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Chapter

Cost-Effective Technical Tips for Agarose Gel Electrophoresis of Deoxyribonucleic Acid

Noboru Sasagawa

Abstract

Agarose gel electrophoresis is one of the most fundamental experiment in biochemistry and/or molecular biology, especially in analyzing deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). Many laboratories do agarose gel electrophoresis almost every day. Besides, sometimes we need to prepare tens of agarose gels at a time for training and/or practices of students. In such situations, the more cost-effective way we have, the much more experiments in laboratories/trainings of students we can achieve. Actually, experiments of using agarose can be achieved in a more inexpensive way. In this manuscript, conditions of agarose gel electrophoresis experiment (agarose, buffer, and equipment) are considered, and achievements of such efforts are described.

Keywords: agarose, electrophoresis, buffer, equipment

1. Introduction

In molecular biology and biochemistry, the size of biomolecules (molecular weight of protein, length of nucleic acids, and so on) is an important key information in the experiment. One of the popular methods for size fractionation of such molecules is electrophoresis. Agarose is often used as the gel structure for electrophoresis to fractionate nucleic acids. Although polyacrylamide gel is also usable, agarose gel is the most major compound for electrophoresis of nucleic acids, because of its easy handling [1–3]. A fractionation effect depends on the pore size in the gel. Generally, nucleic acids for research have rather large size (around 20 to several thousand base pairs), and the pore size of agarose gel is enough for such large molecule (nucleic acids) to be fractionated. The pore size of 1% agarose gel is estimated to be around 200 nm [4]. Basically, 0.3–2.0% (weight per volume in buffer) of agarose is used in electrophoresis [5].

In this manuscript, several cost-effective ways of agarose gel electrophoresis of DNA are explained. On the other hand, a modification to develop a quality of agarose gel electrophoresis is reported; adding and mixing graphene oxide powder in agarose gel enhances a separation quality of electrophoresis [6]. Furthermore, several modifications of agarose gel electrophoresis by adding a special reagent in the agarose gel are proposed [7, 8]. The principle of these modified electrophoresis methods is basically the same as the traditional method described here, and the cost-saving method in this manuscript will also be applicable for such modified methods.

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Agarose gel electrophoresis is a very popular experiment for training of students in educational institutions [9–11]. Cost-effective methods described here should be good news for such institutions, because running costs cannot be ignored in student training practices.

2. Agarose

Agarose is a kind of carbohydrate macromolecules (polysaccharides), also known as a kind of dietary fibers. Agarose is purified from a certain red seaweed *Rhodophyta*. Polysaccharides from *Rhodophyta* mainly consist of agarose and agaropectin. Agaropectin is not capable of forming gels and has to be removed as impurities from the agarose/agaropectin mixture. The quality of agarose depends on such purification steps, and these steps push up agarose commercially much expensive.

2.1 Quality of agarose

In Asian countries, agar is widely known as an ingredient in foods and/or desserts (e.g. mitsumame, yokan, and so on in Japan). Agar is also a well-known gelling reagent for bacteria medium.

"Agarose" and "agar" are sold as different merchandises, but the origin of them is the same; both are made from the same seaweed. It can be said that agar for bacteria medium is a partially and roughly purified form of sea weeds and is of lower quality than agarose for electrophoresis.

In my experience, agar for bacteria medium is quite suitable for a gel electrophoresis reagent. INA AGAR® BA-30 (Ina Food Industry Co., Ltd. (Nagano, Japan)—Funakoshi Co., Ltd. (Tokyo, Japan)) (**Figure 1**, left) is an agar of which grade is for bacteria, but its quality is very good for electrophoresis. The cost for this reagent goes to about 1/5 of standard agarose for electrophoresis. Moreover, Ina agar S-7 (Ina Food Industry Co., Ltd. (Nagano, Japan)) (**Figure 1**, right) is an agar

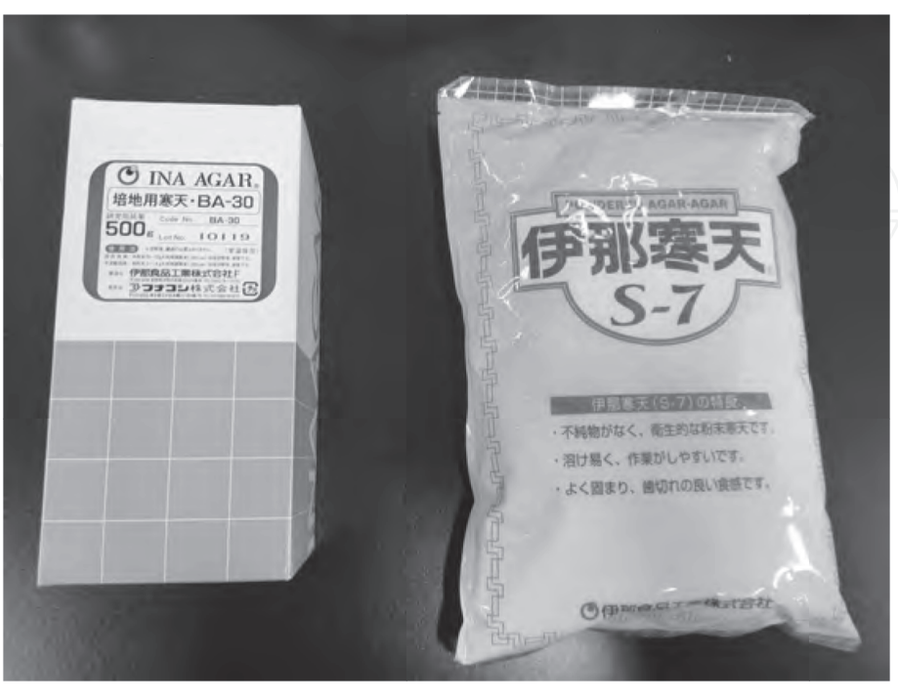

Figure 1.
Agars for not electrophoresis but other use. Left, INA AGAR BA-30 for bacteria medium. Right, INA S-7 agar for cooking.

for cooking, the quality of which is adequate for agarose gel electrophoresis. In this case, the cost is as much as 1/20. Although there is no warranty or trust for results (i.e. a quality test should be done at each package), it is worth doing in each laboratory to test agars for bacteria and/or for cooking. An example of the result by using BA-30 is shown in **Figure 2**, and S7 is shown in **Figure 3**.

Generally, agarose of low purity is more breakable because of its low gel strength. This disadvantage was critical especially when Southern or northern blotting was achieved in the experiment. In recent days, such blotting techniques have given way to the other; for example, polymerase chain reaction (PCR) to see DNA polymorphism and real-time PCR to see gene expressions. The major visualizing way of DNA in agarose gel is to use ethidium bromide (EtBr) or the other DNA intercalators that make fluorescence excited in certain wavelength [12]. Several protocols for staining reagent to intercalate DNA are known; (a) add the reagent in the gel before solidifying, (b) add the reagent in the loading buffer at

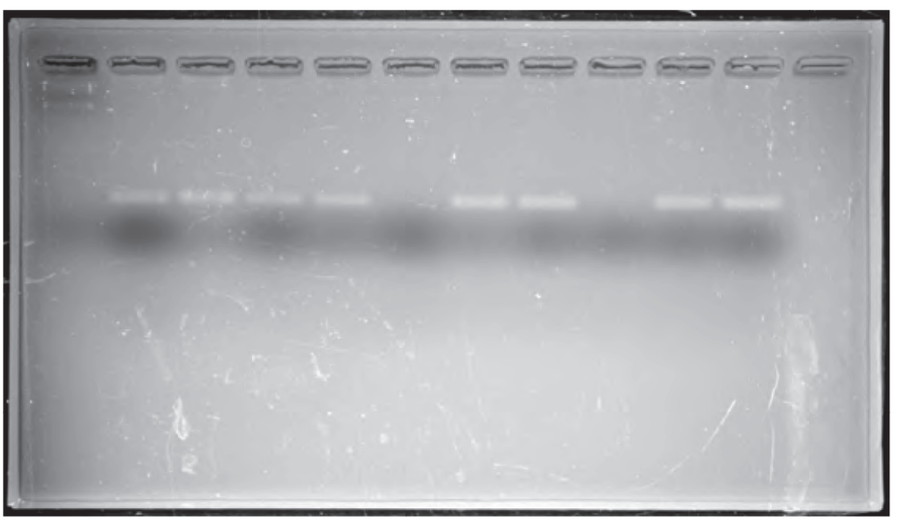

Figure 2.
INA agar BA-30 for agarose gel electrophoresis. 2% weight per volume of agar was applied.

Figure 3.
Result of the electrophoresis by using INA S-7 agar. The gel is 1% weight per volume.

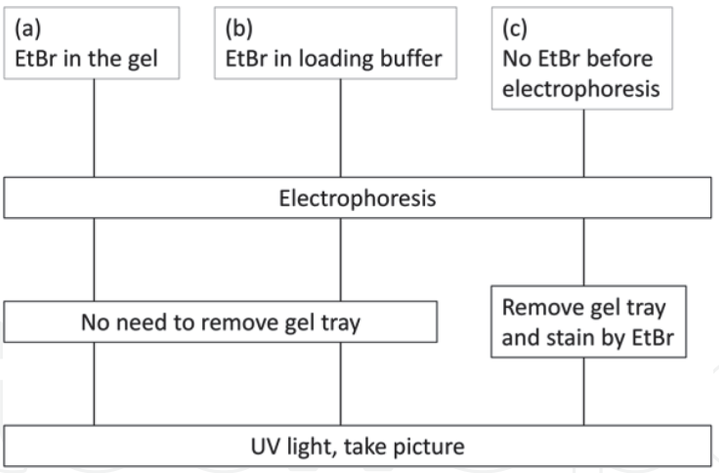

Figure 4.
Scheme for staining and visualizing of DNA in agarose gel electrophoresis.

electrophoresis, and (c) soak the gel in the reagent buffer after electrophoresis (**Figure 4**). In (a) and (b), a photograph of the gel can be taken with a gel tray, when the tray is clear. In such a situation, a low gel strength does not disturb electrophoresis and DNA visualization. Based on these reasons, a low gel strength seems not a too much annoying point when simply doing electrophoresis and taking photographs.

2.2 Recycling and reusing of agarose

It is known that used agarose gel is reusable again and again. Recycling of agarose after electrophoresis is very effective for cost-saving. Several reports are published [13, 14], in which used agarose gels are simply boiled and poured to a gel tray. After cooling to make the recycle gel solid, the recycled gel is enough for applying another electrophoresis. On the other hand, the DNA staining reagent (such as EtBr) still remains in the used agarose gel. EtBr is well known as toxic mutagen [15, 16], so when used and stained gel is boiled, toxic fumes containing

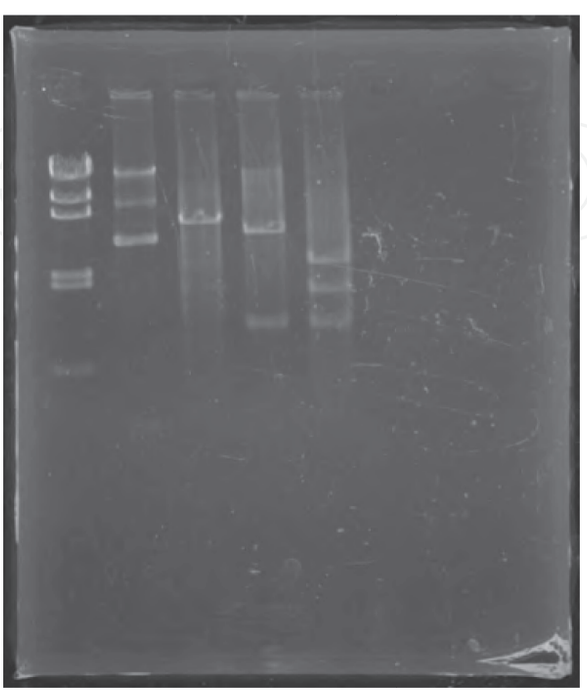

Figure 5.
Recycled gel (1% weight per volume) was applied to the electrophoresis.

EtBr appear. This fume will be hazardous when incorporated through the respiratory system.

To avoid such hazardous fumes, a freeze-and-thaw of used agarose gel is very effective for removing toxic EtBr from the gel [17]. By repeating freeze-and-thaw, the EtBr concentration of used agarose gel dramatically reduces to as much as a negligible level. The result of electrophoresis by recycled agarose is shown in **Figure 5**.

Agarose is hydrolyzed in acidic condition. Therefore, repeating the boiling and melting step in acidic condition might degrade the polymer structure of the agarose. The freeze-and-thaw method mentioned above is free from such a degradation.

3. Electrophoresis buffer

The most standard buffer for agarose gel electrophoresis is TAE buffer (tris, acetic acid, EDTA). TBE (tris, boric acid, EDTA) is the second major buffer. It is said that TBE has an advantage to fractionate small length DNA; in an old sequence analysis, a combination of acrylamide gel and TBE buffer was a standard condition.

When TAE is compared with TBE, the cost of TBE is higher than TAE. This is because of the difference of the price of acetic acid and boric acid.

For RNA electrophoresis, MOPS buffer (MOPS, sodium acetate and EDTA) is another standard, although this buffer is much expensive. Anyhow, daily agarose gel electrophoresis is achieved in a condition of using TAE in standard.

Yet another electrophoresis buffer is SB buffer, which is obtained from sodium borate. The vast majority of SB buffer is the cost, 1/4 of TAE and 1/10 of TBE [18].

In my experience, DNA is well migrated and fractionated in the agarose gel electrophoresis with SB buffer, although small but many air cavities appeared after finishing the electrophoresis. The cavities do not exist when starting the electrophoresis, but they do appear several ten minutes after switching on and/or staining the gel after electrophoresis (**Figure 6**).

3.1 Concentration of buffer

It is a very simple and effective idea of cost-saving that dilution of the buffer is available or not. If 1/2 dilution is available, the cost also will be 1/2. In my experience, 0.5× TAE buffer works fine (**Figure 7**).

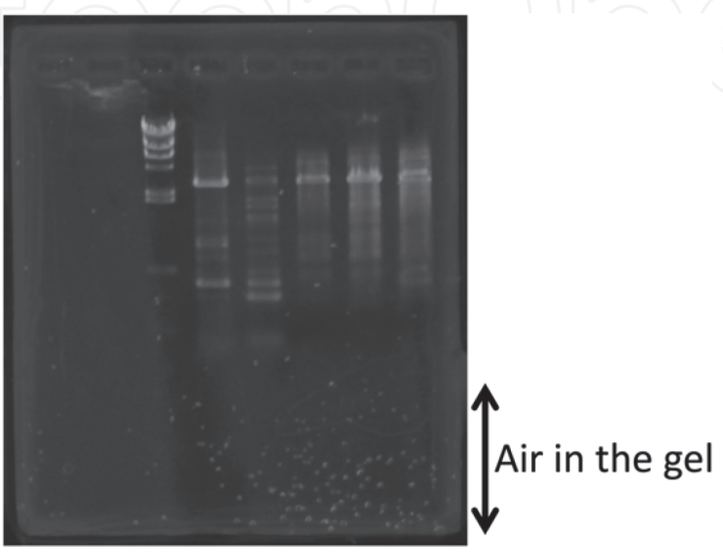

Figure 6.
SB buffer resulted in small but many air cavities in the gel.

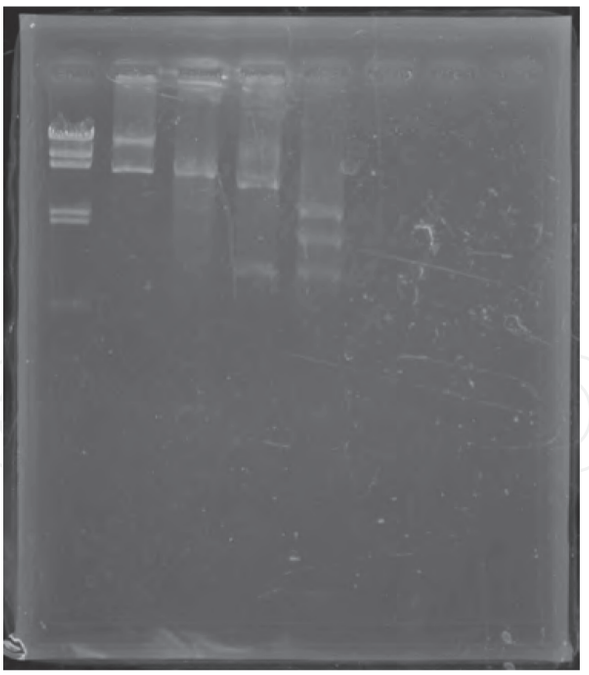

Figure 7.
Agarose gel electrophoresis with 0.5% TAE.

A low concentration of ions in the buffer results in higher resistance in an electric circuit, which leads to heating of the buffer. Therefore, too much dilution of the buffer might result in boiling of buffer and melting of agarose gel.

4. Equipment

There is so much commercial equipment for agarose gel electrophoresis, but unfortunately, they are rather expensive for its purpose; for example, tens of electrophoresis tanks are needed at a time in students' practice, but it is sometimes difficult to buy so many tanks at a time. One of the major reasons of this higher cost is that platinum is used as electrodes in the tank. Platinum is a precious and noble metal, which is very stable and never degraded in electrolysis. The second reason is that the buffer tank of the equipment has a special shape. Generally, the bottom face of the buffer tank has an anti-U-shaped structure (**Figure 8**). The third reason is that the equipment is sold with the special power supply. It seems that no electrophoresis is available without the manufacturer's specified power supply.

4.1 Buffer tank

The anti-U-shaped structure is not always necessary in buffer tank. Basically, a structure of an electrophoresis tank can be much more simple, and we can make it by do-it-yourself (DIY) (**Figure 9**).

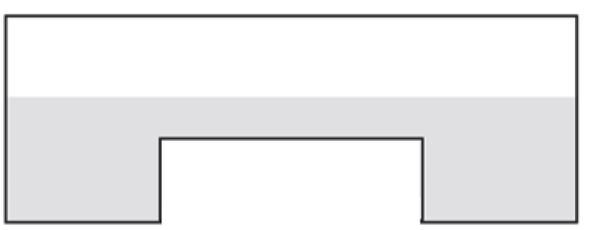

Figure 8.
A horizontal view of typical buffer tank for agarose gel electrophoresis.

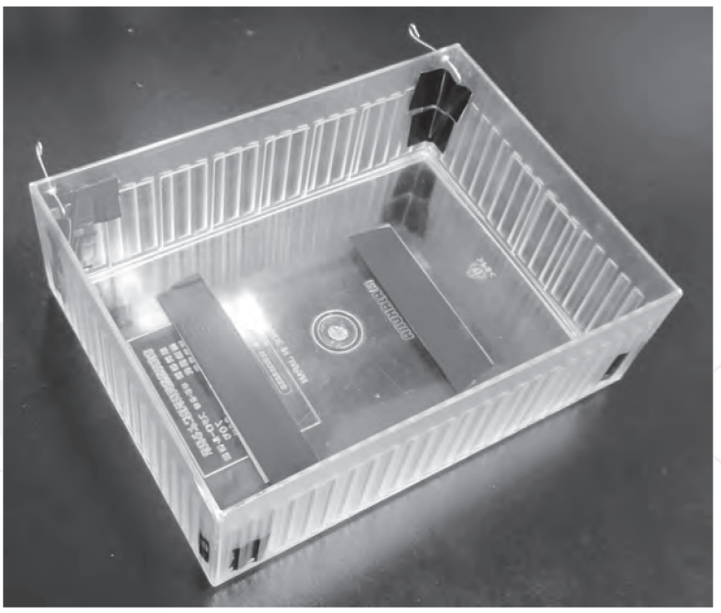

Figure 9.
DIY buffer tank for agarose gel electrophoresis. Note that the basket has a flat bottom, unlike a standard commercial buffer tank.

4.1.1 Making DIY buffer tank

A plastic basket in variety store (so-called 100-yen shops, 99 cents store, Dollar store, etc.) is good enough for electrophoresis tank. Plastic tape is put on the basal plane in three- to fourfold repeatedly, which works as a stopper of the gel during electrophoresis (**Figure 9**).

4.1.2 Electrodes

Although carbon stick like a lead of a pencil works as an electrode, stainless steel wire in hardware stores is a good choice of electrodes for agarose gel electrophoresis. No expensive metal is needed; almost the cheapest one will be worth testing. Wireframe of 1-2 mm in diameter leads to a good result. Wires are run at the bottom corner of the tank, simply put by mending tape (**Figure 10**).

Figure 10.
An inexpensive stainless wire as electrodes of electrophoresis tank. This wire is 1.2 mm in diameter.

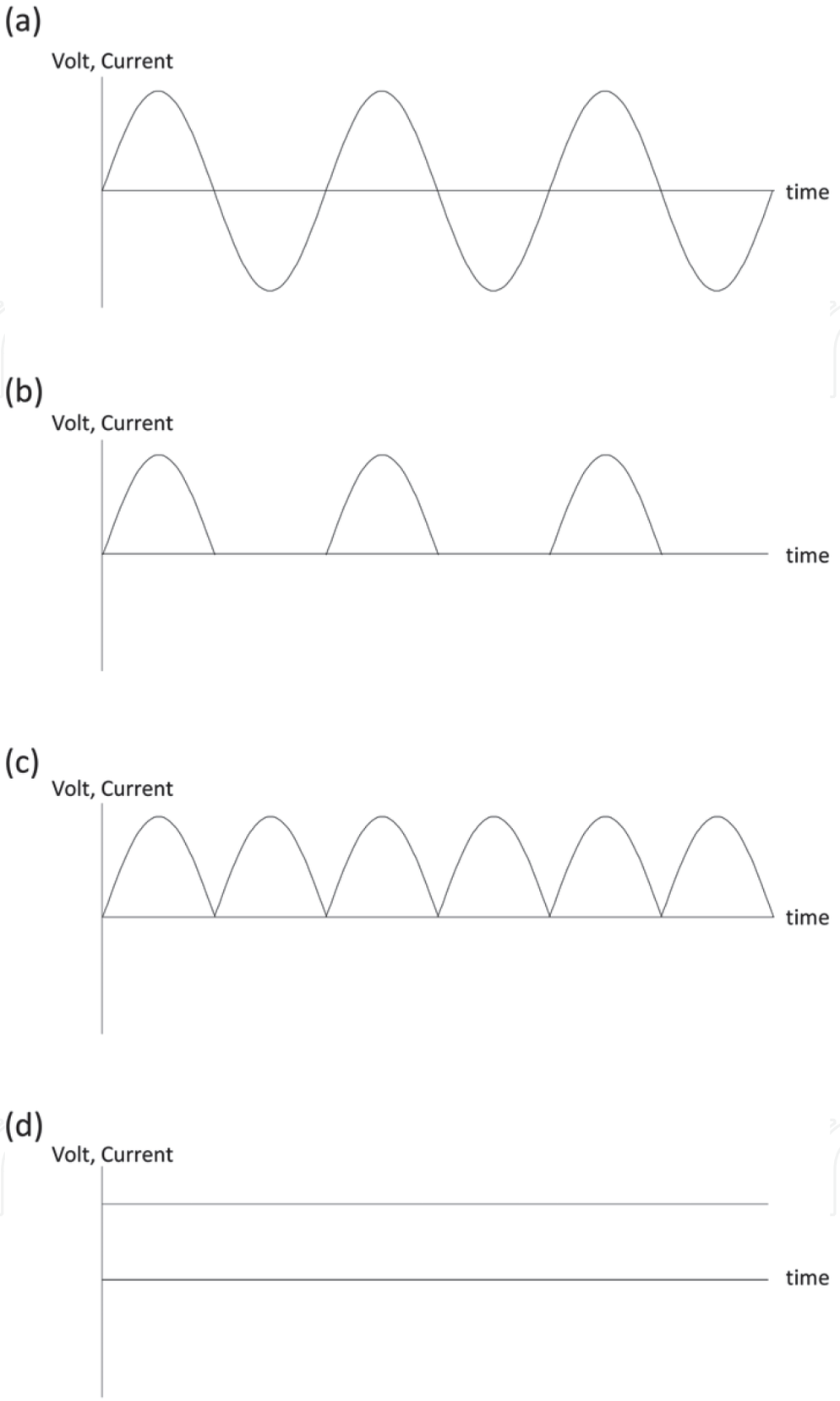

Figure 11.
An alternating current (a), half-wave rectified current (b), full-wave rectified current (c), and a true direct current. (b) and (c) are enough for doing agarose gel electrophoresis, and true direct current like (d) is not needed.

One avoidable notice is that copper is included in the wire as a component of wire. Copper is thought to be toxic, and it is ionized and flow out into the buffer during electrophoresis. Such a wire should be avoided, and it can be easily and clearly determined if copper is ionized because copper ion turns the buffer blue.

4.2 Power supply

Basically, agarose gel electrophoresis is achieved in around 100 volts [19]. In laboratories of molecular biology and biochemistry, power supply for SDS-PAGE is very popular equipment. This supply gives fine and direct current, promising proteins to migrate correctly. Of course, this power supply is also available for use in agarose gel electrophoresis. But actually, such a high-quality direct current is not needed in agarose gel electrophoresis.

Generally, the household electric power is supplied as alternating current (**Figure 11(a)**). This alternating current is not usable as electrophoresis. The current is passed through a diode, and an odd part of the current is picked up (half-wave rectification (**Figure 11(b)**)). A combination of diode enables to make all the alternating current as one direction (full-wave rectification (**Figure 11(c)**)). Half-wave rectified current and full-wave rectified current are a kind of pulsating current, which is not a true direct current (**Figure 11(d)**). It is known that such half-wave or full-wave rectified current is enough for agarose gel electrophoresis [20].

4.2.1 DIY power supply

To make half-wave or full-wave rectified current is not so difficult. **Figure 12** is a diagram showing full-wave rectification from alternating current. Four diodes are needed in this diagram, and they are substituted by one Graetz bridge.

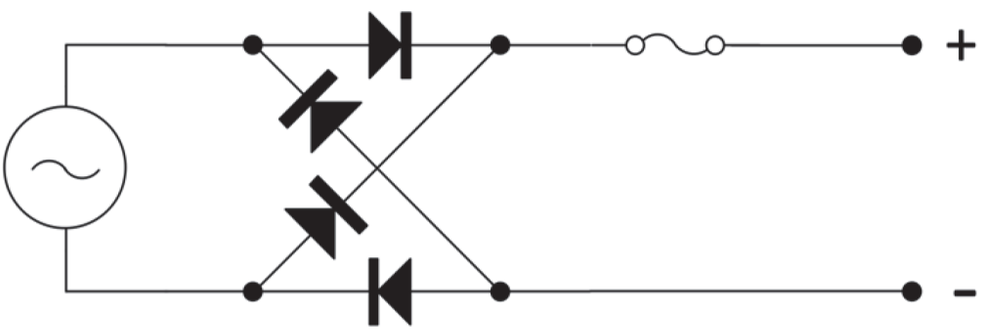

Figure 12.
A simple circuit diagram of full-wave rectification for agarose gel electrophoresis.

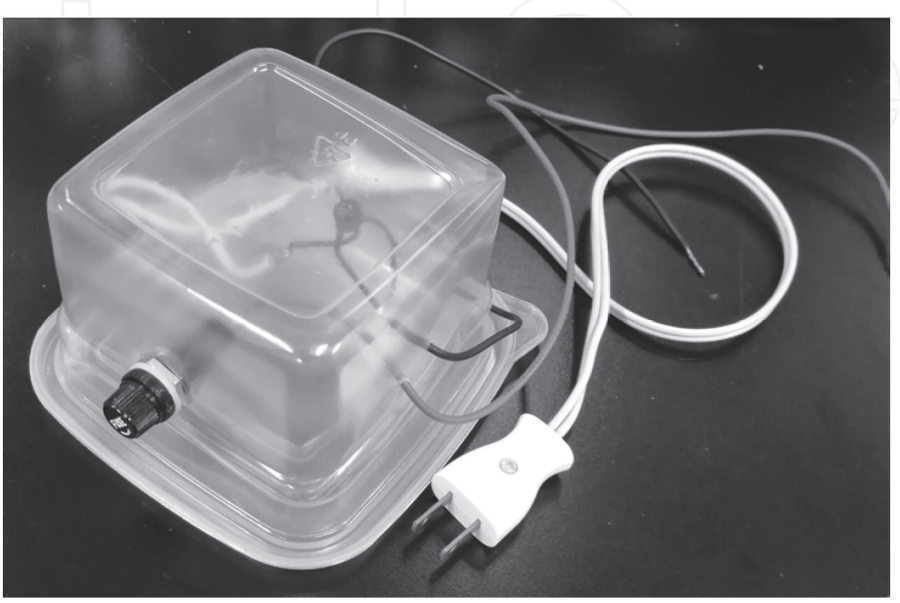

Figure 13.
A DIY power supply based on a diagram of **Figure 12**.

Figure 14.
*A close view of the DIY power supply (***Figure 13***). An inexpensive Graetz bridge (AM1510) is used in the supply.*

Figure 13 is an example of DIY power supply, which is based on the diagram of **Figure 12**. **Figure 14** is a close view of the same DIY power supply, in which only one Graetz bridge is used. A fuse is incorporated in this supply for safety.

5. Concluding remarks

In this manuscript, several technical tips for low-cost agarose gel electrophoresis have been described. The key factor of the tips is agarose (or agar) selection, recycling of agarose, buffer selection, and DIY equipment. Several experiments need a step to recover and isolate fractionated DNA from the agarose gel. In such cases, a high quality of agarose can affect the experiment. Nevertheless, such a high-quality agarose is not always needed for simply checking the band patterns of fractionated DNA. Agarose quality can be changed in its purpose, time, place, and occasion.

Agarose gel electrophoresis is a simple technique. Based on its principle, it can be modified and customized as how much cost you spend to the experiment. Moreover, technical tips described here do not mean downgrading of experiment quality; DNA can migrate and be fractionated as the same way as the standard protocol. The important point is that a calibration test is needed at each reagent and equipment. In my experience, gel strength varies in each product, and concentration of the agar in the gel should be adjusted at each condition.

In this manuscript, the topic has been focused into mainly DNA electrophoresis by agarose gel. RNA is far more sensitive to nuclease (ribonuclease for ribonucleotides) than DNA (deoxyribonuclease for deoxyribonucleotides). This also means that much higher quality of reagents is required for RNA electrophoresis, especially eliminating a contamination of ribonuclease. Moreover, some special technique is required in RNA electrophoresis for denaturation of tertiary structure of single strand RNA. Even though there stand such points to take account of RNA, the

buffer tank and power supply in this manuscript will also be able to work in RNA electrophoresis, because of the same principle of the electrophoresis of nucleic acids.

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Conflict of interest

The authors declare no conflict of interest.

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