described by Yie et al. ***(14)***. Vector pROKII is based on pBIN19, but contains the CaMV 35S promoter and nos termination sequence. For plasmid transfer to *Agrobacterium*, both the triparental mating procedure (modified from **ref. *15***) and the electroporation procedure (modified from **ref. *5***) will be described, because electroporation equipment may not be available in all laboratories.

2. Materials

1. A DNA sample of a cloned cDNA copy of the virus CP gene (e.g., CMV strain O, clone pUCP).
2. DNA of the *Agrobacterium* binary vector (here pROKII; *see also* **Note 1**).
3. Molecular biology reagents for the purification and cloning of DNA (*see* **Note 2**).
4. The appropriate *Agrobacterium* strain (here LBA4404, but *see* **Note 3**).
5. For triparental mating (**Subheading 3.2.2.**): a helper plasmid (pRK 2013) present in *E. coli* (normally HB101) for mobilization of the binary plasmid from *E. coli* to *Agrobacterium*, and nitrocellulose filter circles (2.5 cm diameter, 45 μ pore size). Neither of these materials are necessary if *Agrobacterium* is to be transformed by electroporation.
5. For electroporation (**Subheading 3.2.1.**): modified YEP broth and agar. Prepare by adding 10 g yeast extract, 10 g peptone, 5 g NaCl, and 5 g sucrose to 950 mL distilled water. Bring to pH 7.5 and make to 1 L. Sterilize by autoclaving.
6. Sterilized LB broth and LB agar (*see* Chapter 27).
7. Appropriate filter-sterilized antibiotic solutions (here kanamycin sulfate), 50 mg/mL in dH_2O, and rifampicin (50 mg/mL in methanol) (*see* **Note 4**).
8. Sterilized glycerol.

3. Methods

3.1. Cloning of DNA Fragments Into Plant Transformation Vectors

Throughout this chapter pROKII will be used as an example of a binary vector and pUCP ***(14)*** will be used to provide the virus CP insert. The CP gene may be obtained by digestion of pUCP with *Bam*HI and *Sac*I and inserted into similarly digested pROKII. A non–insert-containing clone should also be produced to provide control transgenic plant lines.

1. Digest approx 10 μg of the cloned virus DNA with the appropriate restriction enzyme to release the CP gene fragment from the cloning vector. For pUCP, this could be *Bam*HI and *Sac*I (*see* **Note 5**).
2. Separate the CP fragment from the vector by agarose gel electrophoresis. The fragment may be visualized by ethidium bromide staining and UV irradiation and purified from the agarose using your preferred technique (*see* **Note 6**).
3. Digest approx 5 μg of the binary vector with the appropriate restriction enzymes (for pROKII, these are *Bam*HI and *Sac*I) and purify the linearized vector from an agarose gel (*see* **Notes 1** and **7**).