Submission ID #: 61904
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18880768

Title: Evaluating the Impact of Hydraulic Fracturing on Streams Using Microbial Molecular Signatures

Authors and Affiliations: Jeremy R. Chen See[1,2], Olivia Wright[1], Lavinia V. Unverdorben[1,2], Nathan Heibeck[1], Stephen M. Techtmann[3], Terry C. Hazen[4,5], and Regina Lamendella[1,2]

[1]Department of Biology, Juniata College
[2]Wright Labs, LLC
[3]Department of Biological Sciences, Michigan Technology University
[4]Biosciences Division, Oak Ridge National Laboratory
[5]Department of Civil and Environmental Engineering, University of Tennessee

Corresponding Author:
Regina Lamendella
lamendella@juniata.edu

Co-Authors:
chensej@juniata.edu
wrighog18@juniata.edu
unverlv16@juniata.edu
heibens18@juniata.edu
smtechtm@mtu.edu
tchazen@utk.edu

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. **Software:** Does the part of your protocol being filmed demonstrate software usage? **N**

3. **Interview statements:** Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees self-record interview statements outside of the filming date. JoVE can provide support for this option.

   NOTE: Videographer filmed the interview, please use those footage

4. **Filming location:** Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **55**

Introduction

1. Introductory Interview Statements

NOTE: Videographer filmed the interview, please use those footage.

REQUIRED:

1.1. **Regina Lamendella**: This protocol can be used to answer the question of whether and how hydraulic fracturing affects bacteria in nearby streams and, by extension, the streams themselves **[1]**.

1.1.1. ~~LAB MEDIA: To be provided by Authors:~~ Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Regina Lamendella**: The main advantage of this technique is its holistic nature, as it takes the researcher from sample collection through data analysis **[1]**.

1.2.1. ~~LAB MEDIA: To be provided by Authors:~~ Named talent says the statement above in an interview-style shot, looking slightly off-camera

Introduction of Demonstrator on Camera

1.3. **Regina Lamendella**: Demonstrating the procedure will be Jeremy Chen See, a technician in my laboratory, and Olivia Wright, an undergraduate student at Juniata College **[1][2]**.

1.3.1. INTERVIEW: Author saying the above

1.3.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera

Protocol

2. Sediment Sample Collection

2.1. To collect sediment samples for nucleic acid extraction, use gloves to submerge a capped, sterile, 50-milliliter conical tube into the stream water from the shore **[1-TXT]**.

2.1.1. WIDE: Talent submerging tube **TEXT: Collect sediment facing upstream if in water**

2.2. While the tube is submerged, remove the cap **[1]** and use the cap to scoop approximately 3 milliliters of sediment from a depth of 1 to 3 centimeters into the tube **[2]**.

2.2.1. Cap being removed

2.2.2. Sediment being collected into tube

2.3. After collecting the sample, dump all but approximately 1 milliliter of water out of the tube **[1]** and use a 1000-microliter pipette to add 3 milliliters of DNA-RNA preservative to the sample **[2]**.

2.3.1. Water being dumped

2.3.2. Talent adding DNA/RNA preservative to sample

2.4. Swirl the capped conical tube for 5 seconds to thoroughly mix the preservative and sample **[1]** and store the sample on ice **[2]**.

2.4.1. Tube being swirled/contents being mixed

2.4.2. Talent placing sample on ice

2.5. Upon return to the lab, store the sample at minus 20 degrees Celsius for 16S (sixteen-S) DNA analysis or minus 70 degrees Celsius for metatranscriptomics RNA analysis **[1]**.

2.5.1. Talent placing sample into freezer

3. Filter Collection

3.1. For filter collection, completely fill and empty an entire, sterile, 1-liter bottle with stream water three times to condition the bottle **[1]** before filling the bottle one final time **[2]**.

3.1.1. WIDE: Talent filling and dumping bottle

3.1.2. Bottle being filled and capped

3.2. On a stable surface, draw a full volume of stream water into a sterile Luer lock syringe **[1]** and connect the syringe to a sterile and DNA-RNA-free, 1.7-centimeter diameter polyethersulfone filter with a 0.22-micron pore size **[2]**.

3.2.1. Talent filling syringe

3.2.2. Talent connecting syringe to filter

3.3. Flush the entire volume of stream water through the filter **[1]**. When the entire sample volume has been filtered in the same manner, draw approximately 20 milliliters of air into the syringe **[2]** and push the air through the filter to remove any excess water from the filter **[3]**.

3.3.1. Talent flushing water through filter

3.3.2. Talent drawing air into syringe

3.3.3. Syringe being depressed/air being pushed through filter

3.4. Next, use a P1000 micropipette to add 2 milliliters of DNA-RNA preservative to the larger opening of the filter while holding the filter horizontally with the tip of the pipette in the barrel of the filter to ensure that the preservative enters the filter **[1-TXT]**.

3.4.1. Preservative being delivered to filter **TEXT: Change tip after each use**

3.5. Then seal the filter with tightly wrapped squares of paraffin film around each opening **[1]** and place the filter into a sterile sample bag on ice **[2]**.

3.5.1. Talent wrapping opening

3.5.2. Talent placing filter into bag on ice

3.6. Upon return from sampling, store the filters for 16S or for metatranscriptomic analysis as demonstrated **[1]**.

3.6.1. Talent placing bag into freezer

4. Nucleic Acid Extraction and Quantification

4.1. Before beginning a sample transfer, clean the work area with 10% bleach and 70% ethanol **[1]**.

4.1.1. WIDE: Talent wiping area, with bleach and ethanol containers visible in frame

4.2. For nucleic acid extraction from a sediment sample, use a flame- and ethanol-sterilized metal tool to transfer approximately 250 micrograms of sample into a microcentrifuge tube **[1-TXT]**.

4.2.1. Talent adding sample to tube, with Bunsen burner and beaker visible in frame *Videographer: Important step* **TEXT: Re-sterilize tool between samples**

4.3. For nucleic acid extraction from a filter sample, use a 70% ethanol- and flame-sterilized vise-grip to break open the filter casing on the sterile surface **[1]** and remove the core from the casing **[2]**. NOTE: 4.3.1 and 4.3.2 were combined

4.3.1. Casing being broken *Videographer: Important step*

4.3.2. Core being removed *Videographer: Important step*

4.4. Use a sterile scalpel slice at the top, bottom, and along the seam of the core **[1]** and use sterile tweezers to fold the filter paper **[2]** before cutting it into small pieces with the scalpel **[3]**.

4.4.1. Core being sliced *Videographer: Important/difficult step*

4.4.2. Filter paper being folded *Videographer: Important/difficult step*

4.4.3. Filter paper being cut into pieces *Videographer: Important/difficult step*

4.5. Then carefully place the filter pieces into a microcentrifuge tube without contacting any unsterilized surfaces **[1]**.

4.5.1. Talent adding pieces to bead tube *Videographer: Important/difficult step*

4.6. For lysis of the cells within either type of sample, subject the tube to a cell disruptor at high speed **[2]**.

4.6.1. Talent disrupting tube

4.7. After at least 5 minutes, centrifuge the samples **[1-TXT]** and transfer the supernatant to a new, sterile, microcentrifuge tube **[2]**.

4.7.1. Talent placing tube(s) into centrifuge **TEXT:** 10,000 g for 30 seconds at room temperature (~20°C)

4.7.2. Talent transferring supernatant to new tube

4.8. Add lysis buffer to the supernatant at a 1:1 ratio **[1-TXT]** and transfer the solution to the provided filter **[2]**.

4.8.1. Talent adding buffer to tube, with buffer container visible in frame **TEXT: See text for all buffer and solution preparation details**

4.8.2. Talent adding solution to filter

4.9. Place the filter into a new microcentrifuge tube **[1]** add 400 microliters of preparation buffer to the tube **[2]**.

4.9.1. Talent placing filter into tube

4.9.2. Talent adding buffer to tube, with buffer container and centrifuge visible in frame as possible

4.10. After centrifugation, add 700 microliters of wash buffer to the tube **[1]** and centrifuge the filter again **[2]**.

4.10.1. Talent adding buffer to tube, with buffer container visible in frame

4.10.2. Talent adding tube to centrifuge

4.11. After discarding the flow through, add 400 microliters of wash buffer to the tube for an additional centrifugation **[1]** and transfer the filter to a new, sterile microcentrifuge tube **[2]**.

4.11.1. Talent adding wash buffer to tube, with wash buffer container visible in frame

4.11.2. Talent adding filter to tube

4.12. To elute the DNA, treat the filter with 50 microliters of DNase-RNase-free water for 5 minutes at room temperature **[1]**. In the meantime, place a three-HRC (H-R-C) filter into a collection tube **[2]** and add 600 microliters of HRC prep solution **[3]**.

4.12.1. Talent adding DNase/RNase-free water to tube

4.12.2. Talent placing filter into tube

4.12.3. Talent adding solution to tube, with solution container visible in frame

4.13. After centrifugation, transfer the filter into a new, sterile microcentrifuge tube **[1-TXT]** and transfer the eluted DNA to the filter for centrifugation **[2-TXT]**.

4.13.1. Talent adding filter to tube **TEXT: 3 min, 8000 x g**

4.13.2. Talent adding DNA to tube **TEXT: 3 min, 16,000 x g**

4.14. The flow through contains the extracted DNA **[1]**.

4.14.1. Shot of flow through in tube

5. DNA 16S rRNA Library Creation

5.1. To create a DNA 16S rRNA library, first use the freshly extracted DNA product for 16S ribosomal RNA amplicon sequencing with a standard PCR protocol **[1]**.

5.1.1. WIDE: Talent adding sample to thermocycler

5.2. Mix 7 microliters of the resulting PCR product and 13 microliters of DNase free water **[1]** and load the PCR solution onto a 2% agarose gel **[2]**. NOTE: 5.2.1 and 5.2.2 were combined

5.2.1. Talent mixing sample in tube

5.2.2. Talent adding sample to gel

5.3. Then run the gel at 90 volts for 60-90 minutes to check for a band size of 386 base pairs as evidence of a successful amplification **[1]**.

5.3.1. LAB MEDIA: Figure 1 *Video Editor: please emphasize bright bands in lanes 4 and 6*

6. DNA 16S rRNA Library Purification

6.1. To purify the DNA 16S ribosomal RNA library, pool 10 microliters of the PCR products that yielded bright bands and 13 microliters of the samples that yielded faint bands in a sterile, microcentrifuge tube **[1]** and load around 150-200 nanograms of each pooled sample into individual wells of a new 2% gel **[3]**.

6.1.1. WIDE: Talent adding product to tube

6.1.2. Talent adding sample to gel

6.2. After running the gel as demonstrated, excise the 386-base pair bands from the gel **[1]** and use a commercial kit to purify the DNA **[2]**.

6.2.1. Band being excised *Videographer: Important step*

6.2.2. Talent opening purification kit, with sample visible in frame

6.3. Then elute the purified DNA with 30 microliters of 10-millimolar Tris (triss)-hydrochloride **[1-TXT]** and pack the purified libraries in dry ice before shipping for next generation sequencing **[2]**.

6.3.1. Talent adding Tris-HCl to tube, with Tris-HCl container visible in frame **TEXT: Tris: (hydroxymethyl)aminomethane**

6.3.2. Talent placing tubes into shipping container

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see? Please list 4 to 6 individual steps.

4.2.-4.5., 6.2.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success? Please list 1 or 2 individual steps from the script above.
4.4., 4.5. (essentially getting the filter paper out of the filter and into the extraction tub). The most important thing is just to take your time and be careful. More specifically, a small protrusion on the filter can be used to turn it so you can cut the paper off. Once the paper is off, it's helpful to use tweezers in addition to the sterile blade to fold the paper. The tweezers can then be used to put the folded paper into the tube. It's vital to make sure you have a firm grip on the paper before trying to put in the tube (to avoid inadvertently dropping it).

Results

7. Results: Representative Hydraulic Fracturing Stream Microbial Molecular Signature Analyses

7.1. In this representative analysis **[1]**, all extractions, except for one, would be dubbed successful **[2]**.

7.1.1. LAB MEDIA: Table 1
7.1.2. LAB MEDIA: Table 1 *Video Editor: please emphasize row 14*

7.2. Bright bands observed following PCR amplification indicate success for the 16S protocol **[2]**.

7.2.1. LAB MEDIA: Figure 1
7.2.2. LAB MEDIA: Figure 1 *Video Editor: please emphasize bright bands in lanes 4 and 6*

7.3. 16S samples should have a minimum of 1000 sequences **[1]**, with at least 5000 being ideal **[2]**, while metatranscriptomics samples should have a minimum of 500,000 sequences **[3]**, with at least 2,000,000 being ideal **[4]**.

7.3.1. LAB MEDIA: Figure 2 *Video Editor: please emphasize red vertical data line*
7.3.2. LAB MEDIA: Figure 2 *Video Editor: please emphasize green vertical data line*
7.3.3. LAB MEDIA: Figure 2 *Video Editor: please emphasize red vertical data line*
7.3.4. LAB MEDIA: Figure 2 *Video Editor: please emphasize green vertical data line*

7.4. Sediment samples from 21 different sites at 13 different streams for 16S and metatranscriptomics analysis are shown **[1]**.

7.4.1. LAB MEDIA: Figure 3A

7.5. Of those 21 sites, 12 were downstream of fracking activity and classified as hydraulic fracturing-positive **[1]** and 9 were either upstream of fracking activity or in a watershed in which fracking was absent and classified as hydraulic fracturing-negative **[2]**.

7.5.1. LAB MEDIA: Figure 3A *Video Editor: please emphasize red data points*
7.5.2. LAB MEDIA: Figure 3A *Video Editor: please emphasize blue data points*

7.6. As assessed by PERMANOVA analysis, the separation observed between the hydraulic fracturing-positive and -negative data **[1]** suggests that the hydraulic fracturing-positive samples were impacted by fracking **[2]**.

7.6.1. LAB MEDIA: Figure 3B
7.6.2. LAB MEDIA: Figure 3B *Video Editor: please separately outline/emphasize blue and red data clusters*

7.7. The most important random forest predictors would reveal which features were most essential for correctly differentiating samples **[2]**.

7.7.1. LAB MEDIA: Figure 3C
7.7.2. LAB MEDIA: Figure 3C *Video Editor: please add/emphasize data points*

7.8. If a taxon is identified as important by the random forest model **[1]**, its antimicrobial resistance profile in hydraulic fracking-positive samples could be compared to its profile in hydraulic fracking-negative samples **[2]**. If they differ greatly, that could suggest that fracking fluid containing biocides entered the stream **[3]**.

7.8.1. LAB MEDIA: Figure 3C *Video Editor: please top right data point*
7.8.2. LAB MEDIA: Figures 3C and 3D *Video Editor: please salmon wedge in Figure 3D*
7.8.3. LAB MEDIA: Figures 3C and 3D

Conclusion

8. Conclusion Interview Statements

NOTE: Videographer filmed the interview, please use those footage.

8.1. **Regina Lamendella**: Microbes are everywhere, so contamination is a huge potential issue with this type of work. The initial sample transfer steps are especially prone to this **[1]**.

8.1.1. ~~LAB MEDIA: To be provided by Authors:~~ Named talent says the statement above in an interview-style shot, looking slightly off-camera (4.2.-4.6.)

8.2. **Regina Lamendella**: If one is interested in microbial biodegradation or other metabolisms, shotgun sequencing of RNA can be used to investigate active microbial gene expression **[1]**.

8.2.1. ~~LAB MEDIA: To be provided by Authors:~~ Named talent says the statement above in an interview-style shot, looking slightly off-camera

8.3. **Regina Lamendella**: This technique allows the standardization of molecular techniques for investigating in situ bacterial communities as well as bioinformatics analyses of bacterial sequence data **[1]**.

8.3.1. ~~LAB MEDIA: To be provided by Authors:~~ Named talent says the statement above in an interview-style shot, looking slightly off-camera