9. As with other plasmids, the antibiotic will depend on the vector used. Where *lacZ* color selection is possible (*see* Chapter 27), include X-gal and IPTG in the medium.
10. In the present example, the enzymes used should be *Bam*HI and *Sac*I. Alternatively, the presence of the insert may be confirmed by PCR amplification using primers able to hybridize to sequences within the CP gene or within the 35S CaMV promoter and the nos terminator. More details of the latter primers are shown in **Subheading 3.3.2.**, and PCR screening is described in Chapters 28, 41, 42, and 48.
11. *A. tumefaciens* may normally be grown in LB agar containing appropriate antibiotics (e.g., rifampicin for strains LBA4404 and pGV3850, which carry the resistance gene on their chromosome). For cells required for electroporation, YEP medium should be used and they should be grown to an absorbance of 0.5–1.0. *Agrobacterium* should be grown between 25 and 30°C; temperatures higher than this may result in loss of the Ti plasmid. A shaking speed similar to that used for *E. coli* (e.g., 200–250 rpm) is acceptable.
12. The DNA concentration in the mix should be approx 1 μg/40 μL cells. It is not necessary to prepare highly purified DNA; clean miniprep DNA prepared by the alkaline lysis method ***(19)*** and resuspended in sterile distilled water is suitable. If excess salt is present in the DNA solution, sparking may occur during the electroporation and this will markedly reduce the transformation efficiency. If such problems are routinely encountered, remove the salt using proprietary clean-up columns or ethanol precipitation.
13. The gene pulser should be set to 25 μF capacitance and 2.5 kV charge, the pulse controller resistance to 200Ω. Successful transformations have also been obtained with 400Ω.
14. Include the antibiotics appropriate to the plasmids/strains used. In this example, use kanamycin (50 μg/mL) for pRK2013 and pROKII, and rifampicin (50 μg/mL) for LBA4404. Cultures in exponential phase will give the greatest number of transconjugant colonies, but stationary phase cultures may be used.
15. Washing the cells removes the antibiotics that might kill the bacteria prior to the conjugation.
16. Pipet the bacteria carefully onto the filters and do not move the plates until the filters have dried. If the suspension runs off, the bacteria can be collected using the disk just before transfer to water. If a laminar flow cabinet is not available, the mixed culture may be centrifuged for 5 min in a microcentrifuge, the supernatant removed, and the pellet placed over the filter or plated directly onto the agar. Control filters, of each culture alone, should also be prepared.
17. It is normally suggested that bacteria be resuspended and diluted in 10 m*M* $MgSO_4$, but sterile distilled water can be used provided that plating is done without undue delay.
18. None of the commonly used *E. coli* strains are resistant to rifampicin; thus, only *Agrobacterium* should grow on these plates. Kanamycin selects for the presence of pROKII sequences in transconjugants; the pRK2O13 plasmid does not repli-