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Protocol

Creation of Baculovirus Display Libraries

Anna R. Mäkelä, Wolfgang Ernst, Reingard Grabherr, and Christian Oker-Blom

INTRODUCTION

The baculovirus expression vector system has been used extensively to produce numerous proteins originating from both prokaryotic and eukaryotic sources. In addition to easy cloning techniques and abundant viral propagation, the system's insect cell environment provides eukaryotic post-translational modification machinery. The baculovirus display vector system provides a number of advantages over prokaryotic systems, allowing the combination of genotype with phenotype, enabling presentation of foreign peptides or even complex proteins on the baculoviral envelope or capsid. Baculoviruses permit larger gene insertions, are easily propagated, and can be grown to high titers. Furthermore, surface modifications of the viral capsid enable specific targeting. This strategy can be used to enhance viral binding and entry to a wide variety of both dividing and nondividing mammalian cells as well as to produce antibodies against the displayed antigen. In addition, the technology should enable modifications of intracellular behavior, i.e., trafficking of recombinant "nanoparticles," a highly relevant feature for studies of targeted gene or protein delivery. It is important to note that, although the viruses do not replicate in mammalian cells, they are not entirely transcriptionally silent. They can also be highly antigenic when used in vivo, limiting their therapeutic use. This protocol describes methods for generating display libraries.

RELATED INFORMATION

An introduction to the theory and background of **Baculovirus-Based Display and Gene Delivery Systems** (Mäkelä et al. 2010a) is available. Additional procedures are presented for the **Determination of Recombinant Baculovirus Display Viral Titer** (Mäkelä et al. 2010b) and for the characterization of these gene delivery vehicles in **Immunofluorescence Analysis of Baculovirus-Displayed Viral Proteins on Infected Insect Cells** (Mäkelä et al. 2010c), **Immunoelectron Microscopy Analysis of Recombinant Baculovirus Display Viruses** (Mäkelä et al. 2010d), and **Monitoring Baculovirus-Mediated Efficiency of Gene Delivery** (Mäkelä et al. 2010e).

MATERIALS

CAUTIONS AND RECIPES: Please see Appendices for appropriate handling of materials marked with <**!**>, and recipes for reagents marked with <**R**>.

Reagents

Alkaline phosphatase, calf intestine

DNA, baculoviral, *Ac*-omega

The vector used in this protocol is a derivative of wild-type Autographa californica *multiple nucleopolyhedrovirus (*Ac*MNPV) containing a unique restriction site (Sce I) downstream from the polyhedrin promoter, allowing linearization and direct ligation to the fragment to be inserted (*Fig. 1*) (Ernst et al. 1994).*

Adapted from *Gene Transfer: Delivery and Expression of DNA and RNA* (ed. Friedmann and Rossi). CSHL Press, Cold Spring Harbor, NY, USA, 2007.

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DNA insert of interest
Ethanol (70%)
Insect cells, *Spodoptera frugiperda* (*Sf*9; ATCC CRL-1711)
IPL-41 powdered medium (pH 6.2 +/- 0.1; ~400 mOsmol) (prepared with serum)
Liposome formulation, cationic (e.g., Cellfectin II; Invitrogen)
Meganuclease, I-Sce I (e.g., Roche Applied Science) and associated buffer
<**!**>Phenol:chloroform
<**R**>Phosphate-buffered saline (PBS)
Store at 4°C.

Equipment

Centrifuge, capable of accommodating volumes >100 mL
Consumable items for adherent cell cultures (e.g., Petri dishes, multiwell plates, T-flasks)
Disposable items (e.g., single-use tubes, sterile filters)
Flasks, spinner, for growing cells in suspension
Hood, laminar flow
Incubator preset to 27°C-28°C
Micropipettor and tips
Waterbath, variable-temperature

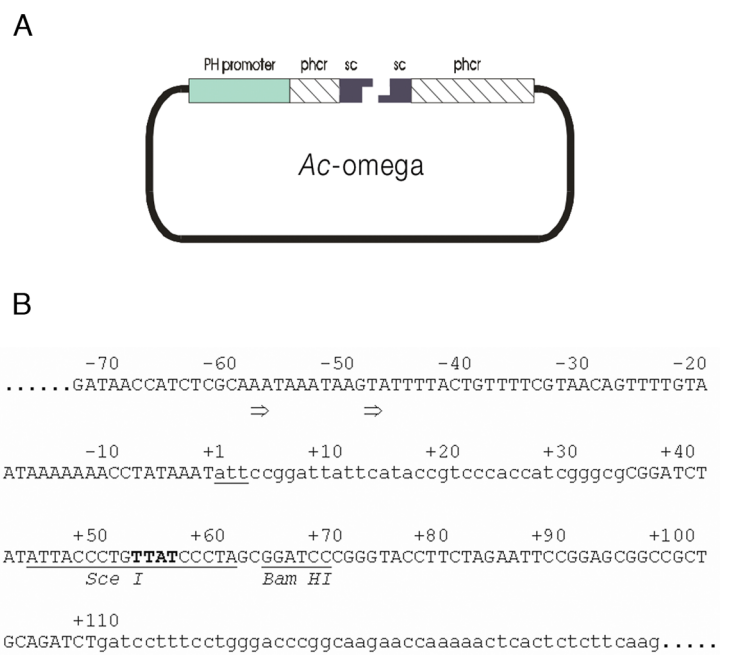

FIGURE 1. (*A*) Schematic of *Ac*-omega containing the Sce I single-cut site (sc) for direct gene insertion of DNA fragments treated with I-Sce I meganuclease. The polyhedrin promoter (PH promoter), polyhedrin-coding residues (phcr), and multiple cloning site in *Ac*-omega are acquired from the transfer vector pVL1393. (*B*) The nucleotide sequence of the *Ac*MNPV derivative *Ac*-omega at the I-Sce I recognition site, including the polyhedrin promoter region and the 5′ and 3′ phcr. The arrows (positions -57 and -48) indicate the 5′ end of the mRNA and the transcription start site, respectively. Natural polyhedrin ATG is mutated to ATT (+1, underlined). The lowercase letters (positions +1 to +35) indicate 5′ phcr. The 18-bp I-Sce I recognition site spans from nucleotides +45 to +62 (underlined). The I-Sce I 3′ nonpalindromic overhang of four bases is also shown (bold). The target-gene insertion site (unidirectional only) is located at position +58. Nucleotides +65 to +110 are derived from the multiple cloning site of transfer vector pVL1393. At position +111, the 3′ polyhedrin-coding region continues with nucleotide +172 of the natural polyhedrin gene (lowercase).

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METHOD

1. Digest 10 µg of *Ac*-omega viral DNA with 50 units of I-Sce I meganuclease in 100 µL of the manufacturer's recommended buffer for 5 h at 37°C.
2. Treat the linearized viral DNA with calf intestine alkaline phosphatase to remove 5′-phosphate groups.
3. Extract the DNA with phenol:chloroform.
4. Precipitate the DNA with 70% ethanol.
5. Ligate the insert of interest to 100-200 ng of the purified, dephosphorylated viral *Ac*-omega DNA (from Step 4) at a molar ratio of 1:40 as described by Ernst et al. (1994).
6. Incubate the ligation mixture overnight at 16°C.
7. Combine the ligation mixture with 20 µL of Cellfectin II (in a total volume of 100 µL). Incubate for 15 min at room temperature.
8. Transfect 2.5×10^6 *Sf9* cells in culture with the liposome/ligation mixture. Incubate for 6 h.
9. Add serum-containing IPL-41 medium. Incubate for 4-6 d at 27°C.
10. Perform plaque assays and analyze cells expressing the foreign protein of interest by fluorescence-activated cell sorting (see, e.g., Ernst et al. 1998).

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