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Soil Viromics Protocol - Emerson Lab v1

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protocol .

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As of April 28, 2022, this is the soil viromics laboratory protocol used in the Emerson group at UC Davis to generate viral DNA from a soil sample for shotgun metagenomic sequencing. By no means did we 'invent' this protocol! It is a mash-up of several previously published protocols, influenced most substantially by Göller et al. 2020, Trubl et al. 2019, Williamson et al. 2005, and, for the ultracentrifugation step, Emerson et al. 2012 (see references below). This version of the protocol appears in Santos-Medellin et al. 2022, which would be an appropriate citation if you follow the procotol precisely, but we recommend citing at least Göller et al. 2020 as well, and for any deviations, please consult and reference the original publications as appropriate.

As part of this protocols.io page, you can find the laboratory protocol (under Steps), list of supplies and reagents (under Materials), and an extensive FAQ document (downloadable PDF under Guidelines & Warnings). Please consult the FAQ document before running the protocol. If you are inclined to deviate from the protocol, please consult the FAQ document first, as we have a number of suggestions in there, as well as info about what we have and have not tried. We will update/clarify as often as we can.

Please note that there is another soil viromics protocol by Trubl et al. available on protocols.io, which has some great suggestions/clarifications in the discussion: https://www.protocols.io/view/soil-viral-extraction-protocol-for-ssdna-dsdna-vir-q26g75qklwz1/v3

References:

Göller et al. 2020 Microbiome: https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-020-0795-2

Trubl et al. 2019 PeerJ: https://peerj.com/articles/7265/

Williamson et al. 2005 AEM: https://journals.asm.org/doi/full/10.1128/AEM.71.6.3119-3125.2005

Emerson et al. 2012 AEM (especially the supplementary methods): https://journals.asm.org/doi/10.1128/AEM.01212-12

Santos-Medellin et al. 2022 bioRxiv: https://www.biorxiv.org/content/10.1101/2022.03.24.485562v1.full

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Joanne Emerson, Sara Geonczy, Christian Santos Medellin, Anneliek Ter Horst, Jane Fudyma 2022. Soil Viromics Protocol - Emerson Lab v1. **protocols.io** https://protocols.io/view/soil-viromics-protocol-emerson-lab-v1-b7nyrmfw

protocol

Christian Santos-Medellín, Katerina Estera-Molina, Mengting Yuan, Jennifer Pett-Ridge, Mary K. Firestone, Joanne B. Emerson. Spatial turnover of soil viral populations and genotypes overlain by cohesive responses to moisture in grasslands. bioRxiv 2022.03.24.485562; doi: https://doi.org/10.1101/2022.03.24.485562

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See FAQ document:

Emerson soil viromics FAQ.pdf

Reagents / kits:

BSA - Bovine Serum Albumin (BSA) DNase- and Protease-free Powder **Fisher Scientific Catalog #BP9706100**

10X PBS Liquid Concentrate **Millipore**

PBS - **Sigma Catalog #6507-4L**

Potassium Citrate Monohydrate **Fisher**

K- citrate - **Scientific Catalog #P222500**

Magnesium sulfate **Sigma –**

$MgSO_4$ - **Aldrich Catalog #M7506**

RQ1 RNase-Free DNase,

DNase - 1,000u **Promega Catalog #M6101**

DNeasy PowerSoil Pro Kit

DNeasy PowerSoil PRO kit - (250) **Qiagen Catalog #Cat No./ID: 47014**

Qubit™ 1X dsDNA HS Assay Kit **Thermo**

Qubit dsDNA HS kit - **Fisher Catalog #Q33231**

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Materials:

Tower filter
Syringe filter
50 mL conicals
Ultracentrifuge tubes
1.5 mL tubes
Filtered pipette tips (10, 200, 1000 µl)
Serological pipette

Equipment:

Orbital shaker

Benchtop centrifuge

Eppendorf™ 5810R Centrifuge
Centrifuge
Eppendorf **02-262-8187**

Sorval centrifuge

Ultracentrifuge

Optima LE-80K Preparative Ultracentrifuge
Ultracentrifuge
Beckman Coulter **8043-30-1192**

PDF

Microcentrifuge

Pipettes

Vortex
Vortex adapter

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Vortex Adapter for 24 (1.5–2.0 ml) tubes
Vortex adapter
QIAGEN 13000-V1-24

Qubit fluorometer

Preparation

1 Prepare PPBS buffer (https://microbiomejournal.biomedcentral.com/articles/10.1186/s40168-020-0795-2) (2% BSA, 10% PBS, 1% K-citrate, 150 mM MgSO4)

1.1 Add PBS, K-citrate, MgSO4 to a 1 L bottle and fill to 1 L with milliQ water. Adjust pH to 6.5 (about 50 µl of 6N
HCl or about 100 µl of 2N HCl). Leave a stirring bar inside the bottle.

1.2 Autoclave bottle with buffer + empty bottle.

1.3 Once buffer bottle reaches room temperature, place it on a magnetic stir plate and slowly add BSA directly into the bottle (avoid foaming, don't agitate too much). Add ~5 g of BSA at a time.

1.4 Filter through a 0.22 µm vacuum filter into the autoclaved empty bottle.

1.5 Store in 4°C fridge (above freezing, not stable long-term, ~3 months, make sure buffer remains clear and
uncontaminated by aliquoting only the amount needed).

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2 Clean ultracentrifuge tubes - scrub, fill with NaOH for at least 1 hour (also soak black caps, but NOT metal red caps),
rinse 3x with MilliQ water, rinse with 100 µl of 0.02 µm filtered UltraPure water

3 Pre-cool the ultracentrifuge rotor: 50.2 Ti (12 samples) or 70Ti (8 samples) at 4 °C (overnight the night before), i.e., leave the rotor in the fridge or cold room.

4 Fresh soil is recommended, but if using frozen soil samples stored at -80°C, place samples in -20°C freezer overnight the night before.

5 Keep a record of how your ultracentrifuge tubes are labeled and which tube will go with which sample.

Virion Purification and Concentration

6 Per sample, weigh 10 g soil into a 50 mL conical tube.

7 Add 9 mL PPBS to 10 g soil.

8 Shake (orbital shaker) at 300 rpm at 4°C for 10 min. If necessary, the shaking can be done at room temperature.

9 **4000 rpm, 4°C, 00:10:00** Centrifuge at 4000 RPM at 4°C. 10m

10 Carefully pour supernatant into a new 50 mL conical & store supernatant at 4°C.

11 Repeat steps 7-10 so that samples have gone through a total of 3x shaking (may need to vortex prior to shaking to loosen up soil; if so, reduce the shaking time by the amount of vortexing time) & 3x centrifuging, and continue to pool supernatant into the same tube as in step 10. Should accumulate ~27 mL supernatant total (less if soils are high in organic matter).

12 Centrifuge conical containing accumulated supernatant for 8 min at 10,000 RCF (g) (7840 RPM) at 4°C.

Transfer supernatant to new 50 mL conical tube.

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13

14 Centrifuge the 50 mL conical tube containing supernatant (from step 13) for 8 min at 10,000 RCF (g) (7840 RPM) at 4°C.

15 (*optional*) Avoiding any pellet at the bottom of the tube, pre-filter the supernatant through a 5 µm filter into a new 50 mL conical tube.

16 Avoiding any pellet at the bottom of the tube, filter the supernatant through a 0.22 µm filter into an ultracentrifuge tube. Depending on the soil, you might need multiple filters, and/or this might be easier if you have pre-filtered (optional step 15 above).

17 Check the mass of all tubes, tare the heaviest one, and add PPBS to the others to equalize weight (within a 0.1 g
margin of error).

18 Ultracentrifuge at 35,000 rpm & 4°C under vacuum. Depending on the rotor, the length of spin will be different (according to sediment coefficient (s) = 58): 70Ti rotor (maximum 8 samples) = 3 hrs, 50.2Ti rotor (maximum 12 samples) = 2 hr 25 min.

19 Before removing tubes from the ultracentrifuge, pay attention to their orientation in the rotor so that you know where to expect the pellet of viral particles (on the lower outside of the tube).

Viral Pellet Resuspension and DNase Treatment

20 Carefully pour or remove supernatant by pipette, taking care not to disturb the viral pellet (which may or may not be visible, but you will often see a 'hole punch-sized' brown or yellow pellet).

21 DNase treatment is recommended, especially for fresh soil, but it may not be possible for some soils (e.g., very dry soil or soils stored frozen). If **not DNase treating samples**, go to first step of DNA Extraction below (and follow special instructions).

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22 If **DNAse treating samples**, resuspend pellet in 100 µl of 0.02 µm filtered UltraPure water into a labeled 2 mL
microcentrifuge tube. Set heat block to 37°C.

22.1 Make master mix (per sample: 10 µl of DNase buffer + 10 µl of DNase).

22.2 Add 20 µl of master mix to resuspended virions. Mix by pipetting.

22.3 Incubate at 37°C for 30 min.

22.4 Add 10 µl of Stop solution. Briefly vortex. Proceed to first step of DNA Extraction (and follow special instructions).

Viral DNA Extraction

23 In principle, many DNA extraction protocols would work at this point. You can try your favorite. We use the QIAGEN PowerSoilPro DNeasy kit.

24 To prepare, aliquot an appropriate amount of each solution. Keep CD2 at 4°C.

25 **Prepare Sample**

25.1 Turn on heat block to 65°C (ours takes about 15 min to reach temperature)

25.2 Spin the PowerBead Pro Tube briefly to ensure that the beads have settled at the bottom.

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25.3 Add up to 800 µl of CD1 to bead tubes.

25.4 (1) **No DNase step**: Scrape pellet from ultracentrifuge tube and place in bead tube. Take 100 µl of CD1, which is already in bead tube, and use it to resuspend whatever is left of the pellet and put into bead tube.

(2) **DNase step**: After stop solution has been added, transfer resuspended pellet to bead tube.

25.5 Vortex briefly to mix.

25.6 Incubate on heat block at 65°C for 10 min.

26 Cell Lysis

26.1 Vortex tubes at maximum speed for 10 min.

26.2 Centrifuge tubes at 15,000 x g (RCF) for 1 min.

26.3 Transfer supernatant to new microcentrifuge tube. (expect 500-600 µl)

27 Inhibitor Removal

27.1 Add 200 µl CD2

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27.2 Vortex 5 sec

27.3 Centrifuge at 15,000 x g (RCF) for 1 min. Avoiding the pellet, transfer up to 700 µl of supernatant to a new
microcentrifuge tube. (expect 500-600 µl).

28 Bind DNA

28.1 Add 600 µl CD3

28.2 Vortex for 5 sec.

28.3 Load 650 µl of lysate to an MB Spin Column.

28.4 Centrifuge at 15,000 x g (RCF) for 1 min.

28.5 Discard the flow-through and repeat previous steps to ensure that all the lysate has passed through the MB
Spin Column.

28.6 Carefully place the MB Spin Column into a new collection tube (provided).

29 Wash

29.1 Add 500 µl EA to the MB Spin Column.

29.2 Centrifuge at 15,000 x g (RCF) for 1 min.

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29.3 Discard the flow-through and place the MB Spin Column back into the same 2 ml collection tube.

29.4 Add 500 µl of C5 onto spin column.

29.5 Centrifuge at 15,000 x g (RCF) for 1 min.

29.6 Discard the flow-through and place the MB Spin Column into a new 2 ml collection tube (provided).

29.7 Centrifuge at 16,000 x g (RCF) for 2 min. Carefully place the MB Spin Column into a new 1.5 ml elution tube
(provided).

30 **Elute DNA**

30.1 Add 50-100 µl of C6 to center of the white filter membrane.

30.2 Centrifuge at 15,000 x g for 1 min. Discard the MB Spin Column. The DNA is now ready for downstream
applications.

DNA Quantification

31 Quantify DNA w/ method of choice (e.g., Qubit, use 2 µl, https://assets.thermofisher.com/TFS-Assets/LSG/manuals/Qubit_dsDNA_HS_Assay_UG.pdf). Store short-term (up to a few weeks) at -20°C after quantification, longer term at -80°C.

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