Technic : Transformation

Goal: Introduce recombinant plasmid DNA (ligation product) into competent *E. coli* XL10 Gold by heat shock, followed by recovery and plating on selective medium.

Procédure:

1. Thaw competent cells. Retrieve XL10 Gold competent cells from the –80 °C freezer and thaw on ice.
2. DNA addition. Add 10 µL of ligation product to 100 µL of thawed competent cells in a sterile tube. Mix gently by flicking; avoid pipetting up and down.
3. Incubation on ice. Keep the mixture on ice for 30 min at 4 °C.
4. Heat shock. Transfer the tube to a 42 °C water bath or heat block for 30 seconds.
5. Recovery on ice. Immediately place the tube back on ice for 2 min.
6. Add 150 µL of LB
7. Outgrowth. Incubate the tube at 37 °C with shaking (200–250 rpm) for 1 hour to allow expression of antibiotic resistance genes.
8. Plate the resuspension onto a prewarmed LB agar plate containing ampicillin. Spread evenly across the surface ("like a crêpe") to ensure well-distributed colonies.
9. Drying. Allow the plate surface to dry briefly at room temperature until no liquid remains visible.
10. Incubation. Place the plate inverted at 37 °C overnight to allow colony growth.

Reagents and Solutions:

- XL10 Gold chemically competent cells (stored at –80 °C)
- Ligation product (DNA of interest)
- LB agar plates supplemented with antibiotic (ampicillin, final concentration 100 µg/mL)

Equipment:

- Sterile microcentrifuge tubes
- Ice bucket
- Water bath or heat block set to 42 °C
- Incubator/shaker set to 37 °C
- Sterile spreaders or glass beads
- Pipettes and sterile tips
- Petri dish rack

Estimated time: ~ 3 hours

Controls and Troubleshooting:

- Verification of bacterial vitality on antibiotic-free agar, if no bacterial growth in selective media, perhaps antibiotic resistance hasn't had time to develop. In this case, incubate longer at step 7.
- Verification of plasmid antibiotic resistance by spreading untransformed bacteria on selective agar