Transformation of chemically competent *E. coli* DH5α cells

Introduction

Protocol extracted from Liljeruhm *et al.*, 2014.

Materials

- Material for transformation of ligation reaction
- Material for transformation of an intact plasmid (positive control)
- Material for negative control
- Chemicallycompetent*E. coli* DH5α

Procedure

Transformation - 50µL of competent cells + 10ng of DNA or 5µL of ligation reaction

1. Turn on a water bath or heating block to 42°C.
2. Heat 950 µL of LB media at 37ºC
3. Thaw competent cells on ice for 15 min
4. Add 10ng of DNA or 5µL of ligation reaction to 50 µL of competent cells. DO NOT mix by pipetting and NEVER vortex competent cells, they will die.
5. Incubate for 30 min on ice. This step allows for the diffusion of DNA across the sample so we do not need to mix.
6. Heat shock for 45 s at 42°C.
7. Incubate for 5 min on ice.
8. Add 950 µL of LB media (pre-heated to 37°C on step 2) to the eppendorf with the competent cells.
9. Incubate for 1–1.5 hr at 37°C, with shaking. (45min without shaking and 45min with shaking)
10. Plating (on agar plates containing the appropriate antibiotic):
    - Positive controls: Mix gently and plate either 100 µL (directly to step 13) or full volume (follow steps 11 and 12)
    - Distribution kit: Plate full volume (follow steps 11 and 12)
    - Ligation reactions: Plate full volume (follow steps 11 and 12)
11. If needed, spin cells down from remaining 900 µL at 4000 rpm for 5 min.
12. Discard 800 µL of the supernatant and resuspend the pellet in the remaining 100 µL. Plate the 100 µL.
13. Pipette the suspension on an agar plate with the appropriate antibiotic and spread it using beads or a spreader.
14. A) For beads:

- Put 5 to 10 beads on the plate.
- Shake the plate so it spreads the suspension until all the inoculum has gone into the agar. Discard the beads (in a beaker, to be sterilized again)

B) For spreader:

- Dip the spreader into 95% ethanol.
- Put it into the flame for a second.
- Let the ethanol burn off outside the flame.
- Spread the bacterial suspension evenly until all the inoculum has gone into the agar.
- Put the plates at 37°C overnight.

References

Liljeruhm, J., Gullberg, E., & Forster, A. C. (2014). Synthetic biology: a lab manual.