Version 3

Working

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Soil viral extraction protocol for ssDNA & dsDNA viruses Version 3

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ABSTRACT

Protocol for resuspending viruses from soils and sediments. See attached file for considerations, adaptations, and references. This version does not include CsCl purification, see version 2 for CsCl information.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

- Trubl, G., Solonenko, N., Chittick, L., Solonenko, S.A., Rich, V.I. and Sullivan, M.B., 2016. Optimization of viral resuspension methods for carbon-rich soils along a permafrost thaw gradient. *PeerJ, 4*, p.e1999.
- Trubl, G., Solonenko, N., Li, YF, Roux, S., Bolduc, B.; Rodríguez-Ramos, J., Carlson, M., Eloe-Fadrosh, E., Rich, V.I., Sullivan, M.B. (Submitted to PeerJ). Shifting from particles to DNA: a protocol to generate viromes from challenging soils.

More protocols on www.protocols.io under Dr. Matthew Sullivan's lab

DOCX

Generate_soil_viromes_protocol_v4.docx

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

GUIDELINES

- Need to record how much buffer is collected for viral counts
- During the manual shaking, make sure the sample is broken up
- The more new tubes the samples touch, the greater the viral loss will be
- For the Amicon step, try not to use new filters. The filters will slowly get clogged and it may take a while depending on how many contaminants (e.g. organics) there are. Using new filters will increase viral loss. The same concept applies to the 0.2 µM filters. The composition of the filter may affect viral recovery.
- For the DNA extraction, use the Qiagen PowerSoil DNA extraction kit
- Need to do Swift library prep. If you want quantitative samples that have both ssDNA and dsDNA viruses
- I have outlined areas where you can stop (store in 4°C overnight)
- This protocol does not include EDTA in the AKC buffer. EDTA interferes with DNase and it mainly used to chelate metals. Don't add unless you have high metal contamination
- You will get contamination from any past or present viruses cultured or used in the lab

BEFORE STARTING

Preparations

- AKC Buffer (Make before and store at 4°C)
- "Amended K-citrate": 1% K-citrate + 10% PBS + 5 mM EDTA (don't add if doing DNase) + 150 mM $MgSO_4$; (per Liter: 10g of k-citrate, 1.44g of Na_2HPO_4 *7H_2O, and 0.24g of KH_2PO_4 brought to pH 7)

- 1% BSA (w/v) in PBS
- Need 2 ml per Amicon filter. Prepare 1% BSA (w/v) in PBS.
- DNase
- Weigh out sample in 50 ml centrifuge tubes

Day 1- Resuspension of Viral-Like Particles

1 In 4°C cold room, add 25 ml of AKC' buffer.

25 ml

NOTE
sample needs to be supersaturated; need to record how much you recover

2 Place on shaker at 400 rpm for 15 minutes at 4°C

00:15:00

3 Vortex tubes for 1 min on the highest setting. (1/3)

00:01:00

4 Shake manually for 30 s at 4°C.

00:00:30

5 Vortex tubes for 1 min on highest setting. (2/3)

00:01:00

6 Shake manually for 30 s at 4°C.

00:00:30

7 Vortex tubes for 1 min on highest setting. (3/3)

00:01:00

8 Shake manually for 30s at 4°C.

00:00:30

9 Centrifuge tubes for 20 minutes at 15,000 g at 4°C to pellet soil and plant debris.

00:20:00

10 Pipet supernatant into a new 50 ml tube.

Day 1- second resuspension

In 4°C cold room, add 25 ml of AKC' buffer.

11 25 ml

NOTE
This needs to be done on the same initial soil material for a total of three resuspensions.

NOTE
sample needs to be supersaturated; need to record how much you recover

Day 1- Resuspension of Viral-Like Particles

12 Place on a shaker at 400 rpm for 15 minutes at 4°C.

13 Vortex tubes for 1 min on the highest setting. (1/3)
00:01:00

14 Shake manually for 30 s at 4°C.
00:00:30

15 Vortex tubes for 1 min on the highest setting. (2/3)
00:01:00

16 Shake manually for 30 s at 4°C.
00:00:30

17 Vortex tubes for 1 min on highest setting (3/3)
00:01:00

18 Shake manually for 30 s at 4°C
00:00:30

19 Centrifuge tubes for 20 minutes at 15,000 g at 4°C to pellet soil and plant debris

20 Pipet supernatant into a new 50 ml tube.

Day 1- third resuspension

21 In 4°C cold room, add 25 ml of AKC' buffer .
25 ml

NOTE

sample needs to be supersaturated; need to record how much you recover

NOTE
This needs to be on the same initial soul material again for a total of three resuspensions

22 Place on a shaker at 400 rpm for 15 minutes at 4°C.

23 Vortex tubes for 1 min on the highest setting. (1/3)
00:01:00

24 Shake manually for 30 s at 4°C.

25 Vortex tubes for 1 min on highest setting (2/3)
00:01:00

26 Shake manually for 30 s at 4°C.

27 Vortex tubes for 1 min on highest setting (3/3)
00:01:00

28 Shake manually for 30 s at 4°C.

29 Centrifuge tubes for 20 minutes at 15,000 g at 4°C to pellet soil and plant debris.

30 Pipet supernatant into a new 50 ml tube.

Day 1- filtration

31 Have all supernatant collected into one tube.

32 Filter supernatant with a 0.22 or 0.45 µm vacuum filter into new 50 ml tubes to remove microbes.

NOTE

0.22 µm may lose larger viruses, but increase microbial contamination

NOTE
use fewer filters to loose fewer viruses; filter type affects viral recovery

33 Store the filtrate at 4°C overnight.

Day 2

34 DNase samples

35 Incubate 2 ml of 1% BSA on Amicon filter for 1 hour
2 ml
01:00:00

36 Centrifuge filters at 1,000 g for 10 min (or until all BSA filters through) .
00:10:00

37 Wash filters by centrifuging filters with 2 ml 1x PBS at 1,000g for 10 min (or until all PBS is filtered through).
2 ml
00:10:00

38 Concentrate samples with Amicon filters (to ~5ml for CsCl purification).

39 Store at 4°C overnight.

Day 4- DNA extraction

40 DNA extraction using PowerSoil DNA extraction kit; you don't need the beads, but still use the bead solution; do alternative heat lysis (see attachemnt for notes)

41 DNA elutes in 100 µl

Day 5

42 Put some DNA into a new tube for a working sample.

43 Check microbial contamination with qPCR 16S analyses (~3 µl).

44 Quantify DNA with PicoGreen or Qubit (~2 µl).

45 NanoDrop for DNA purity (~2 µl).

46 You need 50 µl for shearing if you're using Swift kit (for ssDNA viruses; otherwise Nextera XT for dsDNA viruses).

47 If you need to resize the DNA you can use AMPure beads (same ones that are used in the Swift kit) or a Pippin prep.