Soil viral extraction protocol for ssDNA & dsDNA viruses

Version 2

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Abstract

Protocol for resuspending viruses from soils and sediments.

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Guidelines

- Need to record how much buffer is collected for viral counts
- During the manual shaking, make sure the sample is broken up
- If you can't do the CsCl gradients or you lose too much biomass from it, then you can try and replace it with DNase
- 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
- If you don't care about ssDNA viruses, then get rid of the 1.30 g/cm^3 density layer. Add the 1.5 ml to the other layers; there will be some contamination in the 1.3 layer (See Thurber et al. 2009_Laboratory procedures to generate viral metagenomes)
- 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
- Ultracentrifugation at 24,000 rpm for at least 2 hours (longer for better separation)

Before start

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
- CsCl Density gradients
- Weigh out sample in 50 ml centrifuge tubes

Protocol

Day 1- first resuspension

Step 1.

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

AMOUNT
25 ml Additional info:

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

Day 1- first resuspension

Step 2.

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

DURATION
00:15:00

Day 1- first resuspension

Step 3.

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

DURATION
00:01:00

Day 1- first resuspension

Step 4.

Shake manually for 30 s at 4°C.

DURATION
00:00:30

Day 1- first resuspension

Step 5.

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

DURATION
00:01:00

Day 1- first resuspension

Step 6.

Shake manually for 30 s at 4°C.

DURATION
00:00:30

Day 1- first resuspension

Step 7.

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

DURATION
00:01:00

Day 1- first resuspension

Step 8.

Shake manually for 30s at 4°C.

DURATION
00:00:30

Day 1- first resuspension

Step 9.

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

DURATION
00:20:00

Day 1- first resuspension

Step 10.

Pipet supernatant into a new 50 ml tube.

Day 1- second resuspension

Step 11.

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

AMOUNT
25 ml Additional info:

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

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

Day 1- second resuspension

Step 12.

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

DURATION
00:15:00

Day 1- second resuspension

Step 13.

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

DURATION
00:01:00

Day 1- second resuspension

Step 14.

Shake manually for 30 s at 4°C.

DURATION
00:00:30

Day 1- second resuspension

Step 15.

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

DURATION
00:01:00

Day 1- second resuspension

Step 16.

Shake manually for 30 s at 4°C.

DURATION
00:00:30

Day 1- second resuspension

Step 17.

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

DURATION
00:01:00

Day 1- second resuspension

Step 18.

Shake manually for 30 s at 4°C

DURATION

00:00:30

Day 1- second resuspension

Step 19.

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

DURATION

00:20:00

Day 1- second resuspension

Step 20.

Pipet supernatant into a new 50 ml tube.

Day 1- third resuspension

Step 21.

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

AMOUNT

25 ml Additional info:

NOTES

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

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

Day 1- third resuspension

Step 22.

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

DURATION

00:15:00

Day 1- third resuspension

Step 23.

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

DURATION

00:01:00

Day 1- third resuspension

Step 24.

Shake manually for 30 s at 4°C.

DURATION
00:00:30

Day 1- third resuspension

Step 25.

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

DURATION
00:01:00

Day 1- third resuspension

Step 26.

Shake manually for 30 s at 4°C.

DURATION
00:00:30

Day 1- third resuspension

Step 27.

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

DURATION
00:01:00

Day 1- third resuspension

Step 28.

Shake manually for 30 s at 4°C.

Day 1- third resuspension

Step 29.

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

DURATION
00:20:00

Day 1- third resuspension

Step 30.

Pipet supernatant into a new 50 ml tube.

Day 1- filtration

Step 31.

Have all supernatant collected into one tube.

Day 1- filtration

Step 32.

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

NOTES

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0.22 µm may lose larger viruses, but increase microbial contamination

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

Day 1- filtration

Step 33.

Store the filtrate at 4°C overnight.

Day 2

Step 34.

DNase samples or skip if you do CsCl purification.

Day 2

Step 35.

Incubate 2 ml of 1% BSA on Amicon filter for 1 hour

AMOUNT

2 ml Additional info:

DURATION

01:00:00

Day 2

Step 36.

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

DURATION

00:10:00

Day 2

Step 37.

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

AMOUNT

2 ml Additional info:

DURATION

00:10:00

Day 2

Step 38.

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

Day 2

Step 39.

Store at 4°C overnight.

Day 3- Density gradient ultracentrifugation

Step 40.

CsCl protocol (need ultracentrifuge; SW41 bucket; major viral loss step; some viruses sensitive to CsCl, see Thurber et al. 2009)

NOTES

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Use densities:

1. 20 g/cm^3 (1.2) density layer is 1.0 ml (microbial cells)
2. 30 g/cm^3 (1.3) density layer is 1.5 ml (for ssDNA viruses)
3. 40 g/cm^3 (1.4) density layer is 1.5 ml (for ssDNA & some dsDNA viruses)
4. 50 g/cm^3 (1.5)density layer is 1.5 ml (dsDNA viruses)
5. 65 g/cm^3 (1.65) density layer is 1.0 ml (DNA and other contaminants)

Day 4- DNA extraction

Step 41.

DNA extraction using PowerSoil DNA extraction kit; you don't need the beads, but still use the bead solution; do alternative heat lysis

Day 4- DNA extraction

Step 42.

DNA elutes in 100 µl

Day 5

Step 43.

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

Day 5

Step 44.

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

Day 5

Step 45.

Quantify DNA with PicoGreen or Qubit (2 µl).

Day 5

Step 46.

NanoDrop for DNA purity (2 µl).

Day 5

Step 47.

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

Day 5

Step 48.

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.