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 (same as agar, but omitting bluo-gal and IPTG):
    - 50ug/ml kanamycin
    - 7ug/ml gentamicin
    - 10ug/ml tetracycline
2. Grow the cultures at 37°C with shaking at 240 rpm for ~17 hours.

Day 2: isolate bacmid DNA

3. 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.
4. Spin down the remaining volume of cells 5 min, 4000 × g, 4◦C, and discard the supernatant
5. Resuspend in 300 µL P1 buffer
6. Add 300 µL P2 buffer and mix
7. Add 300 µL **P3** buffer and mix. Precipitate should form.
8. Centrifuge 10 minutes at maximum speed in microfuge
9. 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. Spin at maximum speed in cold microfuge
13. Aspirate supernatant and discard.
14. Wash pellet twice with 1 mL 70% ethanol, spinning in cold centrifuge for 1 minute after each wash and discarding supernatant
15. Discard supernatant
16. Spin again for 1 minute and remove additional liquids
17. Open tube in TC hood and let dry for 30 minutes. Do not over-dry the pellet.
18. 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.
19. 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
20. Store at -20 or -80