9. Centrifuge at 7000*g* at 4°C for 15 min, decant the ethanol, and drain the tube well.
10. Resuspend the pellet in 1 mL H_2O and add 3 mL of 4*M* sodium acetate, pH 6.0, and incubate on ice for 2 h. This step precipitates single-stranded RNA but tends to solubilize protein and DNA contaminants that are insoluble in ethanol.
11. Centrifuge as before, drain the pellet well, and resuspend the pellet in 2 mL H_2O. **Note:** This pellet is more difficult to resuspend than the previous ones, so a larger volume of H_2O is added. Add 2 vol of ethanol, plus 0.05 vol of 3*M* sodium acetate, pH 4.8, incubate at –20°C, and centrifuge as before.
12. Resuspend the pellet in 500 µL H_2O and determine the concentration of RNA by measuring the absorbance at 260 nm and scan the sample from 210–310 nm. 1 OD at 260 nm is equal to a concentration of 40 µg/mL RNA.
13. To check that the RNA is not degraded, analyze a portion on a 1% (w/v) agarose gel prepared in 1X TAE.

4. Notes

1. Hordeivirus particles tend to aggregate because the particles have a strong negative charge and, hence, polycations will interact with them. When performing the virus purification, try to complete it in 1 d, because aggregation of the particles increases as the time used for purification increases.
2. If BSMV is stored in 5% ethylene glycol at –20°C, it remains infectious for many years.

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