E. Coli Heatshock Transformation

This protocol allows the transformation of *E. coli* with a plasmid of interest through heatshock.

Materials

- Competent DH10β *E. coli*
- Plasmid DNA
- LB Media
- LB plates (with antibiotics as needed)

Equipment

- 42ºC water bath
- 37ºC shaking incubator
- Microcentrifuge (Eppendorf centrifuge 5424)
- 2 mL sterile Eppendorf tubes
- Ice bucket
- Bunsen burner
- Pipettes and sterile pipette tips (0.5 - 10, 2-20, 20-200, 100 - 1000 µL

Protocol

1. Thaw competent cells on ice for 15 minutes on ice
2. Add 4 µLof DNA to the cells.
3. Incubate for 30 minutes on ice.
4. In the meantime, prepare LB broth and prewarm it at 37 C until use
5. Set the water bath or shaker to 42 ℃; allow it to heat up
6. After 30 minutes, proceed with the heat shock
7. Put cells on 42 C for 90 seconds
8. Immediately transfer back on ice for 5 minutes
9. Repeat heat shock once more
10. Add in 1 mL of the prewarmed LB
11. Recover the cells for 1 hour at 37ºC
12. In the meantime, if not available yet, prepare LB agar plates with the relevant
13. After 1 hour, plate 100 µLon agar plates
14. In the case of expected low efficiency, spin down the remainder of the cells at 6800 g for 3 minutes (or until a visible pellet can be identified)
15. Discard most of the supernatant by decanting the Eppendorf
16. Resuspend pellet in remaining supernatant and plate as described in step 13. 17.
17. Incubate the plates at 37ºC overnight.

E. Coli Electroporation

This protocol allows the transformation of electrocompetent E. coli with a plasmid of interest through electroporation.

Materials

- Electrocompetent E. coli
- Plasmid DNA
- SOC Outgrowth Medium (NEB B9020S)
- LB plates (with antibiotics as needed)

Equipment

- Electroporator
- 37ºC shaking incubator
- Electroporation Cuvettes
- 2 mL sterile Eppendorf tubes
- Ice bucket
- Bunsen burner
- Pipettes and sterile pipette tips (0.5 - 10, 2-20, 20-200, 100 - 1000 µL

Protocol

1. Set electroporator at 1.8 kV, 200 Ω, 25 µF
2. Put the cuvette and the electrocompetent E. coli on ice
3. Turn on the Bunsen burner and work as stericaly as possible
4. In a sterile Eppendorf tube, mix 1-100 ng of plasmid with 100 µLof electrocompetent cells
5. Put the mixed plasmid and cells in the cuvette
6. Prepare to pipette 900 µLof SOC medium as fast as possible
7. Insert the cuvette and pulse
8. Add the SOC medium and pipette up and down 4 times
9. Transfer the cells back into the Eppendorf tube and incubate at 37 ºC (or 30 ºC for a heat-sensitive plasmid) for 1-2 hours, while shaking
10. Plate 50 µLonto LB plates with antibiotic
11. Spin down the remaining contents for 3 minutes at 6800g
12. Remove 800 µLof supernatant and resuspend with the remaining medium and plate on LB plates with antibiotic
13. Incubate the plates at 37ºC overnight