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Reagent Prep Guide

1. Prior to receiving biologicals from HHMI

Prepare

- Components for media:
  - $CaCl_2$ (100 mM)
  - Cycloheximide (10 mg/mL)
  - Dextrose (40 %)
  - Glycerol (40 %)
- Media:
  - PYCa liquid medium (500 ml)
  - PYCa-agar plates (500 ml)

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.

SEA PHAGES

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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: Microbacterium foliorum *and* Arthrobacter globiformis bacterial strains. For each strain, you will receive *2 x glycerol stocks* 1 x agar slant.
    Bacteriophage: *1 x DMSO stocks of bacteriophage that infect* Microbacterium foliorum *and* Arthrobacter globiformis*, each.*
    Upon receipt, place the agar slant at 4 ˚C and all the remaining stocks at -80 ˚C for indefinite storage.
    Note: Stocks contain a cryoprotectant, glycerol or DMSO, and are suitable for freezing at -80 ˚C. The agar slant cannot be frozen.
  - *A. globuformis* streak plate
    Using the agar slant for A. globiformis, *prepare 2 streak plates. Incubate the streak plates at 37 ˚C for 3 days. A general video protocol for preparing a streak plate is available on the program website: https://seaphages.org/videochannels/12/*
    In this video, the bacteria is obtained from a frozen glycerol stock. When you prepare your streak plate, you will use your agar slant.

Test

- *A. globiformis* 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.*

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3. Preparing Additional Glycerol Stocks

Prepare

- *A. globiformis* liquid culture and glycerol stocks.

  From a single colony, inoculate a 50 ml liquid (PYCa) culture at 37 ˚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 *A. globiformis* glycerol stocks by streak plate

  *Using one of your freshly prepared glycerol stocks, streak 2 plates for individual colonies. Incubate the streak plates at 37 ˚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 (*A. globiformis*). Document your observations, including photographs.*