CHAPTER IV

CONCLUSIONS

1. With the use of only one pair of primers that contain two restriction sites at their 5′ ends, we could generate the hpGUS construct by amplifying the *gus* fragment using these primers, and digesting each end of the PCR product with *Asc*I/*Swa*I and *Bam*HI/*Xba*I in a two-step cloning to put the *gus* sequence in the sense and antisense orientations in pFGC5941, respectively as expected.
2. *Agrobacterium*-mediated transformation was able to introduce a gene sequence consisting of the hpGUS construct driven by the 35SCaMV promoter into tobacco (*Nicotiana tobacum* L. cv. *Virginia Coker*). All putative double transformants were selected on kanamycin-containing media and there were no morphological differences between the control and the hpGUS-expressing double transformants.
3. Integration of the *gus* gene and the hpGUS construct into the genomes of the three putative independent transgenic tobacco plants from each of the control and the hpGUS-expressing double transformants was confirmed by PCR analyses. The hpGUS-expressing double transformants contain both the *gus* gene and the hpGUS construct, but the control plants contain only the *gus* gene.
4. By Southern blot analysis, three putative independent lines of the hpGUS-expressing double transformants were verified to be likely independent.
5. By northern blot analysis, all hpGUS-expressing double transformants show silencing in expression of the *gus* mRNA under *in vitro* growth.
6. The GUS activity level of two hpGUS-expressing double transformant lines examined by both histochemical and spectrophotometric methods was shown to

decrease corresponding with the reduction of *gus* mRNA level examined in northern blot analysis

7. The hpGUS construct can silence expression of the *gus* gene in tobacco at the protein level.