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# A Simple, Efficient, and Economical Method for Recovering DNA from Agarose Gel

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**Abstract:** A simple method of recovering DNA from agarose gel that is fast, inexpensive, and friendly both to operators and environment is described. Two rows of wells are made in an agarose gel, and a DNA sample is loaded into the well nearest to the negative pole for separation by electrophoresis. Recovery is accomplished by pipetting the DNA-containing TAE buffer from the well near the positive pole after target DNA fragments have migrated into the well. A recovery rate of up to 94 $\pm$ 2.3% was observed with this method.

**Keywords:** Agarose gel, Method, DNA recovery

## INTRODUCTION

In many experiments of molecular biology, it is desirable to physically recover target DNA fragments from agarose gel for subsequent use. Consequently, many methods have been developed to accomplish this goal, including hydrolysis of agarose with agarase, electroelution, and extraction of DNA from low-melting agarose.[1] In recent years, various commercial DNA recovery systems have been developed and used widely.

Although these methods claim to meet the goal of recovering DNA from gel, they are subject to a number of limitations. Some methods are not suitable for recovery of a number of different fragments simultaneously, or expensive instruments or reagents may be involved.[2] Many methods are not friendly to

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operators and not environmentally suitable since phenol, chloroform, and other deleterious reagents are required.[3,4] Although commercial DNA extractions system are efficient for recovering DNA in most cases, a low recovery rate may occur when DNA fragments of interest are larger than 10 kb or smaller than 100 kb. Moreover, since the recovery rate may be influenced markedly by the structure of the DNA molecule, a system designed for extraction of linear DNA is not suitable for isolation of super-coiled DNA (www.marligen.com/pdfs/Manuals/GelExt.pdf).

We have developed a method recovering target DNA fragments from gel. The recovery rate is not influenced appreciably by the size and structure of DNA molecules. This method also satisfies the important considerations of simplicity, efficiency, low cost[5] and friendliness to operators and the environment.

## EXPERIMENTAL

### Materials and Methods

Three methods were compared in this study. Method 1 was recovery of DNA from low-melting agarose, which was done according to the method of Ref.[1]. Method 2 was performed based accor3ding to the instructions of a commercial DNA recovery system (www.marligen.com/pdfs/Manuals/GelExt.pdf). The third method was done as follows:

1. An agarose gel (0.8–1.2% agarose containing 0.5 μg EtBr $mL^{-1}$) was prepared, and two rows of wells were made by placing two combs [Fig. 1(A)]. The distance between the two rows of wells is dependent on the size of DNA of interest and adjacent bands. If two or more DNA bands have migrated closely, a longer distance and gel with higher concentration are required for full separation. The well close to the positive pole is used to recover DNA, which is called "Recovery well" (the size of Recovery well is 2 × 5 × 5 mm, W × H × D). The well close to the negative pole is used to load the DNA sample, which is called "Sample well" (the size of Sample well is 1 × 5 × 5 mm, W × H × D). The Recovery well should be larger than the Sample well since diffusion of DNA bands will occur during migration.
2. DNA fragments were separated by electrophoresis in a standard TAE buffer system. The level of TAE buffer was appreciably higher than the top of the gel so that the gel was connected with electric field, but TAE buffer did not flow into the Recovery well, even if it was empty [Fig. 1(B)]. The entire Sample well and Recovery well should be filled with TAE buffer (containing 0.5 μg EtBr $mL^{-1}$) before electrophoresis.

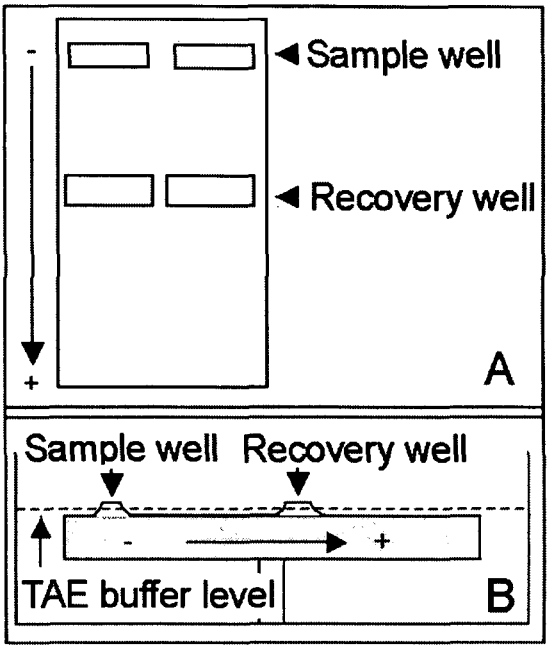


**Figure 1.** Agarose gel for separation and recovery of DNA. (A) Two rows of wells were made in a gel. The well close to the positive pole is used to recover DNA, which is called the "Recovery well". The well close to the negative pole is used to load DNA sample, which is called the "Sample well". The Recovery well should be larger than the Sample well since diffusion of DNA bands will occur during migration. The arrow at the left side of the gel indicates the direction of electrophoresis. (B) The action of surface tension between the comb and melting agarose gel would result in the occurrence of "dikes" at the surface of each well, and the "dike" will keep the TAE buffer out of the Recovery well, even if it was empty. The level of TAE buffer is indicated with a dashed line, and the level should be slightly lower than the top of the Recovery well. The depth of the gel is ~8 mm, depending on the volume of the samples.

3. Electrophoresis was conducted with a voltage of about 10–12 V $cm^{-1}$. The mobility of the target DNA fragment was checked periodically with a long-wave ultraviolet lamp (a means of estimating the time of target DNA migrating into Recovery well is described in this paper).
4. When the target DNA fragment moved into the Recovery well, the electrophoresis was stopped and the DNA-containing TAE solution was pipetted into a microcentrifuge tube. Usually, these four steps are sufficient to compete the DNA recovery.

5. If the DNA fragments need to be ligated into a vector or used as a PCR template, an aliquot (5–10 μL) of DNA-containing TAE buffer may be used directly. Generally, further purification is not necessary.
6. If further concentration and purification are desirable, centrifuge at 1500 × *g* for 1 min and transfer the supernatant into a new microcentrifuge tube so that the small pieces of agarose possibly pipetted with the DNA-containing TAE buffer are removed. Then, ethanol is added to precipitate the DNA. Phenol and chloroform extraction are not necessary.
7. If the DNA fragment migrates out of the Recovery well, stop the electrophoresis and change the direction of the electric field; then, continue to run until the DNA fragment migrates into the Recovery well again.

## RESULTS

### Recovery and Subsequent Use of DNA Fragments

The present method is able to recover various DNA fragments, and the recovery rate is not appreciably dependent on the size and structure of the DNA molecules of interest. Two linear DNA fragments and one super-coiled plasmid DNA were recovered successfully by this method (Fig. 2), and an average recovery rate of up to 94 ± 2.3% was obtained.

The recovered DNA fragments were used in PCR amplification, restriction digestion, or were subcloned into pUC18 plasmids. When the recombinant plasmids were transformed into competent DH5α cells, about $10^3$ of

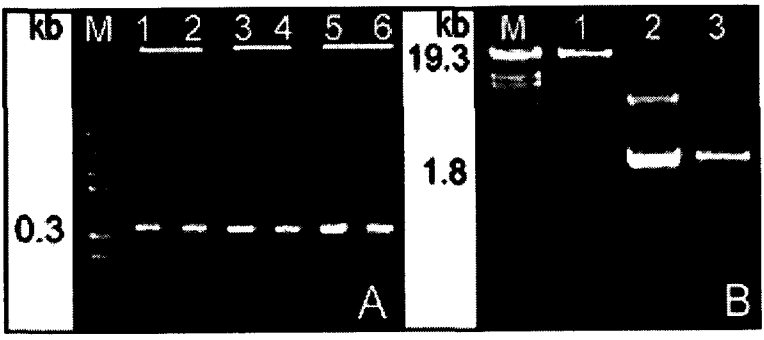


**Figure 2.** Various DNA fragments recovered by the three methods. (A) A 0.3 kb DNA fragment recovered parallel by three methods. Lanes 1 and 2: Commercial recovery system; lanes 3 and 4: the present method; lanes 5 and 6: low-melting agarose method. (B) A 19.3 kb and a 2.69 kb super-coiled DNA recovered by this method. Lanes 1 and 3 are the recovered DNA, lane M and lane 2 are DNA samples before recovery. The super-coiled DNA was observed at 1.8 kb.

the colonies were counted in one plate after 12 h of culture. Recombinant plasmids were prepared and sequenced; the sequencing results indicate the presence of target gene in all preparations.

## Comparison of Recovery Rate, Cost, and Time

In order to compare the recovery rate, cost, and time between the low-melting agarose method, the commercial recovery system, and the present method, a 0.3 kb DNA fragment was recovered in parallel by the three methods. The results show that the recovery rate of the present method is comparable with hat of low-melting agarose method ($P > 0.05$), and higher than that of commercial recovery system [$P < 0.05$, Fig. 3(A)], but the time and cost of

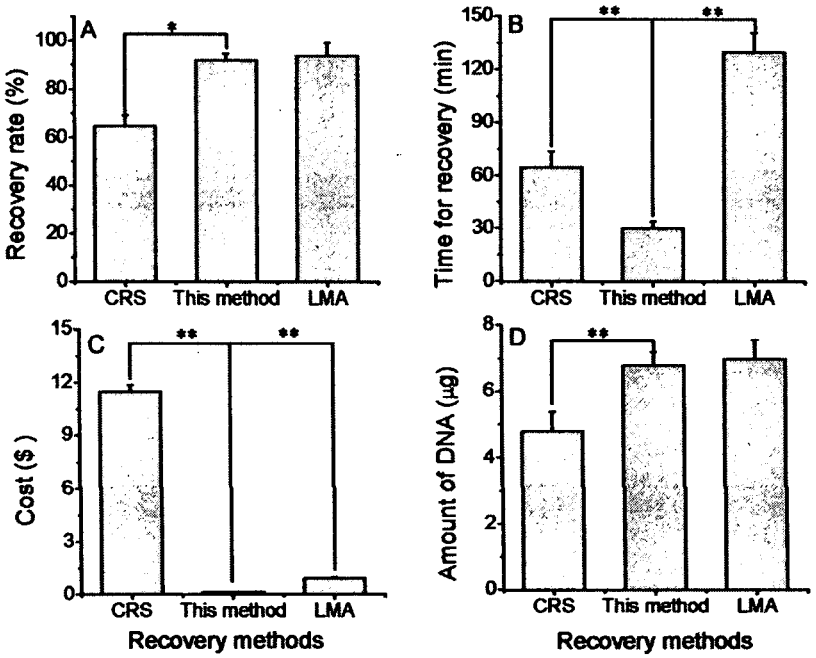


**Figure 3.** Comparison of recovery rate (A), time (B), cost (C), and total amount of recovered DNA (D) between the commercial recovery system (CRS), the present method, and the low-melting agarose method (LMA). The bars of time, cost, and total amount show the sum of three parallel recovery wells for which the size is 2 × 5 × 5 mm (W × H × D). The DNA fragment recovered in this experiment was 0.3 kb in size. The recovery time shown in B includes the time of about 25 min of electrophoresis. Each bar represents the means ± SE (standard error) of three repeats. The single asterisk presents $P < 0.05$, and double asterisks present $P < 0.01$.

this method are significantly shorter and lower than those of other two methods [$P < 0.01$, Fig. 3(B) and (C)]. Similar total recovery DNA was obtained by this method and low-melting agarose method, which were significantly higher than that of the commercial recovery system [$P < 0.01$, Fig. 3(D)].

### *Predicting the Time of DNA Fragment Migrating into* Recovery Well

We developed the following means to estimate the time of target DNA migrating into the Recovery well, since monitoring the mobility of DNA fragments of interest by ultraviolet lamp is a tedious task. The relative mobility of target DNA fragment ($Rf_{DNA}$) is calculated by following formula:

$$Rf_{DNA} = \frac{d_{DNA}}{d_{br}}$$

in which $d_{DNA}$ and $d_{br}$ are the migration distance of target DNA and bromophenol blue, respectively. They are calculated as follows:

$$d_{DNA} = v_{DNA} \cdot t, \quad d_{br} = v_{br} \cdot t$$

In both formulas, $v_{DNA}$ and $v_{br}$ are the migrating speed of target DNA and bromophenol blue, respectively, and $t$ represents the time of electrophoresis. So, the time of target DNA migrating into the Recovery well is obtained by the formula:

$$t_{DNA} = \frac{D_{S-R}}{v_{br} \cdot Rf_{DNA}}$$

in which $D_{S-R}$ is the distance between the Sample well and the Recovery well. For instance, when the speed of bromophenol blue $v_{br}$ is 0.11 cm $min^{-1}$ (1% of agarose gel, standard TAE buffer system, with voltage of 60 V and current of 25 mA), the time of migration into the Recovery well of a 0.42 kb DNA fragment whose $Rf$ was 0.96 (Table 1) was about 23.6 min, if the distance between the Sample well and the Recovery well was 2.5 cm [2.5/(0.11 × 0.96)]. So, the only thing one needs to do is to determine the migration speed of bromophenol blue under a given electrophoresis condition if the time of target DNA migrating into the Recovery well is required to be calculated. $Rf_{DNA}$ values listed in Table 1 may be useful in that calculation.

**Table 1.** $R_f$ values of DNA fragments.

| Linear DNA (kb) | $R_f$ value | Super-coiled DNA (kb) | $R_f$ value |
|---|---|---|---|
| 19.3 | 0.27 ± 0.01 | 7.5 | 0.35 ± 0.01 |
| 7.74 | 0.34 ± 0.01 | 6.7 | 0.39 ± 0 |
| 6.22 | 0.36 ± 0.01 | 5.2 | 0.49 ± 0 |
| 4.25 | 0.40 ± 0.01 | 3.7 | 0.52 ± 0.01 |
| 3.47 | 0.43 ± 0.01 | 3.3 | 0.54 ± 0 |
| 2.69 | 0.48 ± 0.02 | 2.9 | 0.58 ± 0.01 |
| 1.88 | 0.57 ± 0.01 | | |
| 1.49 | 0.64 ± 0.01 | | |
| 0.93 | 0.76 ± 0.01 | | |
| 0.42 | 0.96 ± 0 | | |

*Note*: $R_f$ values represent the means ± SE (standard error) of four tests.

## DISCUSSION

The method described here has substantial advantages in manipulation, cost, and yield over several other published methods. Almost all other methods of DNA recovery need to first run electrophoresis to separate target DNA fragments, and then cut out the gel pieces containing DNA of interest for recovery. But, in the present method, the two steps, separation by electrophoresis and recovery, are combined together and accomplished in one step. These advantages result in a marked reduction of time. A recovery can be finished within 30 min, which include the time of electrophoresis [Fig. 3(B)]. This may be the shortest time according to our knowledge. This method needs no special apparatus or reagents,[2] so it is less expensive (about $0.05 per sample, the cheapest method reported cost $0.12 per sample.[3]). Since no phenol, chloroform, or other toxic materials[6] are necessary, it is friendly both to operators and the environment.

To check the migration of DNA of interest periodically by ultraviolet lamp[7] is relatively tedious and time-consuming, and DNA may be damaged as well by repeated exposure to ultraviolet light. We describe an easy method to predict the time of target DNA fragments migrating into the Recovery well, and our results showed the prediction worked well.

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