Development Of DNA Extraction On Mangrove Leaves

Meighina Atika Istiqomah, Mohammad Basyuni, Poppy Anjelisa Zaitun Hasibuan

Abstract: Extraction to obtain high-quality DNA is a basic principle that must be completed in molecular analysis and is one of the succesfull factors in DNA amplification to be used in genetic character analysis. This research was aimed to determine the effectiveness and purity of DNA concentration with DNA extraction of mangrove leaves of Nypa fruticans, Ceriops tagal, and Rhizophora mucronata. The parameters observed in this study are the value of DNA purity and concentration. The samples analyzed were gill and fin tissues of common carp cells. The spectrophotometric method was used to calculate DNA concentration and purity. This research was conducted in the Laboratory of Parasitology and Laboratory of Central Research, Gadjah Mada University in April 2019. Preparation samples are cell lysis, washing RNA, elucus RNA, isolation cDNA, and NanoVue operation. The purity of DNA was measured from the absorbance ratio (R) of obtained DNA from the results of this study ranging from 1.7 – 1.9. The results of DNA isolation were pure if the ratio was between 1.7 to 2.0. In this research, DNA concentration values obtained were medium (5.15 µg/ml). That good concentration (C) for PCR ranged from 0.5 to 6.5 µg/ml. High total DNA concentration values was diluted to a certain concentration to be used for the PCR amplification. Dilution of DNA concentration was carried out to the concentration of total DNA isolates to reach 5 µg/ml. Based on the purity and concentration of DNA, the DNA was used for the amplification process. The value of concentration DNA was moderate, and high value is Nypa fruticans (5.05), which is almost the same as 5-FU value as control positive (5.15). The high purity of DNA was displayed by the value of ratio absorbance to be 1.7 to 2.0, showed that the sample was not contaminated and met the requirements for further investigation and other purposes such as a starting material for technology of anti-cancer development.

Index Terms: concentration, DNA, isolation, NanoVue, nucleic acids, RNA.

1 INTRODUCTION

Quantification and assessment of DNA/RNA and Protein purity and concentration is the first entry step in most of the molecular biology protocols routinely employed in many labs. Molecular biologists routinely work with DNA. RNA, and have devised some simple, fast spectrophotometric assays for these molecules [1]. With this concerned utilization of the UV absorbance of biological samples to obtain qualitative and quantitative information, especially for nucleic acid [2]. For this purview for quantifying the amount of nucleic acid in preparation are (A) spectrophotometric estimation (B) fluorometric determination and (C) DNA quantification using NanoDrop. Nowadays, UV analysis of DNA/Protein has been the benchmark method for rapid quantification and assaying purity of samples for various purposes. In conventional UV spectrophotometers, the sample is generally placed on the sample beam in a silica cuvette [3]. The absorbance obtained is compared with the standard, so the concentration is obtained. However, when the number of samples is limited, dilution or the use of ultra-low volume cuvettes is required and requires high precision [4]. DNA extraction and purification is the process of compiling DNA from other cell elements. Good extraction is supported by the results of the quantity of DNA, the extract obtained [5]. Information about DNA concentration and purity is required to determine the degree of contamination of a sample and whether the sample is high quality for use at a later stage. Therefore, measurements were made of both quantity and purification of genomic DNA [6]. As development progressed, using NanoVue, a typical sample volume of 2 µl was piped to a nonpolar surface, and then a very short path length of either 0.2 mm or 0.5 mm was made by lowering the sample head to the top of the sample. With a small sample, all common software features of the instrument, including scanning for single or longer wavelengths. [7]. Absorbance measurements absorbance ratios and concentrations can be used. In all these modes, the instrument uses track length. The advantage of this small line is that the instrument can assess very small sample volumes [8]. NanoVue also has a more detailed set of life science applications, including Deoxyribo Nucleic Acid and Ribonucleic Acid, concentration and purity and protein measurement calculations [9]. This study aims to determine the effectiveness and purity of DNA concentration with DNA extraction of mangrove leaves of Nypa fruticans, Ceriops tagal, and Rhizophora mucronata as technology for anti-cancer development.

- *The first author is currently a Magister Student at Magister Degree Program of Pharmacy of Faculty of Pharmacy in Universitas Sumatera Utara, Medan, 20155, Indonesia. E-mail: meighinatikaa@gmail.com*
- *The second Author is a lecturer at Department of Forestry, Faculty of Forestry, Universitas Sumatera Utara, Medan, 20155, Indonesia. E-mail: m.basyuni@usu.ac.id (Corresponding Author)*
- *The third author is a lecturer at the Program Study of Pharmacy, Faculty of Pharmacy in Universitas Sumatera Utara, Medan, 20155, Indonesia. E-mail: poppyanjelisa94@gmail.com*

2. MATERIAL AND METHODS

2.1 Experiment Design

This research was conducted in the Laboratory of Parasitology and Laboratory of Central Research, Gadjah Mada University. This research was conducted in April 2019 using NanoVue.

2.2 RNA Extraction

WiDr cells (7.5 x 10^8 cells/wells) were put in wells with a total of 6 wells then incubated for 24 hours. After that, the cells were given exposure to the test solution, then incubated again for 24 hours, after which the media was discarded and added with new media. The floating and attached cells were collected by giving trypsin 0.025% and then transferred into conical tubes, cells washed each with 1 ml PBS and centrifugation 2500 rpm for 5 minutes, the top layer is removed and the sediment collected and resuspended in PBS and centrifugation 3000 rpm for 180 seconds, remove the up layer and add with 1 mL PBS. Discarded culture medium then washed cells with PBS. PBS was taken, and trypsin added as much as 0.10-0.25%. After the cell is released, add the

medium, and then transfer it to the 15 mL conicle, proceed with suspending cell culture [10].

2.3 Cell Lysis

400 µL RB buffer and 4 µL β-mercaptoethanol (or 8 µl Dithiothreitol 2M was added which has been prepared in a water-free RNase), then resuspended. After that, homogenized the mixture, incubated at room temperature for 5 min. 500 µL was added with 70% ethanol that has been prepared in (free of Dnase and RNase). RB column in the 2 mL tube was transfered to 0,5 L of the mixture to the RB column. The tube was centrifuged with the strength of 15,000 x g for 1 minute, then discarded the filtrate. The remaining mixture was removed in the same RB column and centrifuged with a strength of 15,000 x g for 1 minute. The filtrate was removed and placed the column in 2000 µL tube [10].

2.4 Washing RNA

The RB column added 400 µL buffer W1, a centrifuge with a strength of 15,000 x g for 30 seconds. The filtrate was discarded, then placed it again in the RB column on the 2 mL tube and added 600 µl washing buffer (make sure ethanol has been added) to the RB column. Centrifuged with the strength of 15,000 x g for 30 seconds, then discarded the filtrate. The dried was placed to RB column into the new 1.5 mL microcentrifuge tube. 50 µL of water-free RNase was added to the center of the matrix column. Let stand for a few moments to complete the RNase free water perfectly. Then centrifuged with the strength of 15,000 x g for 60 secs [10].

2.5 Elucus RNA

The DNase 1 reaction in a 1.5 mL (free RNase) microcentrifuge tube was prepared as dispalyed in Table 1.

Table 1 *Preparation of the Dnase Reaction Solution*

Composition	Volume
RNA in RNase is water-free	$(1\text{-}40)^{-3}$ mL
DNase I	$0{,}5^{-3}$ mL/µg RNA
Dnase I reaction buffer	5^{-3} mL
RNase is water-free	add 5^{-2} mL
Total Volume	5^{-2} mL

Carefully the DNAse I reaction solution pippeted in the mixture (do not vortex) and then incubate the microcentrifuge tube at 37ºC for a half hour. The reaction was stopped by adding 0,001 mL of 20.000 µM EGTA (pH = 8.0), then re-incubate the microcentrifuge tube at 65ºC for 600 secs. After that, the RNA sample again was purified by adding 250 µL RB buffer to the DNase I reaction mixture, mixing the vortex wells. All sample mixture was transfered into the new RB centrifuge column with a strength of 15,000 x g for 60 secs, after removing the filtrate [10].

2.6 cDNA

The total RNA (3000 µL) was used to make cDNA, then PCR water was added to a total volume of 12 µL. A total of 8 µL of mixed solution (5 x RT-buffer 4 µL, random primary 1 µL, dNTP 2 µL, and Rever Tra-Ace 1 µL) were added to each tube containing RNA, then resuspended and used for followingPCRs. The PCR product was stored at -20ºC [10].

2.7 NanoVue Operatiom

Firstly, the sample was placed in a vertical position then used a low volume pipette (0-10 µl) to take about two µl of the sample. When issuing very small aliquots, answering the sample was absolutely accurate. Secondly, the sample was made carefully to avoid the object of the middle black dot between the midpoint of the alignment was stable not to left, right, up and down. The emergence of bubbles was prevented into the sample. After the pusher was depressed, the pipette carefully was lifted to still exsist the zone. Thirldy, the position was observed and shaped the drop. If a program has been selected and the Auto-Read function was set to on, the sample reading process begun automatically. Before cleaning the instrument case, the power instrument turned off the power and unpluged the power cable. All external surfaces was rinsed using a soft cloth. Mild liquid detergent was used to duscard scars that were usually difficult to remove.

2.8 Data analysis

Data analysis was performed descriptively quantitative. Data obtained from DNA isolation results using several DNA isolation methods and results amplification. From research, this tape was produced from each of the different isolation methods compared to positive control and negative control to get results the best quality based on DNA and the amount of DNA.

$$C = (\lambda A_{260} \times 50)$$

$$R = \lambda A_{260} - \lambda A_{320} / \lambda A_{280} - \lambda A_{320} \quad (1)$$

3. Result and Discussion

The sample used in this study was mangrove leaves, namely N. fruticans, C. tagal, and R. mucronata. DNA isolation produced DNA isolates both in terms of quality and quantity. Quantification results by calculating the concentration and purity level using a spectrophotometer was shown in Table 2 and Table 3.

Table 2 *Value of DNA Concentration*

Samples	A_{260}	Factor Concentration	C
Nypa fruticans	0.101	50	5,05
Ceriops tagal	0.100	50	5
Rhizophora mucronata	0.100	50	5
5-FU	0.103	50	5,15

Note: C = Concentration (µg/ml)

Good concentration (C) for PCR ranged from 0.5 to 6.5 µg/ml [11]. The high concentration values of total DNA diluted in a certain concentration to be used for the PCR process [12].

Table 3 *Value of DNA Quantity*

Samples	Wavelength			R
	A_{260}	A_{280}	A_{320}	
Nypa fruticans	0.101	0.07	0.035	1.88
Ceriops tagal	0.100	0.075	0.045	1.83
Rhizophora mucronata	0.100	0.075	0.04	1.71
5-FU	0.103	0.075	0.036	1.72

Note: R = Absorbance Ratio

The purity of DNA was depicted from the absorbance ratio (R) of DNA obtained from the results of this study ranging from 1,7 – 1,9. The results of DNA isolation were pure if the ratio between 1,7 to 2,0 [13]. In this research, obtained DNA concentration values were medium, reaching 5,15 µg/ml. DNA purity values determined in each sample was displayed in Table 2. The results indicated that each sample has been pure. DNA isolates was pure when the values ranged from 1,7 to 2,0 and have been incorporating the requirements needed in the molecular analysis [13]. The difference in the concentration of DNA obtained in each sample can be determined by the physical treatment given as well as the ability of extraction buffer in breaking down cells [14]. The process of physical cell destruction was carried out by crushing a sample which can perfect the extraction buffer in breaking down cells. Besides that, the extraction buffer used can determine the concentration of DNA produced. In the remaining stages of protein, fat, and polysaccharide precipitation can be removed by using salt. The final stage of DNA isolation was the stage of DNA washing. Washing DNA was performed to separate other compounds such as solutions and salts that come along with DNA. The DNA washing process was done with the addition of buffer [15]. Dilution of DNA concentration was carried out to the of total DNA concentration isolates to reach 5 µg/ml. Based on the purity and concentration of DNA, then the results of DNA isolation can be used for the amplification process [16]. NanoVue will default to fifty points factors for double-stranded DNA, forty points for RNA, and thirty three points for RNA. It also allows long compensation for dilution - supported by a dilution calculator. Nucleic acid is extracted from cells taken with protein, and purification is widely needed to remove impurities [17].

4. CONCLUSION

The value of concentration DNA was moderate, and high value is N. fruticans, which ws almost the same as 5-FU value can be used a starting material. The purity of DNA of this study revealed that the sample was not contaminated and met the requirements for further investigation of anto-cancer development.

5. CONCLUSION

This research was supported by Penelitian Tesis Magister (PTM) 2019 scheme (11/E1/KP.PTNBH/2019) from the Directorate for Research and Community Service, Ministry of Research, Technology and Higher Education, Republic of Indonesia.

REFERENCES

[1] O. Warburg and W. Cristian, “Isolation and crystallization of enolase,” Biochemische Zeitschri ft, vol. 310, no. 4, pp. 384-421, 1942.

[2] R. Muhammad, Mukrimin and Gusmiaty, "Optimalisasi Teknik Ekstraksi dan Isolasi DNA Tanaman Suren (Tona Sureni Merr.) Untuk Analisis Keanekaragaman Genetik berdasarkan Random Amplified Polymorphic DNA (RAPD),” J. Natur Indones, vol. 14, no. 4, pp. 384-421, 1942.

[3] P. Laird, A. Zijderveld and A. Berns, “Simplified Mammalian DNA Isolation Procedure,” Nucleic Acids Research, vol. 19, no. 15, pp. 4293, 1991.

[4] L. Novita, “Analisis Genetik Karakter Morfoagronomi Jarak Pagar Hasil Pemuliaan Berbasis Pendekatan Kuantitatif Molekuler ,” Thesis, Dept. of Agriculture., Bogor Agricultural Institute., Indonesia, 2013.

[5] U. Amanda , “Isolasi RNA Total dari Mesokarp Buah KELAPA Sawit (Elaeis guineenssis Jacq. Var. Tenera),” Proc. Semin. Nas. Masy. Biodivers Indones), pp. 171-176, Apr. 2015, doi:10.1109/SCIS.2007.367670.

[6] Hasmiwati, “Analisis Genetik Anopheles balabacensis Baisas (Diptera : Culicidae) dari Daerah Bangko (Jambi dan Purworejo (Jawa Tengah) Dengan Random Amplified Polymorfic DNA (RAPD) PCR,” Majalah Kodekteran Andalas, vol. 30, no. 2, pp. 70-80, available at http://repository.unand.ac.id/351/1/Hal_69_vol.30_no.2_2006_Analisis_genetik_nyamuk.doc, Dec. 2006.

[7] S. Kate-Ngam and P.A. Lakote, “A Comparative Study of Different RAPD-PCR Protocols For Genetics Diversity Analysis of Doritis germplasm,” J. Med Microbiol, vol. 44, no. 6, pp. 203-206, 1996.

[8] U. Pich and I Schubert, “Midiprep Method For Isolation of DNA from Plants with A High Content of Polyphenolics,” Nucleic Acid Research, vol. 21, no. 14, pp. 3328, 1993.

[9] A. Lane and S. Bikram, “Isolation of a D-genome Specific Repeated DNA Sequence from Aegilops squarrosa,” Plant Molecular Biology Reporter, vol. 4, no. 2, pp. 102-109, 1986.

[10] Cancer Chemoprevention Research Centre, “Protokol Kultur Sel,” CCRC, http://ccrc.farmasi.ugm.ac.id/wp-content/uploads/03.007.-Subkultur.pdf. 2013

[11] M. Basyuni, S. Baba, and H. Oku. "Microsatellite analysis on genetic variation in two populations of red mangrove Rhizophora mangle L.(Rhizophoraceae) and its implication to conservation." IOP Conference Series: Materials Science and Engineering. Vol. 180. No. 1, pp. 012243, 2017.

[12] J. Grimberg and S. Nawoschik, “A Simple and Efficient non-Organic Procedure for the Isolation of Genomic DNA from Blood,” Nucleic Acid Research, vol. 17, no.20, pp. 8390, 1989.

[13] W. Wilkerson, M. Peter and W. Patrick, "Detection and Lifetime Measurement of Single Molecules in Flowing Sample Streams by Laser-Induced Fluorescence,” Appl. Phys. Lett, vol. 62, pp. 2030, 1993.

[14] J. Sambrook, K. Norrington, and K. Kohno, "S. cerevisiae Encodea an Essential Protein Homologous in Sequence and Function to Mammalian BiP,” Cell, vol. 57, pp. 1223-1226, 1989.

[15] M. Jamilena, C. Ruiz and M. Ruiz, “A Molecular Analysis of the Origin of the Crepis capillaris B Chromosome,” Journal of Cell Science, vol. 107, pp. 703-708, 1994.
[16] S. Ignacimuthu and S. Prakash, “Agrobacterium-mediated Transformation of Chickpea with α-amylase Inhibitor Gene for insect Resistance,” Journal of Bioscience, vol. 131, no. 3 pp. 339-345, 2006.
[17] L. Roger and M. Jennifer, “Ribosomal DNA is frequently concentrated on only one X Chromosome in Permanently Apopmictic Aphids, but This Does Not Inhibit Male Inhibit Male Determination,” Chromosome Research, vol. 4, no. 4 pp. 314-320, 1996.