MAR 09, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.j8nlkw29xl5r/v1

Protocol Citation: Adair Borges 2023. Virome harvesting from cheese microbiomes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkw29xl5r/v1

MANUSCRIPT CITATION:

Protocol status: Working
We use this protocol and it's working

Created: Feb 21, 2023

Last Modified: Mar 09, 2023

PROTOCOL integer ID:
77397

Virome harvesting from cheese microbiomes

Forked from Phage amplification and concentration

In 1 collection

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ABSTRACT

This protocol details how we harvest the viral component, or virome, from cheese rind microbiomes. These viromes can be banked at 4 °C for short-term storage or -80 °C for long-term storage. Alternatively, one can extract DNA or RNA from these viromes for downstream sequencing or chemical analysis.

Keywords: phage, gDNA, virus, virome, microbiome, cheese, cheese rind, storing viromes, viral storage

Virome extraction from cheese microbial community

1 Move a 0.5–1 g (chickpea-sized) sample of cheese rind to a 50 mL conical tube. Add 40 mL SM buffer (bioWORLD 41920012). Try to break up cheese rind by pipetting up and down. Vortexing could help, but don't be overly aggressive because you might destroy the phages.

2 Once the cheese sample is fully suspended, add 1 mL of chloroform to the sample. Close the conical tube, and seal the top with Parafilm. Shake the sample on a platform shaker for 1–2 h at room temperature.

3 Move the samples to a fresh 50 mL conical tube, as chloroform can weaken plastic. Next, spin the sample down at 10,000 x g for 10 min. Move the supernatant to a new conical tube, avoiding the pellet. Spin at 18,000 x g for 20 min. If the supernatant is clear, move to next step. If it is not, continue spinning at 18,000 x g for 10 min intervals.

Virome concentration

4 To concentrate the virome sample, we have used both PEG precipitation and a filter-concentrator-based protocol. The PEG protocol requires an overnight incubation, but the filter-concentrator generally requires more hands-on time. Use whichever phage concentration protocol works best for your circumstances.

Step 4 includes a Step case.

Phage precipitation with PEG

Filter-concentrator based phage prep

step case

Phage precipitation with PEG

PEG prep requires an overnight incubation step at 4 °C.

5 To PEG-precipitate phage, first prepare 5× PEG precipitation solution containing 2.5 M NaCl and 20% w/v PEG8000.

Note

5× PEG solution (500 mL)

5 M NaCl stock solution – 250 mL
PEG8000 – 100 g
DI water – to 500 mL

Stir until dissolved. Sterilize with with 0.22 µm filter. Store at room temperature.

6 Add PEG precipitation solution to the phage lysate to obtain a 1× concentration of 0.5 M NaCl and 4% w/v PEG8000. Mix by inverting the tube several times and refrigerate overnight at 4 °C.

7 After the overnight incubation with PEG precipitation solution at 4 °C, pellet the PEG-precipitated phage particles by centrifuging at 19,000 × g for 60 min at 4 °C. A small white pellet should form at the bottom of the 50 mL conical tube.

8 Remove the supernatant, being careful not to disturb the PEG-precipitated phage pellet.

9 Resuspend the PEG-precipitated phage pellet in 300 µL SM buffer. This sample may be stored for the short term at 4 °C, or -80 °C for long-term storage. If storing at -80 °C, store in 20% glycerol with 1× PBS. You can also extract DNA or RNA directly from these samples.