Jul 21, 2025

Soil Metavirome Protocol

Forked from a private protocol

Microbiology Resource Announcements

DOI
https://dx.doi.org/10.17504/protocols.io.5qpvodyxbg4o/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.5qpvodyxbg4o/v1

External link: https://doi.org/10.1128/mra.00908-25

Protocol Citation: Kelli Feeser, Reid Longley, La Verne Gallegos Graves, Michaeline Albright, Migun Shakya 2025. Soil Metavirome Protocol. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvodyxbg4o/v1

Manuscript citation:
Feeser K, Longley R, Gallegos-Graves LV, Albright M, Shakya M Recovering new viruses from New Mexico soils. Microbiology Resource Announcements 14(11). doi: 10.1128/mra.00908-25

Protocol status: Working

Created: July 21, 2025

Last Modified: July 21, 2025

Protocol Integer ID: 222916

Keywords: Metagenome, Metavirome, Soil, shotgun metagenomic sequencing, metavirome from soil sample, metagenomic sequencing, soil metavirome protocol protocol, extracting bulk metagenome, bulk metagenome, filtered metavirome, soil sample

Abstract

Protocol for extracting bulk metagenome and size-filtered metavirome from soil samples for shotgun metagenomic sequencing.

Soil Collection

1 Soil collection: remove rock and leaf material from soils before using sterilized instruments to collect soil from a 15×15×15 cm area

1.1 Transport soils in cooler back to laboratory for further processing

2 Homogenize soils and sieve with a 2mm sieve

3 Weigh 30g of sieved soil for DNA extraction.

Preparation of Protein Supplemented Phosphate-buffered Saline (PPBS) Elution Buffer

4 Combine the following: 10 mL of 10x phosphate-buffer saline (Fisher scientific), 10g of monohydrate potassium citrate (Spectrum Chemical), 18.05g of anhydrous magnesium sulfate (Fisher Scientific), add sterile water until the volume reaches 1L.

5 Adjust pH to 6.5

6 Add 20g of nuclease and protease-free bovine serum albumin ("BSA", VWR) while stirring the solution on a stir plate.

7 Filter the solution through a 0.22 µm vacuum filter, store at 4 °C.

Size Filtration

8 Split 30g of sieved soil from step 3 into two 50 mL Falcon tubes

9 Suspend soils in 1:1 weight/volume (w/v) PPBS buffer

10 Manually shake for 30s followed by 30s of vortexing

10.1 Repeat step 10 four times

11 Shake suspended samples on an orbital shaker at 300 RPM for 40 minutes at room temperature (RT).

12 Refrigerate samples at 4 °C overnight.

13 The next day, manually shake samples for two minutes

14 Centrifuge samples for 5 min at 5000 RPM at 4 °C

15 Remove supernatant and filter liquid through sterile fine mesh (0.5mm)

16 Resuspend pellet in 1:1 w/v PPBS buffer, using sterile scoopula to physically mix soil with the elution buffer

17 Shake Samples on orbital shaker at 300 RPM for 40 minutes at RT

18 Centrifuge samples for 5 min at 5000 RPM at 4 °C

19 Collect supernatant. Perform pellet resuspension and centrifugation total of three times.

20 Collect supernatant after each centrifugation and combine supernatants for each soil sample

21 Centrifuge combined supernatants three times for 10 min at 5000 RPM.

22 After each centrifugation, transfer supernatant to new tube and discard the pellet.

23 Following centrifugation steps, sequentially filter supernatant liquid through a 25 µm cellulose filter (Cytiva WhatmanTM Qualitative Filter Paper: Grade 4 Circles, Fisher Scientific), 11 µm filter paper (Cytiva WhatmanTM Qualitative Filter Paper: Grade 1 Circles, Fisher Scientific), 3 µm, (Cytiva WhatmanTM Qualitative Filter Paper: Grade 6 Circles, Fisher Scientific), 1 µm (Cytiva Qualitative Grade Plain Filter Paper Circles - P5 Grade, Fisher Scientific), 0.45 µm (FisherbrandTM Disposable PES Filter Units, Fisher Scientific), and finally 0.22 µm syringe-driven PES filters (MilliporeSigma).

23.1 Set aside 550 µL aliquots of the 11 µm filtrate for bulk DNA extraction

24 Store size-filtered liquid overnight at 4 °C.

Viral Concentration

25 Combine 25 ml of the 0.22 µm filtrate with 10 µL of 0.02 µm filtered 0.1g/mL FeCl3 in sterile 26.3 ml polycarbonate tubes

26 Ultracentrifuge for 3 hr at 35,000 RPM under a vacuum.

27 Carefully decant tubes and reserve the supernatant. Do not disturb the pellet.

28 Add 10 µLof FeCl3 solution and ultracentrifuge with the same conditions as step 26.

29 Resuspend pellets in 400 µL of ultra pure water by incubating pellets overnight at 4°C.

30 Following overnight incubation, mix remaining pellet material with sterile scoopula and pipette into new tube

31 Add an additional 200 µL of ultra-pure water. Further mix with scoopula and by pipetting liquid up and down.

DNA Extraction

32 Treat viral enriched samples (step 32) with 15 µL RNase-free DNase (Qiagen, Cat No. 79254) and 60 µL of the accompanying buffer

33 Incubate at RT on a 600 RPM shaker for 15 mins

33.1 Remove tubes and manually invert every two minutes

34 Inactivate DNase by adding 10 µL EDTA, incubate for 10 min at 65 °C

35 Extract bulk community samples (step 23.1) and viral enriched samples (step 35) within 48 hours using DNeasy PowerSoil Kits (Qiagen, Germantown, MD) with the full volume of available sample following manufacturer instructions.

36 Elute viral enriched samples twice in 100 µL of ultra-pure water, elute bulk community samples twice in 50 µL ultra-pure water.

Sample QC

37 Assess DNA concentration using Qubit DNA Assay Kit (ThermoFisher Scientific, Cat. # Q32854)

38 Assess DNA quality by running the sample on an E-Gel 1% agarose gel (ThermoFisher, Cat# G402001) with Lambda DNA/ HindIII Marker (ThermoFisherScientific, Cat. #FER SM0103).

Library Preparation and Sequencing

39 Prepare Illumina libraries with the NEBNext Ultra DNA II Library Preparation Kit (New England Biolabs, Cat. #E7645L).

40 Fragment DNA with a Covaris E220, blunt ends, and add adapters and indexes to the ends of the fragments for sequencing.

41 Elute Illumina libraries in DNA Elution Buffer (Zymo Research, Cat. #D3004-4-10)

42 Assess concentration and size using the Agilent D5000 Assay (Agilent, Cat. #5067-5588, 5067-5589).

43 Accurately quantify library with the Library Quantification Kit – Illumina/Universal Kit (KAPA Biosystems, KK4824).

44 Normalize libraries to the same concentration based on qPCR results.

45 Sequence libraries with the Illumina NextSeq generating paired-end 151 bp reads using the NextSeq 500/550 High Output Kit v2.5 (300 cycles) (Illumina, Cat. #20024908).