FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 68379
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=20855643

Title: Environmental Sampling of Photosynthetic Microbes and Their Viruses: From Field to Lab

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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** , all done
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? **No**

Current Protocol Length
Number of Steps: 17
Number of Shots: 34

Introduction

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

1.1. **Ruben Michael Ceballos:** The goal of these projects is to quantify physiological parameters in viral infections, then map -omics substrates to the observed physiology, leading to models that predict changes in virus-host dynamics.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.4*

Videographer's Note: During Ruben Ceballos's introduction video, I shot a extra take of the first two responses [1.1.1 & 1.2.1]. These extra takes are in file TLV_2862, and I personally think they're better than the original takes.

What are the most recent developments in your field of research?

1.2. **Ruben Michael Ceballos:** With advances in high-throughput sequencing and streamlined bioinformatics workflows, multi-omics analyses are readily accessible, which provides an opportunity to explain physiological phenomena as a function of specific -omics substrates.

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

What technologies are currently used to advance research in your field?

1.3. **Ruben Michael Ceballos:** Using experimental infections, electron microscopy, quantitative PCR, next-generation sequencing, and robust bioinformatics pipelines, network analyses can identify relationships between -omics and physiological variables, which informs the development of predictive models.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.7*

What are the current experimental challenges?

1.4. **Ruben Michael Ceballos:** The biggest challenge is generating pure, single genotype virus stocks with accurate titers so multiplicity of infection can be determined for experimental infections. Titer determination remains an issue in virology.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.5.2*

What significant findings have you established in your field?

1.5. **Ruben Michael Ceballos:** We developed metrics to calculate relative virulence between two viruses infecting a host and one virus infecting different hosts in single-host/single-virus infections. We standardized a workflow that works across virus systems.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.6*

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

Testimonial Questions (OPTIONAL):

Videographer:

- ***Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.***
- ***Also, kindly note that testimonial statements will be presented live by the authors, offering their spontaneous perspectives.***

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Dr. Ruben Michael Ceballos, Associate Professor of Virology**: (authors will present their testimonial statements live)

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Dr. Ruben Michael Ceballos, Associate Professor of Virology**: (authors will present their testimonial statements live)

Protocol

NOTE: Section 2 was drafted from author provided footage

2. Sampling and Processing Marine Microbes and Viruses for Isolation

Demonstrator: Ruben Michael Ceballos and Sobroney Heng

2.1. To begin, perform environmental sampling offshore, along the Southern California coastline **[1-TXT].**

2.1.1. LAB MEDIA: IMG_0003.MOV 00:03 – 00:12

AND
IMG_0004.MOV 00:02 – 00:04
TXT: Deploy boat anchor at the sampling site then record GPS co-ordinates

2.2. Attach a 10-foot-long collapse-resistant rubber flex hose to the outlet of the pump. Plug the transfer pump electrical cord into the inverter generator. Once assembled, prime the transfer pump and test to ensure the pump system is operational **[1]**.

2.2.1. LAB MEDIA: IMG_0011.MOV 00:01 – 00:21

2.3. Secure one end of a 25-meter-long nylon marine rope having a diameter of 1.25 centimeters to the eyelet of a 14-kilogram mushroom anchor **[1]**. Use duct tape to secure the rope to the blue PVC tubing every 3 meters **[2-TXT]**.

2.3.1. LAB MEDIA: IMG_0009.MOV 00:08 – 00:30

2.3.2. LAB MEDIA: IMG_0010.MOV 00:02 – 00:12
TXT: Set suction strainer about 1 m above anchor

2.4. Once a steady flow of water is being transferred, discharge 1 to 2 tube volumes back into the ocean to ensure that water from the desired depth is flowing through the pump **[1]**. Collect water samples in 20 Liter water jugs to transport them to the field station **[2]**.

2.4.1. LAB MEDIA: IMG_0012.MOV 00:00 – 00:11

2.4.2. LAB MEDIA: IMG_0012.MOV 00:12-00:24

2.5. Filter the water sample through a filter cup assembly **[1-TXT].** Then connect the filter cup to a portable air diaphragm vacuum pump using clear vinyl tubing **[2].** Transfer the sample into the filter cup and begin filtration **[3].** When complete, transfer the filtrate into a water collection bottle and label it **[4].**

2.5.1. LAB MEDIA: IMG_0014.MOV 00:11 – 00:20
TXT: Use both 0.45 µm and 0.22 µm filter cup assemblies

2.5.2. LAB MEDIA: IMG_0014.MOV 00:21-00:25

2.5.3. LAB MEDIA: IMG_0015.MOV 00:03 – 00:30

2.5.4. LAB MEDIA: IMG_0016.MOV 00:10-00:22, 00:37-00:44

2.6. Now use a scalpel to cut out the filter from the filter cup assembly **[1].** Cut it in half and transfer into a 50-milliliter centrifuge tube **[2].** Transfer 50 milliliters of the filtered seawater into the same centrifuge tube **[3-TXT].**

2.6.1. LAB MEDIA: IMG_0017.MOV 00:00-00:26

2.6.2. LAB MEDIA: IMG_0018.MOV 00:00-00:11

2.6.3. LAB MEDIA: IMG_0018.MOV 00:12-00:27
TXT: Label and seal the tube

2.7. To concentrate mixed samples, for each sampling depth by site, ship back the ultrafiltrate with a cell debris-laden 0.45-micrometre filter to the laboratory and centrifuge **[1-TXT]**.

2.7.1. LAB MEDIA: IMG_0021.MOV 00:01 – 00:41
TXT: Centrifugation: 2000 x *g*, 10 min, 4 °C

2.8. Transfer 60 milliliters of the filtrate into a spin filter **[1-TXT].** Centrifuge it at 6000 *g* for 10 to 20 minutes to concentrate it **[2].** Repeat centrifugation until a 1.5 to 2-milliliter concentrate is obtained **[3].**

2.8.1. LAB MEDIA: IMG_0036.MOV 00:03-00:12
TXT: Spin Filter: 3000 NWL

2.8.2. LAB MEDIA: IMG_0037.MOV 00:06 – 00:18

2.8.3. LAB MEDIA: IMG_0043.MOV 00:02 – 00:14

2.9. Transfer the resultant concentrate into a 2-milliliter Eppendorf tube for further transmission electron microscopy imaging **[1-TXT].**

2.9.1. LAB MEDIA name: IMG_0044.MOV 00:00-00:22
TXT: Store aliquots at 4 °C (short-term) or 20 °C (long-term)

2.10. Streak environmental samples onto prepared agar plates **[1-TXT]**. To isolate single colonies, use a sterile inoculation loop to transfer an isolate to a new plate **[2].** Use the same loop to pick another colony and transfer into a conical flask containing liquid broth **[3].**

2.10.1. LAB MEDIA: IMG_0023.MOV Timestamps: 00:11-00:16, 00:22-00:30
TXT: Multiple single-genotype pure cultures are developed from mixed cultures to increase the probability of isolating a susceptible and permissive hosts

2.10.2. LAB MEDIA: IMG_0032.MOV 00:09-00:33

2.10.3. LAB MEDIA: IMG_0032. 00:54-01:20

3. Isolation and Characterization of Cyanobacterial Hosts for Environmental Virus Assays

Demonstrator: Sobroney Heng

Videographer's Note: During the shoot the talent mentioned needing to wear goggles for the protocol. One of each take for shot 3.1.1 and 3.2.1 include a wide enough shot to see the talent's face. Please use one of the other takes where the view is tight enough to avoid showing the talent not wearing goggles

3.1. Prepare 10 seed cultures in 20-milliliter culture tubes containing 5 milliliters of BG-11 media **[1].** Add 5 milliliters of a mix of ultrafiltrate from the sample site and BG-11 **[2]**.

3.1.1. Shot of 20 mL culture tubes with BG 11 media.

3.1.2. Talent adding 5 mL of ultrafiltrate with BG-11.

3.2. Pick a single colony isolate from a culture plate with a sterile toothpick **[1].** Then inoculate each tube individually by dropping one toothpick into each tube **[2]**.

3.2.1. Shot of a SCI being picked with a sterile toothpick.

3.2.2. Talent dropping the toothpick into a tube.

3.3. Once positive seed culture growth is detected **[1]**, repeat streak procedures until only a single colony phenotype appears on plates **[2]**.

3.3.1. Shot of a positive seed culture tube.

3.3.2. Talent streaking from the tube onto a plate.

3.4. Once cycles of liquid culture and solid media plate work result in a single cell phenotype, characterize microbial morphotypes using light microscopy **[1-TXT]**.

3.4.1. Talent operating light microscope to visualize sample cells. **TXT: Characterize with TEM and SEM if available**

Videographer's Note: For shot 3.4.1, I included both the SEM and TEM microscopes. The SEM is the smaller first microscope and the TEM is the second one, which is also labeled on the slate. TEM can be found starting at clip number 2892.

AND

SCOPE: SCOPE_single-cell-phenotype_Synechococcus.mp4 00:00-00:23
AND SCOPE_single-cell-phenotype_Synechocystis.mp4 00:00-00:23
Video Editor: Please play both shots side by side
For SCOPE_single-cell-phenotype_Synechococcus.mp4, please label video as "Synechococcus". For SCOPE_single-cell-phenotype_Synechocystis.mp4, please label video as "Synechocystis"

3.5. To detect virus activity, grow putative host lawns of cyanobacteria or microalgae from working stocks on BG-11 agar plates **[1]**. Once individual plaques appear on plates **[2]**, use a sterile loop to scrape a single plaque **[3]**. Swirl the loop in a 10-milliliter seed culture of the host **[4]**.

3.5.1. Talent spreading working stock cells evenly on agar plate using a sterile loop.

3.5.2. Shot of plate with individual plaque.

3.5.3. Talent selecting a distinct plaque and collecting it with a loop.

3.5.4. Talent swirling loop into labelled 10 milliliter culture tube.

3.6. To determine host range, prepare lawns of multiple putative hosts isolated from environmental samples or obtained from other laboratories or culture collections **[1]**.

3.6.1. Shot of prepared separate agar plates for different cyanobacterial strains.

3.7. Generate host growth curves by taking optical density at 730 nanometre readings at regular time intervals from infected and uninfected cultures **[1-TXT]**.

3.7.1. Talent pipetting samples from cultures into cuvettes and placing them into a spectrophotometer. **TXT: Measure OD_{730nm} every 8 h; Verify infection with TEM**

Results

4. Results

4.1. Pure cultures of photosynthetic microorganisms displayed distinct morphologies under light microscopy **[1]**. Based on morphological features **[2]** and 16S rRNA sequencing followed by phylogenetic analysis, the isolate was identified as being a species of cyanobacteria from the family Merismopediaceae **[3].** An alternative isolate showing oval-shaped green cells is suggestive of *Synechococcus* **[4]**.

4.1.1. LAB MEDIA: Figure 2.

4.1.2. LAB MEDIA: Figure 2 B

4.1.3. LAB MEDIA: Figure 5A

4.1.4. LAB MEDIA: Figure 2A and 4A.

4.2. The *Synechocystis*-like strain SWII *(S-W-Two)* formed dense, uniform lawns on BG-11 agar, ideal for plate-based plaque assays **[1]**.

4.2.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the image on the left*

4.3. fSBL14 infection caused visible plaque clearing in spot-on-lawn assays **[1]**, and impeded the growth of the strain SWII in liquid culture **[2]**.

4.3.1. LAB MEDIA: Figure 6A. *Video editor: Highlight the image on the right*

4.3.2. LAB MEDIA: Figure 6B. *Video editor: Highlight the two tubes on the right*

4.4. Growth curves showed that SWII infected with concentrated ϕ *(Phi)* SBL14 suspension had a much slower increase in optical density **[1]** compared to both the diluted phage suspension and the uninfected control **[2]**.

4.4.1. LAB MEDIA: Figure 7A. *Video editor: Trace the red line (concentrated suspension)*

4.4.2. LAB MEDIA: Figure 7A. *Video editor: Trace the blue line (diluted suspension), and the black line (uninfected)*

4.5. Maximum specific growth rate was highest in uninfected SWII cultures **[1]**.

4.5.1. LAB MEDIA: Figure 7B. *Video editor: Highlight the bar corresponding to "Control"*

4.6. Transmission electron microscopy of infected SWII cultures revealed cytopathic effects, including membrane thickening, internal granules, and membrane blebbing **[1]**, which were absent in uninfected controls **[2]**.

4.6.1. LAB MEDIA: Figure 7D.

4.6.2. LAB MEDIA: Figure 7C.

Pronunciation Guide:

- **Environmental**
  - **Pronunciation link:** Britannica Dictionary provides the American pronunciation YouTube+15Encyclopedia Britannica+15YouTube+15
  - **IPA:** /ɪnˌvaɪrənˈmɛntl/
  - **Phonetic Spelling:** in-vy-ruh-NEN-tuhl
- **Photosynthesis**
  - **Pronunciation link:** Britannica Dictionary has the American pronunciation audio Forvo.com+4Forvo.com+4YouTube+4How To Pronounce+1
  - **IPA:** /ˌfoʊtoʊˈsɪnθəsəs/
  - **Phonetic Spelling:** foh-toh-SIN-thuh-suhs
- **Cyanobacteria**
  - **Pronunciation link:** Cambridge Dictionary US provides the American pronunciation YouTube+15Cambridge Dictionary+15YouTube+15
  - **IPA:** /ˌsaɪ.ə.noʊ.bækˈtɪr.i.ə/
  - **Phonetic Spelling:** sigh-uh-nohh-BAK-tuh-REE-uh
- **Virulence**
  - **Pronunciation link:** Cambridge Dictionary US provides the American pronunciation Cambridge Dictionary+2Synonyms.com+2YouTube+6Myefe+6How To Pronounce+6
  - **IPA:** /ˈvɪr.jə.ləns/
  - **Phonetic Spelling:** VIR-yuh-luhns
- **Metamorphosis**
  - **Pronunciation link:** Cambridge Dictionary US provides the American pronunciation Cambridge Dictionary+9Cambridge Dictionary+9Forvo.com+9Cambridge Dictionary+15Forvo.com+15YouGlish+15Collins Dictionary+15Cambridge Dictionary+15Cambridge Dictionary+15
  - **IPA:** /ˌmet.əˈmɔr.fə.sɪs/
  - **Phonetic Spelling:** met-uh-MOR-fuh-sis
- **Anthropology**
  - **Pronunciation link:** Cambridge Dictionary US audio available Cambridge Dictionary
  - **IPA:** /ˌænθrəˈpɑːlədʒi/
  - **Phonetic Spelling:** an-thruh-POL-uh-jee
- **Peristalsis**
  - **Pronunciation link:** Cambridge Dictionary US offers pronunciation Cambridge Dictionary
  - **IPA:** /ˌper.ɪˈstæl.sɪs/
  - **Phonetic Spelling:** peh-ruh-STAL-siss
- **Cyclic**
  - **Pronunciation link:** Cambridge Dictionary provides pronunciation Cambridge DictionaryEncyclopedia Britannica
  - **IPA:** /ˈsaɪklɪk/
  - **Phonetic Spelling:** SYE-klik
- **Microinjection** *(added for technical relevance in your field)*

- **Pronunciation link:** While not found in Cambridge, Merriam-Webster covers "microinjection" (same pattern applies) YouTube+15YouTube+15Cambridge Dictionary+15
- **IPA (American):** /ˌmaɪ.kroʊ.ɪnˈdʒɛk.ʃən/
- **Phonetic Spelling:** MY-kroh-in-JEK-shun

Omics *(commonly used in research contexts)*

- **Pronunciation link:** Not listed in dictionary, but "omics" pronounced like "oh-micks" (standard issue) — “omics” being plural of “-ome” domain analysis.
- **IPA (American approximation):** /ˈoʊmɪks/
- **Phonetic Spelling:** OH-miks