Chapter 5

DNA Purification from Multiple Sources in Plant Research with Homemade Silica Resins

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Abstract

DNA purification is a routine procedure in most plant laboratories. Although different kits are available in the market allowing convenient DNA purification, the cumulative cost of purchasing multiple kits for a laboratory can be staggering. Here, we describe a protocol using homemade silicon dioxide matrix for DNA purification from *Escherichia coli* and *Agrobacterium tumefaciens* cells, PCR and restriction digestion mixtures, agarose gel slices and plant tissues. Compared with the commercial kits, this protocol enables easy DNA purification from diverse sources with comparable yield and purity at negligible expenses.

Key words: DNA purification, Silicon dioxide, *Escherichia coli*, *Agrobacterium tumefaciens*, PCR product, Agarose gel, Plant tissue

1. Introduction

Research in plant molecular biology involves DNA purification on a daily basis. In an attempt to develop a versatile and affordable method that could replace expensive commercial kits in diverse-purpose DNA purification procedures, we explored the silica-based technique which takes advantage of the ability of DNA to bind to silica particles in the presence of chaotropic salt (1). In particular, the cheap chemical compound silicon dioxide was used as the DNA binding matrix, and sodium iodide was added to the cell lysate or DNA-containing solution as the chaotropic salt to facilitate DNA binding to the silica matrix. Nonspecifically bound impurities were eliminated by subsequent washing steps and high-quality DNA was readily eluted from the silica particles with as little as 5 µl water. DNA prepared by this protocol could be directly applied to PCR, restriction digestion, DNA sequencing analysis, or transient expression assays through biolistic bombardment or

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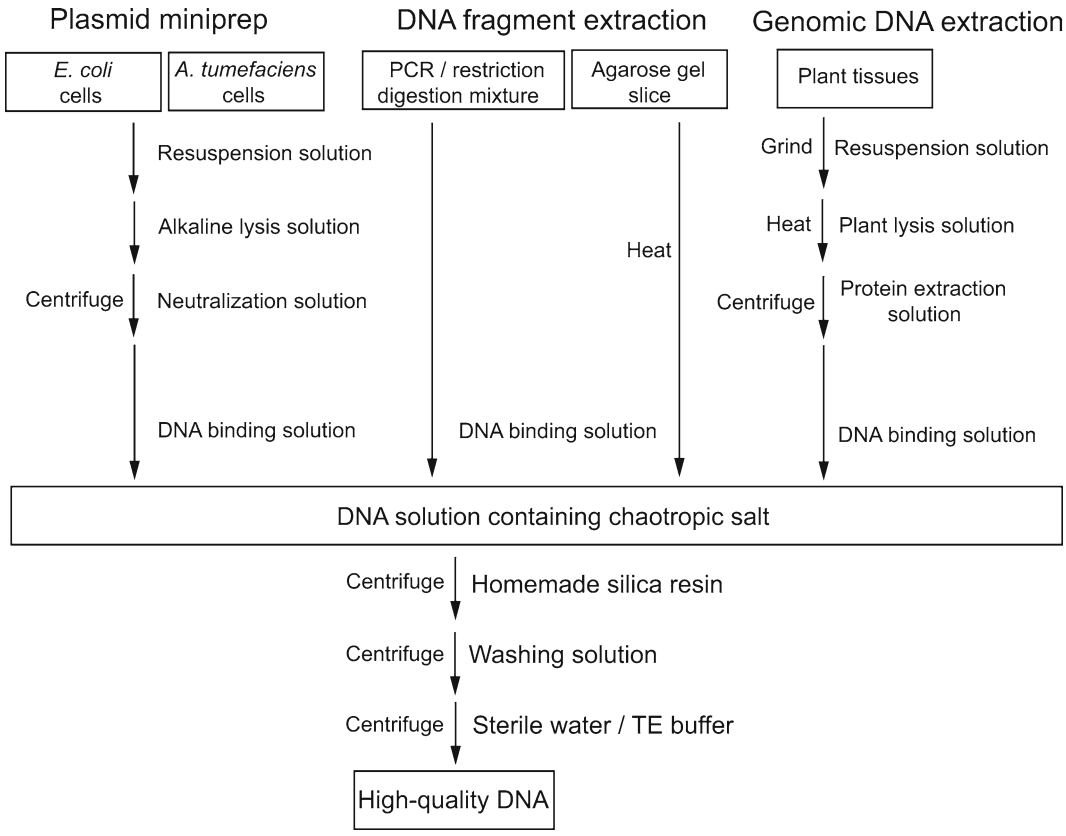

Fig. 1. Flow diagram of the silica protocol for multiple purpose DNA purification in plant research. All procedures are performed at room temperature unless otherwise indicated. The centrifuge step means a 10-s centrifugation at 16,000 × *g*. The heating treatment is conducted at 70°C.

protoplast transfection (2). We have extensively simplified and streamlined this protocol to optimize its time, labor, and cost efficiency for multiple purpose DNA purification (Fig. 1).

2. Materials

All reagents used in this protocol were analytical grade. All solutions were prepared with ultrapure water prepared by purifying deionized water to obtain a sensitivity of 18 MΩ cm at 25°C, and were stored at room temperature unless otherwise stated.

2.1. Bacterial Strain and Growth Medium

1. *Escherichia coli* strain TOP10 or MC1061 and *Agrobacterium tumefaciens* strain GV3101 were used.
2. LB liquid medium: 10 g/l tryptone, 5 g/l yeast extract, and 10 g/l NaCl.

2.2. Plant Tissue

Seven-day-old *Arabidopsis thaliana* (Col-0), *Nicotiana benthamiana* seedlings, and ten-day-old *Zea mays* leaves were used.

2.3. Shared Solutions for Diverse DNA Purification

1. Silica particles: Weigh a 50-ml Falcon tube. Mix 5 g silicon dioxide (Sigma, S5631) with 50 ml sterile water in the Falcon tube and settle the tube upright overnight (see Note 1). Remove the upper fraction containing fine silica particles and resuspend the pellet in 50 ml sterile water. Resettle the tube upright overnight. After discarding the supernatant, weigh the Falcon tube again and calculate the net weight of the remaining silicon dioxide. Resuspend the silica pellet in sterile water to make a final concentration of approximately 100 mg/ml. One milligram of silicon dioxide is able to bind 3–4.5-μg DNA (3). The slurry can be stored at room temperature and be stable for over 12 months.
2. DNA binding solution: 6 M NaI (Sigma, 217638-500G). Filter the solution to remove impurities and store in a dark container at 4°C (see Note 2).
3. Washing solution: 50% (v/v) ethanol, 10 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA.
4. TE buffer: 10 mM Tris–HCl, pH 8.0, 1 mM EDTA.

2.4. Bacterial Lysis Solutions

1. Resuspension solution: 50 mM Tris–HCl, pH 7.5, 10 mM EDTA, 100 μg/ml RNase A. Store at 4°C.
2. Alkaline lysis solution: 0.2 M NaOH, 1% SDS.
3. Neutralization solution: 1.32 M KOAc. Use acetic acid to adjust the pH value to 4.8.

2.5. Plant DNA Extraction Solutions

1. Plant lysis solution: 10% SDS.
2. Protein extraction solution: Phenol:chloroform:isoamyl alcohol (25:24:1, v/v). Cover the solution with a water phase containing β-mercaptoethanol to prevent the oxidation of phenol. Store at 4°C.

3. Methods

All the procedures were carried out at room temperature unless otherwise stated.

3.1. Plasmid Purification from E. coli or A. tumefaciens by Silica Matrix (see Note 3)

1. Grow 2 ml *E. coli* and *A. tumefaciens* cells at 37°C and 28°C, respectively, until the OD600 of the culture reaches 2.0. Collect the cells in a 2-ml round-bottom microfuge tube by centrifugation at 16,000 × *g* for 30 s.

2. Resuspend the cell pellet in 100 µl resuspension solution by brief vortex.
3. Add 100 µl alkaline lysis solution and invert the microfuge tube for five times (see Note 4).
4. Add 100 µl neutralization solution and invert the tube for five times.
5. Centrifuge the tube at 16,000×*g* for 5 min. In the meantime, prepare fresh 1.5-ml microfuge tube and add 500 µl DNA binding solution to each tube.
6. After centrifugation, transfer the supernatant to the prepared 1.5 ml microfuge tube and invert the tube for five times.
7. Add 20 µl silica particles to the tube, mix well, and sit the tube on the rack for 2 min (see Note 5).
8. Pellet the silica particles by a 10-s centrifugation. Pour off the supernatant and gently tap the inverted tube against a pile of Kimwipes to drain the liquid.
9. Wash the silica particles with 500 µl washing solution by vigorous vortex (see Note 6).
10. Repeat steps 8 and 9 (see Note 6).
11. Pellet the silica matrix by a 10-s centrifugation and remove the supernatant by a pipette.
12. Centrifuge for another 10 s and carefully pipette off the residual liquid (see Note 7).
13. Add 40 µl sterile water to resuspend the pellet by brief vortex and place the tube at 70°C for 2 min.
14. Centrifuge the tube at 16,000×*g* for 2 min and transfer 37 µl supernatant containing the eluted plasmid DNA to a fresh tube (see Notes 8 and 9).

3.2. DNA Purification from Solution or Agarose Gel Slice

1. Add 150 µl DNA binding solution to up to 50 µl PCR or restriction digestion mixture in a 1.5-ml microfuge tube and invert the tube for five times. For gel purification, add 300 µl DNA binding solution per 100 mg gel slice and heat the microfuge tube at 70°C for 3 min to dissolve the gel (see Note 10).
2. Add 10 µl silica matrix, mix well, and incubate for 2 min.
3. Pellet the matrix by a 10-s centrifugation and remove the supernatant by a pipette.
4. Wash the matrix in 500 µl washing solution by vigorous vortex.
5. Repeat steps 3 and 4.

6. Pellet the matrix by a 10-s centrifugation and discard the supernatant as much as possible.
7. Centrifuge for another 10 s and pipette off the trace amount of liquid (see Note 7).
8. Resuspend the matrix in 5–30 μl sterile water and place the tube at 70°C for 2 min.
9. Centrifuge the tube at 16,000 × *g* for 2 min and transfer the DNA eluate to a fresh tube (see Note 11).

3.3. Genomic DNA Purification from Plant Tissue

1. Place approximately 10 mg of plant material in a 1.5-ml microfuge tube.
2. Add 200 μl resuspension solution and grind the tissue with a Micro-Grinder homogenizer (Research Products International Corporation) (see Note 12).
3. Add 30 μl plant lysis solution to the homogenate and invert the tube for five times.
4. Place the tube at 70°C for 10 min.
5. Add 250 μl protein extraction solution and vortex the mixture vigorously for 30 s.
6. Centrifuge the tube at 16,000 × *g* for 5 min at 4°C and transfer the upper phase to a fresh tube (see Note 13).
7. Mix with 500 μl DNA binding solution by inverting the tube for five times.
8. Add 20 μl silica matrix and sit the tube for 2 min (see Note 5).
9. Pellet the matrix by a 10-s centrifugation and remove the supernatant by a pipette.
10. Wash the matrix in 1 ml washing solution by vigorous vortex (see Note 6).
11. Repeat steps 9 and 10 (see Note 6).
12. Pellet the matrix by a 10-s centrifugation and pipette off the supernatant.
13. Centrifuge for another 10 s and remove the residual liquid by a pipette.
14. Add 40 μl sterile water to resuspend the pellet and heat the tube at 70°C for 2 min.
15. Centrifuge at 16,000 × *g* for 2 min and transfer 37 μl supernatant containing the eluted genomic DNA to a fresh tube (see Note 8).

4. Notes

1. Instead of settling the tube upright overnight, the larger size silica particles can be pelleted by centrifugation at low speed [e.g., 5 min centrifugation at 400×*g* using a CL2 centrifuge (Thermo Scientific)].
2. It is normal that the DNA binding solution will slowly turn pale yellow upon storage due to oxidation. The solution is usable within 3 months under the indicated storage conditions.
3. This miniprep protocol can also be followed to purify larger amount of DNA (i.e., midiprep or maxiprep) from the samples by scaling up the input of all reagents accordingly.
4. In case of plasmid purification from *A. tumefaciens*, after adding the alkaline lysis solution, let the microfuge tube sit on the rack at room temperature for 5 min to allow a better lysis of *A. tumefaciens* cells.
5. It is normal to see the precipitation of silica particles during incubation.
6. Generally, 10–15 s vortex at the maximal speed of the vortexer is sufficient. During the vortex, the silica particles may still keep in small patches of matrix and it is unnecessary to completely break the silica patches.
7. A 30-s incubation of the microfuge tube at 70°C with the lid open can guarantee a complete elimination of ethanol in the tube. The ethanol contamination at this step may affect subsequent DNA manipulation and lead to overflow when loading sample for DNA electrophoresis.
8. The remaining 3 μl liquid should be abandoned due to a slight contamination by disturbed silica particles. The pelleted silica particles can diffuse into the supernatant after sitting the tube for over 1 min. When large amount of minipreps (e.g., ten samples) are performed at the same time, collect 4–6 DNA eluate at a time and have another 1 min centrifugation before the second round of eluate collection. In practice, the slight silica contamination appears not to affect subsequent DNA analysis such as DNA sequencing. For protection against DNase digestion or pH fluctuations, TE buffer should be used to elute DNA.
9. DNA binding to the silicon dioxide matrix could be efficiently eluted with as little as 5 μl water/TE buffer after heated at 70°C, thus allowing the miniprep DNA to be harvested in sufficiently high concentrations which are necessary for efficient protoplast transfection (4).

10. It is recommended to tap the microfuge tube frequently to facilitate the gel dissolving.
11. The last 2-μl eluate may be abandoned due to a slight contamination by disturbed silica particles.
12. The amount of RNase A included in the resuspension solution is sufficient to remove RNA contamination from DNA prepared from bacterial cells. However, it may not be enough to clean up all the RNA from plant cells. In this case, more RNase A could be added to the resuspension solution for plant DNA purification.
13. When transfer the upper phase, do not touch the middle layer which contains the denatured proteins. It is recommended to sacrifice some upper phase to avoid taking the middle layer accidentally.

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