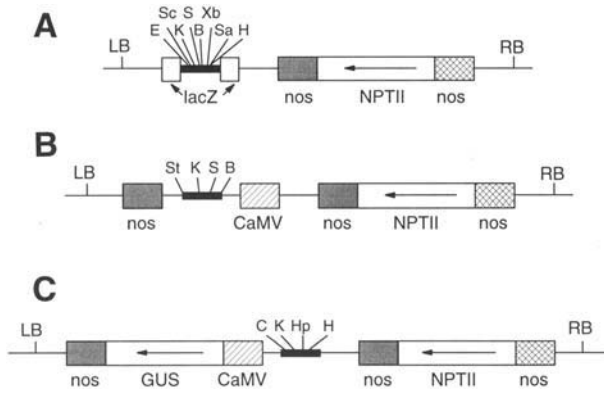

Fig. 1. Examples of types of binary vectors used for *Agrobacterium*-mediated transformation of plant cells. The *npt*II gene provides kanamycin selection for transformed plants, and unique restriction enzyme sites within the multiple cloning sites (mcs, solid bars) facilitate CP gene insertion (only unique sites are shown on this figure). **(A)** pBIN19 ***(9)***, which requires insertion of a CP gene cassette comprising promoter and terminator sequences, but provides color selection from the *lacZ* gene. **(B)** pROKII, which provides transcription regulatory signals flanking the mcs. **(C)** pGA482GG ***(10)***, which includes a GUS marker-gene cassette, but requires a CP gene cassette to be inserted into the mcs. Hatched- and line-filled boxes represent promoters (nos or CaMV, respectively), dot infils the nos terminator. Restriction sites: B, *Bam*HI; S, *Sma*I; K, *Kpn*I; Sc, *Sac*I; H, *Hin*dIII; Hp, *Hpa*I; C, *Cla*I; E, *Eco*RI; Xb, *Xba*I; Sa, *Sal*I.

(10), has proved successful in a number of studies ***(10–12)***. Typical binary vectors are shown in **Fig. 1**.

The artificial T-DNA is transferred to the plant by cocultivation of wounded plant material (generally, leaf disks, *see* Chapter 38) and *Agrobacterium*. The transfer is mediated via the *vir* functions present in *trans* on the Ti plasmid (for a review of *Agrobacterium*-mediated plant transformation, *see* **ref. *13***).

The procedures required to produce CP-expression constructs for *Agrobacterium*-mediated plant transformation can be divided into three steps:

1. Ligation of the virus gene fragment into a plant expression cassette already present in a binary vector, and transformation of *E. coli* with the binary construct.
2. Transfer of the binary construct to *Agrobacterium* by electroporation (method 1) or conjugation (the triparental mating procedure), method 2.
3. Confirmation of the integrity of sequences inserted in *Agrobacterium* by Southern analyses (method 1) and/or the polymerase chain reaction (PCR, method 2).

Cucumber mosaic virus (CMV)-O strain will be used to illustrate the cloning of a plant virus CP gene into the plant binary expression vector pROKII, as