Virome Extraction V.1

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FOOD Micro UCPH

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This protocol is for isolation of bacteriophages from fecal matter. It is based on the protocol used for the $COPSAC_{10}$ cohort and was originally described by Ling Deng in doi:10.3390/v11070667. Extraction is performed using centrifugation and ultrafiltration to isolate viral particles and get rid of contaminants.

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Frej Larsen 2021. Virome Extraction. **protocols.io** https://dx.doi.org/10.17504/protocols.io.byzzpx76

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This protocol is designed for extraction of bacteriophages from fecal samples. The sample should be 50-500mg of fecal material diluted in buffer (2xSM buffer for instance). Sample sizes outside these limits have not been tested and correct sequencing results can not be guaranteed.

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Equipment:

- Pipettors
- Shaking table
- Refrigerated centrifuge
- Ice box

Consumables:

- Crushed ice
- 15 ml centrifuge tubes
- 10 ml pipettes
- 0.45 um PES filters
- SM buffer (200 mM NaCl, 10 mM $MgSO_4$, 50 mM Tris-HCl, pH 7.5)

Fill bucket with crushed ice

Turn on centrifuge and start the fast temp program to reach 4° C

Cool SM buffer in ice bucket

1 Thaw frozen sample in ice bucket

2 Transfer up to **500 mg** of sample to centrifuge tube and add SM buffer to reach a final volume of **5 mL** . Place centrifuge tube back in ice box

3 Homogenize samples by placing ice box on shaking board at **300 rpm, 00:10:00**

4 Centrifuge samples at **5000 rcf, 4°C, 00:30:00** 30m

5 Filter the supernatant through a 0.45 um PES filter into a new centrifuge tube

6 Transfer **2.5 mL** of the sample to the outer chamber of the concentrator

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7 Centrifuge sample in the concentrator at **1500 rcf, 4°C, 00:20:00** 20m

8 Pour off liquid from the inner chamber

9 Repeat the three previous steps until all liquid from the centrifuge tube has passed through the concentrator and the there is no liquid left in the inner chamber

10 Add 100 ul SM buffer to the inner chamber and centrifuge **1500 rcf, 4°C, 00:02:00** 2m

11 Cut off filter from bottom of inner chamber using a sterile scalpel and transfer to new eppendorf tube. Transfer remaining liquid from outer chamber to the eppendofr tube

12 Leave in fridge at least 12 hours and at most for 1 week before proceeding with spotting or DNA/RNA extraction

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