Chlorovirus DNA Miniprep Procedure

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Abstract

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Guidelines

MATERIALS:

1) 60-65°C heat block or water bath
2) Microfuge
3) 1.5 and 2.0 mL microfuge tubes (screw-cap)
4) 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$
5) 10% NP-40 or Triton X-100
6) DNAse I, 2.0 mg/mL in 50 mM Tris-HCl, pH 8.0.
   Store in aliquots at -20°C. Do not refreeze unused material, discard.
7) Proteinase K, 2.0 mg/mL in 50 mM Tris-HCl, pH 8.0.
   Solution can be thawed and frozen several times, but should be discarded after that.
8) 10% Na sarcosyl
9) $CHCl_3$:Isoamyl alcohol (24:1)
10) 500 mM EDTA, pH 8.0
11) 3 M NaOAc
12) 100% EtOH
13) Tris buffer-saturated phenol
14) 10 mM Tris-HCl, pH 8.0, 1 mM EDTA (1X TE)

Protocol

Step 1.
Infect appropriate host with desired *Chlorovirus* species at moi of 0.001-0.01.

Step 2.
Incubate the samples at 25°C for 24-72 hours, with continuous light and shaking.

DURATION
12:00:00

Step 3.
Centrifuge 30 mL of the lysates in the Sorvall SS34 rotor at 5,000 rpm (3,000 rcf), 5 min, 4°C.

DURATION
00:05:00

Step 4.

Save the supernatants.

Step 5.
Add 10% NP-40 (or Triton X-100) to the lysate supernatants for a final concentration of 1%.

Step 6.
Centrifuge the material in a Beckman Ti 50.2 rotor at 15,000 rpm (27,000 rcf_{max}), 75 min, 4°C.

DURATION
01:15:00

Step 7.
Discard the supernatants.

Step 8.
Resuspend the virus pellets with 1.0 mL of 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$.

Step 9.
Transfer 500 µL of the resuspended virus to 1.5 ml screw-cap microfuge tubes.

Step 10.
Add 9 µL of DNAse I and mix.

Step 11.
Incubate at room temperature for 60 min.

DURATION
01:00:00

Step 12.
Add 6.0 µL of 500 mM EDTA, pH 8.0 to the samples and mix.

Step 13.
Add 57 µL of proteinase K and 29 µL of 10% Na sarcosyl and mix.

Step 14.
Incubate the samples at 60-65°C for 60 min.

DURATION
01:00:00

Step 15.
Add 300 µL of buffer-saturated phenol and 300 µL of $CHCl_3$:Isoamyl alcohol (24:1) to the tubes.

Step 16.
Mix by inversion.

Step 17.
Centrifuge in the microfuge at maximum speed for 5 min at 4°C.

DURATION
00:05:00

Step 18.
Remove the upper aqueous layers to clean tubes.

Step 19.
Add 600 µL of $CHCl_3$:Isoamyl alcohol (24:1) to the tubes.

Step 20.
Mix by inversion.

Step 21.
Centrifuge for 5 min at 4°C in the microfuge.

DURATION

00:05:00

Step 22.
Remove the upper aqueous layers to clean tubes and repeat the $CHCl_3$:Isoamyl alcohol extraction 1X.

Step 23.
Place the last extraction into 2.0 mL microfuge tubes.

Step 24.
Add 66 µL of 3 M NaOAc to each tube.

Step 25.
Precipitate the DNAs with 2X volumes (approximately 1350 µL) of 100% EtOH.

Step 26.
Mix well and hold at -20°C overnight.

DURATION
18:00:00

Step 27.
Centrifuge the tubes in the microfuge for 10-15 min at 4°C to pellet the DNAs.

DURATION
00:15:00

Step 28.
Discard the supernatants.

Step 29.
Wash the DNA pellets 1X with 1000 µL of 70% EtOH in the microfuge for 5 min at 4°C.

DURATION
00:05:00

Step 30.
Dry the pellets briefly (10-15 min) in the vacuum desiccator to remove the EtOH.

DURATION
00:15:00

NOTES
Irina Agarkova 14 Apr 2016
Optional: Use speed vac (5 min) instead of vacuum desiccator to remove the EtOH.

Step 31.
Resuspend the DNAs with approximately 60 µL of 1X TE buffer.

Step 32.
If the DNA doesn't go into solution overnight, centrifuge in the microfuge for 15 min at 4°C.

DURATION
00:15:00

Step 33.
Remove the supernatants to clean tubes.

Step 34.
Discard the pellets.

Step 35.
Store the DNAs at 4°C.