Soil viral extraction protocol for ssDNA & dsDNA viruses

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Abstract

Resuspend 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

1. **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

Step 1.

Day 1

1. In 4°C cold room, add 25 ml of AKC buffer (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
3. Vortex tubes for 3 min on highest setting, after each minute 30s manual shaking in 4°C
4. Centrifuge tubes for 20 minutes at 15,000 g at 4°C to pellet soil and plant debris
5. Pipet supernatant into a new 50 ml tube
6. Repeat steps 1-5 two more times on the same initial soil material for a total of three resuspensions. Have all supernatant collected into one tube
7. Filter supernatant with a 0.22 or 0.45 µm vacuum filter (0.22 µm may lose larger viruses, but increase microbial contamination) into new 50 mL tubes to remove microbes (use fewer filters to loose fewer viruses; filter type affects viral recovery).
8. Store the filtrate at 4°C overnight

Day 2

Step 2.

Day 2

1. DNase samples or skip if you do CsCl purification
2. Incubate 2 ml of 1% BSA on Amicon filter for 1 hour. Centrifuge filters at 1,000 g for 10 min (or until all BSA filters through) and then wash filters by centrifuging filters with 2 ml 1x PBS at 1,000g for 10 min (or until all PBS is filtered through).
3. Concentrate samples with Amicon filters (to 5ml for CsCl purification)
4. Store in 4°C overnight

Day 3

Step 3.

Day 3

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

Use densities:

1. 20 g/cm^3 density layer is 1.0 ml (microbial cells)
2. 30 g/cm^3 density layer is 1.5 ml (for ssDNA viruses)

3. 40 g/cm^3 density layer is 1.5 ml (for ssDNA & some dsDNA viruses)
4. 50 g/cm^3 density layer is 1.5 ml (dsDNA viruses)
5. 65 g/cm^3 density layer is 1.0 ml (DNA and other contaminants)

2. Record and combine 1.3-1.52 g/cm^3 fractions and homogenize
3. Amicon concentrate to 500ul
4. Store in 4°C overnight

Day 4

Step 4.

Day 4

1. DNA extraction using PowerSoil DNA extraction kit; you don't need the beads, but still use the bead solution; do alternative heat lysis
2. DNA elutes in 100 µl

Day 5

Step 5.

Day 5

1. Put some DNA into a new tube for a working sample:
    1. Check microbial contamination with qPCR 16S analyses (3 µl)
    2. Quantify DNA with PicoGreen or Qubit (2 µl)
    3. NanoDrop for DNA purity (2 µl)
2. 50 µl needed for shearing if using Swift kit (for ssDNA viruses; otherwise Nextera XT for dsDNA viruses)
3. 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.