4. Ligate the binary vector and CP fragments using T4 DNA ligase and approx 100 ng of the binary vector and a 3–4*M* excess of the CP fragment (in most cases, this will be an approx 20-ng CP fragment; *see* **Note 8**).
5. Transform competent *E. coli* cells with the ligation mixes. Include a transformation control using approx 20 ng of uncut binary vector. Plate the cells onto LB agar containing the appropriate antibiotic (for pROKII, 50 μg/mL kanamycin; *see* **Note 9**).
6. Confirm the presence of the insert by restriction enzyme digestion and agarose gel electrophoresis of small-scale plasmid DNA preparations (*see* **Note 10**).

3.2. Introduction of Plasmids into Agrobacterium

3.2.1. Using Electroporation

1. Grow an overnight culture (10 mL) of the *Agrobacterium* strain, shaking at 28°C (*see* **Note 11**).
2. For each DNA construct to be used, pellet the bacteria from 2 × 1.5-mL aliquots of the culture by centrifugation for 1 min in a microcentrifuge.
3. Wash pellets by resuspension in ice-cold 1 m*M* HEPES/KOH, pH 7.0, followed by centrifugation for 30 s at high speed in the cold.
4. Repeat the washing step twice more.
5. Resuspend the pellets in 0.5 mL ice-cold 10% glycerol; centrifuge as before.
6. Resuspend in 20 μL ice-cold 10% glycerol. Combine the contents of the two tubes.
7. Add binary construct DNA (*see* **Note 12**) and leave the tube on ice for 5 min.
8. Transfer the bacterial mix to an ice-cold electroporation cuvet (Bio-Rad, 0.2 cm electrode gap). Pulse using a Bio-Rad gene pulser with pulse controller (*see* **Note 13**).
9. Dilute with 1 mL YEP medium, incubate shaking for 2 h at 28°C, prior to plating serial dilutions on selective LB agar (for pROKII, 50 μg/mL kanamycin). Incubate at 28°C for 36–48 h.

3.2.2. Using Triparental Mating

1. Grow 10-mL cultures of each of the *E. coli* donor (containing the binary vector construct), and helper strains (HB101 containing plasmid pRK2013), and the appropriate *Agrobacterium* strain to exponential phase (*see* **Notes 11** and **14**). Include also the binary vector culture (the minus insert control).
2. Centrifuge the bacteria at 3000*g* for 10 min; resuspend in 10 mL LB broth (*see* **Note 15**).
3. Mix the three cultures in the ratio of 1 vol of each *E. coli* culture to 2 vol of *Agrobacterium* (e.g., 200:200:400 μL, respectively) in a sterile microcentrifuge tube.
4. Pipet 400 μL of the conjugation mix onto a sterile nitrocellulose circle positioned in the center of a Petri dish containing LB agar. Allow the filters to dry for approx 1.5 h in a laminar flow cabinet. Incubate the dishes and filters at 28°C for 24–36 h *(see* **Note 16**).
5. Place filters in 10 mL of sterile distilled water and shake to resuspend the bacterial cells (*see* **Note 17**).