

13. RNA extraction: Phenol saturated with 10 mM Tris-HCl pH 8.0. Saturated phenol:chloroform (1:1). Distillated ethanol. Sterile 5M NaCl. Sterile dH<sub>2</sub>O. Microcentrifuge tubes.

### 3. Method

#### 3.1. Virus Isolation

1. For the inoculum, grind heavily infected *C. quinoa* leaves in a cold mortar in 50 mM inoculum buffer. About 30 mL of buffer is used for five leaves. This amount of inoculum is sufficient for inoculation of 60 *C. quinoa* plants, which will yield approx 200 g of infected leaves (*see Note 1*) for virus extraction. Be careful to maintain the inoculum on ice at all times. Leaves sprinkled with Celite are mechanically inoculated with a wad of cotton saturated with inoculum. When all the plants are inoculated, wash the leaves with water in order to eliminate excess inoculum and Celite.
2. After inoculation, the plants are maintained for 9–13 d in the greenhouse (*see Note 2*) in the following conditions: 16 h light at 22–24°C, 8 h dark at 16–18°C, with 65–85% humidity. Infected leaves are harvested, weighed, and homogenized in the blender in the presence of 170 mL cold extraction buffer and 100 mL cold CCl<sub>4</sub> for 100 g of leaves (*see Note 3*). Add the leaves little by little at low speed, and, when all the leaves are in the blender, cover it and blend at high speed for 2–3 min to obtain a uniform homogenate.
3. Decant the homogenate into the 0.5-L centrifuge tubes and centrifuge at 9000g for 30 min at 4°C.
4. Filter the supernatant through Miracloth into a big beaker or other recipient. Measure the volume and then add (with stirring) solid NaCl, and then solid PEG, to concentrations of 0.8 and 2%, respectively.
5. Let the mixture precipitate for at least 2 h in the cold room, with gentle stirring.
6. Pour the liquid into the 0.25-L centrifuge tubes and centrifuge at 17,000g for 20 min at 4°C.
7. Resuspend the pellets in 100 mL (total volume) of 10 mM extraction buffer and let the virus resuspend overnight at 4°C, with gentle stirring. The following day, centrifuge the viral suspension in 30-mL centrifuge tubes at 5000g for 20 min (*see Note 4*) to remove debris.
8. Combine the supernatants and repeat the precipitation (0.8% NaCl, 2% PEG) for 2 h at 4°C.
9. Centrifuge the viral suspension in the 30-mL centrifuge tubes at 5000g for 30 min. The pellets are resuspended in 35 mL (total volume) of 10 mM extraction buffer overnight, with gentle stirring at 4°C.
10. The viral suspension is again clarified by 30 min centrifugation at 5000g at 4°C and the supernatant is retained.
11. Two 25-mL ultracentrifuge tubes are each filled with 7 mL of a 20% sucrose cushion, upon which 17.5 mL of the supernatant is overlaid. The tubes are centrifuged for 2 h at 100,000g at 4°C.