Viral 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) Triton X-100
6) DNAse I, 2.0 mg/mL in 50 mM Tris-HCl, pH 8.0. Store in 110 µL 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. Autodigest for 60 min at 37°C before use. Store in 1.0 mL aliquots at -20°C. Can be refrozen unless the material has fallen out of solution.

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) Buffer-saturated phenol

- Preparation: Thaw 100 gm bottles of phenol at 60-65°C. Add a stir bar, 100 mL of 500 mM Tris-HCl, pH 8.0 and 0.1 gm 8-hydroxyquinoline. Stir and allow the phases to separate at 4°C overnight. Aspirate off the upper aqueous layer and add 75 ml of 100 mM Tris-HCl, pH 8.0, 0.2% 2-mercaptoethanol (2-ME) and stir. Allow the phases to separate at 4°C for several hours to overnight and remove the upper aqueous layer. Repeat the 75 mL addition 2X, leaving the final

phase on the phenol. Store at 4°C.

14) 10 mM Tris-HCl, pH 8.0, 1 mM EDTA (1X TE)

Protocol

Step 1.

Infect 60 mL of chlorella with 200 µL of viral single plaque isolates.

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. Save the unused portion of the lysates.

Step 5.

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

Step 6.

Centrifuge the material in Beckman Ti50.2 rotors at 15,000 rpm (27,000 rcfmax), 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 MgCl2.

Step 9.

Transfer 350 µL of the resuspended virus to 1.5 mL screw-cap microfuge tubes and adjust the final volume to 500 µL with 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$.

Step 10.

Add 8.8 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 56.6 µL of proteinase K and 29.0 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 and centrifuge for 5 min at 4°C in the microfuge.

DURATION
00:05:00

Step 21.
Remove the upper aqueous layers to clean tubes and repeat the $CHCl_3$:Isoamyl alcohol extraction 1X.

Step 22.
Place the last extraction into 2.0 mL microfuge tubes.

Step 23.
Add 66 µL of 3 M NaOAc to each tube.

Step 24.
Precipitate the DNAs with 2X volumes (approximately 1350 µL) of 100% EtOH.

Step 25.
Mix well and hold at -20°C overnight.

DURATION
18:00:00

Step 26.
Centrifuge the tubes in the microfuge for 10-15 min at 4°C to pellet the DNAs.

DURATION

00:15:00

Step 27.
Discard the supernatants.

Step 28.
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 29.
Dry the pellets briefly (10-15 min) in the vacuum desiccator or the speed vac (5 min) to remove the EtOH.

DURATION
00:15:00

Step 30.
Resuspend the DNAs with approximately 60 µL of 1X TE buffer. If the DNA doesn’t go into solution overnight, centrifuge in the microfuge for 15 min at 4°C and remove the supernatants to clean tubes.

Step 31.
Discard the pellets.

Step 32.
Store the DNAs at 4°C.