

Selection for Tn mutants

1. First perform transformation and selection on Kanamycin (40) plates.
2. Pick up 50-300 Kanamycin-resistant colonies and combine together.
3. Passage the suspension through a 26G needle 3x
4. Centrifuge for 2 min at 1,500 rpm, 25° C
5. Transfer the supernatant to a new 15 ml conical tube
6. Centrifuge for 2 min at 1,000rpm, 25° C
7. Transfer the supernatant to a new 15 ml conical tube
8. Adjust the density of *M. marinum* using 7H9 to 10⁸/ml
9. Plate 100 µl onto each of the 7H10 plates containing Kanamycin(40)+Sucrose(10%).