MECH step CsCl density gradients

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Abstract

For use in Generating viral metagenomes from the coral holobiont.

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Protocol

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

Step 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).

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

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

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

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

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

Step 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.

DURATION
02:30:00

Step 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.

Step 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).

Step 11.

Diafiltration and buffer exchange were performed to remove CsCl salts.

NOTES

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

Step 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.

Step 13.

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