

transgenic plants with suboptimal resistance to kanamycin, the vectors have been used frequently to produce CP-mediated virus resistance and are not likely to present problems with any commonly used plant species. The complete sequence of pBIN19 is known (16), and an improved version, pBINPLUS has been produced (17). The choice of binary vector may also depend upon the following:

- a. The suitability of restriction enzyme sites (e.g., a multiple cloning site) for the CP gene sequence.
- b. The compatibility of the bacterial selectable marker gene with the *Agrobacterium* strain to be used for plant transformation (many disarmed strains carry some bacterial resistance; e.g., pGV3850 is carbenicillin- and rifampicin-resistant and therefore not suitable for use with binary vectors that are selected for by carbenicillin resistance).
- c. The ability of the binary vector to be maintained in the absence of selection. The latter is of relevance when assessing risk factors, because a low-copy-number vector, which is rapidly lost without selection, has a lower environmental risk than one that is more stable.

Many of the modern binary vectors are more practical, having a wider range of unique enzyme sites to facilitate cloning, either through inclusion of rare restriction enzyme sites and/or decreased vector sequences. A number of versatile vectors have been constructed by Jones et al. (18) that contain different promoters, terminators, and selection cassettes, as well as an array of restriction sites, to facilitate direct insertion of foreign genes or the transfer of foreign-gene cassettes from compatible pUC-based plasmids. Additionally, they allow a choice of orientation of the expression cassette that may be of importance for optimal expression of transgenes (see references within ref. 18). Although the vectors have not been used for CP gene insertion, there is no evidence to suggest that choice of binary vector is important for CP-mediated resistance. Applications for vectors should be directed to the laboratories in which they were developed.

2. Molecular biology protocols used here are described in Chapters 27, 28, and 41–43. Additional information may be obtained from ref. 19.
3. The choice of *Agrobacterium* strain depends on its host range (i.e., the ability of the bacterium to interact with the host plant). As well as the strains described in **Subheading 1**, wide host-range disarmed supervirulent strains, such as AGL1 (20) and EHA105 (21), are available. A survey of the literature should show strains suitable for transformation of particular plant species (for example, see references within Table 3.2 of Grumet [22]). The correct choice of strain is especially important if cereals or grasses are to be transformed (1,23).
4. Some antibiotics are degraded by light, but most solutions are stable if stored at  $-20^{\circ}\text{C}$ . All should be handled with care (use gloves and avoid inhaling the powder), because they may be toxic and/or allergenic.
5. The general procedure outlined is appropriate for cloning all virus CP genes; but since each virus gene has a unique nucleotide sequence, the restriction enzyme sites used will differ. In the example cited, the CP gene is excised with a small