**Media and Reagents for Growing *Mycobacterium smegmatis***

*Mycobacterium smegmatis* mc² 155, smeg, is the host bacterium. It is a common soil organism, non-pathogenic. It grows slowly (colonies in ~ 4 days). It is resistant to carbenicillin (CB), so we always include CB at 50 µg/ml to kill other bacteria. Cyclohexamide (CHX) at 10 µg/ml is added to the media because it kills most molds and yeast. In liquid culture, *M. smegmatis* tends to clump. We grow it from a colony from a plate in 7H9 media with calcium, CB/CHX/ADC, and 0.05% Tween® 80, then subculture this to media without the Tween® for cells for phage infection. We grow it in standard test tubes placed at an angle on a shaker, but for bigger volumes, we use baffled flasks. Most of the phages have a calcium requirement. We always put 1mM CaCl₂ in media and top agar. We standardly use 0.35% top agar in our screens, hopefully to identify bigger phages that might not form plaques on the standard 0.7% agar.

**ADC** (NO HEAT, filter sterilize)
- 60 g dextrose
- 25.5 g NaCl
- 150 g Albumin
- 2850 ml ddH₂O

20% **Tween®** (50°C to dissolve, filter sterilize)
- 20 ml Tween® 80
- 80 ml ddH₂O

**MBTA** (Middlebrook Top Agar) made at 0.7% melted, diluted to 0.35% with 7H9 (plus 2 ml CaCl₂ *)
- 4.7 g 7H9
- 7 g agar
- H₂O to 900 ml

**Phage Buffer** (autoclave or filter sterilize, add 0.1mM CaCl₂ * prior to use)
- 10ml 1M Tris, pH 7.5
- 10 ml 1M MgSO₄
- 4 g NaCl
- 980 ml ddH₂O

**7H9** (autoclave, add antibiotics, ADC, and calcium prior to use)
- 4.7 g 7H9 broth base
- 5 ml 40% glycerol
- 900 ml ddH₂O
**7H10 Plates** (Autoclave, cool to 55° C and add 100 ml ADC and 10 ml 0.1 mM CaCl$_2$, CB, CHX and then pour.)

19g 7H10 agar  
12.5 ml 40% glycerol  
890 ml ddH$_2$O

**CaCl$_2$** Calcium is added to Mycobacterial growth media to ensure adequate calcium is available for necessary cellular metabolic processes. Note that for the most part, we add calcium to obtain a 0.1mM concentration in the final solutions of each media or reagent we use. (Therefore we will add 1ml of the 1mM CaCl$_2$ stock solution to phage buffer, but 2ml of CaCl$_2$ to 7H9 (because it will be used to dilute top agar by 50%).)

**7H9 + CB + CH + ADC + CaCl$_2$ - for liquid culture of smeg**  
100ml 7H9  
10 ml ADC  
100 µl of CB  
100 µl of CHX

250 µl Tween® (For intial sub-culture ONLY!) See direction below.

**Growing Mycobacterium smegmatis mc$^2$155**

When culturing *Mycobacterium smegmatis mc$^2$155*: Start by retrieving a sample from the frozen stock in the -70°C freezer and streaking it on a 7H10 ADC CB CHX plate. Allow to grow for several days. The goal is to produce isolated colonies. Smeg has a distinct colony morphology. When sub-culturing from this plate, pick a tiny (smaller than what you can see) piece from the center of the colony to grow in liquid culture. The liquid media (7H9 ADC CB CHX) has Tween® added to minimize the clumping of the bacterial growth. Once a homogenous culture is obtained, subculture in media without Tween®.

**For growing smeg: Initial transfer from plated smeg**  
(Autoclave, for each L, add 100 ml ADC enrichment and CaCl$_2$ to 1mM, 100 µl carbenicillin (CB) and 100 µl cycloheximide (CHX). Store in fridge after adding ADC.) When growing smeg from plate, add 250 µl 20% Tween® 80 to 100 ml bottle. It is advisable to use a ‘touch’ of the culture from your plate into 1-2 ml of this Tween®-media. Vortex well. Place on shaker for at least 24 hours. Sub-culture from this culture into media without Tween® for phage infection.

**For growing smeg for infections (7H9 without Tween®)**  
Transfer a small amount (1 – 100 µl of the smeg grown in Tween® into a flask that is only filled ~1/5 full of 7H9 + CaCl$_2$ + ADC + CB + CHX (same concentrations as above but no Tween®). Place securely on shaker for 24 hours.