

Submission ID #: 70500

Scriptwriter Name: Pallavi Sharma

Project Page Link: https://review.jove.com/files_upload.php?src=21502548

Title: An optimized protocol for *Candida albicans* infection in *Schmidtea mediterranea* to study fungal pathogenesis and host defense

Authors and Affiliations:

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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? **Yes, it will require the use of a dissecting or stereomicroscope to show images of animals.**

If **Yes**, can you record movies/images using your own microscope camera?

Yes, movies and images can be recorded using the Nikon microscope's camera. The dissecting scope does not have a camera port and needs to be filmed through the eye piece manually.

Authors: Please use your microscope camera to film the scope shots and upload the file to your project page as soon as possible:

https://review.jove.com/files_upload.php?src=21502548

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, we will plan to film in two laboratories at UC Merced. They are less than 400 meters apart.**
4. **Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes.**

Current Protocol Length

Number of Steps: 17

Number of Shots: 29

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Nora Shamoon:** My research focuses on *Candida albicans* infections and how host responses shape whether the outcome is clearance or disease progression.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.10.1*

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Nora Shamoon:** A key challenge is the lack of models for early fungal infection; this protocol uses planarians as a tractable, high-throughput solution.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. **Beryl Arinda:** The planarian system enables the study of disease initiation, progression, clearance, and lethality, with high-resolution measurement of innate immune responses.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.3.2*

VIDEO NOTE: Better answer is the second time she says it.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.4. **Nora Shamooin**: This protocol enables in vivo study of host-pathogen dynamics by integrating imaging and molecular approaches in a genetically tractable host.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Beryl Arinda**: Future applications include high-throughput mutant screening, comparative studies across microbial species, and adaptation for toxicology and drug efficacy testing.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

VIDEO NOTE: These numbers are the same as question 1. I slated them as 5.1 and 5.2

What problem in your field of research does your JoVE article address?

- 1.1. **Nora M. Shamoon, Graduate Student Researcher/ Beryl N. Arinda, Graduate Student Researcher:** (authors will present their testimonial statements live)

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.2. **Nora M. Shamoon, Graduate Student Researcher/ Beryl N. Arinda, Graduate Student Researcher:** (authors will present their testimonial statements live)

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

Preparation and quantification of *Candida albicans* inoculum before infection

Video editor: Please insert the title card to include this information

2. Planarian Colony Preparation for Infection

Demonstrator: Nora M. Shamoon

2.1. To begin, select *Schmidtea mediterranea* planarians approximately 5 millimeters in length, and ensure animals are fully extended before measuring [1].

2.1.1. WIDE: Talent placing the plate containing the sample under microscope.
Videographer: Please film the CU shot as well.

2.2. Avoid using planarians smaller than 5 millimeters, as they may include individuals with blastemas or incomplete development that can produce inconsistent results [1]. Also, exclude planarians larger than 5 millimeters, as larger animals are less suitable for fluorescence immunostaining [2-TXT].

2.2.1. Talent showing planarians smaller than 5 millimeters and keeping them aside.
VIDEO NOTE: Mis-slated as 2.1.2

2.2.2. Talent excluding planarians larger than 5 millimeters from the sample set.

2.3. Examine all planarians under a stereomicroscope before use [1]. Exclude animals with blastemas, tissue lacking pigmentation, or incomplete pigmentation development [2].

2.3.1. WIDE: Talent examining planarians under a stereomicroscope.

2.3.2. **SCOPE**: Talent removing visibly damaged or injured planarians from the sample set.

NOTE: All SCOPE shots are filmed by the videographer

VIDEO NOTE: Under the scope you'll see the rejected planarians in this order: Too Big, Too Small, Incomplete Pigmentation (too light), Injured, Too Dark, and Abnormal.

- 2.4. Transfer healthy planarians meeting all inclusion criteria into 2 milliliters of fresh planarian water per well in a non-tissue culture-treated 6-well polystyrene plate [1].
 - 2.4.1. Talent transferring healthy planarians into wells containing fresh planarian water in a 6-well polystyrene plate.
- 2.5. Assign wells as negative controls with uninfected or mock-treated animals and as positive controls with animals infected with wild-type *Candida albicans* [1].
 - 2.5.1. Talent designating specific wells as negative and positive controls
- 2.6. Remove all water from each well [1] and replace with 2 milliliters of fresh planarian water to ensure uniformity across wells. Allow the animals to equilibrate in the new environment for 1 day before infection [2].
 - 2.6.1. Talent aspirating water from the wells of the plate. VIDEO NOTE: 2.6.1 and 2.6.2 are combined as one clip. The planarians can not sit dry long enough to slate in between steps.
 - 2.6.2. Talent adding fresh planarian water into each well.

3. Systemic Infection Protocol for *Schmidtea mediterranea*-*Candida albicans* Interactions

- 3.1. Using aseptic technique, gently remove all water from the 6-well plate by tilting the plate toward the user [1]. Then, add the calculated volume of planarian water and *Candida albicans* culture to each well [2].
 - 3.1.1. Talent tilting the 6-well plate to remove water under aseptic conditions. NOTE: 3.1.1 and 3.1.2 are combined in a single clip
 - 3.1.2. Talent adding the calculated volume of planarian water/*C. albicans* into each well.
- 3.2. Place the inoculated 6-well plate in a dark, static location at room temperature [1]. Record the date and time of inoculation [2].
 - 3.2.1. Talent placing experimental plates in a separate designated area away from uninfected colonies.
 - 3.2.2. Talent recording the date and time of inoculation. VIDEO NOTE: Close up on the labelling is attached to 3.3.1
- 3.3. Incubate the planarians with *Candida albicans* for up to 72 hours, depending on the desired endpoint [1]. ~~At each time point, gently resuspend the settled *Candida albicans* cells without disturbing the planarians [1].~~
 - 3.3.1. Shot of the 6-well plates kept in the static shelf.
 - ~~3.3.2. Talent showing the resuspension steps.~~ NOTE: This is shown in section 3.4

- 3.4. At the first time point, 24 hours post-infection, tilt the plate slightly toward the user [1]. Then aspirate the liquid containing *Candida albicans* culture using a sterile transfer pipette [2] and gently dispense it into an area of the well free of animals to mix the settled fungal layer [3]. Repeat this step 5–10 times per well to break up the settled fungal layer, avoiding direct contact with planarians [4].
 - 3.4.1. Talent holding a sterile 3 milliliter transfer pipette while tilting the plate slightly.
NOTE: 3.4.1-3.4.4 are combined in a single shot
 - 3.4.2. Talent aspirating planarian water.
 - 3.4.3. Talent dispensing water into a clear area of the well to mix fungal cells.
 - 3.4.4. Close up shot of the settled fungal layer while the talent is adding water
- 3.5. Observe fungal settlement against a dark background before and after the resuspending steps for best visibility [1]. Resuspend uninfected control wells under identical conditions. Repeat this procedure at 48 hours post-infection [2].
 - 3.5.1. WIDE: Talent observing wells against a dark background to assess fungal settlement.
 - 3.5.2. Talent performing resuspension in control wells using the same technique.
- 3.6. At 72 hours post-infection, transfer planarians to fresh wells with 2 milliliters of clean water, minimizing fungal carryover by using minimal liquid [1].
 - 3.6.1. Talent transferring planarians into a new 6-well plate with fresh planarian water.
- 3.7. Once animals are transferred, remove all water [1] and replace it with fresh planarian water [2].
 - 3.7.1. Talent removing water from the wells. **NOTE: 3.7.1-3.7.4 are combined in a single shot**
 - 3.7.2. Talent adding fresh planarian water into each well.
- 3.8. Begin assessing host outcomes at 1-day post-infection and continue daily until the desired experimental endpoint [1].
 - 3.8.1. Talent observing planarians.
- 3.9. Quantify host damage using the standardized 0 to 3 scoring system [1].
 - 3.9.1. TEXT ON A PLAIN BACKGROUND
Score 0: No change from uninfected controls (healthy, asymptomatic).
Score 1: Mild symptom(s).
Score 2: Severe symptom(s).
Score 3: Death.
Perform host symptom evaluation

- 3.10. At 3 days post-infection, gently transfer planarians to a new 6-well plate containing 2 milliliters of fresh planarian water per well while avoiding transfer of residual fungal culture [1].
 - 3.10.1. **SCOPE:** Talent transferring planarians into a clean plate with minimal liquid carryover. **VIDEO NOTE:** This is step 3.6.1. It does not happen under the scope.
- 3.11. Observe animals using light microscopy with a minimum 10 times objective [1]. Record host outcomes using the standardized scoring sheet. Afterwards, perform anti-*Candida* staining to visualize fungal colonization [2].
 - 3.11.1. **SCOPE:** Talent observing planarians under a light microscope. **VIDEO NOTE:** A sick planarian can be seen at 1:30 in video.
 - 3.11.2. Talent recording observations on the standardized scoring sheet.

Results

4. Results

- 4.1. Fungal epithelial colonization was markedly reduced by 4 days post infection [1], and by 7 days post infection, nearly all surface-associated fungi were cleared under ID50 (*I-D-Fifty*) conditions [2].
 - 4.1.1. LAB MEDIA: Figure 3. *Video editor: Zoom in on the specimen labeled 4 dpi and show the reduced number of bright white spots relative to 1 dpi.*
 - 4.1.2. LAB MEDIA: Figure 3. *Video editor: Zoom in on the specimen labeled 7 dpi and show the near absence of bright white spots*
- 4.2. Uninfected controls remained entirely asymptomatic [1]. At approximately 7.0×10^7 *Candida albicans* cells per milliliter, about 30% were asymptomatic [2], 50% were symptomatic [2], and 20% were dead [3].
 - 4.2.1. LAB MEDIA: Figure 4. *Video editor: Highlight the bar for uninfected controls showing only the asymptomatic category*
 - 4.2.2. LAB MEDIA: Figure 4. *Video editor: Highlight the green portion of the bar at 7.0 representing asymptomatic*
 - 4.2.3. LAB MEDIA: Figure 4. *Video editor: Highlight the beige portion of the bar at 7.0 representing symptomatic*
 - 4.2.4. LAB MEDIA: Figure 4. *Video editor: Highlight the orange portion of the bar at 7.0 representing dead*
- 4.3. At approximately 8.0×10^7 *Candida albicans* cells per milliliter, about 30% were asymptomatic [1], 20% were symptomatic [2], and 50% were dead [3].
 - 4.3.1. LAB MEDIA: Figure 4. *Video editor: Highlight the green portion of the bar at 8.0 representing asymptomatic*
 - 4.3.2. LAB MEDIA: Figure 4. *Video editor: Highlight the beige portion of the bar at 8.0 representing symptomatic*
 - 4.3.3. LAB MEDIA: Figure 4. *Video editor: Highlight the orange portion of the bar at 8.0 representing dead*
- 4.4. Median survival declined from about 90% at 7.0×10^7 *Candida albicans* cells per milliliter [1], to about 50% at 8.0×10^7 cells per milliliter [2], and to less than 25% at 8.5×10^7 cells per milliliter [3].
 - 4.4.1. LAB MEDIA: Figure 5. *Video editor: Highlight the bar labeled 7.0 showing a value*

near 90%

- 4.4.2. LAB MEDIA: Figure 5. *Video editor: Highlight the bar labeled 8.0 showing a value near 50%*
- 4.4.3. LAB MEDIA: Figure 5. *Video editor: Highlight the bar labeled 8.5 showing a value below 25%*
- 4.5. Overall survival was about 50% in planarians infected with wild-type *Candida albicans* at approximately 8.0×10^7 cells per milliliter [1], compared to 100% survival in uninfected controls [2].
 - 4.5.1. LAB MEDIA: Figure 6. *Video editor: Highlight the orange survival curve around 50% at the end of the timeline*
 - 4.5.2. LAB MEDIA: Figure 6. *Video editor: Highlight the blue survival curve remaining at 100% across the timeline*

1. Schmidtea
Pronunciation link: No confirmed link found
IPA: /'ʃmɪd.ti:.ə/
Phonetic Spelling: shmɪd·tee·uh
2. mediterranea
Pronunciation link: No confirmed link found
IPA: /,mɛ.dɪ.tə'reɪ.ni.ə/
Phonetic Spelling: meh·duh·tuh·ray·nee·uh
3. planarians
Pronunciation link: <https://www.merriam-webster.com/dictionary/planarian>
IPA: /plə'ner.i.ənz/
Phonetic Spelling: pluh·nair·ee·uhnz
4. blastemas
Pronunciation link: <https://www.merriam-webster.com/dictionary/blastema>
IPA: /blæ'sti:.mə/
Phonetic Spelling: blas·tee·muh
5. fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊ'res.əns/, /flɔ:'res.əns/
Phonetic Spelling: floor·ess·uhns
6. immunostaining
Pronunciation link: <https://www.merriam-webster.com/dictionary/immunostaining>
IPA: /ɪ,mju:.noʊ'steɪ.nɪŋ/
Phonetic Spelling: ih·myoo·noh·stay·ning
7. stereomicroscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereomicroscope>
IPA: /,stɛr.i.ou'maɪ.krə'skoʊp/
Phonetic Spelling: steh·ree·oh·my·kruh·skohp

8. pigmentation
Pronunciation link: <https://www.merriam-webster.com/dictionary/pigmentation>
IPA: /ˌpɪɡ.mənˈteɪ.ʃən/
Phonetic Spelling: pig·muhn·tay·shuhn
9. polystyrene
Pronunciation link: <https://www.merriam-webster.com/dictionary/polystyrene>
IPA: /ˌpɑː.liˈstaɪ.rɪn/
Phonetic Spelling: pah·lee·sty·reen
10. Candida
Pronunciation link: <https://www.merriam-webster.com/dictionary/Candida>
IPA: /ˈkæn.dɪ.də/
Phonetic Spelling: kan·duh·duh
11. albicans
Pronunciation link: <https://www.merriam-webster.com/dictionary/albicans>
IPA: /ælˈbɪ.kænz/
Phonetic Spelling: al·bih·kanz
12. aseptic
Pronunciation link: <https://www.merriam-webster.com/dictionary/aseptic>
IPA: /eɪˈsep.tɪk/
Phonetic Spelling: ay·sep·tik
13. inoculated
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculate>
IPA: /ɪˈnɑː.kjə.leɪ.tɪd/
Phonetic Spelling: ih·nah·kyuh·lay·tuhd
14. resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /ˌriː.səˈspend/
Phonetic Spelling: ree·suh·spend
15. epithelial
Pronunciation link: <https://www.merriam-webster.com/dictionary/epithelial>
IPA: /ˌɛp.əˈθiː.li.əl/
Phonetic Spelling: eh·puh·thee·lee·uhl
16. colonization
Pronunciation link: <https://www.merriam-webster.com/dictionary/colonization>
IPA: /ˌkɑː.lə.nəˈzeɪ.ʃən/
Phonetic Spelling: kah·luh·nuh·zay·shuhn
17. asymptomatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/asymptomatic>
IPA: /ˌeɪ.sɪmp.təˈmæt.ɪk/
Phonetic Spelling: ay·simp·tuh·mat·ik
18. symptomatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/symptomatic>
IPA: /ˌsɪmp.təˈmæt.ɪk/
Phonetic Spelling: simp·tuh·mat·ik

19. equilibrate
Pronunciation link: <https://www.merriam-webster.com/dictionary/equilibrate>
IPA: /ˌiː.kwɪˈlɪ.bri.ɛt/
Phonetic Spelling: ee·kwuh·lih·bree·ayt
20. aspirate
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
IPA: /ˈæs.pəˌreɪt/
Phonetic Spelling: ass·puh·rayt
21. microscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>
IPA: /maɪˈkraɪ.skə.pi/
Phonetic Spelling: my·krah·skuh·pee
22. median
Pronunciation link: <https://www.merriam-webster.com/dictionary/median>
IPA: /ˈmiː.di.ən/
Phonetic Spelling: mee·dee·uhn
23. survival
Pronunciation link: <https://www.merriam-webster.com/dictionary/survival>
IPA: /sərˈvaɪ.vəl/
Phonetic Spelling: ser·vy·vuhl
24. milliliter
Pronunciation link: <https://www.merriam-webster.com/dictionary/milliliter>
IPA: /ˈmɪl.ə.li.t̬ər/
Phonetic Spelling: mil·uh·lee·ter
25. ID50
Pronunciation link: No confirmed link found
IPA: /ˌaɪ.diˈfɪf.ti/
Phonetic Spelling: eye·dee·fif·tee