SHEATECH, A DIVISION OF BIOTECHNOLOGY

BACULOVIRUS DNA INTRODUCTION:

The use of insect cells and lytic baculoviruses for expression of full-length mammalian proteins has been the recent method of choice for many disciplines.[1] *Autographa californica* multiple nuclear polyhedrosis virus (AcMNPV) infects the clonal tissue culture line Sf9 derived from *Spodoptera frugiperda* cells. Expression of the highly abundant polyhedrin gene is non-essential in tissue culture and its strong promoter can be used for the synthesis of foreign gene products. The polyhedrin promoter is maximally expressed very late in infection when the lytic virus is already killing the host cells, giving a reasonable chance for high levels of expression even for certain toxic proteins. Many post-translational modifications are made correctly in insect cells and proteins unable to be expressed in *E. coli* have been successfully expressed in the insect cell system.

The *superBAC* baculovirus DNA, developed and generated in Sheatech, Inc., provides a tool for recombination efficiencies to 100%. The principle of this technique lies in the construction of a modified type of baculovirus DNA, which contains a partial deletion of ORF1629 gene. This *superBAC* baculovirus DNA does not form a viable virus without the full length of ORF1629 gene. Only co-transfection of insect cells with the viral DNA and a complementing transfer vector construct, have ORF1629 gene recovery and generate recombinant virus. All virus plaques are derived from plasmid rescued viruses which contain and express the foreign gene from the plasmid. If it is necessary, the virus may be amplified from a single plaque in a plaque assay.

***SuperBAC_cdc37* baculovirus DNA:** ST003: *superBAC_ced37* baculovirus DNA, total amount 0.5 µg, concentration 0.1 µg/µl.

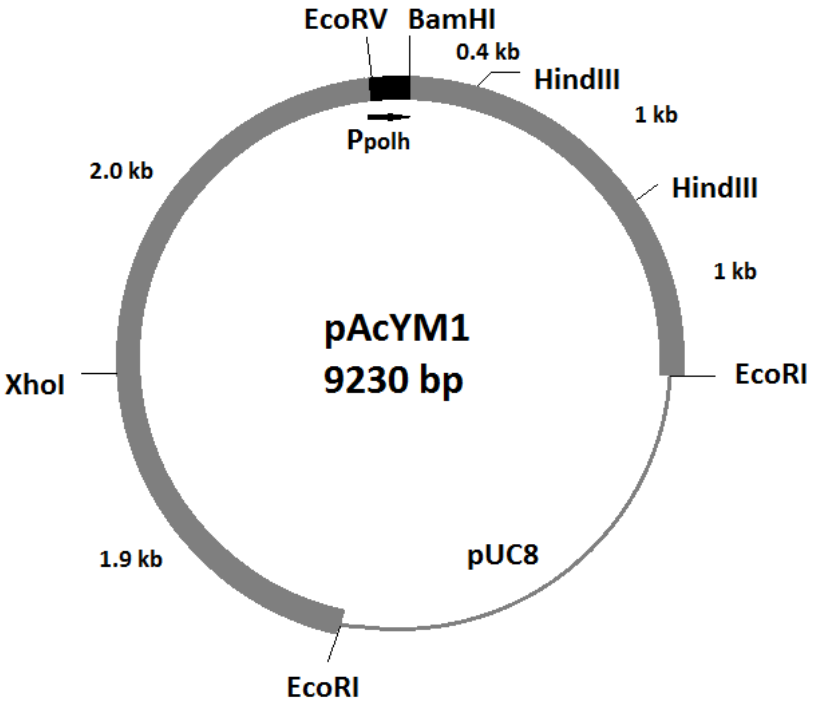
NOTE: Each vial contains materials sufficient for five transfections when used as described in this protocol. The superBAC_PDI was generated in SHEATECH. After homologous recombination, cdc37 folding gene is in virus genome and under the control of virus promoter. It has been published that cdc37 is to help the folding mechanism of gene expression in sf9 insect cells.

Related Products

Product	Cat. No.
pAcYM1	STV01
pAcYM1_L21	STV02
pAc	STV03
pAc_L21	STV04
pAcYM1-polyhedrin baculovirus control vector	STV11
Baculovirus genomic DNA with polyhedrin gene	STV21
superBAC_wt baculovirus DNA	ST001
superBAC_PDI baculovirus DNA	ST002

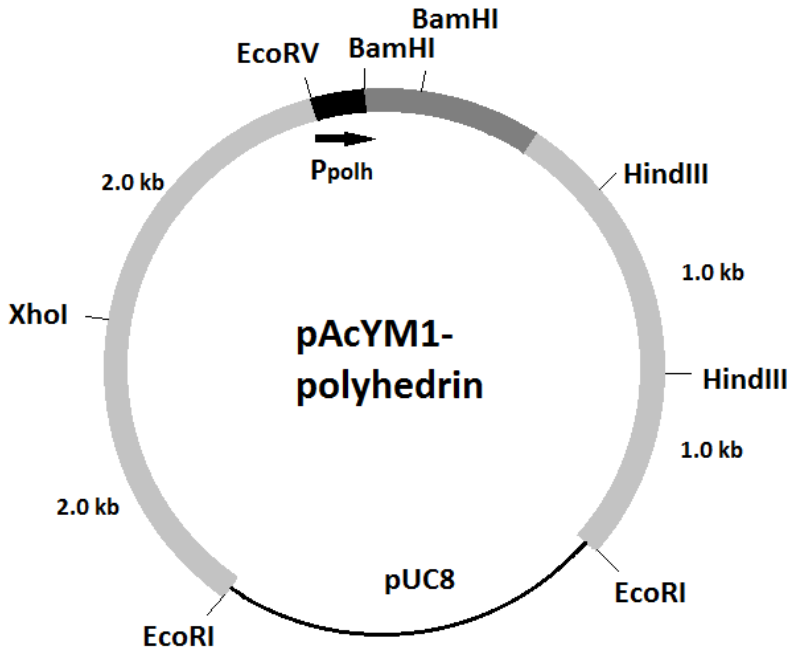
Transfer Vector:

All polyhedrin gene locus-based baculovirus transfer vectors can be used to rescue the ORF1629 gene of the *superBAC* baculovirus DNA. Transfer vectors[1], pAcYM1, pAc, pVL1392, pVL1393, and their derivatives, have been tested and confirmed in Sheatech, Inc. Other polyhedrin gene locus-based vectors, available from the other venders, may also be used for the *superBAC* baculovirus DNA. Following is the restriction enzyme mapping of pAcYM1.



pAcYM1-polyhedrin Baculovirus Control Vector

pAcYM1-polyhedrin is a baculovirus control vector. The polyhedrin gene was amplified from the wild type baculovirus DNA and cloned to pAcYM1 transfer vector. pAcYM1 is a baculovirus transfer vector designed for high level expression of foreign genes under the powerful AcMNPV polyhedrin promoter and contain both 5′ noncoding and 3′ noncoding DNA sequences of polyhedrin gene in pAcYM1. The AcMNPV sequences flanking the gene, 4kb and 2kb, in the transfer vectors allow homologous recombination with the viral DNA to insert the expression cassette into the polyhedrin locus. pAcYM1-polyhedrin is compatible with superBAC derivatives and any baculovirus system that utilizes homologous recombination in sf9 insect cells. This control vector is used to confirm the transfection efficiency and the occlusion body will be identified in the third day co-transfection in sf9 insect cells under microscopy.



Co-transfection by using *superBAC* baculovirus DNA:

- Seed 2 x 10^6 Sf9 cells on a 60 mm tissue culture plate.
- The sf9 insect cells attach firmly to plate for 15 min at room temperature.
- Remove the culture medium from the plate and add 3 ml of SFX serum free medium. Gently rock the plate back and forth to mix to make sure that all areas of the plate are covered with medium. Remove 2.5 ml and 0.5 ml remained in culture plate.

- Mix 0.1 µg of *superBAC* baculovirus DNA, 1 µg of recombinant plasmid DNA containing your gene and add H_2O to 25 µl in a sterile 1.5 ml eppendorf tube.
- Add 20 µl H_2O and 5 µl transfection reagent to DNA mixture tube for 15 min.
- Add 0.5 ml of serum free medium to DNA mixture, mix well and drop-by-drop to the insect cells on the tissue culture plate. After every two or three drops, gently rock the plate back and forth to mix the newly added solution.
- Incubate the plate at 27°C for 1-3 hr.
- After 1-3 hr, add 7 ml fresh TNM-FH medium and incubate the plates at 27°C for 4 to 5 days. Place a sterile water bottle in the bottom of the 27˚C incubator to maintain a humid environment.
- After 4 days, collect the supernatant and infect more cells for amplification. If it is necessary, start with a single virus clone by picking a single plaque from a plaque assay plate.

Positive Control

Note: Confirm the transfection efficiency with positive control vector.

- Seed 2 x 10^6 cells on a 60 mm tissue culture plate.
- After cells have attached, remove the culture medium from the plate and add 3 ml of SFX serum free medium. Gently rock the plate to mix to make sure that all areas of the plate are covered with medium. Remove 2.5 ml and 0.5 ml remained in culture plate.
- Prepare co-transfection positive control by mixing 0.1 µg *superBAC* baculovirus DNA, 1 µg pAcYM1-polyhedrin control vector DNA (STV11) and add H_2O to 25 µl in a sterile 1.5 ml microcentrifuge tube.
- Prepare transfection positive control by mixing 0.1 µg baculovirus genomic DNA with polyhedrin gene (STV21) and add H_2O to 25 µl in a sterile 1.5 ml microcentrifuge tube.
- Add 20 µl H_2O and 5 µl transfection reagent to DNA mixture tube for 15 min.
- Add 0.5 ml of serum free medium to DNA mixture, mix well and drop-by-drop to the insect cells on the tissue culture plate. After every two or three drops, gently rock the plate back and forth to mix the newly added solution.
- Continue co-transfection protocol as above.

Amplification of Recombinant Baculoviruses:

After the co-transfection or after the plaque assay, the recombinant baculovirus must be amplified to obtain a high titer stock solution. It should be infected at a multiplicity of infection (MOI) of 0.1 for virus amplification. The insect cells should be incubated at 27°C for 5 days before harvesting the medium. The medium will

contain at least 10^7 virus particles per ml and should be used for another round of amplification. Two rounds of amplification usually give a virus titer of 2 x 10^8/ml virus particles.

Plaque Assay:

- Seed 2 x 10^6 Sf9 cells/60 mm plate and let cells attach for 15 min at 27°C.
- Make serial dilutions (10^{-3} – 10^{-5}) of your co-transfection virus supernatant with serum free medium. Add 1 ml of diluted viral supernatant to each plate.
- Let infection occur for 1 hour at 27°C and rock the plate back and forth every 15 min.
- In the meantime, prepare a 3% solution of plaque assay agarose in sterile water. Heat mixture in microwave to 60°C and dissolve agarose completely.
- After cooling down to 42°C, add 2 volume of TNM-FH, 10%FCS. Mix well and the final agarose concentration should be 1%.
- Aspirate medium containing the virus inoculum. Overlay cells with 6 ml of the 1% agarose solution by pipetting carefully from one side of the plate. Remove all bubbles using a pipette.
- Let plates sit undisturbed until agarose is completely hardened for 30 min at room temperature. Add 1 ml of TNM-FH, 10% FCS on the top of agarose layer.
- Plates should be kept in a humid atmosphere for 5-7 days at 27°C until visible plaques develop. Plaques can be used for screening to identify the recombinant virus, to determine the virus titer or for virus amplification.
- To amplify virus from a single plaque, mark the plaque by making a dot or circle on the tissue culture plate. Harvest the plaque by taking a plug of the agarose containing the plaque using a 1 ml white tip. Elute the virus particles by rotating the agar plug in 700 µl of TNM-FH medium at 4°C overnight. 100 µl of this recombinant virus-containing medium can be used to further amplify the virus.[1]

Long-term Storage of Recombinant Baculoviruses:

Infective virus particles in high-titer stock solutions can be stored for several weeks without a significant decrease in the virus titer. However, a 10-fold decrease in the virus titer during 6 months storage at 4°C should be expected. For ultimate storage, viral DNA should be isolated from a low-passage virus and stored at –80°C. Whenever necessary, infectious virus particles can be obtained by transfecting the baculovirus DNA into insect cells.

Recombinant Baculovirus DNA Isolation:

- Take the baculovirus-containing medium and remove the insect cell debris by spinning it 10,000 x g for 10 min at 4°C.
- Transfer the supernatant to ultracentrifuge tubes and pellet the virus particles by spinning at 40,000 x g at 4°C for 1 hr.
- Decant the supernatant and resuspend the virus particles in TE Buffer (3×10^{10} virus equivalents per ml).
- Add proteinase K (100 µg/ml), 50 mM EDTA and 0.5% SDS.
- Mix well and incubate solution at 55°C for 2 hours.
- Extract once with one volume of phenol, twice with one volume of phenol/chloroform/isoamyl alcohol mixture (25:24:1[v/v]) and twice with one volume of chloroform. The baculovirus DNA is relatively sensitive to shearing, so extract by inverting the tubes just fast enough to thoroughly mix two phases.
- Add 0.1 volumes of 3 M sodium acetate (pH 4.5) and mix well, add 2 volumes of pure 100% ethanol and invert tubes several times at room temperature.
- Spin down precipitated DNA and wash once with 75% ethanol.
- Air-dry pellet for a couple min and resuspend the baculovirus DNA in appropriate volume of 0.1 x TE buffer.
- Measure OD260, digest the baculovirus DNA with EcoRI or HindIII and run on a 0.6% agarose TAE gel to check the digested DNA fragments. A characteristic restriction fragment pattern should be visible.[1,2,3]
- The baculovirus DNA can be stored at 4°C. It can be transfected into fresh sf9 insect cells. If the virus titer has been low, a new virus stock can be generated from an individual plaque in a plaque assay.

Reference:

1. O'Reilly, D., L. Miller and V. Luckow. *Baculovirus expression vectors*: A laboratory manual. W.H. Freeman and Company, 1992.

2. Ayres, M.D., S.C. Howard, J. Kuzio, M. Lopez-Ferber, and R.D. Possee. 1994. The complete DNA sequence of *Autographa californica* Nuclear Polyhedrosis. *Virology* 202:586-605

3. Kool, M. and J. Vlak. 1992. The structural and functional organization of the *Autographa californica* nuclear polyhedrosis virus genome. *Archives of Virology* 130:1-16.