Co-Transfection (Homologous Recombination) of plasmid DNA and baculovirus DNA into SF9 cells: For commercial baculovirus DNA's use method A and for homemade K/M baculovirus DNA's use either method A or B.

A. 6-well plate method
   1. Plate $2x10^6$ SF9 in 6-well plate wells and allow to adhere for at least 1 hour. Add extra medium if the volume is low. Make one well for a negative control.
   2. Pipet 5uL of linearized baculovirus DNA and 1ug of plasmid DNA into an eppendorf tube. Add 500uL of Transfection Buffer B.
   3. Aspirate the medium from the plated SF9's and gently wash each well with 3mL <u>un-supplemented</u> Grace's medium. Add 500uL Transfection buffer A to each well.
   4. Using a 9" plugged Pasteur pipet, drop the Buffer B/DNA mixture onto the SF9 cell layer, gently rocking the plate as the drops are added. An opalescent precipitate forms during the incubation.
   5. Incubate the cells at 27^oC 4 hours. Longer incubation is toxic to the cells.
   6. Aspirate the DNA mixture and wash each well carefully with 3mL <u>un-supplemented</u> Grace's medium. Add 3mL TMN/FH.
   7. Incubate at 27^oC for 10-14 days, harvesting the supernatant when most of the cells are dead. Sapphire DNA keeps cells alive longer than Baculogold DNA does, and BacVector 3000 is very slow because its efficiency seems to be lower.

B. T25 Flask method
   1. Plate $5x10^6$ SF9 cells per flask, making one extra flask for the negative control.
   2. Follow the method outlined in Section A, using the following volumes and amounts:
      10uL baculovirus DNA at 0.1ug/uL
      2ug plasmid DNA
      1mL Transfection Buffer B
      5mL <u>Un-supplemented</u> Grace's medium wash
      1mL Transfection Buffer A
      5mL <u>Un-supplemented</u> Grace's medium wash
      7mL TMN/FH