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Short Communication: Analysis of Purity and Concentration of Isolated DNA in Making Raw DNA of Rat Species

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ABSTRAK/ ABSTRACT

Analisis kemurnian dan konsentrasi DNA hasil isolasi pada pembuatan DNA baku spesies tikus dilakukan untuk memberikan informasi/ referensi tentang pembuatan DNA baku sehingga baku DNA yang digunakan pada pengujian deteksi DNA spesies tidak hanya bergantung pada baku sintetik yang secara ekonomi memiliki harga yang cukup mahal jika dibandingkan dengan baku yang dibuat secara mandiri. Tujuan dari penelitian ini adalah untuk melihat apakah hasil isolasi DNA yang dilakukan masuk dalam syarat kategori DNA yang baik atau tidak, sehingga dapat digunakan sebagai baku DNA uji. Metode isolasi DNA yang dilakukan adalah metode spin kolom atau centrifuge kolom dengan menggunakan kit ekstraksi Intron Patho Gene-Spin (Viral DNA/RNA). Metode analisis konsentrasi dan kemurnian DNA hasil isolasi dianalisis berdasarkan nilai rata-rata konsentrasi dan kemurnian yang dibaca menggunakan nanophotometer. Berdasarkan hasil penelitian yang dilakukan, diperoleh hasil nilai konsentrasi DNA hasil isolasi berada pada rentang konsentrasi 41.250 ng/µL sampai 42.300 ng/µL, dengan rata-rata konsentrasi DNA hasil isolasi adalah 41.777 ng/µL. Untuk nilai kemurnian DNA hasil isolasi yang dibaca absorbansinya menggunakan nanophotometer pada panjang gelombang A260/A280 diperoleh hasil antara 2.301 - 2.384 dengan rata-rata nilai kemurnian berada pada 2.326. Kesimpulan dari penelitian ini adalah semua sampel yang di ekstrasi menunjukan hasil analisis DNA yang termasuk dalam kategori DNA yang baik.

Analysis of the purity and concentration of isolated DNA in the manufacture of standard rat DNA was carried out to provide information/references about the manufacture of standard DNA so that the DNA standards used in testing species DNA detection do not only depend on synthetic standards which are economically quite expensive when compared to independently made. The purpose of this study was to see whether the results of DNA isolation carried out were included in the requirements of a good DNA category or not, so that it could be used as a standard DNA test. The DNA isolation method used is the spin column method or column centrifuge using the Intron Patho Gene-Spin (Viral DNA/RNA) extraction kit. Analysis method of concentration and purity of isolated DNA was analyzed based on the average value of concentration and purity which was read using a nanophotometer. Based on the results of the research conducted, the results of the isolated DNA concentration values were in the concentration range of 41,250 ng/µL to 42,300 ng/µL, with the average concentration of isolated DNA was 41,777 ng/µL. For the value of the purity of the isolated DNA whose absorbance was read using a nanophotometer at a wavelength of A260/A280, the results were between 2,301 - 2,384 with an average purity value of 2,326. This study concludes that all extracted samples show the results of DNA analysis which are included in the good DNA category.

Kata Kunci: DNA, Tikus, Isolasi, Intron
Keywords: DNA, Rat, Isolation, Intron

1. Introduction

The development of species-specific DNA testing opens up new challenges for DNA-specific molecular testing, where the increasing number of species detected will also create opportunities to provide testing standards. Specific DNA detection often uses standard DNA in testing, so the challenge of providing raw materials that are extracted or isolated from the meat of certain species of animal opens the possibility to do so.

Detection of rat DNA contamination in food products has been carried out on processed beef meatballs detected using PCR (Aminah et al., 2019; Rohman et al., 2021; Septiani, 2021; Suryawan et al., 2020). The success of a molecular analysis using PCR is also supported by several factors, including the DNA extraction technique carried out, the use of appropriate primers, and the use of appropriate test standards. Therefore, the provision of test standards derived from the meat DNA of target species is a serious step in developing the world of species DNA testing. The use of synthetic standards is certainly not a mistake, but the economic considerations of a test standard also need to be considered in conducting a test. The challenge that needs to be considered in the manufacture of DNA raw materials from the meat of the target DNA species is how to produce quality extracted/isolated DNA with good concentration and purity values.

Therefore, this research was conducted to provide an alternative reference in terms of making DNA standards to make standard stock for species DNA testing. As a suggestion for further research, it may be necessary to conduct a comparative test of the use of synthetic standards and native DNA standards as further information in species DNA, where this can provide information for further research on the use of appropriate test standards in conducting tests.

2. Methodology

2.1 Materials

In this study, samples from rat meat were tested 15 times. DNA isolation was performed using the Intron Patho Gene-Spin (viral DNA/RNA) Extraction (cat. 17154.100) isolation kit.

2.2 DNA Isolation

The sample weighing 0.5 g was put into a 2 ml tube and added 700 µL of VL buffer solvent after which it was incubated incubated at 65 ºC for 30 minutes. Then 500 µL of BL buffer was added and then vortexed for 10-15 seconds, then incubated for 1-2 minutes. Transfer all the solution into a spin column then centrifuge at 14,000 rpm for 1 minute (If there is still residue in the 2 mL tube, do the same thing until all the ocean in the 2 mL tube is used up). Discard the liquid in the centrifuge tube then add 500 µL of buffer RW 1 into the spin column then centrifuge at 14,000 rpm for 1 minute. Discard the liquid in a centrifuge tube then add 700 µL of buffer RW 2 and centrifuge at 14,000 rpm for 1 minute. Transfer the spin column to a 1.5 mL microcentrifuge tube and then add 50 µL of EB buffer. Incubated for 1 minute then centrifuged at 14,000 rpm for 2 minutes. Discard the spin column and then calculate the concentration and purity of the extracted DNA using a nano photometer (Intron 2020).

2.3 Analysis of Purity and Concentration of Isolated DNA

The resulting DNA isolation was then continued by analyzing the purity and concentration of DNA using a nano photometer (IMPLEN). Setup tool using the Nucleic acid method, with dsDNA sample type, nano volume 1 µL, nucleic acid factor 50.00, background correction 320 nm, air bubble recognition off, manual dilution factor 1,000 (Sophian, 2021; Sophian et al., 2021).

2.4 Data Analysis

The data obtained were then analyzed by performing statistical tests in the form of an average test of the concentration and purity of the extracted DNA.

3. Results and Discussion

Analysis of concentration and purity was carried out using a nano photometer. The table shows that the concentration values of the extracted samples are in the range of 41.250 ng/µL – 42.300 ng/µL, with an average of 41.777. The purity value measured at wavelength A260/A280 was obtained with a purity range between 2.301 – 2.384, with the average purity value being at 2.326. According to (Abinawanto et al., 2019; Intron, 2020; Matlock, 2015; Qiagen®, 2014), the value of DNA concentration is good if the isolated DNA has a concentration above 20, with the purity value of the DNA extract in the range of 1.8-2.1. In this study, the isolation results have differences in the value of purity, wherein in this study, the value of purity is above 2.1. This is in line with the research conducted by (Sophian, 2021; Sophian et al., 2021), who performed DNA extraction using the Intron Patho Gene-Spin kit (viral DNA/RNA). If the purity value is below 1.8, the isolated DNA may be contaminated with protein, and vice versa, if the purity value is above 2.1, it can be suspected that the DNA isolation carried out is contaminated with RNA. (Eppendorf, 2016).

The Patho Gene-spin DNA/RNA Extraction Kit is designed to quickly and sensitively isolate DNA or RNA from various pathogens such as viruses, bacteria, etc. Or other types of samples which can be fresh or frozen plasma/blood (treated with anticoagulants other than heparin), serum, other cell-free body fluids and pathogen-infected cells/tissues. This kit is specially designed to isolate high-quality nucleic acids from a wide range of pathogens and specimens using a low elution volume that allows for real-time PCR analysis. This kit uses a chaotropic salt in the lysis buffer where this salt immediately inactivates the DNase/RNase to ensure complete DNA/RNA isolation. This kit uses advanced silica gel membrane technology for fast and effective purification of DNA or RNA without organic extraction or ethanol deposition. Furthermore, the buffer conditions were fine-tuned to provide optimal DNA/RNA binding to the column (Intron, 2020).

DNA or RNA is generally a series of nucleotides consisting of 5 types where these five types of nucleotides, if read the absorbance value at wavelength A260/A280, will have different values, for DNA, the purity value 1.8-2.1 is the accumulated value of 4 The types of nucleotides are guanine (1.15), adenine (4.50), cytosine (1.51), and thymine (1.47). while for RNA, thymine will be replaced by uracil which has an absorbance value (4.00). because the absorbance value of uracil is higher than other nucleotides causing the presence of uracil if read at wavelength A260/A280 it will show a purity value that is above 2.1 (University, 2020).

Table 1. Data on DNA Isolation Results

Sample	Nanophotometer Analysis	
	Purity (A260/A280)	Concentration (ng/µL)
1	2.384	41.600
2	2.318	41.600
3	2.301	41.650
4	2.384	41.250
5	2.332	42.300
6	2.311	41.600
7	2.324	42.100
8	2.320	41.800
9	2.314	42.050
10	2.318	41.850
11	2.301	41.750
12	2.311	41.850
13	2.324	41.800

Sample	Nanophotometer Analysis	
	Purity (A260/A280)	Concentration (ng/μL)
14	2.331	41.800
15	2.312	41.650
Average	2.326	41.777

In carrying out the extraction, it is important to know some possible failures when the isolated DNA read on the nanophotometer does not give good results or during the amplification process using PCR. According to (Intron, 2020), the failure of amplification or dimers in the PCR amplification process can be caused by the presence of ethanol carryover. Steps that can be taken to overcome this problem are to Ensure that after the washing Buffer B wash, the column is spun at maximum speed for 1 minute to dry the Patho-Gene spin membrane. In addition, there were other failures such as Little or no nucleic acid in the eluates. This can be caused by several things, namely: Low concentration of pathogen in the sample, Incomplete removal of medium (Cell samples), Step were not followed correctly or the wrong reagent used, DNA/RNA degraded, Too much-starting material and washing A and washing B used in the wrong order.

At the DNA extraction stage, the proteinase K enzyme plays an important role. Proteases interfere with protein by digesting it. Compared with methods that use chemicals, enzymes work quite effectively because they directly target amino acid bonds in protein lysis. The proteinase K enzyme will only be active when incubation occurs at a temperature of 65-70 °C. Therefore, in several studies using this method, it is sometimes necessary to optimize the method before using it. (Sophian, 2021; Sophian et al., 2021) has optimized the incubation period of proteinase K during DNA extraction lysis, from the results of this study it was known that at a temperature of 65 °C, the lysis would work well when incubated for 3 hours or more. If the incubation time is below 3 hours, the lysis process cannot take place completely.

4. Conclusion

Based on the research, it is known that all the extracted samples obtained sample concentration values in the range of 41.250 ng/μL – 42.300 ng/μL, with an average of 41.777, and the value of purity measured at the wavelength A260/A280 obtained results with a purity range between 2.301 – 2.384, with an average purity value of 2.326.

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