Making electrocompetent cells

1. Streak out desired strain from frozen stock and incubate overnight (include drug if present).
    a. DH5α = G184
    b. BL21 = G274
    c. Rosetta = G518
2. Innoculate 5 ml culture with colony from step one (include drug if present) and shake/incubate overnight.
3. Subculture 5 ml into 500 ml of media (include drug if present).
4. Shake/incubate until an OD_{600} of 0.5 is reached (this takes approximately 4 hours).
5. Pour culture into sterile 500 ml centrifuge bottle.
6. Chill culture in icy water for 30 minutes.
7. Centrifuge in GS-3 rotor @ 5,000 rpm for 15 minutes @ 4°C.
8. Pour off supernatant and completely resuspend pellet in 500 ml cold sterile nano-pure water.
9. Centrifuge in GS-3 rotor @ 5,000 rpm for 15 minutes @ 4°C.
10. Pour off supernatant and completely resuspend pellet in 500 ml cold sterile nano-pure water.
11. Centrifuge in GS-3 rotor @ 5,000 rpm for 15 minutes @ 4°C.
12. Pour off supernatant and completely resuspend pellet in 10 ml cold sterile 10% glycerol.
13. Transfer to a chilled 50 ml centrifuge bottle.
14. Centrifuge in SA-600 rotor @ 10,000 rpm for 10 minutes @ 4°C.
15. Pipet off supernatant and completely resuspend in 1 ml cold sterile 10% glycerol.
16. Make aliquots of 50 µl to 250 µl into cold sterile 1.5 ml microfuge tubes.
17. Quick freeze in ethanol bath and store at -80°C.