

Transformation of DH10EMBacVSV

1. Mix 100 ng (maximally 10 µl volume) of the sequenced plasmid with 50-100µl chemical-competent DH10EMBacVSV cells
2. incubated on ice (30 min)
3. 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)
4. Add 500µl prewarmed SOC media.
5. Incubate cells at 37 °C for 4 hours, shaking
6. In this time prepare agar plates containing:
 - kanamycin (50 µg/ml)
 - gentamycin (7 µg/ml)
 - tetracyclin (10 µg/ml)
 - BluoGal (100 µg/ml) or X-gal (500 µg/ml)
 - IPTG (40 µg/ml).
7. Plate on two agar plates, 200µl on one plate, and 20µl cells + 180 µl SOC on the other plate
8. 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
9. Proceed to bacmid preparation for insect cell infection