P1 Transduction

Prepare P1 Lysate

1. Grow o/n culture of strain you want to make P1 lystate of (has selective marker).

2. Dilute o/n culture 1/100 in 10 ml LB + 5 mM $CaCl_2$ + 0.2% glucose.

3. Grow 1 h at 37°C, no shaking. For strains that have to be grown at 32 ° C, grow for 1.5 h.

4. Add 200 µl of P1 lysate (IN THE HOOD) from stock**

5. Incubate at 37°C with shaking for 3 h or until lysis occurs (typically >2 h).

6. Transfer to 15 ml polypropylene tubes (IN THE HOOD) and add 200 µl $CHCl_3$. Mix by vortexing. N.B. do not use polystyrene tubes as these will react with the $CHCl_3$.

7. Centrifuge at full speed for 5 minutes.

8. Collect lysate and store with 200 µl $CHCl_3$. Use in P1 transduction.

**To make more P1 lystate, amplify on strain MG1655 or another “wt” strain

P1 Transduction

1. Label 4 eppendorf tubes # 1-4. Add 0.5 ml LB + 15mM $CaCl_2$ + 30mM $MgCl_2$ to each. Add 0.5 ml LB to tubes #2-4.

2. Add 0.5 ml P1 lystate to tube #1. Transfer 100 µl from tube #1 to tube #2. Mix. Transfer 100 µl from tube #2 to tube #3. Mix.

3. Add 200 µl fresh *E. coli* (recipient) o/n culture to each tube. Incubate 20 min at 37 °C.

4. Pellet cells and resuspend in 1 ml LB + 20 mM sodium citrate. Incubate 1 hour at 37°C, no shaking.

5. Pellet and resuspend in 1ml TM (10mM Tris HCL pH 7.5, 10 mM $MgCl_2$).

6. Pellet and resuspend in 50 µl TM. Plate on selective media (antibiotic marker is being transferred by the P1 lysate). If the donor strain is *lac*$^-$ and the recipient strain is *lac*$^+$ plate onto MacConkey + lactose + antibiotic plates and select only red colonies.

7. Verify strain by PCR.