SOP: RP001

Generation of Recombinant Clone Frozen Stock Protocol

Materials and Reagents
1. LB agar plate (for E.coli stocks) with appropriate antibiotics
2. 7H11 agar plates (for Mycobacterial stocks) with appropriate antibiotics (see SOP M009)
3. Sterile LB broth (for E.coli stocks) with appropriate antibiotics
4. Sterile GAS media (for Mycobacterial stocks) with appropriate antibiotics (see SOP M001)
5. Sterile wood applicator sticks (Fisher Cat. # 01-340)
6. Sterile Cryovials (Fisher Cat. # 07-200-196)
7. Sterile 50% glycerol
8. Streaking loop
9. Bunsen burner
10. 1000μl Pipetman
11. Sterile 1000μl pipette tips
12. Sterile 15 ml Falcon tubes (VWR Cat. # 21008-918)
13. Plate incubator
14. Incubating shaker table

Protocol:
1. _____ Using a sterile loop, streak liquid culture or frozen stock onto agar plate containing appropriate antibiotics, for single colonies.
2. _____ Place plates in partially sealed Ziploc bag, and incubate overnight (2-3 days for M. smegmatis strains) at 37°C, with plates inverted.
3. _____ Add 5-10 ml of liquid medium, with appropriate antibiotics, to a sterile 50 ml Falcon tube.
4. _____ Pick an isolated colony from the plate with a sterile applicator stick and inoculate the liquid culture.
5. _____ Place the lid on the Falcon tube, loosely, and tape down.
6. _____ Place tube in an environmental incubator at 37°C and grow to mid-late log phase (note 1).
7. _____ Add 300 μl of sterile 50% glycerol to a Cryovial (note 2).
8. _____ Add 700 μl of mid-late log phase culture to the Cryovial containing glycerol.
9. _____ Store glycerol stock at –80°C.

Notes:
1. Aerate E. coli at 150 r.p.m. and M. smegmatis strains at 100 r.p.m.
2. For E.coli stocks harboring a pLysS or pLysE plasmid, prepare 8% glycerol stocks vs. the standard 15%.