

DNA EXTRACTION METHOD FOR MOLECULAR DETECTION

METODE EKSTRAKSI DNA UNTUK DETEKSI MOLEKULER

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ABSTRACT

In molecular detection technique such as Loop-Amplification Mediated Polymorphism (LAMP) and Polymerase Chain Reaction (PCR), right upstream preparation of deoxyribonucleic acid (DNA) is very important. Liquid phase extraction and solid phase extraction are some of DNA extraction methods those are available. The purpose of this research was to characterized the method and product of DNA extraction based on DNA purity, DNA visualization, DNA concentration, and processing time of DNA extraction methods. Extraction methods evaluated included phenol-chloroform method (Method A) as liquid phase extraction and Surefood Extraction kit method (Method B) as solid phase extraction. Result showed that Method A could be performed on samples with very low DNA concentrations ranging from 7.00 to 9.45 ng/ μ l with a good purity (1.80 to 2.10). Although, it showed no DNA isolates bands on gel agarose 1% and need \pm 30 hours processing time. Method B had a good performance in extracting sample with high concentration DNA (49.67 to 357.28 ng/ μ l) with a good purity (1.93 to 2.07). This method showed bands for each DNA samples on gel agarose 1% and need about \pm 1 hour processing time. Both methods can be used for sample preparation in molecular analysis including halal authentication purposes.

Keywords: phenol, chloroform, Surefood kit, LAMP.

ABSTRAK

Dalam teknik deteksi molekuler seperti *Loop-Amplification Mediated Polymorphism* (LAMP) dan *Polymerase Chain Reaction* (PCR), pembuatan hulu asam deoksiribonukleat (DNA) sangat penting. Ekstraksi fase cair dan ekstraksi fasa padat merupakan beberapa metode ekstraksi DNA yang tersedia. Tujuan dari penelitian ini adalah untuk mengkarakterisasi metode dan produk ekstraksi DNA berdasarkan kemurnian DNA, visualisasi DNA, konsentrasi DNA, dan waktu pemrosesan metode ekstraksi DNA. Metode ekstraksi yang dievaluasi meliputi metode fenol-kloroform (Metode A) sebagai ekstraksi fasa cair dan metode Ekstraksi Surefood kit (Metode B) sebagai ekstraksi fasa padat. Hasil penelitian menunjukkan bahwa Metode A dapat dilakukan pada sampel dengan konsentrasi DNA sangat rendah berkisar antara 7,00 sampai 9,45 ng / μ l dengan kemurnian yang baik (1,80-2,10). Meski tidak menunjukkan DNA isolat band pada gel agarosa 1% dan membutuhkan waktu pemrosesan \pm 30 jam. Metode B memiliki performa yang baik dalam mengekstraksi sampel dengan DNA dengan konsentrasi tinggi (49,67 sampai 357,28 ng / μ l) dengan kemurnian yang baik (1,93 sampai 2,07). Metode ini menunjukkan band untuk setiap sampel DNA pada gel agarose 1% dan membutuhkan waktu \pm 1 jam. Kedua metode tersebut dapat digunakan untuk preparasi sampel dalam analisis molekuler termasuk tujuan otentikasi halal.

Kata kunci: fenol, kloroform, Surefood kit, LAMP.

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INTRODUCTION

Liquid and solid phase DNA extraction method have a different characteristics in the process and obtained products. Those DNA extraction methods determined the success of molecular detection technique including Loop-Amplification Mediated Polymorphism (LAMP) and Polymerase Chain Reaction (PCR). By characterizing DNA extraction method and its products, we can choose the most suitable one for our research purposes. Factors to be considered when selecting nucleic acid extraction methods for molecular detection include sample background, lysis, appropriate preparation chemical, required detection limits, and requirements for particular application.

In the As previous study, DNA/RNA preparation for molecular detection have been reviewed which reported the various method of nucleic acid extraction¹. Evaluation of DNA and RNA extraction methods was also reported. It showed comparison of some extraction methods². Steps of the methods included extracting, separating, purifying, and concentrating DNA.

The aim of this research was to report the characters of method and products of phenol-chloroform DNA extraction as liquid phase extraction method and Surefood Kit DNA extraction as solid phase extraction method.

MATERIALS AND METHODS

Materials

Porcine tissue, chicken tissue, goat tissue, beef tissue, fish tissue, reagen for phenol-chloroform extraction method, R Biopharm AG (kit SureFood® PREP Basic), agarose, TBE, loading dye, microtube, micropipettes, centrifuge, electrophoresis instrument.

Method A

A total of 250 μ L animal tissue samples were prepared and fed into the microfuge tube and

then 300 μ L STE, 40 μ L SDS 10%, and 20 μ L proteinase-K were incubated at 55°C and homogenized for 2 h. A further 400 μ L of phenol, 400 μ L of CIAA (chloroform isoamyl alcohol), and 40 μ L NaCl 5M were added. Then, it was incubated at room temperature and homogenized for 1 hour. Next it was centrifuged for 5 minutes and the supernatant was taken. Subsequently the sample was incubated at -20 °C for 1 night. Then it was centrifuged for 5 minutes and the alcohol part was removed. Then it was centrifuged for 5 minutes, the alcohol was removed and the tube was dried for 1-2 hours.

Method B

Extraction were done by 50 mg fresh meat using kit manufactured by R Biopharm AG (kit SureFood® PREP Basic) with modification. Initially, sampel were lysed with lysis buffer containing Proteinase-K. DNA was then bind using binding buffer and purified through 2 types of spin filter (clear and yellow) with the aid of washing buffer (pre-wash and wash buffer). DNA was then diluted by elution buffer. The SureFood DNA extraction kit itself basically adopt silica mini column since it is a common approach for DNA isolation.

Gel for Electrophoresis

Gel was made from 0.3 grams of agarose and 30 ml of buffered (0.5 x TBE) solution being heated. The agarose solution was left slightly cold while stirring with a stirrer magnet, then added 1.8 μ l dye of etidium bromide. A total of 5 μ l DNA samples were dissolved in 1 μ l loading dyes. Electrophoresis was carried out for 40 minutes at a constant voltage of 100 volts until the blue bromtimol reaches the bottom of the gel.

RESULT AND DISCUSSION

Result

DNA isolates defined by three categories i.e. DNA purity, DNA visualization, dan DNA

concentration. DNA isolates quality assessment was performed on the basis of DNA purity and the results of DNA visualization on agarose gel qualitatively.

Method A

In method A, the samples used were porcine DNA residues on washed food equipments after washing by different cleaning agents based on sharia. The samples were coded as A to E. The results obtained refer to Table 1.

Table 1 Measurement of DNA concentration and purity of sample in Method A

Sample code	DNA concentration (ng/ μ l)			Purity (λ 260/280)		
	Repetition 1	Repetition 2	Mean \pm SD	Repetition 1	Repetition 2	Mean \pm SD
A	8.80	8.70	8.75 \pm 0.07	2.08	2.12	2.10 \pm 0.03
B	7.80	8.00	7.90 \pm 0.14	1.90	1.70	1.80 \pm 0.14
C	7.10	6.90	7.00 \pm 0.14	1.96	1.97	1.97 \pm 0.01
D	9.20	9.70	9.45 \pm 0.35	1.71	1.92	1.82 \pm 0.15
E	9.50	9.30	9.40 \pm 0.14	1.90	1.92	1.91 \pm 0.01

The DNA concentration of fifth samples ranged from 7.00 to 9.45 ng/ μ l. The purity of samples ranged from 1.80 to 2.10. Visualization of extracted genome DNA based on gel electrophoresis was shown as Figure 1.

The DNA bands from the porcine positive control extraction appeared successfully on 1% agarose gel by electrophoresis, while the DNA bands from the fifth samples did not appear.

Method B

In method B, the samples used were raw meat animal tissue of several species. The samples which were coded as A, B, C, D, and E were porcine, beef, chicken, goat, and fish tissue respectively. The results obtained refer to Table 2.

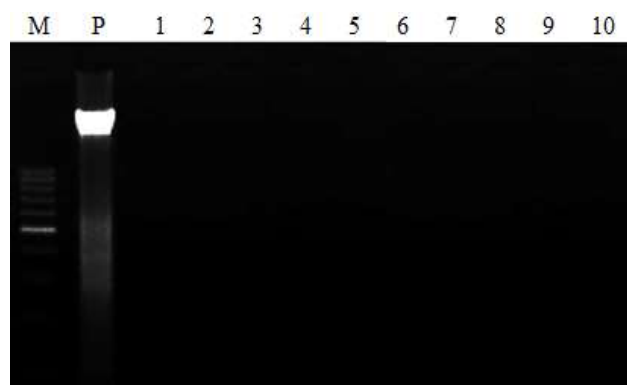


Figure 1. Visualization of extracted genome DNA on 1% agarose gel. M : Marker 100 pb, P : positive control of porcine DNA, 1-2: residue of porcine DNA on sample A, 3-4 : residue of porcine DNA on sample B, 5-6 : residue of porcine DNA on sample C, 7-8 : residue of porcine DNA on sample D, 9-10 residue of porcine DNA on sample E.

Table 2 Measurement of DNA concentration and purity of sample in Method B

Sample	DNA concentration (ng/ μ l)			Purity (λ 260/280)		
	Repetition 1	Repetition 2	Mean \pm SD	Repetition 1	Repetition 2	Mean \pm SD
A	165.64	214.61	190.13 \pm 34.63	1.97	1.95	1.96 \pm 0.01
B	53.18	46.15	49.67 \pm 4.97	1.87	2.03	1.95 \pm 0.11
C	455.76	258.80	357.28 \pm 139.27	2.07	1.99	2.03 \pm 0.06
D	163.20	148.62	155.91 \pm 10.31	2.09	2.04	2.07 \pm 0.04
E	210.56	153.34	181.95 \pm 40.46	1.82	2.03	1.93 \pm 0.15

The DNA concentration of samples ranged from 49.67 to 357.28 ng/ μ l. The purity of samples ranged from 1.82 to 2.07. Visualization of extracted genome

DNA based on gel electrophoresis was shown as Figure 2.

The DNA bands from all samples appeared successfully on 1% agarose gel by electrophoresis.

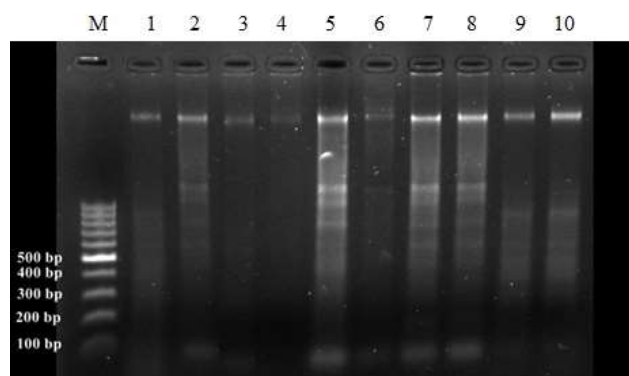


Figure 2 Visualization of extracted genome DNA on 1% agarose gel. M : Marker 100 pb, 1-2: porcine DNA sample, 3-4 : beef DNA sample, 5-6 : chicken DNA sample, 7-8: goat DNA sample, 9-fish DNA sample.

The DNA bands from all samples appeared successfully on 1% agarose gel by electrophoresis.

Discussion

DNA Purity

Both method A and method B resulted ideal purity of DNA isolates, while $\lambda_{260/280}$ of method A was between 1.80 to 2.10 and $\lambda_{260/280}$ of method B was between 1.93 to 2.07. DNA isolates can be said to be pure and have met the requirements for molecular analysis when the ratio $\lambda_{260/280}$ ranges from 1.8 to 2.0^{3,4}. DNA has maximum absorption in λ_{260} and protein has maximum absorption in λ_{280} . So that, the products of both DNA extraction methods can be used for molecular analysis.

DNA Visualization

In method A, the DNA bands from the porcine positive control extraction were successfully visible on 1% agarose gel, but the DNA bands from the fifth samples did not present. The isolate DNA from fifth sample that did not present on the gel can be caused by very small amounts of DNA (<50 ng/ μ l). But, the appearance of band of porcine positive

control and total DNA purity within the normal range indicates that the phenol-chloroform method can be used for molecular analysis especially in sample with low DNA concentration.

In method B, bands appeared in all of samples well, although they had a various thickness. The various band thickness can be cause of different concentration of DNA. Athick or dense and solid DNA band showed a high concentration and good integrity of DNA⁵. It was also showed DNA smear in sample gel agarose visualization. DNA smear showed that DNA was broken/sheared during extraction process⁵.

DNA Concentration

DNA isolation resulted in varying yield from 7.00 to 9.45 ng/ μ l in method A and 49.67 to 357.28 ng/ μ l in method B of DNA concentration. This yield is determined by DNA isolation time and lysis buffer composition. Timing is essential since if it takes too long, there is possibility of DNA precipitation⁶. DNA concentration of the extraction products were not uniform. Therefore, the concentration of DNA that has been obtained is uniformed by dilution, thus DNA needs to be diluted to a certain level concentration. The suggested DNA concentration is 10 ng to 1 μ g DNA per μ l for PCR reaction⁷.

Method Principles

Method A

Method A is defined as phenol-chloroform extraction method. It is categorized as liquid phase extraction method. This is a usual method used for DNA extraction that aims to a high purity isolate¹. The processes included four extraction steps, which were extracting or releasing DNA, separating DNA from protein, purification, and concentrating the DNA¹. Releasing the DNA, as the first step, was a cell lysis that is performed by using Sodium Tris EDTA (STE) and sodium dodecyl sulphate (SDS). SDS is a detergent that is help with lysis by breaking down the membranes. Detergent also reduces the activity of the nuclease which is the DNA degrading

enzyme^{8,9}. Sodium Tris EDTA (STE) roles in inactivating DNase enzymes that can denature isolated DNA, EDTA inactivates nuclease enzymes by binding magnesium and calcium ions required as cofactors of DNase enzyme¹⁰. The second step is separating DNA from protein using proteinase-K. Proteinase-K is one of the primary protease used for DNA separation. It is used to reduce protein background and aid in lysis by digesting membrane or capsid proteins. This protease will break proteins into smaller molecules by cleaving peptide bonds¹¹.

Nucleic acid is a hydrophilic molecule and it is soluble in water. In addition, the protein also contains a hydrophobic residue that causes the protein to dissolve in organic solvents. Based on this nature, there are several deproteinization methods based on the selection of organic solvents. In this research, the method used was phenol - chloroform containing 4% isoamyl alcohol (CIAA). The use of chloroform isoamyl alcohol (CIAA) is based on differences in the nature of organic solvents. Phenols denatures proteins, which stay in the organic phase, whereas the DNA is in the aqueous phase. The addition of chloroform and isoamyl alcohol helps to separate the phases and prevent foaming¹². Chloroform can not mix with water and its ability to deproteinized based on the ability of denatured polypeptide chains to enter or mobilize into phases between chloroform - water. High protein concentrations in the intermediate phase can cause the protein to undergo precipitation. While lipids and other organic compounds will be separated in the chloroform layer¹³. The third step was precipitation using alcohol and high concentration of salt¹⁴. Precipitation also serves to remove chloroform residues derived from the extraction stage. The fourth step was concentrating the DNA by centrifugation and manual manipulation by drying the DNA. According to Thatcher¹, this liquid extraction method produces very clean product, though some molecules or particles can coprecipitate with the DNA. This method need about 30 hours processing time.

Method B

Method B used refers to the extraction methods released by R Biopharm AG (SureFood® PREP Basic kit) by making some modifications. This method of extraction or isolation of DNA used a ready-to-use extraction buffer. In Surefood Kit, filtration tool used is spin filter there were two types of spin filter that was clear spin filter and yellow spin filter. The cell was broken by lysis buffer, then the protein cell component was degraded by using Proteinase-K as protease enzyme. The next step was binding of nucleic acids on spin filter by using buffer bindings, filtering and washing the DNA by washing buffer (pre-wash buffer and wash buffer), at the final stage the DNA was dissolved into elution buffer. The SureFood commercial extraction kit has the same principle as other commercial extractions that use the minicoloumn principle or DNA filtration. DNA extraction using the minicoloumn principle is the most common method of extraction because the results obtained are very good with lesser processing when compared with the phenol-chloroform method and the cost is considerably cheaper when compared to the magnetic bead method¹⁵.

Method B is chategorized as solid phase extraction method. Solid phase extraction method have become the most common method of nucleic acid isolation for several reason. They include minimal hazardous chemicals, fewer and easier manual manipulations, automation capability, and increased throughput. Solid phase methods used for nucleic acid separation include 3 principle technique : size exclusion by gel filtration, ion exchange chromatography by charge-based reversible adsoption, and affinity chromatography¹.

In this research, the kit applied affinity chromatography technique. DNAbinded to silica surfaces under specific binding condition¹⁶. DNA and silica arenegatively charged, the binding is in consequence of adsorption in hydrogen bonding and high ionic strength conditions and that happen as water is removed from the surfaces. The DNA is released when the alcohol or saltis removed and the surfaces are hydrated. Affinity

preparation has four basic steps; lysis, binding, washing, and eluting. Unexpectedly, similar chemicals can be used for lysis and surface-binding. Chaotropic salts can be used for cell lysis and binding to a silica surface¹⁷. A liquid sample passes through by centrifugation (spin filter), pressure (syringe filter), or vacuum. Kits with silica spin filters are fast to perform and do not require hazardous chemicals.

CONCLUSION AND IMPLICATION

The phenol-chloroform method (Method A) can be used for extracting sample with very low DNA concentration (7.00 to 9.45 ng/μl). The extraction product of phenol-chloroform was very clean. The purity of DNA isolates was ideal to be used in molecular analysis (1.80 to 2.10). Although in the DNA visualization, that was no bands appeared in the gel according to the low concentration of DNA isolates. But it need long time process (about 30 hours) and some hazardous chemical were still used. The Surefood Kit method (Method B) were able to extract sample with high concentration DNA samples (49.67 to 357.28 ng/μl) with good DNA purity (1.93 to 2.07), good visualization in gel agarose, and less processing time (1 hour) compared to phenol-chloroform method.

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