Transformation

Protocol:

1. Take competent cells out of -80°C. Thaw the competent cells (100 µl) **on ice** for 5 minutes.

2. Add 5 µl of ligation product to the competent cells and mix gently by stirring the mixture.

3. Incubate the cells on ice for 20 minutes.

4. Heat shock at 42°C for 30 seconds.

5. Put the tubes back on ice for 5 min.

6. Add 200 µl of LB medium into the tube.

7. Incubate the mixture in 37°C shaking incubator for 60 minutes. Shake vigorously (250 rpm).

8. Plate 2 µl, 20 µl and the rest of each cell culture onto three LB agar plate containing appropriate antibiotic respectively*.

   * The concentrations corresponding to the antibiotics used

Antibiotics	**Concentration (µg/ml)**
Ampicillin	100
Chloramphenicol	34

9. Incubate the plates at 37°C overnight.
10. Pick colonies.