Transformation of Escherichia coli

Material

Competent cell

Luria-Bertani liquid medium

DNA

Procedure

1. Remove competent cells from freezer and allow to thaw on ice for 10 min
2. Take care not to disturb the competent E.coli and do not vortex them or pipette them up and down.
3. Add 50 µL of thawed competent cells and then 1 -10 µL of the re-suspended DNA to the labeled tubes. Make sure to keep the competent cells on ice.
4. Incubate the cells on ice for 30 minutes.
5. Heating shock the cells by immersion in pre-heated metal bath at 42°C for 75 seconds. A water bath improves heat transfer to the cells.
6. Incubate the cells on ice for 5 minutes.
7. Add 500µl of Luria-Bertani liquid medium to the tube
8. Incubate the cells at 37°C for 1 hours at a speed of 200 rmp .
9. Prepare several dishes with LB agar and the appropriate antibiotic(s) with the part number, plasmid, and antibiotic resistance. Plate 20 µl to 200 µl of the transformation onto the dishes, and spread.
10. Incubate the plate at 37°C for 12-14 hours
11. Always keep agar plates upside down so that drips of condensation and falling debris do not contaminate them.