

Conjugation Assay Protocol

Overall Goal and generally what we need to do:

- Assay for conjugation of pARO190 from S17-1 to JM109.
- Find the conjugation rate or how much conjugation occurs compared to all cells.
Ex. 1% of cells conjugate(d)?

1. Day 1: Transform pARO190 into S17-1 through electroporation.
2. Day 2: Create liquid cultures of the S17-1 + pARO190 with LB+Carb. Create liquid cultures of JM109 in LB + Nalidixic Acid. Incubate at 37°C

*If there is no Nalidixic Acid, create a stock of 30ml, concentration 30mg/ml.
900mg Nalidixic Acid + 360mg NaOH + 30mL ddH₂O. Filter*

3. Day 3: Take the OD600 of the cultures and note them down
4. Spin at 3000rpm for 2mins in a 4°C centrifuge and then discard the supernatant. Do another spin at 3000rpm for 2mins after resuspending the pellet with LB(-) or PBS (Do this twice). Then resuspend in 5mL of LB(-)
5. Before creating co-cultures in the next step, create plates with dilutions of the S17-1+pARO190 and JM109 with dilutions of 10^{-3} , 10^{-4} , 10^{-5} as to estimate initial bacterial densities in the end. Use Carb plates for S17-1+pARO190 and Nalidixic Acid plates for JM109.
6. Make co-cultures of a 100-fold, and no dilution of both S17-1 and JM109 overnight cultures. Create the co-culture of 5mL for a 2 hour, 5 hour, and 8 hour incubation time, each with the three dilution amounts. Incubate on a gentle shaker (100rpm) at 37°C.
7. Prepare amount needed of Carb, Carb + Nalidixic Acid, and Nalidixic Acid plates by warming them in the 37°C incubator.
8. After incubation of the co-cultures, vigorously mix the co-culture by vortexing. Then make serial dilutions (10^{-2} , 10^{-3} , 10^{-4}). Plate 200 uL of the dilutions onto the
 - a. LB + Carb
 - b. LB + Nalidixic Acid
 - c. LB + Carb + Nalidixic Acid plates.
9. Let them sit for 15mins to settle into the plate. Then incubate the plates overnight
10. The next day, count the cells on each plate. Subtract the amount of cells from the Carb plate by the amount of cells by the Carb + Nalidixic Acid plate and the % of conjugation can be calculated from there.