Cloning of the Human Lactoferrin Gene and Germline Transformation of the Silkworm *Bombyx mori* L. with *a piggyBac*Vector

Yong Soon Kim¹, Bong Hee Sohn¹, Jong Min Lee¹, Sang Uk Lee¹, Myong Gon Kim², Jae
Hak Park³ and Pil Don Kang¹

¹Department of Sericulture and Entomology, NIAST, RDA Suwon 441-100, Korea, ²College of Medicine, Korea University, Seoul 136-701, Korea and ³College of Veterinary Medicine and School of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea

Lactoferrin, an ion-binding 80-kDa glycoprotein, has been suggested to have many biologic activities, such as facilitating ion absorption and having antimicrobial and antiinflammatory effects. Several of these activities are likely to only be facilitated by human lactoferrin because they depend on the binding of human lactoferrin to specific receptor. To produce recombinant human lactoferrin to animal foods using transgenic silkworm, Bombyx mori, we have cloned and sequenced the cDNA encoding for a human lactoferrin(hLf) from the mRNA in mammary tumor line(GI-101). As a result, the 2.5-kb fragment of hLf gene was cloned with pGEM-T vector and than this fragment was sequenced. In the nucleotide sequence analysis, single open reading frame of the 2,136-bp encoding for a polypeptide of 712 amino acid residues was detected. On the other hand, we constructed a recombinant plasmid(pPT-hLf), containing human lactoferrin for germline transformation of the silkworm B. mori L. using a piggyBac transposon-derived vector. A nonautonomous helper plasmid encodes the piggyBac transposase. Approximately 3.6% of individuals in the F0 silkworms expressed GFP. PCR and Southern blot analyses of GFP-positive F0 silkworms revealed that independent insertions occurred frequently. On the basis of these experiments, expression of hLf in G1 generation of transgenic silkworm is now in process.