cate in *Agrobacterium*. Plating of the original bacterial cultures will determine that none are able to grow on the plates containing both antibiotics. If required, transconjugants can be confirmed as *Agrobacterium* by their ability to metabolize 3-keto-lactose, as described by Bernaerts and Deley ***(26)***. To do this, bacteria are grown for 2–3 d on lactose yeast-medium agar plates (10 g lactose, 1 g Difco yeast extract and 20 g LabM agar per 1 L) and the plates flooded with Benedicts reagent. (Dissolve 173 g sodium citrate and 100 g anhydrous sodium carbonate in 850 mL water; then add, while stirring, to 17.4 g copper sulfate dissolved in 100 mL water. Make to 1 L with water, it is not necessary to sterilize this reagent.) A yellow color reaction in the medium around the streak confirms the cultures to be *Agrobacterium*. This method is suitable for all Biotype I strains of *Agrobacterium* (this includes all strains commonly used for plant transformation).

19. *Agrobacterium* cultures survive for only a short time (often less than 1 mo) on LB agar plates at 4°C, and therefore the transconjugant should be stored at –70°C. To do this, prepare an overnight culture of the selected colony, as described in **Subheading 3.2.1.** Mix equal volumes of sterilized glycerol and bacteria to give a 50% glycerol solution. Ensure that the contents of the tube are well mixed, then store at –70°C. Using this glycerol concentration, the bacteria may be sampled without any need for thawing, because the culture remains slightly soft. Cultures stored in this way have remained viable for the past 8 yr in our laboratory. Storage at –20°C is suitable for times of less than 1 yr.
20. Many binary vectors are based on low-copy-number replicons, and therefore the host bacteria should always be grown under appropriate antibiotic selection (in this case, kanamycin).
21. For the present example, *Bam*HI plus *Sac*I digestion will release a 657 bp (CP gene) fragment; *Hin*dIII plus *Eco*RI will release an approx 2-kb fragment, including the promoter, CP, and terminator. The DNA obtained from *Agrobacterium* is often less clean than that obtained from *E. coli*, so that two to five times the usual amount of enzyme should be added and digestion increased to 4–16 h (increase the reaction volume, if necessary). For controls, the original binary vector DNA, plus and minus the insert, should also be digested. Calculate the fragment sizes by comparison with mol-wt markers.
22. The CP gene fragment prepared in **Subheading 3.1., step 2** can be used for preparing the probe. Thoroughly check transconjugants prior to plant transformation, because rearrangement and recombination events may take place during the cloning procedure and multimeric copies of the CP fragment may be inserted into the vector.
23. The primers described here are appropriate for the analysis of all constructs that use the CaMV 35S promoter and the nos terminator. They are sited in regions conserved in all CaMV strains (S. Covey, personal communication) and nos regions analyzed to date.
24. Compare the size of the insert with a CP fragment (e.g., that prepared in **Subheading 3.1.**, **step 2**). Alternative primers may be used; commonly, these have homology with the inserted viral gene sequence or T-DNA border sequences. In