

# Transformation of chemo-competent E.coli KL740 cells

## Materials:

chemo-competent KL740cl 857+cells

LB medium

LB agar plates

## Procedure:

1. Thaw an aliquot of chemo-competent cells on ice.
2. Add 10-50ng plasmid to a 150 $\mu$ L aliquot of chemo-competent KL740cl 857+cells.
3. Incubate for 25min on ice.
4. Incubate cells for 5min at 37°C using a heat block or water bath.
5. Transfer cell suspension into 2mL LB medium in a 13mL culture tube and incubate for 30min at 29°C and 200rpm.
6. Centrifuge cells for 1min at 3000x g at room temperature, remove 1.9mL of the supernatant, and resuspend cell sediment in the remaining medium.
7. Plate at least 30 $\mu$ L of that cell suspension on a pre-warmed LB agar plate containing the appropriate selection marker (and the entire remaining cell suspension on a separate plate as a back-up) and incubate the plate for 16-24h at 29°C.

[Note: This protocol may be shortened by omitting Step B6 and plating an aliquot of the DNA-cell mix directly onto antibiotic-containing LB agar plates.]