariasis is a major disease burden of public health in developing countries, where advanced stages of the disease can lead to the chronic debilitating condition of elephantiasis [2] [3].

Within the genus, *Culex quinquefasciatus* has the greatest impact on human health due to its wide distribution in urban and suburban areas as well as larval tolerance to polluted water reservoirs associated with human and livestock populations [4] [5].

Kemelak Bindung Langit the villages located in East Baturaja sub-district, Ogan Komering Ulu Regency, South Sumatra. Based on research, *Culex* spp are the dominant group of mosquitoes in the Kemelak Bindung Langit area. This is because the environmental conditions are a conducive habitat for the development of these mosquitoes [6].

DNA extraction is one of the important steps in obtaining genetic information and genetic analysis. Good quality DNA is used for activities such as the utilization of molecular markers, genome library creation, and sequencing. The principle of DNA isolation consists of three

stages, namely: wall and membrane lysis cells, DNA separation or purification, and DNA precipitation [7] [8].

The presence of contaminant compounds that are also extracted often inhibits the work of certain enzymes so that they must be avoided [9]. The presence of contaminating compounds can inhibit various processes ranging from DNA cutting, amplification and cloning [10]. Therefore, an extraction method that is able to produce DNA with quantity is needed and good quality.

DNA extraction results are good if the DNA is pure and intact. Measurement of DNA concentration and determination of its purity is an indispensable step in a series of DNA isolation processes. This is done to see the DNA content obtained quantitatively as well as to see contaminants that may still be present from the DNA isolates obtained [11].

The results of the quantitative test using nano drop spectrophotometry were the value of DNA purity at 260/Å280 and the value of DNA concentration. DNA is of good quality if it has a purity of 1.8-2.0 [12] [13].

This study aims to determine the quality, quantity and purity of *Culex* spp mosquito DNA in Kemelak Bindung Langit Village, OKU Regency. So that the sample can be used for further research analysis on Mitochondria D-Loop Sequences in *Culex* mosquitoes.

2. Materials and Methods

Location and Time of Research

Research on DNA isolation of *Culex* spp in Kemelak Bindung Langit Village, Ogan Komering Ulu Regency was carried out from February to March 2021. Sampling was carried out in Kemelak Bindung Langit Village, Ogan Komering Ulu (OKU) South Sumatra in 2019. Identification of mosquitoes was carried out at Baturaja Health Research and Development Center in 2019. The results of the identification of mosquito morphology obtained 4 species of *Culex m*osquitoes, namely *Culex vishnui, Culex quinquefasciatus, Culex tritaeniorhyncus,* and *Culex gellidus.* Samples that have been identified are then put into a tightly closed tube in which camphor is given and stored in a freezer at a temperature of -20 ^{o}C. DNA isolation and PCR were carried out at the Genetics Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Sriwijaya University.

Materials

The tools used in this research are stationery, aspirator, autoclave, petri dish, chambre, vertical electrophoresis, two glass plates for gel molding, erlenmeyer, freezer -20 ^{o}C, measuring cup, hot plate, digital camera, camphor, alcohol cotton, rubber band, gauze, tissue paper, chloroform, nail polish, magnetic stirrer, microscope, nanodrop, paper cup, parafilm, PCR thermocycler, eppendorf micro pipette 100-1000 µL with tip, 500 VA power supply, refrigerator 4 ^{o}C, gloves, micro centrifugation, special comb for wells, 1 mL or 3 mL syringe, analytical balance, tube, UV-transimulator, vortex, water-bath shaker. While the materials used in this study include 1.75 % agarose, aquabidest, 1x TAE buffer, DNA ladder DM3100 ExcelBand™ SmoBio 1 KB, 70 % ethanol, FloroSafe DNA Stain 1 mL, loading dye 6x (Tiagen), 2x My Taq HS Red Mix (Bioline Meridian Bioscience), mosquito DNA sample, Quick-DNA™ Tissue/Insect Miniprep Kit (Zymo Research).

Methods

Mosquitoes Sampling

The method used in the mosquito inventory is carried out using the human bait method, namely the Human landing collection method. The sample was captured using an aspirator, then put into a paper cup and then covered with gauze on top of which was placed cotton that had been fed with mosquito food so that the mosquitoes remained alive until the identification process in the Baturaja Health Research and Development Center laboratory. Mosquito sampling was carried out throughout the night (all night collection) for 12 hours starting from 18.00 – 06.00 with a time ratio of every 40 minutes/hour [14].

Mosquitoes Identification

Mosquitoes identification was carried out at the Entomology Laboratory of the Baturaja Health Research and Development Center. The process of identifying mosquitoes using a microscope and identification book based on the morphological character of each mosquito. Identification was done by observing the similarities between the shape of the antennae, the length of the proboscis and palps, the color of the abdomen and the special characteristics of each species of *Culex* mosquito [15]. The mosquitoes used as research samples were groups of *Culex mosquitoes*, namely *Culex vishnui, Culex quinquefasciatus, Culex tritaeniorhyncus,* and *Culex gellidus.*

DNA Extraction

DNA isolation was carried out using the Quick-DNA ™ Tissue / Insect Miniprep Kit procedure, as follows: First add the mosquito specimen to the ZR Bashing Bead™ Lysis Tube (2.0 mm) then add 750 µL of Bashing Bead™ Buffer to the tube and close the lid tightly. Then secure in a bead beater and process at maximum speed for 10 minutes. After that centrifuged the Lysis ZR Bashing Bead™ Tube (2.0 mm) in microcentrifugation at 10,000 x g for 1 min. Then transfer 400 µL of supernatant to the Zymo-Spin™ III-F Filter in the Collection Tube and centrifuged at 8,000 x g for 1 min. Add 1,200 µL Genomic Lysis Buffer to the filtrate in the Collection Tube from Step 4. Mix well. Next Transfer 800 µL of the mixture from Step 5 to the IICR Zymo-Spin™ Column in a Collection Tube and centrifuge

at 10,000 x g for 1 min. Remove the stream from the Collection Tube and repeat Step. Then add 200 µL of DNA Pre-Wash Buffer to the IICR Zymo-Spin™ Column on the Collection Tube and centrifuge at 10,000 x g for 1 minute. Thereafter add 500 µL g-DNA Wash Buffer to the IICR Zymo-Spin™ Column and centrifuge at 10,000 x g for 1 min. Finally transfer the IICR Zymo-Spin™ Column into a clean 1.5 mL microcentrifugation tube and add 100 µL (minimum 35 µL) of DNA Elution Buffer directly to the column matrix. Centrifugation at 10,000 x g for 30 seconds to elute the DNA.

Quantity and Quality of DNA Isolation

Quantitative measurement of DNA in the form of concentration and purity of DNA using Nanodrop Thermo cycle. The lysis buffer used for DNA isolation was dripped on the nanodrop sensor as much as 1 µL and then run as a blank, then the sensor was cleaned and the DNA isolate sample was dripped with 1 µL and then analyzed using nanodrops. This step was carried out on each sample of DNA isolates. Measurement of DNA concentration with nanodrops was carried out at a wavelength of 260 nm, while protein was measured at a wavelength of 280 nm with pure DNA defined as having an absorbance ratio of 260/280 ranging between 1.6 and 2.0 [16].

Meanwhile, DNA qualitative testing used electrophoresis technique. The product of DNA isolation was separated by using 1.75 % agarose gel electrophoresis technique. The gel is made by mixing distilled water: Buffer TAE 1:9, agarose as much as 1.75 g. The DNA isolation product used was 5 µL with the addition of 2 µL loading dye. As for the marker, 6 µL of the ladder was used with the addition of 2 µL loading dye. Electrophoresis was carried out for 45 minutes at a constant voltage of 80 V. Electrophoresis was carried out by Horizontal electrophoresis. The results of electrophoresis were observed under a UV transilluminator to determine the formed band [17].

3. Results and Discussion

DNA extraction is an important step in molecular engineering. DNA extraction is obtained by damaging or breaking the cell wall, so that the DNA comes out of the cell. The success of DNA isolation depends on the isolation method used. In this study, the method of isolating mosquito DNA was using a modified DNA Zymo Research Quick-DNA™ Tissue/Insect Miniprep Kit. The results of the isolation produced a clear sample. The clear color indicates the presence of DNA at the bottom of the tube. The samples were then tested using electrophoresis qualitatively and quantitatively for purity and concentration using NanoDrop [8].

The results of the isolation of the mosquito genome DNA are shown in Figure 1. Based on Figure 1, the DNA bands produced are clear without any bright degradation *(smears)* and there are no streaks, this indicates that the isolation process is good. Good quality DNA that was not degraded on electrophoresis showed clear bands and no stains [18]. The high quality of the DNA produced cannot be separated from the use of extraction buffers that are cooled before use. The lower the temperature used in the process of destroying plant tissue, the less chance of DNA degradation. The use of pre-cooled extraction buffer was able to produce DNA with good quality [19].

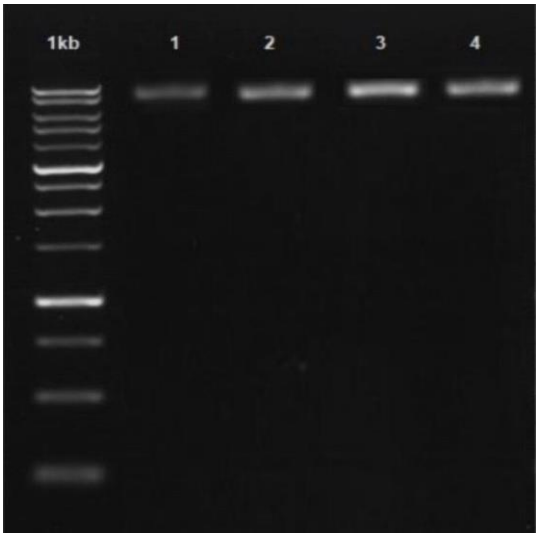

Figure 1: **Figure of Mosquito DNA isolation electropherogram Information: Marker 1 kb, *(1) Culex vishnui, (2) Culex quinquefasciatus, (3) Culex tritaeniorhyncus, (4) Culex gellidus***

Measurement of the concentration and purity of DNA isolates is an important step for a series of DNA isolation processes. The success of primers in amplifying printed DNA is determined by the quality and quantity of DNA. The DNA isolate obtained was then measured for the concentration and purity of DNA using a nanodrop spectrophotometer by comparing the absorbance of nucleic acids at =260 nm and protein at = 280 nm [20]. The working principle of nanodrop spectrophotometry is that pure DNA is able to absorb ultraviolet light due to the presence of purine bases and pyrimidines [13]. Pure DNA can absorb ultraviolet light due to the presence of purine and pyrimidine bases [21]. The double band of DNA can absorb ultraviolet light at a wavelength of 260 nm, while protein or phenol contaminants will absorb light with a wavelength of 280 nm. So that DNA purity can be measured by calculating the absorbance value. The wavelength of 260 nm is divided by the absorbance value of wavelength 280 (A260/A280) and the value of DNA purity is from 1.8 to 2.0, if the absorbance value is less than 1.8, the sample DNA still contain protein contaminants, proteinase is added to remove them. If the

value is more than 2.0, the DNA sample still contains RNA contaminants and ribonuclease is added to remove it [12].

Table 1 : Quantitative Concentration and Purity of *Culex* Mosquito DNA in Kemelak Bindung Langit Village

Code	Species	Konsentrasi (ng/µL)	A260/280
1	*Culex vishnui*	13.6	1.65
2	*Culex quinquefasciatus*	67.3	1.83
3	*Culex tritaeniorhyncus*	21.5	1.99
4	*Culex gellidus*	13.5	1.82

Based on the research conducted, the results of the purity of mosquito DNA are presented in table 1. Most of the mosquitoes have good DNA purity with purity ranging from 1.8 to 2.0. There is only one sample that has a DNA purity below 1.8 namely *Culex vishnui* 1.69. While the other mosquito samples had a purity that ranged above 1.8-2.00. *Culex quinquefasciatus* 1.83, *Culex tritaeniorhyncus* 1.99 and *Culex gellidus* 1.83.

Culex tritaeniorhyncus has the highest DNA purity of 1.99 which indicates that the sample is free from impurities such as protein, fat and carbohydrates and has good quality for use in the next process. While the lowest level of purity of the four samples was found in sample 1 *Culex vishnui* with an absorbance value of 1.69 this was due to the presence of a measurable level of impurity component (protein), suspected in the DNA extraction process the protein was not decomposed completely. Contamination caused by protein can come from cell components that are not lysed during the isolation process so that proteinases need to be added to transfer them back [22].

In addition to looking at the purity of DNA from the nanodrop test concentration of isolated DNA can also be seen. Most of the DNA samples isolated had good concentrations ranging from 10-100 ng/µL. The concentration of template DNA required for PCR activity ranges from 10-100 ng/µL. A high concentration value is not necessarily a high purity value. If the value of 260 which is the value for DNA is high then the concentration value will be high. The contaminant value for DNA purity is influenced by the value of 280 so that even though the DNA concentration value is high it does not mean that the purity value will be high as well [23] [24].

4. Conclusion

Based on the results obtained, it can be concluded that the *Culex* mosquito DNA showed that almost all DNA samples had good quality and quantity, but only one DNA sample had a purity value below 1.8, so it was necessary to add proteinase so that the sample had good quality. Furthermore, the sample from this study can be used for further research analysis on Mitochondria D-Loop Sequences.

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