Isolation and Purification of DNA from Chlorella Viruses

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Abstract

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Guidelines

Materials:

1) *Chlorella* virus, in 50 mM Tris-HCl, pH 7.8

2) 100 mM Tris-HCl, pH 7.4, 10 mM EDTA, 1 M NaCl (10X TEN, pH 7.4)

3) 1.0% Na sarcosyl

4) 10 mM Tris-HCl, pH 8.0, 1 mM EDTA (1X TE)

5) Isopropanol, saturated with CsCl/TE buffer

6) 3 M NaOAc

7) Hoechst dye #33258, 100 µg/mL
8) CsCl gradients: 40-60% (w/w) CsCl gradients made up in SW60 rotor ultra clear tubes, equilibrated with 1X TE, pH 8.0 + 1 µg/mL Hoechst dye (see recipe)

%	gm CsCl	ml 1X TE	µl Hoechst dye (100 µg/mL)	layer (µl)
60	9.0	6.0	60.0	1000
50	7.5	7.5	75.0	1050
40	5.0	7.5	75.0	1050

9) 60% CsCl (w/w): 9.0 gm CsCl, 6.0 ml 1X TE, pH 8.0, 120.0 µL Hoechst dye (100 µg/mL)

Protocol

Step 1.
For each gradient, in 100 x 13 mm tubes, mix together 500 µL of virus, 60 µL of 10X TEN, pH 7.4, and 60 µL of 1% Na sarcosyl.

Step 2.

Add 600 µL of 60% (w/w) CsCl to each tube.

Step 3.
Heat the tubes at 75°C for 15 min.

DURATION
00:15:00

Step 4.
Layer the samples onto pre-formed 40-60% (w/w) CsCl gradients in SW60 rotor tubes (to make a final 30-60% gradient).

Step 5.
Add 1200 µL of the heated virus to each gradient.

Step 6.
Centrifuge the gradients in SW60 rotors at 35,000 rpm, 18 hours, 25°C.

DURATION
18:00:00

Step 7.
Remove the DNA bands from the gradients with a wide bent needle to silicon-coated 30 mL corex tubes.

Step 8.
Extract the Hoechst dye from the DNA by adding an equal volume of CsCl/TE-saturated isopropanol to the DNA solution, mixing gently by inversion, centrifuge for 1 min at 3,000 rpm in the Sorvall to separate the phases, and pipet off the upper isopropanol layer.

DURATION
00:01:00

Step 9.
Repeat last step.

Step 10.
Add 1.0 mL of 3 M NaOAc to each tube and adjust the volume of each tube to 10.0 mL with 1X TE buffer.

Step 11.
Precipitate the DNAs with 2X volumes of 100% EtOH.

Step 12.
Mix well and hold at -20°C overnight.

DURATION
18:00:00

Step 13.
Centrifuge the DNA samples in the Sorvall HB-4 rotor at 10,000 rpm, 20 min, 4°C.

DURATION
00:20:00

Step 14.
Discard the supernatants.

Step 15.
Dry the pellets briefly (10-15 min) in the vacuum desiccator to remove the EtOH.

DURATION
00:15:00

Step 16.

Resuspend the DNA samples with approximately 500 µL of 1X TE buffer.

Step 17.
Determine the DNA concentrations and store the DNAs at 4°C.