Heat-shock transformation

Reagents:

- SOC broth
- LB broth
- LB agar
    - with ampicillin 100 µg/mL
    - with chloramphenicol 37 µg/mL
    - with both antibiotics
- E. coli DH5-α competent cell (stored at -80 °C)

Procedure:

1. Thaw the competent *E. coli* DH5-α on ice (approximately 20-30 mins)
2. (optional) Pre-warm the agar plates with proper antibiotic(s) at 37 °C
3. Aliquot 50 µL of the cell in round-bottom 2 mL tubes
4. Add 5 µL of purified plasmid (conc. should be above 10 pg/µL). If double transforming with two plasmids add 2.5 µL of each primer, for a final volume of 5 µL of DNA added. GENTLY mix by flicking the bottom of the tube with your finger a few times.
5. Leave the competent cell/DNA mixture on ice for 20-30 mins. **Avoid mixing.**
6. Heat shock the cells by placing each transformation tube for 30 sec. into a heating block prewarmed at 42 °C.
7. Put the tubes back on ice for 5 min.
8. Add 950 µL of SOC (or LB) broth (without antibiotic) to the bacteria and incubate at 37 °C, 250 rpm, for 1 hour.
9. Dilute the transformed cell 10-times in LB broth.
10. Plate 50 µL of the diluted transformed cell onto a LB agar plate containing the appropriate antibiotic.
11. Incubate plates at 37°C, 220 rpm overnight.