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Title: Sexual Crosses with the Mucoromycete *Phycomyces blakesleeanus*

Authors and Affiliations:

Luke Stanley, Jesús F. Peña

Organismal Biology and Ecology Department, Colorado College

Corresponding Authors:
Jesús F. Peña jpena2023@coloradocollege.edu

Email Addresses for All Authors:
L. Stanley l_stanley@coloradocollege.edu
Jesús F. Peña jpena2023@coloradocollege.edu

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Filming location:** Will the filming need to take place in multiple locations? **Yes**

   If **Yes**, how far apart are the locations? ~300 ft, just down the hallway to access the incubator

Current Protocol Length
Number of Steps: 13
Number of Shots: 25 (1 Scope)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Jesús F. Peña:** Our lab is interested in exploring the evolution of developmental programs in filamentous fungi. We are curious about what genes participate in the serial morphological transitions that occur during sexual reproduction **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.11.1*

What research gap are you addressing with your protocol?

1.2. **Luke Stanley:** Since fugal mycelia expand radially, different parts of the mycelium make contact at different times and experience asynchronous sexual reproduction. This can complicate gene expression studies that seek to tie gene expression with a particular sexual cell type. Our protocol allows us to limit the number of interacting cells to potentially provide clearer gene expression signal **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1.1*

How will your findings advance research in your field?

1.3. **Jesús F. Peña:** Our protocol will facilitate the extraction of RNA from specific cells during sexual reproduction, allowing us to tie gene function to a specific morphological change **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.6.1*

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Preparing Fungal Spores or Tissue

Demonstrator: Luke Stanley

2.1. To begin, obtain cultures of each *Phycomyces blakesleeanus* */ˌfaɪ.koʊˈmaɪ.siːz ˌbleɪkˈsliː.ə.nəs/)* mating type **[1-TXT]**.

2.1.1. **WIDE:** Talent retrieving labeled culture plates of different *Phycomyces blakesleeanus* mating types. **TXT: *P. blakesleeanus* mating types: NRRL 1555 (-); NRRL 1554 (+); NRRL 1464 (-); and NRRL 1465 (+)**

2.2. Using a scalpel, cut a portion of leading-edge mycelia from the existing cultures **[1]** and place the excised mycelium onto a fresh one hundred percent cornmeal agar or potato dextrose agar plate **[2]**. Incubate the pure cultures for one week at 27 degrees Celsius under a 12-hour light cycle **[3]**.

2.2.1. Talent cutting the leading edge of the mycelium with a scalpel.

2.2.2. Talent placing the cut mycelium piece onto a new agar plate.

2.2.3. Talent placing the plate into an incubator.

2.3. Once sporangiophores are present, flood a sporulating pure culture plate with 0.01 percent Tween 20 in sterilized, deionized water using aseptic technique **[1]**. Then, using a P1000 micropipette, draw 1 milliliter of the Tween 20-spore mixture from the plate into a microcentrifuge tube **[2]**.

2.3.1. Talent adding the sporulating culture plate carefully with Tween 20 solution.

2.3.2. Talent pipetting 1.0 milliliter of spore suspension into a microcentrifuge tube.

2.4. Centrifuge the microcentrifuge tube in a mini centrifuge for 30 seconds **[1]** and carefully decant the supernatant without disturbing the pellet **[2]**.

2.4.1. Talent loading the microcentrifuge tube into a mini centrifuge.

2.4.2. Talent gently pouring off the supernatant.

2.5. If more spores are needed, add another 1 milliliter of the Tween 20 spore mixture into the same microcentrifuge tube and repeat centrifugation **[1]**. After obtaining a suitable amount of spores, decant the supernatant **[2]** and replace it with 500 microliters of

sterile deionized water **[3]**.

2.5.1. Talent adding additional Tween 20 spore mixture into the microcentrifuge tube.

2.5.2. Talent pouring off supernatant.

2.5.3. Talent resuspending the spores with sterile water by pipetting.

2.6. Use a hemacytometer to estimate the concentration of spores **[1]**.

2.6.1. Talent loading a sample of spore suspension onto a hemacytometer for counting.

2.7. Alternatively, use a scalpel to inoculate the crosses directly with leading-edge mycelia from the pure cultures **[1]**.

2.7.1. Talent cutting leading-edge mycelium with a scalpel and transferring it onto a crossing plate. NOTE: 2.7.1, 2.8.1, and 2.8.2 are combined

2.8. Then, using a sterilized razor blade or cork hole borer, excise a portion of the tissue **[1]** and plate the excised inoculum immediately onto a fresh medium **[2]**.

2.8.1. Talent cutting a section of mycelium using a sterilized razor blade or cork hole borer.

2.8.2. Talent placing the excised mycelial tissue onto a fresh agar plate.

NOTE: Move this step (2.9) after 2.6

2.9. Set up the crosses as two-way, four-way, or eight-way crosses as appropriate **[1]**.

2.9.1. Talent arranging culture plates and labeling them according to cross type.

2.10. For a four-way cross, place like mating types opposite each other while neighboring a complementary mating type **[1]**.

2.10.1. Talent arranging culture pieces or spore drops according to the four-way cross. Author's NOTE: 2.10.1 and 211.1 were combined during filming and represent a specific version of 2.7-2.8. Making steps 2.7-2.8 redundant

2.11. Now, place the spores or mycelia of the minus mating types, opposite each other at least 1 centimeter away from the edge of a Petri dish containing potato dextrose agar or cornmeal agar **[1]**.

2.11.1. Talent placing the NRRL 1555 and NRRL 1464 mycelial pieces precisely onto the Petri dish at the specified distance.

2.12. After inoculating, seal the plates with parafilm **[1-TXT]**. Place the plates into a secondary container **[2]**. Incubate the plates at 22 degrees Celsius in the dark **[3]**. Observe the plates daily for evidence of mating and take photographs from underneath the plate during culture growth **[4]**. Trace the growing mycelia over time **[5]**.

2.12.1. Talent sealing the inoculated plates with parafilm. **TXT: Leave the plates unsealed as needed**

2.12.2. Talent placing the plates into a secondary containment box.

2.12.3. Talent placing the container into a dark incubator set at 22 degrees Celsius.

2.12.4. Talent photographing the culture plates.

2.12.5. Talent pointing to a part on the plate.

2.13. Depending on the media type and formulation, if the mycelia begin to make contact, inspect the interacting complementary mating types under a dissecting scope for evidence of mating **[1]**. Take photographs of the interacting mycelia with a camera or smartphone mounted onto the dissecting scope **[2]**.

2.13.1. LAB MEDIA: 2.13.1_view-of-complementary-mycelia-NOT-MATING.MP4 and 2.13.1_view-of-complementary-mycelia-YES-MATING.MP4 *Video editor: Play both videos side by side. Pick timestamps as needed for the length of the VO. Label them as 'Not Mating' and 'Mating' to differentiate.*

2.13.2. Talent taking photographs of the observed mycelial interactions.

Results

3. Results

3.1. At 1-day post-inoculation, strains grown on potato dextrose agar exhibited more yellow pigmentation **[1]** compared to strains grown on cornmeal agar **[2]**.

3.1.1. LAB MEDIA: Figure 3. *Video editor: Highlight lower PDA panel*

3.1.2. LAB MEDIA: Figure 3. *Video editor: Highlight CMA panel*

3.2. At 2 days post-inoculation, mating structures were observed on 100% potato dextrose agar **[1]**, but not on cornmeal agar plates **[2]**. At 3 days post-inoculation, all formulations of potato dextrose agar supported mating, with differing densities of differentiated cell types **[3]**.

3.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight lower PDA panel*

3.2.2. LAB MEDIA: Figure 4. *Video editor: Highlight CMA panel*

3.2.3. LAB MEDIA: Figure 5. *Video editor: Highlight lower PDA panel*

3.3. By 4 days post-inoculation, strains on potato dextrose agar plates exhibited both mating reactions and formation of asexual sporangiophores **[1]**. Strains on cornmeal agar exhibited more diffuse mycelia and fewer sporangiophores compared to potato dextrose agar **[2]**.

3.3.1. LAB MEDIA: Figure 6. *Video editor: Highlight lower PDA panel*

3.3.2. LAB MEDIA: Figure 6. *Video editor: Highlight CMA panel*

3.4. On 100% potato dextrose agar, at 4 days post-inoculation, aerial structures appeared as a mass of undifferentiated cells **[1]**. On 25% potato dextrose agar, cells at the mating site appeared easily distinguishable and predominantly at similar developmental stages **[2]**. On 50% potato dextrose agar, a mix of aerial zygophores and developing progametangia was observed **[3]**.

3.4.1. LAB MEDIA: Figure 7A.

3.4.2. LAB MEDIA: Figure 7C, E.

3.4.3. LAB MEDIA: Figure 7D.