P1 Phage Transduction Protocol

(Eric Klein 3/27/2015)

Note: All steps MUST be performed with filter tips

P1 Lysate Preparation

1. Start overnight culture of donor strain in LB + appropriate antibiotic
2. Dilute overnight culture 1:100 into 5 ml LB + 5 mM $CaCl_2$ + 0.2% glucose
3. Grow for 30-45 minutes or until slightly cloudy
4. Add 50 µl P1 phage
5. Grow culture for 2-4 hours (until culture becomes clear)
6. Add 500 µl chloroform and vortex
7. Spin culture for 10 min at 3000 rpm
8. Transfer 3-4 ml of the supernatant to a sterile vial

Phage Transduction

1. Grow overnight culture (5 ml) of recipient strain
2. Centrifuge the culture at 1500xg for 10 min
3. Resuspend the pellet in 1 ml of 10 mM $MgSO_4$ + 5 mM $CaCl_2$
4. For each transduction, add 100 µl of cells to an eppendorf tube
5. Add 50 µl of P1 lysate and incubate for 30 min at 30°C without shaking (on heat block)
6. Add 1 ml of LB + 10 mM sodium citrate
7. Incubate for 30 min at 30°C without shaking (on heat block)
8. Centrifuge for 2 min at 8000 rpm
9. Resuspend pellet in 100 µl of 1M sodium citrate
10. Plate onto selective LB plates