9. Ammonium carbonate buffer (pH 9.0): 200 m*M* ammonium carbonate, 2% (w/v) sodium dodecyl sulfate (SDS), 2 m*M* disodium EDTA and 200 mg/mL bentonite.
10. Kirby's phenol: Take a 500-g bottle of crystalline phenol and add 50 mL m-cresol, 0.5 g 8-OH quinoline, and 0.1*M* Tris-HCl, pH 8.8, to saturation. Store in a dark bottle at 4°C.
11. Phenol:chloroform (1:1): Mix equal volumes of Kirby's phenol and chloroform and store in a dark bottle at 4°C.
12. 3*M* sodium acetate, pH 4.8: 24.6 g sodium acetate in 75 mL H_2O, pH to 4.8 with glacial acetic acid, and make up to 100 mL with H_2O.
13. TAE: 40 m*M* Tris-acetate, pH 7.5, 1 m*M* EDTA. 20X stock solution: 96.8 g Tris base, 22.8 mL glacial acetic acid, 40 mL 0.5*M* EDTA in 1 L. Dilute to 1X prior to use.

3. Methods

3.1. Virus Purification

Extensive work on hordeivirus purification was initially carried out by Myron Brakke. The purification described here is based on this methodology and incorporates minor modifications to the method described by Jackson and Brakke ***(15)***.

1. Collect BSMV-infected plant tissue, approx 7–10 d postinoculation. Use immediately, or store at 4°C for up to a week (*see* **Note 1**).
2. Cut the leaves into approx 2-cm pieces and extract in borate buffer (300 g tissue: 300 mL buffer). Usually 100 g of tissue are blended in 300 mL of buffer in a Waring blender at high speed for 2 min, or until the tissue is ground into a fine slurry.
3. Squeeze the extract through several layers of cheesecloth to remove the macerated pulp.
4. The filtrate is returned to the blender, another 100 g of tissue are added, and the extraction is repeated. The resulting filtrate is then used for a third round of extraction. The final volume of the filtrate should be 350–400 mL.
5. Centrifuge the filtrate at 3000*g* for 10 min.
6. Remove the supernatant and measure the volume. Then add 0.05 vol of 20% (v/v) Triton X-100 to the supernatant and stir for 10 min at room temperature to dissociate membranes and chloroplast material.
7. Add 7 mL of the 20% (w/v) sucrose pad to twelve 30-mL polycarbonate centrifuge tubes. Carefully layer the tissue extract over the sucrose pad, causing as little disturbance to the sucrose pad as possible. Centrifuge at 180,000*g* for 1 h at 4°C in a fixed-angle rotor.
8. Decant the supernatants, including the sucrose pad, and resuspend each pellet in 4.5 mL of 50 m*M* potassium phosphate buffer, pH 6.8. To ensure that the pellets are suspended, a glass homogenizer is used to disperse the insoluble material into a fine suspension. Combine the resuspended pellets in a beaker and stir at 4°C for 30 min.