ASIAN J TROP BIOTECHNOL
Volume 18, Number 2, December 2021
Pages: 69-72

ISSN: 0216-6887
E-ISSN: 2301-8658
DOI: 10.13057/biotek/c180203

Short Communication: Comparison of two commercial DNA extraction kit to obtain high quality porcine DNA

DIANA TRI WULAN♥, MARCELLA SUTANTA, ALFI SOPHIAN
Special Drug and Food Testing Center, National Center for Drug and Food Testing Development, National Agency of Drug and Food Control Indonesia. Jl. Percetakan Negara No. 23, Jakarta Pusat 10560, Jakarta, Indonesia. Tel. +62-21-4263333, ♥email: diana.wulan@pom.go.id

Manuscript received: 25 October 2021. Revision accepted: 17 November 2021.

Abstract. *Wulan DT, Sutanta M, Sophian A. 2021. Short Communication: Comparison of two commercial DNA extraction kit to obtain high quality porcine DNA. Asian J Trop Biotechnol 18: 69-72.* Comparison of two commercial DNA extraction kits, to obtain high-quality porcine DNA, was carried out to give information about the capabilities of the two types of commercial kits used in isolating DNA. This research was conducted to provide information on the quality of DNA isolated from the manufacture of standard DNA from two different types of extraction kits. The kits used in this study were the Mericon Food Kit and the Life River Kit. The method used in this study was the centrifuge column extraction method. The isolated sample was analyzed for purity and concentration using a nano spectrophotometer. The data obtained was then processed statistically to obtain P-value for two mean T-test. Based on the study results, it was found that the average purity and concentration of DNA extracted with the Mericon Food Kit were 2.17 ng/µL and 139.3 ng/µL. Meanwhile, the average purity and concentration of DNA isolated from the Life River Kit were 1.46 ng/µL and 11.9 ng/µL. Based on the statistical analysis of the P-value for the two mean T-test, it was found that the purity and concentration of the two extraction kits were significantly different.

Keywords: DNA, isolation, kit, purity

INTRODUCTION

Currently, medicinal and food products made from animals, like meat, gelatin, fat, and collagen are growing. Along with these developments, new problems have emerged in finding pork content in food and drug products. The content does not match the product claims. In 2020, the Food and Drug Supervisory Agency in the National Agency of Drug and Food Control Indonesia Annual Report stated that from 120 food samples, 7 samples were positive for porcine DNA. This triggered the testing of porcine content in medicinal and food products to ensure that these products are safe and halal for consumption.

Research to develop methods related to DNA detection in food and drug products has been carried out, such as meatball products (Margawati and Ridwan 2010), soft candy (Fadhlurrahman et al. 2015), removing polysaccharides in fruit (Majumder et al. 2011) and capsule shells (Khayyira et al. 2018). A standard or standard porcine genome is needed in every porcine DNA detection test. Porcine DNA isolation from pork has been done previously, using either traditional methods or extraction kits. Suadi et al. (2020) compared the concentrations and purity of the traditional and kit extraction methods. Based on this research, it is known that the concentration of DNA extraction using the traditional method (phenol-chloroform) is higher than the extraction kit method (Mericon Food Kit). Still, the purity obtained from the traditional method is not satisfactory. In addition, the time required for the extraction process with the traditional method is more extended than using an extraction kit (Suadi et al. 2020).

Therefore, this study aims to provide further information on the quality of the extraction results from two different extraction kit methods through the concentration and purity values of the DNA isolates produced. Furthermore, the purity and concentration of both methods were tested statistically using the T-test. Through this T-test, the two methods carried out have significantly different results (Miller and Miller 2010). Therefore, it is hoped that the results of this study can provide additional information regarding DNA extraction techniques in the manufacture of standard porcine DNA using different types of kits.

MATERIALS AND METHODS

Materials

In this study, samples of porcine meat were tested 24 times. In addition, DNA isolation was carried out using the Dneasy Mericon Food Kit (Qiagen) and the Life River Viral DNA/RNA Isolation Kit (Centrifuge Column).

DNA isolation Mericon Food Kit

DNA isolation using the Mericon Food Kit was carried out by weighing 0.2 gr of pork in a 2 mL centrifuge tube and then added 1 mL of Food Lysis Buffer and 25 µL of Proteinase K. Then the tube was incubated in a 60 °C water bath shaker for 20 minutes, then centrifuged at 14000 rpm

for 5 minutes. After that, 500 µL of supernatant was put into a 2 mL tube filled with 500 µL of chloroform, then centrifuged at 14000 rpm for 15 minutes. Then, 350 µL of the top solution was taken into a 2 mL tube filled with 350 µL of Buffer PB, and then the solution was vortexed until mixed. Then the mixture was transferred to a spin column connected to a 2 mL collection tube, and then centrifuged at 14000 rpm for 1 minute. The solution in the collection tube was then discarded, followed by reusing the collection tube to add 500 µL of Buffer AW2. This was then placed in the centrifuge at 14000 rpm for 1 minute. The solution in the collection tube was discarded and the collection tube reused. Furthermore, the drying process was carried out by centrifugation at 14000 rpm for 1 minute. Then, the spin column was transferred into a 1.5 mL tube, with an added 100 µL of Buffer EB, incubated for 1 minute at room temperature, and centrifuged at 14000 rpm for 1 minute (Qiagen 2020).

DNA isolation Life River Kit

DNA isolation using the Life River Kit was carried out by weighing 0.2 g of pork, then put into a 2 mL centrifuge tube. 20 µL of Proteinase K and 500 µL of Lysis Buffer were added. It was then incubated at 56 °C for 10 minutes. After incubation, the sample was added to 500 µL of absolute ethanol and vortexed for 2 minutes. The solution in the binding column was transferred in the collection tube. Next it was then centrifuged at 12000 rpm for 1 minute. The solution collected in the collection tube was then discarded, with the binding column was placed back in the collection tube. This was then washed with Washing Buffer A and centrifuged at 12000 rpm for 1 minute. Followed by being washed with Washing Buffer W and centrifuged at 12000 rpm for 1 minute. It was then washed again with Washing Buffer and centrifuged at 12000 rpm for 3 minutes. The binding column was then transferred to a 1.5 mL tube, with 50 µL of Elution Buffer added, and then let to stand for 5 minutes at room temperature, before being centrifuged at 12000 x g for 3 minutes (Life River 2020).

Purity and concentration analysis

According to the manual, the analysis of purity and concentration was carried out using a DNA Thermo Scientific NanoDrop Spectrophotometer 2000c Spectrophotometer. The first step using nanodrop is to open the nanodrop software, then pipette 2 µL of ER solution, put it on the needle as a blank, and then measure the blank. Then pipette 2 µL of the sample onto the needle nanodrop and measure its purity and concentration.

Data analysis

The data obtained were then analyzed statistically using Microsoft Excel (Office 365) to calculate the P-value for the two mean T-test.

RESULTS AND DISCUSSION

Extraction results with Mericon Food Kit

Using the Mericon Food Kit, porcine DNA extraction was carried out with 12 replications and tested with a DNA Spectrophotometer to produce the purity and concentration values shown in Table 1.

Based on the table of DNA isolation results with the Mericon Food Kit, the sample concentration values were in the range of 133.7 ng/µL-160.1 ng/µL, with an average of 139.3 ng/µL. The value of sample purity measured at wavelength A260/A280 was in the range 2.15-2.18 with an average of 2.17. According to Sambrook et al. (1989), the purity value of DNA isolate above 2.2 indicates that the DNA extraction results still contain RNA contamination. In contrast, if the purity results show a value less than 1.7, it indicates that the DNA extract still contains protein contamination. In this case, the value of DNA purity extracted with the Mericon Food Kit was within that value range. This is also reinforced by the opinion put forward by Abinawanto et al. (2019) and Sophian (2021b), who revealed the same thing where the range of values is the average value of the 4 nucleotides that make up DNA, namely guanine (1.15), adenine (4.50), cytosine (1.51), and thymine (1.47), where if these four nucleotides were read on a nanophotometer they would produce different absorptions when read at wavelengths A260/A280.

Extraction results with Life River Kit

Porcine DNA extraction using the Life River Kit was carried out with 12 replications and tested with a DNA Spectrophotometer to produce the purity and concentration values (Table 1).

Based on Table 2, the results of DNA isolation with the Life River Kit have a sample concentration value in the range of 11.5 ng/µL-12.5 ng/µL, with an average of 11.9 ng/µL, where the concentration value is smaller when compared to the concentration extracted using Mericon Food Kit. This was due to differences in the Lysis Buffer composition from the two types of extraction kits, which resulted in different concentrations of DNA obtained. In this study, the method used followed the manual kit so that the difference in the volume of the solution and the extraction technique is because the kit used is different.

The value of DNA purity isolated from the Life River Kit was in the range of 1.38 ng/µL-1.64 ng/µL, with an average of 1.46 ng/µL. Less than 1.8 ng/µL, which indicates that the results of the DNA isolate still contain protein contamination. The use of RNA extraction/isolation kits in DNA research was carried out by Sophian et al. (2021) on crab processed food products, the results obtained that the average concentration value was 15.46 with a purity value of 2.240. and also, by Sophian (2021a) on processed salted fish products, which obtained the average concentration value at 25.745 with a purity value of 1.729.

In general, DNA isolation/extraction has the same principle between the types of kits used, it's just that the RNA isolation kit uses an RNA carrier as one of the components of the kit used, but this study tries to carry out the extraction kit's performance with different characters. To obtain information about its ability to isolate DNA in the manufacture of porcine standards.

Table 1. DNA isolation result data with Mericon Food Kit.

No. sample	Nanophotometer analysis	
	Purity (A260/A280)	**Concentration (ng/μL)**
1	2.17	160.1
2	2.18	133.7
3	2.18	136.3
4	2.15	139.3
5	2.17	138.4
6	2.18	138.0
7	2.18	137.9
8	2.15	134.8
9	2.18	138.0
10	2.18	138.4
11	2.17	137.2
12	2.17	139.5
Average	2.17	139.3

Table 2. DNA isolation result data with Life River Kit.

No. sample	Nanophotometer analysis	
	Purity (A260/A280)	**Concentration (ng/μL)**
1	1.64	11.5
2	1.49	11.8
3	1.38	12.5
4	1.43	11.9
5	1.47	11.9
6	1.44	11.9
7	1.39	12.0
8	1.44	12.3
9	1.49	12.0
10	1.47	11.6
11	1.43	12.1
12	1.45	11.8
Average	1.46	11.9

Statistical analysis (T-test)

The results of the purity and concentration values from the nanodrop test were then analyzed statistically using the T-test. According to Miller and Miller (2010), in the T-test if the T-count value is greater than the T-table, the two methods compared are significantly different. On the other hand, if the T-count value is less than the T-table, the two methods compared are not very different. The value of the purity of the DNA isolation results with both kits tested t gave the results of t count of 36.6 and t table of 1.8. From these results, the purity values of the two methods differ significantly. For the concentration value of the nanodrop results of the two kits after the T-test, the T-count data is 63.6 and the T-table is 1.8. Based on the results of the T-test, it is known that if T-count is greater than the T-table, this indicates that the two methods are significantly different. The final elution volume difference is a step adjusted to the method used so that this analysis does not use the same steps and volume for each addition of the solution used. This difference will then be the initial information for more comprehensive optimization of further research.

Discussion

In this study, 2 types of extraction kits were used. In the Life River Kit, there was a slight modification in its use by adding proteinase K to the lysis buffer used. In general, the DNA extraction process consists of three main processes, namely cell lysis, which includes a series of stages of destroying cell membranes with the help of proteinase K was used for degrading/cutting the protein structure and SDS (Sodium Dodecyl Sulfate) bind to membrane cell to denature the macromolecule structure. Then, purification is done by giving phenol and CIAA (Chloroform Isoamyl Alcohol), which binds other macromolecules besides DNA, such as carbohydrates, fats, and proteins. And the last step is precipitation, which is a process carried out to separate DNA and water from other organic materials and draw water is done using alcohol, where the separation occurs when centrifugation is carried out (Kado and Liu 1981).

In the Life River Kit, the extraction process is carried out with a slight modification, namely by adding proteinase K at the time of lysis. This enzyme has an essential role in cell lysis. Proteinase K enzyme has another name, endopeptidase K or protease K is a serine protease with a broad spectrum that is commonly used in DNA extraction. Due to the nature of this enzyme that can digest keratin, this enzyme is often referred to as proteinase K. Mini enzyme was discovered in 1974 isolated from the extract of the fungus Engyodontium (Betzel et al. 1993; Mueller et al. 2004). The isolated DNA was analyzed for purity and concentration using a nanophotometer by measuring the absorbance value at wavelength A260/A280. Analysis at the A260/A280 wavelength is a method that is often used to detect DNA (Matlock 2015; Eppendorf 2016; Koetsier and Cantor 2019).

There are 5 nucleotide compositions that makeup DNA or RNA. If the absorbance at wavelength A260/A280 is read, it will show varying values, namely: guanine (1.15), adenine (4.50), cytosine (1.51), uracil (4.00) and thymine (1.47). So, the result of the analysis (1.8-2.0) is the average value of the components that make up the DNA. Meanwhile, to perform RNA analysis, the range value will be greater than this value because in RNA, one of the constituent components is uracil which when compared with DNA composed by thymine, then uracil has a higher value, namely (4.00), so that if averaged, the purity value will be higher when compared with Leninger's DNA (1975).

Based on the research data, it is known that the average purity of the results of the isolation with the Mericon Food Kit and the Life River Kit is 2.17 ng/μL and 1.46 ng/μL, respectively. The concentration of DNA isolated from the Mericon Food Kit was higher (133.7 ng/μL-160.1 ng/μL) than that isolated from the Life River Kit (11.5 ng/μL-12.5 ng/μL). Based on the results of the T-test on purity and concentration, it was obtained data that T-count is more significant than T-table which indicates that the two methods are significantly different.

ACKNOWLEDGEMENTS

The author would like to thank the head of the Center for the Development of Drug and Food Testing for the support given in conducting this research and for the support of laboratory facilities and infrastructure.

REFERENCES

Abinawanto, Sophian A, Lestari R, Bowolaksono A, Efendi PS, Afnan R. 2019. Analysis of IGF-1 gene in ayam ketawa (*Gallus gallus domesticus*) with dangdut and slow type vocal characteristics. Biodiversitas 20: 2004-2010. DOI: 10.13057/biodiv/d200729.

National Agency for Drug and Food Control Indonesia. 2020. Laporan Tahunan Badan Pengawas Obat dan Makanan 2020. National Agency for Drug and Food Control Indonesia, Jakarta. www.pom.go.id/new. [Indonesian]

Betzel C, Singh TP, Visanji M, Peters K, Fittkau S, Saenger W, Wilson K. S. 1993. Structure of the complex of proteinase with a substrate analogue hexapeptide inhibitor at 2.2-A resolution. J Biol Chem 268 (21): 15854-15858. DOI: 10.1016/s0021-9258(18)82332-8.

Eppendorf. 2016. Nucleic Acid Photometry. Check of Critical Parameters. Eppendorf AG, Hamburg, Germany. eppendorf@eppendorf.com, www.eppendorf.com

Fadhlurrahman, Wardani AK, Widyastuti E. 2015. Deteksi gelatin pada soft candy menggunakan metode PCR-RFLP sebagai salah satu pembuktian kehalalan pangan. J Teknologi Pertanian 16 (2): 81-88. [Indonesian]

Kado CI, Liu ST. 1981. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol 145 (3): 1365-1373. DOI: 10.1128/jb.145.3.1365-1373.1981.

Khayyira AS, Estepane VM, Malik A. 2018. Rapid PCR-based detection optimization of porcine DNA in gelatin capsule shell. Intl J Appl Pharm 10 (6): 217-223. DOI: 10.22159/ijap.2018v10i6.29346.

Koetsier G, Cantor E. 2019. A Practical Guide to Analyzing Nucleic Acid Concentration and Purity with Microvolume Spectrophotometers. New England Biolabs Technical Note, Ipswich, USA. www.neb.com.

Life River. 2020. Viral DNA/RNA Isolation Kit (Centrifuge Column). www.liferiverbiotech.com.

Leninger AL. 1975. Biochemistry, 2nd ED. Worth Publishers, New York.

Majumder DAN, Hassan L, Rahim MA, Kabir MA. 2011. Development of an efficient protocol for genomic DNA extraction from mango (*Mangifera indica*). Nusantara Biosci 3: 105-111. DOI: 10.13057/nusbiosci/n030301.

Margawati ET, Ridwan M. 2010. Pengujian pencemaran daging babi pada beberapa produk bakso dengan teknologi PCR: Pencarian sistem pengujian efektif. Berita Biologi 10 (1): 93-98. [Indonesian]

Mueller A, Hinrichs W, Wolf WM, Saenger W. 1994. Crustal structure of calcium-free proteinase K at 1.5 angstroms resulition. J Biol Chem 269 (37): 23108-23111. DOI: 10.2210/pdb2pkc/pdb.

Matlock B. 2015. Assessment of Nucleic Acid Purity. Technical Note 52646. Thermo Fisher Scientific, Wilmington, USA.

Miller JN, Miller JC. 2010. Statistics and Chemometrics for Analytical Chemistry Sixth edition. Pearson Education Limited, London, UK.

Qiagen. 2020. DNeasy® Mericon® Food Handbook for Extraction of Total Nucleic Acids from a Range of Food Sample Types. www.qiagen.com/shop. Technical Support support.qiagen.com. Website www.qiagen.com.

Sambrook J, Fritsch F, Miniatis T. 1989. Molecular Cloning Laboratory Manual. 3rd Edition. Cold Spring Harbor Laboratory Press, New York, USA.

Sophian A, Purwaningsih R, Muindar, Igirisa EPJ, Amirullah ML. 2021. Short communication: Analysis of purity and concentration of DNA extracted from intron patho gene-spin extraction on crab processed food product samples. Asian J Trop Biotechnol 18: 13-27. DOI: 10.13057/biofar/f190104.

Sophian A. 2021a. Short communication: Analysis of purity and concentration of extracted DNA on salted fish processed food products. Asian J Trop Biotechnol 19 (1): 21-24. DOI: 10.13057/biofar/f190104.

Sophian A. 2021b. Analysis of the results of DNA isolation from chicken feather sampled from the base of the young feathers, the base of the old feathers and the ends of the feathers. Bioeduscience 5 (2): 104-108. DOI: 10.22236/j.bes/526211. [Indonesian]

Suadi Z, Bilung LM, Apun K, Azmi AA. 2020. Efficiency of traditional DNA extraction method in PCR detection of porcine DNA in meat mixtures. J Teknologi 82 (5): 85-90. DOI: 10.11113/jt.v82.14636. [Indonesian]