

**User-developed
protocol**

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 $\geq 10,000$ 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 $\geq 10,000$ 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 $\geq 10,000$ 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 $\geq 10,000$ x g).**

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- 12. Discard flow-through and centrifuge for an additional 1 min to remove residual wash buffer (13,000 rpm or $\geq 10,000 \times g$).**

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 μ l 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.*

QIAGEN handbooks can be requested from QIAGEN Technical Service or your local QIAGEN distributor.

Selected handbooks can be downloaded from www.qiagen.com/literature/handbooks/default.asp.

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from www.qiagen.com/ts/msds.asp.

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