

## Cucumber Mosaic Virus Purification

1. Harvest systemically infected leaf tissue, avoiding stems and major veins, and weigh.
2. Place the fresh tissue in a blender jar. For  $\geq 15$  g of tissue, use a small blender jar. For 15-200 g of tissue use a large blender jar. For small preps use a polytron or a mortar and pestle (Do not add the chloroform until after grinding if using mortar and pestle).
3. For each gram of tissue add, ice cold, 1 ml of Buffer A (to which you have just added the TGA!) and 1 ml of chloroform.
4. Blend at slow speed until all the tissue has passed through the blades, then blend at high speed until smooth (about 30 sec-1 min).
5. Pour into centrifuge bottle, and spin at 15,000 X G (10 K in the GSA rotor) 4°C, for 10 min.
6. Remove the aqueous phase carefully and pass through a dampened miracloth filter, keeping cold.
7. Aliquot into ultracentrifuge rotor tubes. Leave enough space to add a 5 ml cushion. If the volume exceeds the rotor capacity you can concentrate the virus first with a PEG precipitation.
8. Underlay the samples with 5 ml of cold Buffer A + 10% sucrose.
9. Spin in the T1250 rotor at 35,000 rpm for 1.5 hours.
10. Pour off the supernatant and add 3-4 ml of cold Buffer B to the pellets (The volume depends on the size of the pellets, and the number of tubes you plan to spin the following day.)
11. Let the pellets sit overnight at 4°C in Buffer B.
12. Vortex each tube briefly, and combine the tubes into a flask. Add a stirbar, and stir at 4°C for at least 2 hours.
13. Centrifuge the resuspended virus at 7500 X g, 4°C, 10 min.
14. Immediately pour off the supernatant into fresh ultracentrifuge tubes. For small preps you may want to switch to the T1270 rotor for the second spin.

15. Underlay the samples with 5 ml of Buffer C + 10% sucrose.
16. Centrifuge as in step 9.
17. Pour off the supernatant and add 4-5 ml of cold Buffer C. Resuspend the pellets overnight at 4°C.
18. To store as virus, add sterile glycerol to 50%, and store at -20°C.
19. To extract the viral RNA, add an equal volume of VEBA.
20. Extract with an equal volume of Phenol:Chloroform. Use the 50 ml disposable plastic tubes (orange caps are least likely to leak) and the wrist action shaker, for 15 minutes, followed by 5 minutes of centrifugation in the table top centrifuge.
21. Take most of the interface in the first extraction (This amounts to a back extraction).
22. Do at least three extractions.
23. Transfer the final supernatant to a Corex tube, and add 1/10th volume of 3 M NaOAc, and ethanol precipitate with 2.5 volumes of EtOH.
24. Do at least one additional ethanol precipitation, washing the pellet thoroughly with 70% EtOH. The second precipitation can be done in an eppendorf tube. Resuspend the final pellet in 0.1 mM EDTA and store at -20°C. Very clean RNA can easily be resuspended at 10 mg/ml. Freezing and thawing may help get it into solution at high concentrations.

#### CMV purification buffers

Buffer A: 0.5 M Sodium Citrate, pH6.5-7.0  
5 mM EDTA  
0.5% Thioglycolic acid (add just before use)

Buffer B: 5 mM Sodium Borate pH 9.0  
0.5 mM EDTA  
2% Triton X 100

Buffer C: 5 mM Sodium Borate pH 9.0  
0.5 mM EDTA

VEBA: 200 mM Tris pH8.5  
1 M NaCl  
1 % SDS  
2 mM EDTA