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Isolation of a low-copy plasmid from agrobacterium using QIAprep® technology

Steffen Weber, Renate Horn, and Wolfgang Friedt

Institut für Pflanzenbau und Pflanzenzüchtung I, Justus-Liebig-Universität, Giessen, Germany

We have developed an optimized protocol for fast and easy, small-scale purification of low-copy plasmids from agrobacterium. The protocol is suitable for use with the QIAprep® 8 Miniprep Kit and the QIAprep Spin Miniprep Kit, and provides reproducible yields of high-quality plasmid DNA.

Agrobacterium tumefaciens is a Gram-negative bacterium with the capability of transforming eukaryotic cells, and is frequently used as a tool for molecular biologists to transfer cloned DNA into plant cells (1). We have determined the effect of varying the volumes of overnight culture, cell lysis, and DNA elution buffer on the yield and concentration of the single-copy, 14.5-kb, binary plasmid, p35S GUS INT (2), from agrobacterium strain GV2260. Here we describe an optimized protocol for fast and easy, small-scale purification of low-copy plasmids from agrobacterium using the QIAprep 8 Miniprep Kit. This protocol is also suitable for use with the QIAprep Spin Miniprep Kit.

Materials and methods

Grow agrobacteria containing the vector (p35S GUS INT [2]) on YEB plates* with 100 mg/liter rifampicin, 50 mg/liter kanamycin, and 2 ml/liter 1 M $MgSO_4$ for 2 days at 28°C. Inoculate a single colony into 10 ml liquid YEB medium* containing 100 mg/liter rifampicin, 50 mg/liter kanamycin, and 2 ml/liter 1 M $MgSO_4$ (3), and grow the culture overnight at 28°C with 200 rpm shaking to an OD_{600} value of 1.2–1.5. Harvest the cells from a 10-ml aliquot by centrifugation for 15 min at 3500 rpm or 1500 x *g*, and resuspend in 250 µl resuspension buffer P1† containing 0.1 mg/ml RNase A. Add 250 µl lysis buffer P2 to the tube and invert gently 4–6 times to mix. Add 500 µl neutralization buffer N3 to the tube and invert immediately but gently 4–6 times. Centrifuge the lysate for 10 min at maximum speed in a tabletop microcentrifuge (13,000 rpm or ≥10,000 x *g*). Meanwhile, prepare the QIAvac 6S as described in the protocol (*QIAprep Miniprep Handbook*, April 1998, page 23). Transfer the cleared lysates to the wells of the QIAprep 8 strips and apply the vacuum. Wash the membrane once with 1 ml Buffer PB, twice with 1 ml Buffer PE, and dry by applying full vacuum for 5 min. Remove any remaining Buffer PE by vigorously tapping the top plate with the QIAprep strips on a stack of adsorbent paper. To elute the DNA, add 100 µl elution buffer EB prewarmed to 70°C‡ (10 mM Tris·Cl, pH 8.5) directly to the center of the membrane (for QIAprep spin columns elute using 50 µl). Let stand for 1 min, and apply the appropriate vacuum (150–200 mbar).

Results and discussion

We compared the plasmid yields from 5–20 ml overnight cultures and found that 10 ml culture gave the optimum yield and concentration of DNA (Table 1 and Figure 1). Plasmid minipreps from 10 ml overnight culture were also efficiently restriction digested using *Eco*RI and *Hin*dIII (data not shown). In contrast, the 15- and 20-ml overnight-culture volumes did not give significantly higher DNA yield or concentration. This is due to a higher load of cell components in the cleared lysate which can interfere with binding of plasmid DNA to the silica-gel membrane. Furthermore, lysates from 20-ml overnight ▶

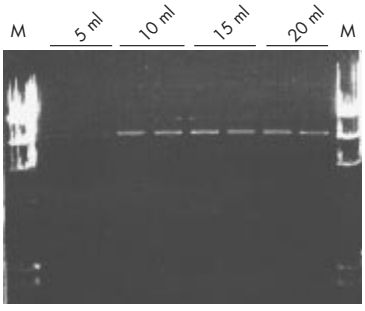

Figure 1 *Agarose gel analysis of duplicate p35S GUS INT minipreps from the indicated overnight-culture volumes of agrobacterium using the QIAprep 8 Miniprep Kit. 6 µl undigested p35S GUS INT was loaded onto a non-denaturing 0.8% agarose gel.* ***M****: lambda–HindIII markers.*

** For 1 liter YEB medium/YEB bactoagar medium: 5 g beef extract, 1 g yeast extract, 5 g peptone, 5 g sucrose, pH to 7.2, and add 18 g bactoagar (optional). Make to 1 liter with H_2O, and autoclave.*

† Depending on the host strain, doubling the volumes of Buffers P1, P2, and P3, or increasing the culture volume to 15 ml, may sometimes enhance plasmid yield.

‡ For plasmids ≤10 kb use room-temperature elution buffer; for plasmids >10 kb use elution buffer at 70°C.

References

1. *Deblaere, R., Bytebier, B., de Greve, H., Deboeck, F., Schell, J., van Montagu, M., and Leemans, J. (1985). Efficient octopine Ti plasmid-derived vectors for agrobacterium-mediated gene transfer. Nuc. Acids Res.* ***13****, 4777.*
2. *Vancanneyt, G., Schmidt, R., O'Connor-Sanchez, A., Willmitzer L., and Rocha-Sosa, M. (1989) Construction of an intron-containing marker gene: Splicing of the intron in transgenic plants and its use in monitoring early events in agrobacterium-mediated plant transformation. Mol. Gen. Genet.* ***220****, 245.*
3. *Sambrook, J. et al., eds. (1989) Molecular cloning: a laboratory manual. 2nd ed., Cold Spring Harbor Laboratory Press.*

Table 1. Effect of overnight-culture volume and elution volume on the yield and concentration of a low-copy plasmid purified from agrobacterium*

Culture volume (ml)	Elution buffer EB volume (µl)	DNA yield (ng)	DNA concentration (ng/µl)
5	75	630	14
	100	780	13
10	75	1035	23
	100	1500	25
15	75	1260	28
	100	1380	23
20	75	1035	23
	100	1440	24

Table 2. Effect of doubling resuspension buffer P1 volume on the yield and concentration of a low-copy plasmid purified from agrobacterium*†

Culture volume (ml)	Resuspension buffer P1 volume† (µl)	DNA yield (ng)	DNA concentration (ng/µl)
5	250	600	10
	500	540	9
10	250	900	15
	500	1380	23
15	250	1440	24
	500	900	15
20	250	1140	19
	500	1200	20

** DNA yield and concentration values were determined using a DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotec). Values represent the mean of three fluorometric measurements from minipreps prepared from two different overnight cultures.*

† The volumes of lysis buffer P2 and neutralization buffer N3 were also doubled such that the final lysis volume was doubled.

‡ See footnote (†) on previous page.

cultures could not be cleared completely, not even with two consecutive centrifugation steps. Since doubling the lysis volume did not lead to higher yields of plasmid DNA (Table 2), the inefficient lysate clearing with 20-ml cultures was probably due to the presence of a higher concentration of cell components such as genomic DNA rather than incomplete lysis. For QIAprep 8 strips, elution with 100 µl elution buffer EB gave an approximately 30% higher DNA yield than elution with 75 µl Buffer EB (Table 1). In contrast, the DNA concentrations were almost identical for both elution buffer EB volumes.

Recommendations for purification of low-copy plasmids and cosmids‡

- Use 10 ml overnight culture
- Resuspend cells in 250 µl Buffer P1 containing 0.1 mg/ml RNase A, and follow the protocols in the *QIAprep Miniprep Handbook* (April 1998) for either the QIAprep 8 Miniprep Plasmid Kit or the QIAprep Spin Miniprep Kit.
- Make sure to include the optional Buffer PB wash step.
- Elute the DNA with elution buffer EB prewarmed to 70°C. For QIAprep spin columns use a 50-µl elution volume, and for QIAprep 8 strips use a 100-µl elution volume.

For a detailed protocol or further information please contact your local QIAGEN Technical Service Department or distributor. ■

Ordering Information

Product	Contents	Cat. No.
QIAprep Spin Miniprep Kit (50)	For 50 high-purity plasmid minipreps: 50 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2-ml)	27104
QIAprep Spin Miniprep Kit (250)	For 250 high-purity plasmid minipreps: 250 QIAprep Spin Columns, Reagents, Buffers, Collection Tubes (2-ml)	27106
QIAprep 8 Miniprep Kit (10)*	For 10 x 8 high-purity plasmid minipreps: 10 QIAprep 8 Strips, Reagents, Buffers, Collection Microtubes (1.2-ml), Caps	27142
QIAprep 8 Miniprep Kit (50)*	For 50 x 8 high-purity plasmid minipreps: 50 QIAprep 8 Strips, Reagents, Buffers, Collection Microtubes (1.2-ml), Caps	27144

** Requires use of QIAvac 6S.*