

Transformation of DH10EMBacVSV

1. Dilute sequenced plasmid to 20 ng/ μ l using SOC. Make sure you have at least 5 μ l.
2. Mix 5 μ l of the diluted plasmid (total of 100 ng) with 50 μ l 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 500 μ l prewarmed SOC media.
6. Incubate cells at 37 °C for 4 hours, shaking
7. In this time prepare agar plates containing:
 - kanamycin (50 μ g/ml)
 - gentamycin (7 μ g/ml)
 - tetracyclin (10 μ g/ml)
 - BluoGal (100 μ g/ml) or X-gal (500 μ g/ml)
 - IPTG (40 μ g/ml).
8. Plate on two agar plates, 200 μ l on one plate, and 20 μ l cells + 180 μ l 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