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Recovery of DNA from Agarose Gel with Home-made Silica Milk

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Abstract: **An usefulness of silica milk made with waste ultraviolet light tube for recovery of DNA fragment from agarose gel was represented. The glass milk is a water suspension of 50% fine silica powder prepared by grinding the crushed waste ultraviolet light tube with a porcelain mortar. It was showed that one microliter of the glass milk could bind more than 1 μg of DNA fragment, and DNA fragment in length from 125 bp to 23 kb could be efficiently recovered from agarose gel. The bound DNA could be eluted from the particle of SiO_2 in the glass milk with a yield of about 60%-80%. The eluted DNA could be used in all manipulations in molecular cloning.**

Key words: **silica milk; DNA recovery; usage of waste UV tube**

CLC number: **Q 781**

Recovering DNA fragment from agarose gel was one of the most useful manipulations in molecular cloning. At present, none of the commonly used methods can satisfactorily eliminate the problems of incomplete separation of DNA from agarose, low yield, high cost, and inconvenience, etc. Vogelstein and Gillespine (1979) devised a glass-binding method to recover the DNA fragment[1]. The method is rapid and convenient, and DNA of wide molecular weight ranges could be isolated in high yield and without degradation. Since then, it had become the most popular method for purifying DNA fragments from agarose gel[2]. But the glass powder they used was flint glass prepared from ground scintillation vials and it was quite expensive. As an alternative, many home-made versions of binding resin had been made by using crushed flint glass, diatomaceous earth, pumice or silica particles from other sources[2-6]. But they cost still highly[3].

We reported here a cheap method for preparing glass powder from waste ultraviolet light tube. The binding capacity of this kind silica particles, the size of recovered DNA, and the yield of recovered DNA were measured. The quality of isolated DNA was proved to be satisfactory for manipalations in molecular cloning.

1 Materials and Methods

1.1 Materials

1.1.1 A waste ultraviolet light tube got from our lab.

1.1.2 Restriction endonucleases and reagents

Restriction endonucleases were purchased from Sino-American Biotechnology Company and Promega Biotech. All other reagents were purchased from market. A saturated solution of NaI/Na_2SO_3 and Neet wash which contains 100 mmol/L NaCl, 1 mmol/L EDTA, 50%EtOH, 10 mmol/L Tris-HCl(pH7.5) were made according to the previous method[3].

1.1.3 Strain and plasmids

Escherisia coli DH5α [supE44ΔlacU169 (φ80lacZΔM15) hsdR17recA1endA1gyrA96thi-1relA1] and plasmid pThiohisE2 which contained the cDNA of HGV-E2 fragment were preserved by our lab. The plasmid pBluescriptII-sk$^+$ was purchased from Stratagene Biotech Company.

E. coli DH5α was maintained on LB plate and

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DH5α harboring the plasmids were maintained on LB plate containing ampicillin 100 mg · L^{-1}.

1.2 Methods

1.2.1 Preparation of home-made silica milk

The preparation of the home-made silica glass milk is described as followings: the crushed pieces of waste ultraviolet light tube were put in a porcelain mortar and ground into fine powder; 10 g of the silica powder was combined with 100 mL ddH_2O in a 250 mL beaker and stirred for 60 min on rotary shaker at 20 r/min; The suspension was allowed to set for 90 min to allow larger particles to sediment; The supernatant was collected and centrifuged for 10 min at 9 000 r/min; The supernatant was discarded and the pellet was resuspended in 50 mL ddH_2O; Concentrated HNO_3 was added to a final concentration of 50% and the suspension was heated close to boiling in a fume hood. The acidified suspension was allowed to cool and centrifuged as above; The pellet was washed with ddH_2O for 4-6 times untill the pH returns to neutral; Then the silica powder was dried at 50℃ and resuspend in ddH_2O to form a 50% silica slurry and store at −20℃. The silica glass milk should be thoroughly mixed till to homogenous before using.

1.2.2 Seperation of DNA fragment

Agarose gel electrophoresis was run in 1 × TAE to seperate the DNA fragments in the sample. The gel was stained with 0.5 mg/L ethidium bromide as described in the standard method[7].

1.2.3 Recovering DNA from agarose gel

Gel slice containing DNA fragment cut from a preparative gels were suspended in 2-3 mL of saturated NaI per gram of agarose gel slice. Incubate at 37-50℃, and mixed frequently until agarose gel was completely dissolved. Add 1 μL of the glass milk per μg of DNA. Incubate in room temperature, mix the suspension occasionally. Spin 10 s at the top speed in a microfuge, remove and discard the supernatant. Wash the glass pellet twice with NaI solution of at least 10 volume of the silica pellet. Spin and wash the pellet 2-3 times with Neet wash of same volume as mentioned above. Dry the pellet well until it did not smell of ethanol. Resuspend pellet in 10 μL of ddH_2O or TE buffer and elute the DNA fragments bound on the silica particles at 50℃ for 10 min. Spin 1 min at the top speed in a high speed microfuge and pool the supernatant containing the eluted DNA.

1.2.4 Preparing competent cells of *E. coli*, transformation and isolation of the plasmid DNA from the transformants

All the manipulations were performed as previously described by Sambrook. *et al*[8].

1.2.5 DNA quantity assay

The content of DNA recovered from agarose gel slice was determined by comparing the brightness of the DNA fragment band with that of λ-phage DNA Hind Ⅲ digests after running an agrose gel eletrophoresis and staining the gel with ethidium bromide.

2 Results

2.1 The Home-Made Silica Glass Milk Could be Used for Efficiently Recovering All Size of DNA Fragments from Agarose Gel Slice

To determine the DNA size binding capability of the home-made silica glass milk, λ-phage DNA Hind Ⅲ digests (0.5 g/L, 2 μL) were separated with a preparative agarose gel. The DNA fragments of λ-phage recovered from agarose gel slices were electrophoresed again. The results were shown in Fig. 1 (lane 1-3). It was found that all DNA fragments in the digests could be recovered from agarose gel slice.

In order to calculate recovering yield, 1 μL of plasmid pThiohisE2 solution was subjected to agarose gel electrophoresis and recovered with the glass milk mentioned above. The amount of the recovered DNA was determined by agarose gel electrophorisis and comparing with a 2 μL of original DNA solution and λ-DNA Hind Ⅲ digests according to the brightness of DNA bands. The result was shown in Fig. 1. It was estimated that 85 ng DNA (lane 4) could be recovered from the agarose gel slice containing 115 ng DNA (lane 5). The recovery yield was 73.9%.

2.2 Binding Capacity of the Silica Glass Milk is 1.06 μg Per Microlitre of the Glass Milk

A serial agarose gel slices containing 5 μg of pThiohisE2 DNA were processed according to the protocol mentioned above. An diagram of agarose electrophoresis of the DNA which was recovered with different volume of the silica glass milk was

represented in Fig. 1(lane 6,7). It was estimated that 1 μL of the silica glass milk could recover 1.06 μg of DNA from agarose gel slice.

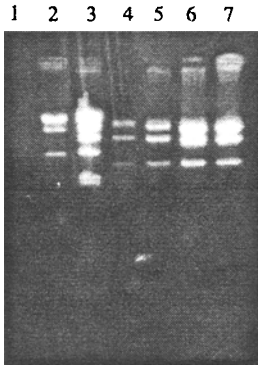

Fig. 1 Size and yield of the DNA fragments recovered from agrose gel slice with the silica glass milk and DNA the binding capacity of silica powder

1:λ-DNA Hind Ⅲ digests recovered(564 bp);2:λ-DNA Hind Ⅲ digests recovered(23.1,9.4,4.4 kb);3: λ-DNA/Hind Ⅲ marker (0.5 g/L,2 μL);4:1 μL of pThiohisE2 DNA recovered(about 85 ng);5:2 μL of pThiohisE2 DNA(about 230 ng);6: DNA of pThiohisE2 recovered with 2 μL of the glass milk; 7:DNA of pThiohisE2 recovered with 1 μL of the glass milk.

2.3 The Recovered DNA Could be Used for all Manipulation in Molecular Cloning

In order to test the quality of the recovered DNA, phagemid pBluescriptII-sk$^+$ DNA was digested with restriction enzyme BamH I into linear moleculars and the lineared DNA was recovered from agarose gel after running an agarose gel electorophoresis. Then lineared DNA was ligated with T4 phage DNA ligase. Competent cells of E. coli DH5α was transformed with the ligated pBluescriptII-sk$^+$ DNA . The number of the transformants was almost equal to that of unprocessed pBluescriptII-sk$^+$ as shown in Table 1. The result suggested that the eluted DNA could be used for all the manipulations in molecular cloning.

Table 1 Transformation efficiency of recircularized linear phagemid pBluescript Ⅱ -SK$^+$DNA

Linear phagemid DNA	Number of the transformunt per μg of the recircularized DNA
recovered from agarose gel with the glass milk	1.39×10^7
pBluescript Ⅱ -SK$^+$EcoR I digests	1.41×10^7

Competent cells of DH5αwere transformed with 10 ng of recircularized linear pBluescript Ⅱ -SK$^+$ DNA obtained either directly from the phagemid-EcoR I digests or from the recovered linear phagemid DNA with the glass milk. The colonies grown on the transformation plates were counted and the transformation efficiency was calculated after incubating the plates for overnight.

3 Discussion

Vogelstein, *et al.* used glass powder prepared from ground scintillation vials to effectively recover DNA from agarose gel[1]. From then on, binding the DNA to silica particles had been a very popular method for purifying DNA from agarose gel[2-6]. We noticed that ultraviolet light tube was made of pure silica. So we had done a serial of experiments to verify that this kind of silica powder could be suitable for efficiently recovering DNA from agarose gel. Moreover, this kind of silica glass milk was prepared from the waste and could greatly lower the cost.

In the procedure of preparing the silica glass milk, it was unavoidably contaminated with Hg/Hg^+ and Fe/Fe^{2+}, which might be deteriorative to further manipulations in molecular cloning. So HNO_3 was used for washing them away.

The procedure mentioned for recovering DNA from agarose gel with silica glass milk was normally used only for the gels run in TAE or Tris-phosphate buffer, however it could also be used for the gel run in TBE buffer. But the borate gel is not easy to be dissolved in the NaI solution. There were two methods to be used for overcoming the problems with TBE gels, 1) add 100 mmol/L Na_3PO_4(pH6.0) into the NaI solution; 2) dissolve 0.1 g of gel slice in 1 mL of NaI solution containing 20% of sorbitol and incubate at room temperature for 20 minutes.

For efficiently recovering DNA from agarose gel, it was important to pay attention to the followings:1) Agarose gel slice should be completely dissolved in the NaI solution; 2) The DNA-silica glass particles mixture should be washed with at least 10 vol. of NaI(or Neet wash)in order to remove residual agarose(or NaI); 3) The washed DNA-silica glass particles should be suspended in about 10 vol. of ddH_2O and incubated at 50℃ for 10 min so that the DNA could be eluted completely. And in order to avoid shearing the high molecular weight DNA, the procedure should be done

gently.

While Vogelstein bound DNA to glass at 25℃, some other authors incubated the mixture on ice[3]. Our result indicated that the incubation temperature was not a critical factor.

By using the home-made silica glass milk, DNA fragment up to 23 kb in length could be recovered from agarose gel. However, the yield of DNA recovery sometimes dropped dramatically when the size of DNA fragment was larger than 6 kb. The major reason might be that one linear DNA fragment might bound to more than one glass particle, which caused DNA to be physically sheared in the procedure. On the other hand, DNA fragments under 100 bp in length could be also recovered with high yield. This technique was suitable for recovering DNA fragments and the recovered DNA was suitable for all the manipulations in molecular cloning. Moreover, DNA binding capacity of the home-made silica glass milk is comparable with that of commercially available one (eg: Geneclean Ⅱ Bio 101, La Jolla, CA, USA). It is about 1.06 μg of DNA that 1 μL of the home-made silica milk can bind. Thus, binding DNA to silica glass milk prepared from UV light tube is an alternatively means of recovering DNA from agarose gel.

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