**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. Autographa californica nuclear polyhedrosis virus (AcNPV) 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. 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 BD BaculoGold™ Linearized Baculovirus DNA from BD Biosciences Pharmingen™ provides a tool for recombination efficiencies close to 100%. The principle of this technique lies in the construction of a modified type of baculovirus DNA, which contains a lethal deletion. This DNA does not code for viable virus. Only co-transfection of insect cells with the viral DNA and a complementing transfer vector construct reconstitutes viable virus. Essentially, 99% of all virus plaques are derived from plasmid-rescued viruses which contain and express the foreign gene from the plasmid. Since the BD BaculoGold baculovirus contains a lacZ gene that is replaced by

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**REFERENCES**

recombination with the plasmid containing the foreign gene, all recombinants will produce colorless plaques on X-gal plates. The small portion of non-recombinant virus plaques (usually less than 1%) will stain blue on X-gal plates. If preferred, the virus may be amplified from a single plaque from a plaque assay.

**BD BaculoGold Linearized DNA**

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
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<tbody>
<tr>
<td>51-21100D BD BaculoGold Linearized DNA</td>
<td>2.5 µg</td>
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<tr>
<td>51-21484P pVL1392-XylE Control Vector</td>
<td>5 µg</td>
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**NOTE:** Each vial contains materials sufficient for five transfections when used as described in this protocol.

### Selection of a Transfer Vector

All polyhedrin gene locus-based baculovirus transfer vectors can be used to rescue the lethality of the BD BaculoGold DNA. Transfer vectors from BD Biosciences Pharmingen include pVL1392, pVL1393 (provided with the BD BaculoGold Transfection Kit), pAcSG2, pAcGP67, pAcUW21, pAcUW51, pAcM2P2 and pAcMP3, and pAcGLHT (GST fusion transfer vectors) and pAcHHT (6xHis fusion transfer vectors). Other vectors on the market may also be used, but have not been tested for compatibility with the BD BaculoGold DNA.

### Co-transfection Using BD BaculoGold

**NOTE:** TNM-FH medium contains 10% fetal calf serum.

- Seed 2 x 10⁶ Sf9 cells (Sf9 insect cells, live, Cat. No. 554763); onto a 60 mm tissue culture plate. Initial cell density should be 50-70% confluent.
- Allow the insect cells to attach firmly to plate (approximately 15 min).
- Remove the culture medium from the plate and add 1 ml of Transfection Buffer A. Make sure that all areas of the plate are covered with Transfection Buffer A, to prevent the cells from drying out.
- Mix 0.5 µg of BD BaculoGold Baculovirus DNA and 2 µg of recombinant plasmid DNA containing your gene, in a sterile 1.5 ml Eppendorf tube.
- Let mixture sit for 5 min before adding 1 ml Transfection Buffer B.
- Mix well.
- Add 1 ml of the Transfection Buffer B/DNA solution 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 with the Transfection Buffer A. During this procedure, a fine precipitate should form making the solution slightly milky.
- Incubate the plate at 27°C for 4 hr.
- After 4 hr, remove the transfection solution from the plate and add 3 ml of TNM-FH insect medium. Rock the plate back and forth several times before once again removing all the medium. Add 3 ml fresh TNM-FH medium and incubate the plates at 27°C for 4 to 5 days. To maintain a humid environment, place a moist paper towel in a dish in the bottom of the 27°C incubator.
- After 4 days, collect the supernatant and infect more BD Biosciences Pharmingen™ Customer/Technical Service • Toll Free 877-232-8995

**Plaque Assay for Insect Cells**

- Seed 7 x 10⁶ Sf9 cells/10 cm plate (70-80% confluent), or 2.5 x 10⁶ Sf9 cells/60 mm plate (70-80% confluent).
- Let cells attach for 5 min at room temperature.
- Make serial dilutions (10⁻⁴ – 10⁻⁷) of your co-transfection virus supernatant. Add 1 ml of diluted viral supernatant to the medium of each plate.
- Let infection occur for 1 hr at room temperature. Rock every 15 min for 1 hr.
- In the meantime, prepare a 2% solution of Plaque Assay Agarose, Cat. No. 554766, in sterile water. Heat mixture in microwave oven to 60°C and dissolve agarose completely. Solution should stay clear (don’t overheat).
- After cooling down to 42°C, add 1 volume of 2X Grace’s Medium (LTI, Cat No. 11667) prewarmed to 42°C. Mix well. The final agarose concentration should be 1%.
- If you will be amplifying your virus from a single plaque, or if color selection is required, add X-gal to the agarose solution to a final concentration of 250 µg/ml. Colorless plaques will be the recombinant BD BaculoGold Linearized Baculovirus DNA.
- Aspirate medium containing the virus inoculum. Overlay cells with 4 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 agarase is completely hardened, approximately 10-15 min.
- 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 agarase containing the plaque using a sterile Pasteur pipet. Elute the virus particles by rotating the agar plug in 700 µl of TNM-FH medium at 4°C overnight. 100 µl of this

**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. To this end, freshly seeded insect cells should be infected at a multiplicity of infection (MOI) of >1. This is usually done by infecting 5 x 10⁶ cells per 10 cm plate (approximately 60% confluent) with 500 µl of transfection supernatant or 100 µl of the plaque pick-up viral supernatant in 15 ml of TNM-FH medium.

The cells should be incubated at 27°C for 3 days before harvesting the medium. At 24 hr past infection, virus-infected cells can be visualized by fluorescence microscopy. The medium will contain at least 10⁷ 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 10⁸/ml virus particles.