Transformation of DH10EMBacVSV/Y

1. Dilute sequenced plasmid to 100 ng/µl using SOC. Make sure you have at least 5 µl.
2. Mix 100 ng of the diluted plasmid with 50 µl chemical-competent DH10EMBacVSV cells (or any other DH10Bac strain).
3. Incubated on ice for 30 min (10 minutes if cells are Mix&Go)
4. Heat shock at 42 °C for 15 seconds and place cells again quickly on ice. (you can skip this step if the cells are Mix&Go)
5. Add 450µl prewarmed SOC media.
6. Incubate cells at 37 °C for 4-5 hours, shaking
7. In this time prepare agar plates containing:
   - kanamycin (50 µg/ml)
   - gentamycin (7 µg/ml)
   - tetracyclin (10 µg/ml)
   - IPTG (40 µg/ml)
   - BluoGal (500 µg/ml) or X-gal (1000 mg/ml)
     - Prepare fresh from powder – don't use frozen DMSO stocks!
     - Media must be buffered to pH 7.0 - 8.0.
     - Bluo-Gal must be used at a final concentration of at least 300 µg/ml in top agar and autoclaved media.
     - Bluo-Gal should be added to autoclaved media after it has cooled to 45-55°C.
     - Both IPTG and bluogal can be spread onto the plate once it has solidified
8. Plate on two agar plates, 100 µl on one plate, and 100 µl of a 1:10 dilution in SOC on the other plate
9. Incubate at 37 °C 24 hours and select for white colonies. Deeper blue and white color colonies become more visible after leaving the plates for an additional day on the bench at room temperature
10. Proceed to bacmid preparation for insect cell infection

Isolation of bacmid DNA

01. Using a sterile pipette tip, inoculate a single, isolated DH10Bac *E. coli* colony into 5 ml of LB medium containing (same as agar, but omitting bluo-gal and IPTG):
    - 50ug/ml kanamycin
    - 7ug/ml gentamicin
    - 10ug/ml tetracycline
02. Grow the cultures at 37°C with shaking at 220 rpm for ~17 hours.
03. Prepare a glycerol stock of each recombinant bacterial clone by mixing 0.5 ml of overnight culture with 0.2 ml of sterile 50% glycerol in a cryovial. Store glycerol stocks at -80°C.
04. Spin down the remaining volume of cells 5 min, 4000 × g, 4◦C, and discard the supernatant
05. Resuspend in 300 µL P1 buffer
06. Add 300 µL P2 buffer and mix
07. Add 300 µL **P3** buffer and mix. Precipitate should form.
08. Centrifuge 10 minutes at maximum speed in microfuge
09. Transfer supernatant to new tube, spin again 10 minutes
10. Transfer supernatant to new tube
11. Add 800 µL isopropanol, invert several times, store on ice for ten minutes
12. Centrifuge the sample for 15 minutes at maximum speed, 4°C
13. Carefully remove the supernatant, taking care to not disturb the pellet.
14. Add 1ml of 70% ethanol and invert the tube several times to wash the pellet
15. Centrifuge for 1 minute, 4°C and discarding supernatant
16. Repeat wash and discarding supernatant
17. Spin again for 1 minute and remove additional liquids
18. Open tube in TC hood and let dry for 30 minutes. Do not over-dry the pellet.
19. Dissolve the DNA pellet in 100ul of sterile water. To avoid shearing, do not mechanically resuspend the DNA. Allow the solution to sit in the tube with occasional gentle tapping of the bottom of the tube.
20. Spin at max speed in cold microfuge. Small pellet will form. Bacmid is in the supernatant. Transfer to a new tube, measure concentration, and label
21. Verify bacmid with PCR (see protocol below)

22. Proceed with insect cell transfection (see protocol below) or store the bacmid at either -20 or -80

Bacmid PCR verification

Check by PCR that your purified bacmids have recombined. Not all white colonies are full length!

Create a 5 µM primer mix:

- 1 µl M13F (CCCAGTCACGACGTTGTAAAACG)
- 1 µl M13R (AGCGGATAACAATTTCACACAGG)
- 18 µl nuclease free water

PCR reaction:

- 1 µl Bacmid DNA (diluted to 100 ng)
- 1 µl primer mix
- 5 µl Q5 hot start high-fidelity 2X master mix
- 3 µl water

Run method:

	Temp	Time
Denature	98 °C	30 sec
Denature	98 °C	10 sec
Anneal	64 °C	20 sec
Extend	72 °C	5 min
25 cycles		
Extend	72 °C	7 min

Empty bacmid will yield a 300 bp fragment.
Recombined bacmid will give a fragment of 2300 bp + the size of the insert.