

Generation of recombinant baculoviruses by co-transfection

(modified from PharMingen, WS, 6-10-96)

For co-transfection prepare at least 10 μ g of highly purified plasmid DNA. *Spodoptera frugiperda* cells are sensitive to some contaminants found in crude plasmid preparations, which cannot be removed by phenol/chloroform extraction or ethanol precipitation. The only consistently reliable method for plasmid purification is cesium chloride gradient centrifugation (we have protocol in the lab). Quickprep miniprep using an isolation filter may work, but DNA **must** be sterile. Impure preparations of plasmid DNA are toxic to the cells, and many cells may lyse shortly after transfection. This results in an apparently lower viral titer. At about 24 hr post-transfection, Sf9/Sf21 cell viability should be greater than 97%. (Dr. Peter Snow (P.S) just use Wizard miniprep in addition of some special precautions.)

Material needed

- * Sf9 cell in log phase (in a spinner flask)
- * 3 tissue culture plates (60 mm)
- * 15 mls TMN-FH insect medium complemented with 10% Fetal Calf Serum * 0.5 μ g BaculoGoldTM DNA (cat.# 21100D)
- * 50 μ l Wildtype AcNPV Virus supernatant (cat. # 21103E)
- * 2 μ g Recombinant Baculovirus Transfer Vector DNA containing your gene
- * 1 ml Transfection Buffer A (Grace's Medium with 10% Fetal Calf Serum)
- * 1 ml Transfection Buffer B (25 mM Hepes pH 7.1, 125 mM CaCl₂, 140 mM NaCl)

1. Set up three plates for each transfection to provide cells for the co-transfection, as well as for the positive and negative control. Seed 2 x 10⁶ Sf9 cells onto each 60 mm tissue culture plates. This should be done on a flat and even surface. Allow the cells to attach firmly which takes usually 15-20 minutes. If cells don't attach after that time, they are either not healthy or wrong plates (e.g., non-coated petri dishes) have been used.
2. If you make Transfection buffer A and B by yourself, make them now. (buffer A: Grace's Medium with 10% FCS, 1% PenStrep, filter buffer B through 0.2 μ m filter by syringe) Precipitate your DNA (routine protocol, with 1/10 3M NaAcetate, pH 5.2, and 2.5 vol of EtOH), spin down, take off supernatant immediately. Speedvac immediately to keep sterile. Redissolve DNA in filtered TE buffer ~50 μ l in the hood.
3. While cells are attaching to the plate, mix 0.5 μ g of viral DNA (e.g., BaculoGold" DNA, P.S only use as less as 0.12 μ g) and 2-5 μ g recombinant baculovirus transfer vector DNA (P.S use up to 5-10 μ g of DNA) in a microcentrifuge tube. Let mixture sit for five minutes at room temperature before adding 1 ml (P.S use 0.75 ml, all of these is to make the vector DNA more concentrated.) of Transfection buffer B (125 mM Hepes, pH 7.1, 125 mM CaCl₂, 140 mM NaCl). Mix well.
4. Label one plate as co-transfection plate. Remove the old medium from the plate and replace it with 1ml (P.S use 0.75 ml) of Transfection Buffer A (Grace's Medium with 10% FCS). Let the medium stream flow down the wall of the plate very slowly so that it doesn't

perturb the cells. Make sure that the entire surface of plate is covered, so the cells won't dry out.

5. Label one plate as a positive control. Aspirate old medium and replace with 3 ml of fresh TMN-FH medium. Add 50 μ l of wildtype AcNPV virus supernatant to the cells.

6. Label one plate as a negative control. Aspirate old medium and replace with 3 ml of fresh TMN-FH medium and add nothing to this plate.

7. Add the 1 ml of Transfection Buffer B/DNA solution drop-by-drop to the co-transfection plate. After every three to five drops, gently rock the plate back and forth to mix the drops with the medium. (Gently rock the plate all the time during this procedure) During this procedure, a fine calcium phosphate/ DNA precipitate should form.

8. Incubate all three plates at 27' C for 5 hours.

9. After 5 hours, remove the medium from the co-transfection plate. Add 4 ml fresh TMN-FH medium and rock the plate back and forth for a couple of times before removing once again all the medium. Add 4 ml of fresh TMN-FH medium and incubate the plate at 27' C for 4-5 days. Keep the positive and negative control at the same place, but don't change the medium.

10. After 4 days, check the three plates for signs of infection. Compare the negative and positive controls to the co-transfection plate. Infected cells will appear much larger than uninfected ones. Infected cells have large nuclei and often float in the medium.

11. After 5 days collect the supernatant of the co-transfection plate. Identify recombinant viruses by plaque screening and/or end-point dilution. The transfection supernatant may be amplified to produce a high-titer stock solution. Alternatively, a plaque purification may be done to start from a single recombinant virus. Optionally, lyse the transfected cells or use an aliquot of the supernatant to check for expression of your protein of interest (depending whether the recombinant protein is secreted or not).

12. The virus supernatant can be stored in the dark at 4'C over a period of up to 6 months.

Note: Instead of Sf9 cells, Sf21 cells may also be used.