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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