P22 Transduction of *Salmonella*

Pre-experiment: Make sure that your strain has the correct O-antigen. P22 will bind to serogroups A, B, and D1.

P22 infection with Donor strain

In this step, you will be infecting your donor strain (harboring some sort of selective marker such as an antibiotic cassette).

1) Inoculate 5 mL of LB broth (supplemented with the correct concentration of your selective agent) with 1 cfu of your donor strain. Grow shaking at 37°C at 200 rpm for 16-20 hr.
   a) *Alternative: You can grow your culture shaking for 8-12 hours and use that the same day for step 2.*
2) Add 200 µL of your overnight/late log-phase culture of your donor strain to 1 mL of $5x10^6$ pfu/mL P22 lysate in a 1.5 mL Eppendorf tube.
3) Grow at 37°C under static conditions, for 16-20 hr.

P22 lysates containing DNA from Donor Strain

In this step, you will harvest the P22 with DNA from your donor strain.

1) Add 80 µL chloroform to 1 mL of your 16-20 hr P22 phage lysate and incubate at 37°C for 20 mins in a heating block.
2) Centrifuge cell debris at 13,000 rpm for 3 min, and transfer supernatant to a clean 1.5 mL eppendorf tube.
3) Spot 15 uL lysate onto a BHI plate to check for live bacteria.
4) Store the lysate at 4°C (note: can be stored for multiple years without a significant loss in infectivity) OR use the lysate to infect a mid-log phase culture of your donor strain.

Transduction of recipient strain with P22 lysates from donor strain

1) Inoculated 5 mL of LB + antibiotics with one cfu of recipient strain and grew shaking at 200 rpm at 37°C.
   a. *Alternative: set up an overnight culture the day before*
2) Sub-culture strains (1:100) and grow bacteria at 37°C (shaking at 200 rpm) to mid-log phase.
3) Add10 uL lysate to 500 uL recipient strain culture. (only do this step if the spot-checks performed above were clear).
4) Incubate at 37°C without shaking in a heating block for 30 mins.
5) Add 500 uL 20 mM EGTA, incubate 1 hour at 37°C with shaking (200 rpm).
6) Centrifuge at 10,000 rpm for 3 min, and discard supernatant with a P1000 pipette.
7) Re-suspend pellet in 500 uL LB.
8) Spread 50 or 100 µL onto selective media (such as LB – kanamycin) supplemented with 10mM EGTA.

Confirm mutation

1) Patch transformed bacteria onto a fresh LB –antibiotic plate supplemented with 10 mM EGTA.
2) Perform colony PCR to confirm recipient strain has the desired mutation.