

# Transgenic Silkworm Research Center

---

## Introduction

Systems for production of useful materials using transgenic organisms have been developed in plants and mammals and such systems are applicable to insects. The ability to produce protein in the domesticated silkworm, *Bombyx mori*, is very high since a large amount of silk protein is produced during the larval stage and extensive knowledge about silkworm science and technology has accumulated in Japan for more than 100 years. About 10 years ago, our institute developed innovative methods for construction of transgenic silkworms for efficient production of useful materials. Based on the fundamental science, transgenic silkworm research was thought to have very high potential for production of new materials as well as development of technology for a new field of agrobiological sciences. In the Transgenic Silkworm Research Center, we have studied the development of new methods for the generation of transgenic silkworm through construction of new vectors and development of new marker genes. We are also studying systems that control expression of the introduced foreign genes in silkworms and are breeding silkworm strains for the production of useful recombinant proteins and new silk fibers.

## Construction of a new injection system using the electric manipulator

Efficient production of transgenic silkworms is essential for continuing the development and application of research in this area. The original method of using glass capillaries may not be appropriate for injection of DNA into silkworm eggs because the eggs possess thick and hard eggshells. Therefore, the injection of DNA into an egg was generally performed by the following processes: first, make a small hole in the eggshell with a tungsten needle; insert a glass capillary into the hole; and supply air pressure to the capillary to push the DNA solution into the egg. During this process, the penetration of the glass capillary into the small hole is a crucial step. To improve this step, we developed a method that uses an electric manipulator to automatically move the position of the hole in the eggshell. This method increased the speed of injection, the hatchability of eggs after injection, and the efficiency of production of transgenic silkworms. We concluded that the new system makes injection much easier compared to the original method for construction of transgenic silkworms.

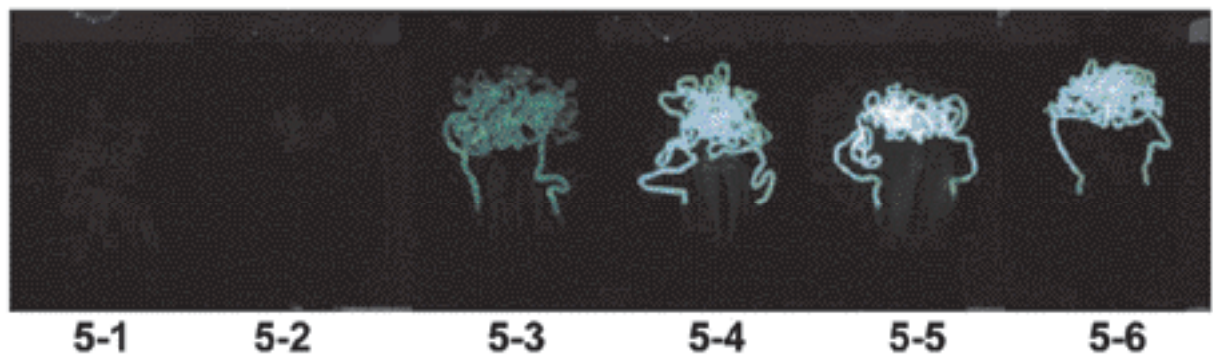


Fig 1. The gene expression system of posterior part of silk glands of the 5th instar larva of the transgenic silkworm. Numbers indicate the stage of the 5th instar.

### **Development of a gene regulation system using the promoter of the fibroin gene of the domesticated silkworm *Bombyx mori* and the Japanese oak silkworm *Antheraea yamamai*.**

A system for expression of transgenes in the posterior silk gland had been developed using the GAL4 strain under the control of the promoter from the fibroin L chain gene of silkworm. This promoter, however, stimulated expression of EGFP driven by the GAL4 in young larvae which apparently also caused an abnormal shape of the posterior silk gland and reduced production of EGFP. To circumvent this problem, we made two constructs of the GAL4 gene — one under the control of the fibroin H chain gene promoter from domesticated silkworm, *Bombyx mori*, and the other under control of the fibroin H chain gene from oak silkworm, *Antheraea yamamai*. We generated transgenic silkworms with these fibroin H chain promoter constructs and investigated the expression pattern of EGFP after crossing with a homozygous UAS-EGFP strain. The expression of EGFP was only observed in the posterior silk gland after the 2nd day of the 5th instar (Fig 1). In contrast to previous transgenic silkworms controlled by the fibroin L chain promoter, the abnormal shape of the silk gland was not observed in transgenic silkworms containing these H chain promoter constructs and production of silk proteins in the silk gland was no different from that of non-transgenic silkworms. The results indicate that the fibroin H chain promoter system is superior for the production of the recombinant proteins compared to the system using the fibroin L chain promoter.

### **Production of modified GAL4 constructs to increase the expression level of the target gene**

To increase transgene expression level, we have shown that modification of the GAL4 construct is effective in experiments using cultured cells. In these experiments, we have shown that the level of expression of the best construct was more than 100 times higher than that of an unmodified GAL. Based on these results, we constructed a vector with the modified GAL4 construct under the control of the sericin gene promoter and then generated

transgenic silkworm lines with the new construct. We confirmed that the lines expressed the target gene in the middle part of the silk gland of transgenic silkworms only during the middle and late stages of the final instar. In addition, we constructed new GAL4 strains that stimulate the expression of a target gene by heat-shock. These strains are expected to be very useful for achieving temporal expression of the transgene.

### **Improvement of a production system for the recombinant proteins in middle part of silk gland**

To develop systems for production of useful recombinant proteins in the middle part of silk glands, we studied the effect of related factors on the production of recombinant proteins in transgenic silkworms. This year, we mainly tested the effects of the exon-intron of sericin 1 and fibrohexamarin genes, tandem repeats and copy numbers. Addition of the exon and intron sequence of sericin 1 gene was not effective in increasing mRNA and protein of the target gene and the level was almost the same as that for the addition of the signal peptide sequence of sericin 1. In contrast, the addition of the exon-intron sequence of fibrohexamarin gene increased target gene mRNA levels and resulted in larger amounts of the target protein. Constructs with tandem repeats and increased copy numbers were effective in increasing the amount of protein produced in the middle silk gland of transgenic silkworms. We obtained transgenic silkworms that produce more than 1 mg of the recombinant protein in a pair of the middle silk glands. From the results, we are constructing more efficient vectors compared to the old ones.

### **Breeding of new silkworm strains for the production of recombinant protein**

To breed new silkworm strains that are adapted for the production of recombinant protein, we repeatedly performed selection and mating between transgenic strains and the strains that produce a large amount of silk protein. The amount of sericin protein produced by the newly bred strains reached more than 75 mg, which is twice that of the original

transgenic strains. The results suggest that the use of the new strain may drastically increase production.

#### **Production of the recombinant opioid receptor protein in transgenic silkworms**

In collaboration with Gunma University, we successfully produced recombinant protein of G protein opioid receptor in transgenic silkworms. The receptor produced by the silkworm possessed native activity of the receptor and the yield was comparable to that of the baculovirus expression system.

#### **Study of the development of recombinant silk produced by transgenic silkworms for practical use and industrialization**

Through collaborations with Toray Industries, Inc., Tokyo University of Agriculture and Technology, Gunma Sericultural Technology Center, Textile Research Center of Gunma, Riken, Amalgaam, Ltd., and Silk Technology unit of NIAS, we studied production of high quality recombinant silks. We successfully produced high quality silks having

different fluorescent colors using transgenic silkworms. Furthermore, we bred strains for mass-rearing of silkworms and harvesting large amounts of the cocoons with the fluorescence color. Then, we performed a mass-rearing of the transgenic silkworms and successfully harvested large amounts of cocoons (Fig 2). We also developed the technology to spin large amounts of fluorescence-colored silks and produced large amounts of the recombinant silks (Fig 3). Tapestries, other textiles and lamp shades were produced from the recombinant silks and evaluated for practical use (Fig 4). In addition, we developed a means to produce thin silk by introducing a gene for an acidic peptide into silkworm. The silk produced by these transgenic silkworms is thinner than that of the original race. We showed the possibility of producing recombinant silk suitable for higher quality of clothes. We also demonstrated that artificial veins assembled from recombinant silks expressing a specific peptide related to the affinity of mammalian cells had better quality compared to those made from natural silk.

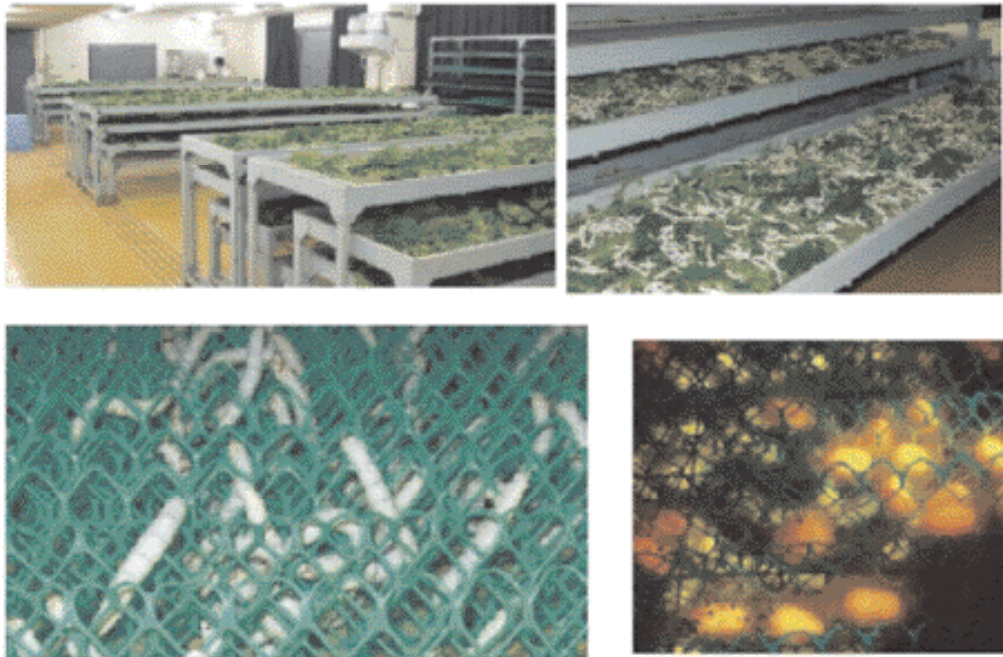


Fig 2. Mass rearing of transgenic silkworms, larvae constructing cocoons, and spun cocoons expressing the orange fluorescent protein.

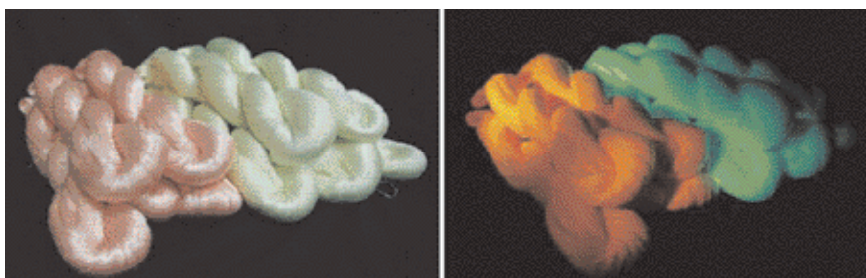


Fig 3. Fluorescent-colored silks.  
Left: under white light. Right: illuminated by blue-LED light (viewed with a yellow

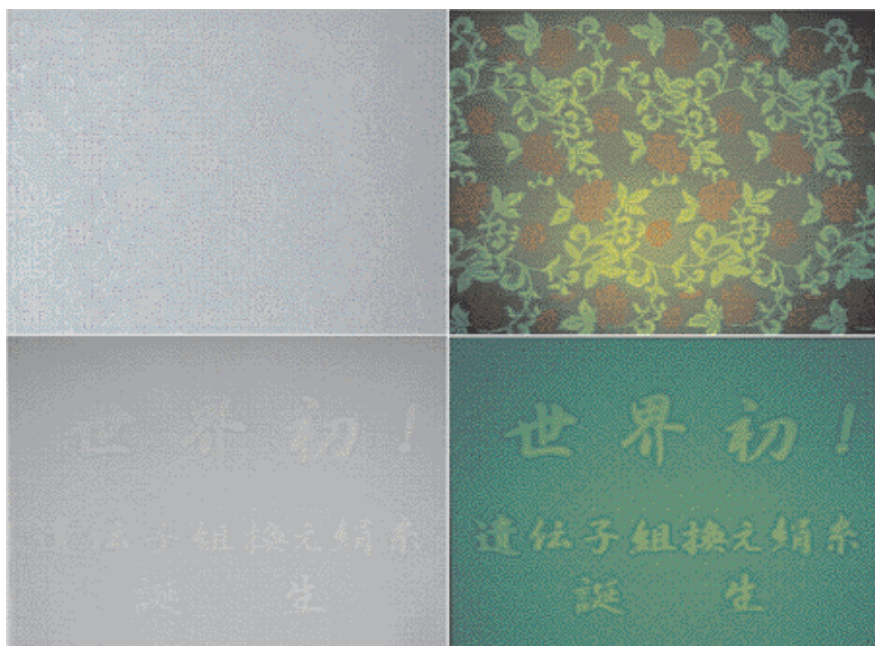


Fig 4. Tapestries (Jacquard) made with fluorescent-colored silks.  
Left: under white light. Right: illuminated by blue-LED light (viewed with a yellow filter); the characters and background pattern are fluorescing.