6. RNA electrophoresis separating buffer (0.5X TBE buffer): 0.045*M* Tris, 0.045*M* boric acid, 0.001*M* EDTA, pH 8.0.
7. Polyethylene glycol (PEG) solution: 30% (w/v) of PEG, average mol wt 8000, in water.

3. Methods

3.1. Virus Propagation

1. Arrange a set of pots filled with a soil mixture (PRO-MIX R6). Equal the top by pressing with another pot.
2. Water the soil.
3. Put 10–20 barley seeds on top and cover with a 1-cm layer of soil. Press with another pot and water.
4. Put pots into greenhouse and keep in a full-light condition, below 25°C. If necessary, use artificial light with a photoperiod of 16 h light/8 h dark.
5. Prepare virus inoculum by grinding 1 g of infected leaves (taken from another plant) with a mortar and pestle in 2 mL of inoculation buffer. Add a 600-mesh Carborundum powder to the inoculum.
6. Dust the two- to three-leaf stage barley plants with Carborundum and inoculate the virus by rubbing each leaf with gloved fingers moistened with inoculum. The leaves must be rubbed hard, but not hard enough to damage the leaves.
7. Using a household sprayer, spray the inoculated plants with water immediately after inoculation to prevent leaf dehydration.
8. Keep the inoculated plants in greenhouse for another 2 wk.

3.2. Virus Purification (see Note 1)

1. Collect those leaves that show systemic symptoms (a mosaic) of BMV infection. Do not collect inoculated leaves.
2. Grind infected leaf tissue in chilled mortar using pestle and crushed glass, with 1 mL extraction buffer per gram of tissue (*see* **Note 1**).
3. Transfer into a centrifuge tube (e.g., Corex glass tube or plastic Oakridge tube) and add 0.2 mL chloroform/g tissue, then emulsify by vortexing for 30 s.
4. Centrifuge for 5 min at 5000*g* in a low speed centrifuge at 4°C. Use a precooled rotor.
5. Remove supernatant, avoiding the interface, and filter it through a coarse filter paper or three layers of Miracloth.
6. Add one-third vol of 30% PEG, stir for 1 min, and leave on ice for 30 min.
7. Collect the precipitate by centrifugation for 10 min at 12,000*g*.
8. Dissolve the pellet in 0.2 mL storage buffer per gram of the original tissue and emulsify again with 0.4 mL of chloroform per 1 mL of virus solution.
9. Centrifuge 5 min at 12,000*g* and discard the supernatant.
10. Leave the virus pellet on ice overnight in 0.2 mL of storage buffer per gram of the original tissue to resuspend the virus.
11. Ultracentrifuge the dissolved pellet for 2 h at 100,000*g*.