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Full Length Research Paper

Recovery of DNA from agarose gel by trap method

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Recovery of DNA from agarose gel electrophoresis is a basic operation during molecular cloning. Circular or linear DNA fragments which vary from 1.5 to 6.5 kb and correspond to 1 kb marker can be recovered from 0.8 to 1.0% agarose gel smoothly with a simple and rapid trap method. The recovery efficiency could be more than 70% and the quality of the recovered DNA is proved to be good enough for future research. Here, we provided another good method to recover DNA from agarose gel besides using commercial kits.

Key words: Trap method, recovery of DNA, agarose gel electrophoresis.

INTRODUCTION

Agarose gel electrophoresis is a simple and efficient way to separate DNA with different numbers of base pair (Brody et al., 2004). Over the years, a variety of recovery methods have been proposed. In these methods, recovery of DNA from agarose gels by electrophoresis onto DEAE-cellulose membranes is a rapid and efficient one (Girvitz et al., 1980; Dretzen et al., 1981). Recovery of DNA with electroelution into dialysis bags (McDonell et al. 1977) is also good for recovery of DNA from gels with high recovery rate, especially for larger fragments. Other good approaches to recover DNA also have been developed, such as FlashGel® System for DNA Recovery (Lonza) etc. We also found a way to recover DNA from gels, which can recover DNA with common laboratory facilities. This way can provide another selection without kits in hand.

After DNA fragments are separated in gel electrophoresis, the target DNA bands are usually cut out to recover. But we can recover DNA by electrophoresing DNA into liquid directly after digging out a trap before the target band and refilling it with the same buffer. When the molecules of DNA move from agarose gel into a well, the velocity of DNA will increase in the well without interaction between gel and DNA (Stellwagen and Stellwagen, 2009). It is possible to recover them from the well directly when the length of the well increase appropriately. The volume of recovered buffer is huge, which is difficult for the micro centrifuge. But our DNA could be extracted from n-butanol. Based on this, DNA fragments could be recovered from gel smoothly, and the yield and quality of recovered DNA is qualified.

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MATERIALS AND METHODS

DNA preparation

TG1, anti-ubiquitin scFv phage (anti-ubiquitin scFv fragment cloned in an ampicillin resistant phagemid vector pIT2) (de Wildt et al., 2000), KM13 helper phage (Kristensen and Winter, 1998) were from Tomlinson I+J scFv phage libraries (Geneservice). Crude anti-ubiquitin scFv plasmid and KM13 plasmid were extracted according to the protocols provided by the manufacturer and Sambrook (2001), and then dissolved in 100 µl TE with 20 µg/ml RNase A.

Electrophoresis analysis of crude plasmid DNA

Preparation of 1% agarose gel (agarose LE, Spain) was done with 0.5×TBE or 1×TAE. 10 µl pIT2 and KM13 was mixed with 6×sample buffer, the samples were loaded to 0.5 cm-width slots and 5 µl 1 kb DNA marker to another slot. A voltage of 10 v/cm was applied for 30 min, and the DNA bands were assayed.

Recovery of DNA from agarose gels by trap method

According to the following trap method, two bands of DNA fragments (approximately 1.5 and 6.5 kb correspond to 1 kb marker

separately) within crude KM13 plasmid and one 3 kb DNA band within crude anti-ubi scFv plasmids were recovered.

Preparation of agarose gel containing trough-shaped plastic film

Trough-shaped plastic film (plastic films came from packing materials of 96-well cell culture plates) was slightly wider than sample slots (for example, if 1 cm-width comb was selected, approximately 1.2 cm-bottom-width trough-shaped plastic film was needed). The sample slots were laid in the middle of the trough-shaped plastic films. The bottom of the trough-shaped films should be deeper than the sample slots to avoid DNA from moving below plastic films. The concentration of 0.8 to 1% gel and 0.5 to 0.8 µg/ml EB (ethidium bromide) was added before gel solidification.

Electrophoresis

Crude plasmid DNA was added to two 1.5 cm-width sample slots (50 µl each slot) and electrophoresis began. When DNA fragments were isolated completely by detection with a portable long-wavelength UV lamp after regular gel electrophoresis, the liquid surface of tank buffer was adjusted to the same level as agarose gel and about 3 to 5 cm-length traps along the trough-shaped plastic films was dug out beyond the target bands. The traps were washed 2 to 3 times with buffer and refilled again. The voltage was set up to 5 v/cm and electrophoresis continued.

Extraction of the buffer containing target DNA fragments from the traps

When DNA bands ran into the buffer of the traps (2 to 6kb plasmid DNA fragments generally continue to be electrophoresed in TBE or TAE for 10 min or so), the power was turned off, and the buffer was taken out from the traps to 15 ml centrifuge tubes with a Pasteur pipette. The volume of the buffer extracted was equal to the length of the trap x width x depth, for example, in our experiments, the recovered volume = 4 x 1.2 x 0.5 cm = 2.4 ml.

Concentration and purification of DNA

At the first concentration, 2 to 3 volumes of pre-chilling (at 4 or -20℃) n-butanol was added to the extracted buffer after adding 200 µl 5 M sodium chloride, spun at 1600 g for 1 min, and the upper phase (n-butanol) (during the previous concentrations, n-butanol may not be removed completely in order to avoid DNA loss) was removed. At the following concentrations, equal volume of n-butanol was added. Using 3 to 5 times of concentrations, the final concentrated solution of about 500 µl was transferred to a 1.5 or 2 ml microcentrifuge tube, and then DNA was extracted with equal volume of phenol/chloroform. The supernatant was transferred into another microcentrifuge tube, and 2 volumes of ethanol were added to the supernatant to precipitate DNA after adding 0.2 volume of ammonium acetate, and then the precipitations was washed with 70% ethanol.

Dissolving DNA

DNA was dissolved in 50 µl TE (pH8.0) without RNase A. When DNA dissolved slowly in TE, it was heated at 65℃ in a water bath for 5 min.

Analysis of plasmid DNA that was recovered by agarose gel electrophoresis

To test the activity of DNA, 0.5 to 0.8 µg/ml EB was added to the 0.8 to 1.0% agarose gel before solidification. The DNA bands were scanned after DNA have migrated to appropriate place under a voltage of 10 v/cm, and 1 kb DNA marker (Axygen) was used to run as a control.

Analysis of the amount of plasmid DNA recovered by trap method

In order to check how much DNA could be recovered by trap method, three kinds of DNA fragments with different mobile velocity extracted from the earlier mentioned steps were used for further analysis, each kind of DNA fragments was mixed well separately, then their contents were assayed with UV spectrophotometer. 50 µl of each kind was taken to 2-cm sample slot, the DNA was recovered again, and their contents were assayed with UV spectrophotometer. Each sample was repeated five times and the recovery efficiency was calculated.

Analysis of the quality of DNA that was extracted by trap method

Plamids (ScFv) were digested with Sa*l*I/No*t*I at the same time. The samples were run on agarose gel.

Data analysis

The percentage of DNA recovered was calculated. The content of DNA was detected with Beckman coulter UV spectrophotometer. The amount of DNA recovered = (the content of DNA after being recovered x the volume of the DNA after being recovered) / (the content of DNA before being recovered x the volume of the DNA before being recovered) x 100%.

RESULTS

Agarose gel electrophoresis of crude plasmid pIT2 and KM13

The result of electrophoresis of crude anti-ubiquitin scFv plasmids and KM13 plasmids extracted according to the protocol is shown in Figure 1. After gel electrophoresis for 30 min, anti-ubiquitin scFv plasmids showed three bands. KM13 plasmids also contained three bands (the latter band contained two kinds of DNA fragments, not separated completely). These bands were different structure plasmids. They showed different velocity of movement.

Recovery of the required plasmid DNA smoothly from gels by trap method

After regular electrophoresis for 30 min in 0.9% gels, the bands of crude KM13 plasmids were isolated (Figure 2a). About 4cm-length traps were dug out before the target

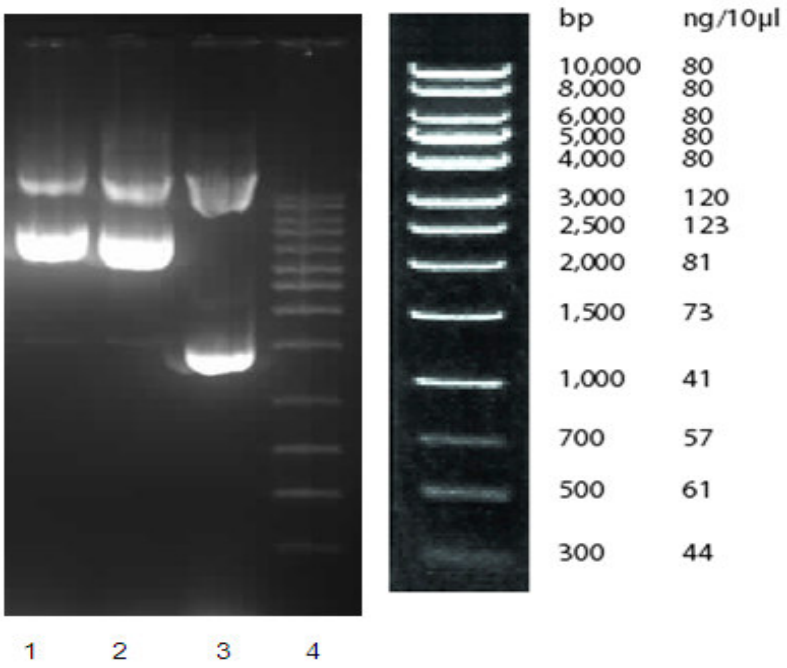

Figure 1. Left figure: gel electrophoresis of crude plasmids. Lanes 1 and 2 are plasmid anti-ubi scFv (10 µl), plasmid KM13 (10 µl) is in lanes 3, and lane 4 is 1 kb DNA marker (5 µl). Right figure: 1 kb DNA marker (10 µl). Buffer: 0.5 x TBE; Voltage: 10 v/cm.

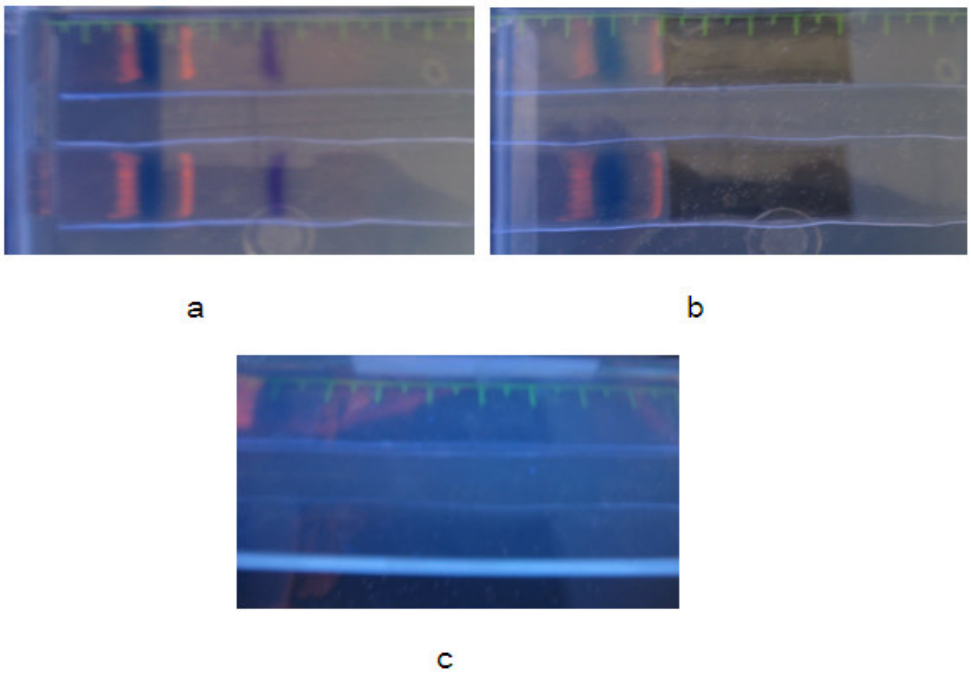

Figure 2. (a): 0.9% agarose gel electrophoresis of crude plasmid KM13, the two bands of DNA were to be extracted. Buffer:0.5 x TBE; Voltage: 10 v/cm; (b): Two 4 cm-length traps were dug out before the front bands were extracted along the plastic grooves with a blade or plastic slice, and the tank buffer level was lowered to gel surface, and after washing the traps, they were refilled with buffer. The voltage was adjusted to 5 v/cm, and electrophoresis was continued; (C): After 4 min electrophoresis, the front DNA band stained with ethidium bromides moved into the traps partly. Electrophoresis buffer: 0.5 × TBE; Voltage: 5 v/cm.

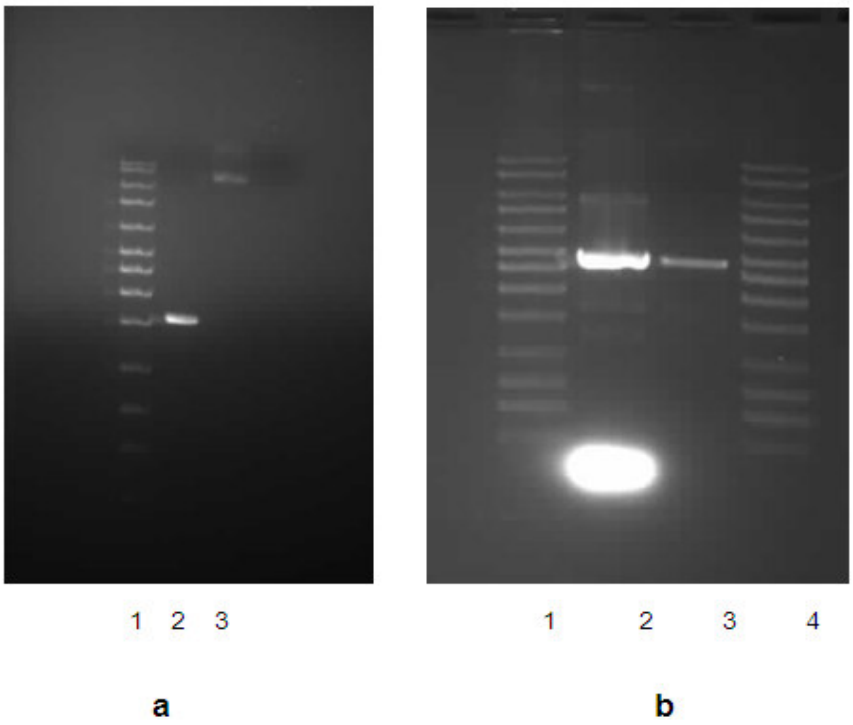

Figure 3. Electrophoresis of DNA bands recovered. (A): Two bands of the DNA extracted from crude plasmid KM13 (electrophoresis for about 50 min). Lane1: 1 kb DNA molecule marker (5 µl); lane 2; the front DNA extracted (5 µl, 77.3 µg/ml); lane 3: the following DNA was extracted (5 µl, 58.3 µg/ml). Buffer: 0.5 x TBE; Voltage: 10 v/cm, 1% gel. (B): Anti-ubi scFv plasmid electrophoresis in 0.8% agarose gel. Lanes 1 and 4: 1 kb DNA marker, 5 µl/lane; lane 2: crude scFv plasmid band, 10 µl; lane 3: anti-ubi scFv plasmid DNA recovered. Buffer: 1 x TAE; Voltage: 10 v/cm.

bands along the trough-shaped plastic film with a blade (or plastic film) (Figure 2b). Then, the traps were refilled with electrophoresis buffer. The voltage of the electric field was adjusted to 5 v/cm and electrophoresis was continued. After electrophoresis for 4 min, most of the DNA fragments of the target bands migrated into the trap (Figure 2c), and electrophoresis continued until the DNA moved into the trap completely. The buffer was pipette out of the traps and transferred into 15 ml centrifuge tubes. The traps were refilled with buffer, and electrophoresis was continued until the following DNA band migrated into the traps completely again. In order to assay whether the target DNA fragments have been recovered, the two kinds of DNA were electrophoresed again, the result is shown in Figure 3a. Like the earlier mentioned steps, DNA was extract within crude anti-ubiquitin scFv plasmids, the result is shown in Figure 3b.

The recovery efficiency of DNA is high by trap method

We calculated the recovery amount of DNA in TAE buffer. The recovery percentage of DNA was from 69.6 to 84.6%, the average recovery percentage of 1.5 kb fragment was 73.3%, of 3 kb was 74.2% and of 6.5 kb was 71.6%.

DNA quality test with restriction enzymes and gel electrophoresis

Crude anti-ubiquitin scFv plasmid is circular DNA (3 kb, which correspond to the 1 kb marker; Figure 4a). They showed about 5.4 kb DNA band after being digested with single enzyme (NotI or SalI; Figure 4a, lanes 2 and 5). When digested with both NotI and SalI, the recovered DNA showed two DNA bands of about 320 bp and 5.1 kb (Figure 4a, lane 3). The results suggested that the recovered DNA fragments were excised effectively with restriction enzymes, and the activity was good.

Recovered 30 µl anti-ubiquitin scFv plasmid DNA after digestion with enzyme SalI (at 37℃ for 1 h) to obtain linear DNA was loaded to 1.5 cm sample slot of 0.8% gel for recovering again, and finally dissolved in 30 µl TE. Then, two plasmid DNA (each 5ul) were taken and

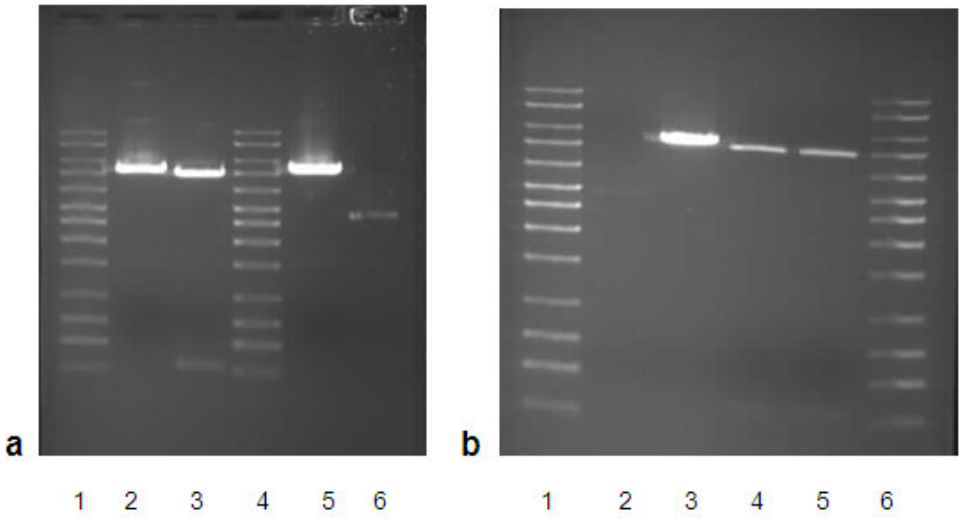

Figure 4. Electrophoresis of DNA recovered after digestion with restriction enzymes. (A): Electrophoresis in 0.8% gel of scFv plasmid DNA recovered after being digested with restriction enzymes *Sal* I and *Not* I . Lanes 1 and 4: 5 µl 1 kb marker; Lane 2: 5 µl DNA excised with restriction enzyme *Not* I ; Lane 3: 5 µl DNA excised with both *Not*I and *Sal*I; Lane 5: 5 µl DNA excised with enzyme *Sal* I ; Lane 6: 5 µl circular DNA recovered without being digested. Buffer: 1 x TAE; Voltage: 10 v/cm. (b): Recovery of linear DNA and analysis with restriction enzymes. Lanes 1 and 5: 1 kb DNA marker; Lane 2: circular plasmid (5 µl) not digested with restriction enzyme; Lane 3: linear plasmid (10 µl) after being digested with *Sal* I ; Lanes 4 and 5: linear plasmids (5 µl each) recovered again and were obtained by digestion of 30 µl circular plasmid anti-ubi scFv plasmids with enzyme *Sal* I and dissolved in 30 µl TE, which were further spliced with enzyme *Not*I. Buffer: 1 x TAE; Voltage: 10 v/cm.

digested with enzyme NotI (at 37℃ for 1 h) (the remaining 20 µl would be used as control group, and 10 µl was loaded to lane 3 in Figure 4b), and electrophoresis continued. Each 5 µl of the two were loaded to lane 4 and lane 5 separately in Figure 4b, another 5 µl circular pIT2 plasmid was added to lane 1 as the control group. The result suggested the linear DNA could also be recovered effectively and can maintain good activity.

DISCUSSION

Agarose gel electrophoresis is an important method to analyze and purify DNA fragments with different migrating speed in gels. In our study, we recovered DNA with different movement velocity which corresponds to DNA marker smoothly by trap method. It is easier, faster and cheaper, no special facilities are needed. Trough-shaped plastic film can be reused. n-Butanol could get rid of EB in agarose gels and concentrate DNA easily (Sambrook and Russel, 2001). Based on our experience, approximately each 5 ml n-butanol can remove about 1 ml buffer and the step for concentrating DNA with n-butanol generally can be done within 20 min. The speed of DNA migrating into the trap is affected by the concentration of the agarose gel, molecular weight, the structure of DNA, buffers characters, voltage, EEO (electroendosmosis), etc (Brody et al., 2004). The speed of DNA fragment is much faster in low concentration gels. So DNA fragment slowed down in higher concentration gels, which means it cannot be recovered entirely. In free solution, the electrophoretic mobility of DNA fragments of 0.4 to 48.5 kb at 25℃ is found to be (3.75 ± 0.04) x 10 – 4 cm 2v-1 in TAE, in TBE it is (4.5 ± 0.04) x 10 – 4 cm 2v-1 (Stellwagen et al., 1977), which is not related to molecular weight of DNA. When the electrophoretic conditions of a voltage of 5 v/cm, 10 min are applied, the movement distance of DNA is about 1.125 or 1.35 cm in free solution. Therefore, for 2 to 6.5 kb DNA fragments, 3 to 5 cm-length trap is enough with the voltage of 5 v/cm. Lower voltage can delay DNA which goes through the trap, and give enough time to recover the DNA entirely. In fact, we can also increase the length of trap to recover larger DNA fragments.

When DNA is migrating in gel, besides Tris+ ion (Stellwagen et al., 2000; Stellwagen and Stellwagen, 2002), agarose gel can also interact with DNA (Stellwagen et al., 2002; Stellwagen and Stellwagen 2009). After the running of DNA into free TBE, boric acid ions can associate with them (Stellwagen et al., 1977 2000) immediately. But in TAE, acetate ions do not interact with them, or else interact very weak. Therefore,, negative charge boric acid ions make DNA to move quickly in TBE (Stellwagen et al., 1977), and final

recovered DNA usually contains associated boric acid ions as a result of this. This makes the DNA to be dissolved in TE incompletely. Sodium ions can compete with Tris+ to bind to DNA in free buffer (Stellwagen et al., 2000, 2005, 2007), so we added sodium chloride to the extracted DNA buffer for recovering.

It is well-known that crude plasmids usually contain different structures of DNA, which has different migration velocity in agarose gel (Mickel et al., 1977; Levene and Zimm, 1987). We had recovered three kinds of DNA fragments with different velocities from agarose gels separately, and for 3 kb DNA fragment, there was a higher yield. Within 6.5 kb fragment extracted, there was partly contaminated DNA following the 6.5 kb fragment, so longer running time was needed to separate different sizes bands. Circular 3 kb DNA fragment recovered which contained SalI/NotI restriction enzyme sites was spliced smoothly with SalI and NotI restriction enzymes, which showed that DNA recovered by trap method worked well. To test whether linear recovered DNA can be further digested by enzymes, we digested circular anti-ubiquitin scFv plasmids with enzyme SalI to obtain linear DNA (Figure 4a), then after linear DNA was recovered by trap method, they were digested with restriction enzyme NotI, the recovered DNA was spliced smoothly again.

The trap method is similar to the method of electroelution into a dialysis bag. It is easier, and the recovery efficiency is high enough for further molecular cloning. Fan et al. (2005) also had tried to recover DNA from agarose gel using a recovery well. They produced a recovery well in the front of the band before electrophoresis, this procedure used randomly, could lead to recover DNA fragment that is not separated completely and disrupt the result of electrophoresis. The width of the recovery well that was produced by using a comb is not enough. If a band in free solution is wider than the recovery well being recovered, the amount of recovered DNA could not be guaranteed, or the process is laborious. In our method, we improve the method; DNA fragments can be completely separated and observed. DNA fragments that move into the trap are more than 70%. Before gel solidification, we put a trough-shaped film in gel, because we found that DNA fragments can run in the bottom gel of the trap partly. It is also convenient to produce a well along the trough-shaped film. Moreover, we use n-butanol to concentrate DNA during recovery of DNA from gels, which helps us recover larger volume of DNA by using microcentrifuge tubes. Of course, we can also omit the concentrated step and extract DNA from larger volume buffer directly with ethanol if condition permits.

CONCLUSIONS

Circular or linear DNA fragments of 1.5 to 6.5 kb can be recovered smoothly from gels with high yields by trap method after electrophoresis without commercial kits. The quality of DNA recovered is high. Repeated recovery of DNA does not decrease the activity of DNA to be digested with restriction enzyme. The trap method is a simple and effective way to recover DNA from agarose gel. Although, we only recovered about 1.5, 3 and 6.5 kb DNA fragments, it is predictable that larger DNA fragments can be recovered effectively by this method as well.

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