SEA PHAGES

Reagent Prep Guide

1. Prior to receiving biologicals from HHMI

Prepare

- Components for media and phage buffer:
  - $CaCl_2$ (100 mM)
  - Cycloheximide (10 mg/mL)
  - Dextrose (40 %)
  - Glycerol (40 %)
  - $MgSO_4$ (1 M)*
  - Tris (base), pH 7.5 (1 M)*
- Media & Phage Buffer:
  - PYCa liquid medium (500 ml)
  - PYCa-agar plates (500 ml)
  - PYCa top agar*
  - Phage Buffer*

Recipe cards for preparing these various reagents, including estimated amounts to be prepared, are provided in the Phage Discovery Instructor's Guide, Appendix B. Video protocols for preparing filter-sterilized solutions as well as agar plates are available on the program website: https://seaphages.org/videochannels/12/

Test

- Media
  - PYCa liquid medium
    Incubate 1 aliquot of prepared media at 30 ˚C for 48 hrs and observe for growth of contaminants.
  - PYCa-agar plates
    Incubate 1 plate per batch of agar at 30 ˚C for 4 days and observe for growth of contaminants.
  - PYCa top agar*
    Incubate an aliquot of solidified top agar, per batch of media prepared, at 30 ˚C for 4 days and observe for growth of contaminants.

***** Prepare or test these items if performing the optional Step 4 of this guide prior to the Phage Discovery workshop in the summer.

SEA PHAGES

Reagent Prep Guide

2. Upon receiving biologicals from HHMI / University of Pittsburgh

Prepare

- Biologicals
  - *Receive bacterial strains and bacteriophage lysates from HHMI/University of Pittsburgh.*
    You will receive the following biologicals from the Hatfull Lab at the University of Pittsburgh:
    Bacteria: 2 x glycerol stocks of Microbacterium foliorum *and* Gordonia rubripertincta, each.
    Bacteriophage: *1 x DMSO stocks of bacteriophage that infect* Microbacterium foliorum *and* Gordonia rubripertincta*, each.*
    Upon receipt, place all stocks at -80 ˚C for indefinite storage.
    Note: These stocks contain a cryoprotectant, glycerol or DMSO, and are suitable for freezing at -80 ˚C.

  - *M. foliorum* streak plate
    Remove one of the M. foliorum *glycerol stocks from the freezer and prepare 2 streak plates. Before returning the glycerol stock to the freezer, mark the glycerol stock tube with a permanent marker so that you can identify which tube has been opened. Incubate the streak plates at 30 ˚C for 3 days. A video protocol for preparing a streak plate is available on the program website: https://seaphages.org/videochannels/12/*

Test

- *M. foliorum* streak plate
  - *Observe the freshly streaked plates daily for colony formation, paying attention to colony morphology. Document your observations, including photographs. Once single colonies are formed, proceed to Preparing Additional Glycerol Stocks.*

SEA PHAGES

Reagent Prep Guide

3. Preparing Additional Glycerol Stocks

Prepare

- *M. foliorum* liquid culture and glycerol stocks.
  From a single colony, inoculate a 50 ml liquid (PYCa) culture at 30 ˚C for 3 days, with shaking, and observe for growth. Once saturated, prepare 5 – 10 glycerol stocks to be frozen. You will come to rely on these stocks for several years.
  Recipe cards preparing a liquid culture and glycerol stocks are provided in the Phage Discovery Instructor's Guide, Appendix B.
  Video protocols for preparing a liquid culture and glycerol stocks are available on the program website: https://seaphages.org/videochannels/12/
  Once you have prepared additional glycerol stocks, follow the instructions below to test your freshly prepared glycerol stocks.

Test

- Test *M. foliorum* glycerol stocks by streak plate
  *Using one of your freshly prepared glycerol stocks, streak 2 plates for individual colonies. Incubate the streak plates at 30 ˚C for 3 days. Observe the freshly streaked plates daily for colony formation, paying attention to colony morphology. Compare your observation to the streak plate prepared using the glycerol stock sent from the program to determine that your freshly prepared stocks are not contaminated with non-host bacteria. Non-host bacteria are likely to have different growth rates and/or colony morphology when compared to your host bacteria (*M. foliorum*). Document your observations, including photographs.*

SEA PHAGES

Reagent Prep Guide

4. Optional: Preparing Additional Bacteriophage Freezer Stocks

Optional

- Prepare additional bacteriophage freezer stocks.

  You will need to prepare additional bacteriophage freezer stocks for use beyond the first few months of offering the SEA-PHAGES course. We recommend that you prepare these additional freezer stocks after you have received HHMI-sponsored training to handle phage at the Phage Discovery training workshops in the summer. However, should you wish to prepare these freezer stocks ahead of the workshop, you may do so by following the instructions below.

Prepare

- Additional freezer stocks (for bacteriophage that infect *M. foliorum*)

  Begin by carefully reviewing the experimental procedures and supplies needed for the following protocols in the Phage Discovery Guide - Protocol 6.2: Serial Dilutions; Protocol 5.3: Plaque Assay; Protocol 6.3: Collecting Plate Lysates.

  Using a sterile pipette tip or stick, scrape a small amount (3-5 μl) of frozen bacteriophage lysate and place it into 100 μl of phage buffer in a clean microcentrifuge tube (note: return the lysate to the freezer to avoid thawing). Then, follow protocol 6.2 to serially dilute the phage sample to the 10^{-8} dilution. Next, using protocol 5.3, plate each dilution for plaques. Incubate plates at 30 ˚C for 1 – 2 days and observe for growth of a bacterial lawn and the formation of plaques. Use protocol 6.3 to flood the plate with high density of plaques to prepare more phage lysate for future use and storage. As you will have yet to receive DMSO or glass beads from the program to prepare freezer stocks of lysates, prepare phage lysates using the same procedure used for preparing glycerol stocks of bacteria. This is described in the recipe card for Preparing Frozen Stocks of Phage Lysates in the Phage Discovery Instructor's Guide, Appendix B. Store these stocks at -80 ˚C.