Transformation of DH10Bac cells

1. Prepare LB agar plates containing 50 ug/mL kanamycin, 7 ug/mL gentamicin, 10 ug/mL tetracycline, 100 ug/mL Bluo-gal, and 40 ug/mL IPTG.
2. Add plasmid DNA to the competent cells and mix gently.
3. Incubate the mixture on ice for 30 minutes.
4. Heat-shock the mixture at 42°C for 45 seconds. Chill on ice for 2 minutes.
5. Dispense 800 µl of S.O.C. medium.
6. Place in shaking incubator at 37°C with for 4-5 hours.
7. Spread 200 µl of culture evenly over the surface of the LB agar plate. Store the rest of the culture at 4°C. Incubate the plates at 37°C for 2 days.
8. Streak white colonies onto a fresh plate. Incubate the plates at 37°C for 2 days.

Solution	Stock concentration	Storage
Gentamicin	10 mg/ml in water	- 20°C
Kanamycin	100 mg/ml in water	- 20°C
Tetracycline	10 mg/ml in 70% ethanol	- 20°C
Bluo-gal	20 mg/ml in DMSO	- 20°C
IPTG	1M	- 20°C

Isolation of bacmid DNA

Day 1: inoculate bacterial cultures

1. Using a sterile pipette tip, inoculate a single, isolated DH10Bac *E. coli* colony into 5 ml of LB medium containing 50ug/ml kanamycin, 7ug/ml gentamicin, and 10ug/ml tetracycline.
2. Grow the cultures at 37°C with shaking at 240 rpm for ~17 hours.

Day 2: isolate bacmid DNA

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.

1. Spin down the remaining volume of cells in 15ml conical tube.
2. Follow Qiagen miniprep protocol up to the addition of N3.
3. Spin at 4°C for 10 minutes.
4. Transfer supernatant to fresh tube and spin again.
5. Gently transfer the supernatant to a 2ml microcentrifuge tube containing equal volume of cold isopropanol. Invert the tube a few times to mix and place on ice for 20 minutes.
6. Centrifuge the sample for 15 minutes at 15,000 rpm at 4°C.
7. Carefully remove the supernatant, taking care to not disturb the pellet. Add 1ml of 70% ethanol and invert the tube several times to wash the pellet.
8. Centrifuge the sample for 10 minutes at 15,000 rpm at room temperature.
9. Remove as much of the supernatant as possible, taking care to not disturb the pellet. Air dry the pellet for 10 minutes at room temperature. Do not over-dry the pellet.
10. 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.
11. Aliquot and freeze in at -80°C.

Transfection of Sf9 cells with bacmid DNA (P1 virus)

1. Culture Sf9 cells to a density of $2x10^6$ cells/ml in SF900II media + 5% FBS + 1% antibiotics.

2. Dilute cells to $1x10^6$ cells/ml.

3. Seed each well of a 6-well plate with 1ml of $1x10^6$/ml cells to achieve ~70% confluence. Allow the cells to attach for 30min at 28°C. Remove the media and wash with 2ml of unsupplemented media once, and add 1ml of unsupplemented media.

4. Prepare Transfection Mix A
    a. Vortex Cellfectin II
    b. Add 64ul Cellfectin II to 600ul unsupplemented media to microcentrifuge tube.

5. Prepare Transfection Mix B
    a. Add 8ug bacmid DNA to 600ul unsupplemented media.
    b. Mix by swirling with pipet DNA to not shear DNA and let incubate for 5 minutes.

6. Add Transfection Mix A to B
    a. Mix by swirling and pipetting only once and incubate for 30min at room temp.

7. Add combined transfection mixture (~210ul) dropwise onto each well and incubate at 28°C for 4 hours.

8. Remove the transfection mixture, wash with 2ml supplemented media, and add 2.5 ml of supplemented media.

9. Incubate cells for 5 days.

10. Harvest media from each well and spin at 1500xg for 5 minutes.

11. If not using immediately, filter using 0.22micron, cover tube with foil and place in cold room.

Making P2 virus

1. Culture Sf9 cells to a density of $2x10^6$ cells/ml in SF900II media + 5% FBS + 1% antibiotics.
2. Add 40ml of cells to a T225 flask and place in 28°C incubator for 30 minutes.
3. Aspirate media and pipet 5ml of P1 virus into flask.
4. Place flask into incubator for 1 hour. Every 20 minutes, rock the flask to cover all the cells with virus.
5. Add 50ml of supplemented media to the flask and place in incubator for 3 days.
6. If not using immediately, filter using 0.22micron, cover tube with foil and place in cold room.

Making P3 virus

1. Culture 500ml of Sf9 cells to a density of $2x10^6$ cells/ml in SF900II media + 5% FBS + 1% antibiotics.
2. Add 50ml of P2 virus and place in incubator shaker for 3 days.
3. Spin down cells at 4000xg for 30 minutes.
4. Filter using 0.22micron, cover bottle with foil and place in cold room.

Making P4 virus

1. Culture 500ml of Sf9 cells to a density of 2x106 cells/ml in SF900II media + 5% FBS + 1% antibiotics.
2. Add 50ml of P3 virus and place in incubator shaker for 3 days.
3. Spin down cells at 4000xg for 30 minutes.
4. Filter using 0.22micron, cover bottle with foil and place in cold room.