User-Developed Protocol:

Isolation of plasmid DNA from *Agrobacterium* using the QIAprep® Spin Miniprep Kit; spin procedure

This procedure has been adapted by customers from the QIAprep® Spin Miniprep Kit Protocol and is for use with the QIAprep Spin Miniprep Kit. It has not been thoroughly tested and optimized by QIAGEN. The procedure has been used successfully for isolation of a single-copy, 14.5 kb, binary plasmid, p35S GUS INT, from *Agrobacterium tumefaciens* strain GV2260 (1). Please be sure to read the QIAprep Miniprep Handbook and the detailed QIAprep Spin Miniprep Kit Protocol carefully before beginning the procedure.

Procedure

1. Grow *agrobacteria* containing the vector on YEB plates for 2 days at 28°C. 
   See last page for medium composition.

2. Inoculate a single colony into 10 ml liquid YEB medium and grow the culture overnight at 28°C with 200 rpm shaking to an $A_{600}$ value of 1.2–1.5.

3. 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. Depending on the host strain, doubling the volumes of Buffers P1, P2, and N3, or increasing the culture volume to 15 ml, may sometimes enhance plasmid yield.

4. Add 250 µl lysis buffer P2 to the tube and invert gently 4–6 times to mix.

5. Add 350 µl neutralization buffer N3 to the tube and invert immediately but gently 4–6 times.

6. Centrifuge the lysate for 10 min at maximum speed in a tabletop microcentrifuge (13,000 rpm or ≥10,000 x $g$).

7. Meanwhile, place a QIAprep Spin Column in a 2 ml collection tube.

8. Transfer the cleared lysates from step 6 to the QIAprep Spin Column by decanting or pipetting.

9. Centrifuge 30–60 s (13,000 rpm or ≥10,000 x $g$). Discard flow-through.

10. Wash the QIAprep Spin Column by adding 0.5 ml of Buffer PB and centrifuging 30–60 s (13,000 rpm or ≥10,000 x $g$). Discard flow-through.

11. Wash the QIAprep Spin Column by adding 0.75 ml of Buffer PE and centrifuging 30–60 s (13,000 rpm or ≥10,000 x $g$).
12. Discard flow-through and centrifuge for an additional 1 min to remove residual wash buffer (13,000 rpm or ≥10,000 x g).

IMPORTANT: Residual wash buffer will not be completely removed unless the flow-through is discarded before this additional centrifugation. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

13. Place the QIAprep Spin Column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl of Buffer EB (10 mM Tris-Cl, pH 8.5) or water to the center of each QIAprep Spin Column, let stand for 1 min, and centrifuge for 1 min.

Medium composition YEB medium (1 liter): To prepare 1 liter YEB medium: In 600 ml water, dissolve 5 g beef extract, 1 g yeast extract, 5 g peptone, 5 g sucrose, and pH to 7.2. For YEB plates, add 18 g bactoagar. Bring volume up to 1 liter with water and autoclave. Add sterile solutions of kanamycin, rifampicin, and MgSO₄ to final concentrations of 100 mg/liter, 50 mg/liter, and 2 mM, respectively (2).

References