manipulation, and therefore an alternative small plasmid (a 10- to 20-kb binary vector) is used that is able to replicate in *Escherichia coli* as well as *Agrobacterium*. Thus, cloning is done in *E. coli* and the final expression cassette in the binary vector is then introduced into *Agrobacterium* containing the *vir* plasmid, using either conjugation (with the help of plasmid pRK2013, which provides the transfer functions for mobilization of plasmids from *E. coli* to *Agrobacterium* ***[4]***) or transformation procedures. In the latter case, electroporation of transformation-competent *Agrobacterium* ***(5)*** is the method of choice, although, in the absence of the appropriate equipment, the less-efficient freeze–thaw method ***(6)*** may be used.

The binary vector represents an artificial T-DNA generally defined by the T-DNA borders (although only the RB is essential for transfer, and binary vectors containing only the RB are transfer competent). Inserted between the borders, there are a selectable marker gene expression cassette for selection of transformed plant cells and a site for cloning of the foreign gene. The latter may either constitute a multiple cloning site (mcs) suitable for direct cloning of an already-constructed foreign gene plant expression cassette or an expression cassette composed of a plant transcriptional promoter, a cloning site(s), and a termination (or polyadenylation) signal. The strong constitutive cauliflower mosaic virus (CaMV) 35S promoter and the CaMV or nopaline synthase (*nos*) poly(A) sequence regions are commonly used for transcription initiation and termination, respectively. Presently, there is no evidence to support the requirement for tissue-specific promoters for CP-mediated protection, even for phloem-limited viruses. Thus, the CaMV 35S promoter is suitable for expression in all dicot plants, but alternative promoters may be required for high levels of protein expression in some monocots ***(7)***.

The neomycin phosphotransferase II gene (*nptII*) is a commonly used selectable marker gene that confers resistance to kanamycin in transformed plants. This has proven useful for solanaceous plants, but less so for the more recalcitrant legumes and monocots, for which hygromycin or the herbicide bialophos are commonly used; in these cases, alternative vectors will be required (e.g., *see* **refs. *1*** and ***8***).

Binary vectors also possess an antibiotic resistance gene for selection of recombinant bacteria. The presence of the *lacZ* gene may facilitate identification of recombinant clones through blue-white color selection (*see* Chapter 27), but is available on only a few binary vectors (e.g., pBIN19; **ref. *9***), and generally not on those that already contain the expression cassette. A reporter gene cassette (commonly encoding β-glucuronidase [GUS]) for identification of transformed plant tissues may also be useful. Few of the vectors used for CP- mediated protection contain the GUS gene, although one, pGA482GG