

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 37⁰C, with plates inverted.
4. ____ Add 5-10 ml of liquid medium, with appropriate antibiotics, to a sterile 50 ml Falcon tube.
5. ____ Pick an isolated colony from the plate with a sterile applicator stick and inoculate the liquid culture.
6. ____ Place the lid on the Falcon tube, loosely, and tape down.
7. ____ Place tube in an environmental incubator at 37⁰C and grow to mid-late log phase (note 1).
8. ____ Add 300 µl of sterile 50% glycerol to a Cryovial (note 2).
9. ____ Add 700 µl of mid-late log phase culture to the Cryovial containing glycerol.
10. ____ Store glycerol stock at -80⁰C.

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%.