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MECH step CsCl density gradients

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Abstract

For use in **Generating viral metagenomes from the coral holobiont**.

1 CsCl solutions were made with solid molecular-grade CsCl (Sigma-Aldrich) dissolved in 0.02 µm filtered (Anotop, Whatman) SM buffer.

2 A 3 mL cushion of 1.6 g mL^{-1} CsCl was added to the bottom of a 13.2 mL UltraClear™ ultracentrifuge tube (Beckman Coulter).

3 Add 2.5 mL of 1.45 g mL^{-1} density on top of the 1.6 g mL^{-1} layer.

4 Add 2.5 mL of 1.3 g mL^{-1} density.

5 Add 2 mL of 1.2 g mL^{-1} density.

6 The density of sample homogenate supernatant was adjusted to 1.12 g mL^{-1} with CsCl.

7 2 mL of sample was placed on top of the layered gradient.

8 Gradients were then centrifuged in an Optima XL-80K ultracentrifuge (Beckman Coulter) in a swinging bucket rotor (SW 41 Ti, Beckman Coulter) for 2.5 h at 40,000 rpm and 4°C.
02:30:00

9 Fractions (0.5 mL) from the gradients were collected in 1.5 mL tubes using an 18 bore gauge needle and luer-lok syringe, puncturing the tube ~1 mL from the bottom.

10 The density of fractions was determined gravimetrically and DNA concentration of each fraction was measured using a Quant-It Picogreen dsDNA High Sensitivity assay kit (Invitrogen, Life Technologies).

11 Diafiltration and buffer exchange were performed to remove CsCl salts.

Note

Their presence may interfere with downstream processing, such as DNA extraction.

12 Fractions containing the nucleic acid peaks were pooled and buffer exchange was performed with Amicon® centrifugal spin columns (30 kDa, Millipore) against 0.02 µm

filtered SM buffer.

13 The diafiltrated sample was then filtered using a 0.2 µm pore size Durapore® syringe filter to remove remaining contaminating bacteria.