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Modified Spin Column-Based RNA Extraction Methods of *Staphylococcus aureus* using PureLink® RNA Mini Kit and Basic Laboratory Instrument

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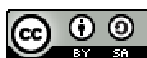
Abstract

RNA extraction is an important process before gene expression assessment at the transcriptomic level. RNA is a sensitive material to environmental factors such as temperature and contaminants, so the RNA extraction process generally requires sophisticated and expensive laboratory instruments. In this study, we extract RNA from *Staphylococcus aureus* bacteria using the PureLink® RNA Mini Kit. The instruments used in this study are basic instruments such as a hand homogenizer and non-thermal centrifuge. The results of RNA extraction were visualized using agarose gel electrophoresis. These results indicate that bacterial RNA extraction can be performed using the PureLink® RNA Mini Kit even with inexpensive basic laboratory instruments.

Keywords

Basic Laboratory Instrument, PureLink® RNA Mini Kit, RNA Extraction, *Staphylococcus aureus*.



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INTRODUCTION

Total RNA extraction from bacterial cells is used to analyze gene expression at the transcriptional level. High-quality total RNA is a prerequisite for a variety of downstream applications (1). RNA quality and quantity are important factors for ensuring the accuracy of gene expression analysis and other RNA-based downstream applications (2,3). The purity and integrity of RNA can impact the accuracy of techniques such as Real-Time quantitative Polymerase Chain Reaction (4). Spin Column-Based is one of the RNA extraction methods that comprise of four stages namely lysis of cells, binding of nucleic acid to silica gel membrane, washing the nucleic acid bound to the silica gel membrane, and elution of the nucleic acid (5).

First, the cell membrane was broken by a lysis solution in order to free nucleic acid from cell. The binding solution that contains buffer solution and ethanol is added to a spin column, and the column is put in a centrifuge. Samples are lysed and homogenized in the presence of guanidinium isothiocyanate, a chaotropic salt capable of protecting the RNA from endogenous RNases (6). The centrifuge forces the binding solution through a silica gel membrane inside the spin column. The nucleic acid will bind to the silica gel membrane as the solution passes through if the pH and salt concentration of the binding solution is optimal. The column is put in a centrifuge again, forcing the wash buffer through the membrane to removes any remaining contaminant from the membrane, leaving only the nucleic acid bound to the silica gel. Lastly, the column is put in a centrifuge again, forcing the elution buffer through the membrane. The elution buffer removes the nucleic acid from the membrane and the nucleic acid is collected from the bottom of the column.

Staphylococcus aureus is an important nosocomial Gram-positive pathogen that causes various infectious diseases in humans, ranging from harmless localized skin lesions to systemic infections, such as endocarditis, pneumonia, and other life-threatening diseases (7). Gram-positive bacteria have a thick peptidoglycan layer and no outer lipid membrane (8). RNA extraction is an important step to assess genetic expression, for example, to determine the effect of the antibiotic compound on the genetic expression of *S. aureus.* Generally, RNA extraction uses commercial kits that permit for fast purification of high-quality nucleic acids. The success of commercial kits largely relies on the spin columns assembled with solid-phase nucleic acid binding material which allows easy binding, washing, and elution of nucleic acids in the purification process (9).

The extraction method should be subject to various considerations such as budget, organism, and in particular, the aim of the

experiment (10). RNA extraction process generally requires sophisticated and expensive laboratory instruments. With limited laboratory instruments, we evaluate RNA extraction using PureLink® RNA Mini Kit and basic instruments such as a hand homogenizer and non-thermal centrifuge.

MATERIALS AND METHODS

Materials used in this study are PureLink® RNA Mini Kit (*Thermo Fisher Scientific, United States*) (comprises of Lysis Buffer, Wash Buffer I, Wash Buffer II, RNase-Free Water, Spin Cartridges, Collection Tubes), 2-Mercaptoethanol, *S. aureus* Culture, and Luria Bertani Broth. The instrument used are hand homogenizer (Mini Hand Homogenizer MT-13K) and non-thermal centrifuge (BR Technologies).

S. aureus Cultivation and Cell Harvesting

Bacterial cultivation and cell harvesting was done based on (11). A one-loop single colony of *S. aureus* from Mannitol Salt Agar and Luria Bertani Agar was transferred to Luria Bertani Broth. Cultured was incubated for 24 hours at 37°C. Culture in Luria Bertani Broth was harvested by centrifuging at 4,000 rpm for 5 minutes and removes supernatant.

RNA Extraction

RNA Extraction was done based on the manufacturer's guide protocol (PureLink® RNA Mini Kit) (5) with modification.

Lysis and Homogenization

Cell pellet was transferred to RNase-free tube. 0.6 mL Lysis Buffer prepared with 2-mercaptoethanol was added to sample using RNase-free pipette tips. Sample was vortexed at high speed until the cell pellet is completely dispersed and the cells appear lysed. Sample was homogenized with hand homogenizer (Mini Hand Homogenizer MT-13K) for 2 minutes.

Binding, Washing, and Elution

One volume 70% ethanol was added to each volume of cell homogenate. Sample was vortexed to mix thoroughly and to disperse any visible precipitate that may form after adding ethanol. Sample up to 700 µL (including any remaining precipitate) was transferred to the Spin Cartridge (with the Collection Tube). Sample was centrifuged at 8,000 rpm for 15 seconds at room temperature. The flow-through was discarded and the Spin Cartridge was reinserted into the same Collection Tube. Steps (transfer to spin cartridge and centrifuge) was repeated until the entire sample is processed. Seven hundred µL Wash Buffer I was added to the Spin Cartridge. Sample was centrifuged at 8,000 rpm for 15 seconds at room temperature. The flow-through and the Collection Tube was discarded.

The Spin Cartridge was placed into a new Collection Tube. Five hundred µL Wash Buffer IIwith ethanol was added to the Spin Cartridge. Sample was centrifuged again at

8,000 rpm for 15 seconds at room temperature. The flow-through was discarded and the Spin Cartridge was reinserted into the same Collection Tube. Steps Wash buffer II was repeated once. The Spin Cartridge was centrifuged at 8,000 rpm for 1-2 minutes to dry the membrane with attached the RNA. The Collection Tube was discarded and the Spin Cartridge was inserted into a Recovery Tube. 50 µL RNase–Free Water was added to the center of the Spin Cartridge. Sample was incubated at room temperature for 1 minute. The Spin Cartridge was centrifuged for 2 minutes at 8,000 rpm at room temperature to elute the RNA from the membrane into the Recovery tube.

Agarose Gel Electrophoresis

The integrity of total RNA isolated and the extent of genomic DNA contamination were analyzed through agarose gel electrophoresis (12). The integrity and size of RNA can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining. Unlike DNA, RNA molecules quickly form secondary and tertiary structures (13). 5 µL sample was mixed with 5 µL nuclease-free water and 2 µL loading dye, then transferred to 1.2% agarose gel well. Electrophoresis was run at 100V for 1 hour. Agarose gel was dipped in Ethidium Bromide for 30 minutes and then visualized on High Performance Ultraviolet Transilluminator (*Thermo Fisher Scientific, United States*).

RESULTS

The visualization of agarose gel under ultraviolet illumination showed RNA indicated by the formation of the single bands pointed by arrow (Figure 1).

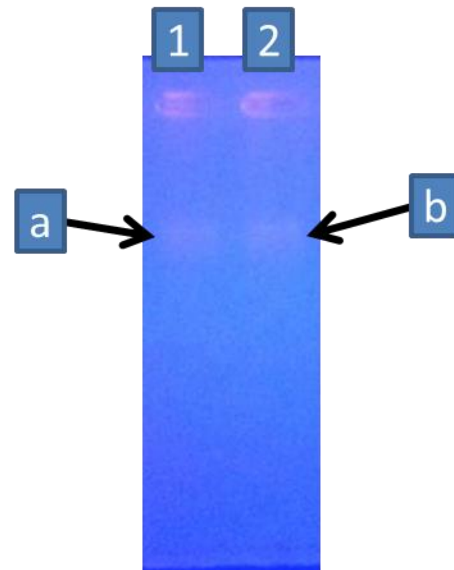

Figure 1. Visualization of Agarose Gel under UV after 3 days stored at -40ºC. visualization is done in duplicate (shown in wells 1 and 2). The bands formed are shown in a and b.

This result indicated that PureLink® RNA Mini Kit with basic laboratory instruments such as a hand homogenizer and non-thermal centrifuge can be used to extract total RNA from *S. aureus* culture.

The result of RNA isolation was stored at -40ºC for 3 days then was visualized again under UV Transilluminator to determine whether the extracted RNA still survived or degraded after the storage period. The results of the second electrophoresis are shown in Figure 2.

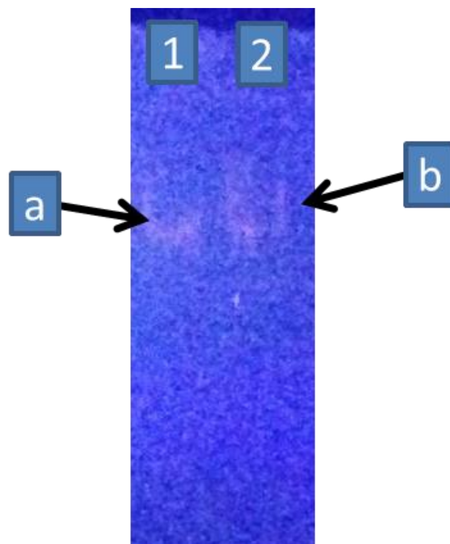

Figure 2. Visualization of Agarose Gel under UV after 3 days stored at -40ºC. visualization is done in duplicate (shown in wells 1 and 2). The bands formed are shown in a and b.

DISCUSSION

The success of RNA isolation is influenced by several factors, including the choice of method and the availability of instruments. Rodriguez *et al*. (10) stated that the extraction method should be subject to various considerations such as budget, organism, and the aim of the experiment. PureLink® RNA Mini Kit is designed for fast purification of high-quality nucleic acids based on the Spin Column-Based method. The PureLink™ RNA Mini Kit provides a simple method for isolating high-quality RNA. Bacterial cells are lysed and homogenized in the presence of guanidine isothiocyanate, and ethanol is added to the sample (1). Guanidine isothiocyanate is a chaotropic salt capable of protecting the RNA from endogenous Rnases (6). The sample is then processed through a spin cartridge containing a clear silica-based membrane to which RNA binds. The binding mechanism of nucleic acid adsorption on the silica surface mainly includes four factors: hydrogen bond, salt bridge and electrostatic force formed between the nucleic acid and the silica surface, and the solubility of nucleic acid (14). Any impurities are effectively removed by subsequent washing. The purified RNA is then eluted in RNase-free water (1). Yang *et al*. (15) also reported that the RNA isolated using e silica spin column-based was relatively less contaminated by protein, polysaccharide, and phenolic compounds or other reagents. The application of high concentration GuSCN (w/v 50%) in subsequent binding and washing buffers also enhanced removing denatured proteins and other inhibitors.

RNA extraction process generally requires sophisticated and expensive laboratory instruments. With limited laboratory instruments, we evaluate RNA extraction using PureLink® RNA Mini Kit and basic instruments such as a hand homogenizer and non-thermal centrifuge. In the manufacturer's protocol (5), it is stated that homogenization can be done with 3 alternative tools, namely the Homogenizer, Syringe and needle, and Rotor-stator. The Homogenizer provides highly consistent results and is more convenient than other homogenization methods (5). Xu *et al*. (16)

stated that the RNA extraction results increased from 35.4–37.6 (Ct values) without homogenization to 37.8–40.2 (Ct values) with homogenization. The lysis step is combined with a homogenization step that includes denaturation with a guanidine-thiocyanate containing buffer to inactivate the RNases released from the cell (17). Wever *et al*. (18) indicate that the important factor that determines the results of RNA extraction is the buffer volume added during homogenization according to the recommendation of the homogenization protocols. The results of this study prove that hand homogenizer can be used for the sample homogenization process in the cell lysis process.

Another factor that affects the RNA extraction results is temperature. A centrifuge is a vital tool in the RNA extraction process. The centrifuge forces the binding solution through a silica gel membrane inside the spin column, forces wash buffer through the membrane to removes any remaining contaminant from the membrane, and forces elution buffer through the membrane (5). In this study, we used a non-thermal centrifuge for the RNA extraction process. RNA extraction in the field reported by Breitler *et al*. (19) can be carried out at high temperatures (25–38°C) using a TRIzol reagent. Abdallah *et al*. (20) also reported that the stability of MERS-CoV RNA on spin columns of RNA extraction kit at room temperature. The results of this study indicate that the use of a non-thermal centrifuge can be an effective alternative for RNA extraction if a thermal centrifuge is not available in the laboratory.

Novelty

This study reports a new RNA isolation strategy using simple laboratory instruments. According to the PureLink® RNA Mini Kit guide, homogenization can use one of 3 alternatives, namely 1. Homogenizer; 2. Syringe and needle; 3. Rotor-stator. Another important process in RNA isolation is centrifugation. In the manufacturer's guide, it is recommended to use a temperature-controlled centrifuge because RNA is sensitive to high temperatures. However, in some laboratories, these sophisticated instruments are not available, so in this study, alternative instruments that are widely available in public laboratories are used, namely non-thermal centrifuge and hand homogenizer. The results of this study indicate that this simple instrument can be used to isolate *S. aureus* RNA. Even after being stored for 3 days at -40°C, the results of electrophoresis still showed that the isolated RNA was not degraded. These results are expected to be an alternative for laboratories with limited instruments in order to practice RNA isolation.

CONCLUSIONS

Based on the results of visualization of agarose gel on the UV transilluminator, we conclude that RNA isolation with the PureLink® RNA Mini Kit can be carried out in a laboratory with basic instruments such as a hand homogenizer and centrifuge without temperature control.

AUTHOR CONTRIBUTIONS

Muhammad Taufiq Hidayat: conceptualization, methodology, formal analysis, investigation, writing - original draft, writing - review & editing, visualization. Endry Nugroho Prasetyo: conceptualization, resources, supervision, project administration, funding acquisition.

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CONFLICT OF INTEREST

There are no conflicts of interest.

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