User-Developed Protocol:

Isolation of plasmid DNA from *Agrobacterium* using the QIAprep® Spin Miniprep Kit; vacuum 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 this 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, prepare the vacuum manifold as described in the *QIAprep Miniprep Handbook*.**
8. **Apply the supernatant from step 6 to the QIAprep Spin Column by decanting or pipetting.**
9. **Switch on vacuum source to draw the solution through the QIAprep Spin Column, and then switch off vacuum source.**
10. **Wash the QIAprep Spin Column by adding 0.5 ml Buffer PB. Switch on vacuum source. After the solution has moved through the column, switch off vacuum.**
11. **Wash the QIAprep Spin Column by adding 0.75 ml Buffer PE. Switch on vacuum source to draw the wash solution through the column, and then switch off vacuum source.**

12. **Transfer the QIAprep Spin Columns to a microcentrifuge tube. Centrifuge for 1 min.**

    IMPORTANT: This extra spin is necessary to remove residual Buffer PE. Residual ethanol from Buffer PE may inhibit subsequent enzymatic reactions.

13. **Place the QIAprep Spin Column in a clean 1.5 ml microcentifuge 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 $MgSO_4$ to final concentrations of 100 mg/liter , 50 mg/liter , and 2 mM, respectively (2).

References

1. *Weber, S., Horn, R., and Friedt W. (1998) Isolation of a low-copy plasmid from agrobacterium using QIAprep technology. QIAGEN News No. 5 1998, 7.*
2. *Sambrook, J., Fritsch, E.F., and Maniatis, T. (1989) Molecular cloning: a laboratory manual. 2nd edition, Cold Spring Harbor, New York,: Cold Spring Harbor Laboratory Press.*