Transformation of *E. coli* by Electroporation

MATERIALS:

- GYT medium (ice cold)
- Glycerol (10% v/v)(molecular biology grade), ice cold
- LB medium, prewarmed to 37˚C
- Plasmid DNA (recombinant plasmid)
- Pure H_2O
- SOB agar plates containing 20mM $MgSO_4$, and the appropriate antibiotic
- SOC medium

METHOD:

1. Inoculate a single colony of E. coli from a fresh agar plate into a flask containing 50mL of LB medium. Incubate the culture overnight at 37˚C with vigorous aeration (250 rpm in a rotary shaker).
2. Inoculate two aliquots of 500mL of prewarmed LB medium in separate 2-liter flasks with 25mL of the overnight bacterial culture. Incubate the flasks at 37˚C with agitation (300 cycles/minute in a rotary shaker). Measure the OD_{600} of the growing bacterial cultures every 20 minutes.
3. When the OD_{600} of the cultures reaches 0.4, rapidly transfer the flasks to an ice-water bath for 15-30 minutes. Swirl the culture occasionally to ensure that cooling occurs evenly. In preparation for the next step, place the centrifuge bottles in an ice-water bath.
4. Transfer the cultures to ice-cold centrifuge bottles. Harvest the cells by centrifugation at 1000g (2500rpm in a Sorvall GSA rotor) for 15 minutes at 4˚C. Decant the supernatant and resuspend the cell pellet in 500mL of ice-cold pure H_2O.
5. Harvest the cells by centrifugation at 1000g (2500rpm in a Sorvall GSA rotor) for 20 minutes at 4˚C. Decant the supernatant and resuspend the cell pellet in 250mL of ice-cold 10% glycerol.
6. Harvest the cells by centrifugation at 1000g (2500rpm in a Sorvall GSA rotor) for 20 minutes at 4˚C. Decant the supernatant and resuspend the cell pellet in 10mL of ice-cold 10% glycerol.
7. Harvest cells by centrifugation at 1000g (2500rpm in a Sorvall GSA rotor) for 20 minutes at 4˚C. Carefully decant the supernatant and use a Pasteur pipette attached to a vacuum line to remove any remaining drops of buffer. Resuspend the pellet in 1mL of ice-cold GYT medium.

8. Measure the OD_{600} of a 1:100 dilution of the cell suspension. Dilute the cell suspension to a concentration of 2 x 10^{10} to 3 x 10^{10} cells/mL (1.0 OD600 = approx. 2.5 x 10^{8} cells/mL) with ice-cold GYT medium.
9. Transfer 40ul of the suspension to an ice-cold electroporation cuvette (0.2cm gap) and test whether arcing occurs when an electrical discharge is applied (see step 16). If so, wash the remainder of the cell suspension once more with ice-cold GYT medium to ensure that the conductivity of the bacterial suspension is sufficiently low (<5 mEq).
10. To use the electrocompetent cells immediately, proceed directly to step 12. Otherwise, store the cells at -70˚C until required. For storage, dispense 40ul aliquots of the cell suspension into sterile, ice-cold 0.5mL microcentrifuge tubes, drop into a bath of liquid nitrogen, and transfer to a -70˚C freezer.
11. To use frozen electrocompetent cells, remove an appropriate number of aliquots of cells from the -70˚C freezer. Store the tubes at room temperature until the bacterial suspensions are thawed and then transfer the tubes to an ice bath.
12. Pipette 40ul of the freshly made (or thawed) electrocompetent cells into ice-cold sterile 0.5mL microcentrifuge tubes. Place the cells on ice, together with the appropriate number of bacterial electroporation cuvettes.
13. Add 10pg to 25ng of the DNA to be electroporated in a volume of 1-2ul to each microcentrifuge tube and incubate the tube on ice for 30-60 seconds. Include all of the appropriate positive and negative controls.
14. Set the electroporation apparatus to deliver an electrical pulse of 25uF capacitance, 2.5kV, and 200 ohm resistance.
15. Pipette the DNA/cell mixture into a cold electroporation cuvette. Tap the solution to ensure that the suspension of bacteria and DNA sits at the bottom of the cuvette. Dry condensation and moisture from the outside of the cuvette. Place the cuvette in the electroporation device.
16. Deliver a pulse of electricity to the cells at the settings indicated above. A time constant of 4.5 milliseconds with a field strength of 12.5kV/cm should register on the machine.
17. As quickly as possible after the pulse, remove the electroporation cuvette and add 1mL of SOC medium at room temperature.
18. Transfer the cells to a 17 x 100mm or 17 x 150mm polypropylene tube and incubate the cultures with gentle rotation for 1 hour at 37C.
19. Plate different volumes (up to 200ul per 90mm plate) of the electroporated cells onto SOB agar medium containing 20mM $MgSO_4$ and the appropriate antibiotic.
20. Store the plates at room temperature until the liquid has been absorbed.
21. Invert the plates and incubate them at 37˚C. Transformed colonies should appear in 12-16 hours.