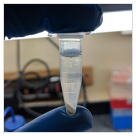

Version 2

Jan 22, 2021

Holobiont Virus Extraction Protocol (Veglia et al.) V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bmr5k586

Alex Veglia

ABSTRACT

The decribed holobiont virus extraction protocol contains five steps: i) Rinse and rest, ii) Tissue separation, iii) Physical cell disruption and homogenization, iv) Homogenate cleaning, and v) Virus particle concentration and extraction validation. These protocol stages were informed by previous virology studies applying common techniques for virus particle and nucleic acid extraction from biotic and abiotic materials (Williamson et al. 2003; Davy et al. 2006; Thurber 2009; Weynberg et al. 2014).The use of polyethylene glycol removed the need for ultracentrifugation making the protocol more practical for trips to the field and is designed to remove some level of impurities (BioVision Research Products).Overall, it is expected that most RNA viruses and double and single-stranded DNA viruses including cyanophages will be retained in the pellet. The speed of the final centrifugation step (Stage 4, Fig. 2) can be altered to target larger virus size classes (e.g. the proposed order *Megavirales*).

DOI

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PROTOCOL CITATION

Alex Veglia 2021. Holobiont Virus Extraction Protocol (Veglia et al.). **protocols.io** https://dx.doi.org/10.17504/protocols.io.bmr5k586 Version created by Alex Veglia

KEYWORDS

Virus, Extraction, Holobiont, Coral Reefs, Invertebrates

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PROTOCOL INTEGER ID

42525

MATERIALS TEXT

MATERIALS

☒ 2 mL centrifuge tubes (autoclavable) **Thermo Scientific**

☒ 1.5 mL centrifuge tube (sterile/autoclavable) **Thomas Scientific Catalog #1148T71**

☒ Disruption Beads for Tissue 1.0mm Bead Size Glass Beads 2.5 g/cc Density 454 Grams **Research Products International (rpi) Catalog #9832**

☒ Disruption Beads for Yeast/Fungi 0.5mm Bead Size Glass Beads 2.5 g/cc Density 454 Grams **Research Products International (rpi) Catalog #9831**

☒ Disruption Beads for Bacteria 0.1mm Bead Size Glass Beads 2.5 g/cc Density 454 Grams **Research Products International (rpi) Catalog #9830**

☒ Disruption Beads for Skin/Plant 2.3mm Bead Size Zirconia/Silica Beads 3.7 g/cc Density 454 Grams **Research Products International (rpi) Catalog #9838**

☒ PEG Virus Precipitation Kit **Contributed by users Catalog #K904**

Rinse and rest

1 First, invertebrate samples taken at reef or from aquarium must be rinsed with sterile seawater to mitigate potential contamination from ambient seawater.

At end of step, organisms sit in sterile beaker with sterile seawater prior to tissue processing step

1.1 *Before beginning the protocol, all bench areas should be sterilized using either 70% ethanol and/or lysol (or both)*

1) Remove reef sourced sea water from sampling container

***wipe down outside of containers and area around drain/waste storage container (if on benchtop)*

↓

2) Refill the sampling container with sterile seawater (0.2 micron (cellulose nitrate membrane) filter and autoclaved)

***wipe down outside of containers and area around drain/waste storage container (if on benchtop)*

3) Now, vigorously shake and/or swirl the sampling container, to remove/loosen any particulates or external mucus sheets from the invertabrate sample, for ~ **00:00:30**

4) After resting for around **00:02:00** , swirl sample once more prior to decanting particulate- or mucus-containing seawater into a waste beaker

5) Repeat Steps 2-4 two more times

6) After final round of rinsing, carefully move the invertebrate sample to a sterile glass beaker containing sterile seawater

OPTIONAL STEP *-> Post-rinse, allow invertebrate to sit in sterile beaker with sterile seawater for 5 to 10 minutes to allow fitlering organisms like sponges or tunicates to "flush out" particles sourced from*

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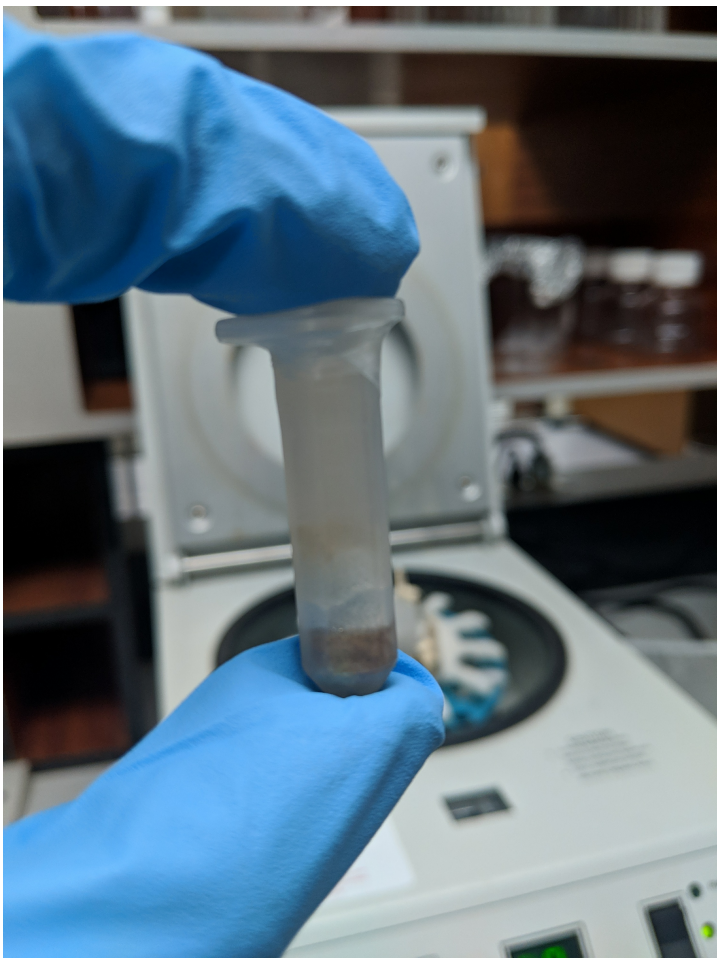
the reef prior to tissue processing

Tissue Separation

2 Depending on the organism, tissue can be seperated/processed in several ways. Generally, with fleshy organisms with or without a skeleton, a sterile razor blade can be used. A scalpel is sufficient as well, especially for softer organisms like sponges or seaslugs.

****This step is usually done in triplicate, for example, three tissue aliquots of 0.2-0.3 mL processes per sampled invertebrate to allow for ample volume of virus particles for downstream manipulation (i.e. isolation)**



Example of 2 mL tube with ~.2-.3 mL of tissue at base, then the glass bead mixture, and the sterile medium at top before homogenization step

2.1 **7)** ~0.2-0.3 mL of tissue was removed from organism using a sterile scalpel or razorblade and placed in a sterile 2 mL centrifuge tube

*** for organisms with very thin tissue layers over a calcium carbonate skeleton, whole samples can be processed using a mortar and pestle*

8) On top of the tissue, 0.5 mL of a sterilized glass bead mixture (e.g. 0.1 g of 0.5 mm beads, 0.15 g of 0.1 mm beads, 0.4 g of 1.0 mm beads and three 1.3 mm beads) is added to the 2 mL centrifuge tube

*** the glass bead mixture should contain three-four different sizes to target a majority of cellular organisms within invertebrate tissue*

9) Fill 2 mL centrifuge tube containing glass beads and invertebrate tissue with sterile seawater to the 2 mL mark

10) Seal the tops of 2 mL centrifuge tubes with parafilm prior to homogenization step to mitigate the chance of them opening and spilling

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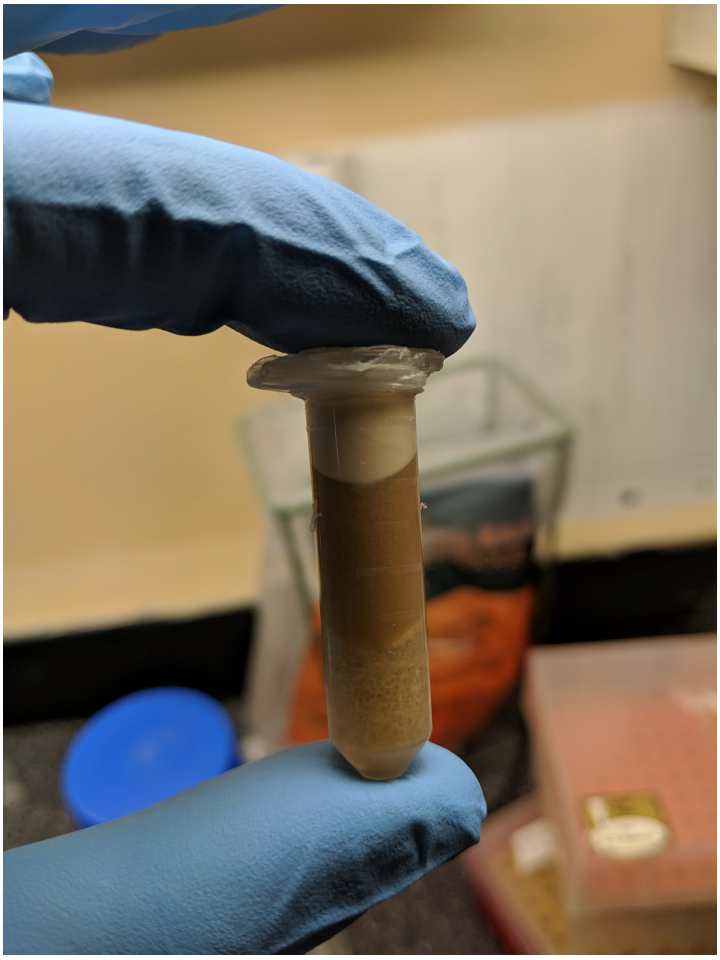
Physical cell disruption and homogenization

3 For the homogenization step a vortex or a FastPrep machine can be used. Between cycles of disruption, be sure to put samples on ice.



Example of what a sample may look like post homogenization

3.1 **11)** Secure the 2 mL centrifuge tubes containing, glass beads + tissue + sterile seawater, to a vortex machine or a FastPrep™ instrument (Thermo Electron Corporation; FP120)

12) Vortex -> On highest setting (10), run vortex for **00:05:00**

OR

FastPrep -> Run for **00:00:20**

13) Let samples sit on ice for **00:01:00**

14) Repeat Steps 12-13 three times (example result is pictured above)

Homogenate cleaning 36m

4 Instead of physical filters being used, cellular debris or any remaining intact cells were then removed from the supergntant using sequential centrifugation steps. Any bench top centrifuge capable of reaching speeds of 3,200 xg and 16,000 x g is ok to use for this step. Ideally, there would be temperature control, however, extractions have been successful without.

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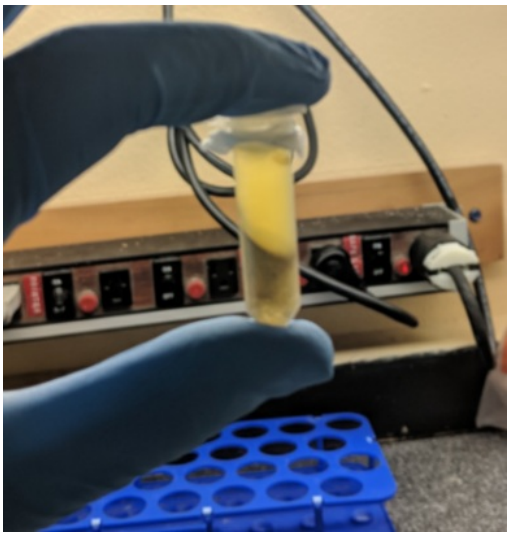

Example of result after first centrifugation step

15) Centrifuge samples for **00:16:00** at **3.200 x g, 4°C**

16) Transfer as much supernatant as possilbe to a new, sterile 1.5 mL centrifuge tube and if low volume, dilute to ~1.2 mL mark with sterile seawater

17) Centrifuge sample again for **00:17:00** at **16.000 x g, 4°C**

18) Transfer supernatant to new, sterile 1.5 mL centrifuge tube and dilute to 1.0 mL mark, with sterile seawater, before moving to the next step

Virus particle concentration and extraction validation

5 To seperate virus particles from the resultant supernatant, polyethylene glycol (5X; BioVision Research Products, CA) is

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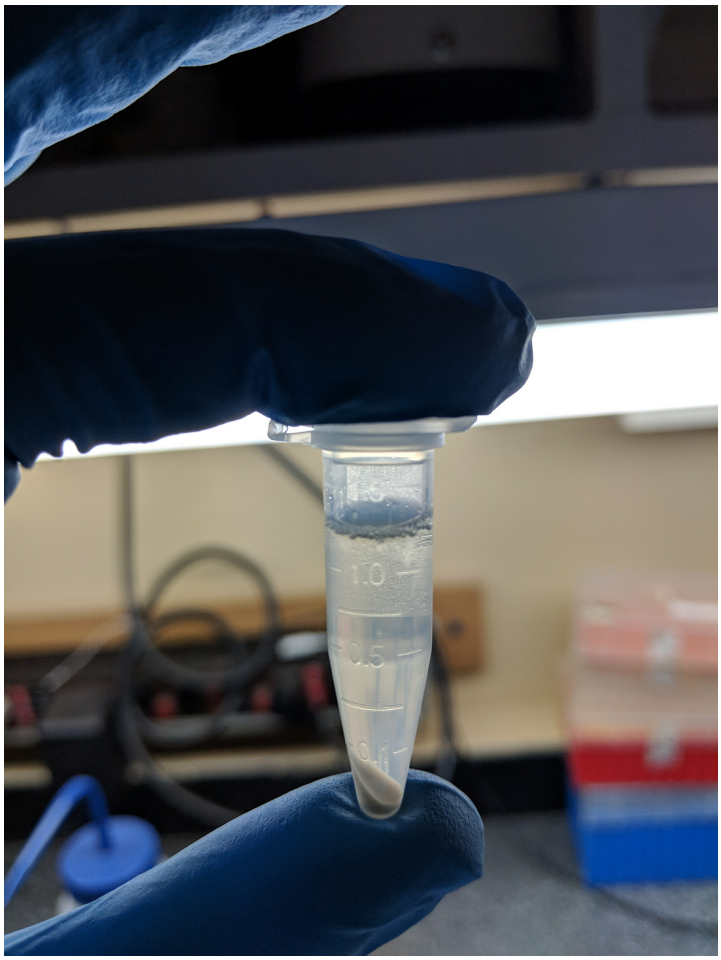
added to solution.



Example of virus pellet formation

19) Add 250-300 ul of PEG (polyethylene glycol 5x; BioVision - Catalog #K904) to the newly tranferred supernatant in the sterile 1.5 mL centrifuge tube.

20) Let the 1.5 mL centrifuge tube with the supernatant + PEG sit overnight (no less than 12 hours) at 4 degrees C

21) After the overnight incubation, centrifuge the sample for **00:30:00** at **3.200 x g, 4°C**

22) Very carefully, remove the supergnatant without disturbing the pellet using a pipette, or by carefully decanting to a waste container.

23) Re-suspend pellet in atleast ~1 mL of sterile seawater or to keep in high concetration, in ~200 ul of virus resuspension liquid (BioVision - Catalog #K904). At this point, depending on size of pellet, this solution can be diluted further to increase the volume usable for isolation efforts.

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