SEA PHAGES

Prep Guide

This document is to guide you in preparing and testing the reagents necessary for your Phage Discovery semester. You can begin this process even before receiving your host strain and positive control phage.

Weeks prior to Phage Discovery Semester	
8 - 6	**Prepare** • Components for media and phage buffer ○ AD Supplement; Glycerol (40%); $MgSO_4$ (1M); Tris (base), pH 7.5 (1M) ○ $CaCl_2$ (100mM) ○ Tween80 (20%); Cycloheximide (10mg/mL); Carbenicillin (50mg/mL) • Media ○ 7H9 liquid medium (neat) ○ L-agar plates ○ 2× or 1× 7H9 top agar **Test** • Media ○ 7H9 liquid medium (neat) *incubate 20 ml per batch of media at 37˚C for 48 hrs and observe for growth of contaminants* ○ 7H9 liquid medium (complete) *prepare 20 ml of 7H9 complete media, incubate at 37˚C for 48 hrs, and observe for growth of contaminants* ○ L-agar plates *incubate 1 plate per batch of agar at 37˚C for 4 days and observe for growth of contaminants* ○ 2× or 1× 7H9 top agar *incubate a 20 ml solidified aliquot of top agar, per batch of media prepared, at 37˚C for 4 days and observe for growth of contaminants*

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Prepare

- Biologicals
  - *M. smegmatis mc^2 155* streak plate
    You will receive a glycerol stock of M. smegmatis *from the Hatfull Lab. Upon receipt, streak bacteria onto 2 plates and incubate plates at 37˚C for 3-5 days. Place the glycerol stock at -80 ˚C for indefinite storage.*
  - D29 bacteriophage frozen stock
    You will receive a liquid lysate of D29 from the Hatfull Lab, which will already contain a cryo-protectant like glycerol or DMSO. Immediately store this lysate at -80˚C.

Test

- M. smegmatis mc^2 155 streak plate
  Observe the freshly streaks plates daily for colony formation.

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Prepare

- Biologicals
  - *M. smegmatis mc^2 155* liquid culture with Tween [P1FF]
    from a single colony, prepare and incubate a 50 ml culture at 37˚C for 3 – 5 days, with shaking, and observe for growth. Once saturated, prepare 5 – 10 glycerol stocks to be frozen.

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Prepare

- Biologicals
  - *M. smegmatis mc^2 155* liquid culture without Tween [P2FF]
    by sub-culturing from P1FF, prepare and incubate a 50 ml culture at 37˚C for 1 – 2 days, with shaking, and observe for growth.

Test

- Biologicals
  - *M. smegmatis mc^2 155* top agar lawn
    prepare a top agar lawn on a L-agar plate using P2FF, and incubate at 37˚C for 1 – 2 days and observe for growth of a bacterial lawn.
  - Mycobacteriophage D29 plaques
    Using a sterile pipette tip, remove a small amount of frozen lysate and dilute it into phage buffer. Prepare a dilution series, and plate for plaques using P2FF and top agar. Incubate plates at 37˚C for 1 – 2 days and observe for growth of a bacterial lawn and the formation of plaques. Flood the plates with high density of plaques to prepare more D29 lysate for use and storage.

2	**Prepare** • Biologicals ○ *M. smegmatis mc^2 155* streak plate [P0FF] ○ *M. smegmatis mc^2 155* liquid culture with Tween [P1FF] ○ *M. smegmatis mc^2 155* liquid culture without Tween [P2FF] *Prepare sufficient amounts of P2FF in the last week before the Phage Discovery semester, as this will be used for your first week of class.*
	The following reagents will only be needed once students have purified their phage and prepared a High Titer Lysate. These reagents should be prepared at least 2 weeks before needed. **Prepare:** • Molecular reagents ○ Rnase A stock ○ Dnase 1 stock ○ Molecular marker ○ Uranyl acetate stain ○ TAE (50X) or TBE (10X), as needed ○ EDTA (500mM)