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 150μL aliquot of chemo-competent KL740cl 857+cells.
3. Incubate for 25min on ice.
4. Incubate cells for 5min at 37℃ using a heat block or water bath.
5. Transfer cell suspension into 2mL LB medium in a13mL culture tube and incubate for 30min at 29℃ and 200rpm.
6. Centrifuge cells for 1min at 3000x g at room temperature，remove1.9mL of the supernatant，and resuspend cell sediment in the remaining medium.
7. Plate at least 30uL 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℃.

[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.]