Brief Report

DOI: 10.5582/bst.2018.01267

A freeze-and-thaw method to reuse agarose gels for DNA electrophoresis

Noboru Sasagawa

Department of Applied Biochemistry, School of Engineering, Tokai University, Hiratsuka, Kanagawa, Japan.

Summary **A novel protocol to reuse agarose following agarose gel electrophoresis was established in this study. By repeated freeze-and-thaw of the agarose gel, ethidium bromide and other buffer components in the gel were safely removed from the gel without generation of any toxic fume. The agarose recovered using this method can be used for further electrophoretic experiments without any issues.**

Keywords: Agarose, agarose gel electrophoresis, ethidium bromide

1. Introduction

It is widely known that agarose gels for electrophoresis are reusable. Used gels can be boiled and melted again, and poured into a template gel tray, as is done for new agarose gels. However, agarose gels are commonly stained with ethidium bromide (EtBr; 3,8-diamino-5-ethyl-6-phenylphenanthridinium bromide, $C_{21}H_{20}BrN_3$). EtBr is a toxic substance that forms fumes and vapor during the boiling and melting steps; this can lead to inhalation of EtBr molecules. Therefore, boiling and melting EtBr-stained agarose gels is not recommended.

Dialysis of used agarose gel can help remove EtBr, but it is not suitable on a day to day basis since 100 L of distilled water is needed for diluting the EtBr in 100 mL of agarose to a final dilution of 10^{-3}. A method consisting of repeated gel washing/dialyzing for reusing agarose gels has been reported (*1,2*). This method reduces the amount of water, but its execution is difficult owing to the fragility of agarose gels, which causes them to break easily. Here, I report a safe, easy, and suitable method for effectively removing EtBr from used agarose gels.

2. Materials and Methods

Standard agarose was purchased from different providers (*e.g.*, STAR Agarose RSV-AGRP from Rikaken, Aichi, Japan). Agarose gels contained agarose 1% (w/v) dissolved in Tris-acetate-ethylenediaminetetraacetic acid buffer (TAE buffer; 40 mM Tris, 40 mM acetic acid, and 1 mM EDTA). Agarose gel electrophoresis was performed at 100 V for 45 min by standard protocol (*3*) using TAITEC PICO-1 (Saitama, Japan). After electrophoresis, the agarose gels were stained with EtBr by incubating them in TAE buffer containing EtBr (0.1 ng/mL final concentration) for 30-60 min. Finally, the gels were photographed.

3. Results and Discussion

The protocol for reusing agarose gels proposed in this paper is summarized in Figure 1. Stained gels were collected in clean paper cartons or plastic bags. When a carton or bag was filled with gels, it was left in a –20°C freezer overnight. Using paper cartons or plastic bags is essential because the glass or plastic bottles crack during freezing. The next day, the containers were settled upside-down on a kitchen mesh or colander on top of a bucket and incubated at room temperature until completely thawed. The resulting solution seeped through the gels, which contains EtBr, can be collected and decontaminated following standard protocols (*4,5*).

Generally, buffers and solutions freeze disproportionally because of freezing-point depression. In a buffer, pure water freezes faster and the concentrated buffer freezes later. In the thaw step, the concentrated buffer elutes first and the pure water elutes later. The "Freeze-out" or "Freeze-concentrate" technique based on this principle has been widely used to concentrate molecules from diluted buffers and solutions (*6*). The

**Address correspondence to:*
Dr. Noboru Sasagawa, Department of Applied Biochemistry, School of Engineering, Tokai University, Hiratsuka, Kanagawa 259-1292, Japan.
E-mail: noboru.sasagawa@tokai.ac.jp

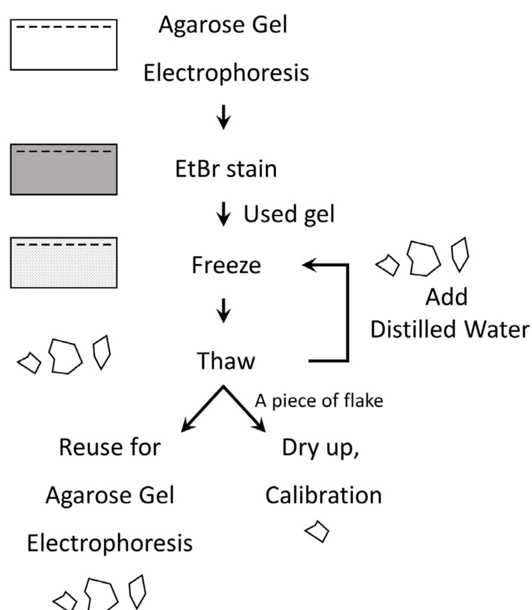

Figure 1. Scheme of the freeze-and-thaw method proposed in this manuscript.

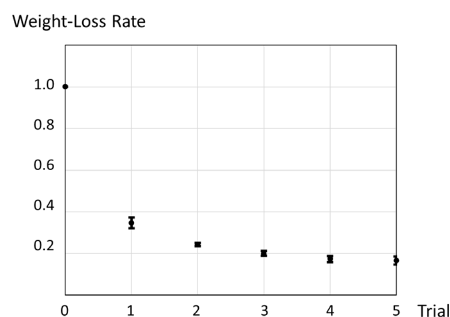

Figure 2. Weight loss of the agarose by freeze-and-thaw. Repeated freeze-and-thaw of agarose gel reduces gel weight but does not completely remove contaminant solutions (n = 3). The thawed agarose gels were placed back in the –20°C freezer without any additional water.

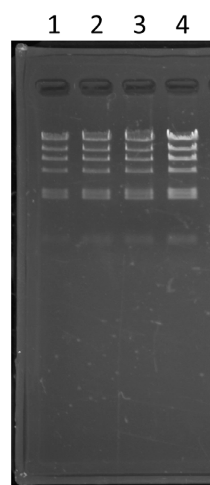

Figure 3. Agarose gel electrophoresis using recycled agarose (1%). Lambda DNA/*Hin* dIII digest was applied for the electrophoresis. DNA at volumes 125, 250, 375, and 500 μg was applied to lanes 1, 2, 3, and 4, respectively.

TAE buffer in the agarose gel freezes in the same way as in the freeze-concentrate technique. This property contributes to the effective removal of EtBr and agarose purification. Another attractive feature is that agarose gel changes its structure to flakes. The freezing step causes ice crystals to form in the agarose gel, destroying its mesh structure resulting in wet flakes of agarose. This "cake-to-flake" conversion causes the agarose gel to reduce its volume. The smaller the volume, the less water is needed for dilution of unnecessary content.

It is well known that DNA is separated from agarose gel by the freeze-squeeze method (*7*). Therefore, the freeze-and-thaw step and "cake-to-flake" conversion are also useful for removing nucleic acids from the agarose gel.

Figure 2 shows the effectivity of the freeze-and-thaw steps to remove solutions from the agarose gels. In this experiment, the thawed agarose gels were subjected to five freeze-and-thaw cycles without adding additional water. In the initial freeze-and-thaw step, a large amount of solution (including water, EtBr, and other molecules) was eluted.

However, repeated freeze-and-thaw steps did not seem to result in complete removal of water. Therefore, adding distilled water to the thawed gel before re-freezing it is the more effective way of removing the buffer and other contaminants. The ratio of the wet weight of the agarose gel before- and after- the initial freeze-and-thaw is 0.35 (s.d. = 0.03), indicating that the freeze-and-thaw step is more effective for removing electrophoresis buffer than simple dialysis. It also means that 5 cycles of freeze-and-thaw result in and EtBr dilution of 10^{-3} when 100 mL of distilled water is added to 100 mL of agarose gel before each re-freezing step. In conclusion, a total of 500 mL of distilled water is needed to reuse 100 mL of agarose gel (200 times more water is necessary to achieve the same level of purification by dialysis). Moreover, no fume or vapor is generated in this method.

Gel flakes are kept in the refrigerator as dumplings. A piece of flake from the dumpling is weighed (wet weight). Then, it is completely dried by simply leaving in the air and weighed again (dry gel weight). This dry-up calibration reveals the agarose concentration in the wet gel flake which enables to calculate the adequate agarose gel concentration for electrophoresis. In my experience, the wet gel flakes contain 3-7% agarose. Based on this calibration step, the residual gel dumpling is boiled, melted, and poured into a bottle in the appropriate concentrations and buffers and then cooled until hard. This melting step is also a good chance for filtrating dust from the agarose. The reused agarose gels can be used for gel electrophoresis, as previously described (*3*), without any difficulties (Figure 3).

In conclusion, I propose a novel protocol for reusing

agarose after agarose gel electrophoresis. This method is simple, free from EtBr toxicity, and very easy to execute. Furthermore, no special equipment is needed to use this method. Although five days are needed to complete the protocol, only a simple freeze-and-thaw process is needed at each step; thus, only 5-10 min are needed for completing each step.

Acknowledgements

I would like to thank Editage (www.editage.jp) for English language editing.

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(Received November 3, 2018; Revised December 27, 2018; Accepted December 29, 2018)