

A *piggyBac*-mediated germline transformation of the silkworm *Bombyx mori* L. with the cellulase gene of the mulberry longicorn beetle, *Apriona germari*

Yong Soon Kim¹, Bong Hee Sohn¹, Kwang Ho Choi¹, Tae Won Goo¹, Pil Don Kang¹, Seok Woo Kang¹, Byung Rae Jin²

¹*Department of Sericulture and Entomology, NIAST, RDA Suwon 441-100, Korea and* ²*College of Natural Resources and Life Science, Dong-A University, Busan 604-714, Korea*

Germline transgenesis was recently achieved in *Bombyx mori* L. after many attempts over a number of years. It was made possible by the use of a combination of the green fluorescent protein (GFP) marker gene cloned in the *piggyBac* transposon under control of the *B. mori* cytoplasmic actin (*BmA3*) promoter. We have cloned and sequenced the cDNA coding the cellulase from the mulberry longicorn beetle, *Apriona germari*, with the polymerase chain reaction (PCR) previously. To express the cellulase gene in the silkworm, we constructed the recombinant plasmid with *piggyBac* vector-mediated germline transformation for target gene. Thus, the recombinant plasmid, pPT-Xcell harboring the cellulase gene of the mulberry longicorn beetle, was constructed for germline transformation of the silkworm. The transformation vector constructs consist of the *piggyBac* inverted terminal repeats flanking a fusion of the *BmA3* promoter and the cellulase gene, the IE2 promoter and the green fluorescent protein (GFP), respectively. A nonautonomous helper plasmid encodes the *piggyBac* transposase. The recombinant plasmid was co-injected into preblastoderm eggs of *B. mori*. Approximately 4.8% of the silkworm larvae in the G1 broods expressed GFP. PCR analyses of GFP-positive G1 silkworms revealed that the marker gene inserted certainly in silkworm genome DNA. In Western blot hybridization, we determined the 30kDa immunoreactive band predicted sizes for the cellulase. On the basis of these experiments, expression of the cellulase gene in G1 generation of transgenic silkworm is

now in process.