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Analysis of Purity and Concentration of DNA Isolated in Dragonfly (*Onychogomphus forcipatus*)

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Abstrak. Analisis kemurnian dan konsentrasi hasil isolasi DNA dilakukan pada menggunakan sampel serangga spesies capung *(Onychogomphus forcipatus)*. Penelituan ini menjadi penting dilakukan karena banyaknya penelitian dibidang molekuer yang membutuhkan teknik isolasi DNA yang baik untuk mendukung keberhasilan penelitiannya. Tujuan penulisan ini untuk memberikan informasi mengenai kualitas DNA hasil isolasi yang dilihat menggunakan parameter kemurnian dan konsentrasi. Metode ekstraksi menggunakan metode *centrifuge colom*. Data hasil isolasi dianalisis *statistic* menggunakan uji rata-rata. Hasil isolasi DNA menunjukkan nilai rata-rata konsentrasi berada pada kisaran 60,10 – 69,95. Analisis kemurnian DNA hasil isolasi yang dibaca pada rasio A260/A280 berada pada rentang nilai 1.817 – 1.929, sedangkan analisis kemurnian pada rasio A260/A230 berada pada rentang 0,760 – 0,822. Berdasarkan hasil penelitian dapat disimpulkan bahwa analisis kemurnian dan konsentrasi DNA dari isolasi sampel capung berada dalam kategori yang baik pada rasio A260/A280, sedangkan untuk kemurnian yang dibaca pada rasio A260/A230 berada di bawah nilai yang dikategorikan baik.

Kata Kunci: *Analisis, DNA, Kemurnian, Konsentrasi, Rasio*

Abstract. *Analysis of the purity and concentration of DNA isolation results was carried out using samples of dragonflies (Onychogomphus forcipatus) species of insects. This research is important because there are many kinds of research in the molecular field that require good DNA isolation techniques to support the success of the research. The purpose of this paper is to provide information about the quality of the isolated DNA seen using the purity and concentration parameters. The extraction method uses the centrifuge column method. The isolated data were analyzed statistically using the average test. The results of DNA isolation showed that the average concentration value was in the range of 60.10 – 69.95. The analysis of the purity of the isolated DNA read at the A260/A280 ratio was in the range of 1.817 – 1.929, while the purity analysis at the A260/A230 ratio was in the range of 0.760 – 0.822. Based on the results of the study, it can be concluded that the analysis of the purity and concentration of DNA from the isolation of dragonfly samples was in a good category at the A260/A280 ratio, while the purity read at the A260/A230 ratio was below the value categorized as good.*

Keywords*:* *Analysis, DNA, Purity, Concentration, Ratio*

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1. INTRODUCTION

Research on the analysis of purity and concentration of isolated DNA carried out on dragonfly species is research that can be used to support other research in the field of species identification. Dragonflies are insects where this species has its challenges in DNA isolation. In conducting molecular-based research, one of the challenges that must be overcome is how to produce good isolated DNA.

Analysis of the concentration and purity of the extracted DNA is the first step and is an important part that must be done for DNA-based molecular testing. The extraction step can be determined from the value of purity and concentration produced during extraction (Corkill & Rapley, 2008). The extraction stage will work according to three main processes, namely the lysis process, or cell wall destruction, separation of DNA from other components such as protein and fat, and the DNA purification process (Sambrook, 1989). The extraction method used is a spin column or centrifuge column extraction method with the Dneasy mericon Food Kit combined with a robotic extraction system where this system works automatically to extract DNA (Sophian, 2021a; Sophian et al., 2021). In conventional extraction methods, it takes a long time to work (Sophian, 2021b).

Therefore, to save time and control the error rate of the researcher, modifications were made to combine these two types of methods. The advantage of this system is that it has more stable extraction results when compared to conventional methods. On the other hand, humans are one of the critical factors in contributing errors to the DNA extraction process. DNA extraction that has been done on dragonflies is using DNeasy Tissue Kits (Phillips & Swanson, 2018), and DNeasy kit (Karthika et al., 2012).

In this study, the extraction is certainly different from what has been done previously where this technique will combine conventional DNA extraction techniques and robotic extraction. One of the difficulties in extracting DNA from dragonflies is that their small bodies dry easily when sampled making DNA isolation opportunities a bit challenging to produce good DNA isolation. The selection of dragonfly species is because dragonflies require a sampling technique that is slightly different from common vertebrate species, whereas, in dragonflies, most of the samples obtained are only the skin of the skeleton where extraction requires a certain level of difficulty. Therefore, this study was conducted to provide information about DNA isolation

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techniques using conventional extraction techniques combined with robotic extraction so that the results of this study can be used as methods that can be adopted in molecular biology research using dragonflies as research objects

2. METHODOLOGY

2.1 Materials

The materials in this study were dragonflies (*Onychogomphus forcipatus*), Nuclease free water, Dneasy Mericon Food Kit extraction kit (50) paint. 69514 (Qiagen).

2.2 Sample Preparation

Weigh the sample weighing 0,5 g, then add 750 µL of Food Lysis Buffer and 10 µL of proteinase K enzyme and then homogenize by vortexing for 10 seconds. The samples were then incubated at 70°C for 30 minutes while occasionally vortexing for 10 seconds (Qiagen, 2014). After the incubation stage is complete, the sample is then removed and the temperature is lowered by allowing it to stand at room temperature for 30 seconds and then put into an ice block/freezer for 10 minutes. After cooling, the sample was then centrifuged at 14000 rpm for 10 minutes (Qiagen, 2014). The sample that has undergone the centrifuge process will then form 2 phases, pipette 500 µL of chloroform into a new 2 ml tube carefully, transfer 200-500 µL of the clear top layer without touching the precipitation that occurs at the bottom of the tube and then put it into the tube containing 500 µL of chloroform and vortexed for 15 seconds and then centrifuged at 14000 rpm for 15 minutes. Take 350 µL of the clear top layer and put it in a new 1,5 mL tube then put it in an automatic extraction device (Qiacube), using the standard method with elution of 60 µL of EB buffer (Qiagen, 2014). The elution DNA can be directly used for the real-time PCR process or stored at -20^{o}C for long storage (Qiagen, 2014).

2.3 Purity and Concentration Analysis

The isolated data was analyzed for the quality of the isolated DNA by evaluating the purity and concentration of DNA obtained using a nano photometer NP80 (IMPLEN), setting method; Nucleic acid, dsDNA type, nano volume mode, sample volume 2 µL, nucleic acid factor 50,00, background correction 320 nm, air bubble recognition off, manual dilution factor 1,000.

2.4 Data analysis

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The data obtained from the nano photometer was then analyzed statistically by comparing the average value of purity and concentration against DNA standards where the purity at the A260/A280 wavelength was in the range of 1.8–2.1, while the concentration was greater than 20 ng/mL (Sophian, 2021c; Sophian et al., 2021, Sophian & Syukur 2021; Matlock 2015; Eppedorf 2016). For analysis, the purity in the A260/A230 wave is in the range of 2,.0-2.22 (Matlock, 2015; Eppedorf, 2016).

3. RESULT AND DISCUSSION

The results of DNA isolation carried out in this study are presented in (Table 1). From the table, it is known that the average concentration value obtained from the isolation carried out was 66,043 ng/µL with a range in the range of 60,10 ng/µL – 69,95 ng/µL. For the analysis of the purity of the isolated DNA, which read the ratio A260/A280, it was in the range of 1,817 – 1,929 with an average purity value of 1,867. As for the purity analysis, which is read at the A260/A230 ratio, it is in the range of values of 0,760 – 0,822 with an average purity value of 0,797.

Tabel 1. DNA analysis nanophotometer data isolated

Sample	Concentration (ng/ui)	Purity	
		A260/280	A260/230
1	65,05	1,851	0,822
2	65,05	1,864	0,821
3	65,45	1,892	0,819
4	66,00	1,880	0,821
5	66,65	1,859	0,818
6	67,60	1,875	0,816
7	60,20	1,899	0,812
8	67,95	1,844	0,776
9	68,00	1,866	0,778
10	60,15	1,906	0,814
11	60,10	1,929	0,814
12	69,40	1,831	0,760
13	69,95	1,834	0,760
14	69,15	1,861	0,763
15	69,95	1,817	0,760
Average	**66,043**	**1,867**	**0,797**

When extracting samples, the lysis technique is a step that must be observed, where at this stage the DNA will be removed from the cell so that good lysis will affect

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the success of DNA isolation (Sophian, 2021d; Sophian et al., 2021). If seen from the data presented in (table 1), the value of the resulting purity is below 2,0 - 2,2, this can be presumed because the extracted DNA was not carried out properly so that the remaining wash buffer used was still carried away when washing at the final stage of the extraction process. This is in line with the opinion put forward by Matlock (2015), which states that if the purity value read at the A260/A230 ratio is below 2,0 – 2,2, this can be caused by several factors, namely carried carbohydrates (often a problem with plants). Residual phenol from nucleic acid extraction, residual Guanidine (often used in column-based kits) and Glycogen are used for precipitation. Meanwhile, if the purity value read at the A260/A230 ratio is above the value 2,0 – 2,2, this can be caused by several factors, namely blank measurement is carried out on a dirty base and using an inappropriate solution for measuring blanks.

In this study, DNA isolation was carried out using a centrifuge column extraction technique with the phenol-chloroform method. This technique is a common technique that is often used to isolate DNA from animal samples or raw material in the form of animal flesh, blood or other animal parts that can be used to isolate DNA. Measurement of the isolated DNA was carried out with a nanophotometer at the ratio A260/A28 and A260/A230. The measurements carried out will include all nucleotide molecules in the sample such as RNA, ssDNA and dsDNA. This is because all these molecules can be absorbed in the A260 wavelength (Matlock, 2015). In the analysis of isolated DNA, absorbance ratios of 260 and 280 were used to assess purity at a value of 1.8 - 2.0 where a value below 1.8 indicates the presence of protein, phenol or other contaminants (Sophian 2021b; Sophian et al., 2021; Wulan et al., 2021).

The main difficulty faced in this research is how to get a sample from the dragonfly being tested, where when sampling is done, dragonflies have different body characteristics from animals in general, whereas insects do not have muscle flesh like mammals or vertebrates in general.The isolation technique used in this study is a conventional isolation technique combined with a robotic technique. One of the reasons why this technique is carried out is to produce a faster DNA isolation time with higher accuracy, whereas by using a robot the pipetting consistency is more stable. This is in line with the research conducted by Sophian (2021b), wherein his research using robotic extraction techniques obtained stable DNA isolation results between the samples tested. In analyzes using samples such as this study, the level

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of difficulty faced when carrying out the extract is how to do the lysis well. This is because, at the time of extraction, the lysis process carried out will contribute to determining the success of the DNA isolation produced. Proper washing and centrifuge techniques are also very important where contamination of phenol and other mineral salts can also be a key indicator of the success of the DNA extraction process.

In terms of the concentration of isolated DNA, when viewed as a whole, it shows a uniform value in the range of concentration values of 60,10 ng/µL - 69,95 ng/µL, where these results indicate that in terms of concentration, the isolated DNA produced will show good results. The value of the concentration of DNA from good isolation can be shown with a concentration value that is above 20 ng/µL (Sophian & Syukur 2021).

4. CONCLUSION

Based on the results of the research conducted, it was found that the purity and concentration analysis carried out on DNA isolated from the dragonfly sample was in the category of good DNA isolation results for the analysis of concentration and purity read at the ratio A260/A280, while for purity read at the ratio A260 /A230 the resulting value is below the value that is categorized as a good DNA purity value.

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