PROCEDURE FOR PREPARING CHEMICALLY COMPETENT (CC) 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).

1. Prepare seed culture. Inoculate in 5-10 mL of LB broth a single colony and incubate overnight (16-20 hours) at 37 °C with 200 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 rpm shaking. Periodically measure the OD_{600}.
4. Once the OD_{600} reaches 0.2-0.4, chill culture on ice for 10 minutes.
5. Pellet cells by centrifugation at 4000 rpm for 30 minutes at 4 °C.
6. Decant supernatant and resuspend cells in 1:4 culture volume with sterile $MgCl_2$ [100 mM]. Incubate on ice for 5 minutes.
7. Pellet cells by centrifugation at 4000 rpm for 20 minutes at 4 °C.
8. Carefully decant supernatant and resuspend cells in 1:4 culture volume with sterile $CaCl_2$ [100 mM]. Incubate on ice for 20 minutes.
9. Pellet cells by centrifugation at 4000 rpm for 20 minutes at 4 °C.
10. Carefully decant supernatant, resuspend cells in 1:40 culture volume with sterile $CaCl_2$ + glycerol [100 mM, 15% *(v/v)*].
11. Aliquot 100 µL into prechilled 1.5 mL Eppendorf tubes.
12. Immerse tubes in liquid nitrogen and store at -80 °C.