
Preface

Baculoviruses, which are a group of viruses that infect invertebrates, were first “discovered” in diseased silk worms in the 1500s, although the viral nature of this disease was not demonstrated until 1947. Subsequently, hundreds of other baculoviruses have been discovered. For example, the *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) was first isolated from the alfalfa looper (i.e., *A. californica*) insect species. AcMNPV is the most widely used and best characterized baculovirus and is known to infect many insect species in addition to *A. californica*, including *Spodoptera frugiperda* (fall armyworm) and *Trichoplusia ni* (cabbage looper). Furthermore, AcMNPV is the baculovirus that is usually used to produce recombinant baculoviruses for subsequent recombinant protein synthesis.

Shangyin Gao and Thomas Grace independently established the first continuous insect cell lines in the late 1950s and early 1960s. In the 1960s and 1970s insect cell culture was primarily used as a model to study insect metabolism and for the *in vitro* synthesis of baculoviruses for potential use in insect control (i.e., as a biopesticide). The widespread use of insect cell culture, however, did not occur until the baculovirus expression vector system (BEVS) was independently developed in the Max D. Summers and Lois K. Miller laboratories in the early 1980s. The BEVS takes advantage of the very strong polyhedrin promoter found in the AcMNPV genome whose natural product (polyhedrin protein) is nonessential in insect cell culture. Thus, the BEVS involves using the polyhedrin promoter to drive foreign protein expression and provides the means to express high levels of recombinant proteins in a relatively short time. Note that there have been many extensions of this basic principle, many of which are described in this book. In brief, some advantages of the BEVS are (1) ease of constructing a recombinant baculovirus (compared to isolating stably transformed cells), (2) potentially high expression levels, and (3) the ability of host insect cells to properly process proteins in a manner similar to mammalian cells. Thus, the BEVS provides a recombinant protein expression system intermediate between bacteria (e.g., *E. coli*) and mammalian cells (e.g., CHO cells) in terms of expression levels, cost, and ability to perform complex protein modifications. The BEVS has become especially popular for small-scale recombinant protein expression in laboratories throughout the world when biologically active proteins are required for research applications. Furthermore, the BEVS is becoming a popular choice for producing commercial farm animal and human vaccines. For example, a subunit/marker vaccine against classical swine fever (“Porcilis Pesti”) produced by MSD Animal Health was released in 1998 for vaccinating pigs, a virus-like particle vaccine against cervical cancer (“Cervarix”) produced by GlaxoSmithKline was released in 2007 for vaccinating human girls, and an annual trivalent flu vaccine (“Flublok”) produced by Protein Sciences was released in 2013 for vaccinating humans.

The third edition of *Baculovirus and Insect Cell Expression Protocols* (the first edition, edited by Christopher D. Richardson, was published in 1995 and the second edition, edited by David W. Murhammer, was published in 2007) was written to provide an updated step-by-step guide to biochemists, molecular biologists, biochemical engineers, and others using the BEVS and/or insect cells for producing recombinant proteins. Furthermore, the third edition of *Baculovirus and Insect Cell Expression Protocols* will provide assistance to scientists

and engineers interested in developing and producing baculovirus insecticides. In both of these cases the procedures involved in producing products at laboratory scale and large scale will be discussed, as well as production in insect larvae.

The third edition of *Baculovirus and Insect Cell Expression Protocols* is divided into seven parts. The first part, entitled "Introduction," contains one chapter that serves as an overview of the major techniques discussed in detail elsewhere in the book. Furthermore, this chapter provides step-by-step procedures involved in quantifying cell growth, baculovirus infection, and cell metabolism. It is strongly recommended that this chapter be read prior to reading your specific chapter(s) of interest. The second part, entitled "Baculovirus molecular biology/development of recombinant baculoviruses," contains three chapters that give an overview of baculovirus molecular biology and methods involved in constructing and isolating recombinant baculoviruses. Moreover, this part contains another chapter that discusses using modified baculoviruses to express genes in mammalian cells ("BacMam"). The third part, entitled "Insect cell culture," contains four chapters that list currently available insect cell lines, methods to isolate new cell lines and develop your own serum-free medium, and routine maintenance and storage of insect cell lines and baculoviruses. The fourth part, entitled "Protein production with recombinant baculoviruses," contains four chapters that discuss small- and large-scale recombinant protein production with the BEVS in both insect and mammalian cell culture and in insect larvae. The other two chapters in this part discuss the large-scale production of virus-like particles and an alternative approach to expressing multicomponent protein complexes. The fifth part, entitled "Recombinant protein production with transformed insect cells," contains a chapter that discusses methods involved in developing stably transformed insect cells for expressing recombinant proteins directly from the insect cell genome, another chapter about improving the protein processing capabilities of host insect cells for use with the BEVS, and a chapter about using *Drosophila* cell lines, which provide an alternative to the lepidopteran insect cell lines used with the BEVS. The sixth part, entitled "Baculovirus development and production for use as insecticides," contains three chapters about the use, production, and characterization of baculoviruses (both wild type and recombinant) for use as biopesticides. The seventh part, entitled "Miscellaneous techniques and applications of the baculovirus/insect cell system," contains three chapters that discuss the use of green fluorescent protein, tubular reactors, and RNAi for research applications. The other two chapters discuss the application of the baculovirus/insect cell system to study apoptosis and generating envelop-modified baculovirus for gene delivery into mammalian cells.

The third edition of *Baculovirus and Insect Cell Expression Protocols* provides the detailed steps required to perform the techniques involved with the use of baculoviruses and insect cell culture and discusses problems that may be encountered. It is hoped that this book will not only aid the user in successfully completing the tasks described herein but also stimulate the development of improved techniques and new applications of baculoviruses and insect cell culture.

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Iowa City, IA, USA

David W. Murhammer

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Murhammer, D.W. (Ed.)

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