

Transformation

This page shows the protocol for the transformation of competent Escherichia coli.

1. Thaw competent cells on ice 5 minutes before use. Label each tube accordingly. Take out an extra tube for the positive control and another one for the negative control.
2. Add 1ul of DNA to each tube.
3. Incubate on ice for 30 minutes. Preheat a thermal block at 42°C. Place SOC media in the block..
4. Heat-shock the bacteria by placing them on the heat block for 90s.
5. Put on ice 5 minutes.
6. Add 300ul of SOC media to each tube.
7. Shake at 37°C and 220rpm for the time necessary:
Ampicillin: 1h, Kanamycin and Chloranphenicol: 1:30h,
Tetracycline: 2h.

8. Add 100ul of the transformed bacteria to a plate of LB with Agar and the respective antibiotic, spread with a Drigalski spatula. Let the medium absorb the culture.
9. Incubate overnight at 37°C.