SOP: PP041.2
Modified: 8-3-16 ASimpson

Large-scale growth of *Mycobacterium smegmatis*

Materials and Reagents:

1. 2 cryovials 1ml frozen stocks of *M. smegmatis*
2. 7H11 + OADC agar plate (SOP M007)
3. 2.8 L glass fernbach flasks containing 1L sterile GAS medium, capped with cotton and cheesecloth plugs wrapped in aluminum foil (SOP M001.1)
4. P-200 pipettor
5. P-200 tips, sterile, aerosol-resistant tips
6. Ziploc bags
7. Biosafety cabinet
8. Inoculation loops, 10 µL
9. Orbital shaker (fits 7 fernbach flasks)
10. Cell scrapers
11. Serological 50mL pipettes
12. Serological pipettor
13. Sterile Milli-Q water
10. 2.5% Vesphene
11. 70% Ethanol
11. Autoclave
12. -80 freezer

Protocol:

1. _____ Thaw the frozen stocks at room temperature (note 1).

2. _____ Inside the biosafety cabinet streak a large 7H11 + OADC agar plate with 200µL bacteria and spread to grow as a lawn (note 2).

3. _____ Incubate at 37ºC until a lawn has formed (1-2 weeks).

4. _____ Inside the biosafety cabinet, use a sterile cell scraper and aseptically transfer each plate of bacterial lawn to a fernbach flask containing GAS media (or 7H9/OADC/tween media). Flame the mouth of the fernbach flask, then replace the cotton plug/foil.

5. _____ Put the flasks in an orbital shaker at 37ºC. Up to seven flasks will fit on the shaker table. Monitor the flasks every ~24 hours (note 3).

6. _____ Inside the biosafety cabinet, harvest cells. Gently swirl a flask and set it down to allow the cells to form into a pile in the center. Once the cells have settled, remove the cotton plug/foil. Transfer all the cells to one fernbach, swirl, and allow the cells to settle in a pile.

7. _____ Transfer the cells into a pre-weighed, 230 mL Falcon tube. Wash cells several times with sterile Milli-Q water. Let cells settle and remove the water. Measure the wet weight of the cells. Note the wet weight, date, your initials, and CSU lot number on the tube.

8. _____ Store cells in a -80 freezer. Optional: γ-irradiation (BSL2 organism).

9. _____ Autoclave the flasks of spent media with vesphene before proceeding with normal glassware cleaning guidelines.

Notes:

1. All open culture is to be done in the hood. Autoclave all used materials and trash. See SP041.1 for hood use guidelines.

2. Use a plate prepared according to SOP M009.1. Plates take 1-2 weeks to show a lawn. Inoculate as many plates as fernbachs to be used later. Optional: Scrape plates to 250 Eflasks for a smaller volume or 250 Eflasks can be used to upscale to fernbachs if desired.

3. Check for any signs of contamination. Four-six days of growth seems to be optimal to hit mid to late log phase. Smeg is very flocculent so any cloudy media will indicate contamination. Optional: Take a 1 ml sample and use the spectrophotometer to check the optical density (OD) level on each flask, however, cells precipitate too much to get an accurate reading even when grown with tween.