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PURIFICATION OF PEANUT STUNT VIRUS

1. Remove infected leaf tissue with a clean razor blade; do not harvest stems or large mid-veins (tobacco).

2. Weigh tissue and place in a chilled blender. Add 2 ml of cold PSV extraction Buffer and 2 ml of cold chloroform for each gram of tissue. Blend on low speed for about one minute, or until all the tissue is drawn into the blades. Blend at high speed for two minutes. Blend at low speed for an additional 30 seconds.

3. Pour the homogenate into a 250 ml plastic bottle, using a funnel. Keep the bottle on ice. Centrifuge at 15,000 X g (10,000 rpm in the SLA-1500 rotor) for 10 minutes, at 4°C.

4. Remove the aqueous phase carefully with a 25 ml pipette, and strain through one layer of damp Miracloth. Be very careful not to take any of the organic phase.

5. Aliquot the extract into ultracentrifuge tubes. Carefully layer 5 ml of sucrose cushion I under the extract in each tube.

6. Centrifuge at 40,000 rpm for 2 hour, 4°C.

7. Remove your tubes from the rotor, and pour the supernatant into the sink. Allow the tubes to drain for 1-2 minutes. Add 3-6 ml of water to each pellet, cover the tubes with Parafilm, and let them sit overnight at 4°C.

8. Briefly vortex your virus pellets in water from the previous day. Add CMV Buffer A to 10% (ie. FC = 50 mM sodium Citrate, 0.5 mM EDTA), and Triton X 100 to 2%.

9. Pour the contents of your tubes into a small erlenmeyer flask and place a stir bar in the flask, cover the flask with Parafilm, and stir in the cold room for 2 hours

10. Pour the virus extract into a COREX tube, and centrifuge at 7500 X g (6550 rpm) in the HB-6 (swinging bucket) rotor for 10 minutes, 4°C. Immediately pour the supernatant into an ultracentrifuge tube. Layer 5 ml of sucrose cushion II under the extract in your tube. Centrifuge as above (Step 6).

11. Resuspend pellet in water; store in 10% CMV Buffer A. The volume depends on the pellet size. Check with Marilyn until you feel confident to judge yourself.

12. Refer to the CMV Purification Protocol (#43.0), steps #18 - #25 for GlycerolStorage and RNA Extraction procedures.

Buffers

<u>Extraction Buffer:</u>

0.1 M Sodium citrate, pH 7
20 mM EDTA
0.1 % thioglycolic acid (add immediately <u>before</u> use)

<u>CMV Buffer A:</u>

0.5 M Sodium citrate pH 7
5 mM EDTA

<u>Sucrose cushion I:</u>

0.1 M Sodium citrate pH 7
1 mM EDTA
10% sucrose

<u>Sucrose cushion II:</u>

50 mM Sodium citrate pH 7
0.5 mM EDTA
10 % sucrose

All buffers should be used cold.