

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.

<p style="text-align: center; color: #4F81BD; font-size: 24px;">Week 1</p>	<p>Prepare</p> <ul style="list-style-type: none"> • Components for media and phage buffer <ul style="list-style-type: none"> ○ CaCl₂ (100 mM) ○ Cycloheximide (10 mg/mL) ○ Dextrose (40 %) ○ Glycerol (40 %) ○ MgSO₄ (1 M); ○ Tris (base), pH 7.5 (1 M) • Media & Phage Buffer <ul style="list-style-type: none"> ○ PYCa liquid medium ○ PYCa-agar plates ○ PYCa top agar ○ Phage Buffer <p>Test</p> <ul style="list-style-type: none"> • Media <ul style="list-style-type: none"> ○ PYCa liquid medium <i>incubate 20 ml from each batch of prepared media at 30 °C for 48 hrs and observe for growth of contaminants.</i> ○ PYCa-agar plates <i>incubate 1 plate per batch of agar at 30 °C for 4 days and observe for growth of contaminants</i> ○ PYCa top agar <i>incubate a 20 ml solidified aliquot of top agar, per batch of media prepared, at 30 °C for 4 days and observe for growth of contaminants</i>
<p style="text-align: center; color: #4F81BD; font-size: 24px;">Week 2</p>	<p>Prepare</p> <ul style="list-style-type: none"> • Biologicals <ul style="list-style-type: none"> ○ <i>M. foliorum</i> streak plate <i>You will receive a glycerol stock of M. foliorum from the Hatfull Lab. Upon receipt, streak bacteria for single colonies on 2 plates and incubate plates at 30 °C for 3 days. Place the glycerol stock at -80 °C for indefinite storage. Video protocols are available at seaphages.org</i> ○ bacteriophage frozen stock <i>You will receive a liquid lysate of a control bacteriophage from the Hatfull Lab, which will already contain a cryo-protectant like glycerol or DMSO. Immediately store this lysate at -80°C.</i> <p>Test</p> <ul style="list-style-type: none"> • <i>M. foliorum</i> streak plate <i>Observe the freshly streaks plates daily for colony formation, paying attention to colony morphology. Document your observations, including photographs.</i>

<p>Week 3</p>	<p>Prepare</p> <ul style="list-style-type: none"> • Biologicals <ul style="list-style-type: none"> ○ <i>M. foliorum</i> liquid culture <i>from a single colony, prepare and incubate a 50 ml culture at 30 °C for 3 days, with shaking, and observe for growth. Once saturated, prepare 5 – 10 glycerol stocks to be frozen. Video protocols are available at seaphages.org</i> <p>Test</p> <ul style="list-style-type: none"> • Biologicals <ul style="list-style-type: none"> ○ <i>M. foliorum</i> top agar lawn & bacteriophage plaques <i>Using a sterile pipette tip, remove a small amount of frozen lysate and dilute it into 100 µl of phage buffer. Prepare a 10-fold dilution series, and plate for plaques using bacterial culture, agar plates, and top agar. Incubate plates at 30 °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 phage lysate for future use and storage. Video protocols are available at seaphages.org</i>
<p>1 month prior to class</p>	<p>Prepare</p> <ul style="list-style-type: none"> • Biologicals <ul style="list-style-type: none"> ○ <i>M. foliorum</i> streak plate ○ <i>M. foliorum</i> liquid culture <p>Test</p> <ul style="list-style-type: none"> • <i>Attempt 5 – 10 Enriched Isolations & Spots Tests. Include a positive control in the spot tests.</i>
<p>2 -weeks prior to class</p>	<p>Prepare</p> <ul style="list-style-type: none"> • Biologicals <ul style="list-style-type: none"> ○ <i>M. foliorum</i> streak plate ○ <i>M. foliorum</i> liquid culture <i>Prepare sufficient amounts of liquid culture in the last week before the Phage Discovery semester, as this will be used for your first week of class. Continue to streak plates and prepare cultures every two weeks for use during the semester.</i>
<p>As needed</p>	<p>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.</p> <p>Prepare:</p> <ul style="list-style-type: none"> • Molecular reagents <ul style="list-style-type: none"> ○ Rnase A stock ○ Dnase 1 stock ○ Molecular marker ○ Uranyl acetate stain ○ TAE (50X) or TBE (10X), as needed ○ EDTA (500mM)