

Transduction of Salmonella with P22

(Anthea's modifications)

Donor:

1. Grow donor standing overnight (~2 mls)
2. Add 0.5 mL of overnight culture to 5 mls LB, incubate 37°C (standing) for 1-2 hours
3. Add 100 uL P22 and incubate standing 37°C standing overnight
4. Add 200 uL chloroform and switch to plastic 15 mL conical tube. Vortex vigorously
5. Centrifuge @ maximum speed on tabletop centrifuge for 10 minutes.
6. Transfer clear liquid to new 15 mL tube and add 200 uL chloroform to store in cold room

Recipient:

1. Grow recipient standing overnight (~2mls)
2. Add 0.5 mL of overnight culture to 5 mLs LB and grow 5-6 hours standing (~ 2×10^8 cells/ml)
3. Add 100 uL of phage to tube
4. Incubate standing 37°C for 10 minutes. At same time, dry a selective plate in the plate-dryer.
5. To dry plate, add 100uL 0.5M EGTA + 100 uL infected bacteria. Spread onto selective plate. Incubate overnight to get colonies.