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VLP Extraction from Fecal Samples V.2

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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 COPSAC₁₀ 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. VLP Extraction from Fecal Samples. **protocols.io**
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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.

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 MgSO₄, 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

- 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 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 Leave in fridge to release VLPs from filter for at least 12 hours and at most for 1 week before proceeding with spotting or DNA/RNA extraction