PROCEDURE FOR PREPARING AND TRANSFORMING ELECTRO-COMPETENT (EC) CELLS

(A) Preparing electro-competent (EC) cells

NOTE: Make sure that all solutions, Eppendorf tubes, centrifuge buckets are prepared, autoclaved and stored in the fridge prior to use. All steps where manipulation of the culture is needed are done under sterile conditions (near a flame). Keep bacterial culture and pellet on ice as much as possible.

1. Prepare seed culture by inoculating a single colony in 5-10 mL of LB broth and incubate overnight (16-20 hours) at 37 °C with 200-250 rpm shaking.
2. Inoculate 400 mL of fresh LB media with 0.05% *(v/v)* of seed culture.
3. Incubate at 37 °C with 200-250 rpm shaking. Periodically measure the OD_{600}.
4. Once the OD_{600} reaches 0.5-0.7, chill culture on ice for 15 minutes.
5. Transfer cells into a sterile and pre-chilled centrifuge bucket.
6. Pellet cells by centrifugation at 4000 rpm for 20 minutes at 4 °C.
7. Decant supernatant and resuspend cells in 5 mL sterile ice-cold ddH_2O.
8. Add 1 culture volume of sterile ddH_2O to the resuspended pellet, ensuring to mix well, then incubate on ice for 5-10 minutes.
9. Pellet cells by centrifugation at 4000 rpm for 20 minutes at 4 °C.
10. Repeat steps 7-9 one more time.
11. Carefully decant supernatant and resuspend cells in 1:10 culture volume with sterile ice-cold ddH_2O + glycerol [10% (*w/v*)].
12. Mix resuspended culture then incubate on ice for 5-10 minutes.
13. Pellet cells by centrifugation at 4000 rpm for 20 minutes at 4 °C.
14. Carefully decant supernatant, resuspend cells in 1:40 culture volume with sterile ice-cold ddH_2O + glycerol [10% (*w/v*)], ensuring to mix well.
15. Aliquot 100 µL into prechilled 1.5 mL Eppendorf tubes.
16. Freeze cells in dry ice and store at -80 °C.

(B) Transforming electro-competent (EC) cells

NOTE: When transforming a ligation reaction, remove residual salts by drop-dialysis or a PCR cleanup. Quantify DNA concentration (if capable) via gel electrophoresis or by nanodrop. Use sterile 1 mm gap electroporation cuvettes (catalog # FB101, Fisherbrand). Keep all components on ice.

1. Thaw electro-competent cells on ice. Add 5 pg – 0.5 µg of DNA (maximum 1 µL) directly into the tube of thawed cells.
2. Mix tube with a pipette or by flicking then transfer contents to a pre-chilled electroporation cuvette.
3. Tap cuvette onto benchtop to settle cells and wipe sides with a KimWipe to remove any residual ice/water. **Ensure the cuvette is completely dry before moving onto the next step**.
4. Set electroporation apparatus to 2.5 kV, 25 µF with a pulse controller set to 200-400 Ω (setting Ec1 for FX lab's apparatus).

5. Place cuvette in sample chamber and apply the pulse. Take note of the time-constant, time constants of ~5 ns are desired.
6. Remove cuvette and immediately add 1 mL of sterile ice-cold LB (or other appropriate media). Resuspend cells in cuvette by mixing with a pipette then transfer to a sterile 1.5 mL Eppendorf tube.
7. Incubate culture at 37 °C with 200-250 rpm shaking for 45 minutes to 1 hour.
8. Centrifuge culture at 8000 g for 1 minute. Decant 900 µL of media and resuspend pellet in the remaining supernatant (~ 100 µL).

NOTE: When transforming a sample for the first time, plate 100 µL of different dilutions (1:100, 1:1000 and 1:1000000) onto different quadrants of the plate. This will allow the identification of the necessary dilution for proper colony resolution.

9. Plate transformants on appropriate antibiotic plate and grow overnight in a 37 °C incubator.