Bacmid transfection (P0)

1. Count insect cells and plate 1 million cells per well in a 6-well plate. If the cells are too dense, dilute them to 0.5-1 cells/ml so that there is enough liquid to cover the bottom of the well.
2. Let incubate for 20 minutes in a humidity chamber with a wet paper towel
3. For each transfection mix:
    a. Tube A – 2 µgr bacmid + 100 µl grace media
    b. Tube B – 6 µl FuGene HD + 100 µl grace media
4. Let tubes incubate for 5 minutes
5. Mix tube A + tube B and incubate for 15 minutes
6. Remove media from cells
7. Add 800 µl grace media to the cells, gently, on the wall of the well, so not to disturb the cells.
8. Add the transfection mix gently to the cells from above, drop-wise
9. Incubate for 4-5 hours
10. Add 2 ml of full media (ESF 921)
11. Incubate for 4-5 days. If using DH10EMBacVSV, monitor infection by fluorescence in the red channel
12. Harvest virus by spinning cells in a conical tube at 4000 rpm. Transfer supernatant to a new, sterile tube. Use all the P0 to infect 30 ml of insect cells at 1E6 cells/ml to generate P1. Incubate for 3-4 days
13. Make P2: use 3 different concentrations (typically 5 µl, 50 µl, and 500 µl) of P1 to infect 200 ml of insect cells at 1E6 cells/ml. Incubate for 3 days.
14. Spin down at 4000 g for 10 minutes. Add 5% FBS and filter sterilize. This is your P2.