Viral DNA Miniprep Procedure

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VERVE Net

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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 MgCl₂
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) CHCl₃: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)

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

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

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

4  Save the supernatants. Save the unused portion of the lysates.

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

6  Centrifuge the material in Beckman Ti50.2 rotors at 15,000 rpm (~27,000 rcfmax), 75 min, 4°C.

7  Discard the supernatants.

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8 Resuspend the virus pellets with 1.0 mL of 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂.

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 MgCl₂.

10 Add 8.8 µL of DNAse I and mix.

11 Incubate at room temperature for 60 min.

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

13 Add 56.6 µL of proteinase K and 29.0 µL of 10% Na sarcosyl and mix.

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

15 Add 300 µL of buffer-saturated phenol and 300 µL of CHCl₃:isoamyl alcohol (24:1) to the tubes.

16 Mix by inversion.

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

18 Remove the upper aqueous layers to clean tubes.

19 Add 600 µL of CHCl₃:isoamyl alcohol (24:1) to the tubes.

20 Mix by inversion and centrifuge for 5 min at 4°C in the microfuge.
21. Remove the upper aqueous layers to clean tubes and repeat the CHCl₃-Isoamyl alcohol extraction 1X.

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

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

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

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

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

27. Discard the supernatants.

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

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

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.

31. Discard the pellets.

32. Store the DNAs at 4°C.

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