

Submission ID #: 70187

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21407083>

Title: DNA Viral Size Fraction Metagenomics for Human Stool Samples

Authors and Affiliations:

Elizabeth R. Denison, Luke S. Hillary, Haley A. Bolanos, Hazel I. Anagu, Joanne B. Emerson

Department of Plant Pathology, University of California, Davis

Corresponding Authors:

Joanne B. Emerson jbemerson@ucdavis.edu

Email Addresses for All Authors:

Elizabeth R. Denison	erdenison@ucdavis.edu
Luke S. Hillary	lhillary@ucdavis.edu
Haley A. Bolanos	habolanos@ucdavis.edu
Hazel I. Anagu	hazelanagu@gmail.com
Joanne B. Emerson	jbemerson@ucdavis.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? **No**
- 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. **No**

Current Protocol Length

Number of Steps: 12

Number of Shots: 25

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. **Liz Denison:** We study environmental and host-associated viral populations, where part of our research aims to better understand the healthy human virome and evaluate common techniques used to study it.
 - 1.1.1. 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.~~

- 1.2. **Liz Denison:** This protocol provides a framework for researchers interested in using metagenomics to study viral communities present in the human gut.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

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

- 1.3. **Liz Denison:** By enriching and sequencing viral DNA from stool, researchers can then use bioinformatics to characterize gut virome diversity and ecology.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Liz Denison:** Since steps like filtration or DNase treatment can bias viral enrichment, it's important to consider methodological choices when interpreting any downstream sequencing results.

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

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.5. **Liz Denison:** The resulting sequencing data can be used for virome analysis, like assembling uncultivated viral genomes and analyzing ecological trends across human biosamples.

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

VIDEO NOTE: This answer was changed.

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

Ethics Title Card

This research has been approved by the Institutional Review Board (IRB) at the University of California, Davis

Protocol

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

2. Processing Human Stool Samples for Virion Purification

Demonstrators: Liz Denison or Haley Bolanos

- 2.1. To begin, obtain a human stool sample in a 50-milliliter conical tube [1] and add 8 milliliters of PPBS buffer directly to the tube [2]. Vortex the tube to thoroughly resuspend the sample [3].
 - 2.1.1. WIDE: Talent placing the 50 ml tube with human stool sample on the work desk inside the fume hood.
 - 2.1.2. Talent adding PPBS buffer into the conical tube containing stool.
 - 2.1.3. Talent vortexing the conical tube to resuspend the contents.
- 2.2. Shake the tubes at 300 revolutions per minute for 10 minutes at 4 degrees Celsius [1].
 - 2.2.1. Talent placing the tubes into the shaker.
- 2.3. Then, centrifuge the samples at 4000 g for 10 minutes at 4 degrees Celsius [1].
 - 2.3.1. Talent placing the tube into the centrifuge.
- 2.4. In a fume hood, carefully decant the supernatant into a new sterile 50-milliliter conical tube [1]. Store the tube at 4 degrees Celsius [2]. Retain the original conical tube containing the pellet and add 8 milliliters of PPBS [3]. Repeat the resuspension, shaking, and centrifugation steps 2 more times [4]
 - 2.4.1. Talent decanting the supernatant into a new sterile 50 milliliter conical tube in a fume hood.

NOTE: Part of the important process is the leftover pellet. Turn at end of clip shows pellet.
 - 2.4.2. Talent placing conical number 2 into cold storage.
 - 2.4.3. Talent adding PPBS to the pellet and vortexing.
 - 2.4.4. Talent placing the tube into the centrifuge.

2.5. After pooling the supernatant into the same tube [1], centrifuge the 50-milliliter conical tube containing the accumulated supernatant at 10000 *g* for 8 minutes at 4 degrees Celsius [2].

2.5.1. Talent adding more supernatant into the same 50 ml tube containing the supernatant from round 1.

2.5.2. Talent placing conical tube into the centrifuge.

2.6. Decant the supernatant into a new 50-milliliter conical tube [1] and centrifuge the tube at 10000 *g* at 4 degrees Celsius for 8 minutes [2].

2.6.1. Talent decanting the supernatant into a new conical tube.

2.6.2. Talent placing conical tube into the centrifuge.

3. Filtration and Ultracentrifugation to Obtain Concentrated Virions

Demonstrator: Liz Denison or Haley Bolanos

3.1. For filtration, stack a 5-micrometer filter on top of a 0.2-micrometer filter [1] and attach them to a single syringe, one by one [2]. Now, sequentially filter the supernatant through these filters [3] and collect the filtrate directly into an ultracentrifuge tube [4].

3.1.1. Talent attaching 5.0 micrometer filter to a 0.20 micrometer filter.

3.1.2. Talent attaching the filters to a syringe.

NOTE: Talent finds it easier to attach each filter to the syringe one at a time, instead of attaching them together first.

3.1.3. Talent adding the supernatant into the syringe for filtration.

3.1.4. Talent filtering the sample into an ultracentrifuge tube.

3.2. Check the mass of all ultracentrifuge tubes containing the samples [1] and pair tubes based on equal mass for ultracentrifugation [2].

3.2.1. Talent weighing ultracentrifuge tubes with samples.

NOTE: Please only use a shot of one of the tubes being weighed, not both. Both tubes should, in theory, weigh the same for the experiment.

3.2.2. Talent arranging tubes into balanced pairs on a rack.

3.3. Ultracentrifuge the samples at 112000 *g* under vacuum for 2 hours and 25 minutes at 4 degrees Celsius [1].

3.3.1. Talent placing ultracentrifuge tubes into the ultracentrifuge.

3.4. Remove the supernatant either by decanting or using a pipette while avoiding disturbance of the pellet, which contains the virions [1]. Then, resuspend the pellet gently [2]. **NOTE: The VO is edited for the additional shot**

3.4.1. Talent carefully removing the supernatant without disturbing the pellet.

Added shot: The pellet is being resuspended

3.5. If required, treat the concentrated virions with DNase in the presence of a suitable buffer [1] at 37 degrees Celsius for 30 minutes [2].

3.5.1. Talent adding DNase master mix to the resuspended pellet.

3.5.2. Talent placing the sample in an incubator.

3.6. Finally, perform DNA extraction using a commercial DNA extraction kit [1] and quantify the extracted DNA using a fluorescence-based method [2].

3.6.1. Talent adding the sample to a DNA purification bead tube.

3.6.2. Talent placing the sample in a fluorometer sample chamber.

Results

4. Results

- 4.1. Application of this protocol to a frozen stool sample processed in technical triplicate used quality-filtered sequences and de novo-assembled contigs to identify viral sequences and estimate their relative abundance from reads mapped to viral contigs [1].

- 4.1.1. LAB MEDIA: Figure 1

- 4.2. Viral reads comprised, on average, 74% of all sequenced reads in stool viromes [1], compared to only 4% in total metagenomes generated from the same sample [2].

- 4.2.1. LAB MEDIA: Figure 1A. *Video editor: Highlight the bars labeled “Stool Virome” showing approximately 74% viral reads*

- 4.2.2. LAB MEDIA: Figure 1A. *Video editor: Highlight the bars labeled “Stool Metagenome” showing approximately 4% viral reads*

- 4.3. High-confidence viral genomes were generated from stool samples, with a mean virus confidence score of 0.97 [1].

- 4.3.1. LAB MEDIA: Figure 1B. *Video editor: Highlight the “Stool Virome” box plot*

- 4.4. Viral contigs assembled from stool virome replicates had significantly higher mean virus confidence scores [1] than those from total metagenomes [2].

- 4.4.1. LAB MEDIA: Figure 1B. *Video editor: Highlight the “Stool Virome” group*

- 4.4.2. LAB MEDIA: Figure 1B. *Video editor: Highlight the “Stool Metagenome” group*

- 4.5. Modified versions of the protocol resulted in successful DNA extraction from the viral size fraction across different buffer types [1], filter sizes [2], and virion concentration methods [3].

- 4.5.1. LAB MEDIA: Figure 2. *Video editor: Highlight A*

- 4.5.2. LAB MEDIA: Figure 2. *Video editor: Highlight B*

- 4.5.3. LAB MEDIA: Figure 2. *Video editor: Highlight C*

1. Vortex
Pronunciation link: <https://www.merriam-webster.com/dictionary/vortex>
IPA: /'vɔːr.teks/
Phonetic Spelling: vor·teks
2. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sen.trə.fjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
3. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /,suː.pə'nei.tənt/
Phonetic Spelling: soo·per·nay·tuhnt
4. Resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /,riː.sə'spend/
Phonetic Spelling: ree·suh·spend
5. Ultracentrifugation
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultracentrifuge>
IPA: /,ʌl.trə'sen.tri.fjuː'geɪ.jən/
Phonetic Spelling: uhl·truh·sen·trih·fyoo·gay·shuhn
6. Virions
Pronunciation link: <https://www.merriam-webster.com/dictionary/virion>
IPA: /'vaɪ.ri.ən/
Phonetic Spelling: vy·ree·on
7. Filtration
Pronunciation link: <https://www.merriam-webster.com/dictionary/filtration>
IPA: /fɪl'treɪ.jən/
Phonetic Spelling: פִּיל·tray·shuhn
8. Ultracentrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultracentrifuge>
IPA: /,ʌl.trə'sen.trə.fjuːdʒ/
Phonetic Spelling: uhl·truh·sen·truh·fyooj
9. DNase
Pronunciation link: No confirmed link found
IPA: /'diː.ɛn.eɪs/
Phonetic Spelling: dee·en·ays
10. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊ'res.əns/
Phonetic Spelling: floor·eh·suhns
11. Fluorometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorometer>
IPA: /flʊ'raː.mə.tər/
Phonetic Spelling: floor·ah·muh·ter

12. Triplicate
Pronunciation link: <https://www.merriam-webster.com/dictionary/triplicate>
IPA: /'trip.lɪ.kət/
Phonetic Spelling: trip·lih·kuht
13. De novo
Pronunciation link: <https://www.merriam-webster.com/dictionary/de%20novo>
IPA: /deɪ 'noʊ.vəʊ/
Phonetic Spelling: day noh·voh
14. Contigs
Pronunciation link: No confirmed link found
IPA: /'kɑːn.tɪɡz/
Phonetic Spelling: kon·tigz
15. Metagenomes
Pronunciation link: No confirmed link found
IPA: /,met.ə'dʒiː.noʊmz/
Phonetic Spelling: met·uh·jee·nohmz
16. Viromes
Pronunciation link: No confirmed link found
IPA: /'vaɪ.ə.roʊmz/
Phonetic Spelling: vy·uh·rohms
17. Genomes
Pronunciation link: <https://www.merriam-webster.com/dictionary/genome>
IPA: /'dʒiː.noʊmz/
Phonetic Spelling: jee·nohmz
18. Replicates
Pronunciation link: <https://www.merriam-webster.com/dictionary/replicate>
IPA: /'rep.lɪ.kət/
Phonetic Spelling: rep·lih·kuht