# Journey of silkworm from Silkroad to Bioroad

Today's Biotechnology based industry faces a bottleneck towards further advancement owing to the lack of proper gene expression systems. Although as time changes the system should change to accommodate the requirements arising. Thus said, with the completion of Human Genome Project, we have come across a Herculean task of trying to understand the function of the proteins being coded by the genes. Since the genome is known now the challenge of understanding the protein function has become daunting. The first steps towards reaching the holy grail of biotechnology, that is, to elucidate all the known proteins in a humans and their inter-relationship, is to develop an efficient expression system to express humanized proteins.

Silkworms have been in use for human needs for centuries. The silk derived from the silkworm is of great economical value. Lately insects and in particular silkworms have attracted the attention for expressing proteins of high value to perform post-translational modifications needed for humanizing a protein for human applications or study. To express desired protein of interest in silkworms various techniques exists and among them baculovirus based expression system (BES) comes foremost. Baculovirus being the ideal vector carrying desired cDNA of interest does not infect or cause any known disease to humans or other livestock other than its host, thus dismaying any biohazard concerns. Thus it suits the purpose and with the advent of bacmid technology, the ability to produce recombinant baculovirus has grown many folds; both Autographa californica nucleopolyhedrosis virus (AcNPV) and Bombyx mori nucleopolyhedrosis virus (BmNPV). AcNPV and BmNPV both belong to the family of baculovirus.

These baculoviruses belonging to various families provide a wide range of options for expressing the protein of interest in various insects. In our laboratory BmNPV based expression system for expressing proteins was improvised using BmNPV bacmid. BmNPV bacmid is capable of replicating in both Escherichia coli and expressing protein of interest in Bombyx mori derived cell lines or silkworms.1) As shown in Fig. 1 this method utilizes the advantage of a bacmid it can be easily prepared and screened in E. coli to produce sufficient DNA for subsequent expression in silkworms. BmNPV based bacmid is easy to create by using a donor vector and injecting into the silkworm the bacmid DNA to express the protein of interest. Once the DNA has been injected, within 1 week's time sufficient amount of protein can be collected from the silkworm hemolymph. Using His-tags or any other desired methods the protein can be further purified and analyzed for its quality. The handling of silkworm is very easy requiring minimum labor and capital investments and the protein derived from its hemolymph surpasses the quality achieved by any known expression system. Recently the silkworm expression system has been automated too enabling to scale up the production levels further.

Up to now, HBeAg, sugar-modified eukaryotic proteins, such as carbohydrate transferases and cell surface receptors, have been produced using B. mori silkworm larvae. This work demonstrates great potential advantages of the BmNPV bacmid system for the large-scale expression of foreign genes in the silkworm as well as preparation of recombinant viruses, especially by direct injection of the recombinant bacmid DNAs, because it has no time consuming steps involved for virus titer-up in insect cells and it is free from biohazard. Furthermore, recent reports showed that the recombinant baculovirus containing mammalian cell-active promoter can exhibit efficient gene transfer of potential vaccines into mammalian cells. Thus, for large-scale preparation of such recombinant viruses, the BmNPV bacmid system using silkworm would be very attractive due to low cost (about 10% of the SF9/Bm cell-based expression), easy to treat and high safety for biohazard. This would be a great breakthrough in production of recombinant eukaryotic proteins and viruses, which will be a powerful tool in a new proteome era.

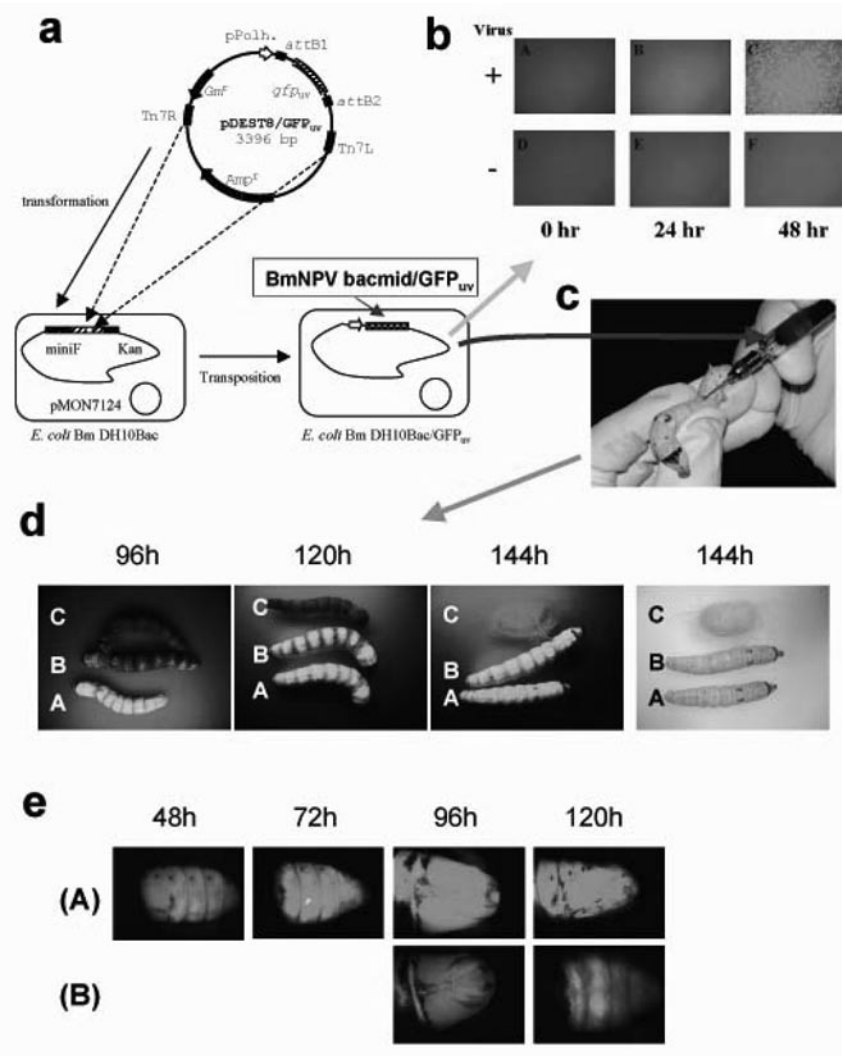


**Fig. 1.**
GFPuv expressions by the BmNPV Bacmid system. The donor plasmid pDEST8/GFPuv carrying the GFPuv gene was transformed into E. coli Bm DH10Bac competent cell for the transposition, and the recombinant DNA obtained was designated BmNPV bacmid/GFPuv (A). B. mori cells were transfected with BmNPV bacmid/GFPuv DNA (B). The photographs of B. mori cell were taken at 0 hr, 24 hr, and 48 hr of posttransfection using UV illuminator in complete darkness. Silkworm and pupae were transfected with BmNPV bacmid/GFPuv DNA (D) and (E) by injecting to silkworm larvae (shown here) or pupae by syringe (C). B. mori larvae were infected by direct syringe injection of BmNPV/GFPuv virus (a in D), BmNPV bacmid/GFPuv DNA (b in D) and mock (c in D). The photographs of the larvae were taken at 96 hr, 120 hr, and 144 hr of postinfection time using UV illuminator in complete darkness. The pupae were infected by needlepoint immersed in BmNPV/GFPuv virus (a in E), and by direct injection of 20 µl BmNPV bacmid/GFPuv DNA using pipette (b in E). The photographs of the larvae were taken at 48hr, 72hr, 96hr, and 120 hr of postinfection time under the fluorescence microscope.

**References**

1. Motohashi, T. et al., Efficient large-scale protein production of larvae and pupae of silkworm nuclear polyhedrosis virus (BmNPV) bacmid system. Biochem. Biophys. Res. Com. **326,** 564-569 (2005).

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